MOLECULAR GENETIC ANALYSIS OF FAMILIAL CONGENITAL HEART DISEASE

by

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ABSTRACT

Development of the human heart is a complex process controlled by multiple genes (in interacting pathways), many of which are still to be determined. Abnormal heart development results in a spectrum of congenital heart disease (CHD), occurring in isolation or part of a syndrome, and with or without a family history, implying a genetic basis in some individuals. In this project I investigated the molecular genetic basis of CHD, in 23 families with non-syndromic CHD. Using autozygosity mapping, I initially investigated the molecular basis of CHD in a single large consanguineous family (CHD1), and identified a region of interest containing a candidate gene (*GDF1*). I proceeded to sequence *GDF1* (and genes in the same developmental pathway - *NODAL*, *CFC1, TDGF1,* and *FOXH1*) in 9 kindreds, but did not identify any pathogenic mutations. I then utilised whole exome sequencing (WES) to identify candidate mutations in potential CHD genes (*GMFG, WNT11* and *DVL2*), and investigated these further by conventional sequencing. A novel *GMFG* nonsense variant was validated in family CHD1 and was absent from ethnically matched controls. Bioinformatics analysis of WES data from 19 affected individuals from 9 kindreds did not identify a frequently mutated candidate gene (or further *GMFG* candidate mutations), though candidate variants in individual kindreds were identified. Further functional analysis using animal models is required to determine the pathophysiological effect of the *GMFG* truncating mutation in cardiac development.

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ABBREVIATIONS

AA	Aortic arch
AD	Autosomal dominant
AR	Autosomal recessive
AS	Aortic stenosis
ASD	Atrial septal defect
ATP	Adenosine triphosphate
AV	Atrioventricular
AVSD	Atrioventricular septal defect
BAV	Bicuspid aortic valve
BGI	Beijing genomics institute
BLAST	Basic local alignment search tool
Bp	Base pairs
BT	Blalock–taussig
CHD	Congenital heart disease / defect
cHTZ	Compound heterozygous
cM	CentiMorgan
CNV	Copy number variant
CoA	Coarctation of the aorta
dbSNP	Comprehensive SNP database
DECIPHER	DatabasE of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources)
DNA	Deoxyribonucleic acid
dNTP	Dideoxynucleotides
DORV	Double outlet right ventricle
ECHO	Echocardiogram
EDTA	Ethylenediaminetetraacetic acid
EVA	Exome variants analysis
FHF	First heart field
FISH	Fluorescent *in situ* hybridisation
H_2O	Water
HLHS	Hypoplastic left heart syndrome
HMZ	Homozygous
HTZ	Heterozygous
IAA	Interrupted aortic arch
IV	Interventricular
Kb	Kilobases
KOMP	Knock out mouse project
LMD	London medical databases
LPM	Lateral plate mesoderm
LV	Left ventricle
LVOTO	Left ventricular outflow tract obstructions
Mb/MB	Megabase
MGI	Mouse genome informatics
miRNA	MicroRNA
NCBI	National centre for biotechnology information

NHS	National Health Service
OMIM	Online mendelian inheritance in man
PA	Pulmonary atresia
PAPVD	Partial anomalous pulmonary venous drainage
PCR	Polymerase chain reaction
PDA	Patent ductus arteriosus
PPAS	Peripheral pulmonary artery stenosis
PS	Pulmonary stenosis
RNA	Ribonucleic acid
RV	Right ventricle
SHF	Second heart field
SNP	Single nucleotide polymorphism
TALEN	Transcription activator-like effector nuclease
TAPVD	Total anomalous pulmonary venous drainage
TBE	Tris-borate/EDTA
TGA	Transposition of the great arteries
TOF	Tetralogy of fallot
TrA	Truncus arteriosus
UCSC	University of California, Santa Cruz
UNISTS	Comprehensive database of sequence tagged sites (STS)
VSD	Ventricular septal defect
WES	Whole exome sequencing
WTSI	Wellcome trust sanger institute
ZFN	Zinc finger nuclease
B	Beta
γ	Gamma

CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction and overview

'With the advent of modern genetic analysis techniques, congenital heart disease research will no longer be limited by sequencing or genotyping capacity' (Andelfinger and Khairy, 2011)

The formation of the heart involves a precisely orchestrated series of molecular and morphogenetic events and subtle changes of this process can have serious consequences in the form of congenital heart disease (CHD). This is a major cause of childhood morbidity and mortality worldwide, and despite advances in clinical and surgical management, understanding the aetiology of CHD remains incomplete. Advances have been made in understanding the molecular aspects of normal heart development, including the identification of transcriptional regulators, signalling molecules, and structural genes. Animal models have significantly aided in identifying the molecular mechanisms of abnormal heart development, however more research needs to be directed in investigating these in humans to better understand the pathogenesis of human CHD. The improved resolution of cytogenetic analysis, common use of fluorescent *in situ* hybridisation (FISH) analysis, and addition of molecular techniques (Comparative Genomic Hybridisation arrays, linkage analysis, whole exome sequencing), have allowed advancements in localising and detecting loci critical for heart development in humans. Identifying and understanding the molecular functions, interactions and pathways involved in heart development, will ultimately benefit families and clinicians to improve clinical diagnosis with diagnostic tests, provide accurate genetic counselling on recurrence risks, and pave the way for potential therapeutic interventions.

1.2 Normal heart development

The cardiovascular system (heart and blood vessels) is the first major organ system to function in the developing embryo. The heart begins to beat at day 22-23 and blood flow begins during the fourth week (Moore and Persaud, 1998). Establishment of left-right asymmetry is also very important for normal heart development.

1.2.1 Primordial heart tube

The primitive streak develops at day 13, and the primordium of the heart is mainly derived from the splanchnic mesoderm which appears around day 18 of embryonic development. Cells from the anterior lateral plate mesoderm (which contributes to most of the visceral organs) fuse at the primitive streak to form a cardiac crescent. As the embryo folds laterally, the cells of the crescent coalesce along the ventral midline to form a single primitive heart tube. The inner cells of the heart tube become the endothelial lining of the heart (endocardium), and an external layer of cells become the muscular wall of the heart (myocardium). As the embryo continues to fold the heart tube comes to lie ventrally to the foregut. The linear heart tube is segmentally patterned into precursors of the atria, ventricles and outflow tracts. It continues to elongate and develop dilatations and constrictions (i.e. truncus arteriosus, bulbous cordis, ventricle, atrium, and sinus venosus). The arterial and venous ends of the heart are fixed by other embryonic structures, and the bulbous cordis and ventricle grow faster than the other nearby regions, resulting in the heart tube bending upon itself (rightward looping) to form a U-shaped bulboventricular

loop. The atrium and sinus venosus end up lying dorsal to the truncus arteriosus, bulbous cordis, and ventricle. This heart looping process is complete by day 28 (Figures 1 & 2) (Moore and Persaud, 1998; Srivastava, 2006).

1.2.2 Partitioning of the primordial heart and chamber development

Partitioning of the atrioventricular (AV) canal, atrium and ventricle all occur concurrently from the middle of the fourth week and are completed by the end of the fifth week. The endocardial cushions form from the dorsal and ventral walls of the AV canal and fuse dividing the AV canal into the right and left AV canals. These AV canals partially separate the primordial atrium from the ventricle.

The primordial atrium is divided into right and left atria by the formation, modification and fusion of two septa (septum primum and septum secundum). The septum primum grows towards the fusing endocardial cushions from the roof of the primordial atrium, thereby partially dividing it into right and left atria. The area between the endocardial cushion and the free edge of the septum primum is called the foramen primum, which eventually closes as the septum primum fuses with the endocardial cushion. Just before the foramen primum closes, perforations in the septum primum appear creating another opening (foramen secundum). The septum secundum then grows from the roof of the atria (to the right of the septum primum) towards the endocardial cushion, gradually overlapping the foramen secundum and the septum primum, resulting in the formation of the foramen ovale which allows blood entering the right

atrium to pass to the left atrium in one direction only (before birth). After birth the foramen ovale closes resulting in a complete partition between the atria.

Division of the primordial ventricle begins with the formation of the muscular primordial interventricular (IV) septum in the floor of the ventricle near its apex. Initially the growth in the IV septum is due to the dilatation of the ventricles on either side of the IV septum, and then active proliferation of myoblasts in the septum results in the final increase in size. The gap between the growing IV septum and endocardial cushion is called the IV foramen, which allows blood to flow between the two ventricles. Proliferation of the mesenchymal cells in the walls of the bulbous cordis results in the formation of the right and left bulbar ridges. Fusion of the right and left bulbar ridges and the endocardial cushion results in the closure of the IV foramen (usually by the end of the seventh week) and the formation of the membranous part of the IV septum. This membranous IV septum develops as an extension of tissue from the endocardial cushion to the muscular IV septum and also merges with the aorticopulmonary septum (see later). This is completed by end of week 7 (Figures 3 & 4) (Moore and Persaud, 1998).

1.2.3 Partitioning of the bulbous cordis and truncus arteriosus

In the fifth week of development, ridges (similar to the bulbar ridges) develop in the truncus arteriosus and are continuous with the bulbar ridges. Both the bulbar and truncal ridges undergo a 180 degree spiralling motion resulting in the formation of a spiral aorticopulmonary septum when the ridges fuse. This

septum creates two arterial channels – the aorta and the pulmonary trunk. This spiralling also twists the pulmonary trunk around the ascending aorta. Therefore once the IV foramen has closed and the membranous IV septum fully formed (see before), the pulmonary trunk communicates with the right ventricle and the aorta communicates with the left ventricle. This is completed by end of week 7 (Figure 5) (Moore and Persaud, 1998).

1.2.4 Valve development

Once partitioning of the truncus arteriosus is almost complete, the semilunar valves begin to develop from three swellings of subendocardial tissue located around the openings of the aorta and pulmonary trunk. These swellings reshape to form three thin-walled cusps. The atrioventricular valves develop in a very similar manner from swellings of the tissue around the AV canals (Moore and Persaud, 1998).

Figure 1: Drawings of views of the developing heart (20-28 days).
Taken from Moore & Persaud (1998)

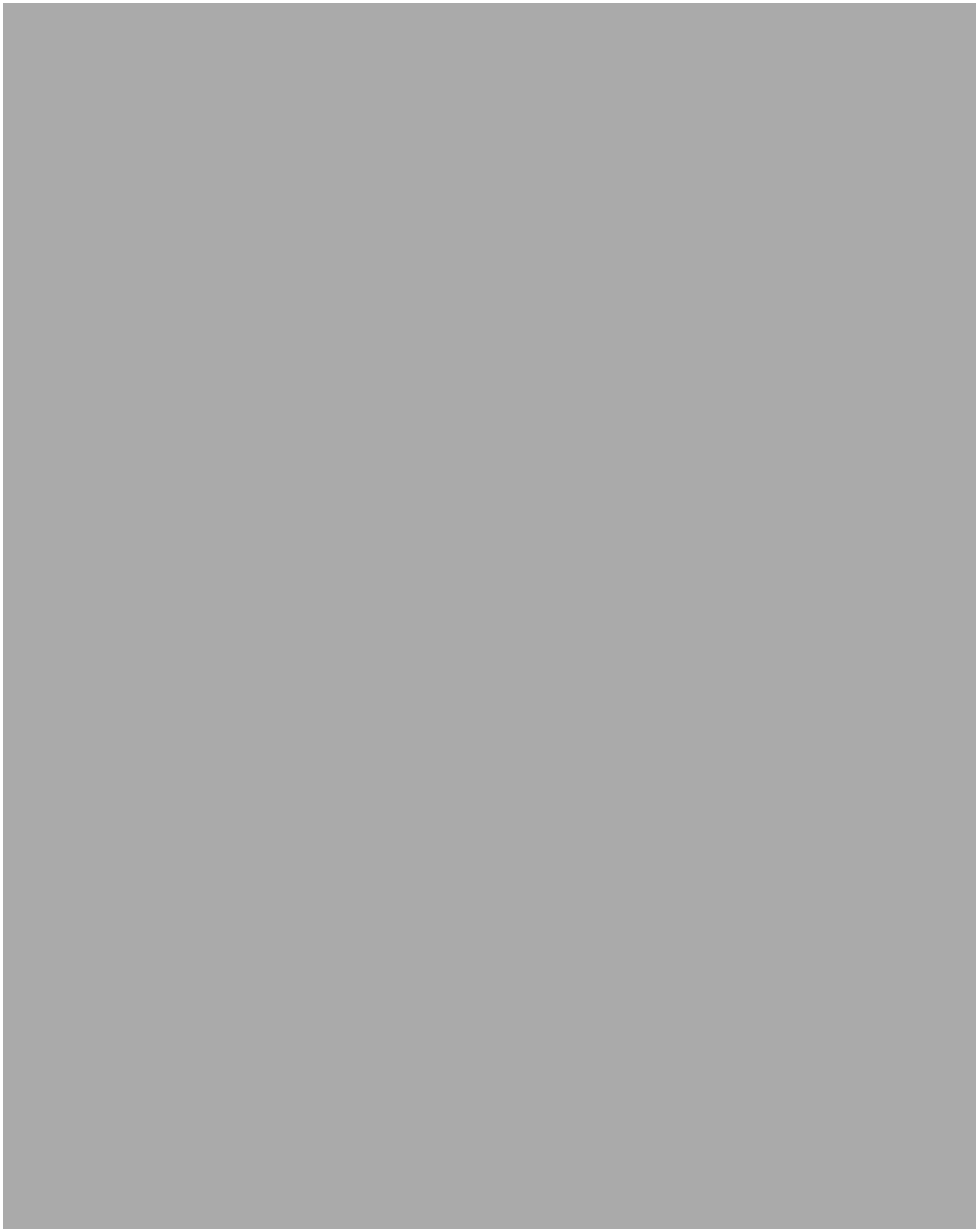


Figure 2: Drawings of ventral views of the developing heart (22-35 days).
Taken from Moore & Persaud (1998)

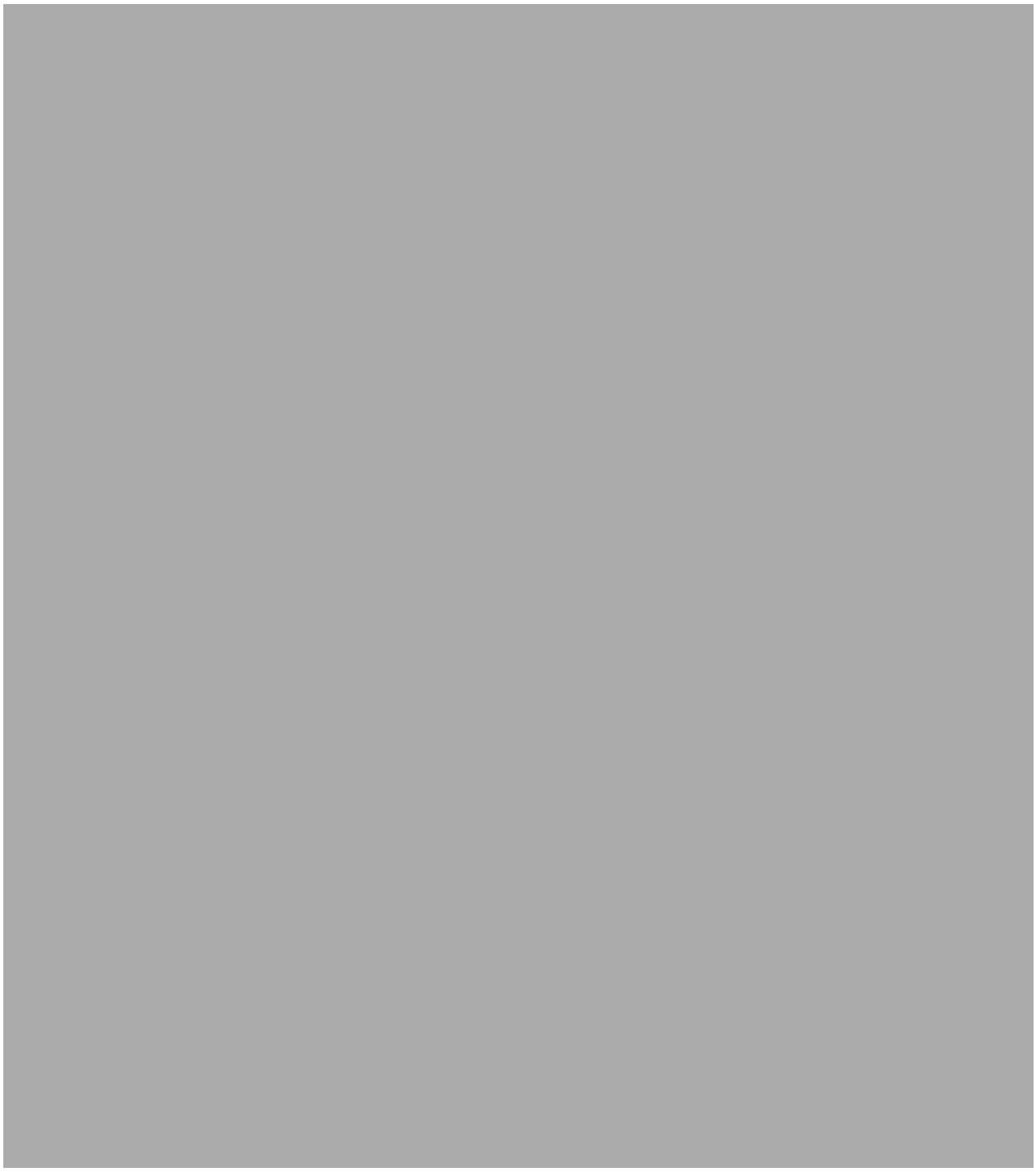


Figure 3: Drawings the heart during the fourth and fifth weeks.
Taken from Moore & Persaud (1998)

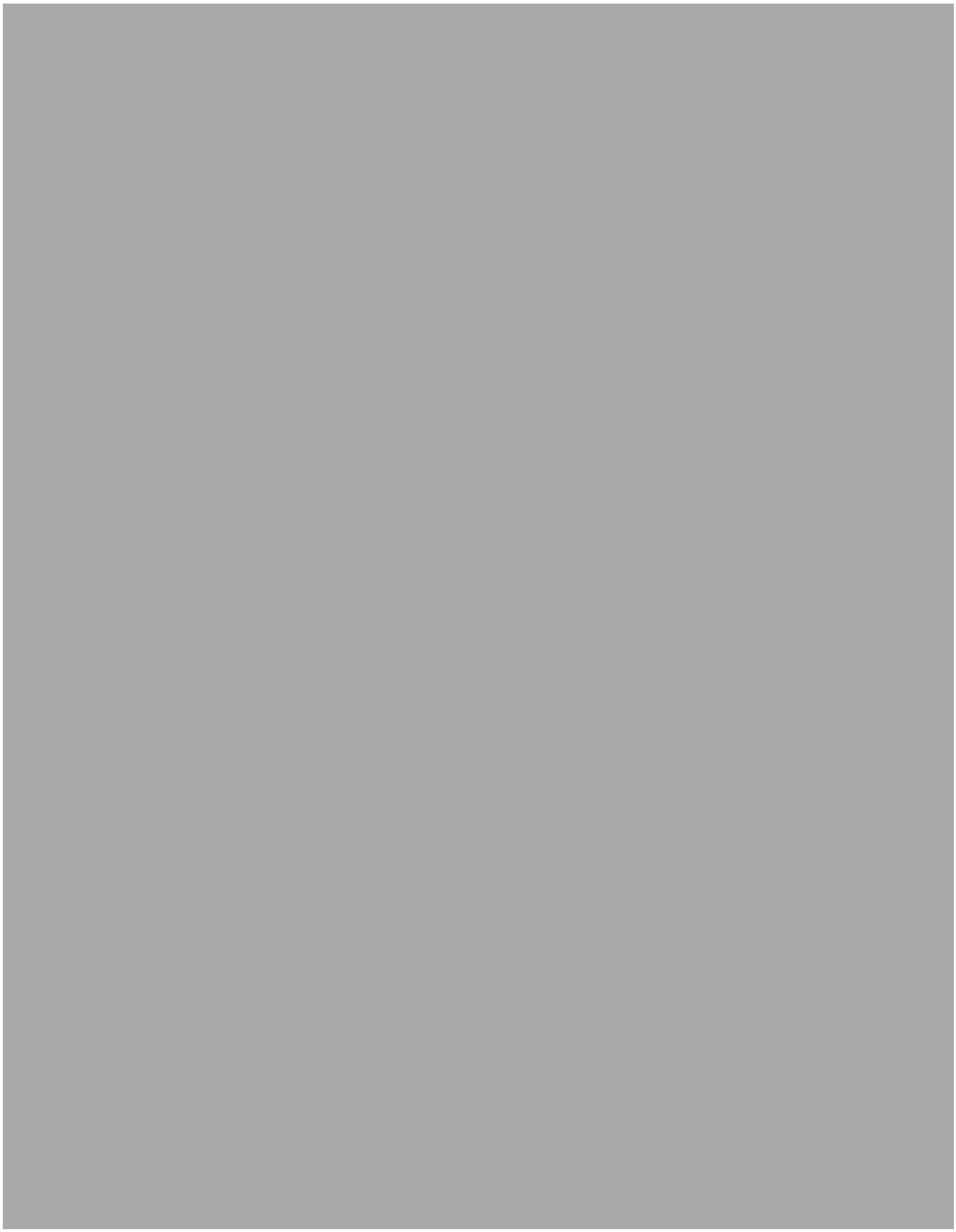


Figure 4: Drawings illustrating partitioning of the primordial atrium, ventricle and atrioventricular canal.
Taken from Moore & Persaud (1998)

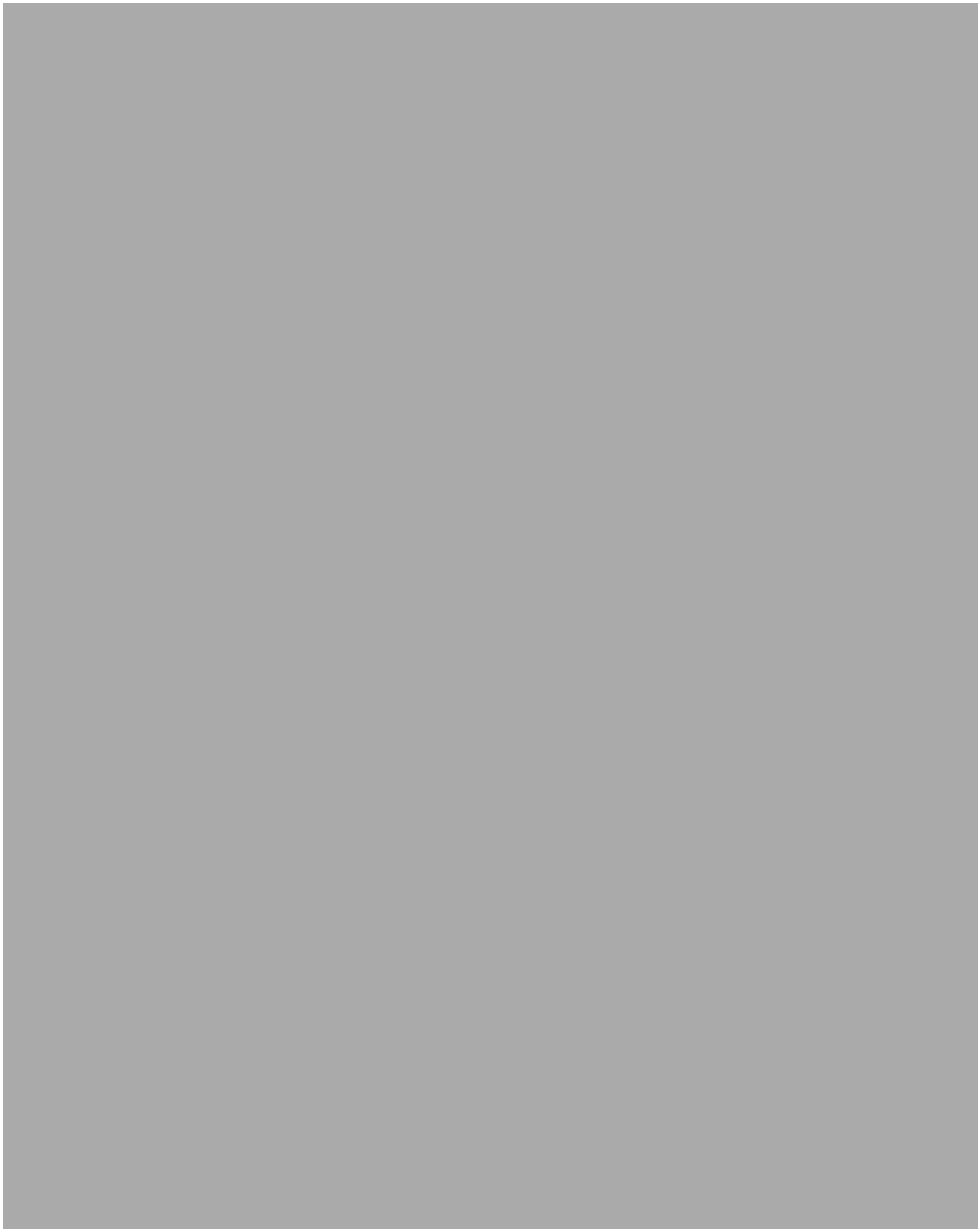
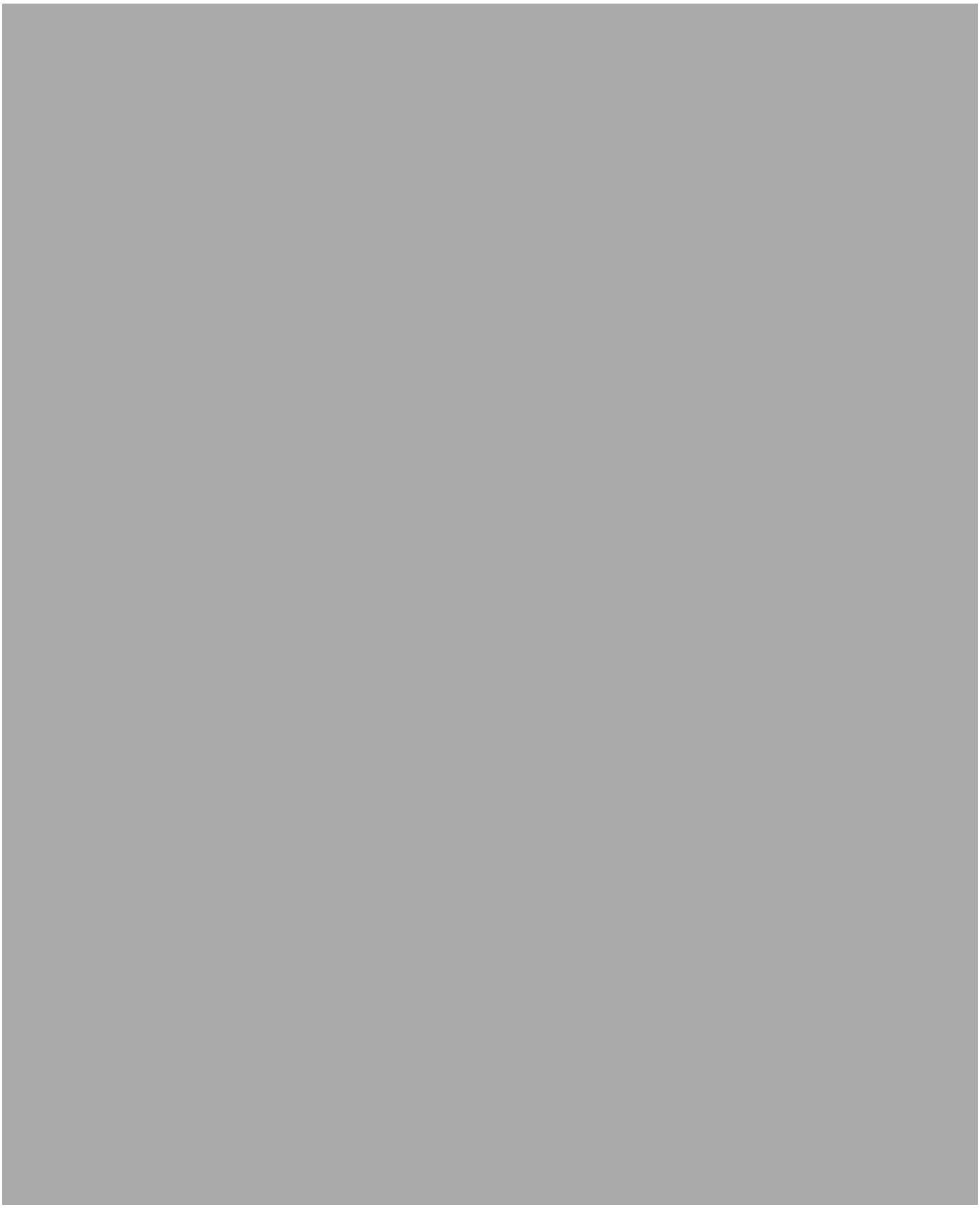


Figure 5: Drawings illustrating partitioning of the bulbous cordis and truncus arteriosus.
Taken from Moore & Persaud (1998)



1.2.5 Normal heart circulation

Deoxygenated blood returns from the body via the superior and inferior vena cavae, which connect to the right atrium. Blood flows from the right atrium through the tricuspid valve into the right ventricle. From the right ventricle, it flows through the pulmonary valve in the right ventricular outflow tract into the main pulmonary artery, which then branches into the left and right pulmonary arteries, taking blood to the respective lungs. Four pulmonary veins (two from each side) return oxygenated blood from the lungs to the left atrium. It then flows to the left ventricle via the mitral valve, and then through the aortic valve in the left ventricular outflow tract into the aorta and to the rest of the body (Figure 9).

1.2.6 Heart fields, Networks and Pathways

1.2.6.1 Heart fields

The complex process of heart development described above requires the contribution of numerous cell types in a precise and coordinated manner. The current model of heart development suggests several distinct groups of myocardial progenitor cells (the First Heart Field and Second Heart Field) which contribute spatially to the development of the mature heart in a highly regulated fashion (Figure 6) (Srivastava and Olson, 2000). The First Heart Field (FHF) consists of splanchnic mesoderm cells in the anterior lateral plate mesoderm, which form the linear heart tube and the left ventricle, via the formation of the cardiac crescent. The Second Heart Field (SHF) consists of undifferentiated myocardial progenitor cells of the pharyngeal mesoderm (medial and caudal to

the FHF), which migrate into the arterial and venous parts of the primitive heart tube and contribute to the development of the outflow tracts, right ventricle, and the inflow region (i.e. the atria). A third heart field has been identified consisting of cells located ventral to the heart tube, which will give rise to the venous pole (i.e. the periphery of inflow tracts) (Andelfinger, 2008). The cardiac neural crest cells represent a subdivision of the cranial neural crest cells (from the dorsal neural tube). They migrate from the neural tube and form the smooth muscle of the great arteries and have a role in the remodelling of the six pairs of pharyngeal arch arteries that later become the ascending aorta, proximal subclavian, carotid, pulmonary arteries, and ductus arteriousus. They also migrate to the outflow tract and contribute to the spiral aortopulmonary septum, separating the truncus into the aorta and pulmonary artery (Jiang et al., 2000; Clark et al., 2006). The final lineage of cardiac precursor cells are derived from the proepicardium (which develops from the mesothelium overlaying the liver bud). They extend towards the primitive heart, attach, and spread over the myocardial surface to form the epicardium and the coronary vessels (Yamagishi et al., 2009).

1.2.6.2 Heart networks and pathways

Experiments in animal models (mice, zebrafish, and frogs) have led to the identification of numerous genes involved in normal heart development. Cells derived from the FHF and SHF appear to be regulated by complex signalling pathways involving members of the bone morphogenetic protein (BMP), sonic hedgehog (SHH), fibroblast growth factor (FGF), Wnt, and Notch proteins, and

a highly conserved network of transcription factors. The core regulators appear to be the transcription factors: NKX2, GATA, TBX, and MEF2, but numerous other transcription factors contribute to heart development by acting as accessory factors to these core regulators (Figure 7) (Srivastava, 2006; Jing-Bin et al., 2009).

The right ventricle is enriched in a transcription factor called Hand2 (or dHAND) which is required for its expansion, and disruption of this factor leads to right ventricular hypoplasia in mice. Right ventricular hypoplasia is also observed in mice lacking *Mef2c* which is a target of *Isl1, Gata4, Foxh1,* and *Tbx20* in the SHF. This suggests these factors are important for SHF cells to expand into the right ventricle. Sonic hedgehog (Shh) regulates the expression of *Tbx1*, through forkhead-containing transcription factors (like Foxc1/Foxc2). *Tbx1* is known to be a central SHF transcriptional regulator which is necessary for the development of the outflow tracts. Mice lacking *Tbx1, Foxc1, Foxc2,* or *Shh* show similar outflow tract anomalies. *Tbx1* also regulates the production of fibroblast growth factors (like *Fgf8*), which activate receptors on adjacent neural crest-derived cells affecting their differentiation, which as described previously are also important in outflow tract development (Srivastava, 2006). *Hand1* (or eHAND) which is closely related to *Hand2*, is expressed in the FHF and mostly in the left ventricle. Its expression depends on Nkx2.5 in the left ventricle suggesting *Nkx2.5* is critical for FHF development. Many of the transcription factors found in the FHF are also present in the SHF (Nkx2.5 and Gata4) implying they also contribute to SHF development (Srivastava, 2006).

The Notch intracellular signalling pathway plays an important role in the development of the endocardial cushions that contribute to valve formation. In humans there are four Notch family receptors (NOTCH1-4) with their ligands encoded by the Jagged (*JAG1* and *JAG2*) and Delta-like (*DLL1, DLL3,* and *DLL4*) gene families. (Jing-Bin et al., 2009); The BMP signalling pathway (via BMP, ALK3, BMPR4, and SMAD6) is involved in valve and outflow tract development (Tan et al., 2012). Details regarding the NODAL and WNT pathways are described later. Disruption of all of these signalling pathways can lead to a variety of genetic causes of CHD (see later).

Figure 6: Frontal views of cardiac precursors of heart development.
Taken from Srivastava (2006)

Figure 7: Pathways regulating cardiac morphogenesis.
Taken from Srivastava (2006)

1.2.7 Left-Right patterning

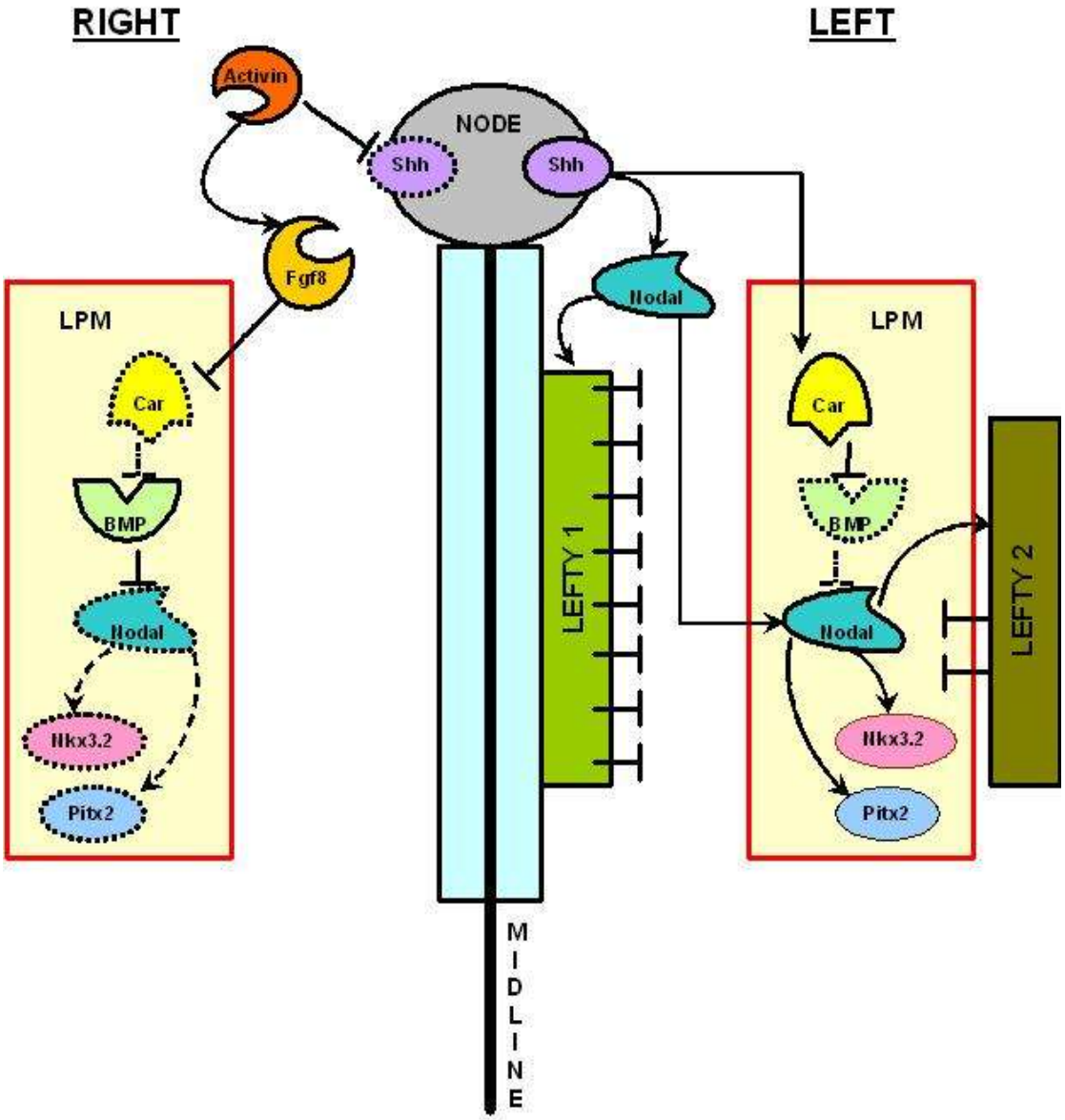
The embryonic body plan is initially symmetric and the first indication of patterning the left-right axis in humans is the rightward looping of the midline heart tube. The proper folding of the straight heart tube aligns the atria with their appropriate ventricles, and the right and left ventricles with the pulmonary trunk and aorta respectively. The normal positioning of the organs on the left or right side of the chest and abdomen relies on normal left-right axis patterning. This intricate change on body plan must rely on highly conserved genetic pathways that control determination of the left-right axis. There are three major steps involved in establishing the left-right axis: (a) node-dependent symmetry breaking, (b) establishment of embryonic midline, and (c) node-dependent signalling in the left lateral plate mesoderm (Clark et al., 2006).

Several signalling molecules have been found to regulate the left-right asymmetry of the embryo. Hensen's node controls the establishment of asymmetric gene expression throughout the lateral plate mesoderm (LPM) (Figure 8). Asymmetric expression of *Shh* at the left nodal region leads to perinodal expression of *Nodal* and *Lefty* in the left lateral plate mesoderm, and of Caronte (*Car*). As *Car* expression extends to the left lateral plate mesoderm, it inhibits Bone Morphogenetic Proteins (BMPs), which normally inhibit Nodal and Lefty activity. Nodal induces the expression of *Nkx3.2* and *Pitx2* (transcription factors) in the left lateral plate mesoderm. On the right side, an Activin receptor mediated pathway inhibits Shh expression and thereby it inhibits expression of *Nodal* and *Lefty* on the right side. This is done by a

pathway that upregulates Fibroblast growth factor 8 (Fgf8) which in turn inhibits *Car* expression. The block of Car activity in the LPM allows BMP to further block any Nodal activity on the right side, and reduces levels of Nkx3.2 and Pitx2. Nodal establishes expression of *Lefty1* in the midline and *Lefty2* in the LPM, which both act as barriers of Nodal to maintain its expression on the left side and prevent spread to the right side. The two pathways on the left and right (Nodal and Activin) ultimately result in a net expression of *Pitx2* on the left side of the embryo and this induces rightward looping of the primitive heart tube, and is also sufficient to establish the left-right asymmetry in the developing heart, lungs, and gut (Kathiriya and Srivastava, 2000; Garg, 2006).

Figure 8: Diagram illustrating components of left-right patterning.
Adapted from (Kathiriya and Srivastava, 2000; Brand, 2003; Shiratori and Hamada, 2006)

Active parts of the pathway are marked in full black lines & arrows, and inactive parts are marked in dotted black lines & arrows. Components with increased expression have full back borders, and those with decreased expression have dotted black borders. LPM: lateral plate mesoderm; Shh: sonic hedgehog; Fgf8: fibroblast growth factor 8; Car: caronte; BMP: bone morphogenetic protein.



1.2.8 Epigenetic factors and heart development

The regulation of cardiac development through complex signalling pathways is well studied and defined (as described above), but a more in depth understanding of the regulatory mechanisms of gene expression is still required. Epigenetics refers to functionally relevant modifications to the genome caused by mechanisms other than changes in the underlying DNA sequence. The most well described epigenetic factors are those affecting gene regulation at the chromatin level. Chromatin is a dynamic structure composed of nucleosomes (147 base pairs wrapped around an octameric core of histone proteins), and the compact organisation requires regulatory mechanisms to alter its structure and prepare genomic loci for recombination, repair, replication, and transcription. Three mechanisms controlling chromatin structure are: (a) nucleosome positioning (by chromatin remodelers), (b) histone modification (by histone modifiers), and (c) DNA methylation (by DNA modifiers) (van Weerd et al., 2011).

Numerous factors in these mechanisms have been described in cardiac development, including evidence based on animal models with CHD, and some have also been identified in human diseases associated with syndromic CHD (Chang and Bruneau, 2012; Vallaster et al., 2012). Chromatin remodelers (containing an ATPase subunit) can restructure nucleosomes (move, eject, destabilise, or alter the composition) via the energy from ATP hydrolysis. Mutations in a chromatin remodeler (*CHD7*) are associated with CHARGE syndrome, an autosomal dominant condition characterised by Coloboma of the

eye, Heart defects, Atresia of the choanae, Retardation of growth and/or development, Genitourinary abnormalities, and Ear abnormalities and deafness (MIM 214800). *CHD7* is essential for orchestrating neural crest cell gene expression programs, the cells which migrate to regulate cardiac outflow tract development (malformations of which are seen in this syndrome) (Bajpai et al., 2010). Histone modifiers (by altering the histone-DNA contacts via covalent modification of histone proteins) loosen or tighten the chromatin to regulate the availability of genes to transcription factors. This can occur via many chemical processes such as methylation, phosphorylation, ubiquitination, and acetylation. Kabuki syndrome (MIM 147920) and Kleefstra syndrome (MIM 610253) are autosomal dominant conditions characterised by a number of congenital defects, including cardiac defects, and mental retardation. They are caused by mutations in histone methyltransferases (*MLL2* – Kabuki syndrome, *EHMT1* – Kleefstra syndrome) which impair histone methylation and effect the expression of target genes (Ogawa et al., 2002; Ng et al., 2010b). It is therefore evident that chromatin regulators (of structure and function) exert their effects in a tissue-specific and time-specific manner, and can control cardiac development.

More recently microRNAs (miRNAs), which are small non-coding RNAs have been shown to have a role in cardiovascular development and function. They are suspected to control cardiac gene expression, due to their expression in specific cardiac cell types and the effects on known cardiac transcription factors with over expression of micro-RNAs in animal models. They are evolutionarily conserved, act at the post-transcriptional level, and can be categorised into

intergenic, intronic and exonic miRNAs. Studies indicate that individual miRNAs may not function as essential on-off switches, but may act as fine tuners of gene expression in cardiac development (Cordes et al., 2010; Chen and Wang, 2012).

1.2.9 Haemodynamic factors and heart development

The pattern of blood flow in the developing heart has been proposed to play a role in heart development, and been termed the ‘no flow-no grow’ hypothesis, suggesting that the amount of flow through a structure can determine the extent of its development (Andelfinger, 2008). Normal blood flow is needed for remodelling and growth of the myocardium and heart valves, as well as the vascular structures. Vascular remodelling during cardiovascular development is an adaptation of the vascular network (through feedback mechanisms in the vascular endothelial cells) to a wide range of both chemical (hypoxia and nutritional requirements) and mechanical cues from blood flow (shear stress and circumferential stretch), resulting in differential expression of vascular developmental genes (Jones, 2011). It has been shown that decreased shear forces during blood flow of a developing zebrafish embryo can result in a number of cardiac phenotypes. Disrupting either the inflow or outflow (altering the shear stress) led to either: (a) collapse and fusion of the walls of the inflow and outflow tracts impairing valve formation, (b) lack of bulbous cordis formation, or (c) lack of heart looping (Hove et al., 2003). Therefore haemodynamic factors appear to be important ‘epigenetic’ modulators of cardiovascular development.

1.3 Congenital heart disease

1.3.1 Definitions

Congenital Anomaly: A structural abnormality of any type, present from birth.

Congenital Malformation: A morphological defect of an organ, part of an organ, or larger region of the body that results from an intrinsically abnormal developmental process (e.g. genetic factors)

Congenital Disruption: A morphological defect of an organ, part of an organ, or larger region of the body that results from an extrinsic breakdown of, or an interference with, an originally normal developmental process (e.g. environmental factors).

Syndrome: A pattern of multiple anomalies thought to be pathogenetically related (e.g. Down syndrome).

Congenital heart disease: A gross structural abnormality of the heart or intrathoracic great vessels that is actually or potentially of functional significance (Mitchell et al., 1971).

1.3.2 Epidemiology

Congenital heart disease or defect (CHD) is the most common birth defect affecting 8 in 1000 live births. It is the leading non-infectious cause of neonatal and infant mortality worldwide. Around 40% are diagnosed within the first year of life, and fifty years ago only ~25% of infants would survive beyond the first year of life, but with early diagnosis and advances in medical and surgical management, the prognosis for many of the different CHDs has improved with time, and now more than 85% will survive to adulthood (Moller et al., 1994).

There are now over 250,000 adults with CHD in the UK, and this number is estimated to be growing by 5% each year (Brickner et al., 2000). When assessing a patient with CHD it is important to determine if there is a family history of CHD (familial CHD) or no family history (sporadic CHD). Then one should identify if it occurs as an isolated finding (non-syndromic) or in association with other congenital anomalies (syndromic).

1.3.3 Classification of congenital heart disease

There are many ways to classify CHD, and traditionally it has been either the physiological or anatomical method. The physiological approach is to divide them into either cyanotic or acyanotic lesions. Cyanotic lesions have the presence of deoxygenated blood in the systemic circulation due to either (a) reduced blood flow to the pulmonary circulation with obligatory right to left shunting, or (b) increased blood flow to the pulmonary circulation leading to increased pulmonary vascular resistance and a late onset right to left shunt. Acyanotic lesions include those with (a) simple left to right shunts, or (b) obstructive outflow or regurgitant valves. The anatomical approach is to divide them into simple or complex lesions. Nowadays clinicians will combine both the physiological and anatomical methods (Table 1).

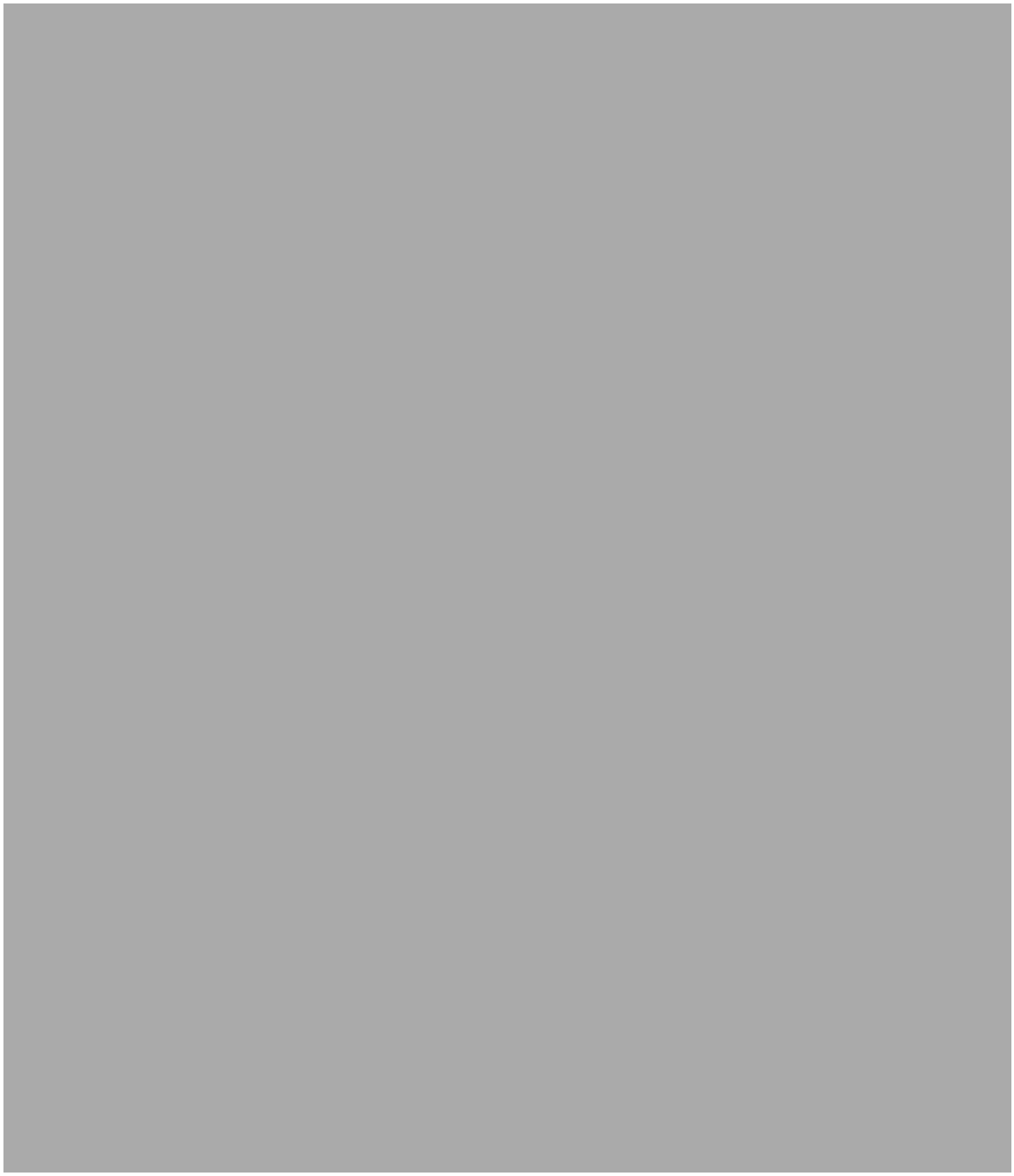
Table 1: Classification of types of CHD.
Adapted from (Wu and Child, 2004)

Left to Right shunt
Atrial septal defect
Ventricular septal defect
Atrioventricular septal defect
Patent ductus arteriosus
Partial anomalous pulmonary venous drainage
Outflow obstruction
Bicuspid aortic valve
Coarctation of the aorta
Pulmonary stenosis
Hypoplastic left heart syndrome
Cyanosis and decreased pulmonary blood flow
Tetralogy of Fallot
Tricuspid atresia
Pulmonary atresia
Hypoplasia of right ventricle
Ebstein's anomaly
Cyanosis and increased pulmonary blood flow
Transposition of the great arteries
Double outlet ventricle
Double inlet ventricle
Truncus arteriosus
Total anomalous pulmonary venous drainage
Cyanosis and increased pulmonary vascular resistance
VSD with Eisenmenger syndrome
ASD with Eisenmenger syndrome
PDA with Eisenmenger syndrome
Anomalies of major blood vessels
Congenitally corrected transposition of the great arteries
Coronary artery anomalies

1.3.4 Description of congenital heart disease

I will now describe some of the common types of CHD, incidences, their clinical presentations and management options (Figure 9) (Hoffman and Kaplan, 2002; Everett and Lim, 2004).

Figure 9: Normal heart and common types of CHD types
All diagrams of the CHD lesions are taken from Everett and Lim (2004).



Atrial Septal Defect (ASD) *– 1 in 1000*

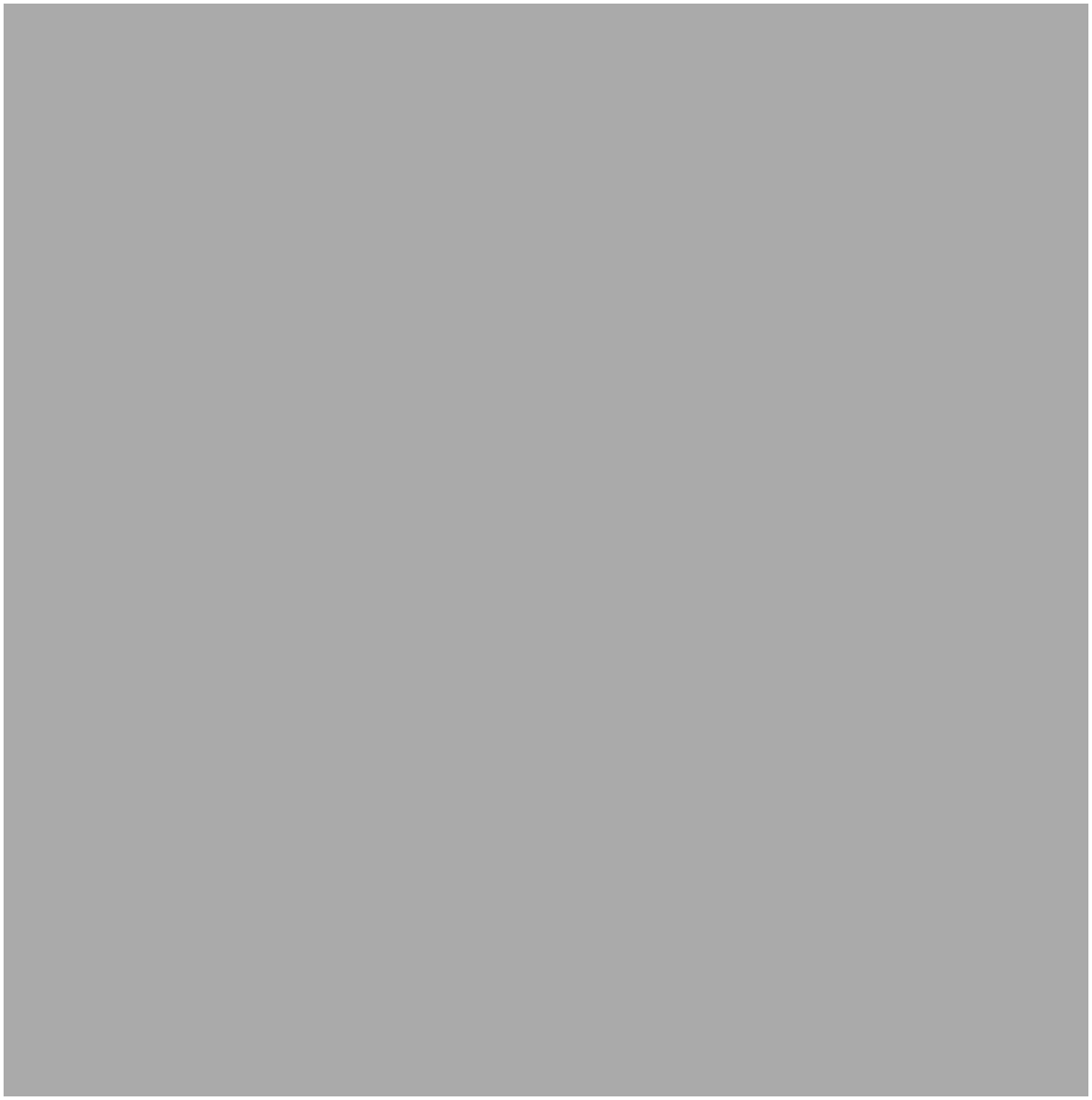
This refers to a communication between the right and left atria and can clinically result in a shunting of blood. Early in life there is little shunting but with time there can be significant left to right shunting leading to enlargement of the right atrium and ventricle, atrial arrhythmias, ventricular dysfunction and increased pulmonary blood flow. If left untreated, with time this can result in increased pulmonary vascular resistance and irreversible pulmonary vascular obstructive disease. These defects are usually closed surgically or by catheter based procedures by the age of 3-4 years.

Ventricular Septal Defect (VSD) – 1 in 300

This refers to a communication between the right and left ventricles. They may be single or multiple and can occur anywhere along the ventricular septum, so are usually classified according to their location. Small defects usually close spontaneously in the first few years of life. Moderate to large VSDs become haemodynamically significant in the first few weeks of life as they can result in shunting of blood from the left ventricle to the right ventricle, leading to excessive pulmonary blood flow and irreversible pulmonary vascular obstructive disease. The vast majority are closed surgically, but some can be closed by catheter based procedures.

Atrioventricular Septal Defect (AVSD) – 1 in 2800

This refers to an abnormality of the endocardial cushions. Therefore in this defect one has malformed mitral and tricuspid valves resulting in a common atrioventricular valve that straddles the ventricular septum. There can be significant left to right shunting of blood at the atrial and ventricular levels leading to excessive pulmonary blood flow and irreversible pulmonary vascular obstructive disease. This requires surgical correction with the optimum time being between 4-6 months of life.



Patent Ductus Arteriosus (PDA) – 1 in 1250

The ductus arteriosus connects the main pulmonary artery to the descending aorta and acts as a shunt in the fetal circulation to bypass the non-aerated lungs, and after birth this duct closes. Persistence of this duct after birth is termed PDA and results in a left to right shunt from the aorta into the pulmonary artery. If this PDA is small the shunt will be insignificant, however if large there will be increased pulmonary artery blood flow and the risks associated with that. In many CHDs (e.g. hypoplastic left heart syndrome, coarctation of the aorta, pulmonary atresia) a PDA is essential to provide pulmonary or systemic blood flow. A PDA may be closed pharmaceutically with indomethacin, but if it does not close may need catheter closure or surgical ligation.

Tetralogy of Fallot (TOF) – 1 in 2400

This is the most common cyanotic heart defect beyond infancy. It refers to a combination of four cardiac lesions: VSD, stenosis of right ventricular outflow tract with associated pulmonary atresia/stenosis, right ventricular hypertrophy, and an enlarged aorta that overrides the VSD. The degree of cyanosis depends on the degree of pulmonary stenosis. These patients experience intermittent spells of extreme cyanosis caused by increased pulmonary stenosis with stress (crying, fever, dehydration) leading to an increased right to left shunt across the VSD. Surgery may involve a palliative procedure at the start with a systemic-pulmonary artery shunt to allow adequate pulmonary blood flow. Complete surgical repair is commonly performed between 4-6 months of age.

Transposition of the Great Arteries (TGA) – 1 in 3200

This is the most common cyanotic CHD presenting in the first week of life. This refers to when the aorta arises from the right ventricle (returning deoxygenated blood to the systemic circulation) and the pulmonary artery arises from the left ventricle (returning oxygenated blood to the pulmonary circulation), resulting in nearly separate, parallel circulations. The existence of a communication between these two circuits (via an ASD, VSD, or PDA) to allow adequate mixing of blood is required for survival. The coronary arteries may also be malpositioned with this defect. Emergency palliative procedures to allow adequate mixing (maintaining patency of ductus arteriosus or patency of foramen ovale) are needed very soon after birth. Surgical correction by an arterial switch procedure is usually performed within the first week of life.

Truncus Arteriosus (TrA) – 1 in 9300

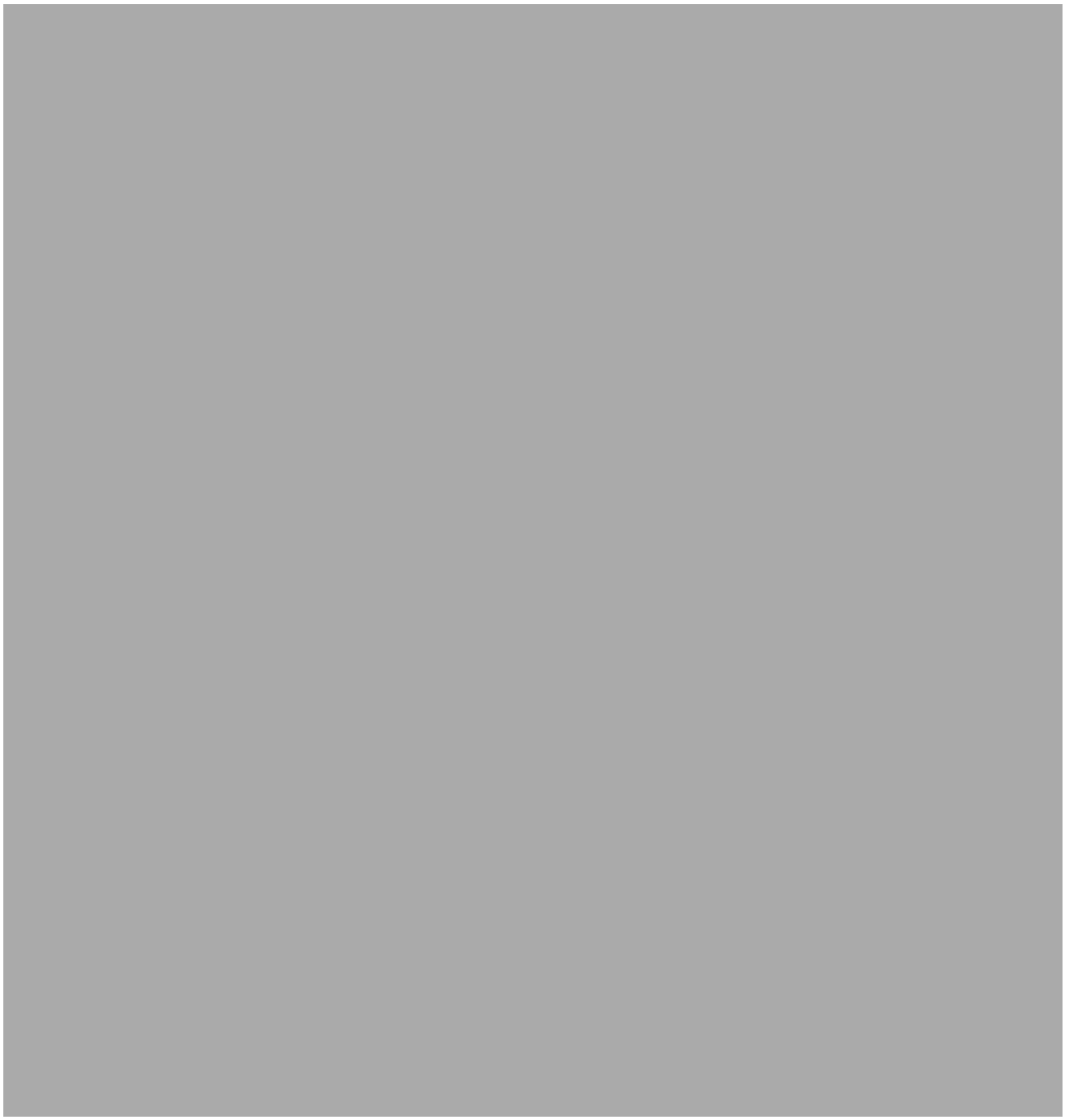
This refers to when the aorta and pulmonary artery leave the heart as a single common trunk. There is usually an anatomically abnormal truncal valve and a co-existing VSD. It can be associated with other cardiac anomalies like right sided aortic arch, aortic arch hypoplasia, coarctation of the aorta, or interrupted aortic arch. There is complete mixing of the systemic and pulmonary circulations with resulting systemic desaturation. Most infants present with congestive heart failure in the first few weeks of life, and may be cyanotic. Prompt surgical repair is the treatment of choice due to the haemodynamic instability and the possibility of irreversible pulmonary vascular disease. This would involve detachment of the pulmonary arteries from the common trunk, closure of the VSD, and an artificial conduit from the right ventricle to the main pulmonary artery.

Above:

1. Truncus Arteriosus (Type I)
2. Abnormal truncal valve (4 leaflets)
3. Ventricular septal defect (VSD)

Double Outlet Right Ventricle (DORV) – 1 in 6400

This refers to when both the aorta and the pulmonary artery arise predominantly from the right ventricle, and is usually associated with a VSD. These children present depending on the exact relationship of the great vessels to the VSD. Definitive therapy is surgical, but in some cases palliative procedures are performed to allow temporary improvement in the medical condition of the child.



Pulmonary Stenosis (PS) – 1 in 1400

This describes a narrowing around the pulmonary valve. In valvular PS the valve leaflets may be thickened and fused at their edges. There is usually post-stenotic dilatation of the pulmonary artery and the valve may be bicuspid. If the narrowing occurs in the pulmonary artery above the valve it is termed supravalvular PS, and this obstruction can be in the main or branch pulmonary arteries (branch pulmonary artery stenosis (PPAS). If the narrowing occurs below the level of the pulmonary valve in the right ventricular outflow tract it is termed subvalvular PS or infundibular stenosis. If less severe these lesions are well tolerated by children and often asymptomatic. Severe stenosis results in increased afterload on the right ventricle and right sided heart failure. Balloon valvuloplasty can be performed in mild cases, stenotic resection for branch pulmonary artery stenosis, or surgical repair in severe cases.

Aortic Stenosis (AS) – 1 in 2500

This describes a narrowing around the aortic valve. In valvular AS the valve leaflets may be thickened or bicuspid. If the narrowing occurs in the proximal aorta above the valve it is termed supravalvular AS. If it occurs at the level of the left ventricular outflow tract it is termed subvalvular AS. These lesions can cause increased afterload on the left ventricle and left ventricular hypertrophy, and subendocardial ischemia (due to increased oxygen demand of the myocardium). Ventricular arrhythmias and sudden death can occur in severe cases. Balloon valvuloplasty is a palliative procedure, but most patients will require a valve transplant.

Coarctation of the Aorta (CoA) – 1 in 2400

This is characterised by a narrowing of the lumen of the aorta, and is frequently associated with a bicuspid aortic valve. The obstruction to aortic blood flow leads to increased afterload of the left side of the heart, and in severe cases can compromise perfusion of organs distal to the point of obstruction. Surgical resection with end-to-end anastamosis is the preferred treatment of choice in the neonatal or infant period.

Above:

SA - subclavian artery
AV - aortic valve

Interrupted Aortic Arch (IAA)

This refers to obstruction/narrowing along the aortic arch between the ascending and descending aorta, and is usually associated with a narrowed left ventricular outflow tract and left ventricular hypoplasia. It presents with shock or congestive heart failure in the first 2 weeks of life (and is similar to CoA). Surgical reconstruction is the only therapeutic option and is performed as soon as the patient is haemodynamically stable.

Hypoplastic Left Heart Syndrome (HLHS) – 1 in 3800

This refers to a small or absent left ventricle with hypoplastic or atretic mitral and aortic valves. It is usually associated with hypoplasia of the ascending aorta and coarctation of the aorta, and an ASD. A PDA is essential for systemic blood flow and coronary perfusion. There is mixing of blood in the right atrium with oxygenated/deoxygenated blood flowing to the rest of the body. Surgical therapy is necessary for the survival of these individuals, even though medical stabilisation is an important part of treatment as well. Surgery would involve staged reconstruction or rarely a heart transplant.

Ebstein's Anomaly – 1 in 8800

This is an abnormality of the tricuspid valve where it is abnormally formed and displaced lower than it should be normally. This displacement causes atrialisation of a portion of the right ventricle, and can lead to a regurgitant tricuspid valve and dilatation of the right atrium if significant. If there is an atrial communication (e.g. ASD) then there is right to left shunting with resulting cyanosis. In most severe cases the infants present at birth with cyanosis and an enlarged heart. Surgical intervention usually requires a palliative shunt to allow adequate pulmonary blood flow, with possible surgical repair of the tricuspid valve at an older age.

Anomalous Pulmonary Venous Drainage (APVD) – 1 in 10600

In this defect at least one (partial - PAPVD) or all (total - TAPVD) pulmonary veins connect anomalously to the right heart. Therefore oxygenated blood from the lungs returns to the right side of the heart rather than to the left side. This oxygenated blood mixes with the deoxygenated blood in the right atrium. There are various forms of this defect. The most common one in PAPVD is when the right upper pulmonary vein drains into the superior vena cava and this is identical in physiology to that of an ASD (left to right shunting), so the greater the number of veins draining anomalously, the larger the shunt. Most patients with PAPVD are usually asymptomatic and identified by chance. Those with TAPVD present with varying degrees of cyanosis. The surgical repair involves replumbing the pulmonary veins to the left atrium,

Laterality Defects

These defects can range from dextrocardia to complex CHDs. Dextrocardia is a term used for when the heart is positioned on the right side of the chest with its apex pointing to the right. It can be associated with a number of CHD and abnormalities of lungs and abdominal organs (e.g. alobar/multilobar lungs, malrotated intestines, asplenia/polysplenia, midline liver). Isolated dextrocardia has no haemodynamic implications and requires no therapy. However if other anomalies are present then surgical treatment may be required.

1.3.5 Management of congenital heart disease

Advances in echocardiography have allowed CHDs to be detected as early as 17-18 weeks gestation, and most are well tolerated during fetal life due the connection with the maternal circulation. It is only after birth when that connection is lost, do many of the CHDs manifest clinically, some will be incompatible with extra uterine life, some clinically apparent within the first few days/weeks of life, and others may remain undetected into adult life. Advances in cardiovascular surgery and intensive care have meant many of the CHDs can be corrected surgically, and in the future fetal cardiac surgery may be a possibility. During adulthood, some patients can develop further complications (e.g. endocarditis, stroke, systemic or pulmonary hypertension, aortic aneurysm or dissection and arrhythmias), and the main causes of death are heart failure and sudden cardiac death. Current guidelines focus on the surveillance and treatment of adults with CHD, aiming to reduce symptoms, and the risk and severity of late complications (Warnes et al., 2008; Marelli et al., 2010).

1.3.6 Aetiology of congenital heart disease

Studies of recurrence and transmission have for a long time provided the hypothesis that CHD is caused by 'multifactorial inheritance' – i.e. due to a combination of both 'genetic' and 'environmental' factors, where the environmental factor interacts with a genetic predisposition in an individual to cause CHD (Nora, 1968). In recent years, however, separate genetic and environmental factors have been identified, but there still remains a large proportion of CHD which has an unknown cause. Understanding the embryology and molecular events ('molecular embryology') allows us to focus on individual modular steps in cardiac development, to determine the aetiology of CHD, as many defects are due to abnormal morphogenesis in specific components of the heart and vessels (Figure 10). A malformation may originate from different embryological mechanisms, and this is known as the 'One heart disease—several mechanisms—several genes' concept (Bajolle et al., 2009). In addition, impairment of these different mechanisms may generate a broad spectrum of heart diseases. These concepts explain the genetic heterogeneity of a single type of CHD and the phenotypic heterogeneity of mutations in a single gene (Figure 11).

Figure 10: Diagram illustrating cardiovascular development with specific structural components.
Taken from Yamagashi et al. (2009)

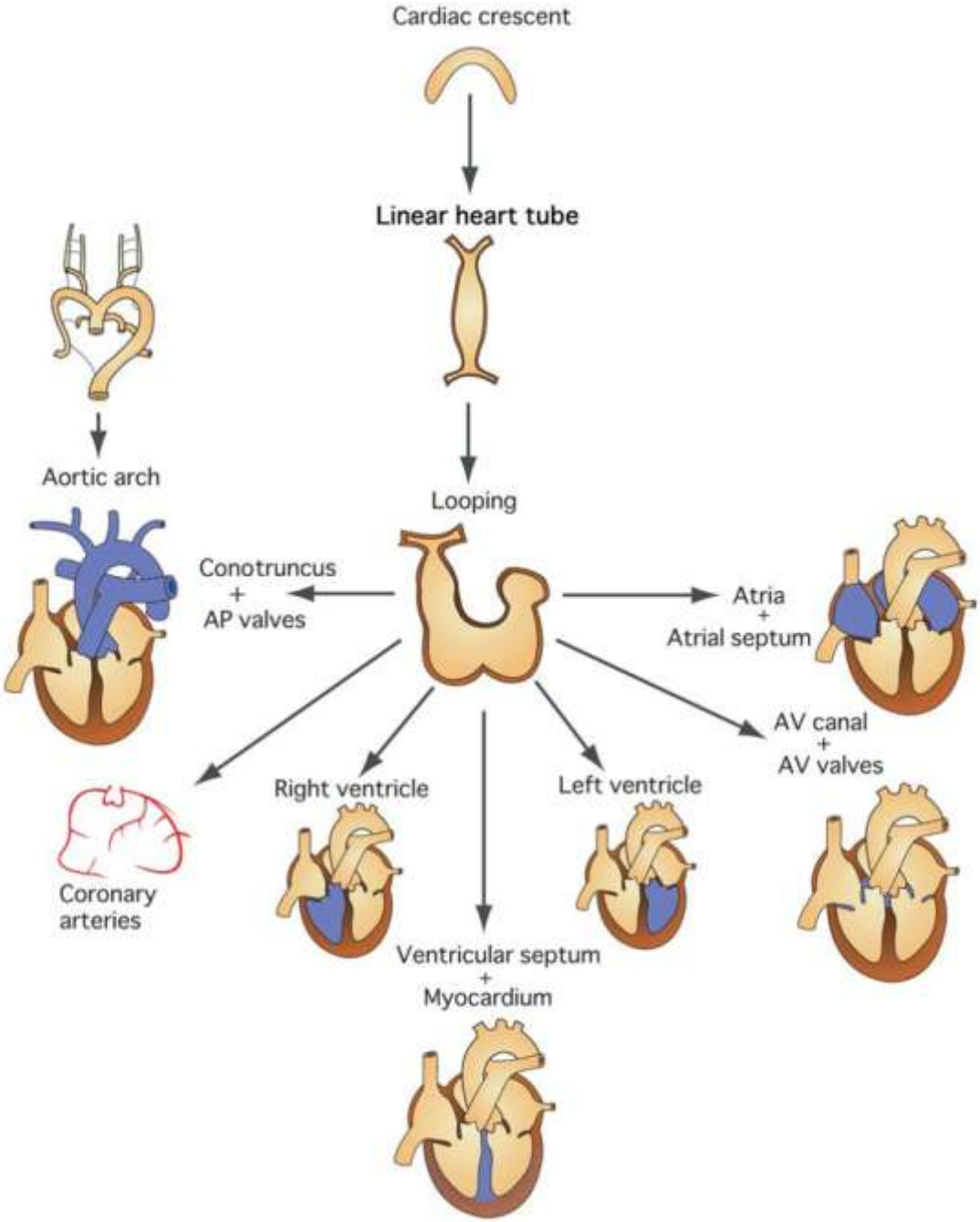
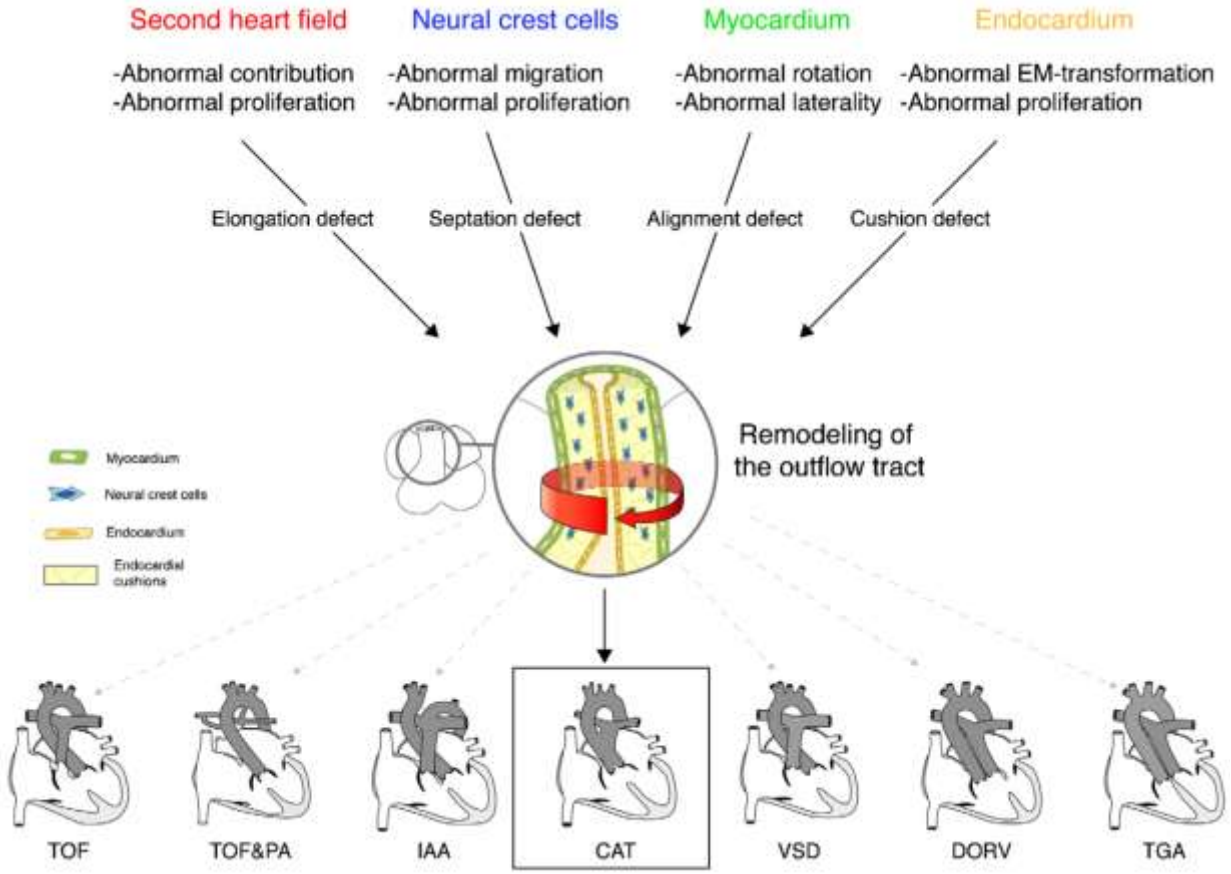


Figure 11: Diagram illustrating the concept of 'One heart disease—several mechanisms—several genes'.
Taken from Bajolle et al. (2009)

A common arterial trunk may result from a participation defect of progenitors from the second cardiac field and/or a migration defect of the neural crest cells and/or a rotation defect of the myocardium and/or a formation abnormality of the endocardial cushions. In addition, impairment of these different mechanisms may generate a broad spectrum of heart diseases affecting the conotruncal region.CAT: common arterial trunk; DORV: double outlet right ventricle; EM: epithelio-mesenchymal; IAA: interrupted aortic arch; TGA: transposition of the great arteries; TOF: tetralogy of Fallot; TOF&PA: tetralogy of Fallot with pulmonary atresia; VSD: sub-arterial ventricular septal defect.



1.3.6.1 Environmental factors

Many environmental agents (termed-teratogens) have been shown to cause developmental disruptions following maternal exposure to them (e.g. drugs, chemicals, radiation, and infections). During development biochemical differentiation usually precedes morphological differentiation, so the period at which structures are sensitive to teratogens is usually a few days before the stage at which they visibly develop. The exact mechanisms by which teratogens disrupt embryogenesis and cause anomalies still remain to be elucidated. A number of environmental factors have been reported to be associated with CHD (Table 2), and the ability to reduce the risk of CHD by modifying environmental interactions is a favourable preventative strategy.

Table 2: Environmental factors and associated CHD (humans).
Adapted from LMD;(Firth et al., 2005; Mahler and Butcher, 2011)

Drugs	
Antifungals	
Bifonazole / Fluconazole	ASD, CHD
Anticonvulsants	
Carbamazepine	CHD
Hydantoin	CHD
Primidone	TOF, PS
Trimethadione	CHD
Valproic acid	VSD, TOF
Immunosuppressants	
Aminopterin / Methotrexate	Laterality defects
Cytarabine / Thioguanine	ASD
Mycophenolate mofetil	PS, TrA, TGA
Recreational	
Alcohol	ASD, VSD, TOF
Cocaine	ASD, VSD, PS, laterality defects
Marijuana	VSD, Ebstein's anomaly
Other	
Carbimazole	CoA
Lithium	Ebstein's anomaly, tricuspid atresia, ASD, CoA, laterality defects
Misoprostol	Laterality defects
Retinoic acid	ASD, VSD, TOF, TGA, TrA, CoA, HLHS
Thalidomide	ASD, VSD, PDA, TOF, TrA, PS
Warfarin	TrA, laterality defects
Organic solvents	
	VSD, PS, HLHS, CoA, TGA, TOF, TAPVD, AVSD, Ebstein's anomaly
Infections	
Cytomegalovirus	VSD, PS
Herpes simplex virus	CHD
Rubella virus	PDA, PPAS, ASD, VSD, PS
Maternal Illness	
Diabetes Mellitus	ASD, VSD, CoA, PDA, HLHS, AVSD, TOF, TGA, TrA, tricuspid atresia, laterality defects
Phenylketonuria	TOF, CoA

1.3.6.2 Genetic factors

The known genetic causes of CHD (syndromic or non-syndromic) can be classified according to underlying genetic mechanism. Syndromic CHD can be caused by chromosomal, single gene, or unknown causes. At the date of submission, CHD had been recorded as a clinical feature in at least one case in 957 syndromes (London Medical Databases). Non-syndromic CHD is more likely to be caused by single gene or unknown causes. These syndromic or non-syndromic cases can be familial or sporadic (i.e. first case in family). Approximately 20% of CHD is associated with a chromosomal abnormality, a congenital syndrome, or a single gene mutation with Mendelian inheritance pattern (Pierpont et al., 2007; Warnes et al., 2008). For the remaining 80% of non-syndromic/non-Mendelian CHD (sporadic) cases the underlying genetic mechanisms are still poorly understood. Table 3 summarises some of the common known chromosomal abnormalities, syndromes (with associated genes if known), and single genes (non-syndromic) associated with each type of CHD in humans.

1.3.6.2.1 Chromosomal

A recent population study identified a chromosomal abnormality in 12.3% of infants with CHD, with some defects more likely to be associated with a chromosome abnormality (e.g. IAA – 69.2%, AVSD – 67.2%, and DORV – 33.3%) (Hartman et al., 2011). Of all children with a chromosomal abnormality around 30% have CHD (Pierpont et al., 2007). The type of CHD can sometimes guide clinicians to test for particular chromosomal abnormalities (even in the

absence of other clinical features). The most common chromosomal abnormalities are trisomies 21, 18, 13, and 22q11.2 deletion. Down syndrome (Trisomy 21) accounts for ~80% of children with CHDs and a chromosomal abnormality (Warnes et al., 2008), and around 40% of children with Down syndrome have CHD, with the most common type being an AVSD. Turner syndrome (45,X) can cause left sided obstructive defects with CoA being the most common (10% of patients) (Mazzanti and Cacciari, 1998). Approximately 15% of patients with TOF or a conotruncal anomaly have a chromosome 22q11.2 deletion. CHD occurs in around 75% of patients with chromosome 22q11.2 deletion (Ryan et al., 1997). Supravalvular AS is seen in up to 75% of patients with Williams syndrome (due to a chromosome 7q11.23 deletion) (Eronen et al., 2002). Screening for chromosomal abnormalities in patients with CHD may lead to an early diagnosis and treatment of extracardiac manifestations, as CHD is often the presenting symptom for these patients. CHD patients with other organ system anomalies and/or mental retardation should raise the suspicion of an underlying chromosomal abnormality.

Cytogenetic deletion/duplication syndromes are rare, and the introduction of microarray analysis to detect small deletions/duplications (copy number variation - CNV) has increased the identification of chromosomal abnormalities associated with CHD. One study identified significant CNVs in 17% of patients with CHD and other birth defects, who had normal standard cytogenetic analysis (Thienpont et al., 2007). Another looked at isolated cases of CHD and suggested CNVs can increase the susceptibility to CHD (as 44% of the CNVs

were also identified in parents with no CHD), and that other modifying factors are needed to manifest the phenotype (Erdogan et al., 2008). Smaller CNVs can provide clues to the localisation of loci critical for heart development and developmental genes. This is best illustrated by the association of CHD and chromosome 8p23.1 deletions, with the subsequent identification of the *GATA4* gene and its role in cardiac development (Pehlivan et al., 1999).

Appendix B: summarises cases of syndomic CHD present on DECIPHER (**D**atabas**E** of **C**hromosomal **I**mbalance and **P**henotype in **H**umans using **E**nsembl **R**esources) database, with a chromosomal abnormality detected by comparative genomic hybridisation array technique.

1.3.6.2.2 Single Gene

Many genes have been identified to be involved in the complex process of heart development in animal models, however few have been shown to be associated with CHD in humans (isolated cases or familial cases). Of the known genes associated with CHD, some cause non-syndromic CHD, and others cause syndromic CHD (Wessels and Willems, 2010). A number of types of CHDs can occur due to mutations in more than one gene (genetic heterogeneity), and mutations in one gene can cause variable CHDs (phenotypic heterogeneity). Many of these CHD genes encode members of the heart developmental programme involving numerous pathways (as described before) with cross communication, ligand-receptor interactions, complex signal transduction

pathways, and a network of transcription factors affecting the expression of cardiac specific genes of heart (Figure 12).

Non-syndromic CHD genes have been identified via genetic studies of familial CHD. One of the first non-syndromic CHD genes identified was *NKX2.5* giving rise to inherited ASD, VSD, TOF, Ebstein's anomaly and cardiac conduction defects (Schott et al., 1998; Benson et al., 1999). Inherited septal defects were then shown to be caused my mutations in *GATA4*, which interacts with NKX2.5 (Garg et al., 2003). Subsequently interactions between GATA4 and TBX5 have also been identified. Therefore it is apparent that a complex interaction between NKX2.5, GATA4, and TBX5 is needed for proper heart septation. *TBX20* gene mutations (another transcription factor within these 3 super-families) have been shown to cause familial ASD, VSD, valve defects and cardiomyopathies (Kirk et al., 2007). *NOTCH1* mutations have been associated with bicuspid aortic valve and AS (Garg et al., 2005).

The more common syndromic CHD genes include: *TBX5, PTPN11*, and *JAG1*. CHD and cardiac conduction defects occur in up to 75% of patients with Holt-Oram syndrome (due to mutations in *TBX5*), and the most common defects include ASDs and VSDs (Basson et al., 1994). The majority (80%) of patients with Noonan syndrome (due to mutations in a number of genes – *PTPN11, KRAS, RAF1, SOS1*) have some form of CHD, typically PS or hypertrophic cardiomyopathy (Burch et al., 1993). More than 90% of patients with Alagille syndrome (due to a chromosome 20p12 deletion or mutations in *JAG1* located

within this chromosome region) have CHDs, typically peripheral pulmonary artery stenosis (PPAS), TOF and/or PS (McElhinney et al., 2002). Detailed genetic studies of chromosome 22q11.2 deletion syndrome have tried to identify candidate genes within the deleted region. *TBX1* has been proposed as a good candidate gene, and the identification of missense mutations in this gene in cases with features of chromosome 22q11.2 deletion syndrome (but normal cytogenetic studies), has further supported this theory (Yagi et al., 2003).

Further support for an underlying genetic mechanism for CHD is seen in studies showing mutations in genes controlling heart development in sporadic cases of CHD, however the frequency of the gene mutations is in <2% of cases. These genes include: *CFC1, CITED2, CRELD1, FOG2, GATA4, GDF1, LEFTYA, NKX2.5, NOTCH1, PROSIT240, TBX1, TBX20*, and *ZIC3* (Kosaki et al., 1999; Bamford et al., 2000; Goldmuntz et al., 2002; McElhinney et al., 2002; Muncke et al., 2003; Pizzuti et al., 2003; Robinson et al., 2003; Ware et al., 2004; Sarkozy et al., 2005; Sperling et al., 2005; Mohamed et al., 2006; Karkera et al., 2007; Kirk et al., 2007). Most of the mutations in these cases are heterozygous mutations and are transmitted from unaffected parents, implying these mutations are partially penetrant. A possible mechanism for CHD in these sporadic cases is the accumulation of rare mutations in a number of the heart development genes (mutational load).

Figure 12: Diagram illustrating the signalling pathways involved in cardiac development in non-syndromic CHD.
Taken from Wessels and Willems (2010)

Disease genes encode proteins in all compartments of the pathways, including ligands (LEFTY2, NODAL, VEGF, GDF1, JAGGED1), receptors (CFC1, TDGF1, ACVR2B, NOTCH1), transcription factors (CITED2, TFAP2B, ZIC3, FOXH1, FOG2, MYOCD, NKX2.5, NKX2.6, GATA4, TBX5), and downstream targets (ACTC1, Myosins, ANF, NOS3).

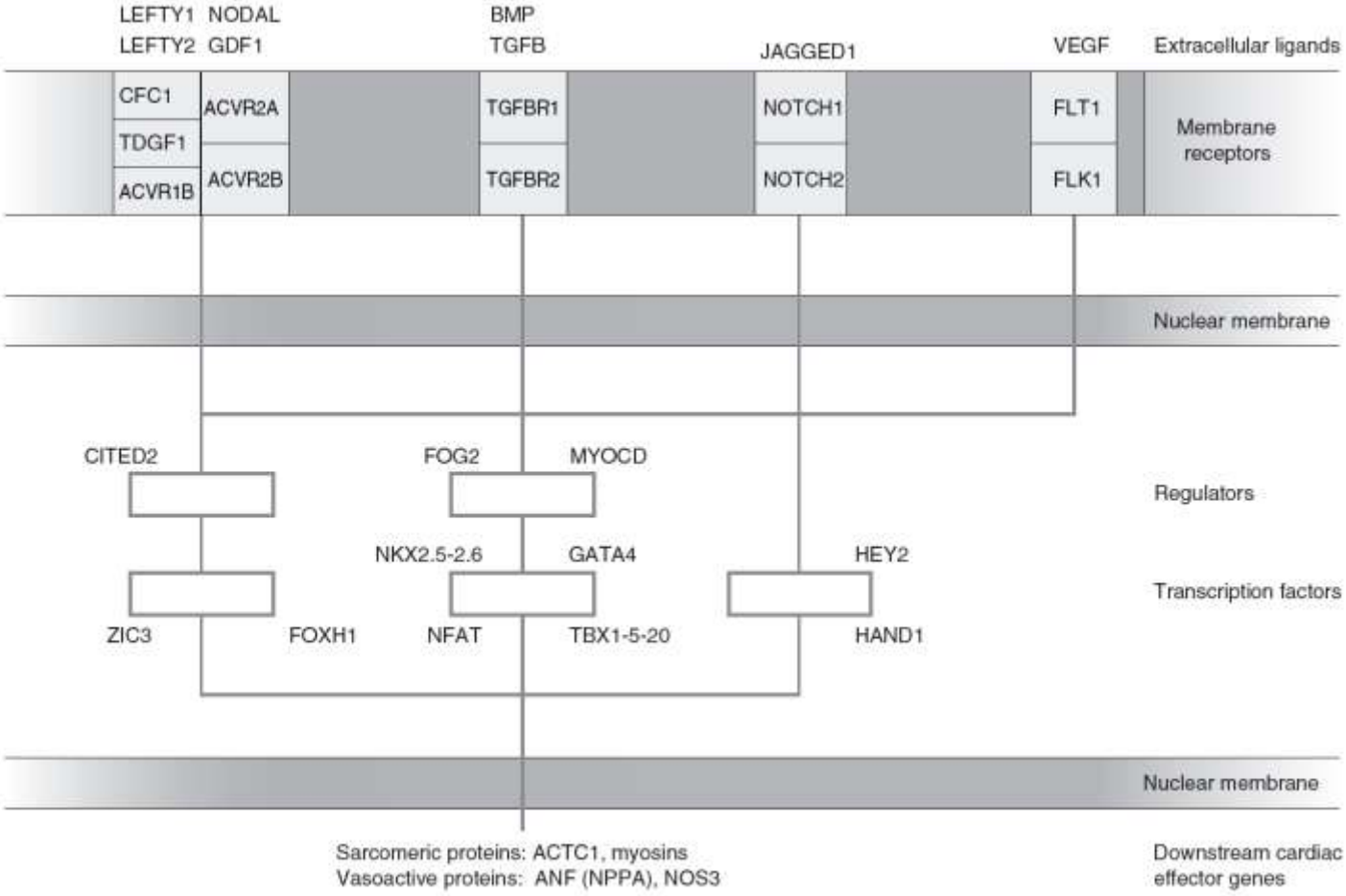


Table 3: Genetics factors and associated CHD (humans).
Adapted from (Johnson et al., 1997; Wimalasundera and Gardiner, 2004; Pierpont et al., 2007; Weismann and Gelb, 2007; Bentham and Bhattacharya, 2008; Bruneau, 2008; Joziasse et al., 2008; Jing-Bin et al., 2009; Barriot et al., 2010; Wessels and Willems, 2010; Ware and Jefferies, 2012).

Type of CHD	Genetic Aetiology with Examples
Septal Anomalies	
***Atrial septal defect* (*secundum*)**	
	Chromosomal
	Deletions of 1, 4, 4p, 5p, 6, 10p, 11, 13, 17, 18, 22
	Trisomies 13, 18, 21
	Klinefelter (XXY)
	Thrombocytopenia-absent radius (1q21.1 deletion)
	Williams (7q11.23 deletion)
	Syndromic (single gene or unknown)
	Axenfeld Rieger (*FOXC1*)
	Cardiofaciocutaneous (*BRAF, KRAS, MEK1, MEK2*)
	CHARGE (*CHD7*)
	Ellis-van Creveld (*EVC, EVC2*)
	Goldenhar
	Holt-Oram (*TBX5*)
	Kabuki (*MLL2, KDM6A*)
	Noonan (*PTPN11, KRAS, SOS1, BRAF*)
	Oculofaciocardiodental (*BCOR*)
	Okihiro (*SALL4*)
	Rubinstein-Taybi (*EP300, CREBBP*)
	Smith-Magenis (*RAI1*)
	Sotos (*NSD1*)
	Townes-Brocks (*SALL1*)
	Non-syndromic (single gene)
	ACTC1
	ALK2
	BMPR2
	CFC1
	CITED2
	GATA4
	GATA6
	MYH6
	MYH7
	MYHBPC3
	NKX2.5
	TBX20
	TLL1
	ZIC3

Atrial septal defect primum) See AVSD	
Ventricular septal defect	
	Chromosomal
	Deletions of many (incl. 4p, 5p, 8p, 10p, 11q, 22q)
	Duplications of many
	Trisomies 8, 9, 13, 18, 21
	Williams (7q11.23 deletion)
	Syndromic (single gene or unknown)
	Carpenter (*RAB23*)
	CHARGE (*CHD7*)
	Frank-Ter-Haar (*SH3PXD2B*)
	Goldenhar
	Holt-Oram (*TBX5*)
	Kabuki (*MLL2, KDM6A*)
	Noonan (*PTPN11*)
	Oculofaciocardiodental (*BCOR*)
	Okihiro (*SALL4*)
	Opitz G (*MID1*)
	Rubinstein-Taybi (*EP300, CREBBP*)
	Townes-Brocks (*SALL1*)
	Ulnar mammary (*TBX3*)
	VACTERL association
	Non-syndromic (single gene)
	ACTC1
	CFC1
	CITED2
	GATA4
	MYHBPC3
	NKX2.5
	NOTCH1
	TBX1
	TBX20

Atrioventricular septal defect (partial / complete)	
	Chromosomal
	Deletions of 3p, 8p, 22q
	Duplications of 10q, 11q, 22q
	Trisomies 13, 18, 21
	Turner (XO)
	Syndromic (single gene or unknown)
	CHARGE (*CHD7*)
	Chondrodysplasias
	Ellis-van Creveld (*EVC, EVC2*)
	Holt-Oram (*TBX5*)
	Hydrolethalus
	Noonan (*PTPN11, KRAS, SOS1*)
	Smith-Lemli-Opitz (*SLOS*)
	Non-syndromic (single gene)
	ALK2
	BMPR2
	CFC1
	CRELD1
	GATA4
	LEFTY2
	NODAL
Conotruncal Anomalies	
Interrupted aortic arch	
	Chromosomal
	Deletions of 10p, 22q
	Trisomy 8
	Non-syndromic (single gene)
	CFC1
	NKX2.5
	TBX1
Truncus arteriosus	
	Chromosomal
	Deletions of 10p, 22q
	Trisomy 8
	Syndromic (single gene or unknown)
	Kleefstra (*EHMT1*)
	Townes-Brocks (*SALL1*)
	Non-syndromic (single gene)
	GATA6
	NKX2.5
	NKX2.6
	TBX1

Tetralogy of Fallot	
	Chromosomal
	Deletions of many (incl. 4p, 8p, 22q)
	Duplications of many
	Trisomies 9, 13, 18, 21
	Alagille (20p12 deletion)
	Cat-eye (22q11 duplication)
	Di George (22q11.2 deletion)
	Syndromic (single gene or unknown)
	Alagille (*JAG1*)
	CHARGE (*CHD7*)
	Okihiro (*SALL4*)
	Townes-Brocks (*SALL1)*
	Numerous other syndromes (see OMIM)
	Non-syndromic (single gene)
	ALDH1A2
	CFC1
	CITED2
	FOG2
	FOXH1
	GATA4
	GDF1
	JAG1
	NKX2.5
	NODAL
	NOTCH1
	TBX1
	TDGF1
Transposition of the great arteries	
	Chromosomal
	Deletions of many
	Trisomies 18, 21
	Di George (22q11.2 deletion)
	Non-syndromic (single gene)
	ALK2
	CFC1
	FOXH1
	GDF1
	LEFTY2
	NKX2.5
	NODAL
	PROSIT240
	THRAP2
	ZIC3

Double outlet right ventricle	
	Chromosomal
	Duplications of 2p, 12p
	Trisomies 9, 13, 18
	Turner (XO)
	Di George (22q11.2 deletion)
	Syndromic (single gene or unknown)
	CHARGE (*CHD7*)
	Frank-Ter-Haar (*SH3PXD2B*)
	Non-syndromic (single gene)
	ALK2
	CFC1
	FOG2
	GATA4
	GDF1
	NKX2.5
	NODAL
	NOTCH1
	PROSIT240
	ZIC3
Pulmonary Outflow Obstruction	
Peripheral pulmonary artery stenosis	
	Chromosomal
	Alagille (20p12 deletion)
	Williams (7q11.23 deletion)
	Syndromic (single gene or unknown)
	Alagille (*JAG1, NOTCH2*)
	LEOPARD (*PTPN11*)
	Noonan (*PTPN11, KRAS, SOS1*)
	Williams (*ELN*)
	Non-syndromic (single gene)
	ELN
	JAG1
Pulmonary valve atresia	
	Chromosomal
	Ring chromosome 9
	Trisomy 9
	Syndromic (single gene or unknown)
	Townes-Brocks (*SALL1*)
	Non-syndromic (single gene)
	CFC1
	NKX2.5
	NODAL

Pulmonary valve stenosis	
	Chromosomal
	Deletions of 1p, 8p, 10p, 22q
	Duplications of 6q, 15q, 19q
	Trisomy 8
	Alagille (20p12 deletion)
	Williams (7q11.23 deletion)
	Syndromic (single gene or unknown)
	Alagille (*JAG1*)
	Cardiofaciocutaneous (*KRAS, BRAF, MEK1, MEK2*)
	Costello (*HRAS*)
	LEOPARD (*PTPN11, RAF1*)
	Neurofibromatosis 1 (*NF1*)
	Noonan (*PTPN11, KRAS, SOS1,BRAF, NRAS*)
	Rubinstein-Taybi (*EP300, CREBBP*)
	Non-syndromic (single gene)
	CITED2
	ELN
	GATA4
	GATA6
	MYOCD
	ZIC3
Aortic Outflow Obstruction	
Aortic valve stenosis	
	Chromosomal
	Deletions of 10q, 11q
	Duplications of 1q, 2p, 2q, 6q, 11q
	Trisomies 13, 18
	Turner (XO)
	Syndromic (single gene or unknown)
	Alagille (*JAG1*)
	Noonan (*PTPN11, KRAS, SOS1*)
	Non-syndromic (single gene)
	ELN
	NKX2.5
	NOTCH1
Supravalvular aortic stenosis	
	Chromosomal
	Williams (7q11.23 deletion)
	Syndromic (single gene or unknown)
	Williams (*ELN*)
	Non-syndromic (single gene)
	NKX2.5

Coarctation of the Aorta	
	Chromosomal
	Deletions of 10p, 11q, 18p
	Duplications of 4p, 4q, 6q, 10p
	Trisomies 8, 9, 13, 18, 21
	Turner (XO)
	Syndromic (single gene or unknown)
	Alagille (*JAG1*)
	Kabuki (*MLL2, KDM6A*)
	Neurofibromatosis 1 (*NF1*)
	Periventricular nodular heterotopia (*FLNA)*
	Non-syndromic (single gene)
	NKX2.5
	NOTCH1
	TBX20
	VEGF
Bicuspid Aortic Valve	
	Chromosomal
	Deletion of 10p
	Duplication of 6q
	Trisomies 13, 18
	Turner (XO)
	Syndromic (single gene or unknown)
	Kabuki (*MLL2, KDM6A*)
	Williams (*ELN*)
	Non-syndromic (single gene)
	NOTCH1
Aortic atresia / Hypoplastic left heart syndrome	
	Chromosomal
	Deletions of 4p, 11q
	Trisomies 13, 18
	Turner(XO)
	Syndromic (single gene or unknown)
	CHARGE (*CHD7*)
	Non-syndromic (single gene)
	NKX2.5
	NOTCH1
	TBX20
	ZIC3

Other defects	
Tricuspid Atresia	
	Chromosomal
	Deletions of 1p, 4p, 22q
	Duplication of 22q
	Non-syndromic (single gene)
	MYH6
Epstein's Anomaly	
	Chromosomal
	Trisomy 21
	Non-syndromic (single gene)
	MYH7
	NKX2.5
Patent Ductus Arteriosus	
	Chromosomal
	Deletions of 4p, 5p
	Trisomies 8, 13, 18,
	Klinefelter (XXY)
	Syndromic (single gene or unknown)
	CHARGE (*CHD7*)
	Mowat-Wilson (*ZEB2*)
	Periventricular nodular heterotopia (*FLNA)*
	Sotos (*NSD1*)
	Non-syndromic (single gene)
	BMPR2
	MYH11
	TBX20
	TFAP2B
Total / Partial Anomalous Pulmonary Venous Drainage	
	Chromosomal
	Trisomy 8
	Non-syndromic (single gene)
	ANKRD1
	BMPR2
	CITED2
	GATA4
	NODAL
	PDGFRA
	ZIC3

Laterality Defects	
	Chromosomal
	Trisomies 13, 18
	? syndromic (single gene)
	ACVR2B
	CFC1
	CITED2
	CRELD1
	FOXH1
	GDF1
	LEFTY2
	NKX2.5
	NODAL
	ZIC3

1.3.7 Genetic counselling in patients with congenital heart disease

With the mainstreaming of clinical genetics services and increased awareness amongst other medical professionals, there are more patients and families with CHD seen by clinical genetics departments for genetic counselling. For many, genetic counselling helps to: (a) identify and understand the genetic basis of their CHD, (b) provide accurate genetic counselling on recurrence risks, and (c) identify and manage extracardiac manifestations (Burchill et al., 2011). Although the majority of cases have an isolated anomaly and are sporadic, some will be syndromic and/or familial, and this will affect the nature of genetic counselling for these individuals. The knowledge and concerns about inheritance of CHD has been evaluated in a population of non-syndromic CHD adult patients, and showed that only a small proportion (33%) recalled receiving information about the inheritance of CHD (and only 18% consulted a clinical geneticist). Only 44% were able to estimate the recurrence risk in the correct range of magnitude. Many had concerns about future children and thought it important that they should be screened for CHD. Insufficient knowledge about the inheritance of CHD meant that 41% desired more information (van Engelen et al., 2011). There are current guidelines recommending genetic counselling as part of the care for adults with CHD (Marelli et al., 2010), but it is clear that this is not implemented widely or meeting the needs of patients.

1.3.7.1 Genetic Assessment of proband

The assessment of any patient with a condition suspected to have a genetic basis, always starts with a detailed family history spanning at least three

generations, including the presence of consanguinity. This can allow the identification of any Mendelian patterns of inheritance. A detailed history and clinical examination will help to identify features suggestive of an underlying genetic syndrome with CHD (e.g. developmental delay, learning difficulties, extracardiac manifestations, behavioural problems, and facial dysmorphism).

1.3.7.2 Genetic testing of proband

Diagnostic genetic testing aims to identify inherited or *de novo* variations that account for CHD. This can be guided by the information collated from the detailed family history and clinical assessment. Various methods currently employed in clinical practice include: karyotyping, fluorescent *in situ* hybridisation (FISH) analysis, microarray analysis, and gene sequencing. Knowledge of the clinical criteria and molecular basis of genetic conditions associated with CHD is an essential prerequisite to genetic testing, and will determine the nature of the test utilised. It is important to be aware that although positive results can be very informative, in cases with a clinical phenotype and family history, a negative result does not disprove a genetic basis.

1.3.7.3 Recurrence risks

The process of evaluating and discussing recurrence risks has to take into account the family history and patient characteristics (nature of defect and extracardiac manifestations). For Mendelian patterns of inheritance this is easily predicted, but one has to account for intra- and inter-familial variability. The challenges lie in sporadic non-syndromic patients with CHD. It has been

estimated that familial recurrence of CHD represents 3-5% of non-syndromic CHD (Calcagni et al., 2007). A prospective multicentre study reported recurrence risks of CHD as 4.1% (with an affected parent) and 2.1% (with an affected sibling). There appeared to be gender differences with more CHD occurring in the offspring of affected women with CHD (5.7%) than affected men (2.2%) (Burn et al., 1998). The recurrence risk for a couple after one affected child is ~3%, but increases to 10% if there are two affected children.

The precise type of CHD is also an important factor in recurrence risks and concordance (identical to CHD seen in index case), and so an accurate diagnosis in the index case is essential to allow accurate counselling on recurrence risk (Gill et al., 2003). For families with one recurrence the exact concordance has been reported as 33%, which increases to 55% in families with two or more recurrences, but overall the exact concordance is 53%. There is wide variation between exact concordance for the various CHD (e.g. 80% for AVSD, 55% for VSD, 43% for TOF, and 15% for CoA) (Gill et al., 2003).

A population based study of 18,000 Danish patients with CHD estimated an individual's risk of CHD given a family history of CHD. They found that the relative risk for the same type of CHD ranged from 3-fold (septal defects) to 80-fold (heterotaxia) in first degree relatives. The overall relative risk for the same type of CHD was 8.15, and for any other type of CHD was 2.68, the latter suggesting that some families have a susceptibility to CHD (Oyen et al., 2009; Oyen et al., 2010). Demographic studies like these and the evolution of

knowledge regarding cardiac development have led to genetic counselling of CHD being based on embryological mechanisms of a heart defect rather than on its anatomy.

As the population of adults with CHD has increased, and these adults have had children themselves, clinicians are now seeing more families with multiply affected individuals with CHD. Many of these families will appear to have an autosomal dominant inheritance pattern, which can significantly increase the risk of CHD to offspring (50%). There are also families with no apparent family history of CHD, but parents have had more than one child with CHD, suggesting an autosomal recessive pattern of inheritance (25% recurrence risk). There is however a great variability in the phenotype between individuals from the same family. These familial recurrences of CHD are a fundamental resource for molecular studies and identification of genes involved in heart development.

1.3.7.4 Screening at risk relatives

Clinical geneticists play a major role in counselling at risk individuals of numerous genetic conditions. In CHD, if the genetic basis is known, clarification of risk can be determined via cascade genetic testing (with appropriate genetic counselling). When the aetiology of CHD is unknown in a patient, cardiac screening of at risk relatives (especially for milder forms of CHD) can be a dilemma for clinicians. Left ventricular outflow tract obstructions (LVOTO) are a group of CHD including: bicuspid aortic valve (BAV), sub/supravalvular AS, mitral valve stenosis, IAA, CoA, and HLHS. They are generally considered to

have high heritability as various LVOTO diagnoses may occur within the same family (Wessels et al., 2005). Screening first degree relatives of patients with LVOTO showed that 20% of these patients had a first degree relative with a cardiac anomaly (most commonly BAV), thereby justifying screening of relatives (Kerstjens-Frederikse et al., 2011).

1.4 Gene identification

1.4.1 Why study the genetics of congenital heart disease?

It is very important to determine an underlying genetic factor in a patient with CHD as there may be: (a) other organ system involvement, (b) prognostic information for clinical outcome, (c) important genetic reproductive risks for the family, and (d) other family members for whom genetic testing may be appropriate.

The research described in this report is primarily focused on the mapping and identification of genes causing CHD, as the molecular mechanisms accounting for the majority of CHDs still remain to be elucidated. By defining the precise mechanisms involved in normal heart development, future targeted strategies at specific stages of the molecular pathway may lead to the development of novel therapeutic options.

1.4.2 Approaches to gene identification

There are a number of methods of identifying a disease-causing gene. The two most popular methods are functional cloning (candidate gene approach) and

positional cloning, but the most efficient and effective way to identify genes is by utilising both strategies, the positional-candidate approach.

1.4.2.1 Functional cloning

Functional cloning requires a detailed knowledge of the phenotype of a particular disease and the possible biological mechanisms causing it. In this approach candidate genes can be selected based on information about their function, tissue expression pattern, role in known developmental pathways, homologies to other genes, and/or animal models. These genes are then screened for mutations within them. So for CHD one would select genes that control the formation and development of the heart. Prior to positional cloning, this was the main approach for the identification of disease causing genes.

Chromosomal abnormalities, such as deletions or translocations, in which patients have very similar phenotypes, may help to identify a locus for that condition. An example would be the occurrence of CHD in 60% of patients with deletion of chromosome 8p (8p23.1 in particular) (Pehlivan et al., 1999). *GATA4* is located at this locus and was therefore screened in other patients with CHD and mutations identified (Garg et al., 2003). Knockout mouse models of *Fog2* developed Tetralogy of Fallot (TOF) phenotypes, which led to the identification of mutations in *FOG2* in patients with TOF (Pizzuti et al., 2003).

New methods in this category include whole exome sequencing technology, which sequences the entire coding region of the genome in an individual. This

method may identify variants in numerous genes, which could then be filtered depending on their function and role in biological processes, and any animal models.

Once gene identification has linked a specific phenotype to a particular biological pathway, this approach of functional cloning allows selection of further candidate genes from the same pathway that may cause identical or related conditions. This is exemplified by the identification of genes in the RAS-MAPK signal transduction pathway (*PTPN11, SOS1, RAF1, KRAS, HRAS, BRAF, MEK1,* and *MEK2*) that cause a spectrum of genetic syndromes (Noonan syndrome, LEOPARD syndrome, Costello syndrome, and Cardiofaciocutaneous syndrome) with many overlapping clinical features including CHD (Weismann et al., 2005).

Functional cloning has been greatly aided by analysis of mouse mutants which have been generated by systematic mutagenesis programs. Completion of the mouse genome project has aided this approach (Waterston et al., 2002). Human-mouse homologies may be particularly valuable, as mutations in specific genes may often cause similar phenotypes.

1.4.2.2 Positional cloning

Positional cloning studies requires affected individuals from families with Mendelian disorders, by utilising linkage analysis to identify and define a portion on a chromosome (locus) which must contain the disease-causing gene

(Wicking and Williamson, 1991). Once a disease locus is identified, candidate genes within this locus can be selected for mutational screening (as described above). The Human Genome Project (Venter et al., 2001) has greatly facilitated this approach to gene identification, by providing physical and genetic maps enabling one to locate markers and genes within a specific chromosomal region. Public databases such as 'NCBI', 'Ensembl' and 'UCSC Genome Bioinformatics site' include detailed information about numerous polymorphic markers spread throughout the genome, genetic maps based on these markers, a physical map based on latest genomic sequence information, a rapidly expanding map of expressed sequences, and information management and search tools. A successful example of this approach in CHD was the identification of the *NKX2.5* gene, which was mapped to the chromosome region 5q35 in numerous large families with an autosomal dominant form of CHD (ASD, VSD, TOF, and subvalvular AS), with the subsequent identification of three different pathogenic mutations (Schott et al., 1998).

The clinical recognition of deletion-specific syndromes (e.g. 4p [Wolf-Hirschhorn], 5p [Cri du chat], 22q11.2 [Di George]) supports the theory that haploinsufficiency for some genes in the deleted region has a direct effect on specific developmental processes. Chromosome maps of deletions and duplications associated with 47 different congenital malformations have been created, using detailed clinical and cytogenetic information from over 3000 patients (Brewer et al., 1998; 1999). They analysed the frequency of deleted/duplicated bands with each malformation to identify any malformation

specific regions/bands. They were able to identify a number of highly significant associated bands for various heart defects (e.g. 4q31 and 22q11 for VSD; 20p13-11 and 22q11 for PS; 11q23-23 for AS and HLHS; 2q22 and 22q11 for TrA). These maps may help to facilitate the identification of genes important in human development, and there have been several successes to localising malformation-specific genes (e.g. Holoprosencephaly-*SHH* gene and Aniridia-*PAX6* gene).

1.4.3 Linkage, genetic markers, and maps

A haplotype is a set of closely linked alleles on a chromosome (either genes or DNA polymorphisms) which are inherited as a block unit. Haplotypes are used extensively in genetic mapping and population studies. All genetic mapping depends on the behaviour of chromosomes at meiosis. Loci that are on the same chromosome will travel together unless there is a crossover between paired homologous chromosomes separating the two loci (recombination). This can happen more often to loci that are widely separated from each other (or on different chromosomes), but rarely to loci close together (Morgan, 1911). The chance of recombination is a measure of the distance between them, and usually loci separated by recombination in 1% of meioses are defined as being 1centiMorgan (cM) apart. This genetic distance is not the same as physical distance (measured in bp, kb, or Mb of DNA). Some chromosome regions have a higher frequency of cross over than others. The order of loci should still be the same in genetic and physical maps, but the spacing may be different, and on average 1cM corresponds to 1Mb, although the relationship varies across the

genome. Linkage between two loci is defined as occurring when recombination events occur less that 50% of the time, with a resultant recombination fraction of <0.5 (Ott and Bhat, 1999). This indicates that the loci are on the same chromosome and close to each other.

Genetic mapping in humans looks at a large number of loci and types them for genetic markers. Markers must be: (a) inherited in a Mendelian manner, (b) sufficiently polymorphic, (c) relatively easy to type, and (d) available across the whole genome at close intervals. DNA polymorphisms can satisfy these requirements and the two well known and used types are microsatellites and single nucleotide polymorphisms (SNPs). In my project I have utilised both types of markers.

Microsatellite markers are short tandem repeats of 2, 3 or 4 bases (e.g. [CA]n sequences) that are present in all people at the same specific chromosomal location, but the size of the repeat unit can vary from person to person (i.e. polymorphic). They can be analysed by polymerase chain reaction (PCR) of a segment of DNA containing the microsatellite and determining the size (using a fluorescent label and a DNA sequencing machine). These markers are present approximately every 30,000 bases in the genomic sequence (Stallings et al., 1991), and can be very informative. Many successful mapping projects have been achieved by the use of microsatellites to conduct genome-wide scans (Aligianis et al., 2005).

SNPs are now much more widely used in genetic mapping studies in the study of complex multifactorial diseases, diseases with Mendelian inheritance and copy number variants (CNVs). They are usually less informative as there are likely to be only two alternative nucleotides at a polymorphic site. However they are abundant in the human genome, can be typed with high throughput technology such as microarrays ('SNP-chips'), and allow researchers to obtain high resolution mapping information. The first SNP array for genome wide scan was produced by Affymetrix (GeneChip® 10K Xba Array) containing 11,555 SNP markers (Affymetrix Inc, Santa Clara, CA). Since 2004, several successful mapping projects have been accomplished using this 10K array (Gissen et al., 2004; Janecke et al., 2004). Currently, the Affymetrix® Genome-Wide Human SNP Array 6.0 contains more than 1.8 million genetic markers (>906,600 SNPs and >946,000 probes for the detection of copy number variation).

Therefore genetic mapping studies now have a dense framework of markers available which are all mapped relative to one another and in defined chromosomal locations (Maps). A number of genetic and physical maps of the genome have been developed, and these maps are fundamental tools for mapping and gene identification. Examples of genetic maps that have been constructed over the last two decades include the Généthon map (a microsatellite marker-based linkage map) (Weissenbach et al., 1992; Dib et al., 1996) and the Marshfield map. The Marshfield map was based on the genotyping of around 8,000 microsatellite markers in eight large, three generation families from the Centre d'Etude du Polymorphisme Human (CEPH)

collection containing 188 meioses (Broman et al., 1998). More recent maps include those generated by deCODE Genetics in Iceland, with substantially improved resolution of up to five times the resolution of previous maps (Kong et al., 2002). This map was based on the genotyping of 5,136 microsatellite markers, in 146 nuclear families containing 1,257 meioses. The construction of these genetic maps and intermediate physical maps significantly contributed to sequencing the entire human genome in the Human Genome Project (Venter et al., 2001). Recent advances in SNP genotyping have also facilitated the construction of a detailed human haplotype map of over 3.1 million SNPs (International HapMap consortium 2007). Once markers have been typed, the genotypes of each marker must be aligned to the pedigrees and checked for co-segregation with the disease being studied.

1.4.4 Autozygosity mapping

1.4.4.1 Introduction

It has been recognised that searching for homozygous regions by descent in consanguineous families offers a powerful strategy for mapping autosomal recessive disorders. The development of genetic maps allowed researchers to reintroduce this concept and formulate a technique called ‘homozygosity mapping‘ for the mapping of autosomal recessive disorders in consanguineous kindreds (Lander and Botstein, 1987). As a locus can be homozygous by virtue of parental descent from a common ancestor, and thus autozygous, this methodology is also termed autozygosity mapping (Mueller and Bishop, 1993). One of the first disorders to be mapping this way was alkaptonuria (Pollak et al.,

1993) and over the last 17 years a multitude of disorders have been successfully mapped this way (Aligianis et al., 2005; Morgan et al., 2006a).

1.4.4.2 Consanguinity

Successful autozygosity mapping relies on the identification of consanguineous families in which there are multiple affected children with an identical disorder. Consanguinity is defined as a union between couples who are related as second cousins or closer. A second cousin couple share 1/32 of their autosomal genes. Their children have identical gene copies at 1/64 of all loci. A first cousin couple share 1/8 of their autosomal genes. Their children have identical gene copies at 1/16 of all loci.

Consanguineous marriages are common practice in a number of communities worldwide. The effect of a consanguineous marriage depends on the frequency and nature of all recessive diseases in that population. Documented effects include increased infant mortality, congenital malformations, learning difficulties, blindness, hearing difficulties and metabolic disorders. An average increase of infant mortality by 4.4% was shown amongst offspring of first cousins compared with unrelated controls (meta-analysis of numerous studies mainly from developing countries) (Bittles and Neel, 1994). However some of these disorders may be successfully treated in developed countries but lead to death in undeveloped countries (partially skewing these figures). In unrelated parents, the prevalence of serious congenital abnormalities and genetic disorders is 2-2.5% (Congenital Anomalies Register) and around 4% in some longer term

studies. These risks are doubled for children from first cousin relationships. A study performed in Birmingham in 1993 found 0.4% of Northern European couples were related, whereas amongst the British Pakistani couples, 69% were related and 57% were first cousins. The birth prevalence of all congenital and genetic disorders was 7.9% in the children of the British Pakistani couples, almost double that of children from the Northern European couples (4.3%). The prevalence of recessive disorders was 3.0–3.3% in the British Pakistani children (~10 times higher than in the Northern European children-0.28%) (Bundey and Alam, 1993).

These genetic risks are recognised within these communities but there are many social advantages that outweigh these risks. A consanguineous marriage is believed to strengthen family ties, minimise the costs of dowry payments, and remove any uncertainties that arise through marriage to an individual outside the family or community. Consanguineous couples usually marry and have children earlier with larger family sizes, thereby increasing the likelihood of expression of a recessive disorder. The clinical approach has always been to offer improved access to genetic counselling, and identify at risk individuals and perform cascade testing of extended family members for carrier status of recessive conditions.

1.4.4.3 Consanguinity and congenital heart disease

The relationship between the incidence of CHD and the degree of consanguinity has been tested in a study of the largest cohort (1500 patients) reported to date

(Chehab et al., 2007). The relative contributions to this cohort from consanguineous families were: 19.4% (first degree cousins), 5.9% (second degree cousins), and 2.1% (other parental consanguinity). In comparison the contributions from consanguineous families in the control group without CHD were: 14.4% (first degree cousins), 5.9% (second degree cousins), and 3.6% (other parental consanguinity), showing statistical difference between the two groups. However the rate of consanguinity varied greatly between types of CHD. The defects with a high incidence of consanguineous parents were ASD, TOF, and valvular AS, supporting the role of autosomal recessive genes in these lesions. Many other studies have also reported a significant higher proportion of parental consanguinity amongst patients with CHD (Gatrad et al., 1984; Gev et al., 1986; Badaruddoza et al., 1994; Becker and Al Halees, 1999; Bassili et al., 2000; Becker et al., 2001; Nabulsi et al., 2003; Ul Haq et al., 2011; Shieh et al., 2012). Some very important factors have been highlighted, that could more accurately assess the impact of consanguinity on health, and could have improved the outcome of many of the above studies (Bittles, 2011). These factors include: more accurate diagnosis and classification of CHD type, improved matching of cases and controls, and clarification of type of consanguinity (e.g. cousin-cousin, uncle-niece).

The role of consanguinity has been established in many autosomal recessive diseases, but it may also play a part in structural malformations, including CHD. This may seem peculiar in relation to the recent advances in molecular genetics of CHD, especially as autosomal dominant families with ASD and mutations in

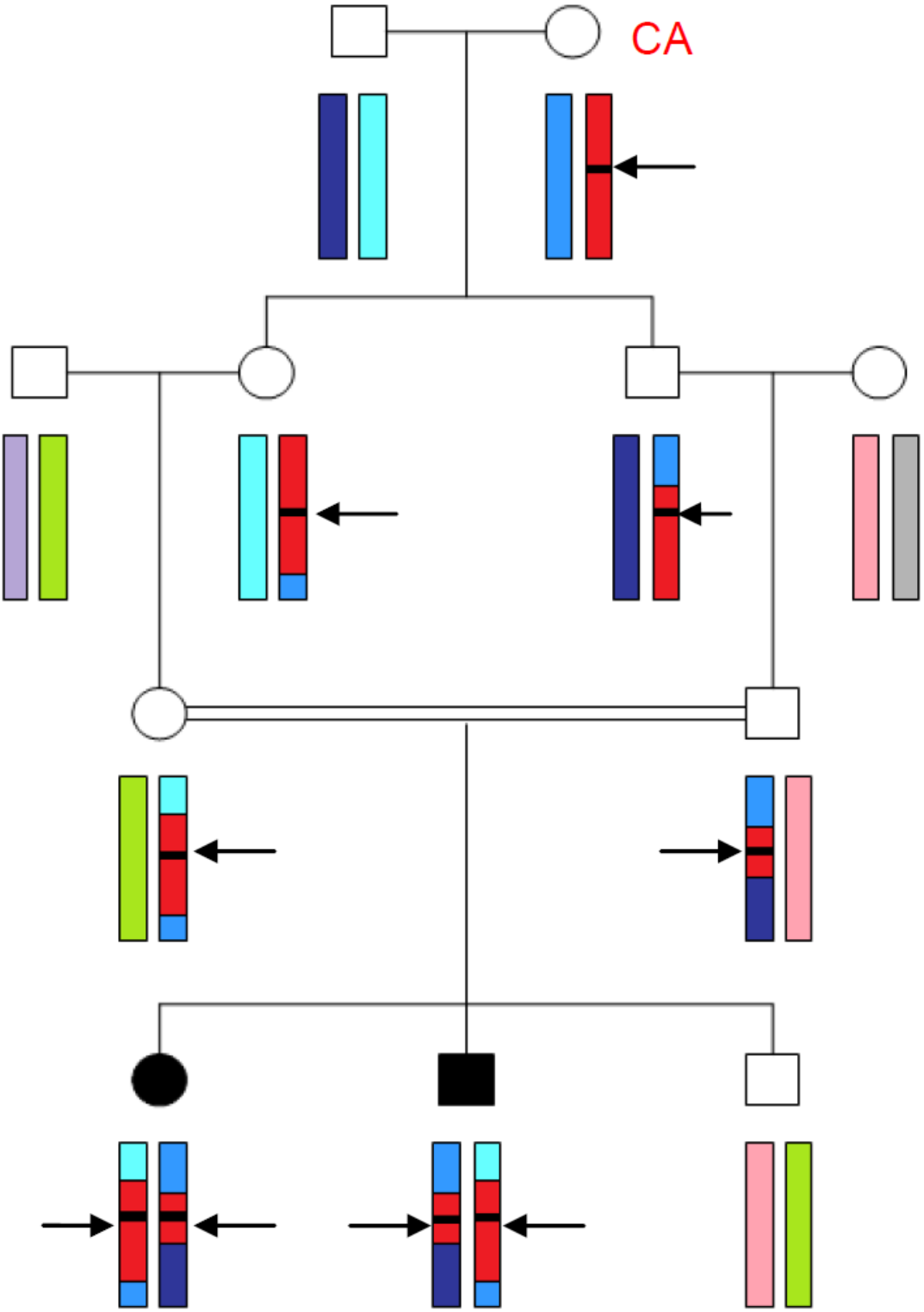
NKX2.5 and *GATA4*, and aortic valve anomalies and *NOTCH1* mutations have been reported (Schott et al., 1998; Garg et al., 2003; Garg et al., 2005). It is still unknown if genes responsible for CHD in autosomal dominant families may also be implicated in autosomal recessive families.

1.4.4.4 Principles of autozygosity mapping

Figure 13 illustrates an example of a pedigree of a consanguineous family with two children affected with an autosomal recessive condition. The disease allele has been passed down through successive generations from the common ancestor (CA). The affected children have thus inherited both copies of this disease allele from their parents, and are thus homozygous-by-descent for this gene. In addition, the chromosomal segment surrounding the gene is also homozygous (shaded in red). Recombination events during meiosis lead to a reduction in the size of the homozygous chromosomal segment (red) over successive generations. Disease loci in consanguineous families are thus normally (but not always) located in such homozygous regions of the genome. These homozygous regions can be identified using genome-wide SNP arrays and microsatellite markers. Once homozygous regions are identified, the region of interest can be examined for candidate genes for sequencing.

Figure 13: The principle of autozygosity mapping.

The specific mutation of a disease gene (indicated by the black arrow) can be passed on from a common ancestor (CA) to offspring and the product of a consanguineous marriage, can result in affected offspring CA.



1.4.4.5 Advantages / disadvantages of autozygosity mapping

Autozygosity mapping and positional candidate gene analysis is a highly effective strategy for gene identification in consanguineous families, much more than the identification of recessive genes in non-consanguineous families. A single affected child from a consanguineous marriage provides as much information as a non-consanguineous family with three affected children. Autozygosity mapping represents a highly efficient and effective approach for generating disease gene localisation even in the presence of locus heterogeneity.

Over the past decade, Professor Maher's research group have developed a major interest in autozygosity mapping studies and identified a large number of novel genes for autosomal recessive disorders including genes for: Achromatopsia (*GNAT2*)(Aligianis et al., 2002), ARC syndrome (*VPS33B, VIPAR*)(Gissen et al., 2004; Cullinane et al., 2010), Beckwith-Wiedemann syndrome (*NLRP2*)(Meyer et al., 2009), Faisalabad histiocytosis/3 Rosai-Dorfman disease (*SLC29A3*)(Morgan et al., 2010), Fowler syndrome (*FLVCR2*)(Meyer et al., 2010), Hermansky-Pudlak syndrome (*BLOC1S3*)(Morgan et al., 2006b), Immunodeficiency syndromes (*TRAC*)(Morgan et al., 2011), Infantile neuraxonal dystrophy (*PLA2G6*)(Morgan et al., 2006a), Infantile parkinsonism (*DAT*)(Kurian et al., 2009), Martsolf syndrome (*RAB3GAP2*)(Aligianis et al., 2006), Meckel-Gruber syndrome (*MKS3*)(Smith et al., 2006), Multiple pterygium/Fetal akinesia syndrome (*CHRNG, RAPSN, DOK7*)(Morgan et al., 2006c; Vogt et al., 2008; Vogt et al.,

2009), Trichohepaticenteric syndrome (*TTC37*)(Hartley et al., 2010), and Warburg MICRO syndrome (*RAB3GAP1*)(Aligianis et al., 2005).

Many of the recessively inherited diseases are rare and it can be very difficult to ascertain a sufficient number of patients with the same phenotype to perform autozygosity mapping studies. If family sizes are small it is less likely for there to be more than two affected siblings within a sibship, and so a solution would be to set up collaborative projects between centres. A number of families will have a ‘private mutation‘ for a genetic disorder, and so it can be difficult to find other families who have mutations in the same gene. Sometimes autosomal recessive diseases in consanguineous families may be caused by compound heterozygous mutations, and in this situation the disease locus may not be within a homozygous region, and will not be detected with genome-wide scans for homozygous regions. Autosomal recessive diseases can also be extremely heterogeneous with more than one genetic locus for the disease, so it is important to identify any previously mapped disease loci. Linkage studies performed in one large consanguineous family with multiple affected individuals can be much more powerful than using several different families with one or two affected individuals. Once linkage is established in that large family, other smaller consanguineous families can be investigated for linkage to the same region. Such approaches may help to overcome this problem of locus heterogeneity.

1.4.5 DNA sequencing

The development of Sanger sequencing provided an enormous leap forward in molecular genetics, and has had a great impact in understanding the genetic basis of various diseases. The finished sequence of the human genome in 2004 was facilitated by the automation of this process (Levy et al., 2007). The advent of high throughput machines has led to a significant decrease in time and cost of Sanger sequencing ('First-Generation Sequencing'), and this has been further reduced by the introduction of massive parallel sequencing techniques ('Next-Generation Sequencing') (Brenner et al., 2000). The latter method has the ability to sequence the entire human genome in days at a fraction of the previous costs, and parallelises the sequencing process, producing thousands or millions of sequences at once. With advances in bioinformatics tools to analyse the data and the production of portable sized machines, it is possible that we will enter an era of bringing the sequencer to the bedside to impact medical management of patients (Marian, 2011).

1.5 Conclusion

I have given an overview of the complex nature of heart development and congenital heart disease (CHD). The specific advances which are altering our understanding of the aetiology of CHD include the: (a) detection of subtle chromosomal abnormalities due to high resolution chromosomal analysis, (b) delineation of the genetic defect in familial CHD via linkage analysis and reverse genetics, and (c) advances in animal genetics allowing detailed studies of animal models of CHD. All these methods should provide further information on this complex physiological process and how mutations in heart development genes can cause CHD.

For families with CHD, the identification of a genetic cause is very beneficial, as it allows the clinician to explain the exact genetic mechanism and pathogenesis to the family, and accurately counsel them regarding recurrence risks. It guides further investigations to identify other associated organ system involvement, and early intervention if needed to reduce co-morbidities. It also provides an opportunity to evaluate extended family members to accurately assess the risk of CHD to them and their offspring.

1.6 Aim of project

The principal aim of the project was to identify novel CHD genes by investigating familial cases of non-syndromic CHD. I was particularly interested in consanguineous families likely to have recessively inherited CHD (in order to utilise an autozygosity mapping approach), but also decided to collect non-consanguineous families that might have other types of inheritance, as these would be investigated for genes identified in the consanguineous families.

CHAPTER 2

GENERAL METHODS

2.1 Acquisition and assessment of patients and controls

2.1.1 Patient recruitment

In this project, familial cases of non-syndromic CHD were being selected for molecular genetic analysis, therefore families who fulfilled the following criteria were recruited:-

a) Consanguineous, one or more affected children with non-syndromic CHD
b) Non-consanguineous, two or more affected children with non-syndromic CHD
c) Outflow tract CHD anomalies (e.g. TOF / TGA / LVOTO) and an autosomal dominant pattern of inheritance.

I used a number of different strategies to identify families suitable for the project. Families were initially identified from those previously seen by the West Midlands Clinical Genetics department (regional service) for genetic counselling. I manually reviewed all 490 files disease coded with CHD (electronically) to select familial cases of CHD. Of these 490 files, 54 files were identified to be familial CHD and were then reviewed in more detail to clarify if they fulfilled the above criteria for familial non-syndromic CHD. Only 30 of these files were deemed suitable to take to the next stage in which these families were contacted by letter or telephone and invited to participate in the project. Of these 30 families, 5 refused, 11 did not ever respond, and 14 accepted the invite and were recruited (see later).

To identify further families from around the UK, I designed a project flyer/advert (Appendix C1) which i sent via email to clinicians from all the regional Clinical Genetics departments, and a selection of paediatric cardiologists with a special interest in CHD (including the tertiary paediatric cardiology service in the West Midlands). A total of 3 families were recruited after referrals from the above group. At the same time I directly liaised with some of the UK CHD patient support groups (e.g. Grown Up Congenital Heart Disease Patient Association, Children's Heart Federation, Heartline, and CHD-UK). They all kindly posted a link advertising the research project on their websites (Appendix C2), and I was also invited to lecture at their patient information conferences (Chapter 8). A number of families subsequently telephoned me for further information and I eventually visited and recruited 6 further families to the project. In total 23 families were recruited via the above processes from July 2009 to March 2011 (see Appendix A for timeline).

2.1.2 Consent and ethics approval

I was involved in the process of ethics approval applications, which also consisted of designing patient information sheets and informed consent forms (Appendix C3). The project was approved by the LREC (Local Research Ethics Committee), and informed consent was obtained from all participants and all clinical research adhered to principles outlined by the Declaration of Helsinki (see Appendix A for timeline).

2.1.3 Clinical assessment and acquisition of blood/saliva samples

I designed a designed a proforma for assessing all families (Appendix C4), in which a detailed family history and clinical assessment was performed, including a full medical history and search for dysmorphic and extra cardiac features to rule out syndromic causes of CHD. The medical notes were examined for details of CHD type, disease evolution and surgery performed. Blood or saliva samples (for DNA extraction) were taken from all available affected individuals, parents and any unaffected siblings. Routine cytogenetic analysis and FISH testing for 22q11.2 deletion syndrome (by the NHS West Midlands Regional Genetics Laboratory, UK) was also performed in one affected individual from each family.

2.1.4 DNA from control subjects

Control DNA samples were kindly donated by the West Midlands Regional Genetics Laboratory for assessing population frequencies of identified genomic variants. The surnames of the families were used to select which control panel they fitted into, after which they were anonymised.

2.2 Materials

2.2.1 Chemical reagents

Acetamide	Sigma
Agarose	Bioline
2X BiomixTM Red	Bioline
dNTPs (diluted from 100mM to a working stock of 2mM)	Bioline

EDTA (ethylenediaminetetraacetic acid)	Sigma
Ethanol	Fisher Scientific
Ethidium bromide	Sigma
GC rich solution of FastStart Taq DNA Polymerase Kit	Roche
Genescan-500 LIZ Size Standard	Applied biosystems
Hi-Di Formamide	Applied biosystems
HyperladderTM I (Separation range 200 – 10000bp)	Bioline
MicroCLEAN®	Web Scientific
Primers	Sigma
10X TBE Electrophoresis Buffer (diluted to 1X)	Geneflow
Water (distilled-RNase/DNase free, dH2O)	Invitrogen

2.2.2 Kits

Affymetrix Genome-Wide SNP 5.0 Array	Affymetrix Ltd
BigDye Terminator Cycle Sequencing Kit version 3.1	Applied biosystems
BigDye 5X Sequencing Buffer (25nM Tris pH 8.7, 4mM MgCl2)	Applied biosystems
Puregene Genomic DNA Purification Kit	Gentra systems

2.3 Molecular genetic investigation

2.3.1 DNA extraction

DNA extraction was kindly performed by the West Midlands Regional Genetics Laboratory. DNA was extracted from cells using the Puregene Genomic DNA Purification kit according to the manufacturer‘s instructions. Extracted genomic

samples were then quantified by spectrophotometry (by measurement of A260:A280 ratios).

2.3.2 SNP genotyping arrays

Autozygosity mapping strategies were utilised to establish disease loci and identify novel disease-causing genes in consanguineous families. In order to identify regions of common homozygosity and/or genomic copy number variants, a genome-wide scan using the Affymetrix SNP 5.0 Array (Affymetrix UK Ltd) was undertaken in affected children and their unaffected siblings.

This microarray technology allows the simultaneous genotyping of a subject‘s DNA for >500,000 single nucleotide polymorphisms (SNPs). SNP genotyping was kindly performed by Louise Tee (a research laboratory technician), according to the manufacturer‘s instructions (Affymetrix GeneChip Human Mapping SNP 5.0 Assay Manual).

In brief, 250ng genomic DNA was digested with Sty 1 and 250ng genomic DNA was digested with Nsp 1. The Sty and Nsp adaptor molecules were then ligated to the product accordingly. PCR reactions were set up for each sample (Sty-3 reactions, Nsp-4 reactions) using a universal PCR primer and the PCR products run on a 1.5% agarose (product sizes ranged from 200-1100bp). The PCR products were then pooled and cleaned up using magnetic beads (Ampure). The amplified DNA was then fragmented (to create products of <200bp), then labelled and hybridised to the SNP 5.0 chip (Affymetrix) for 16-18 hours.

Washing and staining of the arrays was then performed on a fluidics station (Affymetrix) and the chips were subsequently scanned using a gene-chip scanner (Affymetrix GeneChip Scanner 3000) using Affymetrix Genechip Command Console software. Data analysis was then undertaken using Genotyping Console v3.0.2 software to derive SNP genotypes, marker order and linear chromosomal location.

2.3.3 Microsatellite marker genotyping

2.3.3.1 Primers for PCR

The details of all microsatellite markers (and their UNISTS identification number) used for genotyping and fine mapping are tabulated in Table 6. Forward and reverse primer sequences for known microsatellite markers were obtained from each marker's entry on the UNISTS database. Di- tri- and tetra-nucleotide markers with high heterozygosity scores were selected using NCBI UNISTS and Ensembl genome browsers. Fluorescent dyes (FAM-blue, HEX-yellow, TET-green) were added to the 5' end of the forward primer.

2.3.3.2 PCR amplification for microsatellite marker genotyping

PCR amplification was performed in 10µl reactions as follows:-

- 1.0µl genomic DNA (20ng/µl)
- 5.0µl BiomixTM Red 1X
- 3.6µl dH_2O
- 0.2µl (2.0pmol) forward primer (tagged with fluorescent dye)
- 0.2µl (2.0pmol) reverse primer

BiomixTM Red is a premixed, pre-optimised 2X solution designed for high throughput PCR applications. It contains an ultra-stable *Taq* DNA polymerase and an inert red dye, permitting easy visualisation and direct gel loading.

After an initial denaturation at 95^{o}C for 5 min, a standard PCR protocol was followed: 30 cycles of 95^{o}C for 30s, annealing at 55^{o}C for 30s and extension at 72^{o}C for 30s, followed by a final extension step at 72^{o}C for 5 min. Each set of PCR reactions included a negative control (in which dH_2O was added instead of DNA) to check for contamination.

2.3.3.3 Analysis of microsatellite marker PCR products

Markers run on the ABI 3730 DNA Analyzer were initially diluted 1:15 with dH_2O. 1µl of the diluted PCR product was added to 10µl Hi-Di Formamide and 0.04µl Genescan-500 LIZ size standard. PCR product sizes were determined using Genemapper v4.0 software (Applied Biosystems). Scored genotypes were assembled as haplotypes and analysed for evidence of linkage.

2.3.4 Gene sequencing

2.3.4.1 Primers for sequencing candidate genes

All annotations and physical positions are recorded as in NCBI Genome 37.1 build. The DNA template for each gene sequenced in this report was downloaded from the Ensembl database. All coding transcripts for each gene were examined and intronic primer pairs flanking the exonic coding sequence, for exon-specific PCR amplification of the genomic exons and flanking exon-

intron boundaries were designed for exon-specific PCR amplification, either manually or using ExonPrimer and Primer3 software (Tables 7 & 8).

2.3.4.2 PCR amplification for gene sequencing

Standard conditions for PCR amplification were used. PCR amplification was performed in 25µl reactions as follows:-

- 5.0µl genomic DNA (20ng/µl)
- 12.5µl BiomixTM Red 1X
- 6.5µl dH_2O
- 0.5µl (5.0pmol) forward primer
- 0.5µl (5.0pmol) reverse primer

For GC-rich fragments, PCR amplification was performed in 25µl reactions:-

- 5.0µl genomic DNA (20ng/µl)
- 12.5µl BiomixTM Red 1X
- 5µl GC rich solution
- 1.5µl dH_2O
- 0.5µl (5.0pmol) forward primer
- 0.5µl (5.0pmol) reverse primer

PCR conditions were an initial denaturation of 95^{o}C for 5 min followed by 30 cycles of 45s denaturation at 95^{o}C, 45s annealing at 50-65^{o}C (temperature optimised specifically for each amplification reaction) and 1 min extension at 72^{o}C. This was followed by a final extension step at 72^{o}C for 5 min. Each set of

PCR reactions included a negative control (in which dH_2O was added instead of DNA) to check for contamination.

2.3.4.3 Agarose gel electrophoresis

PCR products were checked on 1.5% horizontal agarose gels to separate the PCR products and to ensure that the PCR reaction had worked without contamination. The agarose gels were made by melting agarose with 1X TBE in a domestic 600W microwave oven, then cooling the mixture before adding ethidium bromide (0.5g/ml final concentration) and casting the gel in a gel casting tray. The PCR products were loaded directly. Loading buffer was not required with the use of BiomixTM Red as the mix is of sufficient density to sink to the bottom of each well in the gel. A DNA sizing ladder (Hyperladder I) was added to lane 1 to check for correct PCR product size. Separation of DNA was achieved by electrophoresis at 60-140V for 30-120 min (depending on the gel size and PCR product size). The bands were visualised using Ethidium Bromide and a UV transilluminator (254nm wavelength).

2.3.4.4 PCR product clean-up

The PCR product was cleaned up (to remove unwanted dNTPs and primer) by using MicroCLEAN. 2.5µl MicroCLEAN was added to 2.5µl of PCR product, incubated at room temperature for 5 min and then centrifuged at 4000rpm at 20^oC for 40 min. The supernatant was removed by briefly spinning the plate upside down at 500rpm at 20^oC for 30s. The pellet was left to air-dry and then reconstituted with 4.5µl dH_2O.

2.3.4.5 Cycle sequencing

The purified PCR product was then sequenced in both forward and reverse directions using relevant primers. Each 10µl reaction was set up as follows:-

- 4.5µl purified PCR product
- 0.5µl BigDye Reaction Mix
- 2.0µl 5X Sequencing buffer
- 1.0µl dH_2O
- 2.0µl forward or reverse primer (4pmol)

The cycling conditions were 96^{o}C for 3 min followed by 30 cycles of 96^{o}C (30s), 50^{o}C (15s) and 60^{o}C (4 min).

2.3.4.6 Sequencing reaction clean-up preparation

Sequencing reactions were cleaned up to remove any incorporated dye terminators. The EDTA method of precipitation was used. To the 10µl sequencing reaction, 1µl EDTA (250nM) was added and mixed before adding 30µl of absolute ethanol. This was incubated for 5-10 min at room temperature and then centrifuged for 20 min at 2000rpm (20^{o}C). The supernatant was removed by briefly spinning the plate upside down to a maximum of 400rpm. 90µl of 70% ethanol was then added, mixed and the plate centrifuged again for a further 10 min at 2000rpm (20^{o}C). The supernatant was discarded by inverting the plate and spinning to 400rpm. The pellet was left to air dry.

2.3.4.7 Preparation and analysis of sequencing reactions

Pellets were resuspended in 10µl Hi-Di Formamide and then denatured for 5 minutes before snap-chilling on ice. Sequencing reactions were run on the ABI 3730 DNA Analyzer. Analysed sequences were then downloaded using ‘Chromas‘ software, printed and assessed manually for mutations.

2.3.5 Whole exome sequencing

DNA (1-3µg) was sheared to 100-400 bp using a Covaris E210 or LE220 (Covaris, Woburn, MA, USA). Sheared DNA was subjected to Illumina paired-end DNA library preparation and enriched for target sequences (Agilent Technologies; Human All Exon 50 Mb - ELID S02972011) according to manufacturer's recommendations (Agilent Technologies; SureSelectXT Automated Target Enrichment for Illumina Paired-End Multiplexed Sequencing). Enriched libraries were sequenced using the HiSeq platform (Illumina) as paired-end 75 base reads according to manufacturer's protocol. This methodology was utilised by both our collaborative groups at the Beijing Genomics Institute (BGI) (Beijing, China) and the Wellcome Trust Sanger Institute (WTSI) (Hinxton, UK).

2.3.6 Assessment of Mutation Pathogenicity

Variants identified by molecular genetic investigations were evaluated by:

(a) determining the predicted effect on the gene product using bioinformatics resources (PolyPhen-2, SIFT) (e.g. putative missense mutations were

inspected to determine if they affect amino acids that are evolutionarily conserved and if they have a deleterious effect on protein function)

(b) identifying if candidate genes are known to be involved in cardiac development and/or members of developmental pathways (Ensembl, GeneDistiller, OMIM, PubMed, UCSC Genome Bioinformatics)

(c) identifying animal models of disease associated with the candidate gene (Mouse Genome Informatics, PubMed)

(d) demonstrating appropriate segregation within a family

(e) demonstrating absence in >360 ethnically matched control chromosomes.

2.4 Website addresses for internet resources

A number of websites were utilised for various aspects of experimental design and data analysis (Table 4).

2.5 Timescale for all processes within the project

See Appendix A for a summary of the timescale for all the processes involved within the project.

Table 4: Web based resources.

1000 Genomes	http://www.1000genomes.org
Affymetrix	http://www.affymetrix.com/estore
BioGRID	http://thebiogrid.org
BLAST	http://blast.ncbi.nlm.nih.gov/Blast.cgi
CHDWiki	http://homes.esat.kuleuven.be/~bioiuser/chdwiki/index.php/Main_Page
dbSNP	http://www.ncbi.nlm.nih.gov./projects/SNP
DECIPHER	https://decipher.sanger.ac.uk/application
Ensembl	http://www.ensembl.org
ExonPrimer	http://ihg2.helmholtz-muenchen.de/ihg/ExonPrimer.html
Gene cards	http://www.genecards.org
Gene Distiller 2	http://www.genedistiller.org
Homozygosity Mapper	http://www.homozygositymapper.org
KOMP	https://www.komp.org
London Medical Databases	http://www.lmdatabases.com
Mouse Genome Informatics	http://www.informatics.jax.org
NCBI	http://www.ncbi.nlm.nih.gov
NCBI Nucleotide	http://www.ncbi.nlm.nih.gov/sites/nuccore
OMIM	http://www.ncbi.nlm.nih.gov/sites/entrez?db=OMIM
PolyPhen-2	http://genetics.bwh.harvard.edu/pph2
Primer3	http://frodo.wi.mit.edu/primer3
PubMed	http://www.ncbi.nlm.nih.gov./pubmed
SIFT	http://sift.jcvi.org
Splice Site Prediction by Neural Network	http://www.fruitfly.org/seq_tools/splice.html
UCSC	http://www.genome.ucsc.edu
UK10K	http://www.uk10k.org
UNISTS	http://www.ncbi.nlm.nih.gov./unists

CHAPTER 3

RESULTS

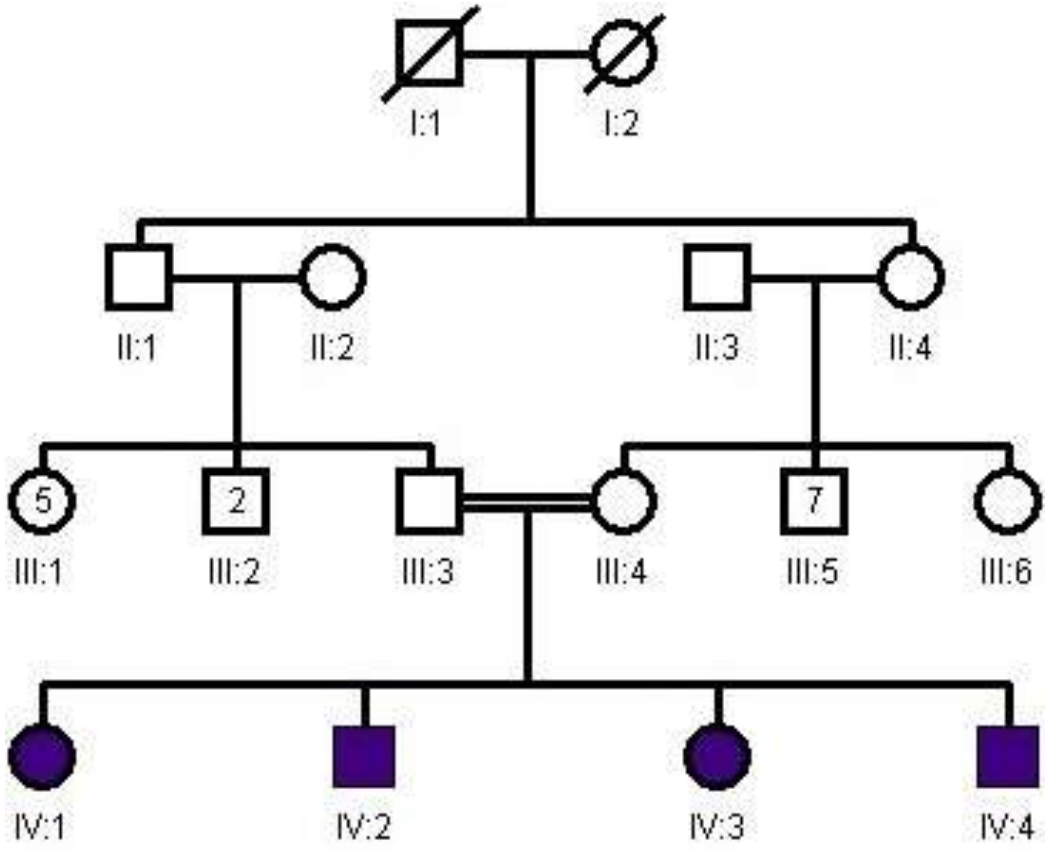
3.1 Clinical studies of familial CHD cohort

In this project, molecular genetic analysis was undertaken in consanguineous and non-consanguineous families containing individuals affected with a form of non-syndromic CHD. A total of 23 families who fulfilled our criteria were visited, clinically examined, and recruited into the project. The ethnic backgrounds of the families were: Pakistani (4), Indian (1), Irish (2), and White British (16). A total of 58 affected individuals were identified, of which consent and blood/saliva (and in one case tissue) samples were taken from 41 available affected individuals (~71%) (highlighted in blue in Table 5). Consent and blood/saliva samples were also taken from 64 unaffected family members (siblings, parents and offspring). Despite appearing to be a non-syndromic familial CHD cohort, to exclude frequent chromosomal abnormalities, routine cytogenetic analysis and FISH testing for 22q11.2 deletion syndrome was performed in one affected individual from all families and the results were normal in all cases. We did not use microarray analysis in each family as they were deemed to be non-syndromic CHD, and therefore less likely to have a chromosome deletion/duplication, and funding was not available to perform testing in the NHS laboratory for our cases.

3.1.1 Clinical phenotype of each individual CHD family

For each individual family, details of the family history (simplified pedigree diagram), clinical phenotypes, and results of routine cytogenetics and FISH studies are described below. Appendix D summarises the data collected fo each family using the recruitment proforma.

3.1.1.1 Clinical phenotype of family CHD1

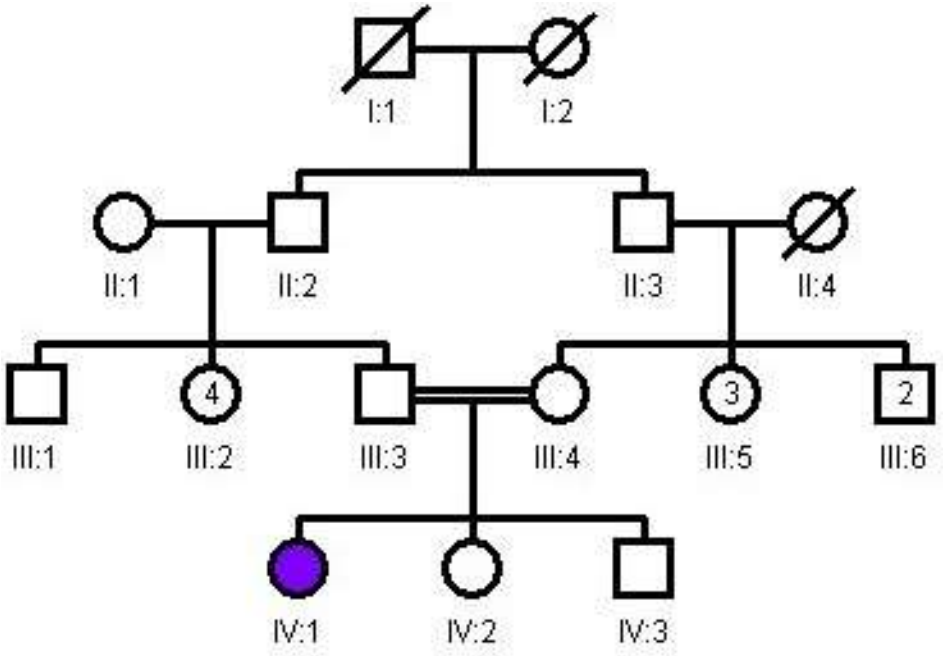


This is a large consanguineous Pakistani family (parents of affected siblings are first cousins) with suspected autosomal recessive inheritance of CHD. IV:1 was diagnosed at birth with TOF (absent pulmonary valve type) due to a murmur on clinical examination, and she had corrective surgery at 3 months of age. IV:2 was born with a right sided cleft lip and palate, pyloric stenosis, trachea-oesophageal fistula with oesophageal atresia, right sided talipes, and a right undescended testicle. The trachea-oesophageal fistula with oesophageal atresia was repaired by 1 day of age, and the cleft lip and palate were repaired at 3 months and 2 years of age respectively. He had a normal renal ultrasound and ophthalmology assessment. At the age of 6 years he collapsed with loss of consciousness and diagnosed with VSD which was surgically closed at that

time. IV:3 was diagnosed antenatally with VSD, but a postnatal ECHO clarified this as TOF (absent pulmonary valve type), and she had corrective surgery at 5 years of age. IV:4 was diagnosed antenatally with TOF (absent pulmonary valve type), which was confirmed on a postnatal ECHO, and he had corrective surgery at 2 years of age.

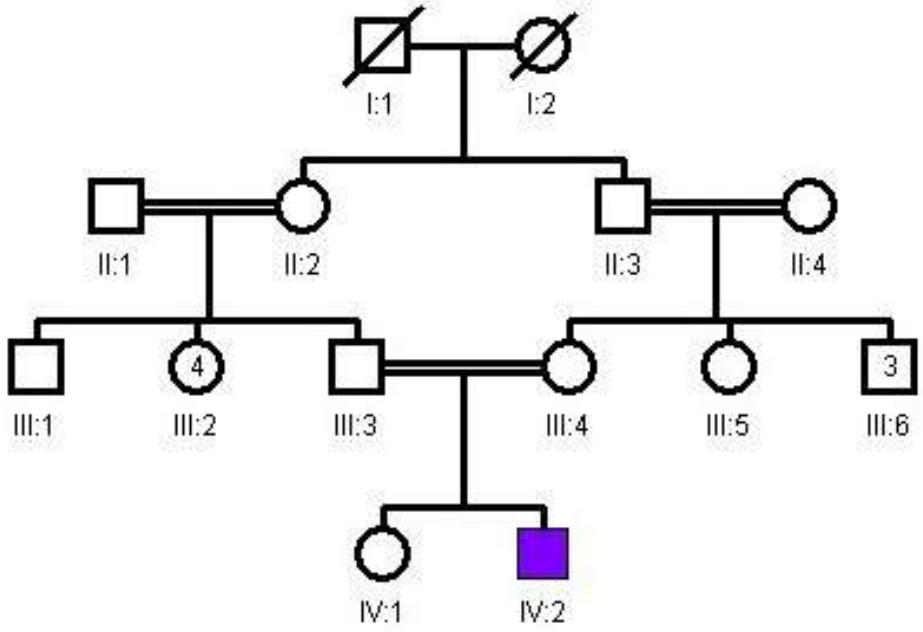
Clinical history and examination of IV:1, IV:3 and IV:4 did not identify any features suggestive of syndromic CHD. No dysmorphic features were noted in IV:2, but in light of his clinical features, a syndromic form of CHD could not be excluded (though there were no obvious diagnoses that counted for his problems). Samples were taken from all affected siblings and their parents. Routine cytogenetic analysis and FISH testing for 22q11.2 deletion in IV:2 and IV:4 was normal. A chromosomal abnormality in IV:2 was further excluded with a normal array CGH (performed using the Affymetrix 2.7M Cytogenetics microarray by the NHS West Midlands Regional Genetics Laboratory).

3.1.1.2 Clinical phenotype of family CHD2



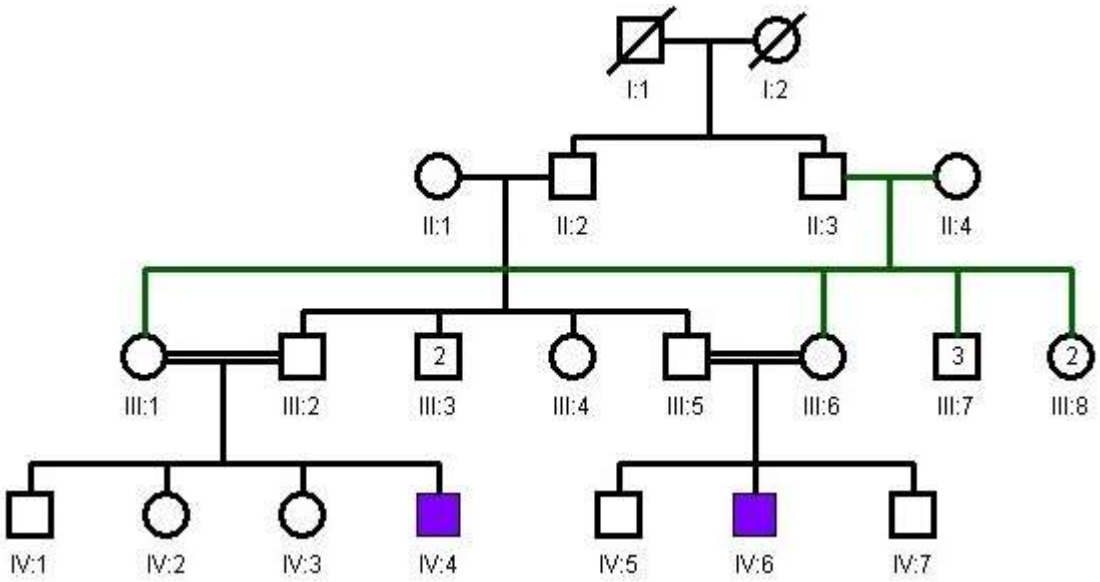
This is a consanguineous Pakistani family (parents of IV:1 are first cousins) with suspected autosomal recessive inheritance of CHD. IV:1 was diagnosed with pulmonary atresia (PA) and VSD (TOF spectrum) at birth, and had a Blalock–Taussig (BT) shunt inserted and corrective surgery at 6 days and 1 year of age respectively. Clinical history and examination of IV:1 did not identify any features suggestive of syndromic CHD. Samples were taken from IV:1, both unaffected siblings (IV:2 and IV:3), and their parents. Routine cytogenetic analysis and FISH testing for 22q11.2 deletion in IV:1 was normal.

3.1.1.3 Clinical phenotype of family CHD3



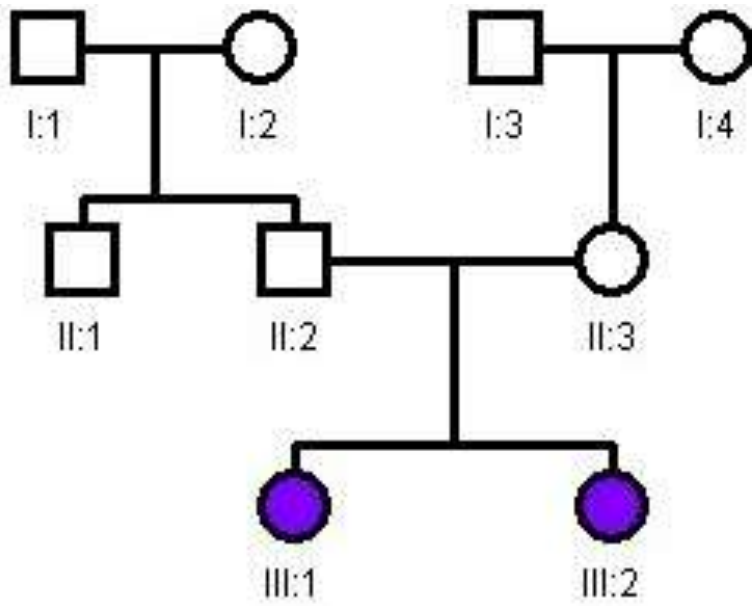
This is a consanguineous Pakistani family (parents of IV:2 are first cousins) with suspected autosomal recessive inheritance of CHD. The maternal and paternal grandparents of IV:2 are also distantly related. IV:2 was diagnosed at 2 years of age with AS and branch pulmonary artery stenosis due to a murmur on clinical examination, but has not required any surgical interventions yet. Clinical history and examination of IV:2 did not identify any features suggestive of syndromic CHD. Samples were taken from IV:2, the unaffected sibling, and their parents. Routine cytogenetic analysis and FISH testing for 22q11.2 and 7q11.23 deletions in IV:2 was normal.

3.1.1.4 Clinical phenotype of family CHD4



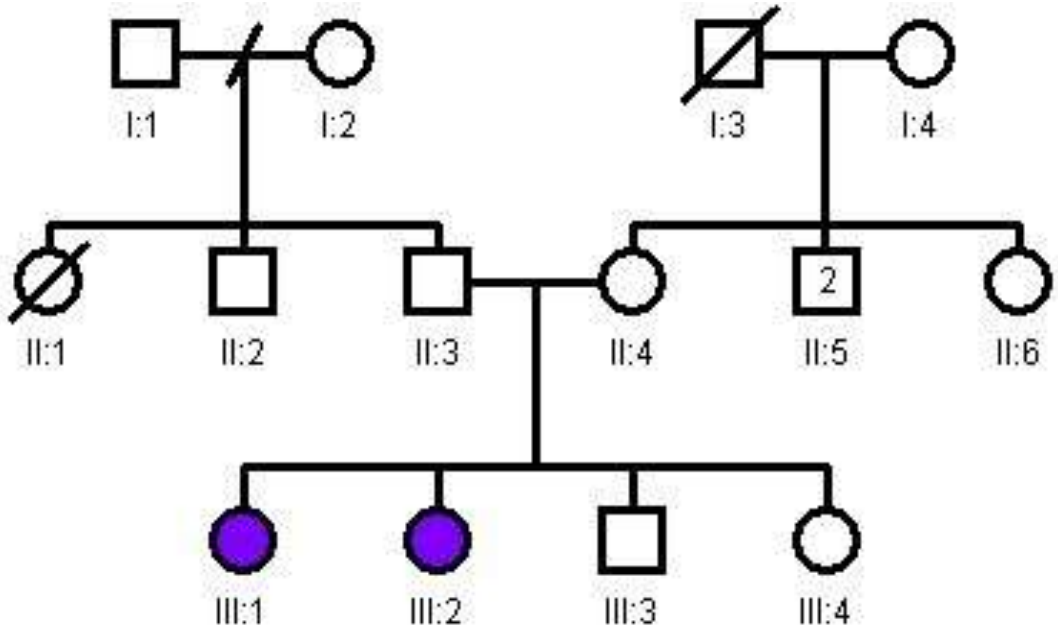
This is a large consanguineous Pakistani family with suspected autosomal recessive inheritance of CHD. There are two branches of the family affected with CHD, and the parents of both affected individuals are first cousins. IV:4 was diagnosed at birth with DORV and VSD and surgically corrected at 1 month of age. IV:6 was diagnosed with a congenitally corrected TGA, VSD and Ebstein's anomaly at 6 weeks of age due to a murmur on clinical examination. He had a double switch procedure and VSD closure at 9 months of age. Clinical history and examination of IV:4 and IV:6 did not identify any features suggestive of syndromic CHD. Samples were taken from both affected individuals, all unaffected siblings (IV:1, IV:2, IV:3, IV:5, and IV:7), and both sets of parents. Routine cytogenetic analysis and FISH testing for 22q11.2 deletion in IV:6 was normal.

3.1.1.5 Clinical phenotype of family CHD5



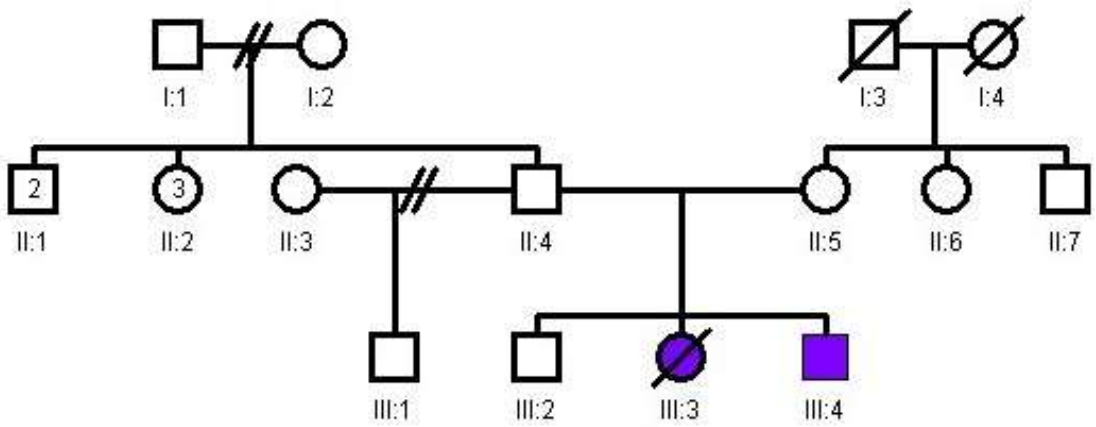
This is a non-consanguineous White British family with suspected autosomal recessive inheritance of CHD. III:1 was diagnosed at 2 weeks of age with VSD and RV hypoplasia due to a murmur on clinical examination. She had a pulmonary artery band, cavopulmonary shunt, and then a Fontan procedure at 9 years of age. Due to the history her sister (III:2) was diagnosed antenatally with a heart defect and a postnatal ECHO confirmed ASD and RV hypoplasia, but she has not required any surgical intervention yet. Clinical history and examination (III:1 and III:2) did not identify any features suggestive of syndromic CHD. Samples were taken from the affected siblings and their parents. Routine cytogenetic analysis and FISH testing for 22q11.2 deletion in III:1 was normal.

3.1.1.6 Clinical phenotype of family CHD6



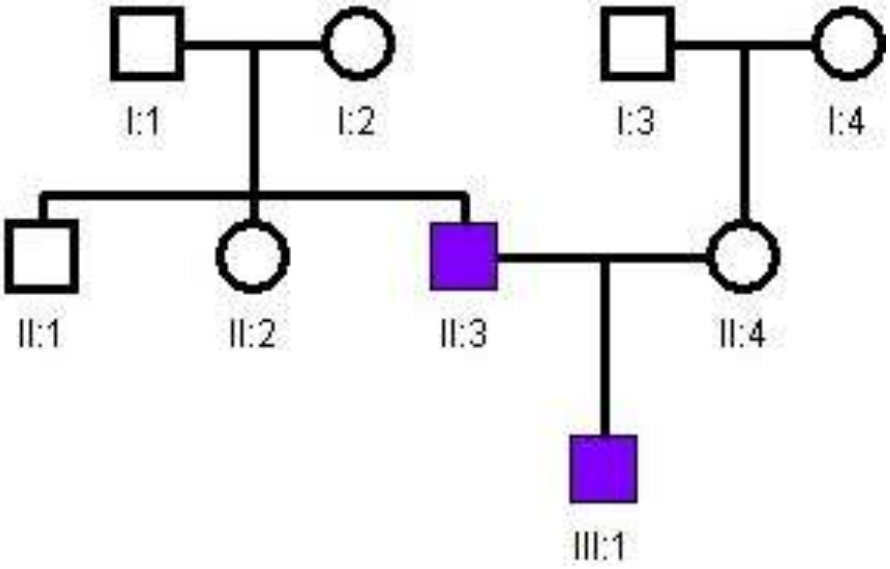
This is a non-consanguineous White British family with suspected autosomal recessive inheritance of CHD. The eldest of four siblings (III:1) was diagnosed with TOF and right sided AA at 3 months of age due to cyanosis and a heart murmur on examination. She had a Waterston shunt inserted at 2 years of age, and subsequently had corrective surgery at 3 years of age. Her sister (III:2) was diagnosed antenatally with a heart defect, and a postnatal ECHO confirmed TOF. She had a BT shunt inserted and subsequently corrective surgery at 7 months and 2 years of age respectively. Clinical history and examination (III:1 and III:2) did not identify any features suggestive of syndromic CHD. Samples were taken from the affected siblings, both the unaffected siblings (III:3 and III:4), and their parents. Routine cytogenetic analysis and FISH testing for 22q11.2 deletion in III:1 was normal.

3.1.1.7 Clinical phenotype of family CHD7



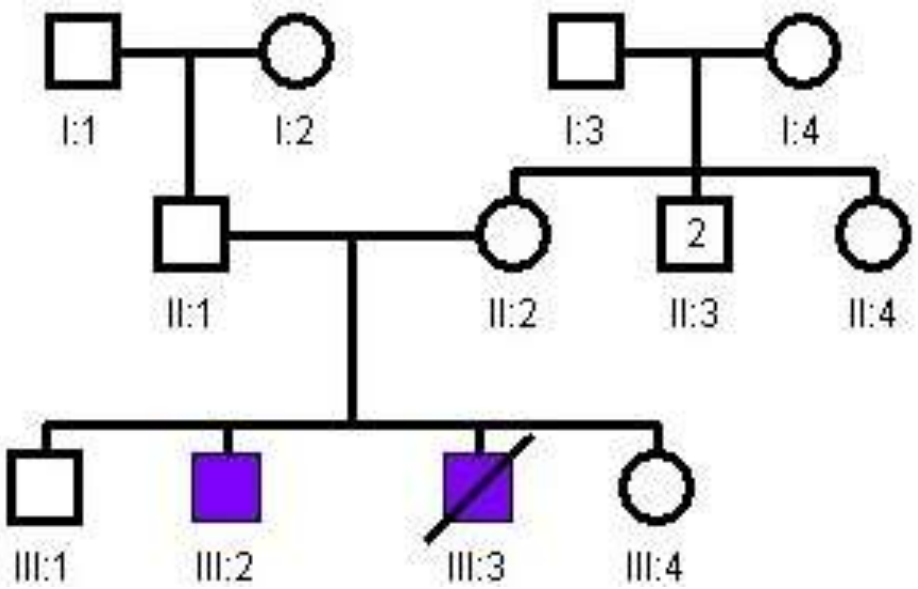
This is a non-consanguineous White British family with suspected autosomal recessive inheritance of CHD. The second eldest of three siblings (III:3) was diagnosed with TAPVD at 13 days of age due to cyanosis and feeding problems. She required multiple surgical procedures but died at 4 months of age due to post operative complications. Her sibling (III:4) was also diagnosed with TAPVD at 10 days of age due to cyanosis and feeding problems, and had corrective surgery at 11 days of age. Clinical history and examination of III:4 did not identify any features suggestive of syndromic CHD. Samples were taken from III:4, unaffected sibling (III:2), and their parents. Routine cytogenetic analysis and FISH testing for 22q11.2 deletion in III:4 was normal.

3.1.1.8 Clinical phenotype of family CHD8



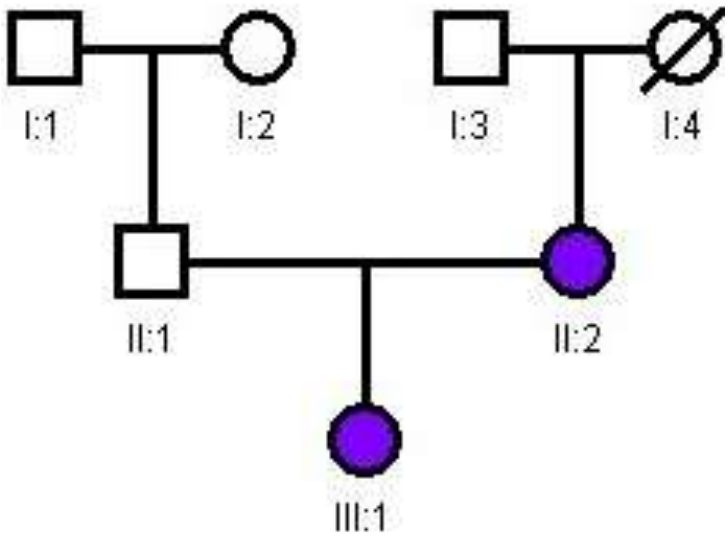
This is a non-consanguineous White British family with suspected autosomal dominant inheritance of CHD. The father (II:3) was diagnosed at 4 years of age with TOF due to cyanotic spells, and had corrective surgery at 4 years of age. His son (III:1) was diagnosed with a heart defect on antenatal scans and a postnatal ECHO confirmed TOF. He had a pulmonary artery patch at 3 months of age, and corrective surgery at 8 years of age. Clinical history and examination of II:3 and III:1 did not identify any features suggestive of syndromic CHD. Samples were taken from the affected father, affected son, and unaffected mother (II:4). Routine cytogenetic analysis and FISH testing for 22q11.2 deletion in III:1 was normal

3.1.1.9 Clinical phenotype of family CHD9



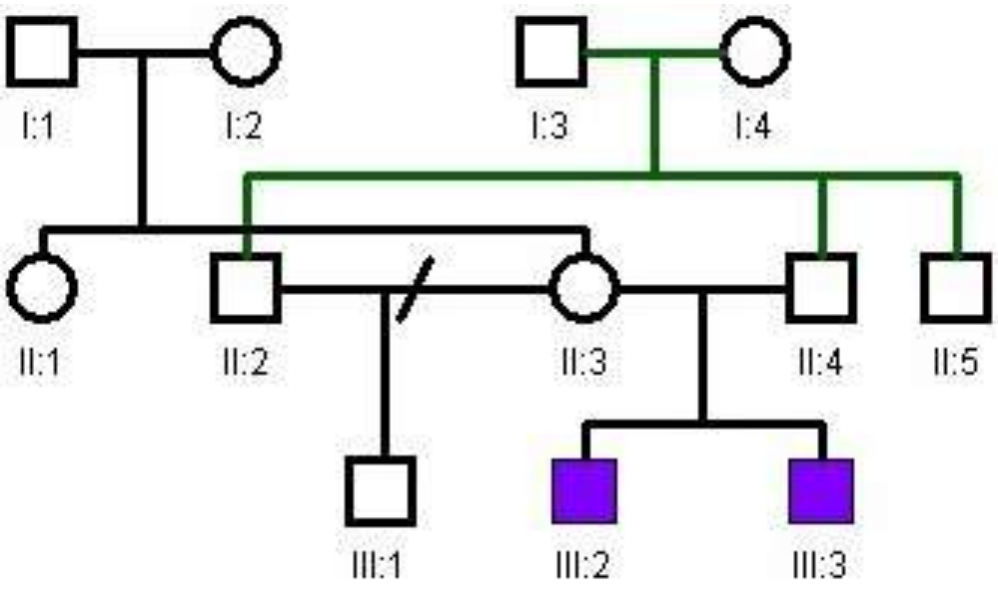
This is a non-consanguineous White British family with suspected autosomal recessive inheritance of CHD. The second eldest of four siblings (III:2) was diagnosed with TGA, VSD, and PS at birth, and had an arterial switch procedure at 2 weeks of age. His sibling (III:3) was also diagnosed with TGA, VSD, and PS at birth, but died due to post operative complications after he had an arterial switch procedure at 1 week of age. Both unaffected siblings had a normal ECHO. Clinical history and examination of III:2 did not identify any features suggestive of syndromic CHD. Samples were taken from III:2, two unaffected siblings (III:1 and III:4), and their parents. Routine cytogenetic analysis and FISH testing for 22q11.2 deletion in III:2 was normal.

3.1.1.10 Clinical phenotype of family CHD10



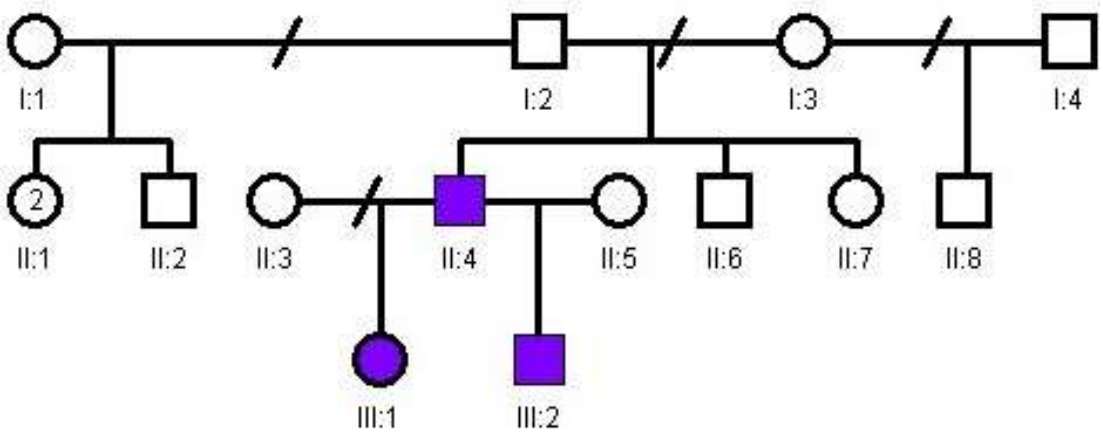
This is a non-consanguineous White British family with suspected autosomal dominant inheritance of CHD. The daughter (III:1) was diagnosed at 6 weeks of age, due to a murmur on clinical examination, with a complex CHD consisting of dextrocardia, left atrial isomerism, AVSD, PS, RV hypoplasia, right sided AA, and bilateral superior vena cavae (SVC). She had palliative surgery with a BT shunt inserted at 6 months of age, and then a cavopulmonary shunt and Fontan procedure at 18 months and 9 years of age respectively. She also has abdominal situs inversus. Subsequently an ECHO in both parents identified that her mother (II:2) (at age 33 years) had a congenitally corrected TGA and dextrocardia, but normal situs of abdominal organs. She has never required any surgical inventions. Clinical history and examination of II:2 and III:1 did not identify any features suggestive of syndromic CHD. Samples were taken from the affected mother, affected daughter, and unaffected father. Routine cytogenetic analysis and FISH testing for 22q11.2 deletion in III:1 was normal.

3.1.1.11 Clinical phenotype of family CHD11



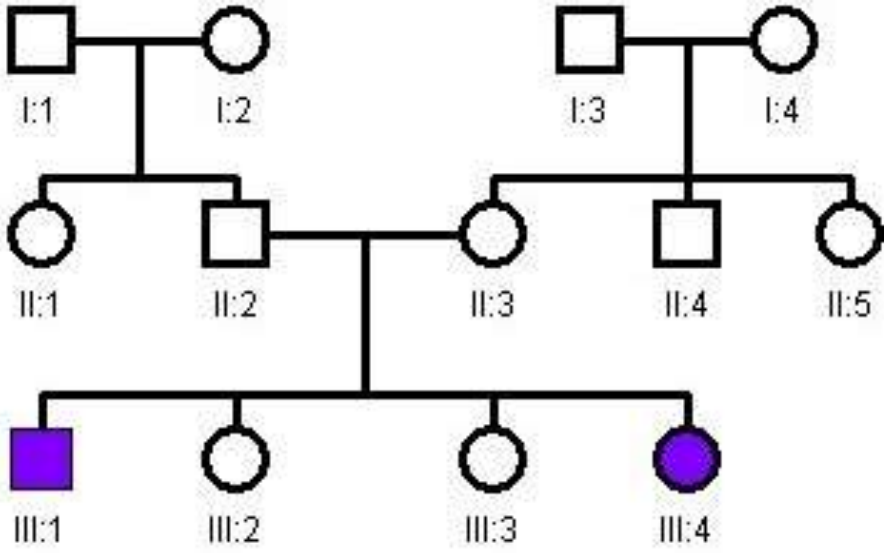
This is a non-consanguineous White British family with suspected autosomal recessive inheritance of CHD. III:2 was diagnosed with VSD at 4 months of age due to a murmur detected on clinical examination, and is awaiting a surgical closure when older. His younger brother (III:3) was diagnosed with AS, BAV, and mild CoA at 5 weeks of age due to feeding problems. He had a balloon dilatation for the AS at 5 weeks of age. Both parents and the unaffected half brother had a normal ECHO. Clinical history and examination (III:2 and III:3) did not identify any features suggestive of syndromic CHD. Samples were taken from the affected siblings, their parents, and the unaffected half sibling (III:1). Routine cytogenetic analysis and FISH testing for 22q11.2 deletion in III:2 was normal.

3.1.1.12 Clinical phenotype of family CHD12



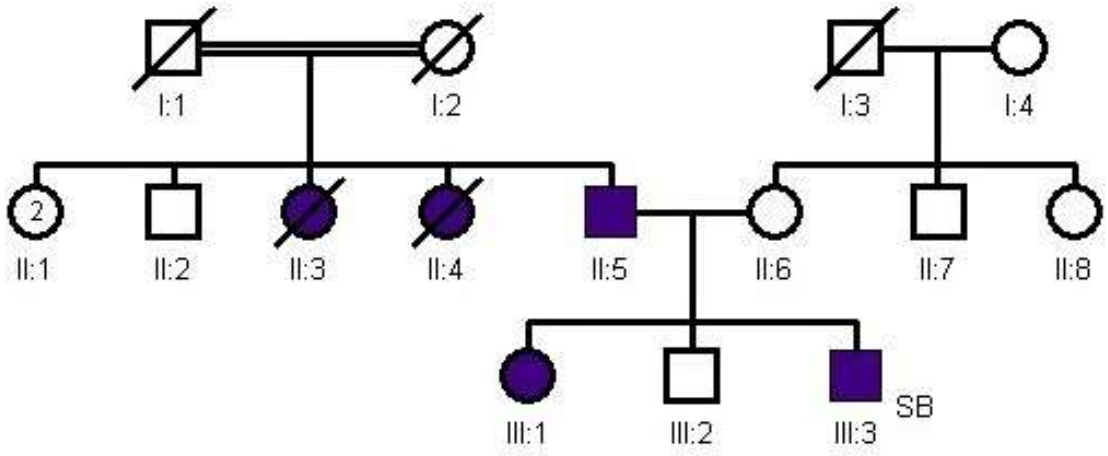
This is a non-consanguineous White British family with suspected autosomal dominant inheritance of CHD. The father (II:4) was diagnosed with TOF and ASD at birth due to cyanosis, and surgery was performed to correct the TOF and ASD at 16 months and 7 years of age respectively. His daughter (III:1) was also diagnosed with TOF and ASD after a murmur was detected on her routine 8 week baby check, and had corrective surgery at 15 months of age. His son (III:2) had an ECHO at 2 weeks of age due to the family history and was diagnosed with VSD, ASD, and PDA, which were surgically corrected at 4 months of age. Clinical history and examination (II:4, III:1, and III:2) did not identify any features suggestive of syndromic CHD. Samples were taken from II:4 only and routine cytogenetic analysis and FISH testing for 22q11.2 deletion was normal.

3.1.1.13 Clinical phenotype of family CHD13



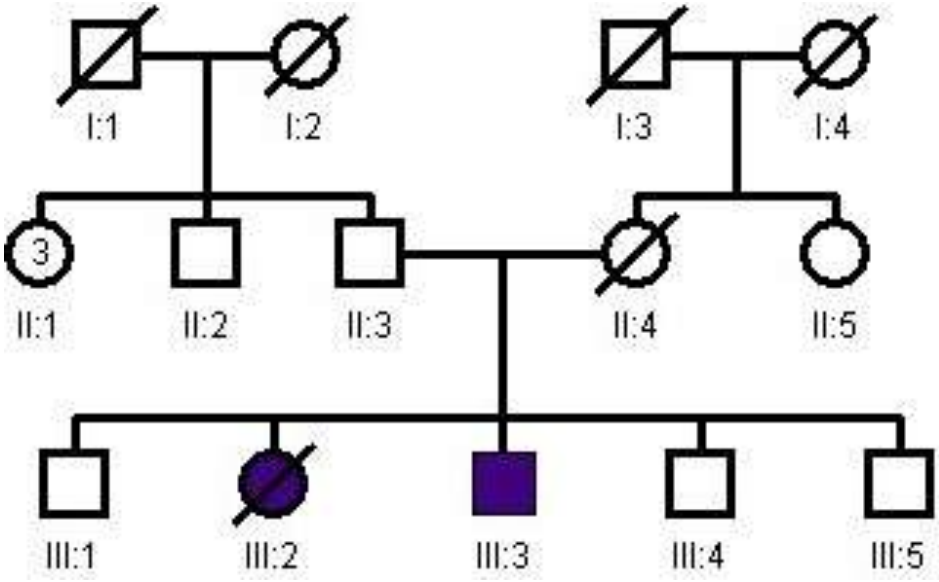
This is a non-consanguineous White British family with suspected autosomal recessive inheritance of CHD. The eldest of four siblings (III:1) was diagnosed with TGA, VSD and PS at 10 days of age due to a murmur on clinical examination and feeding problems. He had a BT shunt inserted at 2 weeks of age, and subsequently had a Norwood procedure and Fontan procedure at 1 year and 6 years respectively. His youngest sister (III:4) was diagnosed antenatally (due to the family history) with TGA, and had corrective surgery (via an arterial switch procedure) at 2 weeks of age. Both the unaffected siblings had a normal ECHO in the antenatal and postnatal period. Clinical history and examination (III:1 and III:4) did not identify any features suggestive of syndromic CHD. Samples were taken from the affected siblings, both unaffected siblings (III:2 and III:3), and their parents. Routine cytogenetic analysis and FISH testing for 22q11.2 deletion in III:1 was normal.

3.1.1.14 Clinical phenotype of family CHD14



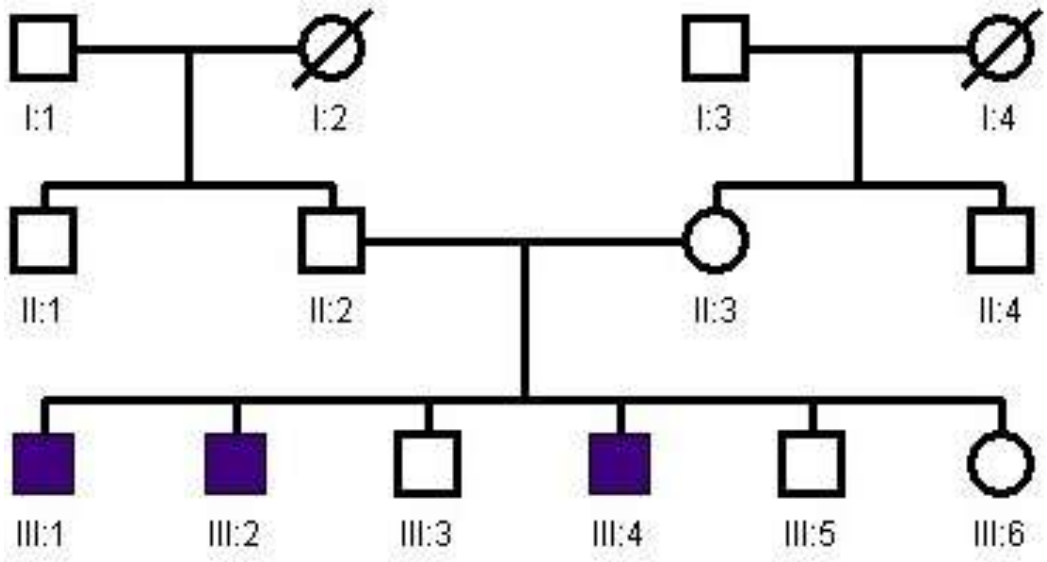
This is a non-consanguineous White British family with suspected autosomal dominant inheritance of CHD. The eldest daughter (III:1) was diagnosed with CoA, VSD, and BAV at 6 weeks of age due to a murmur on clinical examination and feeding problems. The CoA and VSD were surgically corrected at 4 months and 8 months of age respectively. Her youngest brother (III:3) had an antenatal ultrasound diagnosis of HLHS, and was a stillbirth at 26 weeks gestation. A post mortem confirmed HLHS, and identified CoA and BAV. After his birth both parents had an ECHO and the father (II:5) (aged 42 years) was diagnosed with BAV. There was a family history of CHD affecting two sisters (II:3 and II:4) on the paternal side of the family. Unfortunately they died within the first week of life in the 1940's and therefore details were unavailable for further clarification. The parents of II:3, II:4, and II:5 were distantly related (3rd cousins). Clinical history and examination (II:5 and III:1) did not identify any features suggestive of syndromic CHD. Samples were taken from III:1, unaffected sibling (III:2), their affected father (II:5) and unaffected mother. Routine cytogenetic analysis and FISH testing for 22q11.2 deletion in III:1 was normal.

3.1.1.15 Clinical phenotype of family CHD15



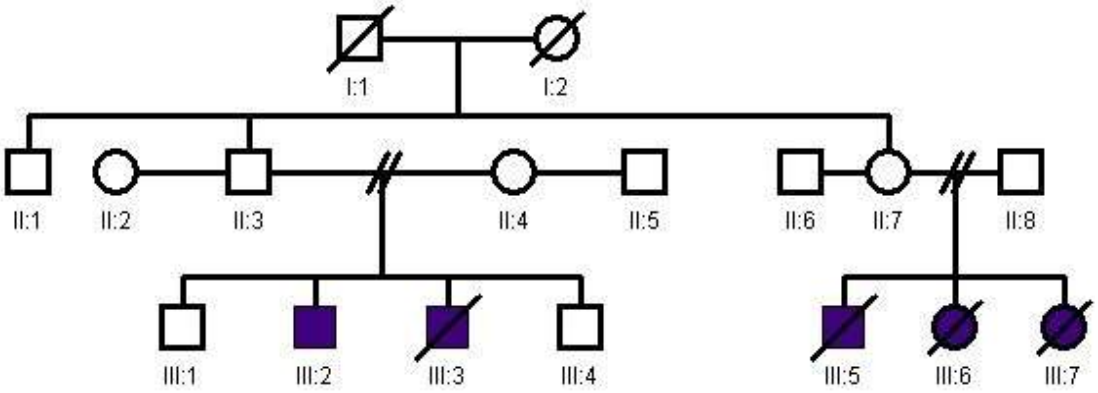
This is a non-consanguineous White British family with suspected autosomal recessive inheritance of CHD. The second eldest of five siblings (III:2) was diagnosed with TGA at birth, but unfortunately died at 3 years of age due to post operative complications. Her sibling (III:3) was diagnosed with TGA, VSD, and ASD at 3 weeks of age due to feeding problems and a cardiac arrest. He had a Mustard procedure at 3 weeks of age and then an arterial switch procedure at 4 years of age. Clinical history and examination of III:3 did not identify any features suggestive of syndromic CHD. Samples were taken from III:3, two unaffected siblings (III:1 and III:5), and their father. Routine cytogenetic analysis and FISH testing for 22q11.2 deletion in III:3 was normal.

3.1.1.16 Clinical phenotype of family CHD16



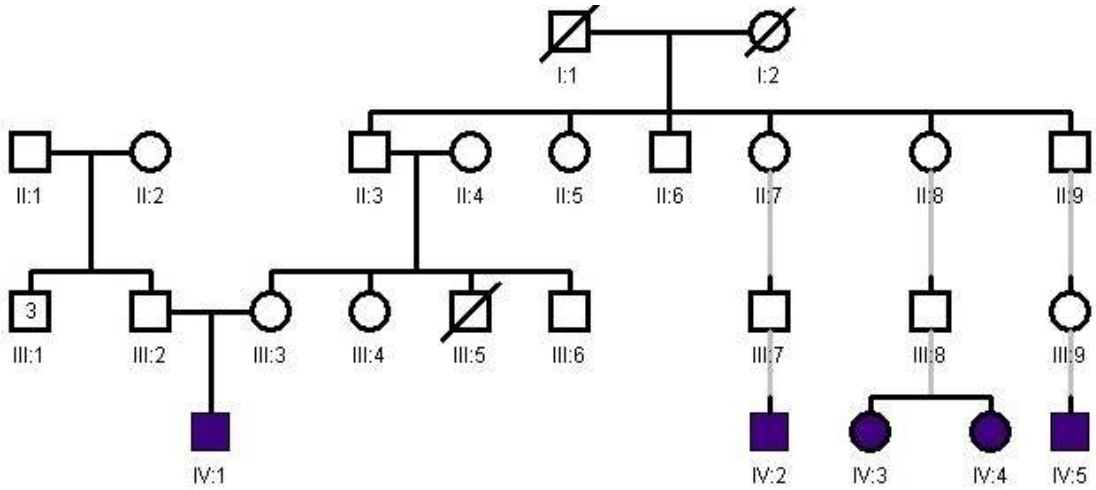
This is a non-consanguineous White British family with suspected autosomal recessive inheritance of CHD. The eldest of six siblings (III:1) was diagnosed with TOF at birth due to cyanosis. He had a pulmonary artery band inserted at 2 weeks of age and the VSD was repaired at 2 years of age. Due to his history all his subsequent siblings had an ECHO at birth. III:2 was diagnosed with CoA, BAV, and VSD, and had surgical correction of the CoA at 9 days of age, but the VSD closed spontaneously at 1 year of age. III:4 was diagnosed with ASD which closed spontaneously by 6 months of age. The other three siblings and both parents had a normal ECHO. Clinical history and examination of III:1, III:2, and III:4 did not identify any features suggestive of syndromic CHD. Samples were taken from all three affected siblings, unaffected siblings (III:3, III:5, and III:6), and their parents. Routine cytogenetic analysis and FISH testing for 22q11.2 deletion in III:1 was normal.

3.1.1.17 Clinical phenotype of family CHD17



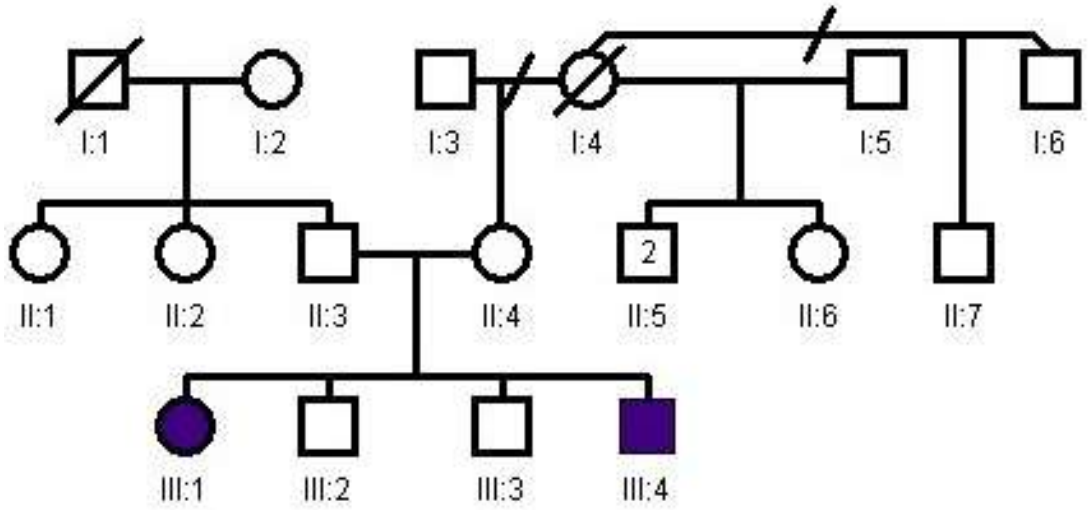
This is a large non-consanguineous White British family with suspected autosomal recessive inheritance of CHD. There are two branches of the family affected with CHD. III:2 was diagnosed with CoA at 4 days of age due to feeding problems, and had surgery at 5 days of age, but required further surgical intervention at 4 years of age. His sibling (III:3) was diagnosed with HLHS and CoA in the neonatal period but died at 4 days of age before surgery. They had three cousins with CHD from their paternal side of the family. III:5 died at 2 days of age and a post mortem identified HLHS. III:6 presented at 2 days of age with severe cardiac failure and died at 5 days of age. A post mortem identified HLHS, CoA and VSD. III:7 was diagnosed with TGA, VSD and ASD at 2 weeks of age and had a pulmonary artery band fitted at 1 month of age. She then had a Fontan procedure at 8 years of age but died post operatively. Clinical history and examination of III:2 did not identify any features suggestive of syndromic CHD. Samples were taken from III:2, one unaffected sibling (III:4), and their father (II:3). I obtained stored tissue from the post-mortem of III:6 for DNA extraction, and samples from her mother (II:7). Routine cytogenetic analysis and FISH testing for 22q11.2 deletion in III:2 was normal.

3.1.1.18 Clinical phenotype of family CHD18



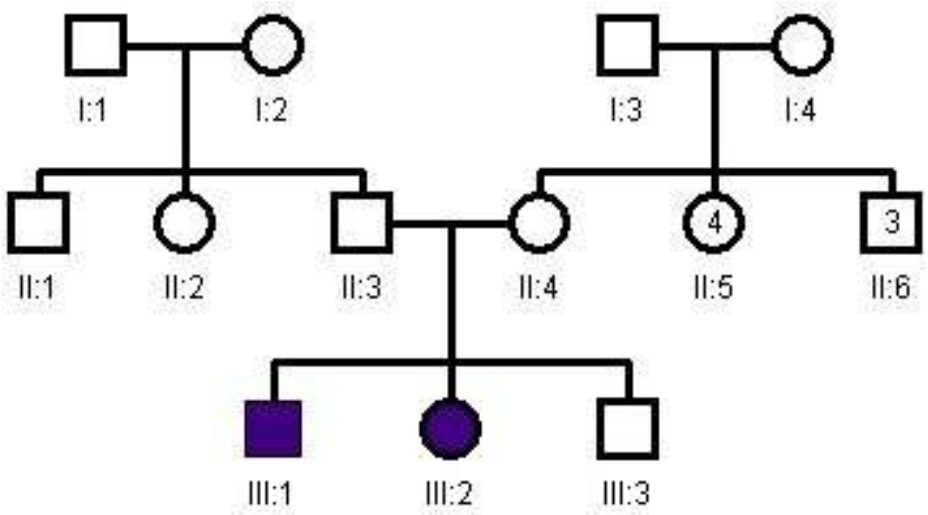
This is a non-consanguineous Irish family with an unknown inheritance of CHD. The parents reported to come from the same town in Ireland. Full details were unavailable for four affected individuals (IV:2, IV:3, IV:4, and IV:5) but were based on the history given by the parents of IV:1, who was diagnosed at birth with HLHS due to cyanosis and feeding problems. He had a Norwood type procedure and Bidirectional Glenn procedure at 1 week and 5 months of age respectively. Clinical history and examination of IV:1 did not identify any features suggestive of syndromic CHD. Samples were taken from IV:1 and his parents. Routine cytogenetic analysis and FISH testing for 22q11.2 deletion in IV:1 was normal.

3.1.1.19 Clinical phenotype of family CHD19



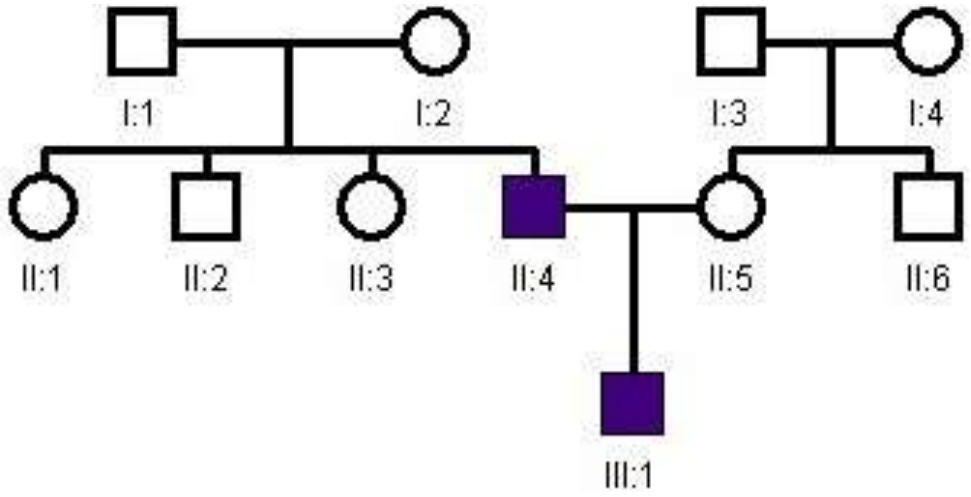
This is a non-consanguineous White British family with suspected autosomal recessive inheritance of CHD. The eldest of four siblings (III:1) was diagnosed antenatally with HLHS, and had stages I and II of the Norwood procedure at 4 days and 14 months of age respectively. Her youngest brother (III:4) was also diagnosed antenatally with HLHS, and had stages I and II of the Norwood procedure at 4 days and 5 months of age respectively. Clinical history and examination of III:1 and III:4 did not identify any features suggestive of syndromic CHD. Samples were taken from III:4, one unaffected sibling (III:2) and their parents. Routine cytogenetic analysis and FISH testing for 22q11.2 deletion in III:4 was normal.

3.1.1.20 Clinical phenotype of family CHD20



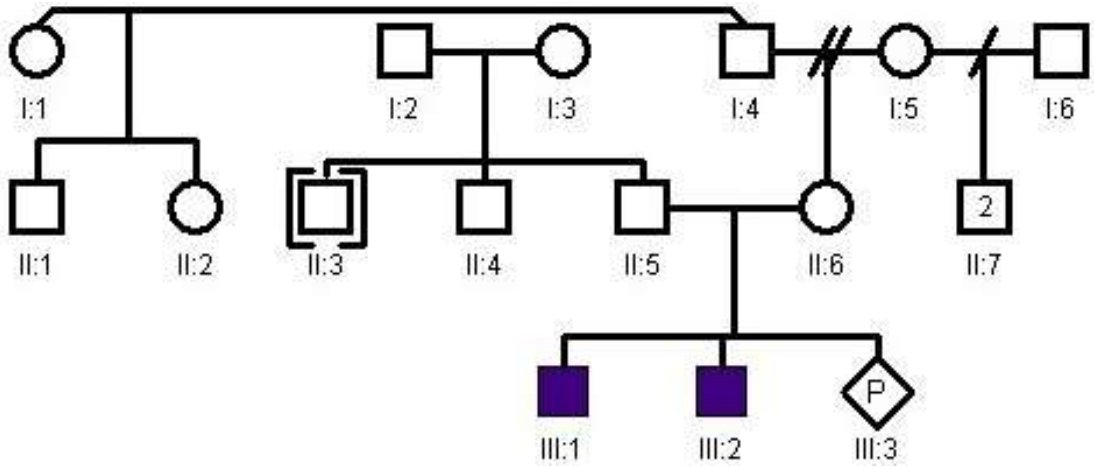
This is a non-consanguineous Indian family with suspected autosomal recessive inheritance of CHD. The eldest of three siblings (III:1) was diagnosed antenatally with a heart defect and postnatal ECHO confirmed tricuspid atresia and VSD. He had a pulmonary artery band at 3 weeks of age, and then a cavopulmonary shunt and Fontan procedure at 18 months and 5 years of age respectively. His sister (III:2) was also diagnosed antenatally with a heart defect and postnatal ECHO confirmed TGA, VSD, RV hypoplasia, and AA hypoplasia . She had stages I and II of the Norwood procedure at 1 day and 4 months of age respectively. The youngest sibling and both parents had a normal ECHO. Clinical history and examination of III:1 and III:2 did not identify any features suggestive of syndromic CHD. Samples were taken from both affected siblings and their parents. Routine cytogenetic analysis and FISH testing for 22q11.2 deletion in III:2 was normal.

3.1.1.21 Clinical phenotype of family CHD21



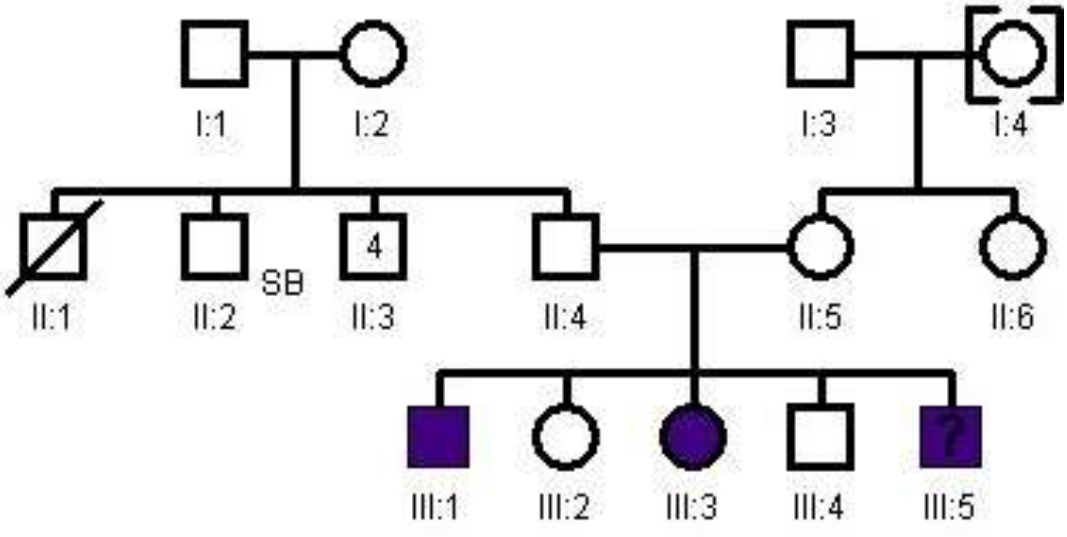
This is a non-consanguineous White British family with suspected autosomal dominant inheritance of CHD. The father (II:4) was diagnosed at 6 weeks of age with CoA and BAV due to cyanosis and feeding problems. He had corrective surgery at 18 months of age. His son (III:1) was diagnosed with a heart defect on antenatal scans, and a postnatal ECHO confirmed HLHS. He had stages I and II of the Norwood procedure at 3 days and 3 months of age respectively. Clinical history and examination of II:4 and III:1 did not identify any features suggestive of syndromic CHD. Samples were taken from both the affected father and son. Routine cytogenetic analysis and FISH testing for 22q11.2 deletion in III:1 was normal

3.1.1.22 Clinical phenotype of family CHD22



This is a non-consanguineous White British family with suspected autosomal recessive inheritance of CHD. The eldest of three siblings (III:1) was diagnosed with HLHS on antenatal scans. He had stage I of the Norwood procedure at 1 day of age, and then a cavopulmonary shunt and Fontan procedure at 4 months and 4 years of age respectively. His brother (III:2) was diagnosed with VSD after a murmur was detected on his routine 8 week baby check, and has not required any surgery. Both parents had a normal ECHO, and antenatal scans of the expected baby (III:3) were normal. Clinical history and examination of III:1 and III:2 did not identify any features suggestive of syndromic CHD. Samples were taken from both affected siblings and their parents. Routine cytogenetic analysis and FISH testing for 22q11.2 deletion in III:1 was normal.

3.1.1.23 Clinical phenotype of family CHD23



This is a non-consanguineous Irish family with suspected autosomal recessive inheritance of CHD. Two siblings (III:1 and III:3) were diagnosed with AS and sub aortic stenosis at 6 weeks of age due to a murmur on clinical examination, and neither have required any surgical intervention yet. Two other siblings (III:2 and III:4) and both parents had a normal ECHO. More recently the youngest sibling (III:5) has been diagnosed with sub aortic stenosis, despite having a normal ECHO at birth. Clinical history and examination of III:1 and III:3 did not identify any features suggestive of syndromic CHD. Samples were taken from both affected siblings (III:1 and III:2) and their parents. Routine cytogenetic analysis and FISH testing for 22q11.2 deletion in III:1 was normal.

3.1.2 Overview of clinical phenotype of familial CHD cohort

The 23 families recruited were clinically and genetically heterogeneous (Table 5). Thus there were 4 consanguineous and 13 non-consanguineous families with a familial pattern of non-syndromic CHD that could be consistent with autosomal recessive inheritance. In addition there were 5 non-consanguineous families with non-syndromic CHD in which the pattern of CHD within the family might suggest autosomal dominant inheritance, and 1 non-consanguineous family in which the pattern of CHD was undeterminable. According to the family histories the types and numbers of CHD seen in affected individuals are as follows: ASD (7), VSD (17), AVSD (1), PDA (1), TOF (11), TGA (10), DORV (1), PS (4), AS (5), CoA (8), BAV (6), HLHS (9), TAPVD (2), Ebstein's anomaly variants (2), RV hypoplasia (4), laterality defects (2), and undetermined CHD (6). Some individuals had more than one type of CHD making their phenotype quite complex. Although there are a good range of CHDs in our cohort, outflow tract anomalies (e.g. TOF, TGA, LVOTO) seem to be more prominent. A number of families (16/23) had more than one individual with the same CHD (concordance), such as TOF (families CHD1/6/8/12), TGA (families CHD9/13/15), left sided obstructive lesions (families CHD14/17/19/21/23), RV hypoplasia/Ebstein's anomaly spectrum (families CHD5/20), TAPVD (family CHD7], or laterality defects (family CHD10).

Table 5: Summary of clinical phenotype of familial CHD cohort.
(Φ = consanguineous, blue = samples obtained)

FAMILY	INHERITANCE	PEDIGREE No.	SEX	TYPE OF CHD
CHD 1 Φ	AR	*IV:1*	F	TOF spectrum (absent PV)
		IV:2	M	VSD
		IV:3	F	TOF spectrum (absent PV)
		IV:4	M	TOF spectrum (absent PV)
CHD 2 Φ	? AR	*IV:1*	F	TOF spectrum (PA)
CHD 3 Φ	? AR	*IV:2*	M	AS, PPAS
CHD 4 Φ	AR	*IV:4*	M	DORV, VSD
		IV:6	M	TGA, VSD, Ebstein's anomaly
CHD 5	AR	*III:1*	F	VSD, RV hypoplasia
		III:2	F	ASD, RV hypoplasia
CHD 6	AR	*III:1*	F	TOF, RT AA
		III:2	F	TOF
CHD 7	AR	*III:3*	F	TAPVD
		III:4	M	TAPVD
CHD 8	AD	*II:3*	M	TOF
		III:1	M	TOF
CHD 9	AR	*III:2*	M	TGA, VSD, PS
		III:3	M	TGA, VSD, PS
CHD 10	AD	*II:2*	F	TGA, dextrocardia
		III:1	F	LT atrial isomerism, RT AA, AVSD, PS, RV hypoplasia, bilateral SVCs, dextrocardia
CHD 11	AR	*III:2*	M	VSD
		III:3	M	AS, BAV, CoA
CHD 12	AD	*II:4*	M	TOF, ASD
		III:1	F	TOF, ASD
		III:2	M	ASD, VSD, PDA
CHD 13	AR	*III:1*	M	TGA, VSD, PS
		III:4	F	TGA
CHD 14	AD	*II:3*	F	CHD
		II:4	F	CHD
		II:5	M	BAV
		III:1	F	CoA, BAV, VSD
		III:3	M	HLHS, CoA, BAV
CHD 15	AR	*III:2*	F	TGA
		III:3	M	TGA, VSD, ASD
CHD 16	AR	*III:1*	M	TOF
		III:2	M	VSD, CoA, BAV
		III:4	M	ASD

CHD 17	AR	*III:2*	M	CoA
		III:3	M	HLHS, CoA
		III:5	M	HLHS
		III:6	F	VSD, CoA, HLHS
		III:7	F	TGA, VSD, ASD
CHD 18	? AR/AD	*IV:1*	M	HLHS
		IV:2	M	Septal defect
		IV:3	F	Univentricular heart
		IV:4	F	Septal defect
		IV:5	M	Septal defect
CHD 19	AR	*III:1*	F	HLHS
		III:4	M	HLHS
CHD 20	AR	*III:1*	M	Tricuspid Atresia, VSD
		III:2	F	TGA, VSD, RV hypoplasia, AA hypoplasia
CHD 21	AD	*II:4*	M	CoA, BAV
		III:1	M	HLHS
CHD 22	AR	*III:1*	M	HLHS
		III:2	M	VSD
CHD 23	AR	*III:1*	M	AS, sub-AS
		III:3	F	AS, sub-AS
		III:5	M	sub-AS

3.1.3 Discussion of clinical phenotype of familial CHD cohort.

Familial cases of CHD imply a genetic factor and are suitable for further molecular analysis. Although there are no reports of genes associated with autosomal recessive non-syndromic CHD, as autozygosity studies are powerful in identifying autosomal recessive disease genes, it is reasonable to utilise a similar tool for families with autosomal recessive non-syndromic CHD. It is plausible that these 'apparently' autosomal recessive families are actually families with autosomal dominant CHD with reduced penetrance in the parents, however to disprove this theory we need to identify gene variants in affected individuals and in one of their clinically unaffected parents.

Clinical heterogeneity is clearly observed in the familial cohort recruited, as the numbers would be too small if a particular sub group of CHD were selected. Despite the various methods of ascertaining suitable families for the project, outflow tract anomalies (e.g. TOF, TGA, LVOTO) seem to be more prominent in the cohort. This could be partially explained by ascertainment bias for two reasons. Firstly, I noticed that a number of the initial families I recruited had outflow tract anomalies, so I added a third category for recruitment (families with outflow tract anomalies and any inheritance pattern). Secondly, families with certain types of CHD may be less enthusiastic to identify the cause for the CHD within the family, due to the minimal impact of the disease. These families are likely to have isolated structural CHDs that are asymptomatic, incidental findings, or sometimes require little medical or surgical intervention (e.g. ASD, VSD).

From our familial cohort it is apparent that some CHDs show exact concordance, and imply genetic factors in their aetiology. Overall the exact concordance is 70% (16/23) in our cohort which is higher than that seen in previous reports where overall the exact concordance was 53% (Gill et al., 2003). This is more than likely to be due to the sample being studied, as our cohort is based on familial cases, whereas Gill et al. (2003) performed a study of recurrence in all cases of CHD (sporadic and familial). Of note in our cohort, are the families with more than one child with TOF (families CHD1/6), TGA (families CHD9/13/15), or left sided obstructive lesions (families CHD17/19/23), which imply an autosomal recessive inheritance pattern. Studies of familial recurrence of TOF have shown a 2.5-3% recurrence risk, and families have shown a horizontal pattern of recurrence, further suggesting a possible autosomal recessive genetic mechanism (Sanchez-Cascos, 1978; Miller and Smith, 1979; Wulfsberg et al., 1991; Digilio et al., 1997). Familial recurrence of TGA is reported to be 1-1.8% (Digilio et al., 2001), but some studies have shown this to be as high as 5.2% (Piacentini et al., 2005). As seen in our cohort, the different types of left sided obstructive lesions (HLHS, CoA, AS, and BAV) may even segregate together within the same family, and previously autosomal dominant and recessive patterns of inheritance have been observed in families with left sided obstructive lesions (Loffredo et al., 2004; McBride et al., 2005; Wessels et al., 2005).

During the diagnostic process in genetic counselling it is important to exclude different diagnoses within the same family, which may account for varying

clinical features despite some overlap, before assuming the same aetiology and variation in expression of the phenotype. This issue was seen in family CHD1, where despite having four affected siblings with CHD, it was clear that one of the siblings (IV:2) could have an alternate genetic diagnosis and aetiology that accounts for all his other congenital malformations. This created an analytical dilemma with regards to further molecular genetic studies, and therefore a decision was made to include or exclude him at each point in the process of molecular genetic analysis of this family.

3.2 Molecular genetic analysis: autozygosity mapping and conventional sequencing studies (family CHD1)

This large consanguineous family contained three siblings who were concordant for the same type of CHD (TOF spectrum) and one sibling with CHD and other congenital malformations. As this appeared to be the most suitable family for further genetic studies, I started with traditional autozygosity mapping approaches.

3.2.1 Autozygosity mapping

3.2.1.1 Genome wide scan

In order to identify recessively inherited candidate CHD genes using an autozygosity mapping strategy, genome-wide SNP genotyping (with the Affymetrix SNP 5.0 microarray) was performed in all four 'affected' individuals (IV:1, IV:2, IV:3, and IV:4) from this family. Prior to further molecular studies it was decided to analyse the results of genetic studies under the analytical conditions that individual IV:2 was a phenocopy and might have a different genotype to the other siblings affected by CHD. The SNP genotyping results were analysed to identify regions of homozygosity >2Mb (common to IV:1, IV:3, and IV:4) and five possible regions of extended homozygosity were detected on: chromosomes 2 (2.4Mb), 10 (5.9Mb), 13 (13.1Mb), 16 (16.4Mb), and 19 (28.9Mb).

3.2.1.2 Microsatellite marker analysis

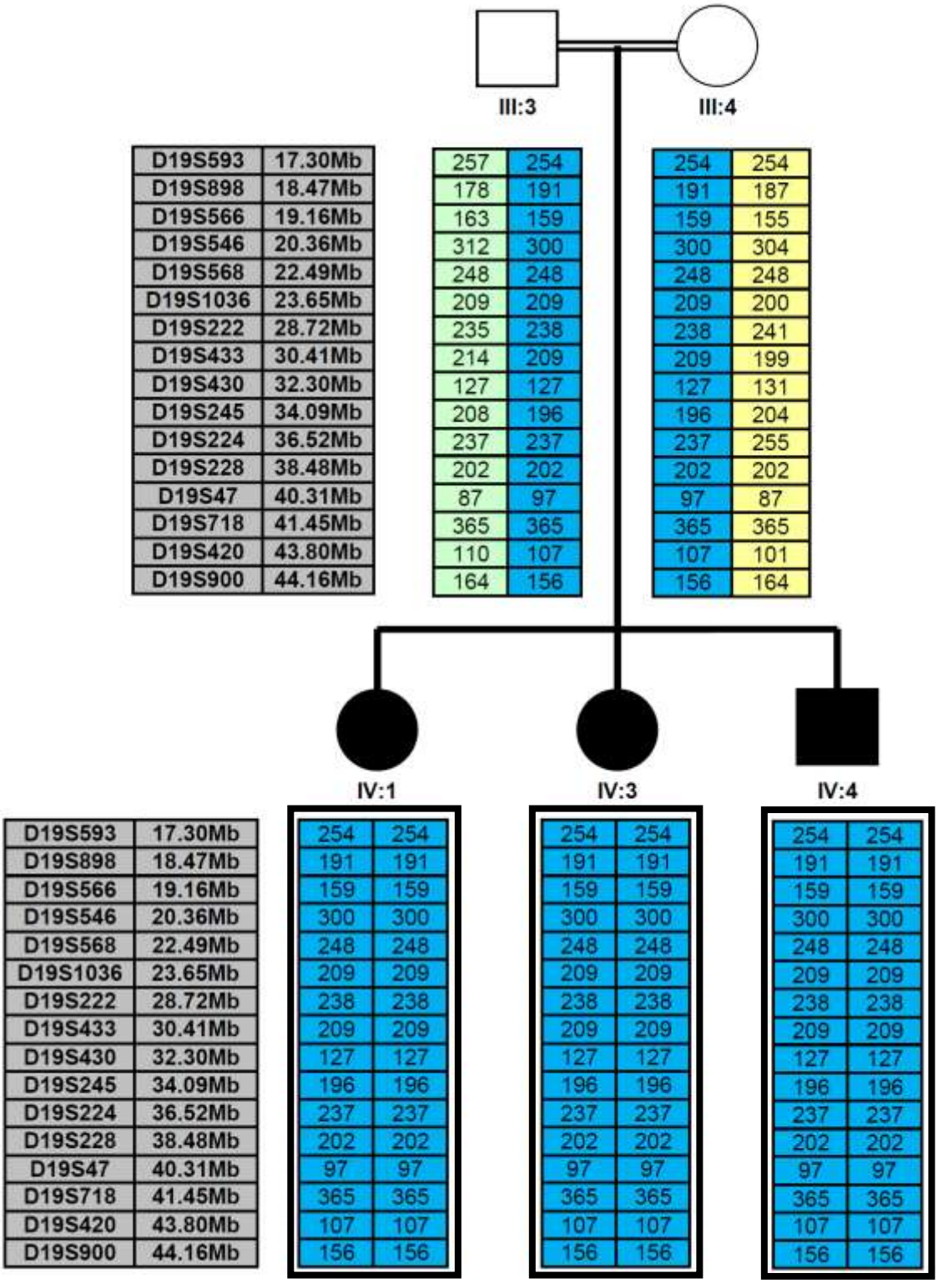
In order to confirm or refute and then refine the five candidate homozygous regions identified from SNP genotyping in individuals IV:1, IV:3, and IV:4, each of these regions was further evaluated using polymorphic microsatellite markers mapping to these regions of interest (Table 6). Linkage to the candidate regions on chromosome 2, 10, 13, and 16 was excluded by segregation of the microsatellite marker alleles. Genotyping of the family with microsatellite markers within the interval on chromosome 19 confirmed linkage but did not refine the homozygous region detected by the SNP array (19:16,925,673-45,862,515bp) (Figure 14).

Table 6: Microsatellite markers used for linkage analysis of the homozygous regions identified in the affected siblings of family CHD1.
(N) = novel

Marker	Start Position (bp)	End Position (bp)	Approximate Size (bp)
D2S2238	40178312	40178496	185-203
D2S2328	40678813	40678952	132-156
D2S2272	40896211	40896420	205-213
D10S37.9(N)	37942703	37942742	194
D10S38.4(N)	38444001	38444050	222
D10S39.0(N)	39045056	39045102	240
D13S153	48890820	48890954	89-121
D13S788	51892627	51892894	240-270
D13S1228	53749089	53749357	264-268
D13S1807	55106096	55106291	186-218
D13S803	57750278	57750439	139-163
D13S233	59448112	59448200	89-109
D16S46.3(N)	46392429	46392475	105
D16S46.6(N)	46615154	46615203	215
D16S46.8(N)	46890039	46890082	210
D19S593	17308106	17308361	252-288
D19S898	18473781	18473960	178-200
D19S566	19162094	19162254	139-169
D19S546	20360262	20360586	306
D19S568	22495143	22495401	249-275
D19S1036	23652821	23653029	204-216
D19S222	28725945	28726177	233-241
D19S433	30417027	30417232	195-225
D19S430	32302506	32302635	127-135
D19S245	34098157	34098361	187-211
D19S224	36528072	36528329	240-262
D19S220	38431554	38431826	267-291
D19S228	38489538	38489695	201-209
D19S47	40314188	40314286	88
D19S718	41458419	41458759	340-376
D19S420	43808799	43809061	95-117
D19S900	44167407	44167579	141-177
D19S559	45330223	45330408	162-198

Figure 14: Microsatellite marker analysis on chr 19 in family CHD1.

The affected children are shaded in black. Microsatellite markers are positioned according to physical distance (MB). Haplotypes for these markers are shown and the disease associated haplotypes are boxed in blue.



3.2.2 Candidate gene analysis from autozygosity mapping

3.2.2.1 Candidate gene selection

Having identified a linked homozygous region I then considered likely candidate CHD genes from within this region. As the microsatellite markers could not refine this candidate region, which contained 664 genes (Appendix E), this involved evaluating each gene for whether it had already been implicated in the pathogenesis of CHD or a CHD-related disorder. I therefore prioritised candidate genes according to: (a) putative function (Ensembl, OMIM, PubMed, UCSC Genome Bioinformatics), (b) published literature (PubMed), and (c) information from model organism genomic databases (MGI).

Initially growth differentiation factor 1 (*GDF1*, chromosome 19:18,979,361-19,006,953bp)(highlighted in yellow - Appendix E) was considered the most promising candidate gene as it encodes a transforming growth factor beta superfamily (TGFβ) protein involved in left-right axis development. Mutations in this gene have been associated with CHD in both humans and mouse models, and other members of this protein family have also been linked to CHD. I therefore proceeded to undertake mutation analysis of GDF1 in this family.

3.2.2.2 Mutational analysis of candidate genes

3.2.2.2.1 Mutational analysis of GDF1 gene

GDF1 sequencing and mutational analysis (using primer pairs in Table 7) was undertaken in all affected individuals (IV:1, IV:2, IV:3, and IV:4) of family CHD1, but no pathogenic mutations were detected. Not all pathogenic mutations can

be detected by sequencing exons and flanking intronic regions but I did not detect any PCR failures which might suggest homozygous exon deletions, and no copy number abnormalities were apparent on the Affymetrix SNP 5.0 arrays performed on IV:1, IV:3, and IV:4. Nevertheless, I considered the possibility that although family CHD1 might harbour a pathogenic *GDF1* mutation that was not detectable by direct sequencing of exons and flanking sequences, other familial CHD kindreds might harbour a detectable mutation. I therefore proceeded to undertake *GDF1* mutation analysis in one affected individual from other CHD families with a similar cardiac outflow tract phenotype, who had been recruited into the project by this time. Mutational analysis in these nine other CHD families (CHD 2/5/6/7/8/9/10/12/13) did not detect any *GDF1* mutations.

3.2.2.2.2 Mutational analysis of NODAL pathway genes

One of the reasons *GDF1* was highlighted as a candidate CHD gene within the homozygous region on chromosome 19 in CHD1, was because of the known interaction between GDF1 and components of the NODAL pathway. Previous studies have implicated NODAL pathway genes in cardiac development and mutations in NODAL pathway genes (e.g. *NODAL* and *FOXH1*) have been reported in human CHD patients (especially those with outflow tract anomalies (see discussion). I therefore proceeded to perform mutation analysis (using primer pairs in Table 7) of four further NODAL pathway genes (*NODAL, CFC1, TDGF1, and FOXH1*) in individual IV:1 from family CHD1, and one affected individual from the same nine CHD families studied for *GDF1* mutations (CHD 2/5/6/7/8/9/10/12/13). However no pathogenic mutations were detected.

Table 7: Primers used for sequencing of coding regions of GDF1, NODAL, CFC1, TDGF1, and FOXH1 in selected familial CHD cohort (CHD 1/2/5/6/7/8/9/10/12/13).

Gene / Exon	Primer Pairs (5' – 3')	PCR Product Size (bp)	Annealing Temperature (oC)
GDF1			
7	CTCAGCCCACTGGTCCC CCGAAGTTGCTAGTAGCCTG	524	60
8.1	AGCCCCAGCGTTCACCTTCCTCC CACCAGCAGCAGCGAGGCCTC	485	60
8.1 (alt)	AGCCCCAGCGTTCACCTTCCTCC ACCTCGCGGAAGCTCAC	607	60
8.2	CTCGCAACGCCTCATGG ATCACCAAGACTGAGGGGC	622	60
NODAL			
Pre-1	CAGAGAGCCACGAGATCACA TAACTGCCCACTGTTTGCTG	200	62
1	TGAGGCCCAGGATATAAGGG CACAGCACTTCCCGAGTCC	333	62
2.1	GTGACACTGACTGAGGCTGG CACCCAGCTGCCTCTGC	500	62
2.1 (alt)	AAGGTAAGGCCTCCAGCAAG AGGTGGACCCACCCAGC	542	60
2.2	CTGGGGCCCTGGAGAAG AGCAAAGCTAGAGCCCTGTC	465	62
3	TTGCACTCAGGAACTGACTTTAAC TTTGCCCCTCTCTGTTTCTC	372	58
CFC1			
1-2	ACTCGTCCATTCTGTGTCCC ACCGCCGTTATGTTTCTCTC	626	60
3	GCATTACAGATCATCAATTTGGG GGTCCTAAACTCTGAGTCCGC	363	60
4-5	TGATTTTACTGCCTCCCCTG ACTGTGGATCGGTATGGAGG	678	60.3
4-5 (alt)	GCTGATTTTACTGCCTCCCC CACTGGAAGATGCACTGTGG	693	60
6	AGCAGGCGTTTCTATTGCAC CCAGTGCTTCAGCTTACGG	335	60

TDGF1			
1	TTTTCCTTTGGCTGTTTTGG GCAGCTCTTTTAAGGCTTGAG	229	58
1 (alt)	ATTGCCATTTTCGCTTTAGG GCAGCTCTTTTAAGGCTTGAG	258	58
2-3	GGTGTTAACTTGTAAGGTTTTATTTCC TGTTGGAAAGATACTCAGGAGAC	405	58
4-5	GAATTGCCCTTGCACTTTTC CCCCAAGGCAACTACGTAAC	437	58
6	AGCAGGATGAACTGCCAGAG AGCAGCAGCCTTTACTGGTC	249	58

FOXH1			
1	CTTCTACACTGCCCCACCG CTTGAACCTGGAAGTGGGTG	312	60
2	CCTGAGCCCGGTAGTGG CCTTGGATGCTCAGGACTTC	254	50
2 (alt)	CCCTGAGCCCGGTAGTG CCTTGGATGCTCAGGACTTC	255	48.5
3.1	GGAAGTCCTGAGCATCCAAG GCTCTGGGGAGAGGGTTG	518	60
3.2	CTCTGTGGCCCCTCTGC CCTGTCAACAAGGTGGGG	513	60

3.2.3 Discussion of autozygosity mapping and candidate gene analysis

3.2.3.1 Autozygosity mapping analysis

Autozygosity mapping has been used as a powerful tool in investigating the genetic basis for many autosomal recessive disorders (as discussed in the introduction). In family CHD1 with a suspected autosomal recessive pattern of inheritance for non-syndromic CHD, it aided in identifying regions of homozygosity for further analysis. The dilemma faced when interpreting the results was whether to include sibling IV:2 to identify candidate regions. Although variable expression of disease is common in many autosomal dominant disorders, it is less common in autosomal recessive disorders, where the phenotype is usually consistent. The decision was made that he has an alternate genetic diagnosis and aetiology that accounts for all his other congenital malformations, as none of these were present in the other three siblings, and therefore he was excluded from autozygosity mapping analysis.

One of the disadvantages of autozygosity mapping is the possibility of identifying large regions of homozygosity that are not always possible to refine using microsatellite markers. This proved to be the case for the region on chromosome 19 which included a large number of genes (664 genes). Conventional methods of identifying candidate genes using the various tools previously described can, if lucky, help to identify a promising candidate. It was fortunate that *GDF1* was the one promising candidate gene in our linked region.

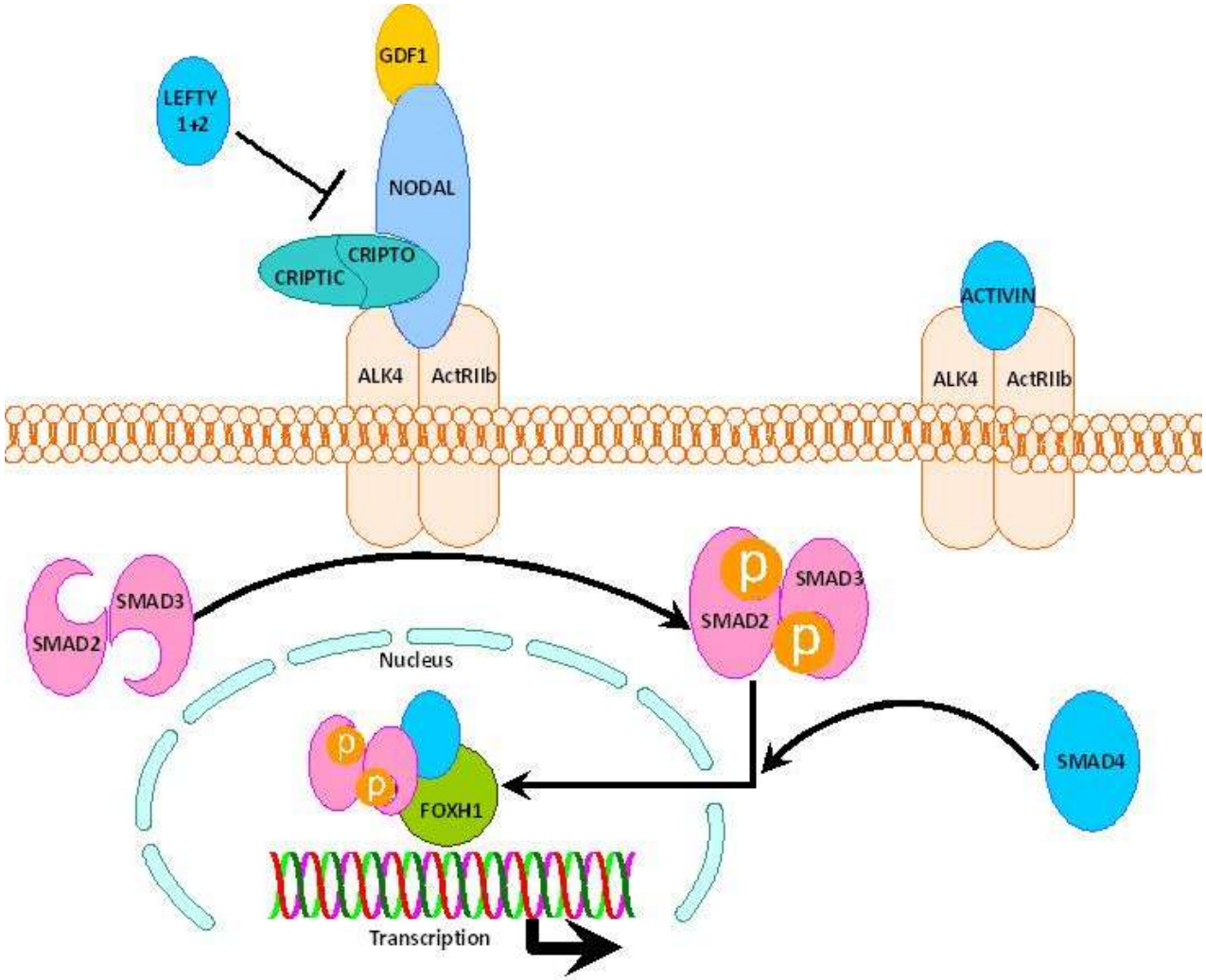
3.2.3.2 Mutational analysis of *GDF1* and *NODAL* pathway genes

GDF1 is a gene belonging to the transforming growth factor beta superfamily (TGFβ), and is expressed throughout the embryo and then in the primitive node, ventral neural tube, and intermediate and lateral plate mesoderm. Knockout mice (with a homozygous deletion) had laterality defects (annular pancreas, malformed spleen, bilateral right sidedness of lungs, randomised heart positioning) and heart malformations ranging from ASD, VSD, AVSD, and TGA (Rankin et al., 2000). *GDF1* has been found to be an upstream regulator of other heart development genes (*NODAL, LEFTY1, LEFTY2,* and *PITX2*), and acts early in embryogenesis to establish laterality. Similarities in the expression domains and mutant phenotypes of *Gdf1* and *Nodal* suggests that GDF1 plays a role in signal transfer to the lateral plate mesoderm from the node by interacting with Nodal (Rankin et al., 2000). It functions as a co-ligand for Nodal and its expression is necessary and sufficient to initiate asymmetric expression of Nodal in the lateral plate mesoderm (Tanaka et al., 2007).

As described in the introduction the Nodal pathway of genes is important in left-right body patterning. Reduced Nodal signalling has been shown to lead to several abnormalities of left-right patterning including CHD in mice (Lowe et al., 2001). NODAL and GDF1 signal via an Activin/TGFβ-like pathway through several Activin-like receptors (e.g. type I Activin receptor [Alk4] and type II Activin receptor [ActRIIB]) (Figure 15). The EGF-CFC (epidermal growth factor-Cripto/FRL-1/Cryptic) proteins are essential for signalling by Nodal and GDF1. *Criptic* (*CFC1*) and *Cripto* (*TDGF1*) (the mammalian homologues of EGF-CFCs)

are required for mesoderm and endoderm induction and for proper left-right axis formation (Gritsman et al., 1999; Yan et al., 1999; Shen and Schier, 2000; Bianco et al., 2002; Schier, 2003). Mice deficient in *Criptic* showed right pulmonary isomerism, AVSD, randomisation of cardiac looping, and TGA (Gaio et al., 1999). Cripto and Criptic act as co-receptors for Nodal and GDF1 to bind and activate Activin-like receptors (Alk4 and ActRIIB). Activin itself however does not need EGF-CFC co-receptors, despite using the same receptors as Nodal and GDF1 (Mathews et al., 1992). The NODAL activated receptor complex phoshorylates intracellular receptor regulated Smads (Smad2/3), and the Smad2/3 complex then binds to Smad4 which results in direct DNA binding or interaction with other transcription factors (e.g. *Foxh1*) (Saijoh et al., 2000). The transcription factor (FOXH1) has a role in establishing a positive feedback loop by binding to Nodal and Lefty2, thereby controlling Nodal gene expression and signalling in embryogenesis (Saijoh et al., 2000; Yamamoto et al., 2001; Norris et al., 2002). It is likely that GDF1 also relies on FOXH1 activity as *FOXH1* mutant embryos showed similar malformations as those which were *Gdf1*-/- ; *Nodal*+/- (Yamamoto et al., 2001).

Figure 15: Diagram illustrating essential components of NODAL signalling.
Adapted from (Cheng et al., 2004; Roessler et al., 2008)



Eight different *GDF1* mutations were found in 8/375 sporadic cases of CHD, with phenotypes very similar to the mouse models (TOF, TGA, DORV) (Karkera et al., 2007). *CFC1* mutations have been found in a number of human cases of left right asymmetry and CHDs (including TGA, AVSD, and DORV) (Bamford et al., 2000; Goldmuntz et al., 2002). Screening the *NODAL* gene in a large cohort of 269 sporadic cases with laterality defects and cardiac looping anomalies identified seven different variants in a total of 14 patients (Mohapatra et al., 2009).

Mutational screening studies of parts of the Nodal pathway in sporadic cases of CHD have identified variants in the following genes: *NODAL, GDF1, TDGF1, FOXH1,* and *CFC1*. Some of the variants were specific to the family but others were common population variants, with reduced bioassay activity, implying that these latter variants act as modifiers and may contribute to the overall CHD phenotype (Roessler et al., 2008; Roessler et al., 2009). In cases of TGA with a family history of concordant or disconcordant CHD, screening the NODAL pathway/laterality genes identified variants in *FOXH1, ZIC3,* and *NODAL* in two patients, where one patient had a mutation in *FOXH1* and ZIC3 (De Luca et al., 2010). This further supports the theory of a 'multiple hit' genetic model whereby independently inherited gene variants act together to influence the cardiac phenotype. Roessler et al. (2009) implied that NODAL pathway defects are detectable in 5-10% of individuals with conotruncal CHDs (higher than chromosome 22q11.2 deletion – 5%), and suggest that future studies of the

molecular causes of CHD should entail analysis of entire signalling pathways rather than individual genes.

Due to the developmental pathway involved, reports in human CHD, animal models, and the possible role in the pathogenesis of CHD, I further investigated selected genes from the NODAL pathway (*NODAL, CFC1, TDGF1,* and *FOXH1*) in relevant families in our project but did not detect mutations or potentially pathogenic variants.

3.3 Molecular genetic analysis: whole exome sequencing (family CHD1)

The absence of a detectable *GDF1* mutation in family CHD1 suggested that another gene in the chromosome 19 candidate region might harbour pathogenic mutations. However, the candidate interval contained another 663 genes and none of these had been previously implicated in CHD. Hence prioritising candidate genes and sequencing them individually was likely to be a long manually laborious and time consuming process. Furthermore, though the chromosome 19 homozygous region was the only candidate interval confirmed by microsatellite marker studies, it was possible that the pathogenic mutation might reside in a gene that was contained in a smaller region of homozygosity (<2Mb). It was even possible that the family, despite being consanguineous, might harbour compound heterozygous mutations or even a non-autosomal recessive mechanism of inheritance.

It was therefore decided that the most efficient approach was to proceed to "whole exome sequencing" (WES) and a DNA sample from the affected individual IV:4 was sent to the Beijing Genomics Institute (BGI)(Beijing, China) for exome sequencing (June 2010). Subsequently further exome sequencing of the two remaining affected siblings (IV:1 and IV:3), and affected individuals from 8 other families in the familial CHD cohort, was performed at the Wellcome Trust Sanger Institute (WTSI)(Hinxton, UK). Therefore the results of WES are reported in two stages.

3.3.1 Whole exome sequencing (WES) at BGI

Our first collaboration was with BGI where the WES laboratory work and read analysis for individual IV:4 from family CHD1 was performed. They used the SureSelect automated target enrichment technology (Agilent) with a 94.3% overall coverage of the whole exome (read depth 1X) but this was only 68.4% coverage with a read depth of 10X.

The raw data was transferred to Birmingham (September 2010) and all variants were annotated as novel or as being reported on the dbSNP (but not the 1000 Genomes) databases. The annotation also included the functional category of the variants and whether they were heterozygous or homozygous variants. A total of 61,421 variants were identified, of which 10,776 were reported to be novel (September 2010) as they were not present in the dbSNP database.

3.3.2 Candidate gene analysis from WES

The BGI WES data provided a large number of variants (61,421) and using Excel (Microsoft) I manually analysed the variants using various filtering strategies. From this large number, only 2,945 variants remained after I selectively filtered out all dbSNPs, and included only potentially pathogenic variants, by focusing on those annotated as functionally significant, such as nonsynonymous (nonsense and missense) and splice acceptor and donor site, anticipating that synonymous variants were less likely to be pathogenic.

I then reviewed all these variants to remove those reported on the 1000 Genomes database, to result in truly novel variants. As the family history and parental consanguinity suggested autosomal recessive inheritance of CHD in family CHD1, I focussed my attention on homozygous variants to assess them for mutation pathogenicity, and was left with a total of 103 variants in 95 different genes (8 nonsense, 93 missense, and 2 splice site). I proceeded to annotate each variant for pathogenicity to determine the predicted effect on the gene product using bioinformatics resources (PolyPhen-2, SIFT). I used a functional approach to prioritise candidate genes according to: (a) putative function (Ensembl, OMIM, PubMed, UCSC Genome Bioinformatics), (b) published literature (PubMed), and (c) information from model organism genomic databases (MGI). Appendix F summaries the list of novel, homozygous, functionally significant variants, and the results of various interpretation approaches to identify candidate genes (published literature, mouse model databases, putative function, and pathogenicity software programmes).

As well as analysis of variants throughout the exome, I also performed a genomic approach to prioritise candidate genes (with variants) limited to the homozygous region on chromosome 19. In total there were 959 variants from the raw data received, which were then narrowed down to only 4 variants (1 nonsense and 3 missense) using the filtering strategies of novel, homozygous, and functionally significant (highlighted in orange - Appendix F).

3.3.2.1 Candidate gene selection and analysis

3.3.2.1.1 Analysis of *WNT11* and *DVL2* genes

Two members of the Wnt pathway demonstrated candidate homozygous missense mutations by second generation sequencing of the affected individual IV:4 from family CHD1. The missense mutation in *WNT11* (c.519T>A, p.D173E, exon 3) occurred in a highly conserved nucleotide and amino acid and analytical software predicted it to be probably damaging and affect protein function. *WNT11* (chromosome 11:75,897,369-75,921,803bp) is part of the Wnt gene family that encode a set of signalling molecules thought to play an important role in embryonic development, with embryonic lethal mouse models. The missense mutation in *DVL2* (c.152C>T, p.A51V, exon 1), occurred in a highly conserved nucleotide and moderately conserved amino acid, however analytical software predicted it to be benign and to be tolerated at protein function level. *DVL2* (chromosome 17:7,128,660-7,137,868bp) is part of a gene family which encodes proteins homologous to the Drosophila dishevelled (dsh) protein and it is suggested that they function in Wnt signalling, with mouse models displaying cardiac outflow tract anomalies, similar to those in family CHD1. As both of these genes are members of the Wnt pathway and well known for their role in murine cardiac development, I further evaluated the variants in both of these genes with Sanger sequencing.

Despite the functional evidence supporting *WNT11* and *DVL2* as candidate CHD genes, Sanger sequencing (using primers in Table 8) did not reveal the putative variants in either IV:4, the affected siblings (IV:1 and IV:3), the

phenocopy (IV:2), or the parents. They were therefore considered to be false positive findings and no further studies in control samples or other CHD families were performed.

3.3.2.1.2 Analysis of *GMFG* gene

Within the chromosome 19 homozygous region only one gene (*GMFG*) contained a putative homozygous novel truncating mutation (c.70C>T, p.Arg24X, exon 2). Glia maturation factor gamma (*GMFG*) (chromosome 19:39,819,010-39,826,726bp) has been reported to have a putative role in cell differentiation and proliferation but has not previously been linked to cardiac development. Nevertheless as the only gene with a homozygous nonsense mutation in the candidate region in this family it was selected for further analysis (highlighted in blue - Appendix E and orange - Appendix F).

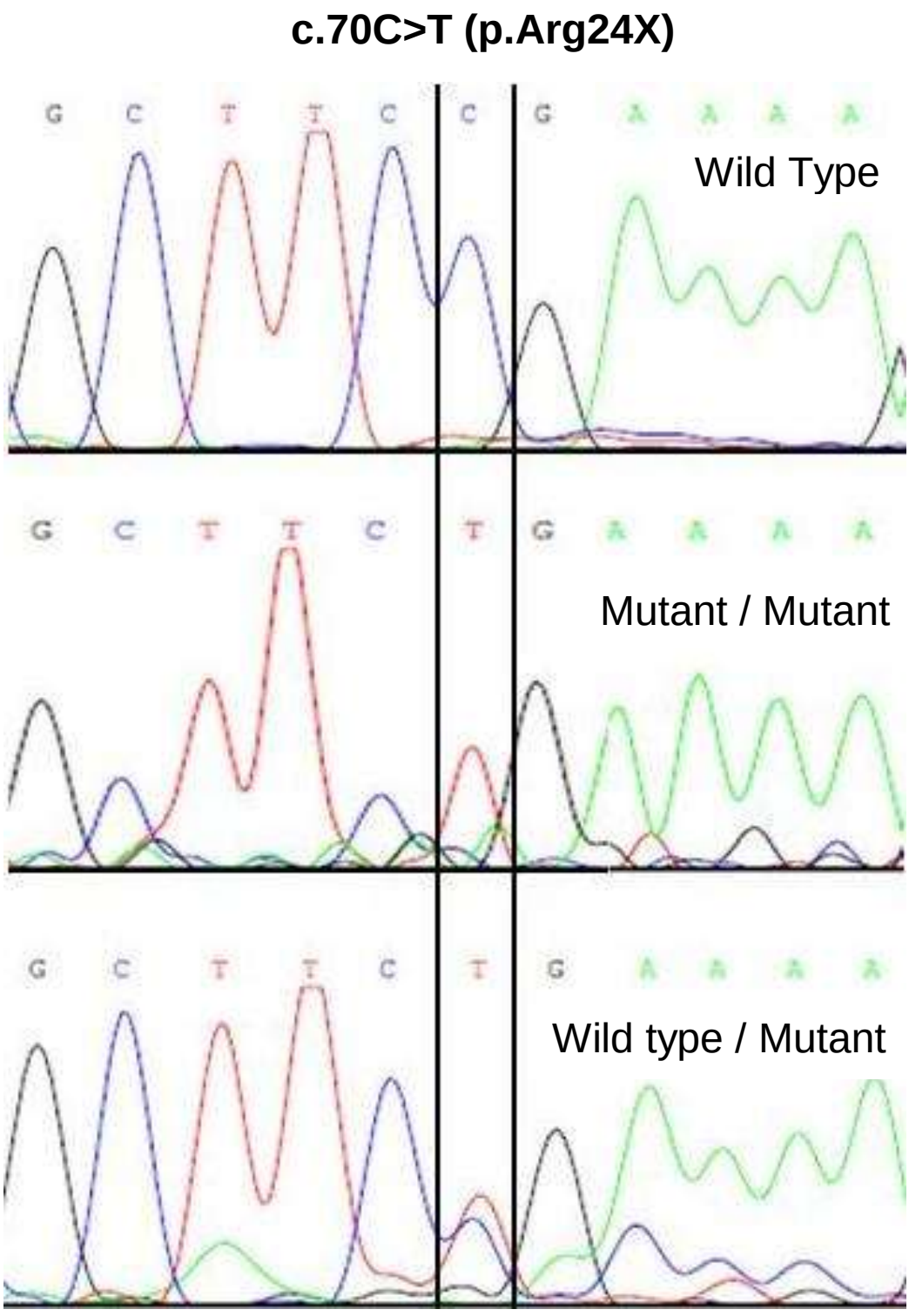
I performed Sanger sequencing analysis (using primers in Table 8) of *GMFG* was undertaken in all affected siblings (IV:1, IV:3, and IV:4), the phenocopy (IV:2), and both unaffected parents from family CHD1, to validate the variant identified from WES (Arg24X). This variant was confirmed by re-sequencing analysis and segregation analysis. All affected individuals were homozygous for this nonsense mutation, and both parents were heterozygous carriers (Figure 16). Interestingly sibling IV:2 was neither heterozygous or homozygous for this variant. Due to the availability, sequencing of *GMFG* was only undertaken in ~200 ethnically matched control chromosomes, and this did not identify this mutation in any controls.

Table 8: Primers used for sequencing of selected exons of GMFG, WNT11 and DVL2 in family CHD1.

Gene / Exon	Primer Pairs (5' – 3')	PCR Product Size (bp)	Annealing Temperature (oC)
GMFG			
2-3	AGAAACAGGTTTGGGGAAGG TTGGTTCTCCCAGTCTCACC	408	60
WNT11			
3	CGAAGGTGGAGGACCTCTG GCCCTTTTCTGGCCAATG	417	60
DVL2			
1	GCCATTAGCCCTTTGGG AAAGTAGACGTGTGCCCCTC	326	58

Figure 16: Sequencing analysis of GMFG in family CHD1.

Confirmation of the Arg24X mutation via segregational analysis.
An affected child is homozygous
An unaffected parent is a heterozygous carrier



3.3.3 Discussion of WES at BGI and candidate gene analysis

The analysis of the results of WES in an individual (IV:4) with familial CHD clearly demonstrates both the advantages and challenges of the large data sets generated by this technology. Whereas Sanger sequencing of individual genes from the homozygous candidate region would have taken years to complete, WES provided a wealth of sequencing data on genes not only within the candidate region but also across the genome. Not all exons are represented in "whole exome sequencing" data but the major challenge for interpreting such data is how to prioritise further investigation knowing that both false positive and false negative variant calling may occur. I therefore adopted two approaches. Firstly I took a "functional approach" and looked for putative mutations in genes whose function suggested that they might be implicated in cardiac development (*WNT11* and *DVL2*). Secondly I took a "genomic approach" in which I put the emphasis on the likelihood of a variant in a gene mapping within a candidate region being pathogenic, even if there was no known functional evidence to implicate the gene in cardiac development.

3.3.3.1 "Functional approach" to WES data analysis: *WNT11* / *DVL2*

The Wnt proteins are a large family of secreted signalling molecules that regulate crucial aspects of development, including cell-fate specification, proliferation, survival, migration and adhesion. These effects are mediated via two Wnt pathways, the canonical and non-canonical pathways (Figure 17) (Flaherty and Dawn, 2008). The canonical pathway is activated by Wnt proteins binding to a co-receptor complex of frizzled (Fzd) and lipoprotein receptor

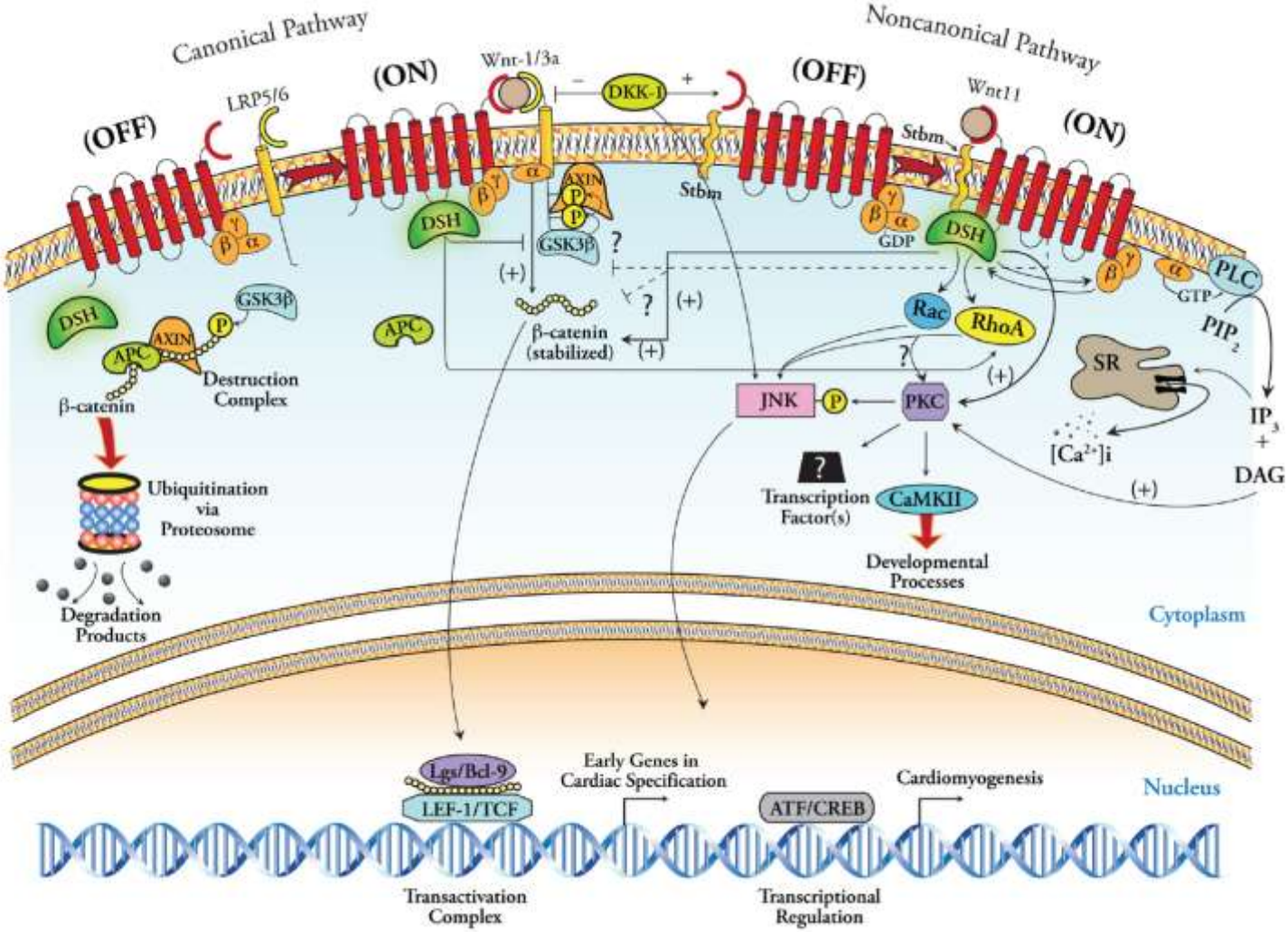
related proteins (Lrp5/6). Binding activates the intracellular protein, dishevelled (Dvl), resulting in inactivation of the protein complex that includes glycogen synthase kinase 3 (Gsk3), axin, and adenomatosis polyposis coli (APC). This complex normally phosphorylates β-catenin and targets it for degradation. Wnt stimulation inhibits the Gsk3/axin/APC complex leading to high levels of β-catenin accumulation in the nucleus, where it complexes with the LEF/TCF family of DNA binding proteins to activate the transcription of Wnt target genes. The non-canonical pathway (also known as the planar cell polarity pathway) is mediated through either of the Ca2+/protein kinase C (PKC) or RhoA/JNK pathways. In Ca2+/PKC signalling, Wnt binding activates the heterotrimeric G-protein dependent activity of Fzd receptors, and intracellular Ca2+ signalling, as well as Ca2+-dependent protein kinases, such as protein kinase C (PKC) and calmodulin-dependent protein kinase II (CaMKII). In RhoA/JNK signalling, Wnt proteins through the Dvl protein activate Rho family GTPases (RhoA and Rac), and subsequently JNK, their downstream effector (Cohen et al., 2008).

The *Wnt* gene family is critical for the developmental process of organogenesis, including specifically cardiogenesis. Canonical Wnt signals appear essential for the proliferation of SHF cells (Ai et al., 2007; Kwon et al., 2007), whereas the non-canonical pathway is thought to be necessary for the polarised migration of myocardial cells required in the outflow tract septum (Henderson et al., 2006). *Wnt11* acts via the non-canonical pathway and its expression overlaps with the first and second heart fields, and in the outflow tract region (Zhou et al., 2007). A critical function for *Wnt11* is in shaping embryonic ventricular cardiomyocyte

spatial organisation and differentiation during heart development (Flaherty and Dawn, 2008; Nagy et al., 2010). This is through regulation of the expression of the *Gata4, Nkx2.5, Mef2C,* and *ANP* genes, which are transcribed at reduced levels compared with controls in *Wnt11*-deficient hearts (Nagy et al., 2010).

Dishevelled functions in Wnt signalling downstream to the canonical and non-canonical pathways (Figure 17). In mammalian species, three Dishevelled isoforms are expressed, termed Dvl1, Dvl2, and Dvl3 (Nemer, 2008). At present no human diseases have been linked to defects in *DVL1, DVL2* or *DVL3*. All three of the murine *Dishevelled* genes are widely expressed in embryonic and adult tissues suggesting that there may be functional redundancy among the three genes. Mice carrying null mutations in *Dvl2* have been found to be perinatally lethal because of defects in cardiac morphogenesis, specifically, outflow tract septation defects, indicating *Dvl2* as a key mediator in conotruncal development (DeLaughter et al., 2011).

Figure 17: Diagram illustrating Wnt/Dvl signalling.
Taken from Flaherty and Dawn (2008)



Pitx2 (also part of the Nodal pathway) is a downstream target of the canonical Wnt pathway, and mice that are null for *Pitx2* show cardiac outflow tract anomalies. These anomalies are similar to those seen in *Dvl2* mutants, and there is a marked decrease in *Pitx2* expression in cardiac neural crest cells migrating to the cardiac outflow tract in *Dvl2* mutants. This suggests that *Pitx2* is a downstream target of Dvl2 (and Wnt) mediated pathways (Kioussi et al., 2002). *Pitx2* mutants also have downregulated *Wnt11* expression in the mesoderm of the second heart field and in the outflow tract. As *Wnt11* mutants show the same cardiac defects as *Pitx2* mutants, Wnt11 seems like a critical downstream effector of Pitx2 and a direct target of the canonical pathway in the developing heart (Zhou et al., 2007). The similar phenotypes seen with *Pitx2, Wnt11* and *Dvl2* mutants highlight the interactions that occur between the canonical and non-canonical Wnt signalling pathways.

It is clear that many components of the Wnt/Dvl/Pitx2 pathway are dose dependent and involved in cardiac development (especially the outflow tract), and there is an overlap with the Nodal pathway. It is possible that the polygenic model of CHD in humans may be explained by variants in numerous genes within these pathways, and future studies investigating genetic causes of CHD in humans should screen for some or all of the genes in these pathways.

Unfortunately the variants in *WNT11* and *DVL2* identified from WES in family CHD1 were not verified by Sanger sequencing and therefore are likely to be false positive results. This highlights the issue of data analysis from WES and

some of the challenges of false positive results, which can lead to unnecessary and time consuming validation work in the laboratories.

3.3.3.2 "Genomic approach" to WES data analysis: *GMFG*

The homozygous nonsense mutation (Arg24X) in *GMFG* in family CHD1 was shown to segregate with affected individuals in the family and with parents as heterozygous carriers. Sibling IV:2 did not have this variant on segregational analysis, but if a homozygous Arg24X mutation had been identified in IV:2, *GMFG* may have been dismissed as a significant candidate gene, due to the variable phenotype. These findings therefore may in fact provide further evidence for this gene as a candidate in this family, and confirm the suspicion of an alternative genetic aetiology for the CHD and malformations seen in IV:2.

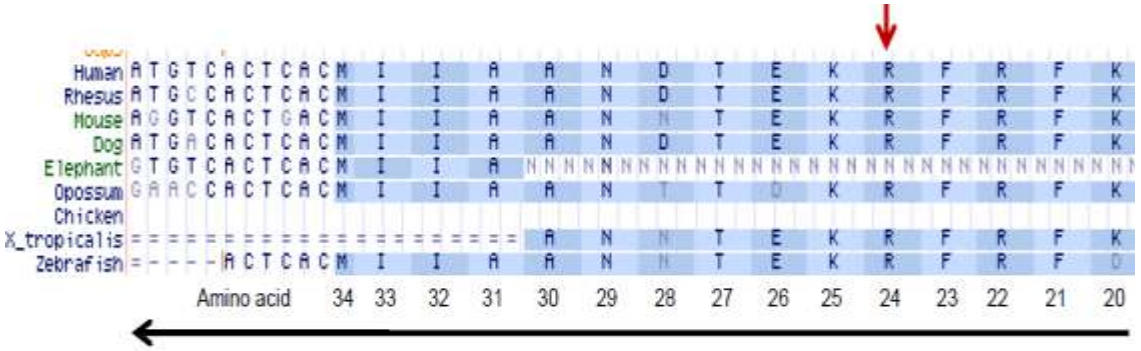
A more recent review of international SNP databases revealed the Arg24X variant has been identified in one individual from African ancestry in the 1000 Genomes project. Our family is from a South Asian origin (Pakistan), which is a population poorly represented in these international SNP databases and therefore there is a potential concern that this might represent a rare polymorphic variant that is present in the South Asian population (though it was not detected in 200 ethnically matched controls). Nevertheless, previous experience within our research group, suggests that pathogenic recessive mutations can be an incidental finding in studies of normal control individuals. We identified a homozygous deletion in the *PLCB1* gene in a family with infantile epilepsy and did not detect the mutation in ethnically matched control

samples, however a heterozygous deletion with similar breakpoints had been reported in 1/540 HapMap chromosomes (www.sanger.ac.uk/resources/downloads/human/hapmap3.html) (Kurian et al., 2010). Nevertheless, since a homozygous *PLCB1* mutation was reported as a cause of infantile epileptiform encephalopathy, a further family with a related disorder (malignant migrating partial seizures in infancy (MMPEI)) has also been reported to have a homozygous deletion in the *PLCB1* gene, confirming the validity of the original report of a pathogenic mutation (Poduri et al., 2012). Though I did not identify the *GMFG* nonsense mutation in other affected families within our cohort (see section 3.4), the finding of a carrier for this candidate mutation in a population control suggests that mutation analysis of *GMFG*, and particularly for the Arg24X mutation should be performed in large cohorts of CHD patients.

GMFG was first isolated and shown to have 82% identity with human glia maturation factor beta (*GMFB*) (Asai et al., 1998). They examined the tissue distribution of *GMFG* mRNA and detected predominant expression in human lung, heart, and placenta, and trace expression in brain, liver, skeletal muscle, kidney, and pancreas. The cDNA length of *GMFG* is 646 bases and encodes a protein of 142 amino acids. The amino acid sequence is highly conserved amongst species (Figure 18), with 95% homology between human and mouse, and 98% between human and bovine. The function of *GMFG* is relatively unclear, but *GMFB* has been well studied and was initially identified as a growth and differentiation factor acting on neurons as well as glia in the vertebrate brain. A new aspect of the intracellular functions of *GMFB* showed that protein

kinase A-phosphorylated GMFB is a potent inhibitor of the extracellular signal-regulated kinases (ERK1 and ERK2), a subfamily of mitogen-activated protein (MAP) Kinases (Zaheer and Lim, 1996).

Figure 18: Diagram illustrating the amino acids (including R24) are highly conserved through evolution.
Taken from UCSC



Expression of rat *GMFG* is predominantly in the thymus, testis, and spleen (Tsuiki et al., 2000). These tissues contain cells going through rapid differentiation and proliferation, thereby suggesting *GMFG* may play a role in cellular differentiation and growth, through intracellular signalling mechanisms (similar to GMFB, as described above). *GMFG* has been show to play an important role in maintaining haematopoietic stem cells, and regulating haematopoietic lineage development (Shi et al., 2006). They showed that *GMFG* expression is high in blood, thymus, spleen, fetal liver and lung. *GMFG* was identified as a novel factor in actin cytoskeleton reorganisation and showed it is expressed preferentially in microvascular endothelial and inflammatory cells (Ikeda et al., 2006). During mouse embryogenesis, *GMFG* was expressed as early as embryonic day 9.5, predominantly in blood islands of the yolk sac, in endothelial and hematopoietic cells, and possibly in the angioblast precursors to

these lineages. This implies that *GMFG* has a function in embryonic vasculogenesis as well as in haematopoiesis. Inflammation and angiogenesis take place actively in the pathophysiology of cardiovascular disease, and *GMFG* expression has been shown to be significantly increased in cardiac ischemia/reperfusion tissues, suggesting a role for *GMFG* in cardiovascular diseases, and a novel approach to modulate these diseases (Ikeda et al., 2006).

Actin cytoskeleton reorganisation is a fundamental process for actin-based cellular functions such as cytokinesis, phagocytosis, and chemotaxis. Deficient actin cytoskeleton reorganisation causes impaired endothelial cell migration, reduces macrophage phagocytosis, and results in defective lymphocyte development and activation. Actin cytoskeleton reorganisation therefore plays an essential role in angiogenesis and immune system function. They showed how overexpression of *GMFG* enhanced actin-based cellular functions such as migration and tube-formation in endothelial cells. GMFG has been shown to be distributed along the axis of movement of migrating T-lymphocytes, with predominance in the leading edge of the cells. Inhibition of its expression reduced cellular basal and chemokine induced migration (Lippert and Wilkins, 2012). This provides further evidence for GMFG in cell movement via actin cytoskeleton remodelling, and has therefore been classified as a member of the actin-depolymerizing factor homology (ADF-H) family. Interestingly the Arg24X mutation lies in the ADF-H domain (residues 7-140) and may result in nonsense

mediated decay preventing the expression of a truncated or erroneous protein (with only part of this domain).

The pattern of blood flow in the developing heart is hypothesised to determine the extent of cardiac development (as described in the introduction). As *GMFG* has been shown to be involved in angiogenesis and actin based cell migration, there may be a clear pathogenic mechanism for CHD in cases of *GMFG* loss of function. It is known that understanding the haemodynamics during heart development can be important for the correction of CHD, as shown by the improvement of left ventricular function and prevention of development of HLHS by in utero surgical interventions for severe aortic stenosis (Selamet Tierney et al., 2007).

3.3.4 Conclusions

I have demonstrated that *GMFG* is a potential candidate gene for autosomal recessive non-syndromic CHD. Further sequencing analysis of *GMFG* in other isolated and familial cases of CHD, and demonstrating the functional effects of the mutation and role of *GMFG* in heart development in animal models, is required before this finding can be considered conclusive.

3.4 Molecular genetic analysis: whole exome sequencing (familial CHD cohort)

3.4.1 Whole exome sequencing (WES) at WTSI

The availability of “whole exome sequencing” data from a single affected individual from family CHD1 illustrated the potential for using exome sequencing to identify candidate novel CHD genes in kindreds with familial CHD. However a major limitation to my project was the cost of exome sequencing which meant that only a single individual (IV:4) was analysed. Though there was relatively strong genetic evidence for GMFG inactivation causing CHD in Family CHD1, there was the possibility that the exon containing the mutation in the responsible gene might not have been captured or sequenced in sufficient depth to detect the “real pathogenic mutation”.

Subsequently we established our second collaboration with Dr Matt Hurles (Human Genetics Division, Wellcome Trust Sanger Institute [WTSI], Hinxton, UK), which enabled WES on the 2 remaining affected siblings from CHD1 (IV:1 and IV:3) and a total of 17 affected siblings from a further 8 CHD families (CHD 4/5/6/13/16/20/22/23). These families were chosen on the basis of multiple affected individuals in one generation, implying an autosomal recessive inheritance pattern.

The DNA samples from a total of 19 individuals from the 9 CHD families were sent in batches throughout 2011 (February, May, and August). They performed the WES laboratory work and read analysis using a newer version of the SureSelect automated target enrichment technology (Agilent) compared to BGI,

with a 100% overall coverage of the whole exome (read depth 1X), which fell to only 92% coverage with a read depth of 10X.

The final complete raw dataset for all families was transferred to Birmingham (August 2011) and all variants were annotated as novel or as being reported on the dbSNP, 1000 Genomes, and UK10K databases. The annotation also included the functional category of the variants, whether they were heterozygous or homozygous variants, and details of the predicted effect on the gene product using SIFT and PolyPhen prediction programmes.

3.4.2 Candidate gene analysis from WES

As had been observed with the WES data from BGI for individual IV:4 (Family CHD1), WES can reveal a vast number of variants and therefore a structured approach was required to interrogate the huge dataset that was provided by the WTSI WES collaboration. As the WTSI analysis was performed after the BGI analysis fewer variants were classed as novel (dated February 2012) as more data was available in the dbSNP, 1000 Genomes, and UK10K databases. Using the Exome Variants Analysis (EVA) software programme and Excel (Microsoft), I manually analysed the variants using various filtering strategies. As performed with the BGI WES data, I selectively filtered out all dbSNPs, and included only potentially pathogenic variants (i.e. nonsense, missense, and splice acceptor and donor site).

Prior to receiving the data I decided that I would analyse the data in a variety of ways so that I would be able to address the evidence available for a variety of different genetic scenarios. For example, even assuming an autosomal recessive model of inheritance, it was possible that in any particular family CHD might result from a mutation in a known CHD gene (that could have been associated with the type of CHD observed in the family or a different type of CHD) or a novel CHD gene. In preparation for this next step in the analysis I assembled a list of candidate CHD genes (similar to Table 3) by analysing data from a large number of sources.

I then used a functional approach to prioritise candidate genes according to: (a) putative function (Ensembl, OMIM, PubMed, UCSC Genome Bioinformatics), (b) published literature (PubMed), (c) information from model organism genomic databases (MGI), and (d) protein-interaction resources (to identify potential interactions with known CHD genes)(BioGRID).

3.4.3 WES data for families with "autosomal recessive" inheritance of CHD

Firstly I will summarise the relevant sequence information for each of the families analysed at the WTSI individually and then the overview analysis. As multiple affected individuals in all families had been sequenced, I was able to filter in variants that were only present in all affected individuals. For individual families, the data was analysed in light of the inheritance pattern in that particular family and the presence or absence of consanguinity. Thus in consanguineous families I expected to find homozygous variants (over

compound heterozygous variants) in a candidate gene. In non-consanguineous families either compound heterozygous variants or homozygous variants might be detected. The WES data analysis for all the CHD families studied is summarised in Table 9. Appendix G tabulates all the novel, functionally significant variants with the results of pathogenicity software programmes, for each family studied using WES.

3.4.3.1 Family CHD1

The WTSI dataset for family CHD1 revealed that a total of 64,675 variants were shared by both affected individuals (IV:1 and IV:4), and 6,490 of these were reported to be novel, as they were not present in the dbSNP databases. Using the various filtering strategies (described previously), only 1,376 potentially pathogenic variants remained. As the family history and parental consanguinity suggested autosomal recessive inheritance of CHD, I focussed my attention on homozygous variants and was left with a total of 59 variants (1 nonsense, 49 missense, and 9 splice site/indels) in 48 genes. When compared to the BGI dataset (with 95 different genes containing homozygous variants), there were only five genes in common, but only three of them had the same variant identified previously (i.e. *GMFG*, *NDUFA13* and *KIA1683*). Interestingly these three genes are within the homozygous region identified on autozygosity mapping, and none have been associated with cardiac development. The *GMFG* mutation (but not the false positive variants -*WNT11* and *DVL2*) from the BGI dataset was detected in the WTSI data in all affected siblings (confirming previous segregational analysis). The only variants in known CHD genes were

the compound heterozygous variants in *EVC* (Ellis-Van Creveld syndrome) and *NOTCH1* (non-syndromic CHD, mainly aortic disease). Interestingly a homozygous missense variant in *MESP1* was identified, and mice with targeted mutations in this gene die in the embryonic period with CHD (MGI). *Mesp1* is throught to promote cardiovascular differentiation during embryonic development, and lineage tracing in mice showed it to represent the earliest marker of cardiovascular progenitors, including derivatives of the primary and second heart fields (Bondue and Blanpain, 2010).

3.4.3.2 Family CHD4

The WTSI dataset for family CHD4 revealed that a total of 52,463 variants were shared by both affected individuals, 4,019 of these were reported to be novel, and only 732 were potentially pathogenic variants. As the family history and parental consanguinity suggested autosomal recessive inheritance of CHD, I was left with a total of 53 homozygous variants (1 nonsense, 44 missense, and 8 splice site/indels) in 37 genes. Compound heterozygous variants were identified in the *MLL3* gene, which is closely related to *MLL2* (Kabuki syndrome), but not known to be involved in heart development. Compound heterozygous variants were also identified in *NOTCH1* (non-syndromic CHD. Without further analysis they can not be excluded as potentially disease causing. There were no variants in other known CHD genes or any of the candidate genes analysed in family CHD1 (*GDF1, WNT11, DVL2* and *GMFG*).

3.4.3.3 Family CHD5

The WTSI dataset for family CHD5 revealed that a total of 59,691 variants were shared by both affected individuals, 3,742 of these were reported to be novel, and only 522 were potentially pathogenic variants. As the family history suggested autosomal recessive inheritance of CHD, I focussed my attention on both homozygous variants and compound heterozygous variants, due to absence of consanguinity. There were a total of 30 homozygous variants (29 missense, and 1 splice site/indels) in 21 genes. There were a total of 492 heterozygous variants (4 nonsense, 442 missense, and 46 splice site/indels), and further analysis showed 42 genes had more than one heterozygous variant. The same two compound heterozygous variants seen in family CHD4 in *MLL3* and *NOTCH1* were identified in this family. There were no variants in other known CHD genes or any of the candidate genes analysed in family CHD1 (*GDF1, WNT11, DVL2* and *GMFG*).

3.4.3.4 Family CHD6

The WTSI dataset for family CHD6 revealed that a total of 63,010 variants were shared by both affected individuals, 7,477 of these were reported to be novel, and only 2,204 were potentially pathogenic variants. As the family history suggested autosomal recessive inheritance of CHD, I focussed my attention on both homozygous variants and compound heterozygous variants, due to absence of consanguinity. There were a total of 42 homozygous variants (39 missense, and 3 splice site/indels) in 33 genes. There were a total of 2,162 heterozygous variants (69 nonsense, 1,986 missense, and 107 splice

site/indels), and further analysis showed 236 genes had more than one heterozygous variant. The same two compound heterozygous variants seen in family CHD4 in *MLL3* and *NOTCH1* were identified in this family. There were also three heterozygous missense variants identified in *TBX20* (non-syndromic CHD). There were no variants in other known CHD genes or any of the candidate genes analysed in family CHD1 (*GDF1, WNT11, DVL2* and *GMFG*).

3.4.3.5 Family CHD13

The WTSI dataset for family CHD13 revealed that a total of 64,176 variants were shared by both affected individuals, 4,335 of these were reported to be novel, and only 554 were potentially pathogenic variants. As the family history suggested autosomal recessive inheritance of CHD, I focussed my attention on both homozygous variants and compound heterozygous variants, due to absence of consanguinity. There were a total of 36 homozygous variants (31 missense, and 5 splice site/indels) in 24 genes. There were a total of 518 heterozygous variants (7 nonsense, 462 missense, and 49 splice site/indels), and further analysis showed 53 genes had more than one heterozygous variant. The same two compound heterozygous variants seen in family CHD4 in *MLL3* and *NOTCH1* were identified in this family. There were no variants in other known CHD genes or any of the candidate genes analysed in family CHD1 (*GDF1, WNT11, DVL2* and *GMFG*).

3.4.3.6 Family CHD16

The WTSI dataset for family CHD16 revealed that a total of 49,664 variants were shared by all three affected individuals, 5,894 of these were reported to be novel, and only 1,808 were potentially pathogenic variants. As the family history suggested autosomal recessive inheritance of CHD, I focussed my attention on both homozygous variants and compound heterozygous variants, due to absence of consanguinity. There were a total of 29 homozygous variants (27 missense, and 2 splice site/indels) in 20 genes. There were a total of 1,779 heterozygous variants (52 nonsense, 1,642 missense, and 85 splice site/indels), and further analysis showed 193 genes had more than one heterozygous variant. The same two compound heterozygous variants seen in family CHD4 in *MLL3* and *NOTCH1* were identified in this family. Two out of the three heterozygous missense variants identified in *TBX20* in family CHD6 were also present. There were no variants in other known CHD genes or any of the candidate genes analysed in family CHD1 (*GDF1, WNT11, DVL2* and *GMFG*).

3.4.3.7 Family CHD20

The WTSI dataset for family CHD20 revealed that a total of 57,734 variants were shared by both affected individuals, 3,617 of these were reported to be novel, and only 608 were potentially pathogenic variants. As the family history suggested autosomal recessive inheritance of CHD, I focussed my attention on both homozygous variants and compound heterozygous variants, due to absence of consanguinity. There were a total of 30 homozygous variants (26 missense, and 4 splice site/indels) in 19 genes. There were a total of 578

heterozygous variants (8 nonsense, 538 missense, and 32 splice site/indels), and further analysis showed 42 genes had more than one heterozygous variant. The same two heterozygous variants seen in family CHD4 in *MLL3* (but not *NOTCH1*) were identified in this family. There were no variants in other known CHD genes or any of the candidate genes analysed in family CHD1 (*GDF1, WNT11, DVL2* and *GMFG*).

3.4.3.8 Family CHD22

The WTSI dataset for family CHD22 revealed that a total of 64,117 variants were shared by both affected individuals, 3,929 of these were reported to be novel, and only 594 were potentially pathogenic variants. As the family history suggested autosomal recessive inheritance of CHD, I focussed my attention on both homozygous variants and compound heterozygous variants, due to absence of consanguinity. There were a total of 42 homozygous variants (35 missense, and 7 splice site/indels) in 26 genes. There were a total of 552 heterozygous variants (6 nonsense, 498 missense, and 48 splice site/indels), and further analysis showed 49 genes had more than one heterozygous variant. None of the variants in *MLL3, NOTCH1* and *TBX20* seen in other families were seen in this family. There were no variants in other known CHD genes or any of the candidate genes analysed in family CHD1 (*GDF1, WNT11, DVL2* and *GMFG*).

3.4.3.9 Family CHD23

The WTSI dataset for family CHD23 revealed that a total of 59,906 variants were shared by both affected individuals, 3,728 of these were reported to be novel, and only 527 were potentially pathogenic variants. As the family history suggested autosomal recessive inheritance of CHD, I focussed my attention on both homozygous variants and compound heterozygous variants, due to absence of consanguinity. There were a total of 51 homozygous variants (47 missense, and 4 splice site/indels) in 35 genes. There were a total of 476 heterozygous variants (7 nonsense, 425 missense, and 44 splice site/indels), and further analysis showed 48 genes had more than one heterozygous variant. The same two heterozygous variants seen in family CHD4 in *MLL3 (*but not *NOTCH1*) were identified in this family. There were no variants in other known CHD genes or any of the candidate genes analysed in family CHD1 (*GDF1, WNT11, DVL2* and *GMFG*).

Table 9: Summary of the numbers of variants identified in the familial CHD cohort studied via WES at WTSI.

'Novel' = not in SNP databases (dbSNP, 1000 Genomes, UK10K) HMZ = homozygous HTZ = heterozygous
'Pathogenic variants' = non-synonymous (nonsense and missense), splice site, and indels

CHD FAMILY	1	4	5	6	13	16	20	22	23
No. affected siblings sequenced	2	2	2	2	2	3	2	2	2
Total No. variants	64675	52463	59691	63010	64176	49664	57734	64117	59906
No. 'novel' variants	6490	4019	3742	7477	4335	5894	3617	3929	3728
No. 'pathogenic' variants	1376	732	522	2204	554	1808	608	594	527
No. HMZ variants	59	53	30	42	36	29	30	42	51
nonsense	*1*	*1*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
missense	*49*	*44*	*29*	*39*	*31*	*27*	*26*	*35*	*47*
splice site/indels	*9*	*8*	*1*	*3*	*5*	*2*	*4*	*7*	*4*
No. genes with HMZ variant	48	37	21	33	24	20	19	26	35
No. HTZ variants	1317	679	492	2162	518	1779	578	552	476
nonsense	*21*	*6*	*4*	*69*	*7*	*52*	*8*	*6*	*7*
missense	*1217*	*635*	*442*	*1986*	*462*	*1642*	*538*	*498*	*425*
splice site/indels	*79*	*38*	*46*	*107*	*49*	*85*	*32*	*48*	*44*
No. genes with >HTZ variant	143	58	42	236	53	193	42	49	48

3.4.4 Overview analysis of WES data for families with "autosomal recessive" inheritance of CHD

Table 9 summarises the numbers of variants in each family using all the various filtering strategies, and Appendix G tabulates all the homozygous and compound heterozygous pathogenic variants identified in each family. The candidate genes (*GDF1, WNT11, DVL2,* and *GMFG*) evaluated in family CHD1 (from previous molecular studies) were reviewed in the datasets from the additional 8 CHD families (CHD4/5/6/13/16/20/22/23) but no candidate mutations were identified.

There was the possibility that there might be: (a) a 'major' gene accounting for CHD in the majority of the families (with phenotypic variability resulting from allelic heterogeneity or stochastic or modifier effects), or (b) extreme locus heterogeneity with CHD in each of the families resulting from mutations in a different gene. Therefore inspection of the datasets was undertaken to represent a variety of scenarios. Firstly I queried if homozygous or compound heterozygous mutations (or allelic variants) in known CHD genes might account for all or a subset of families (e.g. families of a common ethnic origin), but no evidence was found to favour this hypothesis. Secondly I queried if homozygous or compound heterozygous mutations (or allelic variants) in novel CHD genes might account for all or a subset of families (e.g. families of a common ethnic origin). When the data from both consanguineous families (Pakistani origin) was analysed together, 16 genes were identified in which both families harboured a homozygous variant. When the data from all the non-consanguineous families was analysed together, 172 genes were identified in

which more than one family harboured a compound heterozygous variant. When the data from all families was combined, 41 genes were identified in which more than one family (consanguineous and non-consanguineous) harboured a homozygous variant, and 246 genes were identified in which more than one family harboured either a homozygous variant or a compound heterozygous variant (Table 10). Appendix H contains the individual lists of genes from the above analyses across families.

Table 10: Summary of the no. of genes identified via different analytical methods across the familial CHD cohort studied via WES at WTSI.

The lists of genes from the different analytical methods are in Appendix H
HMZ = homozygous,
cHTZ = compound heterozygous (>1 variant in the gene)

ANALYSIS TYPE	NO.
No. genes with HMZ variants in > 1 consanguineous family	**16**
No. genes with HMZ variants in > 1 family (consanguineous or non-consanguineous)	**41**
No. genes with cHTZ variants in >1 non-consanguineous family	**172**
No. genes with HMZ or cHTZ variants in > 1 family (consanguineous or non-consanguineous)	**246**

3.4.5 Discussion of WES at WTSI

The WTSI WES datasets became available towards the end of the project and the analysis of the data was still ongoing at the time that this thesis was written. The analysis within a family and across all families was complicated (especially without bioinformatics tools), and still requires more time before making any firm conclusions.

The analysis performed so far identified some interesting points for discussion. Firstly, there were some genes (not known to be linked to CHD) common across the families, where a number of the variants in these genes (homozygous or compound heterozygous) were the same across the families (e.g. *MESP2* [family CHD1/4/5/13/22] and *RYR* [family CHD4/13/20/23]), implying either polymorphisms or false positives from WES (e.g. indel/frameshift variants), especially as none seemed like good candidate CHD genes.

Secondly, I identified a number of variants (missense and indels) in genes associated with known Mendelian disorders, but the families did not display any clinical features of these conditions (e.g. *CUL7* – 3M syndrome [family CHD16], *MESP2* – Spondylocostal dysostosis, *OPA1* – autosomal dominant optic atrophy [family CHD16], *PKHD1* – autosomal recessive polycystic kidney disease [family CHD6/16], and *POLG* – mitochondrial depletion syndrome [family CHD16]). Without further characterisation of these variants it poses a dilemma on the information given back to the families, and highlights one of the pitfalls and ethical issues relating to use of WES.

Thirdly, in a number of families I identified variants (compound heterozygous missense only) in genes associated with syndromic or non-syndromic CHD or cardiac disease, like cardiomyopathy). Examples include: *EVC* [family CHD1], *NOTCH1* [family CHD1/4/5/6/13/16], *TBX20* [family CHD6/16], and *TTN* [family CHD1/6/16/20/23]. There were also some potential candidate genes due to homology to known CHD genes (e.g. *MLL3* - CHD4/5/6/13/16/20/23). However

for both these scenarios, all of these variants were the same across those families, and without further clarification and laboratory analysis, it is hard to conclude on their significance. As the families were from various ethnic backgrounds there would be less evidence for a founder mutation effect or a common disease mutation.

Due to limitations of time and funding I was not able to fully analyse or characterise many of these variants identified, but further data from population studies, animal models, and large cohorts of CHD patients will help to narrow down and identify potential candidate genes. Nevertheless, it seems likely that the eventual conclusion of the analysis will be that “clinically autosomally recessively inherited CHD” demonstrates extreme genetic heterogeneity as there was no evidence that a substantial fraction of the families might be accounted for by a single gene, or indeed, multiple genes in a single pathway. It might be argued that focussing on specific subgroups of families that have an identical phenotype that is concordant within the family might facilitate gene discovery. However, ascertaining a significant number of such families is challenging and there are multiple examples of instances in which mutations in a single CHD gene can result in variable forms of CHD.

3.4.6 Conclusions

From the data available at the time of writing up the thesis, I would suggest that the absence of other candidate mutations (apart from that in *GMFG*) in family CHD1, despite WES data on three affected siblings, supports the case for *GMFG* representing a novel CHD gene. It should be noted that frameshift variants are more likely to be false positive calls than nonsense variants, which can be seen by the presence of the same frameshift variant in a number of families from different ethnic backgrounds (e.g. *RYR* and *MESP2*). With the proviso that second generation sequencing may result in false positive variants (e.g. *WNT11* and *DVL2*), other potential candidate genes include *MLL3* (numerous families) and *MESP1* (family CHD1). Therefore the variants within these genes (and those seen in *NOTCH1, TBX20*) would be the ones I would initially prioritise for validation and further analysis.

CHAPTER 4

GENERAL DISCUSSION

4.1 General discussion

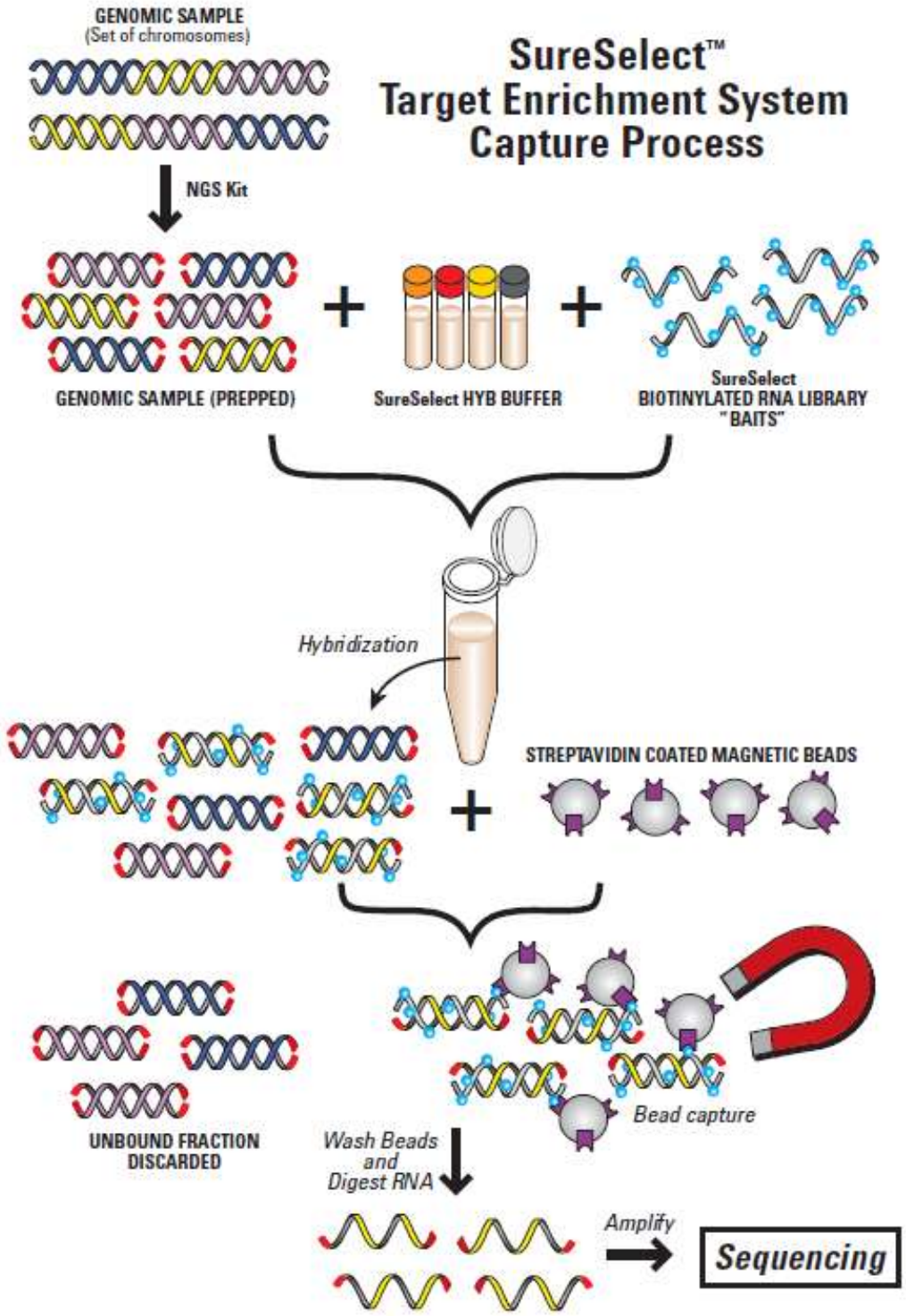
Advances in genetic analysis methodologies in CHD are very likely to lead to a shift in medical management of these patients. Currently diagnostic and therapeutic approaches are based on the anatomical and physiological differences between a normal heart and that seen in CHD. Declining mortality rates due to improved surgical repair, of even complex CHDs, has changed the population demographics of CHD patients, with an enlarging 'Grown Up Congenital Heart disease' (GUCH) population. The lack of knowledge of the underlying aetiology of many patients with CHD, reduces our ability to predict disease course and outcome, or to develop primary and secondary intervention.

4.2 Whole exome sequencing (WES)

Linkage analysis and sequencing large numbers of candidate genes in a disease interval can be time consuming and expensive (as seen before). Whole exome sequencing (also known as targeted exome capture) has revolutionalised human genetics since its initial demonstration to identify uncommon variants and disease genes in both rare Mendelian and more complex diseases (Ng et al., 2009a). To date the majority of disease causing mutations in human genetic diseases have been located in or around the coding sequences (exons) of genes. Sequencing of the entire exome (~1% of genome), rather than the entire human genome, is therefore a well justified and efficient strategy to search for causes of rare Mendelian disorders. Variants in non-coding sequences are rarely detected due to various technical issues and are more likely to have neutral or weak effects on phenotypes, even in well

conserved non-coding sequences. The exome therefore represents a highly enriched subset of the genome in which to search for variants with large effect sizes.

Figure 19: Whole exome sequencing using a target-enrichment method. *Taken from Agilent Technologies (www.agilent.com)*



4.2.1 Principles of whole exome sequencing

The technical platforms used to carry out exome sequencing are: (1) DNA microarrays and magnetic bead based systems for the enrichment of the exome DNA, and (2) next-generation sequencing technologies. Target-enrichment methods allow to selectively capture genomic regions of interest from a DNA sample prior to sequencing. Several target-enrichment strategies have been developed, but in-solution capture methods were used in my project. To capture genomic regions of interest using in-solution capture, a pool of custom oligonucleotides (bait probes) is synthesised and generally biotinylated. The bait probes are mixed with fragmented genomic DNA, and the desired fragments hybridise to baits in solution. Streptavidin beads are then added to allow physical separation. The bead-bait complexes can be pulled down and washed to clear excess material. The beads are then removed and the genomic fragments can be sequenced allowing for selective DNA sequencing of genomic regions (e.g. exons) of interest (Figure 19). The next step in any analysis is to map sequence reads, calibrate base qualities, and call variants. Variants can then be tabulated using Excel spreadsheets and manually analysed with varying filtration strategies, however with advances in bioinformatics these processes are becoming more automated.

All next-generation sequencing approaches require every base in a sample to be sequenced several times for two reasons. Firstly, despite the high sequencing accuracy for each individual nucleotide, the very large number of nucleotides in the exome means that if an individual exome is only sequenced

once, there will be a significant number of sequencing errors. Secondly, reads are not distributed evenly over an entire exome, because the reads sample the exome in a random and independent manner, therefore some bases will be covered more and some may be very less than the average value. The 'depth' in sequencing refers to the number of times a nucleotide is read during the sequencing process. So a depth of 10x means on average each base has been read by 10 sequences. Therefore you need multiple observations per base (increasing the sequencing accuracy) to come to a reliable base call and help distinguish between sequencing errors and true SNPs.

4.2.2 Data filtering strategies

The major challenge with this methodology of gene discovery is the vast number of variants identified in an individual exome (typically ~20,000) (Ng et al., 2009a). Many of these variants will be found in databases of common variants (e.g. dbSNP, 1000 genomes, in-house databases), and these can essentially be filtered out of the data, on the assumption that variants which are common in the population are unlikely to cause rare Mendelian diseases. This can help to narrow down causal variants in cases with the disease.

Another layer of data filtering is based on the potential effect of the variant on protein structure and function, and also by conservation scores. These can be determined by computerised online tools (e.g. PolyPhen-2 and SIFT) with the rationale that mutations which are disruptive to proteins and/or at more conserved sites are more likely to be pathogenic. These tools have limited

specificity and sensitivity, and should be used in conjunction with other strategies and not as a stand-alone filter (Wei et al., 2010).

Candidate genes can then be selected on the basis of the presumed inheritance of the disorder. For autosomal dominant disorders, the candidate gene must have one mutation per individual, and for autosomal recessive disorders, the candidate gene must have homozygous/compound heterozygous mutations. By pooling together data from unrelated individuals with the same disorder, the number of candidate genes can be reduced, with the assumption that all affected individuals will have mutations in the same gene. Further selection of candidate genes relies on traditional methods of phenotypes in animal models and literature reviews of confirmed or suspected function of the genes.

Ideally these data filtering strategies and candidate gene selection processes could be automated with bioinformatics tools, but despite the rapid advances in sequencing technologies, the tools to analyse the data are still undeveloped, complex and both resource and time consuming.

4.2.3 Gene identification in Mendelian disorders

Publications from many groups have demonstrated the power of exome sequencing for gene identification and Mendelian disease analysis (Choi et al., 2009; Ng et al., 2009a; Ng et al., 2009b; Bolze et al., 2010; Edvardson et al., 2010; Hoischen et al., 2010; Johnston et al., 2010; Lalonde et al., 2010; Otto et

al., 2010; Rehman et al., 2010; Sirmaci et al., 2010; Ng et al., 2010a; Ng et al., 2010b).

In consanguineous families (with multiple autozygous regions) and non-consanguineous families with patterns of recessive disease, the new practice is to perform WES to identify homozygous and compound heterozygous mutations. Unpublished studies, from Professor Maher's group, of exome resequencing in recessive disorders (n=29) in 5 consanguineous families are also encouraging and have demonstrated the feasibility of this approach for establishing the genetic basis of Mendelian disorders. Though differentiating disease causing mutations from rare polymorphic variants can be challenging, the increasing availability of publically accessible data on genome variation has facilitated greatly the differentiation of mutations from infrequent non-pathogenic variants (e.g. 1000 Genomes Project and the UK10K study). In addition, the combination of autozygosity mapping data with exome sequencing data can enable candidate mutations to be prioritised more easily. Thus in the most recent exome sequencing studies (by Professor Maher's group) in consanguineous families with recessive disorders each proband (n=14) harboured on average 40 previously unreported non synonymous variants (nonsense, frameshift, splice-site or missense) but only 1 in 4 of these mapped within a previously identified autozygous interval.

4.2.4 Advantages / disadvantages of whole exome sequencing

The trend is now towards cheaper and higher throughput DNA sequencing, and the molecular basis of many of the remaining Mendelian disorders will be quickly elucidated. This technique allows the identification of not only causal variants but also modifier variants in Mendelian diseases. The molecular basis of many Mendelian disorders has been known for many years, but the trends in research with WES can now focus on modifying factors/variants that account for variable expression and incomplete penetrance of diseases.

The challenges are no longer limitations in technology, but the interpretation of variants and specifically identifying the causal variants. This is particularly difficult in common autosomal dominant diseases where several novel and known variants are identified co-segregating with the disease phenotype. In contrast causal variants may be more easily identified in autosomal recessive diseases or rare autosomal dominant diseases (due to *de novo* mutations). Advances are therefore required in analytical software programmes and bioinformatics tools. Distinguishing benign polymorphisms and disease causing variants can be difficult in certain ethnic populations whose genetic variation has not been fully characterised, and therefore more population based studies need to be conducted (e.g. 1000 Genomes Project).

There are many reasons for failure of this method in correctly identifying the genetic basis of disease in a patient or family. The major problem in studying one individual with a rare disorder is that one can never be entirely certain that

a suspected disease gene is the correct gene, until a second unrelated individual with a similar phenotype is found to have a mutation in the same gene (Ng et al., 2010a; Robinson et al., 2011). If the depth of coverage is poor for a particular nucleotide there is an increased risk of false positives. For genetically heterogeneous disorders (like CHD), there may be a number of different genes accounting for the disorder in a particular study group, thereby making this a difficult group to study for gene identification. The strategy of identifying variants/genes shared between a number of unrelated affected individuals can have false-positive results, if candidate genes have a large coding sequence, and so there is a higher chance of an unrelated sequence variant being present amongst different individuals. Another issue is the number of false negative results due to incomplete penetrance of disease, type of variant, and poor coverage. If a causal variant is present in phenotypically affected and unaffected individuals (not fully penetrant) it may be filtered out. Synonymous variants are also usually filtered out, but some may induce exon skipping and potentially still be pathogenic. Typically reasonable coverage is up to around 90% of the exome using most current technologies, and so mutations in regions of the exome that are poorly covered by this technology will be missed (high GC content), and a candidate gene may therefore be falsely removed from further analysis. The analysis of our familial CHD cohort benefited from improvements in coverage of the WES technology, allowing very good coverage of all families studied (including family CHD1). These continuous competitive improvements between companies in the design of various platforms for WES will help to improve the coverage even further with time.

The issues with false positive and false negative results were clearly highlighted in the investigation of the genetic basis of Kabuki syndrome using WES. Initially the *MUC16* gene was identified as a candidate gene as it had a novel variant (non-synonymous, splice site, frameshift indels) in all 10 cases screened with WES, but this was considered a false positive due to the size of the gene. By using less stringent criteria for variant identification and the notion that it could be a genetically heterogeneous disorder, the *MLL2* gene was finally identified as the causative gene with a novel significant variant in 7/10 cases. Despite an average coverage of 96% of the *MLL2* gene with WES, using conventional Sanger sequencing they then identified frameshift indels mutations (not detected by WES) in 2/3 of the remaining cases (Ng et al., 2010b).

The WES dataset from our familial CHD cohort illustrates a number of possible false positives. There were a number of genes that harboured numerous variants (mainly missense), sometimes exceeding five variants, in a number of the families (e.g. *MUC, PRAMEF, IGHV, TRBV, ZNF* genes). There were some genes in which the same variants (homozgygous or compound heterozygous) were seen in a number of families (e.g. *MESP2* and *RYK*), however they did not seem like candidate genes on further analysis of the literature and animal models. The recurrence of the same variants in a number of genes across families, could suggest common mutations, but are more likely to be accounted for by false positives/polymorphisms.

Current testing strategies would also miss mutations in non-coding sequences acting as distant enhancers or regulatory elements, which can be associated with genetic disorders. A good example of alterations in regulatory elements and human genetic disease is the *PAX6* gene and aniridia. Submicroscopic *de novo* deletions of 11p13 were reported, located more than 11kb from the 3-prime end of the *PAX6* gene, in unrelated patients with sporadic aniridia (Lauderdale et al., 2000). The clinical features were indistinguishable from cases with mutations in the coding region of *PAX6*, suggesting that remote 3-prime regulatory elements are required for initiation of *PAX6* expression. More recently over 6000 candidate enhancer sequences were identified directly from fetal and adult human heart tissue. They were significantly enriched near genes implicated in heart development, function and disease, and some drove reproducible reporter gene expression in the heart (May et al., 2012). As I can not exclude the effects of variants in enhancer elements in our familial CHD cohort recruited, future strategies would include whole genome sequencing to identify variants in the non-coding regions.

There are also ethical implications relating to the vast amount of data produced from this technology and what information is transmitted back to the patients and families. Standards for consenting, reporting and counselling patients and families will be required in the future due to the complexity of the information obtained, especially with the possibilty of identifying ‘incidental’ mutations in genes causing other Mendelian disorders for which the patient may be a carrier or at risk of developing (as seen in our dataset). As the technology is

transferred from the research environment to mainstream clinical practice, this area of genomics will become a major tool and challenge for medical practice in the 21st century.

4.3 Animal Models

The successful completion of the Human Genome Project and advances in technologies associated with forward genetics, has directed biological research into the reverse genetics era where strategies for deciphering the function of each gene are being developed. Emerging technologies are promising for detecting genomic alterations in CHD (microarrays and next generation sequencing), however evidence for the pathogenicity of a gene variant or the function and role of a gene in cardiac development, can be a short-coming of these technologies. Bioinformatics tools can predict the effect of a variant on protein coding, structure and possible alterations in function. Testing the *in vivo* effects of these variants is challenging but important, and can be mediated through animal studies which allow the pathophysiological effects to be determined. This can be particularly beneficial when numerous variants have been identified in genes not known to be associated with cardiovascular development, or in cases where family studies are lacking or inclusive (Smith et al., 2009). In order to study the role of *GMFG* and the effects of the mutation identified in family CHD1 we have set up collaborations to establish animal models in both mice (Sanger Institute, UK) and zebrafish (University of Birmingham, UK).

4.3.1 Mouse (Mus musculus)

Mouse models of cardiac development have been extensively studied as it shares similar embryogenesis, anatomy, and physiology with humans. Comparative genomics have shown that many genes, proteins and regulatory elements have high homology and are highly conserved between mouse and human (Gregory et al., 2002). Now that the entire mouse genome is known, it is possible to knock out, mutate, misexpress, and replace every gene. The two main methods utilised in mice are gene trapping and gene targeting. Gene trapping is a high throughput and random mutation technique, allowing large numbers of mouse genes to be knocked out, but is not as specific as gene targeting. Gene targeting (via homologous recombination) can be used to knock out genes or introduce alterations at specific locations in the genome (knock-in) (Guan et al., 2010). Transgenic mice, via the use of multiple fluorescent proteins, can also be used to study cell-cell interactions, whole body imaging (to track cell movement/proliferation during development), and the cell cycle in real time (Miller, 2011). Transgenic mouse models have not only changed our understanding of normal and abnormal heart development, but are also being used to identify new avenues for medical therapy (Snider and Conway, 2011). The Knock Out Mouse Project (KOMP) with its repositories, was set up to develop, archive, and distribute a comprehensive library of embryonic stem cells with null mutations in every protein coding gene in the mouse (Lloyd, 2011). There are a number of practical disadvantages of mouse models including: small numbers of embryos per mouse, inability to monitor embryos, extensive laws regarding their use, and high costs of breeding (Moon, 2006).

4.3.2 African clawed frog (*Xenopus*)

The *Xenopus* has many similarities with humans genetically and anatomically, and has been used in studying vertebrate heart development. The *Xenopus* embryo develops externally and is relatively large, making it more accessible to surgical manipulation, and can develop in the absence of a functional cardiovascular system during embryogenesis. It has a unique ability to heal after microsurgery, allowing excision and culture of targeted regions and the subsequent analysis of downstream transcriptional effects of exogenous agents. Transgenic procedures have been used for promoter and enhancer analyses and introducing heritable modifications into the frog genome. Transgenesis has the advantage of tightly controlling the spatial and temporal expression of a transgene with a tissue specific manner. The most commonly used method of studying protein depletion is through morpholino oligonucleotides (see below). Numerous genes involved in cardiac development and heart defects have been well studied in the *Xenopus* (Kaltenbrun et al., 2011).

4.3.3 Zebrafish (*Danio rerio*)

Zebrafish have become an excellent genetic and embryonic model system to study cardiovascular development and diseases (Bakkers, 2011). Similar to *Xenopus* they also have the advantage of not being fully dependent on a functioning cardiovascular system during embryogenesis, as oxygen can still reach tissues through passive diffusion. Avian and mammalian embryos would die rapidly in the absence of a functioning cardiovascular system, and therefore

zebrafish with severe cardiac defects can be analysed. They are very cost effective as they require minimal equipment, can produce large numbers of progeny, and develop very rapidly with organogenesis complete 72hrs post fertilisation. They are externally fertilised and transparent allowing easy manipulation and observations (Yelon, 2001). There are many techniques to perform loss-of-function studies, and the use of antisense morpholino oligonucleotides is the most frequent. These are short chains of 25 nucleotides which act via a steric blocking mechanism, either translation initiation or modifying pre-RNA splicing. They do not always efficiently knock down the gene of interest (lack of specificity) and can be prone to off-target effects (Nasevicius and Ekker, 2000). Zinc finger nuclease (ZFN) and Transcription Activator-Like Effector Nuclease (TALEN) technologies are new techniques that allow specificity of the target gene with fewer off-target effects. Each ZFN monomer comprises of 3-6 zinc finger motifs which bind to 9-18bp of target DNA (i.e. 3bp per zinc finger). The TALEN has a highly conserved 33-35 amino acid transcription activator-like effector repeat domain, which binds to a single bp of DNA. These effector domains can be ligated together to create extended arrays that then bind to longer DNA sequences. The ZFNs or TALENs can then be fused with the nuclease domain of the *Fok*1 restriction enzyme, and functioning as dimers can create targeted double strand DNA breaks. These breaks are then repaired via non-homologous end joining recombination, creating insertion /deletion mutations and often premature termination (Moore et al., 2012). The limitations of ZFNs are that there may not be any target sites in small genes, and that a lot of work is needed to select the proper ZFN which will recognise

and cleave the target sequence (Urnov et al., 2010). TALENs have higher targeting ranges and success rates for mutagenesis compared to ZFNs, but due to the relative newness of this technique, the reasons for failure remain largely unknown (Kiefer, 2011; Moore et al., 2012). Despite their complexities, these emerging techniques do allow specific gene disruptions in a tissue specific manner, and due to their success in zebrafish are being employed in other animal models. The overall advantages of the nucleases and zebrafish, may make this the preferred animal model in the future.

4.4 Complex inheritance of CHD

As clinical management drives towards long term outcomes, it is evident that phenotypically similar cases can have very different outcomes (taking into consideration effects of clinical variables like surgical techniques). It is therefore very likely that genetic factors contribute to this difference, and research is now directed to identifying genetic variants involved in pathogenesis or outcome of CHD.

The complex pathogenesis of CHD, genetic heterogeneity, and the multifactorial theory of inheritance, makes it difficult to identify genetic causes of CHD. Variable expression and incomplete penetrance of CHD has been demonstrated in familal cases of CHD, as seen in *NKX2.5* and *GATA4* mutations (Schott et al., 1998; Benson et al., 1999; Garg et al., 2003). The strong familial disposition and presence of discordant phenotypes observed suggests that the model of compound heterozygous hits in different genes, or

multiple hits, in related developmental pathways could occur in the CHD population. Population studies have shown a high heritability of many forms of CHD implying an oligogenic model of inheritance (Oyen et al., 2009). Roessler *et al.*, (2008) highlighted how multiple variants in genes in a developmental pathway (Nodal/TGFB), acting in a cumulative and incremental manner, may cause human CHD. A multiple gene analysis method in large CHD patient cohorts has therefore been shown to be more useful in identifying variants whose combined effect results in a phenotype (Granados-Riveron et al., 2012). It is also possible polymorphisms in epigenetic factors (e.g. chromatin remodelers) may affect the penetrance and resulting phenotypes of disease-causing mutations in genes involved in cardiac development. Variants in all these complex networks could act in a syngergistic or antagonistic manner to provide a buffering effect on the phenotype (Schlesinger et al., 2011). Identifying new genes associated with CHD should therefore involve the analysis of all interacting factors, upstream regulators, and downstream effectors of the currently known genes linked to CHD.

4.5 Future Direction

Limitations in funding and time prevented any future developments of this project, but as highlighted in this thesis, there are a number of possible future directions. Familial cases of CHD (like many other congenital defects) are a valuable source to undertake genetic research and identify the genetic basis of CHD. Identification and recruitment of more familial cases of CHD will aid the discovery of the molecular basis for CHD. Conventional autozygosity mapping in further consanguineous families may help to identify any new candidate regions/genes. Whole exome sequencing in familial CHD (sometimes in conjunction with autozygosity mapping in selected families) will aid in identifying new candidate genes. Collaboration with large CHD research networks will allow molecular analysis in sporadic cases of CHD for mutations in candidate genes or variants that increase the susceptibility to CHD (multifactorial model). Sequencing parent-child trios in cases of severe isolated (non-familial) CHD, may aid in identifying novel cardiac genes by filtering the data for *de novo* variants. With improvements in cost and time, whole genome sequencing will become the next tool for investigating genetic diseases (including CHD) to allow the detection of both coding and non-coding sequence variants. Methods of identifying and studying epigenetic factors and miRNAs will further elucidate their role not only in the pathogenesis but management of CHD. Finally functional studies in animal models will characterise the pathogenicity of mutations in genes/regulatory regions via their molecular, physiological, and anatomical effects.

CHAPTER 5

CONCLUSIONS

In this project I identified and recruited a cohort of familial non-syndromic CHD, which are rare but a very good resource for identifying candidate genes involved in CHD. Genome wide scans and microsatellite analysis identified one region of homozygosity on chromosome 19 in one family (family CHD1). *GDF1* a candidate gene in this region was sequenced for mutations, as well as other genes in the same developmental pathway (*NODAL, CFC1, TDGF1*, and *FOXH1*), in family CHD1 and in affected members of other CHD families with a similar phenotype (outflow tract anomalies), but no pathogenic mutations were identified. Whole exome sequencing in family CHD1 identified variants in numerous genes, some of which were good candidate genes for cardiac development (*DVL2* and *WNT11*), but the variants were not confirmed by conventional sequencing and therefore further studies were not performed (false positives). Despite no mutations being identified in any of these selected genes in the families studied with WES, I feel it would be important to consider these developmental pathways (WNT and NODAL) and these genes in any future studies of familial and sporadic cases of CHD. Our results imply that there are further genes to be identified that may explain these familial cases of CHD.

The benefit of combining autozygosity mapping and whole exome sequencing is highlighted with the identification of a novel candidate gene, not previously thought to be involved in cardiac development (*GMFG*) in family CHD1. WES identified a homozygous nonsense mutation in this gene, which is located in the same homozygous region on chromosome 19. Segregational analysis within the

family and negative sequence analysis in >200 ethnically matched control chromosomes implies this is a potential candidate gene. Further functional studies in animal models (mouse and zebrafish) are being undertaken with our collaborators, and will be required to determine the phenotypic effects of the identified mutation, and the role of this gene in cardiac development. Mutational analysis of this gene in large cohorts of CHD sporadic/familial cases is also needed, but the absence of variants in this gene in the other CHD families studied could implicate this gene as a rare cause of CHD (<1%).

I have shown the power of emerging genetic technologies, like whole exome sequencing, in the search for new candidate genes for diseases with a genetic basis. Although a powerful tool for variant detection with both cost and time saving benefits, there are many hurdles to overcome before conclusive results can be obtained. Advances in bioinformatics tools are required to aid in the interpretation of the vast amount of data generated. It is only once we have mastered the ability to distinguish significant from insignificant variants, can we utilise this technology in everyday practice to tailor medical management of patients dependent on their genotype.

The issues researchers in CHD will face in the coming years are highlighted in the discussion, and in order to be successful in identifying new genetic determinants of CHD, one will require: (a) accurate phenotyping of patients (and their family members), (b) improved training of researchers in applying innovative genetic techniques, (c) advances in bioinformatics tools to interpret

data from exome/genome sequencing, and (d) large collaborative networks to interpret rare alleles (through genotyping of cases and controls).

Advances in diagnosis and management of CHD has improved the survival of many patients but has not reduced the incidence of this birth defect. More information is needed about the genetic and molecular pathways involved in heart development and specific advances that are changing our understanding of CHD aetiology include: (a) detection of chromosomal microdeletions and translocations by higher resolution chromosomal analysis and FISH, (b) delineation of the genetic defects in familial CHD through linkage analysis, and (c) advances in animal genetics allowing studies of animal models of CHD. Both genetic heterogeneity and phenotypic heterogeneity imply the existence of modifying factors in CHD, which are being described by the emerging evidence of multiple-hit models of variants in related developmental pathways, and epigenetic factors in heart development. An improved understanding of the genetic and molecular controls of normal heart development will allow for: (a) accurate genetic counselling, (b) identification of possible targets of gene therapy, and (c) development of possible methods of CHD prevention in future generations.

CHAPTER 6

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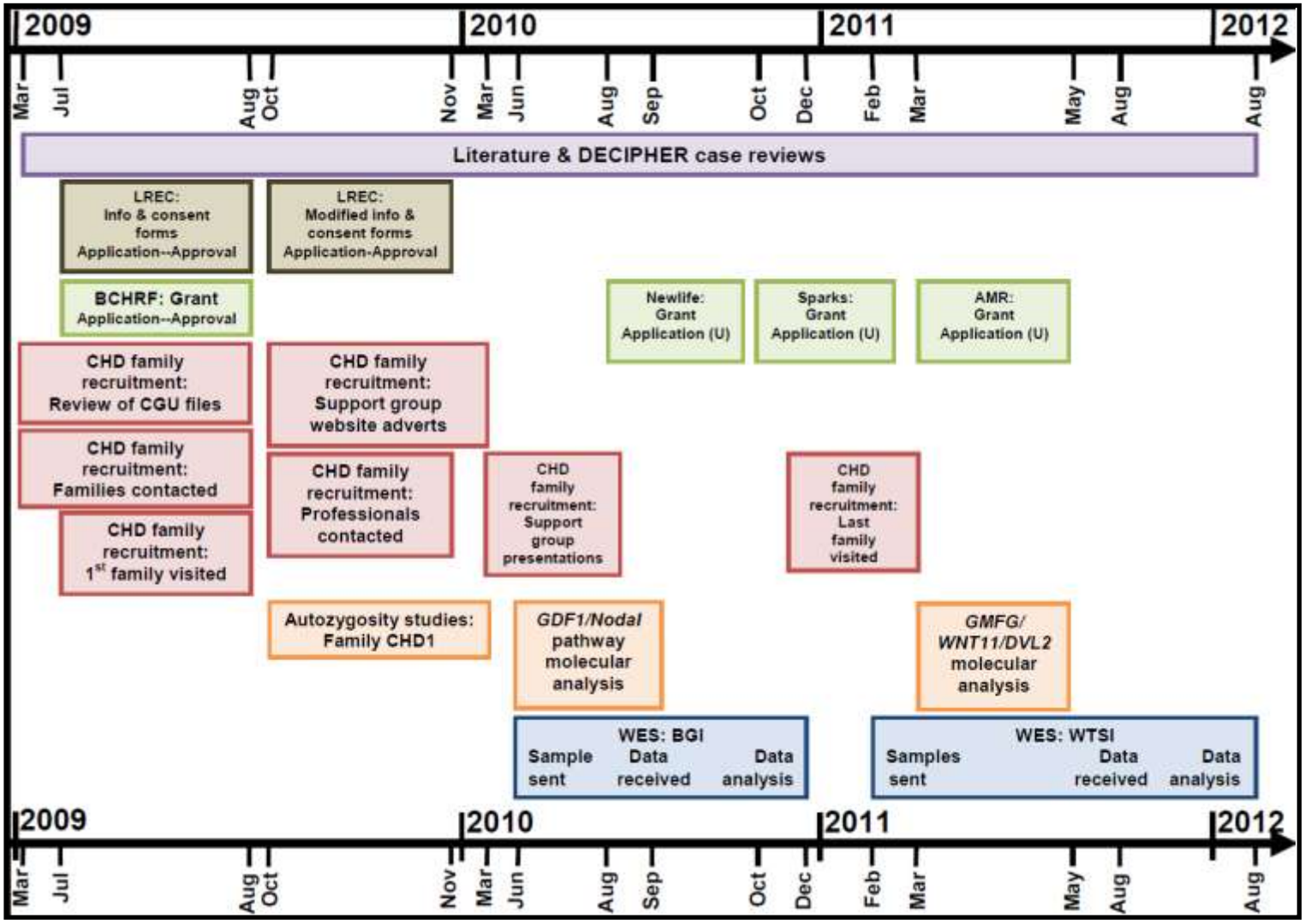
CHAPTER 7

APPENDICES

APPENDIX A: Timeline of CHD project



APPENDIX B: Types of CHD and other clinical features associated with chromosomal abnormalities registered on DECIPHER (DatabasE of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources)

(www.decipher.sanger.ac.uk)

ACC: agenesis of corpus callosum; BW: birth weight; CNV = Copy number variation; DD: developmental delay; EAM: external auditory meatus; IUGR: intrauterine growth retardation; MI: mitral incompetence; SN: sensorineural; TeF: tracheo-esophageal fistula; USPF: upslanting palpebral fissures; TA: tricuspid atresia; TI: tricuspid incompetence **Red = Deletion** **Green = Duplication**

CHR ID	KARYOTYPE	SIZE OF CNV (Mb)	TYPE OF CHD	OTHER CLINICAL DETAILS
1	del(1)(p13.3;p13.3)	2.76	PDA	DD, hypotonia, seizures, lower limb mesomelia, small penis, early puberty, hand/foot anomalies, sacral dimple, dysmorphic
1	del(1)(p36.12;p36.12)	2.88	PDA, CoA/IAA	DD, hypospadias, arthrogryposis, bifid thumb
1	del(1)(p36.13;p36.13)	1.50	ASD	DD, hypotonia, larngeal/tracheal anomaly, feeding problems, dysmorphic
1	del(1)(p36.13;p36.32)	12.54	AVSD	DD, short stature, microcephaly, scoliosis, speech delay, submucous cleft palate, feeding problems, dysmorphic
1	del(1)(p36.21;p36.23)	5.63	PS	DD, short stature, larngeal/tracheal anomaly, SN deafness, undescended testes, speech delay, feeding problems, recurrent infections, dysmorphic
1	del(1)(p36.31;p36.32)	2.83	VSD	DD, microcephaly, hypotonia, inguinal hernia, undescended testes, sacral dimple, macrocornea, dysmorphic
1	del(1)(q24.3;q31.2)	20.95	ASD	DD, short stature, obesity, deafness, vertebral fusion, undescended testes, constipation, finger/toe anomalies, auricular pits, ptosis, hypermetropia, eczema, dysmorphic
1	del(1)(q41;q41)	2.33	VSD	DD, cleft palate
1	del(1)(q41;q42.12)	5.25	VSD	diaphragmatic hernia, cleft palate, talipes, dysmorphic
1	del(1)(q42.3;q44)	12.81	PDA, CoA/IAA	biliary atresia, absent gallbladder, hypogonadism, cleft palate, hemivertebrae, ACC, encephalocele, hypopituitarism, overlapping fingers, rocker bottom feet, dysmorphic
1	del(1)(q42.3;q44)	12.72	AVSD	DD, ACC
1	dup(1)(p12;p13.2)	3.98	PS	DD, ear anomalies, dysmorphic
1	dup(1)(p31.1;p31.1)	0.15	PS	DD, microcephaly, squint, hypotonia
1	dup(1)(q21.1;q21.1)	2.00	ASD	DD, short stature, sleep problems, inguinal hernia, dysmorphic
2	del(2)(p15;p15)	0.00	AS	DD, tall stature, pectus excavatum, scoliosis, inguinal hernia, small testes, flat feet,

				dysmorphic
2	del(2)(p16.3;p16.3)	0.12	Ebstein's, TI, ASD	obesity, DD, macrocephaly, hydrocephalus, ACC, delayed myelination, deafness, small testes/scrotum/penis, joint laxity, perthes hip, dysmorphic
2	del(2)(q22.2;q22.3)	4.30	AS	DD, ACC, seizures, squint
2	del(2)(q22.2;q23.3)	5.39	PS	DD, microcephaly, behaviour problems, small hands/feet, dysmorphic
2	del(2)(q22.3;q23.1)	0.34	ASD, VSD	DD, joint laxity, fetal pads, dysmorphic
2	dup(2)(p14;p14)	4.16	VSD	DD, short stature, microcephaly, SN deafness
2	dup(2)(p15;p16.1)	0.92	ASD	DD, speech delay, short stature, toe syndactyly, dysmorphic
2	dup(2)(p24.1;p25.3)	21.20	AS	DD, macrocephaly, short stature, scoliosis
2	dup(2)(q12.2;q13)	3.86	AVSD	DD, deafness, squint, recurrent infections, feeding problems, oligodontia, small feet, dysmorphic
2	dup(2)(q33.1;q33.3)	6.66	CoA/IAA	DD, dysmorphic
3	del(3)(p12.3;p12.3)	1.15	VSD, PS	thumb anomaly
3	del(3)(p22.2;p22.2)	1.62	TGA	short stature, craniosynostosis, small penis, nipple anomalies, triphalangeal thumbs, rocker-bottom feet, ear anomalies, dysmorphic
3	del(3)(p25.3;p26.1)	3.29	AVSD	DD, microcephaly, seizures, deafness, horseshoe kidneys, sacral dimple, toe post-axial polydactyly, dysmorphic
3	del(3)(p25.2;p25.3)	3.16	CHD	DD, short stature
3	dup(3)(q26.13;q26.3)	10.11	AS, CoA/IAA	DD, symphalangism, scoliosis, choanal atresia, deafness, coarse hair, dysmorphic, microtia
3	dup(3)(q29;q29)	1.77	ASD, AVSD	coloboma, lipodystrophy, dysmorphic
4	del(4)(p16.2;p16.3)	3.09	PS	DD, short stature, microcephaly, febrile convulsions, small kidneys, hypospadias, dysmorphic
4	del(4)(q21.21;q21.23)	3.12	ASD	DD, high BW, hypotonia, speech delay, cleft uvula, cleft palate, urine reflux, small hands/feet, dysmorphic
4	del(4)(q21.23;q23)	15.29	ASD	hypotonia, autism, small/undescended testes, ptosis, arthrogryposis
4	del(4)(q28.3;q31.23)	10.16	VSD, PS	thumb anomaly
4	del(4)(q35.1;q35.2)	6.73	VSD	ACC, hypospadias, small penis, cleft lip/palate
4	dup(4)(p16.3;p16.3)	0.19	ASD	DD, short stature, microcephaly, seizures, choanal atresia/stenosis, anaemia, prominent clitoris, macroglossia, rocker-bottom feet, dysmorphic
4	dup(4)(q12;q12)	0.17	AVSD	DD, feeding problems
4	dup(4)(q32.3;q32.3)	0.38	PS	short stature, auricular pits, ptosis, dysmorphic

5	del(5)(p13.2;p13.2)	0.18	VSD	undescended testes, feeding problems, ear helix anomaly
5	del(5)(q23.1;q31.1)	12.60	ASD	DD, cleft palate, talipes, ear anomalies, glossoptosis,
5	del(5)(q31.1;q31.2)	1.72	PDA	DD, hypotonia, tracheal/laryngeal anomaly, coarse features
5	del(5)(q35.1;q35.1)	0.16	TOF	dysmorphic
5	dup(5)(p12;p13.2)	6.62	PDA	DD, pectus excavatum, auricular tags, dysmorphic
5	dup(5)(p15.31;p15.33)	6.55	PDA, MI	DD, short stature, seizures, cerebellar ataxia, hirschsprung, adrenal hyperplasia, uterine fibroid, hypoplastic breasts, scoliosis, premature ageing, hirsutism, small hands
5	dup(5)(q11.2;q11.2)	0.22	PDA	DD, microcephaly, seizures, speech delay, patchy pigmentation, small teeth, dysmorphic, ? Cardiomyopathy
5	dup(5)(q15;q15)	4.31	VSD	DD, craniosynostosis, joint laxity
6	del(6)(p21.1;p21.1)	2.41	MI	DD, hydrocephalus, hypotonia, speech delay, joint laxity, dysmorphic
6	del(6)(q24.3;q25.1)	2.45	ASD	DD, short stature, ear/helix anomalies, dysmorphic
6	del(6)(q25.2;q25.3)	1.60	VSD, AVSD, PDA	deafness, craniosynostosis, broad thumbs, clinodactyly, brachydactyly, prominent forehead, malocclusion teeth
6	del(6)(q25.3;q25.3)	2.62	ASD	DD, asymmetrical skull, lung cysts, wide spaced nipples, small ears, abnormal nails, dysmorphic
6	dup(6)(q22.31;q22.31)	0.78	TGA	microphthalmia, hypospadias
7	del(7)(p12.1;p15.1)	21.92	VSD	diabetes, diastasis recti, hydrocele testis, flexible joints, broad thumbs, large tongue, dysmorphic
7	del(7)(p12.3;p14.1)	8.28	VSD	DD, cortical atrophy, squint, hypermetropia, toe syndactyly, broad thumbs, bifid hallux
7	del(7)(p13;p14.2)	8.04	ASD, AS	DD, macrocephaly, umbilical hernia, hirsutism, febrile convulsions, feeding problems, recurrent infections, ear anomalies, finger skin syndactyly, dysmorphic
7	del(7)(p14.1;p14.3)	5.36	ASD	low BW, short stature, microcephaly, hypotonia, cleft palate, clinodactyly
7	del(7)(q11.21;q22.1)	35.55	VSD	DD, microcephaly, hydrocephalus, inguinal hernia, cleft palate, hand/foot ectrodactyly, small ears, dysmorphic
7	del(7)(q22.1;q22.2)	3.40	ASD	DD, hypotonia
7	del(7)(q36.3;q36.3)	0.08	TOF	DD
7	dup(7)(p21.3;p21.3)	0.41	PI, single ventricle	overriding toes, toe syndactyly, borad nasal tip
8	del(8)(p23.1;p23.1)	1.01	CHD	no

8	del(8)(p23.1;p23.1)	3.77	AVSD, PS, TAPVD	DD, hyperactive, urine reflux
8	del(8)(p23.1;p23.1)	3.65	ASD	diaphragmatic hernia, undescended testes
8	del(8)(p23.1;p23.1)	3.78	CHD	no
8	del(8)(p23.1;p23.1)	3.31	AVSD, PS, MI	DD, diaphragmatic hernia, low set ears
8	del(8)(p23.1;p23.1)	3.77	ASD, VSD, PS, MI	DD, speech delay
8	del(8)(p23.1;p23.1)	5.31	ASD	DD, microcephaly, undescended testes, synophyrys, large ears, dysmorphic
8	del(8)(q21.3;q22.1)	3.60	PS	DD, USPF, prominent nasal bridge
8	del(8)(q22.2;q23.1)	4.92	PDA	DD, microcephaly, hyperactive, undescended testes, hypospadias, hiatus hernia, pyloric stenosis
8	dup(8)(p22;p22)	0.56	CoA/IAA	no
9	del(9)(p21.1;p21.1)	0.06	AI	DD, short stature, dolicocephaly, patchy skin pigmentation, recurrent infections, speech delay, cleft palate, dysmorphic
9	del(9)(p22.3;p23)	6.78	TI	DD, deafness, cystic hygroma, dysmorphic
9	del(9)(p23;p24.3)	11.34	ASD, VSD, PDA	female genitalia anomaly, submucous cleft palate, thumb anomaly, dysmorphic
9	del(9)(p24.2;p24.3)	2.46	AVSD	DD, low BW, deafness, micrognathia, single palmar crease, feeding problems
9	del(9)(q31.3;q32)	2.05	VSD	DD, cortical atrophy, joint laxity, hypermetropia
9	del(9)(q34.3;q34.3)	3.01	VSD, CoA/IAA	DD, hypotonia, pyramidal signs, asymmetrical limbs, dysmorphic
9	del(9)(q34.3;q34.3)	2.20	ASD, VSD	DD, hypotonia, microcephaly, multiple renal cysts, small penis, undescended testes, hypospadias, recurrent infections
9	dup(9)(q31.1;q33.1)	17.24	VSD, TA	DD, speech delay, undescended testes, cyclic vomiting, dysmorphic
9	dup(9)(q31.3;q32)	3.05	VSD, TA	DD, speech delay, undescended testes, cyclic vomiting, dysmorphic
10	del(10)(p11.23;p11.23)	0.99	PDA	DD, autism, pyramidal signs, short metacarpals, small ears, squint, scoliosis
10	del(10)(p12.1;p12.1)	0.50	PDA	DD, short stature, hypotonia, speech delay, squint
10	del(10)(p15.3;p15.3)	0.15	CHD	microcephaly, IUGR, short stature, cleft uvula, dysmorphic, speech delay
10	del(10)(q21.1;q21.1)	0.17	VSD, PDA	DD, microcephaly, short stature, hyperactive, oligodontia, toe syndactyly, blepharophimosis
10	del(10)(q21.1;q21.2)	4.95	PS	DD, autism, dysmorphic

10	del(10)(q24.31;q24.32)	0.55	PS	DD, short stature, obesity, deafness, cleft lip/palate, microphthalmia, urine reflux, soft skin, short phalanges,
10	del(10)(q25.2;q26.11)	6.70	PDA	DD, autism, ptosis, seizures, hypoplastic toes, dysmorphic
10	del(10)(q25.3;q25.3)	0.33	VSD	DD, radio-ulnar synostosis, dysmorphic
10	dup(10)(q23.33;q25.1)	17.19	VSD	DD, short stature, macrocephaly, behaviour problems, speech delay, pectus excavatum, constipation
11	del(11)(p11;q12.1)	3.46	VSD	DD, short stature, microcephaly, hyperactive, speech delay, undescended testes, inguinal hernia, finger skin syndactyly, supernumerary nipples, dysmorphic
11	del(11)(q22.3;q23.1)	5.47	VSD, PS	DD, hypotonia, ACC, hydrocephalus, cleft uvula, auricular pits, abnormal labia
12	del(12)(p13.2;p13.2)	1.28	AI	DD, high palate, expressionless face
12	del(12)(q13.3;q14.2)	6.03	PS	DD, speech delay, pectus excavatum
12	del(12)(q13.3;q14.3)	10.12	ASD	DD, short stature, autism, feeding problems, sacral dimple, speech delay, dysmorphic
12	del(12)(q21.31;q21.33)	6.90	VSD, PS	thumb anomaly
12	dup(12)(q14.1;q14.1)	0.33	PDA	DD, pleural effusion/chylothorax, hydronephrosis, ureteral anomaly, hypospadias, hydrocele testis, speech delay, auricular tags+pits, squint, flat feet, dysmorphic
12	dup(12)(q24.23;q24.31)	3.48	VSD	DD, craniosynostosis, joint laxity
13	del(13)(q33.2;q34)	9.63	TOF	DD, microcephaly, speech delay, myopia
13	del(13)(q14.11;q14.11)	0.16	TOF	no
13	dup(13)(q13.2;q13.2)	0.60	CoA/IAA	excessive skin folds, dysmorphic
14	del(14)(q11.2;q11.2)	1.62	CHD	DD, hypotonia, dysmorphic
14	del(14)(q21.3;q21.3)	0.16	VSD	undescended testes, feeding problems, ear helix anomaly
14	del(14)(q22.1;q23.1)	5.65	TGA	anophthalmia, renal dysplasia, finger post-axial polydactyly, hypotonia, ear anomaly, absent EAM, sacral dimple,
14	del(14)(q32.31;q32.33)	4.21	ASD	DD, hypotonia, sleep apnoea, feeding problems
14	del(14)(q32.33;q32.33)	1.07	ASD	ACC, cleft palate, dysmorphic
14	dup(14)(q12;q12)	1.21	PS	DD, pyramidal signs, ACC, speech delay, dysmorphic
14	dup(14)(q32.12;q32.33)	10.65	PDA	laryngeal anomalies, DD, hypotonia, microcephaly, sleep disorder, recurrent infections, feeding problems, dysmorphic

15	del(15)(q21.1;q22.2)	10.56	CoA/IAA	DD, ACC
15	dup(15)(q13.3;q13.3)	0.49	ASD	DD, microcephaly, squint
15	dup(15)(q26.1;q26.3)	8.56	AVSD	DD, ACC
16	del(16)(p13.3;p13.3)	0.04	ASD	DD, oligodactyly, cleft uvula, flexion deformities, dysmorphic
16	del(16)(q22.3;q22.3)	1.19	PDA	DD, short stature, dysmorphic
16	del(16)(q22.3;q23.1)	0.17	PS	DD, hypotonia, tall stature, small ears, dysmorphic
16	del(16)(q23.3;q23.3)	0.13	ASD, AS	cystic hygroma, oedema, arthrogryposis, microphthalmia, ear anomalies, talipes, finger skin syndactyly, dysmorphic
16	del(16)(q24.1;q24.1)	1.49	PDA	abnormal placement anus, oesophageal atresia/stenosis
16	del(16)(q24.2;q24.3)	1.09	VSD	cardiomyopathy, DD, DW malformation, dysmorphic
16	del(16)(q24.3;q24.3)	0.18	AVSD, MI	low BW, short stature, microcephaly, clinodactyly
16	dup(16)(p11.1;q22.3)	37.19	VSD	DD, hypotonia, deafness, auricular pits, finger post-axial polydactyly, inguinal hernia, scoliosis, bound tongue, squint, macula pigmentary anomaly, small feet
16	dup(16)(p12.3;p13.11)	1.90	ASD	renal agenesis, ureteral anomaly, primary amenorrhoea, facial nerve palsy, choanal atresia/stenosis, coloboma, ear anomaly, vestibular anomaly, squint
16	dup(16)(p12.3;p13.11)	2.73	ASD	larngeal/tracheal anomaly, narrow chest, absent ribs, bifid thumb, hypoplastic/absent tibia, hemivertebrae, abnormal sacrum, talipes, scoliosis, auricular pits
16	dup(16)(q24.2;q24.3)	2.00	ASD	DD
17	del(17)(p13.3;p13.3)	2.09	PDA, PS	DD, short stature, coloboma, cleft palate, arachnodactyly, feeding problems, dysmorphic
17	del(17)(p13.3;p13.3)	2.12	TOF	DD, short stature, sacrosciatic notch, undescended testes, dysmorphic
17	del(17)(q21.31;q21.31)	0.46	PS	DD, hypotonia, ACC, wide spaced nipples, dislocated elbows, brittle nails, overriding toes, dysmorphic
17	del(17)(q25.3;q25.3)	1.11	ASD, VSD	DD, speech delay, scoliosis, cleft tongue, oligodontia, brachydactyly, dysmorphic
17	dup(17)(p13.1;p13.3)	6.95	VSD	DD, short stature, ptosis, feeding problems, immunoglobulin abnormality, dysmorphic
17	dup(17)(q12;q12)	1.80	ASD	DD, peter's anomaly, microphthalmia, glaucoma, cleft palate, female pseudohermaphroditism, multiple renal cysts
17	dup(17)(q12;q12)	1.84	AVSD	short stature, microcephaly, nasal speech, hypospadias, pectus excavatum, scoliosis, broad neck, bound tongue, ear anomaly, unusual hair pattern, dysmorphic
17	dup(17)(q24.3;q25.3)	11.43	TOF	DD
18	dup(18)(p11.21;p11.32)	14.00	ASD,	low BW, toe syndactyly, sacral dimple, dysmorphic

			CoA/IAA	
18	dup(18)(p11.32;p11.32)	1.11	TOF	DD, short stature, sacrosciatic notch, undescended testes, dysmorphic
19	del(19)(p13.11;p13.11)	0.96	TOF	DD, hand/foot ectrodactyly, skin depigmentation, hand pre-axial polydactyly, bifid nails, speech delay, small penis, squint, dysmorphic
19	del(19)(p13.2;p13.2)	3.42	ASD	DD, sleep disorder, behavioural problems, hypospadias, nasolacrimal duct anomaly, bound tongue, sacral dimple, dysmorphic
19	del(19)(p13.3;p13.3)	2.79	VSD	DD, microcephaly, hypotonia, inguinal hernia, undescended testes, sacral dimple, macrocornea, dysmorphic
19	del(19)(p13.3;p13.3)	0.58	ASD	DD
19	dup(19)(p13.11;p13.12)	1.77	PDA, ASD	DD, microcephaly, short stature, speech delay, finger skin syndactyly, sacral dimple, dysmorphic
19	dup(19)(p13.3;p13.3)	0.01	ASD	short stature, thin, speech delay, hypothyroidism, recurrent infections, microtia
20	dup(20)(p11.23;p13)	20.24	ASD, VSD, PDA	female genitalia anomaly, submucous cleft palate, thumb anomaly, dysmorphic
20	dup(20)(q13.32;q13.33)	4.81	VSD, CoA	DD, hypotonia, pyramidal signs, asymmetrical limbs, dysmorphic
20	dup(20)(q13.33;q13.33)	0.00	VSD	kidney anomalies, DD, short stature, asymmetrical skull, deafness, osteopetrosis/sclerosis, abnormal placement anus, thumb/toe anomaly, dysmorphic
21	dup(21)(q21.1;q21.1)	1.69	HLH	broad neck
22	del(22)(q11.21;q11.21)	2.52	TOF	meningocele
22	del(22)(q11.21;q11.21)	2.75	CoA/IAA	DD, psychotic behaviour, obesity, dysmorphic
22	del(22)(q11.21;q11.21)	2.72	VSD	DD, short stature, speech delay, facial cleft, coloboma, cleft palate, 4 limb post-axial polydactyly, dysmorphic
22	del(22)(q11.21;q11.22)	1.39	ASD, VSD	short stature, microcephaly, anal atresia/stenosis
22	del(22)(q11.21;q11.22)	0.15	CHD	DD, deafness, heterochromia, CAL, patchy depigmentation skin, hypertension, hypothyroidism, pyramidal signs
22	del(22)(q11.21;q11.23)	1.94	VSD	DD, low BW, obesity, duodenal anomalies, speech delay, USPF
22	dup(22)(q11.1;q11.22)	5.60	duplicated/RtAA	horseshoe kidneys, TeF, laryngeal/tracheal anomaly, deafness, coloboma
22	dup(22)(q11.21;q11.21)	0.00	TA	DD, hydronephrosis, short neck, cleft lip/palate, auricular pits, dysmorphic
22	dup(22)(q12.3;q13.33)	18.22	VSD	ACC, hypospadias, small penis, cleft lip/palate

X	del(X)(p11.23;p11.23)	0.20	AVSD	dysmorphic
X	del(X)(p22.13;p22.2)	2.88	TOF	DD, hypotonia, seizures, cataracts
X	del(X)(p22.2;p22.31)	1.18	ASD	DD, retinal anomalies, skin anomalies, urethral valves, small penis, nystagmus, dysmorphic
X	dup(X)(p21.3;p21.3)	0.22	VSD	abdominal situs inversus, DD, hypotonia, microcephaly, pyramidal signs, seizures,
X	dup(X)(p22.31;p22.31)	0.58	TI	DD, short stature, autism
X	dup(X)(p22.31;p22.31)	1.52	ASD	DD, microcephaly
X	dup(X)(p22.33;p22.33)	0.57	AS	DD, cleft palate, dysmorphic
X	dup(X)(q28;q28)	2.08	ASD	DD, microcephaly, hypothyroidism, ptosis

APPENDIX C1:
Flyer designed to send to medical professionals and patient support groups to recruit families into the project

UNIVERSITY OF BIRMINGHAM

INVITATION TO TAKE PART IN A RESEARCH PROJECT TO IDENTIFY THE GENES THAT CAUSE INHERITED CONGENITAL HEART DISEASE

BACKGROUND

Heart development is a complex process and errors in normal development lead to congenital heart disease (CHD). Many genes are known to be involved in heart development but these cannot explain all the causes of CHD. Hence we would like to identify new genetic causes of CHD.

WHO WOULD BE SUITABLE TO PARTCIPATE?

We would like to hear from three types of families. Those with:

A) **Two or more children in the family with CHD (the affected children can be brothers or sisters or cousins), but their parents do not have CHD**
B) **One or more children in the family with CHD, and their parents are blood relatives (e.g. cousins)**
C) **Two or more individuals in the family with outflow tract CHD anomalies (e.g. Transposition of the Great Arteries, Tetralogy of Fallot, or on that spectrum) and any pattern of inheritance.**

Such families could be very helpful for identifying more CHD genes.
(Of note we may extend our research to other families at a later stage).

DO YOU HAVE TO TAKE PART AND WHAT WOULD BE REQUIRED?

Taking part in the project is simple and entirely voluntary. If you are interested in taking part but change your mind then you can withdraw at any time. If you agree to take part we will ask you and other members of your family to provide a small blood sample (taken through a needle from a vein in your arm) and give permission for us to obtain medical details from your children's cardiology unit.

WILL THIS PROJECT BENEFIT YOUR FAMILY?

We cannot guarantee to discover anything that will directly benefit your family. However, if we identify a CHD gene that might be relevant to your family we will contact you (unless you ask us not to) to let you know what gene tests might be available. New gene tests would be helpful for identifying which families might be at risk of having further children with CHD and for improving our understanding of how the heart develops.

WHO DO YOU CONTACT FOR MORE DETAILS?

If you have any questions or concerns about this project, please contact one of the following:

Dr. Chirag Patel **Prof. Eamonn Maher**

APPENDIX C2:
Adverts placed on patient support group websites to recruit families into the project

children's heart FEDERATION

free info line 0808 808 5000
9:30 - 16: 30 Monday - Friday

Welcome
About Us
How We Help
Support Us
Personal Stories
News & Events

MAKE A DONATION

newsletter sign up

facebook

Welcome

Hot News!

To receive out of hours media contact information for the Children's Heart Federation, please enter your email address into our **out of hours media enquiry form**.

CHF have just finished designing a new set of posters to help raise awareness of our organisation and the work we do. They are available for download in either **A3** or **A4** size. Put one up wherever you can!

Participants are required to take part in a University of Birmingham research project to identify the genes that cause inherited congenital heart disease. You can view the **CHD study invitation here**.

Join CHF for **an afternoon at the London Children's Ballet** on 23/04/2010.

To find out about the CHF teen forum summer adventure, watch the video at our brand new **"brighthearts" page**.

Calling **grandparents and parents** to support others coming to terms with a child's heart defect.

The Children's Heart Federation is the leading children's heart charity and the umbrella body for voluntary organisations working to meet the needs

The GUCH Patients Association Blog

Grown Up Congenital Heart Patients Association

Supporting Young People and Adults with Congenital Heart Disease

New research project into genetics at Birmingham

28 January 2010, 5:55 pm

Invitation to take part in a research project to identify the genes that cause inherited congenital heart disease

Background

Heart development is a complex process and errors in normal development lead to congenital heart disease (CHD). Many genes are known to be involved in heart development but these cannot explain all the causes of CHD. Hence we would like to identify new genetic causes of CHD.

Who would be suitable to partcipate?

We would like to hear from two types of families. Those with:

- Two or more children in the family with CHD (the affected children can be brothers or sisters or cousins), but their parents do not have CHD
- One or more children in the family with CHD, and their parents are blood relatives (e.g. cousins) Such families could be very helpful for identifying more CHD genes. (Of note we may extend our research to other families at a later stage).

Do you have to take part and what would be required?

Taking part in the project is simple and entirely voluntary. If you are interested in taking part but change your mind then you can withdraw at any time. If you agree to take part we will ask you and other members of your family to provide a small blood sample (taken through a needle from a vein in your arm) and give permission for us to obtain medical details from your children's cardiology unit.

Will this project benefit your family?

We cannot guarantee to discover anything that will directly benefit your family. However, if we identify a CHD gene that might be relevant to your family we will contact you (unless you ask us not to) to let you know what gene tests might be available. New gene tests would be helpful for identifying which families might be at risk of having further children with CHD and for improving our understanding of how the heart develops.

For more information on this or to enquire about volunteering please contact:

Dr. Chirag Patel

APPENDIX C3:
Patient information sheets and consent forms used to recruit families into the project

INVITATION TO TAKE PART IN A PROJECT
TO INCREASE RESEARCH INTO THE CLINICAL AND GENETIC FEATURES OF:
CONGENITAL HEART DISEASE

You are invited to take part in a research project. Before you decide whether or not you wish to take part it is important for you to understand why the project is being done and what it will involve if you take part. Please read the following information carefully. Discuss it with your friends and relatives if you wish. Ask us if there is anything you don't understand or if you would like more information. You will be given as much time as you want to make a decision.

WHAT ARE WE AIMING TO DO?

We would like to study the gene(s)/genetic factors involved in **CONGENITAL HEART DISEASE**, and may be the cause the condition in you and/or your family.

WHY ARE WE ASKING FOR YOUR HELP?

We are seeking your help as we need to investigate the genes/genetic factors of many individuals and/or families with exactly the same condition. The cells in your blood carry a complete set of your genetic material (genes), which we can study. We would like to store some of the genetic material in the laboratory because it may take some years and many experiments to understand the genetic factors that cause the condition in you and/or your family.

WHAT WILL YOU HAVE TO DO?

We will ask you and other members of your family to consider giving us a small blood sample **(e.g. 10mls/2 teaspoons)** taken through a needle from a vein in your arm. Usually this only causes brief discomfort and occasionally a small bruise. The sample will be used to obtain the genetic material from the white cells. Sometimes we may use alternative samples to obtain genetic material (e.g. saliva, mouth swab, tissue samples from biopsies, surgery or stored pathology specimens). We will ask some questions about your medical and family history. We may want to ask for your permission to look at your medical records. We will ask for your permission to put information about your family onto a computer database to store the information. We will remove all personal details, such as names and addresses, so your family cannot be recognized from it.

WILL THIS PROJECT BENEFIT YOU OR YOUR FAMILY?

We cannot guarantee to discover anything that will directly benefit you or your family. However, we hope to find the gene(s)/genetic factors causing the condition in you or your family. If this happens then genetic tests may be available for your relatives to find out if they are at risk of developing the condition. In this case we will inform you (unless you indicate you would not wish to be contacted). We hope that in the long term future, this research could lead to improved treatments for the condition.

DO YOU HAVE TO TAKE PART?

Your taking part in this project is voluntary. If you would prefer not to take part you do not have to give a reason. You may also withdraw from the project at any time. This will not affect your or your family's medical care. If this research leads to the development of a new treatment or medical test, you will NOT benefit financially from this. If in the future you lose the ability to understand the research or die, you may still want us to carry on the research (unless your family states otherwise).

WHO WILL KNOW ABOUT YOU TAKING PART?

The information collected about you during the course of the research project will be kept strictly confidential and you will not be identifiable from it. Any results arising from this research work will be kept strictly confidential. If any research results are published in medical articles as a result of this project all personal details will be removed so that your family cannot be recognized from it. With your permission your G.P. will be told that you have agreed to be involved in the project.

WHAT OTHER INFORMATION MAY BE PRODUCED BY THE RESEARCH?

You will NOT be told about any genetic alterations which are identified as a by-product of this research that are not relevant to you or your family's illness. In some cases the genetic information produced by studying your genetic material may be placed in an electronic archive with no connection to your name or other personal identifier. This archive will only be accessible to appropriate doctors and researchers who have been approved by a committee set up to ensure the results are only used to advance scientific and medical understanding. Although there is a theoretical possibility that you could be identified by the deposited information (e.g. if you are entered into another independent genetic study), this is extremely unlikely.

WHO DO YOU CONTACT WITH ANY CONCERNS?

If you have any questions or concerns about this project, please contact Prof. Eamonn Maher on [redacted] **or the Patient Advise & Liaison Service (PALS) on 0121 627 2747**. Alternatively you can write to the following contact addresses:

Prof. Eamonn Maher

[redacted]

Birmingham Women's NHS
NHS Foundation Trust

UNIVERSITY OF BIRMINGHAM

Eamonn Maher

PRINCIPAL INVESTIGATOR:
PROFESSOR EAMONN MAHER

Family no:
Patient Identification Number:

CONSENT FORM

Title of Project: Molecular Pathology of Human Genetic Disease

A PROJECT TO STUDY THE CLINICAL AND GENETIC FEATURES OF:
CONGENITAL HEART DISEASE

Name of Researcher: Prof. Eamonn Maher

Please initial boxes

1. I confirm that I have read and understand the information sheet for the above study and have had the opportunity to ask questions. ☐

2. (a) I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

   (b) I give you permission to continue to use the samples and information collected as part of this research in the event of my losing capacity or dying unless my next of kin requests otherwise. ☐

3. I understand that relevant sections of any of my/ my child's medical notes and data collected during the study, may be reviewed by individuals from the project team, from regulatory authorities or from the NHS trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

4. (a) I agree to allow medical information about my family to be entered on a confidential computer database. ☐

   (b) In addition, in some cases the genetic information produced by studying my/ my child's DNA may be placed in an electronic archive with no connection to my/ my child's name or other personal identifier. I understand that this archive will only be accessible to appropriate doctors and researchers who have been approved by a committee set up to ensure the results are only used to advance scientific and medical understanding. Although there is a theoretical possibility that I/ my child could be identified by the deposited information (e.g. if I/ my child is entered into another independent genetic study), this is extremely unlikely. ☐

   (c) If further medical information is requested by members of the project team, I agree to be contacted again for this purpose. ☐

5. (a) I agree to provide a sample, or for..... (my child) to provide a sample, which will be stored and may be used for genetic research studies appropriate to my/ my family's condition. I understand that any results arising from this research work will be kept strictly confidential. ☐

(b) I understand that the techniques used are NOT suitable for diagnostic testing for known genetic defects, and that I will NOT be told about any genetic alterations which are identified as a by-product of this research that are not relevant to my/ my family's illness. This will not affect my/ my child's access to clinically approved genetic advice and testing through other doctors caring for me in any way. ☐

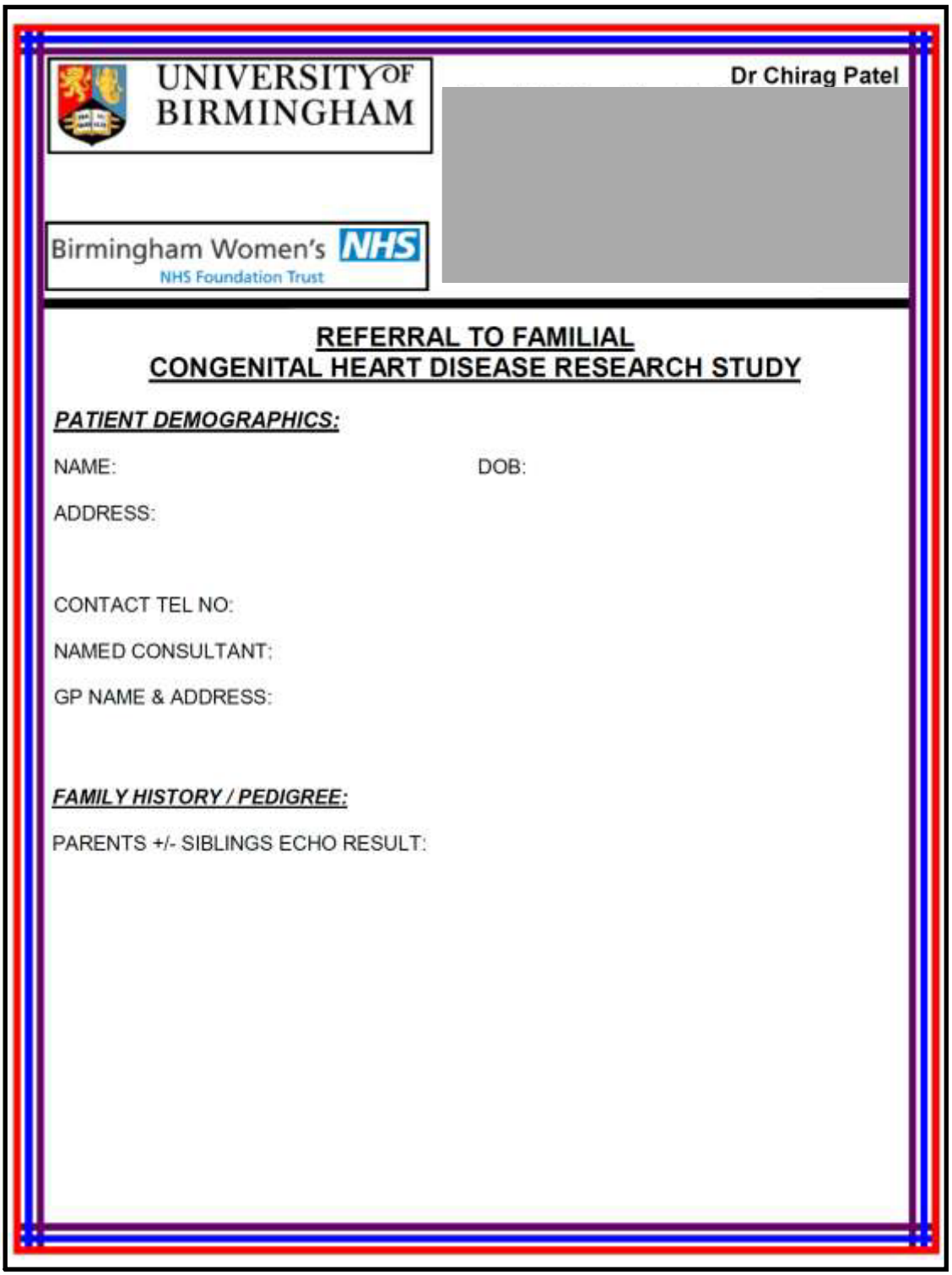
(c) If a genetic test becomes available as a result of medical research on my/ my family's sample(s) I would like to have the opportunity to discuss the implications of these findings with appropriate medical experts. ☐

6. I understand that I/ my child will not benefit financially if this research leads to the development of a new treatment or medical test. ☐

Name of Participant	Date	Signature (if signing on behalf of Participant state your name & relationship to them)
Name of Person taking consent (if different from researcher)	Date	Signature
Researcher	Date	Signature

(1 for participant; 1 for researcher; 1 for hospital notes)

APPENDIX C4:
Proforma used for assessment of families recruited into the project



UNIVERSITY OF BIRMINGHAM

Dr Chirag Patel

[redacted]

Birmingham Women's NHS
NHS Foundation Trust

REFERRAL TO FAMILIAL CONGENITAL HEART DISEASE RESEARCH STUDY

PATIENT DEMOGRAPHICS:

NAME: DOB:

ADDRESS:

CONTACT TEL NO:

NAMED CONSULTANT:

GP NAME & ADDRESS:

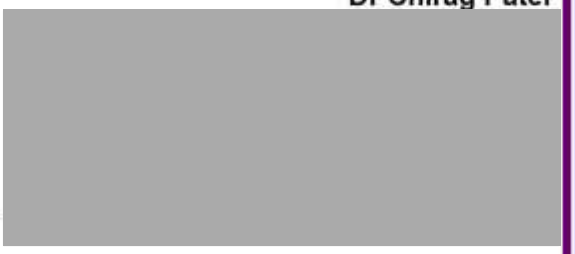
FAMILY HISTORY / PEDIGREE:

PARENTS +/- SIBLINGS ECHO RESULT:

UNIVERSITY OF BIRMINGHAM

Birmingham Women's NHS
NHS Foundation Trust

Dr Chirag Patel



REFERRAL TO FAMILIAL CONGENITAL HEART DISEASE RESEARCH STUDY

CLINICAL DETAILS:

TYPE OF HEART LESION:

AGE OF DIAGNOSIS:

SURGICAL INTERVENTION (& AGE):

OTHER MEDICAL PROBLEMS:	YES / NO
OTHER CONGENITAL ANOMALIES:	YES / NO
GROWTH PROBLEMS:	YES / NO
BEHAVIOUR PROBLEMS:	YES / NO
DEVELOPMENTAL DELAY/ LEARNING DIFFICULTIES:	YES / NO
DYSMORPHIC FEATURES:	YES / NO
NEURO PROBLEMS:	YES / NO

GENETIC INVESTIGATIONS:

KARYOTYPE:	YES / NO	RESULT:
FISH 22q11 DELETION:	YES / NO	RESULT:

COMMENTS / DETAILS:

APPENDIX D: Data collected for each CHD family using the recruitment proforma

Dx= Diagnosis, DD= developmental delay, LD= learning difficulties, M= male, F= female, D= deceased, AN= antenatal,
Y= yes, N= no, NA= not available/applicable, ND= not done, NEG= negative,
RT= right, CL/P= cleft lip & palate, ToF= tracheo-oesophageal fistula, OA= oesophageal atresia, UDT= undescended testis

FAMILY	ETHNIC ORIGIN	PEDIGREE NUMBER	SEX	AGE	TYPE OF CHD	AGE OF DX	AGE OF SURGERY	CONGENITAL ANOMALIES	DD / LD	BEHAVIOUR / NEURO PROBLEMS	GROWTH PROBLEMS	DYSMORPHIC FEATURES	SAMPLE TAKEN	KARYOTYPE	FISH 22q11 DELETION
CHD 1	Pakistani	IV:1	F	12yrs	TOF spectrum (absent PV)	Birth	3mths	N	N	N	N	N	Y	ND	ND
		IV:2	M	10yrs	VSD	6mths	6mths	RT CL/P, ToF, OA, PyS, RT UDT, RT talipes	N	N	N	N	Y	46,XY (normal aCGH)	ND
		IV:3	F	4yrs	TOF spectrum (absent PV)	AN & Birth	5yrs	N	N	N	N	N	Y	ND	ND
		IV:4	M	2yrs	TOF spectrum (absent PV)	AN & Birth	2yrs	N	N	N	N	N	Y	46,XY	NEG
CHD 2	Pakistani	IV:1	F	19yrs	TOF spectrum (PA)	Birth	6days & 1yr	N	N	N	N	N	Y	46,XX	NEG
CHD 3	Pakistani	IV:2	M	3yrs	AS, PPAS	2yrs	ND	N	N	N	N	N	Y	46,XY	NEG
CHD 4	Pakistani	IV:4	M	16yrs	DORV, VSD	Birth	1mth	N	N	N	N	N	Y	ND	ND
		IV:6	M	17yrs	TGA, VSD, Ebstein's anomaly	6wks	9mths	N	N	N	N	N	Y	46,XY	NEG
CHD 5	White British	III:1	F	10yrs	VSD, RV hypoplasia	2wks	9yrs	N	N	N	N	N	Y	46,XX	NEG
		III:2	F	5yrs	ASD, RV hypoplasia	AN & Birth	ND	N	N	N	N	N	Y	ND	ND
CHD 6	White British	III:1	F	24yrs	TOF, RT AA	3mths	2yrs & 3yrs	N	N	N	N	N	Y	46,XX	NEG
		III:2	F	22yrs	TOF	AN & Birth	7mths & 2yrs	N	N	N	N	N	Y	ND	ND
CHD 7	White British	III:3	F	D	TAPVD	13days	NA	NA	NA	NA	NA	NA	NA	NA	NA
		III:4	M	10yrs	TAPVD	10days	11days	N	N	N	N	N	Y	46,XY	NEG
CHD 8	White British	II:3	M	50yrs	TOF	4yrs	4yrs	N	N	N	N	N	Y	ND	ND
		III:1	M	18yrs	TOF	AN & Birth	8yrs	N	N	N	N	N	Y	46,XY	NEG

FAMILY	ETHNIC ORIGIN	PEDIGREE NUMBER	SEX	AGE	TYPE OF CHD	AGE OF DX	AGE OF SURGERY	CONGENITAL ANOMALIES	DD / LD	BEHAVIOUR / NEURO PROBLEMS	GROWTH PROBLEMS	DYSMORPHIC FEATURES	SAMPLE TAKEN	KARYOTYPE	FISH 22q11 DELETION
CHD 9	White British	III:2	M	17yrs	TGA, VSD, PS	Birth	2wks	N	N	N	N	N	Y	46,XY	NEG
		III:3	M	D	TGA, VSD, PS	Birth	1wk	NA	NA	NA	NA	NA	NA	NA	NA
CHD 10	White British	II:2	F	44yrs	TGA, dextrocardia	33yrs	ND	N	N	N	N	N	Y	ND	ND
		III:1	F	13yrs	LT atrial isomerism, RT AA, AVSD, bil. SVCs, PS, RV hypoplasia, dextrocardia	6wks	6mths, 18mths, & 9yrs	abdominal situs inversus	N	N	N	N	Y	46,XX	NEG
CHD 11	White British	III:2	M	5yrs	VSD	4mths	ND	N	N	N	N	N	Y	46,XY	NEG
		III:3	M	2yrs	AS, BAV, CoA	5wks	5wks	N	N	N	N	N	Y	ND	ND
CHD 12	White British	II:4	M	31yrs	TOF, ASD	Birth	16mths & 7yrs	N	N	N	N	N	Y	46,XY	NEG
		III:1	F	5yrs	TOF, ASD	8wks	15mths	N	N	N	N	N	ND	NA	NA
		III:2	M	7mths	ASD, VSD, PDA	2wks	4mths	N	N	N	N	N	ND	NA	NA
CHD 13	White British	III:1	M	11yrs	TGA, VSD, PS	10days	2wks, 1yr, & 6yrs	N	N	N	N	N	Y	46,XY	NEG
		III:4	F	4yrs	TGA	AN & Birth	2wks	N	N	N	N	N	Y	ND	ND
CHD 14	White British	II:3	F	D	CHD	Birth	ND	NA	NA	NA	NA	NA	NA	NA	NA
		II:4	F	D	CHD	Birth	ND	NA	NA	NA	NA	NA	NA	NA	NA
		II:5	M	56yrs	BAV	42yrs	ND	N	N	N	N	N	Y	ND	ND
		III:1	F	21yrs	CoA, BAV, VSD	6wks	4mths & 8mths	N	N	N	N	N	Y	46,XX	NEG
		III:3	M	D	HLHS, CoA, BAV	AN & PM	ND	NA	NA	NA	NA	NA	NA	NA	NA
CHD 15	White British	III:2	F	D	TGA	Birth	NA	NA	NA	NA	NA	NA	NA	NA	NA
		III:3	M	39yrs	TGA, VSD, ASD	3wks	3wks & 4yrs	N	N	N	N	N	Y	46,XY	NEG
CHD 16	White British	III:1	M	24yrs	TOF	Birth	2wks & 2yrs	N	N	N	N	N	Y	46,XY	NEG
		III:2	M	20yrs	VSD, CoA, BAV	Birth	ND	N	N	N	N	N	Y	ND	ND
		III:4	M	15yrs	ASD	Birth	ND	N	N	N	N	N	Y	ND	ND

FAMILY	ETHNIC ORIGIN	PEDIGREE NUMBER	SEX	AGE	TYPE OF CHD	AGE OF DX	AGE OF SURGERY	CONGENITAL ANOMALIES	DD / LD	BEHAVIOUR / NEURO PROBLEMS	GROWTH PROBLEMS	DYSMORPHIC FEATURES	SAMPLE TAKEN	KARYOTYPE	FISH 22q11 DELETION
CHD 17	White British	III:2	M	30yrs	CoA	4days	5days & 4yrs	N	N	N	N	N	Y	46,XY	NEG
		III:3	M	D	HLHS, CoA	Birth	ND	NA	NA	NA	NA	NA	NA	NA	NA
		III:5	M	D	HLHS	PM	ND	NA	NA	NA	NA	NA	NA	NA	NA
		III:6	F	D	HLHS, VSD, CoA	PM	ND	NA	NA	NA	NA	NA	Y	ND	ND
		III:7	F	D	TGA, VSD, ASD	2wks	1mth & 8yrs	NA	NA	NA	NA	NA	NA	NA	NA
CHD 18	Irish	IV:1	M	5mths	HLHS	Birth	1wk & 5mths	N	N	N	N	N	Y	46,XY	NEG
		IV:2	M	NA	Septal defect	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		IV:3	F	NA	Univentricular heart	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		IV:4	F	NA	Septal defect	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		IV:5	M	NA	Septal defect	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
CHD 19	White British	III:1	F	4yrs	HLHS	AN & Birth	4days & 14mths	N	N	N	N	N	ND	NA	NA
		III:4	M	5mths	HLHS	AN & Birth	4days & 5mths	N	N	N	N	N	Y	46,XY	NEG
CHD 20	Indian	III:1	M	5yrs	Tricuspid Atresia, VSD	AN & Birth	3wks, 18mths, & 5yrs	N	N	N	N	N	Y	46,XY	NEG
		III:2	F	9mths	TGA, VSD, RV hypoplasia, AA hypoplasia	AN & Birth	1day & 4mths	N	N	N	N	N	Y	ND	ND
CHD 21	White British	II:4	M	38yrs	CoA, BAV	6wks	18mths	N	N	N	N	N	Y	46,XY	NEG
		III:1	M	8mths	HLHS	AN & Birth	3days & 3mths	N	N	N	N	N	Y	ND	ND
CHD 22	White British	III:1	M	4yrs	HLHS	AN & Birth	1day, 4mths, & 4yrs	N	N	N	N	N	Y	46,XY	NEG
		III:2	M	14mths	VSD	8wks	ND	N	N	N	N	N	Y	ND	ND
CHD 23	Irish	III:1	M	10yrs	AS, sub-AS	6wks	ND	N	N	N	N	N	Y	46,XY	NEG
		III:3	F	7yrs	AS, sub-AS	6wks	ND	N	N	N	N	N	Y	ND	ND
		III:5	M	4yrs	sub-AS	4yrs	ND	N	N	N	N	N	ND	NA	NA

APPENDIX E: Genes in Homozygous region on Chromosome 19 in family CHD1 (GChr37 Build)

Genetic Location (start)	Genetic Location stop	Gene Symbol	Description
16925673		Start of HMZ region	
16830787	16928774	NWD1	NACHT and WD repeat domain containing 1
16940218	16991164	SIN3B	SIN3 homolog B, transcription regulator (yeast)
16987750	16989811	LOC100288045	hypothetical protein LOC100288045
16999826	17002830	F2RL3	coagulation factor II (thrombin) receptor-like 3
17003762	17137625	CPAMD8	C3 and PZP-like, alpha-2-macroglobulin domain containing 8
17160571	17186343	HAUS8	HAUS augmin-like complex, subunit 8
17186591	17324104	MYO9B	myosin IXB
17326155	17330638	USE1	unconventional SNARE in the ER 1 homolog (S. cerevisiae)
17337055	17340028	OCEL1	occludin/ELL domain containing 1
17342694	17346865	LOC100287051	hypothetical protein LOC100287051
17342694	17356151	NR2F6	nuclear receptor subfamily 2, group F, member 6
17360849	17375544	USHBP1	Usher syndrome 1C binding protein 1
17378232	17390162	C19orf62	chromosome 19 open reading frame 62
17392454	17398454	ANKLE1	ankyrin repeat and LEM domain containing 1
17402940	17414282	ABHD8	abhydrolase domain containing 8
17416477	17417652	MRPL34	mitochondrial ribosomal protein L34
17420337	17434107	DDA1	DET1 and DDB1 associated 1
17434034	17445638	ANO8	anoctamin 8
17448356	17453540	GTPBP3	GTP binding protein 3 (mitochondrial)
17462257	17463157	LOC100287090	hypothetical protein LOC100287090
17462264	17488137	PLVAP	plasmalemma vesicle associated protein
17513755	17516384	BST2	bone marrow stromal cell antigen 2
17530912	17536140	FAM125A	family with sequence similarity 125, member A
17547267	17559370	TMEM221	transmembrane protein 221

17566234	17571725	NXNL1	nucleoredoxin-like 1
17581300	17616977	SLC27A1	solute carrier family 27 (fatty acid transporter), member 1
17622432	17632097	PGLS	6-phosphogluconolactonase
17634110	17664648	FAM129C	family with sequence similarity 129, member C
17666511	17693965	GLT25D1	glycosyltransferase 25 domain containing 1
17700379	17700918	RPL21P130	ribosomal protein L21 pseudogene 130
17712137	17799401	UNC13A	unc-13 homolog A (C. elegans)
17830303	17845324	MAP1S	microtubule-associated protein 1S
17862336	17899366	FCHO1	FCH domain only 1
17905919	17924385	B3GNT3	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 3
17927322	17932320	INSL3	insulin-like 3 (Leydig cell)
17935591	17958841	JAK3	Janus kinase 3
17970731	17974124	RPL18A	ribosomal protein L18a
17973397	17973529	SNORA68	small nucleolar RNA, H/ACA box 68
17982782	18005983	SLC5A5	solute carrier family 5 (sodium iodide symporter), member 5
18043824	18054794	CCDC124	coiled-coil domain containing 124
18062111	18109930	KCNN1	potassium intermediate/small conductance calcium-activated channel, subfamily N, member 1
18111944	18124911	ARRDC2	arrestin domain containing 2
18141221	18144330	LOC440514	citrate synthase, mitochondrial-like
18151047	18152054	LOC646666	apolipoprotein A-I binding protein pseudogene
18159439	18159944	RPS18P13	ribosomal protein S18 pseudogene 13
18170371	18197697	IL12RB1	interleukin 12 receptor, beta 1
18208603	18262499	MAST3	microtubule associated serine/threonine kinase 3
18264016	18281343	PIK3R2	phosphoinositide-3-kinase, regulatory subunit 2 (beta)
18284579	18288927	IFI30	interferon, gamma-inducible protein 30
18304040	18307552	MPV17L2	MPV17 mitochondrial membrane protein-like 2
18307611	18314874	RAB3A	RAB3A, member RAS oncogene family
18318771	18359010	PDE4C	phosphodiesterase 4C, cAMP-specific (phosphodiesterase E1 dunce homolog, Drosophila)
18360946	18363235	LOC729966	similar to hCG2001482
18367906	18385319	KIAA1683	KIAA1683

18390563	18392432	JUND	jun D proto-oncogene
18395976	18396355	RPL39P38	ribosomal protein L39 pseudogene 38
18417717	18434001	LSM4	LSM4 homolog, U6 small nuclear RNA associated (S. cerevisiae)
18451408	18480763	PGPEP1	pyroglutamyl-peptidase I
18496968	18499986	GDF15	growth differentiation factor 15
18498887	18516324	LOC100287132	hypothetical protein LOC100287132
18501954	18508415	LRRC25	leucine rich repeat containing 25
18530136	18545459	SSBP4	single stranded DNA binding protein 4
18545625	18548943	ISYNA1	inositol-3-phosphate synthase 1
18553473	18632918	ELL	elongation factor RNA polymerase II
18642568	18654383	FKBP8	FK506 binding protein 8, 38kDa
18650365	18653005	LOC100288150	hypothetical protein LOC100288150
18668604	18680188	C19orf50	chromosome 19 open reading frame 50
18682614	18688270	UBA52	ubiquitin A-52 residue ribosomal protein fusion product 1
18699495	18703147	C19orf60	chromosome 19 open reading frame 60
18704047	18717660	CRLF1	cytokine receptor-like factor 1
18723682	18731849	TMEM59L	transmembrane protein 59-like
18747838	18781302	KLHL26	kelch-like 26 (Drosophila)
18794425	18893143	CRTC1	CREB regulated transcription coactivator 1
18893583	18902114	COMP	cartilage oligomeric matrix protein
18942744	18979039	UPF1	UPF1 regulator of nonsense transcripts homolog (yeast)
18979361	19006953	GDF1	growth differentiation factor 1
18979361	19006953	LASS1	LAG1 homolog, ceramide synthase 1
18979361	19006953	LASS1-GDF1	LAG1 longevity assurance homolog 1 (S. cerevisiae), growth differentiation factor 1 transcription unit
19010323	19030199	COPE	coatomer protein complex, subunit epsilon
19030494	19039436	DDX49	DEAD (Asp-Glu-Ala-Asp) box polypeptide 49
19040010	19052041	HOMER3	homer homolog 3 (Drosophila)
19101697	19144380	SFRS14	splicing factor, arginine/serine-rich 14
19144403	19153687	LOC100288184	hypothetical protein LOC100288184
19144471	19168980	ARMC6	armadillo repeat containing 6

19174808	19223697	SLC25A42	solute carrier family 25, member 42
19230430	19249267	TMEM161A	transmembrane protein 161A
19256376	19281098	MEF2B	myocyte enhancer factor 2B
19256376	19303400	LOC729991-MEF2B	LOC729991-MEF2B readthrough transcript
19287713	19303400	LOC729991	hypothetical protein LOC729991
19303008	19312678	RFXANK	regulatory factor X-associated ankyrin-containing protein
19312224	19314238	NR2C2AP	nuclear receptor 2C2-associated protein
19322782	19363061	NCAN	neurocan
19366450	19373596	HAPLN4	hyaluronan and proteoglycan link protein 4
19375174	19384074	TM6SF2	transmembrane 6 superfamily member 2
19387322	19431307	SF4	splicing factor 4
19431630	19469563	KIAA0892	KIAA0892
19496642	19619741	GATAD2A	GATA zinc finger domain containing 2A
19545872	19545967	MIR640	microRNA 640
19625028	19626469	TSSK6	testis-specific serine kinase 6
19626540	19639073	NDUFA13	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 13
19639720	19648393	YJEFN3	YjeF N-terminal domain containing 3
19649074	19657468	CILP2	cartilage intermediate layer protein 2
19672522	19729439	PBX4	pre-B-cell leukemia homeobox 4
19698415	19699248	PHF5CP	PHD finger protein 5C pseudogene
19734464	19739039	LPAR2	lysophosphatidic acid receptor 2
19740285	19754455	GMIP	GEM interacting protein
19756007	19774503	ATP13A1	ATPase type 13A1
19779663	19791138	ZNF101	zinc finger protein 101
19821281	19843921	ZNF14	zinc finger protein 14
19855363	19856658	LOC100287160	hypothetical protein LOC100287160
19861426	19863215	LOC100130292	similar to sorting nexin 18
19867180	19887222	LOC284440	hypothetical LOC284440
19903520	19932560	ZNF506	zinc finger protein 506
19937823	19938285	LOC100288250	hypothetical LOC100288250

19938863	19948229	ZNF56	zinc finger protein 56
19953229	19960434	LOC100288321	similar to zinc finger protein 253
19976714	20004293	ZNF253	zinc finger protein 253
20011787	20045766	ZNF93	zinc finger protein 93
20047657	20067681	LOC100128975	similar to zinc finger protein 626
20115227	20150277	ZNF682	zinc finger protein 682
20142085	20145144	LOC100288389	hypothetical LOC100288389
20162863	20174094	LOC100129544	similar to BCL2/adenovirus E1B 19kD-interacting protein 3
20188803	20231977	ZNF90	zinc finger protein 90
20235789	20236359	RPS16P10	ribosomal protein S16 pseudogene 10
20244181	20249926	LOC100288458	hypothetical LOC100288458
20278083	20308984	ZNF486	zinc finger protein 486
20349076	20361381	LOC100288487	hypothetical LOC100288487
20368457	20370504	LOC284441	actin-related protein 2 pseudogene
20371834	20379200	LOC100129684	similar to BCL2/adenovirus E1B 19kD-interacting protein 3
20403962	20414581	LOC100129265	similar to Zinc finger protein 66
20493212	20493826	LOC100131306	similar to BCL2/adenovirus E1B 19kD-interacting protein 3
20508134	20522519	LOC100288623	similar to zinc finger protein 208
20574520	20607762	ZNF826	zinc finger protein 826
20579240	20579322	MIR1270	microRNA 1270
20720320	20748620	ZNF737	zinc finger protein 737
20802745	20844402	ZNF626	zinc finger protein 626
20874148	20879252	LOC100131603	similar to zinc finger protein 107
20944462	21094143	ZNF66	zinc finger protein 66
21106080	21133503	ZNF85	zinc finger protein 85
21143864	21146231	KRT18P40	keratin 18 pseudogene 40
21203497	21241653	ZNF430	zinc finger protein 430
21264971	21307897	ZNF714	zinc finger protein 714
21324840	21368805	ZNF431	zinc finger protein 431
21332392	21333261	RPL7AP10	ribosomal protein L7a pseudogene 10

21332392	21333261	RPL7AP68	ribosomal protein L7a pseudogene 68
21416108	21416500	RPL36AP51	ribosomal protein L36a pseudogene 51
21473963	21512212	ZNF708	zinc finger protein 708
21541735	21571384	ZNF738	zinc finger protein 738
21579921	21610297	ZNF493	zinc finger protein 493
21688437	21721079	ZNF429	zinc finger protein 429
21832470	21841410	LOC400682	similar to hCG1776607
21906843	21950430	ZNF100	zinc finger protein 100
21933547	21936240	LOC641367	cyclin Y-like pseudogene
21983282	21983998	LOC100129976	similar to BRI3-binding protein
21987752	22018970	ZNF43	zinc finger protein 43
22148897	22193745	ZNF208	zinc finger protein 208
22178393	22181653	LOC100132200	similar to BCL2/adenovirus E1B 19kD-interacting protein 3
22235266	22273905	ZNF257	zinc finger protein 257
22320778	22322900	PCGF7P	polycomb group ring finger 7 pseudogene
22361903	22379753	ZNF676	zinc finger protein 676
22382880	22386813	LOC100128854	similar to BCL2/adenovirus E1B 19kD-interacting protein 3
22481766	22485151	LOC100288830	hypothetical LOC100288830
22485979	22495580	LOC100130518	similar to Zinc finger protein ENSP00000350085
22496619	22499978	LOC100287226	similar to hCG1773661
22551386	22551896	RPL34P34	ribosomal protein L34 pseudogene 34
22573899	22605148	ZNF98	zinc finger protein 98 (F7175)
22630686	22643433	LOC100288899	hypothetical LOC100288899
22644946	22655682	LOC441843	similar to zinc finger protein 208
22714890	22716293	LOC100128139	hypothetical LOC100128139
22728770	22786235	LOC440518	similar to Golgin subfamily A member 8A/B (Golgi autoantigen golgin-67) (88 kDa Golgi protein) (Gm88 autoantigen)
22786822	22791303	LOC100129536	similar to testis expressed 264
22817239	22850428	ZNF492	zinc finger protein 492
22867595	22869129	ZNF849P	zinc finger protein 849 pseudogene
22882154	22882493	RPL34P33	ribosomal protein L34 pseudogene 33

22939007	22952784	ZNF99	zinc finger protein 99
22954394	22957226	LOC100128849	similar to BCL2/adenovirus E1B 19kD-interacting protein 3
23026880	23041725	LOC646864	similar to hCG36734
23108179	23178656	LOC645118	similar to BCL2/adenovirus E1B 19kD-interacting protein 3
23157936	23185989	ZNF728	zinc finger protein 728
23305988	23307378	LOC100289129	hypothetical LOC100289129
23308643	23310602	LOC126506	sorting nexin 6 pseudogene
23311198	23329586	ZNF730	zinc finger protein 730
23404789	23415422	ZNF724P	zinc finger protein 724 pseudogene
23444850	23445906	LOC100132815	hypothetical protein LOC100132815
23540498	23578269	ZNF91	zinc finger protein 91
23567332	23568095	LOC100128845	similar to Cdc42 effector protein 3
23674864	23676177	ZNF725	zinc finger protein 725
23778968	23779895	LOC100131479	similar to hCG1645503
23780109	23780451	RPS27P29	ribosomal protein S27 pseudogene 29
23835708	23870017	ZNF675	zinc finger protein 675
23921997	23941693	ZNF681	zinc finger protein 681
23945816	24010937	RPSAP58	ribosomal protein SA pseudogene 58
24015084	24015272	ZNF67P	zinc finger protein 67 pseudogene
24060209	24065223	LOC100132393	hypothetical LOC100132393
24092390	24118276	ZNF726	zinc finger protein 726
24225742	24250053	LOC100289269	hypothetical LOC100289269
24269976	24312654	ZNF254	zinc finger protein 254
24344995	24346249	LOC100101266	hepatitis A virus cellular receptor 1 pseudogene
28267033	28294967	LOC100131141	similar to hCG2041551
28281401	28284848	LOC148189	hypothetical LOC148189
28296332	28318506	LOC642290	similar to solute carrier family 25 (mitochondrial carrier; citrate transporter), member 1
28383387	28393759	LOC100132081	similar to ladinin 1
28427672	28475650	LOC100131694	similar to CG14939
29218572	29282883	LOC100129507	similar to mannosidase, alpha, class 1A, member 2

29456040	29460055	LOC148145	hypothetical LOC148145
29698167	29704136	UQCRFS1	ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1
29903721	30016659	LOC284395	hypothetical protein LOC284395
29997873	29999948	LOC100288823	hypothetical protein LOC100288823
30017491	30055097	VSTM2B	V-set and transmembrane domain containing 2B
30097201	30106707	POP4	processing of precursor 4, ribonuclease P/MRP subunit (S. cerevisiae)
30156327	30166384	PLEKHF1	pleckstrin homology domain containing, family F (with FYVE domain) member 1
30189793	30206452	C19orf12	chromosome 19 open reading frame 12
30302901	30315219	CCNE1	cyclin E1
30412089	30412559	LOC126170	similar to TRIM5/CypA fusion protein
30433425	30506616	C19orf2	chromosome 19 open reading frame 2
30447213	30447920	RPL9P32	ribosomal protein L9 pseudogene 32
30528245	30529197	TAF2GL	TAF2G-like gene
30863328	31048965	ZNF536	zinc finger protein 536
31640783	31641313	DKFZp566F0947	hypothetical LOC94023
31765851	31840190	TSHZ3	teashirt zinc finger homeobox 3
32836514	32878573	ZNF507	zinc finger protein 507
32897031	32975236	DPY19L3	dpy-19-like 3 (C. elegans)
33072104	33078331	PDCD5	programmed cell death 5
33087907	33166102	ANKRD27	ankyrin repeat domain 27 (VPS9 domain)
33126984	33127381	RPS12P31	ribosomal protein S12 pseudogene 31
33166313	33169206	RGS9BP	regulator of G protein signaling 9 binding protein
33182653	33200106	LOC100288796	hypothetical protein LOC100288796
33182867	33204702	NUDT19	nudix (nucleoside diphosphate linked moiety X)-type motif 19
33210679	33281714	TDRD12	tudor domain containing 12
33321417	33360683	SLC7A9	solute carrier family 7 (cationic amino acid transporter, y+ system), member 9
33369904	33462869	CCDC123	coiled-coil domain containing 123
33454414	33454771	RPL31P60	ribosomal protein L31 pseudogene 60
33463148	33467974	C19orf40	chromosome 19 open reading frame 40
33469498	33555794	RHPN2	rhophilin, Rho GTPase binding protein 2

33571786	33621318	GPATCH1	G patch domain containing 1
33622998	33666705	WDR88	WD repeat domain 88
33667963	33668036	TRNAT-AGU	transfer RNA threonine (anticodon AGU)
33685599	33699773	LRP3	low density lipoprotein receptor-related protein 3
33699570	33716756	SLC7A10	solute carrier family 7, (neutral amino acid transporter, y+ system) member 10
33790936	33793320	CEBPA	CCAAT/enhancer binding protein (C/EBP), alpha
33793763	33795963	LOC80054	hypothetical LOC80054
33832109	33832890	RPS3AP50	ribosomal protein S3a pseudogene 50
33851203	33852371	AKR1B1P7	aldo-keto reductase family 1, member B1 pseudogene 7
33864413	33870793	LOC100288824	hypothetical protein LOC100288824
33864609	33873592	CEBPG	CCAAT/enhancer binding protein (C/EBP), gamma
33877861	34012801	PEPD	peptidase D
34080073	34080634	RPL21P131	ribosomal protein L21 pseudogene 131
34112861	34264414	CHST8	carbohydrate (N-acetylgalactosamine 4-0) sulfotransferase 8
34287751	34306666	KCTD15	potassium channel tetramerisation domain containing 15
34378039	34399296	LOC100288860	hypothetical protein LOC100288860
34513470	34514355	RPS4P20	ribosomal protein S4X pseudogene 20
34583381	34584271	RPS4P21	ribosomal protein S4X pseudogene 21
34617877	34618328	LOC100128110	similar to coiled-coil-helix-coiled-coil-helix domain containing 2
34663352	34720420	LSM14A	LSM14A, SCD6 homolog A (S. cerevisiae)
34745456	34846471	KIAA0355	KIAA0355
34787071	34787689	RPL29P33	ribosomal protein L29 pseudogene 33
34856069	34891236	GPI	glucose phosphate isomerase
34895303	34917073	PDCD2L	programmed cell death 2-like
34919268	34960798	UBA2	ubiquitin-like modifier activating enzyme 2
34972880	34992085	WTIP	Wilms tumor 1 interacting protein
35024259	35024991	RPS26P55	ribosomal protein S26 pseudogene 55
35032110	35038293	LOC100129800	similar to zinc finger protein 181
35084346	35085490	SCGBL	secretoglobin-like
35125467	35128440	LOC100130632	similar to ZNF304 protein

35136567	35136746	LOC100130342	hypothetical LOC100130342
35168567	35177302	ZNF302	zinc finger protein 302
35224203	35225024	LOC100288954	hypothetical LOC100288954
35225480	35233774	ZNF181	zinc finger protein 181
35248979	35264134	ZNF599	zinc finger protein 599
35353888	35358033	LOC401913	like ABO blood gp (transferase A, a-1-3-N-acetylgalactosaminyltransferase; transferase B, a-1-3-galactosyltransferase)
35417807	35436076	ZNF30	zinc finger protein 30
35447258	35454953	ZNF792	zinc finger protein 792
35491246	35517373	GRAMD1A	GRAM domain containing 1A
35521534	35531353	SCN1B	sodium channel, voltage-gated, type I, beta
35531410	35557475	HPN	hepsin
35549963	35597208	LOC100128675	hypothetical LOC100128675
35596873	35598150	LOC731797	similar to Fc fragment of IgG binding protein
35606732	35615228	FXYD3	FXYD domain containing ion transport regulator 3
35615416	35617602	LOC100288990	hypothetical protein LOC100288990
35615417	35626178	LGI4	leucine-rich repeat LGI family, member 4
35629732	35633954	FXYD1	FXYD domain containing ion transport regulator 1
35634154	35645205	FXYD7	FXYD domain containing ion transport regulator 7
35645627	35660785	FXYD5	FXYD domain containing ion transport regulator 5
35715704	35719628	FAM187B	family with sequence similarity 187, member B
35739559	35758867	LSR	lipolysis stimulated lipoprotein receptor
35759896	35770718	USF2	upstream transcription factor 2, c-fos interacting
35773410	35776046	HAMP	hepcidin antimicrobial peptide
35783038	35804707	MAG	myelin associated glycoprotein
35820079	35838264	CD22	CD22 molecule
35842455	35843357	FFAR1	free fatty acid receptor 1
35849488	35851391	FFAR3	free fatty acid receptor 3
35862262	35863302	GPR42P	G protein-coupled receptor 42 pseudogene
35873074	35874957	EEF1AL5	eukaryotic translation elongation factor 1 alpha-like 5 pseudogene

35898650	35899549	LOC100128682	hypothetical protein LOC100128682
35940617	35941609	FFAR2	free fatty acid receptor 2
35978228	35981356	KRTDAP	keratinocyte differentiation-associated protein
35988122	36004560	DMKN	dermokine
36014271	36019253	SBSN	suprabasin
36017724	36019213	LOC100289024	hypothetical protein LOC100289024
36024314	36036221	GAPDHS	glyceraldehyde-3-phosphate dehydrogenase, spermatogenic
36036545	36038428	TMEM147	transmembrane protein 147
36041095	36054560	ATP4A	ATPase, H+/K+ exchanging, alpha polypeptide
36103646	36116251	HAUS5	HAUS augmin-like complex, subunit 5
36119980	36128587	RBM42	RNA binding motif protein 42
36132647	36135773	ETV2	ets variant 2
36139155	36149684	COX6B1	cytochrome c oxidase subunit Vib polypeptide 1 (ubiquitous)
36157715	36169367	UPK1A	uroplakin 1A
36159135	36164193	LOC100132622	hypothetical LOC100132622
36203830	36207940	ZBTB32	zinc finger and BTB domain containing 32
36208921	36229779	MLL4	myeloid/lymphoid or mixed-lineage leukemia 4
36230151	36233351	TMEM149	transmembrane protein 149
36233428	36236336	U2AF1L4	U2 small nuclear RNA auxiliary factor 1-like 4
36236494	36237903	PSENEN	presenilin enhancer 2 homolog (C. elegans)
36239512	36245420	LIN37	lin-37 homolog (C. elegans)
36245470	36247930	HSPB6	heat shock protein, alpha-crystallin-related, B6
36246281	36247960	LOC100289054	hypothetical protein LOC100289054
36249044	36260077	C19orf55	chromosome 19 open reading frame 55
36266476	36279724	SNX26	sorting nexin 26
36280496	36288739	LOC644050	similar to gene model 1082, (NCBI)
36290892	36304201	PRODH2	proline dehydrogenase (oxidase) 2
36290896	36297504	LOC100289119	hypothetical protein LOC100289119
36316274	36342739	NPHS1	nephrosis 1, congenital, Finnish type (nephrin)
36347810	36358048	KIRREL2	kin of IRRE like 2 (Drosophila)

36359401	36370699	APLP1	amyloid beta (A4) precursor-like protein 1
36379143	36391552	NFKBID	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, delta
36393382	36395173	HCST	hematopoietic cell signal transducer
36395303	36399186	TYROBP	TYRO protein tyrosine kinase binding protein
36428022	36436097	LRFN3	leucine rich repeat and fibronectin type III domain containing 3
36486101	36487214	LOC644096	hypothetical protein LOC644096
36494002	36499672	C19orf46	chromosome 19 open reading frame 46
36500022	36505141	ALKBH6	alkB, alkylation repair homolog 6 (E. coli)
36505563	36523775	CLIP3	CAP-GLY domain containing linker protein 3
36525887	36545664	THAP8	THAP domain containing 8
36540048	36540123	RNY5P10	RNA, Ro-associated Y5 pseudogene 10
36545783	36596012	WDR62	WD repeat domain 62
36602105	36604563	LOC728361	similar to OVO homolog-like 1
36604611	36606206	POLR2I	polymerase (RNA) II (DNA directed) polypeptide I, 14.5kDa
36605888	36616849	TBCB	tubulin folding cofactor B
36630918	36641255	CAPNS1	calpain, small subunit 1
36641824	36643771	COX7A1	cytochrome c oxidase subunit VIIa polypeptide 1 (muscle)
36673187	36705986	ZNF565	zinc finger protein 565
36705504	36729676	ZNF146	zinc finger protein 146
36827162	36870078	ZFP14	zinc finger protein 14 homolog (mouse)
36882861	36909550	ZFP82	zinc finger protein 82 homolog (mouse)
36912439	36913799	LOC644189	similar to peroxisomal long-chain acyl-coA thioesterase
36936021	36980463	ZNF566	zinc finger protein 566
36980535	36981942	LOC728752	similar to chromosome 14 open reading frame 126
36987815	36989118	LOC401914	similar to C-terminal binding protein 2
37001594	37019170	ZNF260	zinc finger protein 260
37034517	37096178	ZNF529	zinc finger protein 529
37096189	37098647	LOC100289147	hypothetical protein LOC100289147
37096221	37119499	ZNF382	zinc finger protein 382
37128283	37157739	ZNF461	zinc finger protein 461

37180302	37212226	ZNF567	zinc finger protein 567
37239025	37253350	ZNF850P	zinc finger protein 850 pseudogene
37264026	37267978	LOC728485	hypothetical protein LOC728485
37308330	37329284	ZNF790	zinc finger protein 790
37341260	37370471	ZNF345	zinc finger protein 345
37382253	37407190	ZNF829	zinc finger protein 829
37392621	37393060	RPL31P61	ribosomal protein L31 pseudogene 61
37407234	37442450	ZNF568	zinc finger protein 568
37464463	37488621	LOC100289218	similar to zinc finger protein 30 homolog
37569382	37620662	ZNF420	zinc finger protein 420
37640990	37663615	ZNF585A	zinc finger protein 585A
37675722	37701451	ZNF585B	zinc finger protein 585B
37717366	37734574	ZNF383	zinc finger protein 383
37825580	37855357	HKR1	GLI-Kruppel family member HKR1
37862059	37883966	ZNF527	zinc finger protein 527
37892306	37894748	LOC100289217	hypothetical protein LOC100289217
37902059	37958339	ZNF569	zinc finger protein 569
37959982	37976242	ZNF570	zinc finger protein 570
37997841	38034239	ZNF793	zinc finger protein 793
38055155	38085673	ZNF571	zinc finger protein 571
38089275	38104996	ZNF540	zinc finger protein 540
38123389	38146313	ZFP30	zinc finger protein 30 homolog (mouse)
38158650	38183216	ZNF781	zinc finger protein 781
38184187	38184465	LOC100129085	similar to HSPC297
38187276	38210691	ZNF607	zinc finger protein 607
38229188	38270200	ZNF573	zinc finger protein 573
38282412	38284715	LOC728533	similar to CG10103
38293667	38294692	LOC100128948	similar to zinc finger protein 345
38307890	38310933	LOC644554	hypothetical LOC644554
38313745	38346630	LOC728853	similar to RIKEN cDNA 4930432E11

38375472	38397317	WDR87	WD repeat domain 87
38397868	38699009	SIPA1L3	signal-induced proliferation-associated 1 like 3
38701649	38720317	DPF1	D4, zinc and double PHD fingers family 1
38741877	38747172	PPP1R14A	protein phosphatase 1, regulatory (inhibitor) subunit 14A
38755202	38783108	SPINT2	serine peptidase inhibitor, Kunitz type, 2
38794200	38806606	YIF1B	Yip1 interacting factor homolog B (S. cerevisiae)
38794804	38795646	C19orf33	chromosome 19 open reading frame 33
38810484	38819654	KCNK6	potassium channel, subfamily K, member 6
38826443	38861589	C19orf15	chromosome 19 open reading frame 15
38865190	38874464	PSMD8	proteasome (prosome, macropain) 26S subunit, non-ATPase, 8
38874992	38878668	GGN	gametogenetin
38877054	38878674	LOC100289362	hypothetical protein LOC100289362
38880939	38886871	SPRED3	sprouty-related, EVH1 domain containing 3
38893775	38899728	FAM98C	family with sequence similarity 98, member C
38899698	38916945	RASGRP4	RAS guanyl releasing protein 4
38924340	39078204	RYR1	ryanodine receptor 1 (skeletal)
39078281	39108643	MAP4K1	mitogen-activated protein kinase kinase kinase kinase 1
39109722	39127595	EIF3K	eukaryotic translation initiation factor 3, subunit K
39138327	39221170	ACTN4	actinin, alpha 4
39220832	39235114	CAPN12	calpain 12
39261608	39264157	LGALS7	lectin, galactoside-binding, soluble, 7
39279850	39282394	LGALS7B	lectin, galactoside-binding, soluble, 7B
39292311	39303740	LGALS4	lectin, galactoside-binding, soluble, 4
39306062	39322497	ECH1	enoyl Coenzyme A hydratase 1, peroxisomal
39306914	39322636	LOC100289396	hypothetical protein LOC100289396
39327028	39342979	HNRNPL	heterogeneous nuclear ribonucleoprotein L
39358470	39368894	RINL	Ras and Rab interactor-like
39369200	39390361	SIRT2	sirtuin (silent mating type information regulation 2 homolog) 2 (S. cerevisiae)
39390615	39399534	NFKBIB	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, beta
39399655	39402798	LOC643669	similar to mCG2980

39405906	39421536	SARS2	seryl-tRNA synthetase 2, mitochondrial
39421348	39423660	MRPS12	mitochondrial ribosomal protein S12
39432042	39466380	FBXO17	F-box protein 17
39514661	39523198	FBXO27	F-box protein 27
39574945	39601478	PAPL	iron/zinc purple acid phosphatase-like protein
39616420	39670046	PAK4	p21 protein (Cdc42/Rac)-activated kinase 4
39687604	39692522	NCCRP1	non-specific cytotoxic cell receptor protein 1 homolog (zebrafish)
39693562	39694906	SYCN	syncollin
39734272	39735611	IL28B	interleukin 28B (interferon, lambda 3)
39745556	39745909	LOC441849	similar to selenoprotein X, 1
39759157	39760732	IL28A	interleukin 28A (interferon, lambda 2)
39786965	39789312	IL29	interleukin 29 (interferon, lambda 1)
39797457	39805976	LRFN1	leucine rich repeat and fibronectin type III domain containing 1
39819010	39826726	GMFG	glia maturation factor, gamma
39832484	39847707	LOC100289492	hypothetical protein LOC100289492
39833108	39875542	SAMD4B	sterile alpha motif domain containing 4B
39876269	39881679	PAF1	Paf1, RNA polymerase II associated factor, homolog (S. cerevisiae)
39881963	39891203	MED29	mediator complex subunit 29
39897487	39900045	ZFP36	zinc finger protein 36, C3H type, homolog (mouse)
39902808	39902900	TRNAI-UAU	transfer RNA isoleucine (anticodon UAU)
39903750	39919055	PLEKHG2	pleckstrin homology domain containing, family G (with RhoGef domain) member 2
39923847	39926618	RPS16	ribosomal protein S16
39930212	39934173	LOC723805	interleukin-like
39936186	39967310	SUPT5H	suppressor of Ty 5 homolog (S. cerevisiae)
39971052	39981528	TIMM50	translocase of inner mitochondrial membrane 50 homolog (S. cerevisiae)
39989557	39999121	DLL3	delta-like 3 (Drosophila)
40005753	40011326	SELV	selenoprotein V
40006086	40009448	LOC100289327	hypothetical protein LOC100289327
40021630	40023494	EID2B	EP300 interacting inhibitor of differentiation 2B
40025103	40025661	LOC100129080	similar to Teratocarcinoma-derived growth factor 1

40029446	40030838	EID2	EP300 interacting inhibitor of differentiation 2
40030894	40045990	LOC390930	similar to lectin, galactoside-binding, soluble, 13
40079595	40079765	RPS29P24	ribosomal protein S29 pseudogene 24
40081621	40081791	RPS29P25	ribosomal protein S29 pseudogene 25
40088475	40088642	RPS29P26	ribosomal protein S29 pseudogene 26
40093169	40098114	LGALS13	lectin, galactoside-binding, soluble, 13
40129325	40133041	LOC100129935	lectin, galactoside-binding, soluble, 14 pseudogene
40135142	40135510	LOC100289395	similar to ribosomal protein S29
40146581	40151287	LOC148003	similar to lectin, galactoside-binding, soluble, 14
40170014	40176361	LOC400696	lectin, galactoside-binding, soluble, 14-like
40188546	40188713	RPS29P27	ribosomal protein S29 pseudogene 27
40194946	40200088	LGALS14	lectin, galactoside-binding, soluble, 14
40221895	40228668	CLC	Charcot-Leyden crystal protein
40267234	40276775	LEUTX	leucine twenty homeobox
40315990	40324841	DYRK1B	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1B
40325093	40337054	FBL	fibrillarin
40353963	40440533	FCGBP	Fc fragment of IgG binding protein
40357458	40364320	LOC100289588	hypothetical protein LOC100289588
40448563	40449659	LOC440525	proline rich 13 pseudogene
40477073	40487353	PSMC4	proteasome (prosome, macropain) 26S subunit, ATPase, 4
40502943	40523514	ZNF546	zinc finger protein 546
40529287	40531409	LOC390933	similar to FLJ36874 protein
40540264	40556239	ZNF780B	zinc finger protein 780B
40575059	40596845	ZNF780A	zinc finger protein 780A
40627230	40628137	LOC100127969	similar to vomeronasal 1 receptor 1
40697651	40721482	MAP3K10	mitogen-activated protein kinase kinase kinase 10
40697821	40721169	LOC100289661	hypothetical protein LOC100289661
40721965	40724306	TTC9B	tetratricopeptide repeat domain 9B
40728115	40732597	CNTD2	cyclin N-terminal domain containing 2
40736224	40791265	AKT2	v-akt murine thymoma viral oncogene homolog 2

40788450	40788548	MIR641	microRNA 641
40826970	40854293	C19orf47	chromosome 19 open reading frame 47
40854332	40884390	PLD3	phospholipase D family, member 3
40885178	40896094	HIPK4	homeodomain interacting protein kinase 4
40899671	40919271	PRX	periaxin
40928408	40931932	SERTAD1	SERTA domain containing 1
40946748	40950282	SERTAD3	SERTA domain containing 3
40953691	40971725	BLVRB	biliverdin reductase B (flavin reductase (NADPH))
40973126	41082365	SPTBN4	spectrin, beta, non-erythrocytic 4
41082757	41097305	SHKBP1	SH3KBP1 binding protein 1
41099072	41135725	LTBP4	latent transforming growth factor beta binding protein 4
41171810	41196556	NUMBL	numb homolog (Drosophila)-like
41197434	41222790	ADCK4	aarF domain containing kinase 4
41223008	41246765	ITPKC	inositol 1,4,5-trisphosphate 3-kinase C
41246761	41255828	C19orf54	chromosome 19 open reading frame 54
41256779	41271294	SNRPA	small nuclear ribonucleoprotein polypeptide A
41281300	41283395	MIA	melanoma inhibitory activity
41284171	41302847	RAB4B	RAB4B, member RAS oncogene family
41305048	41314337	EGLN2	egl nine homolog 2 (C. elegans)
41314400	41317297	CYP2T2P	cytochrome P450, family 2, subfamily T, polypeptide 2 pseudogene
41324580	41332676	CYP2F1P	cytochrome P450, family 2, subfamily F, polypeptide 1 pseudogene
41349443	41356352	CYP2A6	cytochrome P450, family 2, subfamily A, polypeptide 6
41381344	41388657	CYP2A7	cytochrome P450, family 2, subfamily A, polypeptide 7
41397124	41406057	CYP2G1P	cytochrome P450, family 2, subfamily G, polypeptide 1 pseudogene
41414136	41533990	CYP2A7P1	cytochrome P450, family 2, subfamily A, polypeptide 7 pseudogene 1
41430172	41456563	CYP2B7P1	cytochrome P450, family 2, subfamily B, polypeptide 7 pseudogene 1
41497204	41524301	CYP2B6	cytochrome P450, family 2, subfamily B, polypeptide 6
41556357	41568349	CYP2G2P	cytochrome P450, family 2, subfamily G, polypeptide 2 pseudogene
41594368	41602099	CYP2A13	cytochrome P450, family 2, subfamily A, polypeptide 13
41620353	41634281	CYP2F1	cytochrome P450, family 2, subfamily F, polypeptide 1

41640624	41643258	CYP2T3P	cytochrome P450, family 2, subfamily T, polypeptide 3 pseudogene
41675766	41676071	RPL36_7_1657	ribosomal protein L36 pseudogene
41699115	41713444	CYP2S1	cytochrome P450, family 2, subfamily S, polypeptide 1
41725108	41767671	AXL	AXL receptor tyrosine kinase
41748142	41748214	TRNAK38P	transfer RNA lysine 38 (anticodon UUU) pseudogene
41768391	41813811	HNRNPUL1	heterogeneous nuclear ribonucleoprotein U-like 1
41816094	41830788	CCDC97	coiled-coil domain containing 97
41836651	41859816	TGFB1	transforming growth factor, beta 1
41860326	41870037	B9D2	B9 protein domain 2
41869871	41889988	TMEM91	transmembrane protein 91
41892275	41903256	EXOSC5	exosome component 5
41903704	41930910	BCKDHA	branched chain keto acid dehydrogenase E1, alpha polypeptide
41931264	41934635	B3GNT8	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 8
41937224	41945810	ATP5SL	ATP5S-like
41949063	41950670	C19orf69	chromosome 19 open reading frame 69
42011984	42014104	LOC644330	tropomyosin 3 pseudogene
42026588	42028305	PLEKHA3P1	pleckstrin homology domain containing, family A member 3 pseudogene 1
42082531	42093197	CEACAM21	carcinoembryonic antigen-related cell adhesion molecule 21
42107611	42107858	CEACAMP3	carcinoembryonic antigen-related cell adhesion molecule pseudogene 3
42111265	42112093	LOC100286885	hypothetical LOC100286885
42125344	42133442	CEACAM4	carcinoembryonic antigen-related cell adhesion molecule 4
42144529	42145267	LOC100286912	hypothetical LOC100286912
42177235	42192096	CEACAM7	carcinoembryonic antigen-related cell adhesion molecule 7
42212530	42234437	CEACAM5	carcinoembryonic antigen-related cell adhesion molecule 5
42259398	42276113	CEACAM6	carcinoembryonic antigen-related cell adhesion molecule 6 (non-specific cross reacting antigen)
42300534	42315591	CEACAM3	carcinoembryonic antigen-related cell adhesion molecule 3
42333722	42334991	LOC645001	similar to heterogeneous nuclear ribonucleoprotein A1
42341150	42348508	LYPD4	LY6/PLAUR domain containing 4
42349086	42356398	DMRTC2	DMRT-like family C2
42363988	42375484	RPS19	ribosomal protein S19

42381190	42385439	CD79A	CD79a molecule, immunoglobulin-associated alpha
42387267	42411604	ARHGEF1	Rho guanine nucleotide exchange factor (GEF) 1
42412380	42417071	LOC390937	similar to hCG2040171
42460833	42463528	RABAC1	Rab acceptor 1 (prenylated)
42470734	42498382	ATP1A3	ATPase, Na+/K+ transporting, alpha 3 polypeptide
42502473	42569957	GRIK5	glutamate receptor, ionotropic, kainate 5
42580290	42585719	ZNF574	zinc finger protein 574
42592650	42636630	POU2F2	POU class 2 homeobox 2
42702752	42721813	DEDD2	death effector domain containing 2
42724492	42732353	ZNF526	zinc finger protein 526
42734338	42746736	GSK3A	glycogen synthase kinase 3 alpha
42747003	42747458	LOC100132272	hypothetical LOC100132272
42751717	42759309	ERF	Ets2 repressor factor
42788817	42799949	CIC	capicua homolog (Drosophila)
42801185	42806723	PAFAH1B3	platelet-activating factor acetylhydrolase, isoform Ib, subunit 3 (29kDa)
42806284	42814973	PRR19	proline rich 19
42817477	42829214	TMEM145	transmembrane protein 145
42829761	42882921	MEGF8	multiple EGF-like-domains 8
42856948	42880182	LOC100286981	hypothetical protein LOC100286981
42891171	42894444	CNFN	cornifelin
42905666	42931578	LIPE	lipase, hormone-sensitive
42912383	42914974	LOC100289577	hypothetical protein LOC100289577
42932695	42947136	CXCL17	chemokine (C-X-C motif) ligand 17
42989139	42989871	LOC732229	similar to zinc finger, AN1-type domain 5
43011458	43032639	CEACAM1	carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein)
43064619	43064907	CEACAMP2	carcinoembryonic antigen-related cell adhesion molecule pseudogene 2
43084395	43099082	CEACAM8	carcinoembryonic antigen-related cell adhesion molecule 8
43135957	43136496	CEACAMP1	carcinoembryonic antigen-related cell adhesion molecule pseudogene 1
43158203	43167268	CEACAMP5	carcinoembryonic antigen-related cell adhesion molecule pseudogene 5
43174130	43175013	RPS10P28	ribosomal protein S10 pseudogene 28

43225794	43244668	PSG3	pregnancy specific beta-1-glycoprotein 3
43256839	43269831	PSG8	pregnancy specific beta-1-glycoprotein 8
43289863	43290773	CEACAMP6	carcinoembryonic antigen-related cell adhesion molecule pseudogene 6
43325963	43327433	LOC100289650	hypothetical protein LOC100289650
43341149	43359870	PSG10	pregnancy specific beta-1-glycoprotein 10
43371358	43383871	PSG1	pregnancy specific beta-1-glycoprotein 1
43406240	43422043	PSG6	pregnancy specific beta-1-glycoprotein 6
43406244	43530631	PSG11	pregnancy specific beta-1-glycoprotein 11
43428284	43441330	PSG7	pregnancy specific beta-1-glycoprotein 7
43453655	43454567	CEACAMP7	carcinoembryonic antigen-related cell adhesion molecule pseudogene 7
43544526	43545426	CEACAMP8	carcinoembryonic antigen-related cell adhesion molecule pseudogene 8
43568362	43586814	PSG2	pregnancy specific beta-1-glycoprotein 2
43600601	43601512	CEACAMP9	carcinoembryonic antigen-related cell adhesion molecule pseudogene 9
43671895	43690688	PSG5	pregnancy specific beta-1-glycoprotein 5
43696854	43709790	PSG4	pregnancy specific beta-1-glycoprotein 4
43724140	43725051	CEACAMP10	carcinoembryonic antigen-related cell adhesion molecule pseudogene 10
43757435	43773682	PSG9	pregnancy specific beta-1-glycoprotein 9
43787588	43788498	CEACAMP11	carcinoembryonic antigen-related cell adhesion molecule pseudogene 11
43807354	43807650	CEACAMP4	carcinoembryonic antigen-related cell adhesion molecule pseudogene 4
43853208	43853700	PRG1	p53-responsive gene 1
43857825	43867480	CD177	CD177 molecule
43875887	43883275	CD177P	CD177 molecule pseudogene
43892763	43922767	TEX101	testis expressed 101
43964946	43969831	LYPD3	LY6/PLAUR domain containing 3
43979255	44008985	PHLDB3	pleckstrin homology-like domain, family B, member 3
44005790	44009073	LOC100287012	hypothetical protein LOC100287012
44010871	44031396	ETHE1	ethylmalonic encephalopathy 1
44037340	44040290	ZNF575	zinc finger protein 575
44047464	44079730	XRCC1	X-ray repair complementing defective repair in Chinese hamster cells 1
44078790	44079899	LOC100287043	hypothetical protein LOC100287043

44081344	44086271	LOC390940	similar to R28379_1
44088519	44100287	IRGQ	immunity-related GTPase family, Q
44100544	44104587	ZNF576	zinc finger protein 576
44111376	44124014	ZNF428	zinc finger protein 428
44116253	44118650	LOC100170229	hypothetical protein LOC100170229
44126522	44143991	CADM4	cell adhesion molecule 4
44150248	44174502	PLAUR	plasminogen activator, urokinase receptor
44220214	44224173	IRGC	immunity-related GTPase family, cinema
44235301	44259142	C19orf61	chromosome 19 open reading frame 61
44270685	44285409	KCNN4	potassium intermediate/small conductance calcium-activated channel, subfamily N, member 4
44300079	44324808	LYPD5	LY6/PLAUR domain containing 5
44331473	44353050	ZNF283	zinc finger protein 283
44376515	44388116	ZNF404	zinc finger protein 404
44416781	44429558	ZNF45	zinc finger protein 45
44455397	44471752	ZNF221	zinc finger protein 221
44488355	44502477	ZNF155	zinc finger protein 155
44507077	44518072	ZNF230	zinc finger protein 230
44529494	44537263	ZNF222	zinc finger protein 222
44555520	44559299	LOC100286904	hypothetical protein LOC100286904
44556164	44572142	ZNF223	zinc finger protein 223
44576297	44591623	ZNF284	zinc finger protein 284
44598497	44612477	ZNF224	zinc finger protein 224
44617548	44637255	ZNF225	zinc finger protein 225
44645710	44664462	ZNF234	zinc finger protein 234
44669249	44681838	ZNF226	zinc finger protein 226
44716691	44741421	ZNF227	zinc finger protein 227
44764076	44779468	ZNF233	zinc finger protein 233
44790501	44809178	ZNF235	zinc finger protein 235
44794791	44795083	MRPS15P2	mitochondrial ribosomal protein S15 pseudogene 2
44801257	44801511	LOC100131388	similar to NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 3, 9kDa

44830706	44860856	ZFP112	zinc finger protein 112 homolog (mouse)
44875990	44876685	LOC100286969	hypothetical LOC100286969
44889808	44905777	ZNF285A	zinc finger protein 285A
44930426	44952665	ZNF229	zinc finger protein 229
44968405	44977566	ZNF285B	zinc finger protein 285B
44979856	45004574	ZNF180	zinc finger protein 180
45010211	45033548	CEACAM20	carcinoembryonic antigen-related cell adhesion molecule 20
45041045	45060150	FLJ41856	hypothetical LOC388550
45116956	45138792	LOC147710	hypothetical LOC147710
45147098	45169429	PVR	poliovirus receptor
45174724	45187627	CEACAM19	carcinoembryonic antigen-related cell adhesion molecule 19
45202358	45213986	CEACAM16	carcinoembryonic antigen-related cell adhesion molecule 16
45251978	45263301	BCL3	B-cell CLL/lymphoma 3
45281126	45303903	CBLC	Cas-Br-M (murine) ecotropic retroviral transforming sequence c
45312338	45324678	BCAM	basal cell adhesion molecule (Lutheran blood group)
45349393	45392485	PVRL2	poliovirus receptor-related 2 (herpesvirus entry mediator B)
45394477	45406946	TOMM40	translocase of outer mitochondrial membrane 40 homolog (yeast)
45409039	45412650	APOE	apolipoprotein E
45411802	45431701	LOC100129500	hypothetical LOC100129500
45417921	45422606	APOC1	apolipoprotein C-I
45430017	45434450	APOC1P1	apolipoprotein C-I pseudogene 1
45445495	45448751	APOC4	apolipoprotein C-IV
45449243	45452818	APOC2	apolipoprotein C-II
45458638	45496599	CLPTM1	cleft lip and palate associated transmembrane protein 1
45504712	45541452	RELB	v-rel reticuloendotheliosis viral oncogene homolog B
45542298	45574214	SFRS16	splicing factor, arginine/serine-rich 16
45574758	45579688	ZNF296	zinc finger protein 296
45582518	45594782	GEMIN7	gem (nuclear organelle) associated protein 7
45596431	45649730	LRRC68	leucine rich repeat containing 68
45631783	45632340	EIF5AP3	eukaryotic translation initiation factor 5A pseudogene 3

45655161	45661984	NKPD1	NTPase, KAP family P-loop domain containing 1
45666186	45681485	TRAPPC6A	trafficking protein particle complex 6A
45682003	45685058	BLOC1S3	biogenesis of lysosomal organelles complex-1, subunit 3
45715879	45737469	EXOC3L2	exocyst complex component 3-like 2
45754842	45808541	MARK4	MAP/microtubule affinity-regulating kinase 4
45809671	45810899	LOC100287143	hypothetical protein LOC100287143
45809671	45826134	CKM	creatine kinase, muscle
45835887	45836446	RPS16P9	ribosomal protein S16 pseudogene 9
45841038	45842639	LOC100287076	similar to malignancy-associated protein
45843998	45854778	KLC3	kinesin light chain 3
45854649	45873845	ERCC2	excision repair cross-complementing rodent repair deficiency, complementation group 2
45862515		**End of HMZ region**	

APPENDIX F: Analysis of whole exome sequencing data from BGI in family CHD1: Homozygous variants

POLYPHEN: Prediction of mutation pathogenicity, scores range from 0.000 (most probably benign) to 0.999 (most probably damaging)
SIFT: Amino acid substitution is predicted to be damaging if score is <= 0.05, and tolerated if score is > 0.05
Blue = nonsense **Yellow = splice site** **Green = missense**
Orange = genes in candidate region on chr19
Purple = genes with variants predicted to be highly pathogenic or mouse models of CHD

CHR ID	GENE NAME	mRNA POSITION	CODON CHANGE	CODON NO.	RESIDUE CHANGE	FUNCTION OF MUTATION	MOUSE MODELS	POLYPHEN conserved nucleotide	POLYPHEN conserved amino acid	POLYPHEN prediction	SIFT prediction of effect of amino acid substitution	SIFT prediction of pathogenicty
chr3	CCNL1	997	GGA=>TGA	333	G=>*	nonsense	no data					
chr7	RSPH10B2	961	CGA=>TGA	321	R=>*	nonsense	no data					
chr7	NPC1L1	2608	GGA=>TGA	870	G=>*	nonsense	no data					
chr10	UTF1	1020	TGC=>TGA	340	C=>*	nonsense	no data					
chr13	P2RY5	520	GAA=>TAA	174	E=>*	nonsense	no data					
chr14	POTEG	1498	CAG=>TAG	500	Q=>*	nonsense	no data					
chr17	RASD1	355	CAG=>TAG	119	Q=>*	nonsense	no data					
chr19	GMFG	70	CGA=>TGA	24	R=>*	nonsense	no data					
chr2	FAM128B					spliceSite	no data					
chr3	LIMD1					spliceSite	no data					
chr1	PLEKHN1	1642	GCC=>TCC	548	A=>S	missense	no data	weak	weak	0	tolerated	0.67
chr1	WDR8	1148	CCG=>CTG	383	P=>L	missense	no data	high	high	0.999	affect protein function	0
chr1	CHD5	4901	CAG=>CGG	1634	Q=>R	missense	no data	high	high	0.26	tolerated	0.38
chr1	PLEKHG5	3058	GGC=>AGC	1020	G=>S	missense	no data	weak	weak	0	tolerated	1
chr1	KLHL21	43	CCC=>ACC	15	P=>T	missense	no data	high	mod	0.002	tolerated	0.6
chr1	PRAMEF4	457	GTA=>ATA	153	V=>I	missense	no data	weak	weak	0	tolerated	0.5
chr1	PRAMEF10	1402	GTG=>CTG	468	V=>L	missense	no data	weak	weak	0	tolerated	0.5
chr1	PRAMEF10	1012	GAG=>CAG	338	E=>Q	missense	no data	weak	weak	0.914	tolerated	0.32
chr1	PRAMEF7	187	CGC=>TGC	63	R=>C	missense	no data	weak	weak	0.001	tolerated	0.16
chr1	PRAMEF5	578	CGC=>CAC	193	R=>H	missense	no data	weak	weak	0	tolerated	0.48
chr1	SH2D5	953	AGG=>ATG	318	R=>M	missense	no data					
chr1	MUTYH	3	ATG=>ATA	1	M=>I	missense	no data					
chr1	HRNR	3385	GGC=>AGC	1129	G=>S	missense	no data	weak	high	unk	affect protein function	0
chr1	UBE2Q1	223	CTG=>ATG	75	L=>M	missense	no data	high	mod	0.005	affect protein function	0
chr1	FMN2	3302	CCT=>CTT	1101	P=>L	missense	no data	weak	weak	unk	affect protein function	0

chr2	OTOF	2858	ACG=>ATG	953	T=>M	missense	no data	weak	mod	0.153	affect protein function	0.04
chr2	ABCG5	40	GGT=>AGT	14	G=>S	missense	no data	weak	mod	0.011	tolerated	0.65
chr2	FBLN7	116	CAG=>CGG	39	Q=>R	missense	no data					
chr2	IMP4	61	GCG=>ACG	21	A=>T	missense	no data	weak	weak	unk	tolerated	0.38
chr2	MGC50273	254	GCC=>GTC	85	A=>V	missense	no data					
chr3	ITIH1	1927	TTC=>CTC	643	F=>L	missense	no data	weak	high	0.01	affect protein function	0
chr3	CCDC37	506	CGG=>CAG	169	R=>Q	missense	no data	weak	high	0.837	affect protein function	0.03
chr4	MFSD7	920	CGG=>CAG	307	R=>Q	missense	no data	weak	mod	0.91	tolerated	0.11
chr4	IDUA	1210	GAA=>AAA	404	E=>K	missense	lysosomal storage disorder	high	mod	0.548	affect protein function	0.02
chr4	TACC3	1919	AGC=>AAC	640	S=>N	missense	no data	high	high	0.285	affect protein function	0.02
chr5	BTNL8	1429	GCA=>ACA	477	A=>T	missense	no data	weak	weak	0.003	tolerated	1
chr6	PGBD1	360	GAG=>GAT	120	E=>D	missense	no data	weak	mod	0.933	affect protein function	0
chr6	OR2H1	110	CTG=>CCG	37	L=>P	missense	no data	weak	high	0.956	affect protein function	0
chr6	HSPA1L	1007	ATT=>ACT	336	I=>T	missense	no data	high	high	0.622	affect protein function	0
chr6	AIM1	503	AGC=>AAC	168	S=>N	missense	no data	weak	weak	0.956	affect protein function	0
chr6	C6orf170	1085	GCA=>GTA	362	A=>V	missense	no data	high	mod	0.765	affect protein function	0.02
chr7	GTF2IRD1	2362	CCT=>ACT	788	P=>T	missense	embryonic lethal, abnormal angiogenesis, neural tube defects	high	high	0.141	tolerated	0.23
chr7	HIP1	1245	GAT=>GAG	415	D=>E	missense	skeletal malformations, hematopoeitic anomalies	high	weak	0	tolerated	1
chr8	DUB3	1481	CGG=>CCG	494	R=>P	missense	no data	weak	weak	0	tolerated	0.21
chr8	DUB3	1319	CCC=>CTT	440	P=>L	missense	no data	weak	weak	0	tolerated	1
chr8	DUB3	1268	CGC=>CAC	423	R=>H	missense	no data	weak	weak	0	tolerated	0.76
chr8	DUB3	1070	TGT=>TCT	357	C=>S	missense	no data	weak	weak	0.003	tolerated	0.85
chr8	DUB3	994	GAC=>AAC	332	D=>N	missense	no data	weak	weak	0	tolerated	1
chr8	ZNF517	1427	CGC=>CAC	476	R=>H	missense	no data	weak	high	0	affect protein function	0
chr9	DMRTA1	578	GCG=>GTG	193	A=>V	missense	no data	weak	mod	0	tolerated	0.72
chr9	TESK1	1606	CGG=>TGG	536	R=>W	missense	no data	weak	high	0.984	affect protein function	0

chr9	ANKRD20A3	2017	GCC=>ACC	673	A=>T	missense	no data	weak	weak	0	tolerated	1
chr10	RET	1118	GCG=>GTG	373	A=>V	missense	lethal, neurogenic defects, abnormal GU system	weak	mod	0	tolerated	0.5
chr10	SYNPO2L	871	CCC=>TCC	291	P=>S	missense	no data	weak	mod	0.007	affect protein function	0
chr11	IGHMBP2	2042	GAG=>GGG	681	E=>G	missense	no data	weak	mod	0.068	tolerated	0.06
chr11	WNT11	519	GAT=>GAA	173	D=>E	missense	lethal, ? heart devl in humans	high	high	0.999	affect protein function	0
chr12	NOC4L	164	CGT=>CAT	55	R=>H	missense	no data	mod	mod	0.99	tolerated	0.13
chr15	GOLGA6	481	CGC=>TGC	161	R=>C	missense	no data					
chr15	STARD5	16	GCA=>ACA	6	A=>T	missense	no data	weak	mod	0.103	tolerated	0.94
chr15	MESP2	554	GGG=>GCG	185	G=>A	missense	perinatal lethal, absent segmental somites	weak	weak	0.075	affect protein function	0
chr15	MESP2	556	CAG=>GAG	186	Q=>E	missense	perinatal lethal, absent segmental somites	weak	weak	0.011	affect protein function	0
chr16	RHBDF1	560	GCC=>GTC	187	A=>V	missense	no data	weak	mod	0.004	tolerated	0.07
chr16	TPSAB1	8	AAT=>AGT	3	N=>S	missense	no data	weak	weak	unk	tolerated	0.75
chr16	TPSAB1	9	AAT=>AGC	3	N=>S	missense	no data	weak	weak	unk	tolerated	0.75
chr16	TELO2	533	GAG=>GTG	178	E=>V	missense	embryonic lethal	weak	weak	0	tolerated	0.5
chr16	NDUFB10	424	GCC=>ACC	142	A=>T	missense	no data	high	mod	0.062	tolerated	0.05
chr16	PKD1	361	AAC=>GAC	121	N=>D	missense	renal cysts	high	high	0.98	tolerated	0.8
chr16	BTBD12	2323	CAC=>TAC	775	H=>Y	missense	no data					
chr16	ATMIN	503	AGT=>AAT	168	S=>N	missense	no data	high	mod	0.001	tolerated	0.33
chr16	CDH15	1304	CAC=>CGC	435	H=>R	missense	no data	weak	weak	0	tolerated	1
chr16	CPNE7	1133	GGC=>GAC	378	G=>D	missense	no data	high	high	0.985	affect protein function	0
chr17	DVL2	152	GCG=>GTG	51	A=>V	missense	CHD OFT+++	high	mod	0.07	tolerated	0.31
chr17	LGALS9B	550	GTG=>ATG	184	V=>M	missense	no data	weak	weak	0.013	affect protein function	0.04
chr17	GHDC	1561	GCC=>ACC	521	A=>T	missense	no data	weak	high	0.871	tolerated	0.06
chr17	FMNL1	2555	AAG=>AGG	852	K=>R	missense	no data	high	high	unk	affect protein function	0
chr17	ICAM2	7	TCT=>CCT	3	S=>P	missense	no data	weak	weak	0	tolerated	0.39
chr17	CBX2	428	CCG=>CTG	143	P=>L	missense	skeletal malformations	high	high	0.999	affect protein function	0
chr19	DAZAP1	574	GAC=>AAC	192	D=>N	missense	no data	high	high	unk	affect protein function	0

chr19	REXO1	2623	GCC=>ACC	875	A=>T	missense	no data	weak	weak	0	tolerated	0.55
chr19	FAM108A1	557	GGC=>GCC	186	G=>A	missense	no data	high	high	0.176	affect protein function	0.04
chr19	CSNK1G2	248	GCC=>GTC	83	A=>V	missense	no data	high	high	0.102	affect protein function	0
chr19	DOT1L	1357	TAC=>CAC	453	Y=>H	missense	embryonic lethal, poor yolk sac angiogenesis	high	mod	0.006	tolerated	0.14
chr19	INSR	2941	GCT=>TCT	981	A=>S	missense	no data	high	high	0.208	affect protein function	0
chr19	FBN3	6952	GCT=>ACT	2318	A=>T	missense	no data	weak	mod	0.002	tolerated	0.29
chr19	DNMT1	2575	GAC=>TAC	859	D=>Y	missense	embryonic lethal, lack of imprinting at several loci	high	mod	0.995	affect protein function	0
chr19	CDC37	275	CGC=>CAC	92	R=>H	missense	no data	high	weak	0.941	tolerated	0.1
chr19	RGL3	1402	CGC=>TGC	468	R=>C	missense	no data	weak	mod	0.999	tolerated	0.08
chr19	OR7A10	806	CAC=>CCC	269	H=>P	missense	no data	weak	weak	0.986	tolerated	0.28
chr19	CYP4F22	484	GCC=>ACC	162	A=>T	missense	no data	high	high	0.174	affect protein function	0
chr19	KIAA1683	394	GCC=>ACC	132	A=>T	missense	no CHD	high	high	0.445	affect protein function	0
chr19	NDUFA13	200	GTG=>GCG	67	V=>A	missense	embryonic lethal, absent organogenesis	weak	weak	0.151	affect protein function	0.02
chr19	SPTBN4	4166	CGC=>CAC	1389	R=>H	missense	neurological phenotype	high	high	0.037	affect protein function	0.02
chr19	ZFP28	107	CCA=>CGA	36	P=>R	missense	no data	weak	mod	0.048	tolerated	0.11
chr19	ZIM2	902	CCT=>CTT	301	P=>L	missense	no data	weak	high	unk	affect protein function	0
chr19	ZNF530	1079	TTT=>TGT	360	F=>C	missense	no data	high	high	1	affect protein function	0
chr20	THBD	203	GCC=>GTC	68	A=>V	missense	no data	weak	high	0.883	tolerated	0.09
chr20	LAMA5	9826	GTA=>ATA	3276	V=>I	missense	no data	weak	mod	0.563	tolerated	0.08
chr20	LAMA5	1487	TGC=>TAC	496	C=>Y	missense	embryonic lethal with brain, limb, kidney defects	high	mod	1	affect protein function	0
chr20	LIME1	845	AGC=>AAC	282	S=>N	missense	no data	weak	mod	0.683	tolerated	0.29
chr21	ERG	739	GGC=>TGC	247	G=>C	missense	no data	high	weak	0.137	tolerated	0.19
chr22	RHBDD3	160	CTG=>GTG	54	L=>V	missense	no data	high	high	0.094	affect protein function	0.04
chr22	SFI1	2678	CGC=>CAC	893	R=>H	missense	no data	weak	mod	0.001	tolerated	0.49
chr22	SGSM3	1187	GTT=>GCT	396	V=>A	missense	no data					

APPENDIX G: Analysis of whole exome sequencing data from WTSI

POLYPHEN: Prediction of mutation pathogenicity, scores range from 0.000 (most probably benign) to 0.999 (most probably damaging)
SIFT: Amino acid substitution is predicted to be damaging if score is <= 0.05, and tolerated if score is > 0.05
Blue = nonsense **Yellow = splice site** **Green = missense** **Purple = genes for ?further investigation**

CHD1: Homozygous variants

CHR ID	NUCLEOTIDE POSITION	GENE NAME	REF SEQUENCE	ALTERNATE SEQUENCE	AMINO ACID NO.	RESIDUE CHANGE	FUNCTION OF VARIANT	POLYPHEN	SIFT
19	39826105	GMFG	G	A	24	R/*	STOP_GAINED	.	.
10	46321904	AGAP4	C	T	260	R/H	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0.02)
10	51464656	AGAP7	G	C	600	D/E	NON_SYNONYMOUS_CODING	probably_damaging(0.995)	tolerated(0.06)
10	51465650	AGAP7	C	T	269	R/Q	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0.03)
9	117110115	AKNA	T	G	556	H/P	NON_SYNONYMOUS_CODING	probably_damaging(0.979)	tolerated(0.07)
9	66553727	ENSG00000170161	T	C	60	V/A	NON_SYNONYMOUS_CODING	benign(0)	.
15	22709060	ENSG00000185182	C	G	147	E/Q	NON_SYNONYMOUS_CODING	benign(0.313)	tolerated(0.08)
15	30696517	ENSG00000186399	C	T	501	R/H	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	deleterious(0.02)
15	30696515	ENSG00000186399	C	A	502	A/S	NON_SYNONYMOUS_CODING	possibly_damaging(0.862)	tolerated(0.17)
15	82932518	ENSG00000215749	T	C	502	E/G	NON_SYNONYMOUS_CODING	benign(0.003)	tolerated(0.24)
15	82932561	ENSG00000215749	T	C	488	S/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.737)	deleterious(0)
15	82932833	ENSG00000215749	C	A	467	V/F	NON_SYNONYMOUS_CODING	possibly_damaging(0.951)	deleterious(0)
1	146215113	ENSG00000232637	G	A	84	H/Y	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.05)
9	46386902	ENSG00000237198	G	A	34	T/I	NON_SYNONYMOUS_CODING	probably_damaging(0.998)	deleterious(0)
10	51859751	FAM21A	C	A	521	S/Y	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.13)
10	47915891	FAM21B	C	A	270	S/Y	NON_SYNONYMOUS_CODING	benign(0.004)	tolerated(0.13)
9	97080827	FAM22F	C	G	565	G/R	NON_SYNONYMOUS_CODING	benign(0.424)	tolerated(0.07)
15	82637079	GOLGA6L10	C	T	336	R/Q	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(1)
7	74212311	GTF2IRD2	G	T	61	H/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
6	31324931	HLA-B	A	C	3	L/R	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
6	31324547	HLA-B	G	C	88	N/K	NON_SYNONYMOUS_CODING	benign(0.192)	deleterious(0.02)
13	53217540	HNRNPA1L2	T	C	305	Y/H	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0)
19	18177378	IL12RB1	C	T	486	R/Q	NON_SYNONYMOUS_CODING	benign(0.009)	tolerated(0.35)
19	18377956	KIAA1683	C	T	131	A/T	NON_SYNONYMOUS_CODING	possibly_damaging(0.93)	deleterious(0)

19	54725835	LILRB3	G	C	175	R/G	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.32)
19	7051376	MBD3L2	G	A	124	G/S	NON_SYNONYMOUS_CODING	possibly_damaging(0.6)	tolerated(0.19)
1	3431168	MEGF6	G	A	267	R/C	NON_SYNONYMOUS_CODING	probably_damaging(0.972)	tolerated(0.14)
15	90294245	MESP1	C	G	73	G/A	NON_SYNONYMOUS_CODING	probably_damaging(0.959)	tolerated(0.43)
7	100637605	MUC12	A	C	1254	K/T	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195512186	MUC4	T	C	2089	I/V	NON_SYNONYMOUS_CODING	benign(0.028)	.
3	195510515	MUC4	C	T	2646	A/T	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510341	MUC4	A	G	2704	S/P	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510310	MUC4	T	G	2714	Y/S	NON_SYNONYMOUS_CODING	unknown(0)	.
1	145327548	NBPF10	A	G	1369	N/D	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
1	148023040	NBPF14	G	C	162	S/C	NON_SYNONYMOUS_CODING	possibly_damaging(0.939)	deleterious(0.02)
1	148741720	NBPF16	T	C	69	F/S	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
19	19626998	NDUFA13	T	C	67	V/A	NON_SYNONYMOUS_CODING	possibly_damaging(0.547)	tolerated(0.1)
1	248737259	OR2T34	C	G	267	R/P	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
1	2434460	PLCH2	G	T	1038	V/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.67)
2	130832358	POTEF	T	C	896	H/R	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
14	20010235	POTEM	A	G	393	V/A	NON_SYNONYMOUS_CODING	benign(0.006)	tolerated(1)
1	147955256	PPIAL4A	A	G	30	L/P	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
1	13695787	PRAMEF19	C	G	324	G/A	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
1	13695685	PRAMEF19	G	A	358	S/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.68)
1	13696022	PRAMEF19	A	G	246	W/R	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.25)
22	18901004	PRODH	C	T	166	R/Q	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.6)
19	43528921	PSG11	C	G	118	G/R	NON_SYNONYMOUS_CODING	probably_damaging(0.982)	deleterious(0.04)
19	41056166	SPTBN4	C	T	212	P/L	NON_SYNONYMOUS_CODING	possibly_damaging(0.533)	deleterious(0.01)
7	72436652	TRIM74	A	G	13	W/R	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.29)
19	35434387	ZNF30	G	A	174	E/K	NON_SYNONYMOUS_CODING	benign(0.43)	tolerated(0.05)
19	13318672	CACNA1A	CCTGCTG	C	2330-2331	QQ/-	NON_SYNONYMOUS_CODING	.	.
2	187559029	FAM171B	A	ACAG	43-44	-/Q	NON_SYNONYMOUS_CODING	.	.
15	90320134	MESP2	AGGGCAGGGGCAG	A	183-186	GQGQ/-	NON_SYNONYMOUS_CODING	.	.
18	30352057	ENSG00000228835	GCGCCGGCC	G	119-121	.	FRAMESHIFT_CODING	.	.
21	43298954	PRDM15	C	CG	88	.	FRAMESHIFT_CODING	.	.
7	100371476	ZAN	T	TG	1923	.	FRAMESHIFT_CODING	.	.
3	75790810	ZNF717	G	GT	38	.	FRAMESHIFT_CODING	.	.
1	1022582	C1orf159	C	A	119	A/S	SPLICE_SITE:NON_SYNONYMOUS_CODING	possibly_damaging(0.606)	tolerated(0.07)
3	75790427	ZNF717	C	T	43	A/T	SPLICE_SITE:NON_SYNONYMOUS_CODING	possibly_damaging(0.85)	tolerated(0.4)

CHD1: Compound heterozygous variants

CHR ID	NUCLEOTIDE POSITION	GENE NAME	REF SEQUENCE	ALTERNATE SEQUENCE	AMINO ACID NO.	RESIDUE CHANGE	FUNCTION OF VARIANT	POLYPHEN	SIFT
2	148676128	ACVR2A	T	A	310	L/Q	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.29)
2	148676144	ACVR2A	A	C	315	K/N	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
10	127724751	ADAM12	T	G	834	R/S	NON_SYNONYMOUS_CODING	benign(0.028)	tolerated(0.42)
10	127708330	ADAM12	C	T	868	R/Q	NON_SYNONYMOUS_CODING	benign(0.142)	tolerated(0.53)
10	127787029	ADAM12	T	C	321	I/V	NON_SYNONYMOUS_CODING	probably_damaging(0.992)	tolerated(0.43)
10	4879755	AKR1E2	C	A	188	F/L	NON_SYNONYMOUS_CODING	benign(0.055)	deleterious(0.03)
10	4875551	AKR1E2	A	C	73	T/P	NON_SYNONYMOUS_CODING	benign(0.426)	deleterious(0)
4	114275541	ANK2	G	A	1890	E/K	NON_SYNONYMOUS_CODING	probably_damaging(0.991)	.
4	114275548	ANK2	A	C	1892	H/P	NON_SYNONYMOUS_CODING	probably_damaging(0.996)	.
5	94030836	ANKRD32	C	A	999	T/N	NON_SYNONYMOUS_CODING	benign(0.144)	deleterious(0.01)
5	94030832	ANKRD32	G	A	998	E/K	NON_SYNONYMOUS_CODING	probably_damaging(0.955)	deleterious(0)
2	97808524	ANKRD36	A	G	285	I/V	NON_SYNONYMOUS_CODING	benign(0.296)	tolerated(1)
2	97845632	ANKRD36	T	C	566	I/T	NON_SYNONYMOUS_CODING	possibly_damaging(0.767)	tolerated(0.23)
2	97808515	ANKRD36	G	A	282	E/K	NON_SYNONYMOUS_CODING	probably_damaging(0.959)	tolerated(1)
2	97808510	ANKRD36	G	A	280	G/D	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.85)
12	101368637	ANO4	A	C	191	Y/S	NON_SYNONYMOUS_CODING	probably_damaging(0.983)	tolerated(0.39)
12	101368625	ANO4	G	A	187	R/K	SPLICE_SITE:NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(1)
22	39498015	APOBEC3H	C	G	171	R/G	NON_SYNONYMOUS_CODING	benign(0.057)	deleterious(0.01)
22	39498005	APOBEC3H	T	A	167	D/E	NON_SYNONYMOUS_CODING	benign(0.18)	tolerated(0.35)
22	39498012	APOBEC3H	A	G	170	S/G	NON_SYNONYMOUS_CODING	probably_damaging(0.984)	deleterious(0)
22	39498014	APOBEC3H	T	G	170	S/R	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
12	14613915	ATF7IP	T	A	882	V/E	NON_SYNONYMOUS_CODING	possibly_damaging(0.837)	deleterious(0.01)
12	14613917	ATF7IP	C	A	883	Q/K	NON_SYNONYMOUS_CODING	possibly_damaging(0.882)	deleterious(0.04)
11	64666179	ATG2A	A	G	1338	S/P	NON_SYNONYMOUS_CODING	benign(0.403)	deleterious(0.02)
11	64666176	ATG2A	A	G	1339	S/P	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
11	64666182	ATG2A	C	G	1337	A/P	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
11	108175543	ATM	A	G	1880	T/A	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.7)
11	108175544	ATM	C	G	1880	T/R	NON_SYNONYMOUS_CODING	benign(0.001)	deleterious(0.03)
16	84495404	ATP2C2	A	C	705	T/P	NON_SYNONYMOUS_CODING	benign(0.269)	deleterious(0.01)
16	84456242	ATP2C2	T	C	110	V/A	NON_SYNONYMOUS_CODING	possibly_damaging(0.949)	deleterious(0)
20	3565431	ATRN	C	A	956	Q/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.72)
20	3565356	ATRN	T	G	931	C/G	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
10	121436478	BAG3	A	G	471	E/G	NON_SYNONYMOUS_CODING	benign(0.145)	deleterious(0.04)
10	121436483	BAG3	C	G	473	R/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.939)	deleterious(0)
1	147092352	BCL9	G	T	797	L/F	NON_SYNONYMOUS_CODING	possibly_damaging(0.91)	tolerated(0.13)
1	147092353	BCL9	C	T	798	R/W	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.19)
5	137503739	BRD8	G	A	224	S/F	NON_SYNONYMOUS_CODING	probably_damaging(0.996)	deleterious(0.01)
5	137503737	BRD8	C	A	225	G/C	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.06)
10	128192708	C10orf90	T	G	307	H/P	NON_SYNONYMOUS_CODING	benign(0.002)	deleterious(0.01)

10	128192711	C10orf90	A	C	306	V/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.819)	deleterious(0)
11	76256857	C11orf30	T	C	779	I/T	NON_SYNONYMOUS_CODING	possibly_damaging(0.689)	deleterious(0)
11	76171068	C11orf30	A	C	184	R/S	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.33)
12	112622338	C12orf51	T	G	3056	T/P	NON_SYNONYMOUS_CODING	benign(0.187)	.
12	112688167	C12orf51	A	C	822	V/G	NON_SYNONYMOUS_CODING	probably_damaging(0.979)	.
12	112688164	C12orf51	T	C	823	E/G	NON_SYNONYMOUS_CODING	probably_damaging(0.98)	.
20	18433273	C20orf12	T	G	179	T/P	NON_SYNONYMOUS_CODING	benign(0.005)	tolerated(0.15)
20	18433277	C20orf12	T	G	25	H/P	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0)
6	48036327	C6orf138	A	C	5	V/G	NON_SYNONYMOUS_CODING	benign(0.235)	deleterious(0.02)
6	48036051	C6orf138	A	C	97	V/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.493)	deleterious(0)
11	76795985	CAPN5	G	A	18	R/Q	NON_SYNONYMOUS_CODING	benign(0.056)	tolerated(0.41)
11	76796027	CAPN5	T	C	32	F/S	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
17	77807917	CBX4	T	TGCCGCC	508	A/AAA	NON_SYNONYMOUS_CODING	.	.
17	77808584	CBX4	T	C	286	E/G	NON_SYNONYMOUS_CODING	benign(0.372)	deleterious(0.01)
17	77808570	CBX4	A	G	291	S/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.727)	deleterious(0.04)
17	77808572	CBX4	C	G	290	R/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.94)	tolerated(0.14)
2	219893096	CCDC108	T	G	494	T/P	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0.01)
2	219883763	CCDC108	C	A	113	L/F	NON_SYNONYMOUS_CODING	unknown(0)	.
13	37012869	CCNA1	A	G	252	E/G	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
13	37012872	CCNA1	T	G	253	V/G	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
17	45234417	CDC27	A	G	235	I/T	NON_SYNONYMOUS_CODING	benign(0.004)	tolerated(0.19)
17	45234303	CDC27	G	C	273	A/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.783)	tolerated(0.07)
17	45214528	CDC27	A	T	635	Y/N	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
9	131186822	CERCAM	A	C	154	Y/S	NON_SYNONYMOUS_CODING	benign(0.099)	tolerated(0.13)
9	131185203	CERCAM	C	G	7	A/G	NON_SYNONYMOUS_CODING	benign(0.22)	tolerated(0.38)
17	7796794	CHD3	A	C	293	I/L	NON_SYNONYMOUS_CODING	benign(0)	.
17	7796803	CHD3	T	C	296	S/P	NON_SYNONYMOUS_CODING	benign(0.001)	.
16	839702	CHTF18	C	T	198	A/V	NON_SYNONYMOUS_CODING	benign(0.004)	deleterious(0.03)
16	846830	CHTF18	A	G	857	E/G	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0.04)
19	42795827	CIC	G	C	939	G/A	NON_SYNONYMOUS_CODING	benign(0.106)	tolerated(0.44)
19	42795826	CIC	G	C	939	G/R	NON_SYNONYMOUS_CODING	possibly_damaging(0.765)	deleterious(0.03)
16	10995954	CIITA	A	C	181	T/P	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.05)
16	11001733	CIITA	G	A	747	R/Q	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.33)
12	122812699	CLIP1	A	T	46	M/K	NON_SYNONYMOUS_CODING	benign(0.03)	tolerated(0.05)
12	122812697	CLIP1	C	T	47	E/K	NON_SYNONYMOUS_CODING	possibly_damaging(0.918)	deleterious(0.03)
12	122845694	CLIP1	C	A	273	A/S	NON_SYNONYMOUS_CODING	probably_damaging(0.998)	tolerated(0.06)
12	122812709	CLIP1	C	T	43	E/K	SPLICE_SITE:NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.33)
22	19213150	CLTCL1	C	A	652	V/F	NON_SYNONYMOUS_CODING	benign(0.419)	deleterious(0)
22	19213138	CLTCL1	C	A	656	G/C	NON_SYNONYMOUS_CODING	probably_damaging(0.996)	deleterious(0)
7	147926842	CNTNAP2	A	C	177	T/P	NON_SYNONYMOUS_CODING	probably_damaging(0.961)	deleterious(0.04)
7	147183121	CNTNAP2	A	C	589	T/P	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
6	56044835	COL21A1	C	A	61	V/F	NON_SYNONYMOUS_CODING	possibly_damaging(0.775)	deleterious(0.02)
6	56044818	COL21A1	G	T	66	N/K	NON_SYNONYMOUS_CODING	probably_damaging(0.982)	tolerated(0.26)
2	9599739	CPSF3	T	A	593	I/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.51)

2	9599742	CPSF3	G	A	594	R/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.93)
1	207726161	CR1	G	T	572	Q/H	NON_SYNONYMOUS_CODING	possibly_damaging(0.475)	tolerated(0.57)
1	207755348	CR1	C	T	1318	H/Y	SPLICE_SITE:NON_SYNONYMOUS_CODING	possibly_damaging(0.611)	tolerated(0.09)
8	68062165	CSPP1	G	A	703	S/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.64)
8	68062160	CSPP1	T	A	701	N/K	NON_SYNONYMOUS_CODING	benign(0.153)	tolerated(0.98)
6	132030560	CTAGE9	G	A	533	T/M	NON_SYNONYMOUS_CODING	benign(0.062)	tolerated(0.07)
6	132030966	CTAGE9	G	C	398	L/V	NON_SYNONYMOUS_CODING	probably_damaging(0.973)	deleterious(0.01)
10	126678128	CTBP2	T	A	433	T/S	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
10	126678251	CTBP2	C	A	392	V/L	NON_SYNONYMOUS_CODING	benign(0.103)	tolerated(0.12)
10	126714884	CTBP2	G	A	482	T/M	NON_SYNONYMOUS_CODING	probably_damaging(0.998)	deleterious(0.02)
16	50828259	CYLD	C	A	866	T/K	NON_SYNONYMOUS_CODING	possibly_damaging(0.847)	deleterious(0)
16	50828255	CYLD	G	A	865	E/K	NON_SYNONYMOUS_CODING	possibly_damaging(0.945)	deleterious(0.01)
15	51766636	DMXL2	G	A	1736	A/V	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(1)
15	51766637	DMXL2	C	A	1736	A/S	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.57)
5	13754366	DNAH5	C	T	3501	E/K	NON_SYNONYMOUS_CODING	benign(0.004)	tolerated(0.31)
5	13754379	DNAH5	C	A	3496	L/F	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
6	56422200	DST	C	A	4318	V/L	NON_SYNONYMOUS_CODING	benign(0.221)	.
6	56422262	DST	T	C	4297	E/G	NON_SYNONYMOUS_CODING	probably_damaging(1)	.
2	55071278	EML6	C	A	314	D/E	NON_SYNONYMOUS_CODING	probably_damaging(0.994)	tolerated(0.8)
2	54952332	EML6	T	G	45	V/G	NON_SYNONYMOUS_CODING	probably_damaging(0.997)	deleterious(0)
16	15457629	ENSG00000183793	T	A	314	T/S	NON_SYNONYMOUS_CODING	benign(0.158)	tolerated(0.82)
16	15457628	ENSG00000183793	G	T	314	T/N	NON_SYNONYMOUS_CODING	benign(0.348)	tolerated(0.39)
7	100549540	ENSG00000228273	A	C	41	S/R	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.86)
7	100550327	ENSG00000228273	C	T	303	T/I	NON_SYNONYMOUS_CODING	possibly_damaging(0.45)	tolerated(0.43)
18	11619510	ENSG00000257513	A	C	351	*/E	STOP_LOST	.	.
18	11619549	ENSG00000257513	T	G	338	K/Q	NON_SYNONYMOUS_CODING	probably_damaging(0.998)	deleterious(0.03)
12	132445256	EP400	A	C	31	H/P	NON_SYNONYMOUS_CODING	unknown(0)	.
12	132445273	EP400	T	C	37	S/P	NON_SYNONYMOUS_CODING	unknown(0)	.
12	132534944	EP400	C	G	2425	T/R	NON_SYNONYMOUS_CODING	unknown(0)	.
1	16474927	EPHA2	T	G	257	I/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.08)
1	16474932	EPHA2	A	C	255	V/G	NON_SYNONYMOUS_CODING	benign(0.006)	deleterious(0)
4	5733216	EVC	A	C	150	H/P	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.07)
4	5754777	EVC	G	A	438	R/Q	SPLICE_SITE:NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.31)
2	62066569	FAM161A	C	T	524	E/K	NON_SYNONYMOUS_CODING	probably_damaging(0.977)	tolerated(0.41)
2	62066566	FAM161A	G	T	525	Q/K	NON_SYNONYMOUS_CODING	probably_damaging(0.98)	tolerated(0.24)
1	179783089	FAM163A	T	G	90	V/G	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.15)
1	179783095	FAM163A	C	G	92	A/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.481)	tolerated(0.1)
15	41043735	FAM82A2	C	G	138	R/P	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.15)
15	41029923	FAM82A2	A	C	376	V/G	SPLICE_SITE:NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
5	150925475	FAT2	G	C	1738	A/G	NON_SYNONYMOUS_CODING	benign(0.287)	tolerated(0.44)
5	150948147	FAT2	T	G	116	T/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.491)	tolerated(0.14)
11	92616488	FAT3	T	C	624	L/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.542)	tolerated(0.12)
11	92616485	FAT3	A	C	623	N/T	NON_SYNONYMOUS_CODING	probably_damaging(0.978)	tolerated(1)
7	100187851	FBXO24	A	C	65	T/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.462)	deleterious(0)

7	100192051	FBXO24	A	C	266	D/A	NON_SYNONYMOUS_CODING	possibly_damaging(0.907)	deleterious(0.03)
3	99568956	FILIP1L	A	C	98	S/A	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.27)
3	99567984	FILIP1L	A	G	422	S/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.462)	tolerated(0.13)
9	117995	FOXD4	G	C	42	A/G	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.06)
9	117411	FOXD4	C	G	237	A/P	NON_SYNONYMOUS_CODING	probably_damaging(0.981)	deleterious(0.02)
20	29628328	FRG1B	C	G	110	I/M	NON_SYNONYMOUS_CODING	benign(0.191)	deleterious(0.03)
20	29632672	FRG1B	C	T	163	L/F	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0.01)
4	48559517	FRYL	G	T	231	Q/K	NON_SYNONYMOUS_CODING	benign(0.042)	deleterious(0.02)
4	48559529	FRYL	A	C	227	W/G	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0.01)
14	39601195	GEMIN2	G	T	223	E/*	STOP_GAINED	.	.
14	39601192	GEMIN2	C	T	222	L/F	NON_SYNONYMOUS_CODING	probably_damaging(0.958)	deleterious(0.01)
15	34678933	GOLGA8A	C	T	60	A/T	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.63)
15	34673973	GOLGA8A	C	T	513	R/Q	NON_SYNONYMOUS_CODING	benign(0.003)	tolerated(1)
1	37319269	GRIK3	G	A	387	R/W	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
1	37319270	GRIK3	C	A	386	L/F	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.75)
14	106757786	IGHV2-26	G	A	74	S/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.6)
14	106757799	IGHV2-26	C	A	70	A/S	NON_SYNONYMOUS_CODING	probably_damaging(0.955)	tolerated(0.17)
14	106573354	IGHV3-11	A	G	77	I/T	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
14	106573357	IGHV3-11	G	T	76	T/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.62)
14	106573358	IGHV3-11	T	A	76	T/S	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
14	106573378	IGHV3-11	T	G	69	Y/S	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.39)
14	106573352	IGHV3-11	A	T	78	Y/N	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.23)
14	106866406	IGHV3-38	A	C	116	Y/*	STOP_GAINED	.	.
14	106866414	IGHV3-38	C	T	114	A/T	NON_SYNONYMOUS_CODING	benign(0.008)	tolerated(0.09)
14	106866408	IGHV3-38	A	T	116	Y/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.462)	tolerated(0.06)
14	107113742	IGHV3-64	C	T	118	R/K	NON_SYNONYMOUS_CODING	benign(0.002)	tolerated(0.18)
14	107113745	IGHV3-64	G	A	117	A/V	NON_SYNONYMOUS_CODING	benign(0.003)	tolerated(0.06)
14	107034847	IGHV5-51	C	T	78	R/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.37)
14	107034854	IGHV5-51	C	A	76	D/Y	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.24)
14	107034869	IGHV5-51	A	C	71	Y/D	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.32)
14	107034873	IGHV5-51	G	C	69	I/M	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.22)
14	107034874	IGHV5-51	A	C	69	I/S	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.41)
14	107034846	IGHV5-51	T	G	78	R/S	NON_SYNONYMOUS_CODING	benign(0.136)	tolerated(0.44)
2	90139156	IGKV1D-16	C	T	18	P/S	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.46)
2	90139485	IGKV1D-16	G	A	86	G/S	NON_SYNONYMOUS_CODING	benign(0.226)	deleterious(0.02)
1	117122285	IGSF3	G	GTCC	1041	D/ED	NON_SYNONYMOUS_CODING	.	.
1	117159050	IGSF3	C	T	25	V/I	NON_SYNONYMOUS_CODING	benign(0.003)	tolerated(0.68)
14	105174274	INF2	T	G	25	V/G	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.09)
14	105180963	INF2	C	T	1155	A/V	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0)
3	4725969	ITPR1	G	A	1159	G/E	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.22)
3	4725973	ITPR1	T	A	1160	N/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.94)
3	4725976	ITPR1	C	A	1161	N/K	NON_SYNONYMOUS_CODING	benign(0.004)	tolerated(0.94)
16	15696466	KIAA0430	A	G	1296	F/S	NON_SYNONYMOUS_CODING	benign(0.014)	.
16	15696475	KIAA0430	G	A	1293	P/L	NON_SYNONYMOUS_CODING	benign(0.014)	.

16	15696509	KIAA0430	G	A	1282	P/S	NON_SYNONYMOUS_CODING	benign(0.014)	.
1	209796950	LAMB3	A	C	753	V/G	NON_SYNONYMOUS_CODING	benign(0.001)	deleterious(0.04)
1	209791293	LAMB3	T	G	1004	T/P	NON_SYNONYMOUS_CODING	probably_damaging(0.954)	deleterious(0.01)
1	152777625	LCE1C	A	C	110	S/R	NON_SYNONYMOUS_CODING	unknown(0)	.
1	152777627	LCE1C	T	C	110	S/G	NON_SYNONYMOUS_CODING	unknown(0)	.
13	76287349	LMO7	A	T	86	D/V	NON_SYNONYMOUS_CODING	probably_damaging(0.991)	deleterious(0.01)
13	76287354	LMO7	C	G	88	R/G	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0.01)
12	57592066	LRP1	C	T	3137	T/M	NON_SYNONYMOUS_CODING	probably_damaging(0.976)	tolerated(0.06)
12	57550649	LRP1	A	C	503	T/P	NON_SYNONYMOUS_CODING	probably_damaging(0.991)	tolerated(0.2)
2	141259399	LRP1B	A	C	2841	C/G	NON_SYNONYMOUS_CODING	probably_damaging(0.995)	deleterious(0)
2	141625828	LRP1B	G	A	1330	L/F	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0.01)
17	62892071	LRRC37A3	G	C	435	H/Q	NON_SYNONYMOUS_CODING	benign(0.003)	tolerated(0.66)
17	62892031	LRRC37A3	C	T	449	V/I	NON_SYNONYMOUS_CODING	benign(0.048)	tolerated(0.14)
7	20198700	MACC1	G	T	428	D/E	NON_SYNONYMOUS_CODING	benign(0.003)	tolerated(0.57)
7	20198702	MACC1	C	T	428	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.604)	tolerated(0.18)
1	117944954	MAN1A2	T	A	150	I/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.96)
1	117944964	MAN1A2	C	A	153	N/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.96)
5	71490953	MAP1B	G	A	608	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.792)	tolerated(0.27)
5	71490955	MAP1B	C	A	608	D/E	NON_SYNONYMOUS_CODING	possibly_damaging(0.792)	tolerated(0.67)
3	152174078	MBNL1	G	C	277	R/P	NON_SYNONYMOUS_CODING	benign(0.013)	tolerated(0.33)
3	152174076	MBNL1	A	C	272	T/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.512)	tolerated(0.11)
9	123476542	MEGF9	ACGGCGG	A	22-24	AAV/V	NON_SYNONYMOUS_CODING	.	.
9	123384940	MEGF9	C	G	336	L/F	NON_SYNONYMOUS_CODING	probably_damaging(0.961)	deleterious(0.05)
11	12225829	MICAL2	G	T	99	L/F	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0.03)
11	12225830	MICAL2	C	T	100	R/C	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
11	12315177	MICALCL	A	G	67	R/G	NON_SYNONYMOUS_CODING	benign(0.09)	deleterious(0)
11	12315180	MICALCL	C	G	68	R/G	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
10	129901721	MKI67	G	T	2794	Q/K	NON_SYNONYMOUS_CODING	possibly_damaging(0.806)	tolerated(0.13)
10	129901702	MKI67	G	T	2800	A/D	NON_SYNONYMOUS_CODING	probably_damaging(0.996)	deleterious(0)
7	131128354	MKLN1	G	T	430	A/S	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.74)
7	131128350	MKLN1	G	T	428	L/F	NON_SYNONYMOUS_CODING	possibly_damaging(0.69)	tolerated(0.08)
8	17611546	MTUS1	T	C	591	T/A	NON_SYNONYMOUS_CODING	probably_damaging(0.996)	tolerated(0.08)
8	17601155	MTUS1	G	C	749	R/G	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
7	100646199	MUC12	T	C	4119	F/L	NON_SYNONYMOUS_CODING	possibly_damaging(0.662)	.
7	100645825	MUC12	C	A	3994	T/K	NON_SYNONYMOUS_CODING	probably_damaging(0.992)	.
7	100636722	MUC12	G	C	960	V/L	NON_SYNONYMOUS_CODING	unknown(0)	.
7	100637008	MUC12	C	A	1055	A/E	NON_SYNONYMOUS_CODING	unknown(0)	.
7	100637547	MUC12	G	A	1235	A/T	NON_SYNONYMOUS_CODING	unknown(0)	.
7	100637839	MUC12	C	G	1332	P/R	NON_SYNONYMOUS_CODING	unknown(0)	.
19	9002623	MUC16	C	T	39	R/H	NON_SYNONYMOUS_CODING	benign(0.011)	tolerated(0.24)
19	9002636	MUC16	C	T	35	V/I	NON_SYNONYMOUS_CODING	benign(0.051)	tolerated(0.37)
19	9002612	MUC16	T	G	43	K/Q	NON_SYNONYMOUS_CODING	benign(0.055)	tolerated(0.23)
19	8961997	MUC16	C	A	1101	L/F	NON_SYNONYMOUS_CODING	possibly_damaging(0.448)	deleterious(0)
19	9002659	MUC16	C	T	27	G/E	NON_SYNONYMOUS_CODING	possibly_damaging(0.582)	tolerated(0.19)

19	9002597	MUC16	C	T	48	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.866)	deleterious(0)
19	9002660	MUC16	C	T	27	G/R	NON_SYNONYMOUS_CODING	probably_damaging(0.998)	tolerated(0.5)
3	195510636	MUC4	C	G	2605	Q/H	NON_SYNONYMOUS_CODING	benign(0.391)	.
3	195507062	MUC4	C	T	3797	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195510649	MUC4	G	A	2601	A/V	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195510622	MUC4	G	T	2610	P/H	NON_SYNONYMOUS_CODING	probably_damaging(0.991)	.
3	195510350	MUC4	C	G	2701	D/H	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510396	MUC4	A	C	2685	H/Q	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510415	MUC4	G	T	2679	S/Y	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510509	MUC4	A	C	2648	S/A	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510553	MUC4	G	A	2633	A/V	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510611	MUC4	G	T	2614	L/I	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510613	MUC4	C	T	2613	S/N	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510614	MUC4	T	C	2613	S/G	NON_SYNONYMOUS_CODING	unknown(0)	.
11	1018095	MUC6	G	A	1569	P/L	NON_SYNONYMOUS_CODING	benign(0.014)	deleterious(0.01)
11	1017466	MUC6	T	C	1779	R/G	NON_SYNONYMOUS_CODING	benign(0.142)	tolerated(0.57)
11	1017693	MUC6	T	A	1703	H/L	NON_SYNONYMOUS_CODING	probably_damaging(0.978)	tolerated(0.82)
11	1018042	MUC6	A	G	1587	S/P	NON_SYNONYMOUS_CODING	probably_damaging(0.979)	deleterious(0.01)
11	1017655	MUC6	G	A	1716	P/S	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.14)
11	1016554	MUC6	C	T	2083	A/T	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.6)
11	1016565	MUC6	C	T	2079	S/N	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.09)
11	1017596	MUC6	T	G	1735	Q/H	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.18)
11	1018207	MUC6	T	C	1532	T/A	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.28)
11	1018552	MUC6	G	A	1417	P/S	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.73)
11	1018561	MUC6	T	C	1414	S/G	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.07)
11	1019464	MUC6	G	T	1281	Q/K	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(1)
11	1024858	MUC6	T	G	1071	T/P	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.14)
11	69062844	MYEOV	C	T	8	T/I	NON_SYNONYMOUS_CODING	unknown(0)	.
11	69062879	MYEOV	C	T	20	R/C	NON_SYNONYMOUS_CODING	unknown(0)	.
1	145367800	NBPF10	G	A	586	D/N	NON_SYNONYMOUS_CODING	benign(0.009)	deleterious(0.03)
1	145302775	NBPF10	T	G	330	Y/D	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(1)
9	139413211	NOTCH1	T	G	311	T/P	NON_SYNONYMOUS_CODING	benign(0.004)	deleterious(0)
9	139413097	NOTCH1	T	G	349	T/P	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
17	9143043	NTN1	A	G	525	T/A	NON_SYNONYMOUS_CODING	benign(0.004)	tolerated(0.47)
17	9143044	NTN1	C	G	525	T/R	NON_SYNONYMOUS_CODING	benign(0.004)	tolerated(0.43)
1	228430947	OBSCN	C	G	998	A/G	NON_SYNONYMOUS_CODING	benign(0.003)	.
1	228434292	OBSCN	C	G	1274	A/G	NON_SYNONYMOUS_CODING	benign(0.006)	.
1	228464168	OBSCN	G	C	2080	V/L	NON_SYNONYMOUS_CODING	probably_damaging(0.998)	.
11	35016535	PDHX	C	T	129	A/V	NON_SYNONYMOUS_CODING	probably_damaging(0.989)	deleterious(0)
11	35016479	PDHX	G	A	110	M/I	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
7	95216415	PDK4	C	A	298	L/F	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
7	95222084	PDK4	T	TGG	136-137	.	FRAMESHIFT_CODING	.	.
8	110463218	PKHD1L1	C	A	2064	Q/K	NON_SYNONYMOUS_CODING	benign(0.023)	tolerated(1)
8	110455187	PKHD1L1	A	T	1469	Y/F	NON_SYNONYMOUS_CODING	possibly_damaging(0.799)	tolerated(0.33)

8	110455184	PKHD1L1	C	T	1468	S/F	NON_SYNONYMOUS_CODING	probably_damaging(0.982)	deleterious(0.01)
2	159519467	PKP4	T	G	757	V/G	NON_SYNONYMOUS_CODING	benign(0.389)	deleterious(0)
2	159519458	PKP4	A	G	754	E/G	NON_SYNONYMOUS_CODING	probably_damaging(0.991)	tolerated(0.08)
19	4511679	PLIN4	C	T	751	A/T	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.26)
19	4512933	PLIN4	C	A	333	V/L	NON_SYNONYMOUS_CODING	benign(0.08)	tolerated(0.08)
1	208252715	PLXNA2	A	C	826	C/G	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
1	208272311	PLXNA2	G	C	537	C/W	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
1	208272313	PLXNA2	A	C	537	C/G	SPLICE_SITE:NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
2	131414338	POTEJ	G	A	669	A/T	NON_SYNONYMOUS_CODING	benign(0.011)	deleterious(0.01)
2	131414878	POTEJ	A	C	849	T/P	NON_SYNONYMOUS_CODING	probably_damaging(0.988)	tolerated(0.31)
19	42596242	POU2F2	G	A	399	Q/*	STOP_GAINED	.	.
19	42596244	POU2F2	C	A	443	L/F	NON_SYNONYMOUS_CODING	possibly_damaging(0.45)	deleterious(0)
1	12887211	PRAMEF11	T	A	257	I/F	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.59)
1	12887253	PRAMEF11	T	C	243	M/V	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.36)
1	12887121	PRAMEF11	C	T	287	D/N	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.64)
1	12837570	PRAMEF12	G	A	427	G/E	NON_SYNONYMOUS_CODING	benign(0.004)	tolerated(1)
1	12837548	PRAMEF12	G	A	420	A/T	NON_SYNONYMOUS_CODING	benign(0.007)	tolerated(0.62)
1	12837564	PRAMEF12	G	T	425	C/F	NON_SYNONYMOUS_CODING	possibly_damaging(0.446)	tolerated(0.7)
1	12837669	PRAMEF12	G	T	460	G/V	NON_SYNONYMOUS_CODING	probably_damaging(0.988)	deleterious(0)
6	106546559	PRDM1	A	G	4	E/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.878)	.
6	106546576	PRDM1	A	C	10	T/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.901)	deleterious(0)
1	186276217	PRG4	C	A	322	P/T	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0.03)
1	186276229	PRG4	A	T	326	T/S	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.68)
1	186276358	PRG4	C	A	369	P/T	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0.01)
1	186276370	PRG4	G	T	373	A/S	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.38)
19	40901647	PRX	A	G	871	V/A	NON_SYNONYMOUS_CODING	benign(0.019)	tolerated(0.26)
19	40901648	PRX	C	G	871	V/L	NON_SYNONYMOUS_CODING	benign(0.404)	tolerated(0.24)
10	27702786	PTCHD3	A	C	132	W/G	NON_SYNONYMOUS_CODING	benign(0.003)	tolerated(0.36)
10	27700857	PTCHD3	A	C	364	V/G	SPLICE_SITE:NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
1	117487406	PTGFRN	C	T	34	S/L	NON_SYNONYMOUS_CODING	benign(0.031)	tolerated(0.17)
1	117492036	PTGFRN	A	C	211	D/A	NON_SYNONYMOUS_CODING	benign(0.044)	tolerated(0.15)
1	117491894	PTGFRN	C	G	164	R/G	NON_SYNONYMOUS_CODING	benign(0.282)	tolerated(0.67)
3	191179127	PYDC2	T	C	59	F/S	NON_SYNONYMOUS_CODING	benign(0.29)	deleterious(0)
3	191179129	PYDC2	A	C	60	T/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.817)	tolerated(0.06)
2	87205020	RGPD1	G	T	810	R/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
2	87205031	RGPD1	T	C	814	C/R	NON_SYNONYMOUS_CODING	possibly_damaging(0.85)	tolerated(0.78)
13	25416217	RNF17	G	A	841	D/N	NON_SYNONYMOUS_CODING	benign(0.019)	tolerated(0.12)
13	25451175	RNF17	C	G	1542	R/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.751)	tolerated(0.07)
7	4259872	SDK1	C	A	139	Q/K	NON_SYNONYMOUS_CODING	benign(0.253)	tolerated(0.31)
7	4247821	SDK1	A	C	17	T/P	NON_SYNONYMOUS_CODING	probably_damaging(0.975)	tolerated(0.11)
18	12948136	SEH1L	A	C	6	S/R	NON_SYNONYMOUS_CODING	benign(0.004)	tolerated(0.55)
18	12948134	SEH1L	G	C	5	R/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.778)	deleterious(0.02)
17	48626182	SPATA20	A	C	109	T/P	NON_SYNONYMOUS_CODING	probably_damaging(0.957)	deleterious(0.02)
17	48628510	SPATA20	C	A	496	P/H	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)

1	16258610	SPEN	C	G	1959	R/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.536)	tolerated(0.05)
1	16203071	SPEN	G	A	260	S/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.767)	tolerated(0.1)
1	16262471	SPEN	A	C	3246	T/P	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0.02)
16	2812468	SRRM2	T	G	647	S/A	NON_SYNONYMOUS_CODING	unknown(0)	.
16	2816107	SRRM2	C	G	1112	P/A	NON_SYNONYMOUS_CODING	unknown(0)	.
6	36489582	STK38	G	A	107	Q/*	STOP_GAINED	.	.
6	36489585	STK38	C	A	106	V/F	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
6	33410691	SYNGAP1	T	C	729	S/P	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0.01)
6	33410683	SYNGAP1	G	C	726	R/P	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0.01)
1	152084213	TCHH	C	G	494	E/Q	NON_SYNONYMOUS_CODING	unknown(0)	.
1	152084216	TCHH	C	G	493	E/Q	NON_SYNONYMOUS_CODING	unknown(0)	.
7	100228635	TFR2	T	C	297	E/G	NON_SYNONYMOUS_CODING	benign(0.002)	.
7	100228633	TFR2	G	C	168	A/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.849)	.
12	83251115	TMTC2	T	G	137	V/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.926)	deleterious(0)
12	83251120	TMTC2	C	G	139	R/G	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
9	117838699	TNC	G	T	944	Q/K	NON_SYNONYMOUS_CODING	benign(0.002)	tolerated(0.91)
9	117849170	TNC	G	T	280	N/K	NON_SYNONYMOUS_CODING	benign(0.003)	deleterious(0.05)
9	117838707	TNC	G	T	941	P/Q	NON_SYNONYMOUS_CODING	probably_damaging(0.994)	deleterious(0.01)
2	218713552	TNS1	C	T	438	R/Q	NON_SYNONYMOUS_CODING	benign(0.404)	tolerated(0.3)
2	218696268	TNS1	G	A	970	R/W	SPLICE_SITE:NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
7	142498738	TRBC2	C	G	5	N/K	NON_SYNONYMOUS_CODING	benign(0.002)	tolerated(0.34)
7	142498735	TRBC2	A	C	4	K/N	NON_SYNONYMOUS_CODING	benign(0.111)	tolerated(0.08)
7	142008750	TRBV3-1	A	T	75	I/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.24)
7	142008783	TRBV3-1	A	G	86	K/E	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142008675	TRBV3-1	A	G	50	T/A	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(1)
7	142008727	TRBV3-1	G	T	67	S/I	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.4)
7	142008785	TRBV3-1	A	C	86	K/N	NON_SYNONYMOUS_CODING	benign(0.001)	deleterious(0.02)
7	142008718	TRBV3-1	T	C	64	I/T	NON_SYNONYMOUS_CODING	benign(0.002)	deleterious(0.01)
7	142008672	TRBV3-1	G	A	49	D/N	NON_SYNONYMOUS_CODING	benign(0.006)	tolerated(0.61)
7	142008745	TRBV3-1	T	C	73	L/P	NON_SYNONYMOUS_CODING	probably_damaging(0.993)	tolerated(0.27)
7	142045680	TRBV4-2	T	C	70	F/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.67)
7	142045693	TRBV4-2	C	T	74	T/I	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.36)
7	142180566	TRBV6-5	A	T	98	L/Q	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.22)
7	142180567	TRBV6-5	G	C	98	L/V	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.06)
7	142180573	TRBV6-5	T	C	96	R/G	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.12)
7	142180590	TRBV6-5	G	T	90	T/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142180593	TRBV6-5	G	T	89	T/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.44)
7	142180596	TRBV6-5	G	A	88	S/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.31)
7	142180618	TRBV6-5	T	C	81	N/D	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.78)
7	142180633	TRBV6-5	G	T	76	Q/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142180635	TRBV6-5	T	G	75	D/A	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.22)
7	142180641	TRBV6-5	A	G	73	I/T	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.91)
7	142180704	TRBV6-5	G	T	52	S/Y	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142180737	TRBV6-5	T	A	41	Q/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.16)

7	142180578	TRBV6-5	G	A	94	P/L	NON_SYNONYMOUS_CODING	benign(0.002)	tolerated(0.17)
7	142180588	TRBV6-5	C	G	91	E/Q	NON_SYNONYMOUS_CODING	benign(0.005)	tolerated(0.2)
7	142180647	TRBV6-5	G	T	71	A/D	NON_SYNONYMOUS_CODING	benign(0.08)	tolerated(0.18)
7	142247230	TRBV7-3	C	T	76	D/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.38)
7	142247236	TRBV7-3	C	G	74	A/P	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.31)
7	142247271	TRBV7-3	G	A	62	P/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142247380	TRBV7-3	T	A	26	T/S	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.87)
7	142099548	TRBV7-8	A	G	85	F/S	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142099581	TRBV7-8	A	G	74	L/P	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.35)
7	142099512	TRBV7-8	T	G	97	K/T	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.14)
22	38120532	TRIOBP	G	A	657	D/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.28)
22	38120563	TRIOBP	T	C	667	I/T	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.75)
22	38120575	TRIOBP	A	G	671	N/S	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.9)
22	38120573	TRIOBP	G	C	670	E/D	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.75)
22	38120524	TRIOBP	G	T	654	R/L	NON_SYNONYMOUS_CODING	benign(0.192)	tolerated(0.3)
2	179544685	TTN	C	CTCT	9928	E/EE	NON_SYNONYMOUS_CODING	.	.
2	179634421	TTN	T	G	2917	T/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.539)	.
2	179419226	TTN	A	C	20676	I/M	NON_SYNONYMOUS_CODING	possibly_damaging(0.789)	.
2	179440352	TTN	A	C	14563	C/G	NON_SYNONYMOUS_CODING	probably_damaging(0.969)	.
2	179621111	TTN	C	T	3527	A/T	NON_SYNONYMOUS_CODING	unknown(0)	.
22	18640569	USP18	G	C	47	A/P	NON_SYNONYMOUS_CODING	benign(0.002)	tolerated(0.2)
22	18640566	USP18	AGG	A	46	.	FRAMESHIFT_CODING	.	.
6	41774681	USP49	T	G	14	Q/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.883)	tolerated(0.06)
6	41774685	USP49	C	G	13	A/P	NON_SYNONYMOUS_CODING	probably_damaging(0.986)	deleterious(0.01)
6	33423203	ZBTB9	G	C	109	R/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.936)	tolerated(0.38)
6	33423200	ZBTB9	T	C	108	L/P	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
16	87448068	ZCCHC14	T	G	382	T/P	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.15)
16	87448079	ZCCHC14	C	G	378	R/P	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.13)
8	102213947	ZNF706	A	T	8	I/N	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0)
8	102213962	ZNF706	C	G	3	R/P	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0)
19	52887918	ZNF880	A	G	362	N/S	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
19	52887974	ZNF880	G	A	381	E/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
19	52887982	ZNF880	A	T	383	K/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.56)
19	52887950	ZNF880	A	G	373	I/V	NON_SYNONYMOUS_CODING	benign(0.003)	tolerated(0.25)

CHD4: Homozygous variants

CHR ID	NUCLEOTIDE POSITION	GENE NAME	REF SEQUENCE	ALTERNATE SEQUENCE	AMINO ACID NO.	RESIDUE CHANGE	FUNCTION OF VARIANT	POLYPHEN	SIFT
1	51584465	C1orf185	C	T	84	Q/*	STOP_GAINED	.	.
1	13448180	PRAMEF13	A	G	432	L/P	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.34)
10	51225724	AGAP8	G	C	420	L/V	NON_SYNONYMOUS_CODING	probably_damaging(0.99)	tolerated(0.12)
17	58499989	C17orf64	C	A	12	D/E	NON_SYNONYMOUS_CODING	benign(0.011)	tolerated(0.42)
2	96551974	ENSG00000174501	A	G	54	L/P	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
1	146215113	ENSG00000232637	G	A	84	H/Y	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.05)
7	74212311	GTF2IRD2	G	T	61	H/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
12	123335170	HIP1R	A	G	202	T/A	NON_SYNONYMOUS_CODING	unknown(0)	.
6	31324931	HLA-B	A	C	3	L/R	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
6	31324547	HLA-B	G	C	88	N/K	NON_SYNONYMOUS_CODING	benign(0.192)	deleterious(0.02)
19	50832152	KCNC3	T	C	63	D/G	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(1)
19	7051376	MBD3L2	G	A	124	G/S	NON_SYNONYMOUS_CODING	possibly_damaging(0.6)	tolerated(0.19)
19	7032648	MBD3L5	G	A	124	G/S	NON_SYNONYMOUS_CODING	benign(0.266)	tolerated(0.23)
19	9048362	MUC16	A	G	11090	V/A	NON_SYNONYMOUS_CODING	unknown(0)	.
19	9088228	MUC16	G	T	1196	P/H	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195512186	MUC4	T	C	2089	I/V	NON_SYNONYMOUS_CODING	benign(0.028)	.
3	195514471	MUC4	A	G	1327	M/T	NON_SYNONYMOUS_CODING	benign(0.057)	.
3	195514768	MUC4	T	C	1228	D/G	NON_SYNONYMOUS_CODING	benign(0.144)	.
3	195506704	MUC4	T	C	3916	D/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195507494	MUC4	C	T	3653	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195514733	MUC4	C	A	1240	A/S	NON_SYNONYMOUS_CODING	possibly_damaging(0.533)	.
3	195506914	MUC4	G	A	3846	P/L	NON_SYNONYMOUS_CODING	possibly_damaging(0.931)	.
3	195514948	MUC4	G	A	1168	P/L	NON_SYNONYMOUS_CODING	probably_damaging(0.975)	.
3	195510601	MUC4	A	G	2617	V/A	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510610	MUC4	A	G	2614	L/P	NON_SYNONYMOUS_CODING	unknown(0)	.

1	145327548	NBPF10	A	G	1369	N/D	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
1	145359049	NBPF10	A	G	2997	K/E	NON_SYNONYMOUS_CODING	benign(0.049)	tolerated(1)
1	148023040	NBPF14	G	C	162	S/C	NON_SYNONYMOUS_CODING	possibly_damaging(0.939)	deleterious(0.02)
1	148741720	NBPF16	T	C	69	F/S	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
1	248737259	OR2T34	C	G	267	R/P	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
1	248802469	OR2T35	C	T	31	V/I	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.2)
19	15586705	PGLYRP2	G	A	259	T/M	NON_SYNONYMOUS_CODING	possibly_damaging(0.884)	deleterious(0)
1	89273249	PKN2	A	G	610	R/G	NON_SYNONYMOUS_CODING	probably_damaging(0.959)	deleterious(0)
2	130832358	POTEF	T	C	896	H/R	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
2	131221622	POTEI	C	T	665	M/I	NON_SYNONYMOUS_CODING	possibly_damaging(0.539)	deleterious(0)
14	20010235	POTEM	A	G	393	V/A	NON_SYNONYMOUS_CODING	benign(0.006)	tolerated(1)
1	13448184	PRAMEF13	C	T	431	A/T	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.35)
1	13448185	PRAMEF13	G	C	430	F/L	NON_SYNONYMOUS_CODING	benign(0.006)	tolerated(0.65)
1	13695685	PRAMEF19	G	A	358	S/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.68)
1	13695787	PRAMEF19	C	G	324	G/A	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
1	13696090	PRAMEF19	T	C	223	K/R	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.47)
22	18901004	PRODH	C	T	166	R/Q	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.6)
2	108479432	RGPD4	G	A	805	A/T	NON_SYNONYMOUS_CODING	possibly_damaging(0.67)	tolerated(0.2)
1	40318469	TRIT1	C	T	85	R/H	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.55)
19	21366170	ZNF431	G	A	355	R/Q	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.32)
9	123476542	MEGF9	ACGGCGG	A	22-24	AAV/V	NON_SYNONYMOUS_CODING	.	.
18	31319034	ASXL3	ACAGAACATAAGGAGT	A	556-561	TEHKES/T	NON_SYNONYMOUS_CODING	.	.
11	66512290	C11orf80	G	GGGC	26	G/GA	NON_SYNONYMOUS_CODING	.	.
22	37964408	CDC42EP1	CCAGCGCCTGCTGCAAACCCCT	C	253-260	PAPAANPS/P	NON_SYNONYMOUS_CODING	.	.
15	90320134	MESP2	AGGGCAGGGGCAG	A	183-186	GQGQ/-	NON_SYNONYMOUS_CODING	.	.
18	30352057	ENSG00000228835	GCGCCGGCC	G	119-121	.	FRAMESHIFT_CODING	.	.
21	43298954	PRDM15	C	CG	88	.	FRAMESHIFT_CODING	.	.
3	133969437	RYK	A	AG	19-20	.	SPLICE_SITE:FRAMESHIFT_CODING	.	.

CHD4: Compound heterozygous variants

CHR ID	NUCLEOTIDE POSITION	GENE NAME	REF SEQUENCE	ALTERNATE SEQUENCE	AMINO ACID NO.	RESIDUE CHANGE	FUNCTION OF VARIANT	POLYPHEN	SIFT
2	97808524	ANKRD36	A	G	285	I/V	NON_SYNONYMOUS_CODING	benign(0.296)	tolerated(1)
2	97845632	ANKRD36	T	C	566	I/T	NON_SYNONYMOUS_CODING	possibly_damaging(0.767)	tolerated(0.23)
2	97808515	ANKRD36	G	A	282	E/K	NON_SYNONYMOUS_CODING	probably_damaging(0.959)	tolerated(1)
2	97783875	ANKRD36	T	C	91	L/P	NON_SYNONYMOUS_CODING	probably_damaging(0.983)	deleterious(0)
2	97808510	ANKRD36	G	A	280	G/D	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.85)
22	39498015	APOBEC3H	C	G	171	R/G	NON_SYNONYMOUS_CODING	benign(0.057)	deleterious(0.01)
22	39498005	APOBEC3H	T	A	167	D/E	NON_SYNONYMOUS_CODING	benign(0.18)	tolerated(0.35)
11	64666179	ATG2A	A	G	1338	S/P	NON_SYNONYMOUS_CODING	benign(0.403)	deleterious(0.02)
11	64666182	ATG2A	C	G	1337	A/P	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
5	137503739	BRD8	G	A	224	S/F	NON_SYNONYMOUS_CODING	probably_damaging(0.996)	deleterious(0.01)
5	137503737	BRD8	C	A	225	G/C	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.06)
17	56386386	BZRAP1	G	T	1416	P/Q	NON_SYNONYMOUS_CODING	possibly_damaging(0.948)	deleterious(0)
17	56386402	BZRAP1	A	G	1411	S/P	NON_SYNONYMOUS_CODING	probably_damaging(0.995)	deleterious(0)
17	56386383	BZRAP1	C	CTT	1417	S/KS	FRAMESHIFT_CODING	.	.
12	112622338	C12orf51	T	G	3056	T/P	NON_SYNONYMOUS_CODING	benign(0.187)	.
12	112688167	C12orf51	A	C	822	V/G	NON_SYNONYMOUS_CODING	probably_damaging(0.979)	.
19	16614150	C19orf44	T	G	345	V/G	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.53)
19	16614154	C19orf44	C	G	346	D/E	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.95)
17	45234417	CDC27	A	G	235	I/T	NON_SYNONYMOUS_CODING	benign(0.004)	tolerated(0.19)
17	45234303	CDC27	G	C	273	A/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.783)	tolerated(0.07)
17	45214528	CDC27	A	T	635	Y/N	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
9	135947051	CEL	G	A	690	G/E	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
9	135947056	CEL	C	G	692	P/A	NON_SYNONYMOUS_CODING	benign(0.015)	tolerated(0.92)
17	7796794	CHD3	A	C	293	I/L	NON_SYNONYMOUS_CODING	benign(0)	.
17	7796803	CHD3	T	C	296	S/P	NON_SYNONYMOUS_CODING	benign(0.001)	.
12	122812697	CLIP1	C	T	47	E/K	NON_SYNONYMOUS_CODING	possibly_damaging(0.918)	deleterious(0.03)
12	122812709	CLIP1	C	T	43	E/K	SPLICE_SITE:NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.33)
22	19223229	CLTCL1	T	C	320	K/R	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(1)
22	19213150	CLTCL1	C	A	652	V/F	NON_SYNONYMOUS_CODING	benign(0.419)	deleterious(0)
8	68062165	CSPP1	G	A	703	S/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.64)
8	68062160	CSPP1	T	A	701	N/K	NON_SYNONYMOUS_CODING	benign(0.153)	tolerated(0.98)
10	126678128	CTBP2	T	A	433	T/S	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
10	126678177	CTBP2	G	T	416	N/K	NON_SYNONYMOUS_CODING	benign(0.01)	tolerated(0.46)
15	51766636	DMXL2	G	A	1736	A/V	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(1)
15	51766637	DMXL2	C	A	1736	A/S	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.57)
2	96614324	ENSG00000174501	C	T	439	R/H	NON_SYNONYMOUS_CODING	possibly_damaging(0.923)	deleterious(0.01)
2	96610395	ENSG00000174501	C	CA	490-491	.	FRAMESHIFT_CODING	.	.
12	10658524	ENSG00000180574	T	G	8	V/G	NON_SYNONYMOUS_CODING	benign(0.004)	tolerated(0.07)
12	10658616	ENSG00000180574	C	A	39	Q/K	NON_SYNONYMOUS_CODING	probably_damaging(0.994)	deleterious(0.01)
16	15457629	ENSG00000183793	T	A	314	T/S	NON_SYNONYMOUS_CODING	benign(0.158)	tolerated(0.82)
16	15457628	ENSG00000183793	G	T	314	T/N	NON_SYNONYMOUS_CODING	benign(0.348)	tolerated(0.39)

2	132021023	ENSG00000188219	G	A	665	M/I	NON_SYNONYMOUS_CODING	possibly_damaging(0.539)	deleterious(0)
2	132021946	ENSG00000188219	G	A	973	G/D	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
19	56283297	ENSG00000229292	G	A	43	D/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.52)
19	56284090	ENSG00000229292	G	A	137	A/T	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.41)
19	56283289	ENSG00000229292	A	G	40	K/R	NON_SYNONYMOUS_CODING	benign(0.39)	tolerated(0.51)
9	46386995	ENSG00000237198	C	A	3	R/M	NON_SYNONYMOUS_CODING	benign(0.013)	tolerated(0.36)
9	46386902	ENSG00000237198	G	A	34	T/I	NON_SYNONYMOUS_CODING	probably_damaging(0.998)	deleterious(0)
9	46386956	ENSG00000237198	C	G	16	R/P	NON_SYNONYMOUS_CODING	probably_damaging(0.998)	deleterious(0)
9	46386806	ENSG00000237198	C	T	66	R/Q	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.75)
11	92616488	FAT3	T	C	624	L/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.542)	tolerated(0.12)
11	92616485	FAT3	A	C	623	N/T	NON_SYNONYMOUS_CODING	probably_damaging(0.978)	tolerated(1)
10	135440159	FRG2B	T	A	30	T/S	NON_SYNONYMOUS_CODING	possibly_damaging(0.94)	tolerated(0.45)
10	135440222	FRG2B	C	T	9	D/N	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	tolerated(0.17)
1	37319269	GRIK3	G	A	387	R/W	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
1	37319270	GRIK3	C	A	386	L/F	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.75)
8	21983120	HR	C	G	511	A/P	NON_SYNONYMOUS_CODING	benign(0.255)	tolerated(0.23)
8	21973850	HR	G	C	1157	P/R	NON_SYNONYMOUS_CODING	possibly_damaging(0.441)	tolerated(0.29)
22	23247142	IGLJ3	T	C	30	F/L	NON_SYNONYMOUS_CODING	unknown(0)	.
22	23247169	IGLJ3	T	G	39	W/G	NON_SYNONYMOUS_CODING	unknown(0)	.
22	23247170	IGLJ3	G	T	39	W/L	NON_SYNONYMOUS_CODING	unknown(0)	.
3	4725969	ITPR1	G	A	1159	G/E	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.22)
3	4725976	ITPR1	C	A	1161	N/K	NON_SYNONYMOUS_CODING	benign(0.004)	tolerated(0.94)
17	39346592	KRTAP9-1	ACCT	A	152-153	TC/S	NON_SYNONYMOUS_CODING	.	.
17	39346433	KRTAP9-1	A	C	99	T/P	SPLICE_SITE:NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.08)
1	152777625	LCE1C	A	C	110	S/R	NON_SYNONYMOUS_CODING	unknown(0)	.
1	152777627	LCE1C	T	C	110	S/G	NON_SYNONYMOUS_CODING	unknown(0)	.
17	35298121	LHX1	G	C	204	K/N	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
17	35298125	LHX1	A	C	206	T/P	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
4	151509276	LRBA	G	A	2085	T/I	NON_SYNONYMOUS_CODING	possibly_damaging(0.909)	deleterious(0.03)
4	151236754	LRBA	T	G	2551	D/A	NON_SYNONYMOUS_CODING	probably_damaging(0.996)	tolerated(0.3)
7	151927025	MLL3	A	G	987	Y/H	NON_SYNONYMOUS_CODING	probably_damaging(1)	.
7	151932997	MLL3	C	T	892	G/R	NON_SYNONYMOUS_CODING	probably_damaging(1)	.
19	9005678	MUC16	G	A	83	P/L	NON_SYNONYMOUS_CODING	benign(0)	deleterious(0)
19	9005645	MUC16	T	C	94	Q/R	NON_SYNONYMOUS_CODING	benign(0.002)	tolerated(0.21)
19	9005649	MUC16	G	C	93	Q/E	NON_SYNONYMOUS_CODING	benign(0.002)	tolerated(1)
19	9005679	MUC16	G	C	83	P/A	NON_SYNONYMOUS_CODING	benign(0.002)	deleterious(0)
19	9005714	MUC16	A	C	71	V/G	NON_SYNONYMOUS_CODING	benign(0.015)	tolerated(0.31)
19	9005721	MUC16	C	G	69	D/H	NON_SYNONYMOUS_CODING	benign(0.02)	tolerated(0.14)
19	9002612	MUC16	T	G	43	K/Q	NON_SYNONYMOUS_CODING	benign(0.055)	tolerated(0.23)
19	9005697	MUC16	C	T	77	A/T	NON_SYNONYMOUS_CODING	benign(0.138)	tolerated(0.19)
19	9005706	MUC16	T	C	74	R/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.847)	tolerated(0.65)
19	9002597	MUC16	C	T	48	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.866)	deleterious(0)
3	195513846	MUC4	C	G	1535	M/I	NON_SYNONYMOUS_CODING	benign(0.012)	.
3	195506387	MUC4	G	T	4022	P/T	NON_SYNONYMOUS_CODING	benign(0.025)	.
3	195513847	MUC4	A	G	1535	M/T	NON_SYNONYMOUS_CODING	benign(0.042)	.
3	195506549	MUC4	A	G	3968	S/P	NON_SYNONYMOUS_CODING	benign(0.044)	.

3	195506590	MUC4	G	A	3954	P/L	NON_SYNONYMOUS_CODING	benign(0.057)	.
3	195505910	MUC4	T	C	4181	S/G	NON_SYNONYMOUS_CODING	benign(0.095)	.
3	195505945	MUC4	A	G	4169	V/A	NON_SYNONYMOUS_CODING	benign(0.095)	.
3	195506137	MUC4	A	G	4105	V/A	NON_SYNONYMOUS_CODING	benign(0.095)	.
3	195506150	MUC4	T	C	4101	S/G	NON_SYNONYMOUS_CODING	benign(0.095)	.
3	195506215	MUC4	C	G	4079	S/T	NON_SYNONYMOUS_CODING	benign(0.095)	.
3	195508343	MUC4	A	T	3370	S/T	NON_SYNONYMOUS_CODING	benign(0.095)	.
3	195508777	MUC4	A	G	3225	V/A	NON_SYNONYMOUS_CODING	benign(0.095)	.
3	195508790	MUC4	T	C	3221	S/G	NON_SYNONYMOUS_CODING	benign(0.095)	.
3	195506364	MUC4	G	C	4029	H/Q	NON_SYNONYMOUS_CODING	benign(0.118)	.
3	195506473	MUC4	A	G	3993	V/A	NON_SYNONYMOUS_CODING	benign(0.137)	.
3	195505859	MUC4	T	C	4198	T/A	NON_SYNONYMOUS_CODING	benign(0.182)	.
3	195506099	MUC4	T	C	4118	T/A	NON_SYNONYMOUS_CODING	benign(0.182)	.
3	195506987	MUC4	T	C	3822	T/A	NON_SYNONYMOUS_CODING	benign(0.182)	.
3	195508667	MUC4	T	C	3262	T/A	NON_SYNONYMOUS_CODING	benign(0.182)	.
3	195510228	MUC4	C	G	2741	E/D	NON_SYNONYMOUS_CODING	benign(0.182)	.
3	195506582	MUC4	C	T	3957	D/N	NON_SYNONYMOUS_CODING	benign(0.183)	.
3	195505836	MUC4	G	C	4205	H/Q	NON_SYNONYMOUS_CODING	benign(0.204)	.
3	195505909	MUC4	C	T	4181	S/N	NON_SYNONYMOUS_CODING	benign(0.204)	.
3	195506149	MUC4	C	T	4101	S/N	NON_SYNONYMOUS_CODING	benign(0.204)	.
3	195507228	MUC4	G	C	3741	H/Q	NON_SYNONYMOUS_CODING	benign(0.204)	.
3	195508716	MUC4	A	C	3245	H/Q	NON_SYNONYMOUS_CODING	benign(0.204)	.
3	195508789	MUC4	C	T	3221	S/N	NON_SYNONYMOUS_CODING	benign(0.204)	.
3	195515017	MUC4	A	G	1145	V/A	NON_SYNONYMOUS_CODING	benign(0.231)	.
3	195506569	MUC4	A	G	3961	V/A	NON_SYNONYMOUS_CODING	benign(0.243)	.
3	195507428	MUC4	T	A	3675	T/S	NON_SYNONYMOUS_CODING	benign(0.352)	.
3	195508787	MUC4	G	T	3222	L/I	NON_SYNONYMOUS_CODING	benign(0.352)	.
3	195510194	MUC4	G	C	2753	L/V	NON_SYNONYMOUS_CODING	benign(0.352)	.
3	195506076	MUC4	C	G	4125	Q/H	NON_SYNONYMOUS_CODING	benign(0.391)	.
3	195510636	MUC4	C	A	2605	Q/H	NON_SYNONYMOUS_CODING	benign(0.391)	.
3	195508661	MUC4	A	G	3264	S/P	NON_SYNONYMOUS_CODING	benign(0.421)	.
3	195508709	MUC4	A	G	3248	S/P	NON_SYNONYMOUS_CODING	benign(0.421)	.
3	195506533	MUC4	C	A	3973	S/I	NON_SYNONYMOUS_CODING	possibly_damaging(0.473)	.
3	195505849	MUC4	G	A	4201	A/V	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195505897	MUC4	G	A	4185	A/V	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195506650	MUC4	G	A	3934	A/V	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195506746	MUC4	G	A	3902	A/V	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195506963	MUC4	C	T	3830	A/T	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195507062	MUC4	C	T	3797	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195507155	MUC4	C	T	3766	A/T	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195507193	MUC4	G	A	3753	A/V	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195507385	MUC4	G	A	3689	A/V	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195507398	MUC4	C	T	3685	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195507434	MUC4	C	A	3673	A/S	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195507446	MUC4	C	T	3669	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195508070	MUC4	C	T	3461	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.

3	195508500	MUC4	G	C	3317	D/E	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195508502	MUC4	C	T	3317	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195508668	MUC4	G	C	3261	D/E	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195510649	MUC4	G	A	2601	A/V	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195505906	MUC4	G	A	4182	T/I	NON_SYNONYMOUS_CODING	possibly_damaging(0.607)	.
3	195506213	MUC4	T	G	4080	T/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.607)	.
3	195506983	MUC4	G	A	3823	T/I	NON_SYNONYMOUS_CODING	possibly_damaging(0.607)	.
3	195507443	MUC4	T	G	3670	T/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.607)	.
3	195507475	MUC4	G	C	3659	T/R	NON_SYNONYMOUS_CODING	possibly_damaging(0.607)	.
3	195507827	MUC4	G	A	3542	L/F	NON_SYNONYMOUS_CODING	possibly_damaging(0.607)	.
3	195508678	MUC4	G	T	3258	S/Y	NON_SYNONYMOUS_CODING	possibly_damaging(0.607)	.
3	195506602	MUC4	G	C	3950	T/S	NON_SYNONYMOUS_CODING	possibly_damaging(0.717)	.
3	195511285	MUC4	T	C	2389	D/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.748)	.
3	195511286	MUC4	C	T	2389	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.748)	.
3	195511525	MUC4	T	C	2309	D/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.748)	.
3	195511526	MUC4	C	T	2309	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.748)	.
3	195512117	MUC4	C	T	2112	A/T	NON_SYNONYMOUS_CODING	possibly_damaging(0.748)	.
3	195506501	MUC4	G	A	3984	P/S	NON_SYNONYMOUS_CODING	possibly_damaging(0.768)	.
3	195507604	MUC4	C	G	3616	R/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.811)	.
3	195514844	MUC4	C	G	1203	V/L	NON_SYNONYMOUS_CODING	possibly_damaging(0.817)	.
3	195506516	MUC4	T	A	3979	T/S	NON_SYNONYMOUS_CODING	possibly_damaging(0.862)	.
3	195507445	MUC4	T	A	3669	D/V	NON_SYNONYMOUS_CODING	possibly_damaging(0.862)	.
3	195506117	MUC4	G	T	4112	P/T	NON_SYNONYMOUS_CODING	possibly_damaging(0.864)	.
3	195507461	MUC4	G	A	3664	P/S	NON_SYNONYMOUS_CODING	possibly_damaging(0.864)	.
3	195515314	MUC4	A	G	1046	F/S	NON_SYNONYMOUS_CODING	possibly_damaging(0.873)	.
3	195506146	MUC4	A	G	4102	L/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.881)	.
3	195507694	MUC4	A	G	3586	L/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.881)	.
3	195508786	MUC4	A	G	3222	L/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.881)	.
3	195506294	MUC4	T	C	4053	N/D	NON_SYNONYMOUS_CODING	possibly_damaging(0.9)	.
3	195506507	MUC4	C	T	3982	A/T	NON_SYNONYMOUS_CODING	possibly_damaging(0.916)	.
3	195506522	MUC4	C	A	3977	A/S	NON_SYNONYMOUS_CODING	possibly_damaging(0.916)	.
3	195505870	MUC4	G	A	4194	P/L	NON_SYNONYMOUS_CODING	possibly_damaging(0.931)	.
3	195508018	MUC4	G	A	3478	P/L	NON_SYNONYMOUS_CODING	possibly_damaging(0.931)	.
3	195506990	MUC4	C	G	3821	D/H	NON_SYNONYMOUS_CODING	possibly_damaging(0.934)	.
3	195507614	MUC4	C	G	3613	D/H	NON_SYNONYMOUS_CODING	possibly_damaging(0.934)	.
3	195508238	MUC4	C	G	3405	D/H	NON_SYNONYMOUS_CODING	possibly_damaging(0.934)	.
3	195508670	MUC4	C	G	3261	D/H	NON_SYNONYMOUS_CODING	possibly_damaging(0.934)	.
3	195515414	MUC4	T	C	1013	S/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.943)	.
3	195506554	MUC4	G	A	3966	A/V	NON_SYNONYMOUS_CODING	probably_damaging(0.959)	.
3	195507605	MUC4	G	A	3616	R/C	NON_SYNONYMOUS_CODING	probably_damaging(0.965)	.
3	195515008	MUC4	C	G	1148	G/A	NON_SYNONYMOUS_CODING	probably_damaging(0.972)	.
3	195513826	MUC4	G	A	1542	P/L	NON_SYNONYMOUS_CODING	probably_damaging(0.975)	.
3	195515449	MUC4	A	T	1001	V/E	NON_SYNONYMOUS_CODING	probably_damaging(0.979)	.
3	195515435	MUC4	C	T	1006	A/T	NON_SYNONYMOUS_CODING	probably_damaging(0.985)	.
3	195513398	MUC4	C	T	1685	G/S	NON_SYNONYMOUS_CODING	probably_damaging(0.99)	.
3	195510622	MUC4	G	T	2610	P/H	NON_SYNONYMOUS_CODING	probably_damaging(0.991)	.

3	195508336	MUC4	C	T	3372	G/D	NON_SYNONYMOUS_CODING	probably_damaging(0.994)	.
3	195510910	MUC4	G	T	2514	P/H	NON_SYNONYMOUS_CODING	probably_damaging(0.997)	.
3	195510310	MUC4	T	G	2714	Y/S	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510341	MUC4	A	G	2704	S/P	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510347	MUC4	T	C	2702	T/A	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510350	MUC4	C	G	2701	D/H	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510373	MUC4	T	C	2693	D/G	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510374	MUC4	C	T	2693	D/N	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510389	MUC4	A	G	2688	S/P	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510396	MUC4	A	C	2685	H/Q	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510492	MUC4	C	A	2653	Q/H	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510611	MUC4	G	T	2614	L/I	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510613	MUC4	C	T	2613	S/N	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510614	MUC4	T	C	2613	S/G	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195505742	MUC4	G	C	963	H/D	SPLICE_SITE:NON_SYNONYMOUS_CODING	possibly_damaging(0.662)	tolerated(0.09)
11	1018095	MUC6	G	A	1569	P/L	NON_SYNONYMOUS_CODING	benign(0.014)	deleterious(0.01)
11	1016957	MUC6	T	G	1948	R/S	NON_SYNONYMOUS_CODING	benign(0.034)	tolerated(0.84)
11	1017466	MUC6	T	C	1779	R/G	NON_SYNONYMOUS_CODING	benign(0.142)	tolerated(0.57)
11	1017307	MUC6	G	A	1832	P/S	NON_SYNONYMOUS_CODING	benign(0.34)	tolerated(0.21)
11	1016919	MUC6	C	T	1961	R/K	NON_SYNONYMOUS_CODING	possibly_damaging(0.496)	tolerated(0.33)
11	1017693	MUC6	T	A	1703	H/L	NON_SYNONYMOUS_CODING	probably_damaging(0.978)	tolerated(0.82)
11	1018042	MUC6	A	G	1587	S/P	NON_SYNONYMOUS_CODING	probably_damaging(0.979)	deleterious(0.01)
11	1016554	MUC6	C	T	2083	A/T	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.6)
11	1016565	MUC6	C	T	2079	S/N	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.09)
11	1018207	MUC6	T	C	1532	T/A	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.28)
11	1018552	MUC6	G	A	1417	P/S	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.73)
17	12659746	MYOCD	G	A	397	D/N	NON_SYNONYMOUS_CODING	benign(0.002)	tolerated(0.71)
17	12666520	MYOCD	C	A	792	S/R	NON_SYNONYMOUS_CODING	benign(0.073)	tolerated(0.16)
17	12661424	MYOCD	A	G	694	D/G	NON_SYNONYMOUS_CODING	benign(0.153)	tolerated(0.44)
9	139413211	NOTCH1	T	G	311	T/P	NON_SYNONYMOUS_CODING	benign(0.004)	deleterious(0)
9	139413097	NOTCH1	T	G	349	T/P	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
1	228430947	OBSCN	C	G	998	A/G	NON_SYNONYMOUS_CODING	benign(0.003)	.
1	228434292	OBSCN	C	G	1274	A/G	NON_SYNONYMOUS_CODING	benign(0.006)	.
1	248436582	OR2T33	T	C	179	T/A	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.25)
1	248436638	OR2T33	A	G	160	V/A	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.4)
7	95216407	PDK4	G	A	301	S/F	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
7	95216415	PDK4	C	A	298	L/F	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
8	110455187	PKHD1L1	A	T	1469	Y/F	NON_SYNONYMOUS_CODING	possibly_damaging(0.799)	tolerated(0.33)
8	110455184	PKHD1L1	C	T	1468	S/F	NON_SYNONYMOUS_CODING	probably_damaging(0.982)	deleterious(0.01)
12	106820987	POLR3B	C	T	314	L/F	NON_SYNONYMOUS_CODING	benign(0.088)	deleterious(0)
12	106820975	POLR3B	C	T	310	L/F	SPLICE_SITE:NON_SYNONYMOUS_CODING	benign(0.102)	deleterious(0)
6	106546559	PRDM1	A	G	4	E/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.878)	.
6	106546576	PRDM1	A	C	10	T/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.901)	deleterious(0)
9	33798075	PRSS3	T	C	150	F/S	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
9	33798574	PRSS3	G	A	182	S/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
2	85571228	RETSAT	G	C	265	A/G	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.43)

2	85571225	RETSAT	T	C	266	E/G	NON_SYNONYMOUS_CODING	benign(0.048)	tolerated(0.28)
2	87205020	RGPD1	G	T	810	R/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
2	87205031	RGPD1	T	C	814	C/R	NON_SYNONYMOUS_CODING	possibly_damaging(0.85)	tolerated(0.78)
12	109017692	SELPLG	G	A	147	A/V	ESSENTIAL_SPLICE_SITE:NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.52)
12	109017693	SELPLG	C	T	147	A/T	ESSENTIAL_SPLICE_SITE:NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.55)
19	51920115	SIGLEC10	C	T	171	E/K	NON_SYNONYMOUS_CODING	benign(0.124)	tolerated(0.42)
19	51920112	SIGLEC10	C	T	172	E/K	NON_SYNONYMOUS_CODING	benign(0.16)	tolerated(0.77)
7	142498738	TRBC2	C	G	5	N/K	NON_SYNONYMOUS_CODING	benign(0.002)	tolerated(0.34)
7	142498735	TRBC2	A	C	4	K/N	NON_SYNONYMOUS_CODING	benign(0.111)	tolerated(0.08)
7	142008783	TRBV3-1	A	G	86	K/E	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142008675	TRBV3-1	A	G	50	T/A	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(1)
7	142008785	TRBV3-1	A	C	86	K/N	NON_SYNONYMOUS_CODING	benign(0.001)	deleterious(0.02)
7	142008718	TRBV3-1	T	C	64	I/T	NON_SYNONYMOUS_CODING	benign(0.002)	deleterious(0.01)
7	142008672	TRBV3-1	G	A	49	D/N	NON_SYNONYMOUS_CODING	benign(0.006)	tolerated(0.61)
7	142045680	TRBV4-2	T	C	70	F/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.67)
7	142045693	TRBV4-2	C	T	74	T/I	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.36)
7	142168414	TRBV5-4	G	C	103	D/E	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142168466	TRBV5-4	A	C	86	L/R	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.24)
7	142180566	TRBV6-5	A	T	98	L/Q	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.22)
7	142180585	TRBV6-5	C	T	92	D/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.79)
7	142180590	TRBV6-5	G	T	90	T/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142180593	TRBV6-5	G	T	89	T/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.44)
7	142180596	TRBV6-5	G	A	88	S/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.31)
7	142180618	TRBV6-5	T	C	81	N/D	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.78)
7	142180633	TRBV6-5	G	T	76	Q/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142180635	TRBV6-5	T	G	75	D/A	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.22)
7	142180641	TRBV6-5	A	G	73	I/T	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.91)
7	142180704	TRBV6-5	G	T	52	S/Y	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142180737	TRBV6-5	T	A	41	Q/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.16)
7	142180578	TRBV6-5	G	A	94	P/L	NON_SYNONYMOUS_CODING	benign(0.002)	tolerated(0.17)
7	142180588	TRBV6-5	C	G	91	E/Q	NON_SYNONYMOUS_CODING	benign(0.005)	tolerated(0.2)
7	142180647	TRBV6-5	G	T	71	A/D	NON_SYNONYMOUS_CODING	benign(0.08)	tolerated(0.18)
7	142099548	TRBV7-8	A	G	85	F/S	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142099581	TRBV7-8	A	G	74	L/P	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.35)
7	142099512	TRBV7-8	T	G	97	K/T	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.14)
7	142099533	TRBV7-8	T	C	90	E/G	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.36)
7	142099537	TRBV7-8	G	T	89	P/T	NON_SYNONYMOUS_CODING	benign(0.003)	tolerated(0.39)
6	41774681	USP49	T	G	14	Q/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.883)	tolerated(0.06)
6	41774685	USP49	C	G	13	A/P	NON_SYNONYMOUS_CODING	probably_damaging(0.986)	deleterious(0.01)
6	33423203	ZBTB9	G	C	109	R/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.936)	tolerated(0.38)
6	33423200	ZBTB9	T	C	108	L/P	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
16	87448068	ZCCHC14	T	G	382	T/P	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.15)
16	87448079	ZCCHC14	C	G	378	R/P	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.13)
19	56701618	ZSCAN5B	C	T	356	A/T	NON_SYNONYMOUS_CODING	benign(0.043)	tolerated(0.64)
19	56703359	ZSCAN5B	C	G	150	A/P	NON_SYNONYMOUS_CODING	probably_damaging(0.975)	tolerated(0.27)

CHD5: Homozygous variants

CHR ID	NUCLEOTIDE POSITION	GENE NAME	REF SEQUENCE	ALTERNATE SEQUENCE	AMINO ACID NO.	RESIDUE CHANGE	FUNCTION OF VARIANT	POLYPHEN	SIFT
9	66553727	ENSG00000170161	T	C	60	V/A	NON_SYNONYMOUS_CODING	benign(0)	.
1	146215113	ENSG00000232637	G	A	84	H/Y	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.05)
10	81609619	FAM22E	T	G	632	S/A	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	74212311	GTF2IRD2	G	T	61	H/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
1	145327548	NBPF10	A	G	1369	N/D	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
1	148741720	NBPF16	T	C	69	F/S	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
1	248737259	OR2T34	C	G	267	R/P	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
2	130832358	POTEF	T	C	896	H/R	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
1	13695685	PRAMEF19	G	A	358	S/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.68)
1	13696054	PRAMEF19	G	A	235	S/F	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.69)
1	13695787	PRAMEF19	C	G	324	G/A	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
1	13696022	PRAMEF19	A	G	246	W/R	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.25)
1	13696047	PRAMEF19	A	T	237	D/E	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.24)
1	13329354	PRAMEF3	T	C	309	K/E	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
1	13329350	PRAMEF3	A	T	310	V/E	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.46)
15	82932518	ENSG00000215749	T	C	502	E/G	NON_SYNONYMOUS_CODING	benign(0.003)	tolerated(0.24)
14	20010235	POTEM	A	G	393	V/A	NON_SYNONYMOUS_CODING	benign(0.006)	tolerated(1)
3	195512186	MUC4	T	C	2089	I/V	NON_SYNONYMOUS_CODING	benign(0.028)	.
1	146409969	NBPF12	A	C	263	I/L	NON_SYNONYMOUS_CODING	benign(0.036)	deleterious(0)
1	145359049	NBPF10	A	G	2997	K/E	NON_SYNONYMOUS_CODING	benign(0.049)	tolerated(1)
2	108479432	RGPD4	G	A	805	A/T	NON_SYNONYMOUS_CODING	possibly_damaging(0.67)	tolerated(0.2)
9	40705798	FAM75A3	C	A	1152	P/Q	NON_SYNONYMOUS_CODING	probably_damaging(0.968)	tolerated(0.1)
16	65561	WASH4P	G	C	385	A/G	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0.04)
3	195510526	MUC4	A	G	2642	L/P	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510610	MUC4	A	G	2614	L/P	NON_SYNONYMOUS_CODING	unknown(0)	.
14	20020201	POTEM	G	A	7	S/L	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0)
6	1611802	FOXC1	G	GGGC	374-375	-/G	NON_SYNONYMOUS_CODING	.	.
15	90320134	MESP2	AGGGCAGGGGCAG	A	183-186	GQGQ/-	NON_SYNONYMOUS_CODING	.	.
15	31776210	OTUD7A	TGGC	T	696	A/-	NON_SYNONYMOUS_CODING	.	.
21	43298954	PRDM15	C	CG	88	.	FRAMESHIFT_CODING	.	.

CHD5: Compound heterozygous variants

CHR ID	NUCLEOTIDE POSITION	GENE NAME	REF SEQUENCE	ALTERNATE SEQUENCE	AMINO ACID NO.	RESIDUE CHANGE	FUNCTION OF VARIANT	POLYPHEN	SIFT
10	51464656	AGAP7	G	C	600	D/E	NON_SYNONYMOUS_CODING	probably_damaging(0.995)	tolerated(0.06)
10	51465650	AGAP7	C	T	269	R/Q	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0.03)
14	105415264	AHNAK2	G	A	2175	S/L	NON_SYNONYMOUS_CODING	benign(0.104)	tolerated(0.13)
14	105415265	AHNAK2	A	T	2175	S/T	NON_SYNONYMOUS_CODING	benign(0.104)	tolerated(0.32)
2	97808524	ANKRD36	A	G	285	I/V	NON_SYNONYMOUS_CODING	benign(0.296)	tolerated(1)
2	97845632	ANKRD36	T	C	566	I/T	NON_SYNONYMOUS_CODING	possibly_damaging(0.767)	tolerated(0.23)
2	97808515	ANKRD36	G	A	282	E/K	NON_SYNONYMOUS_CODING	probably_damaging(0.959)	tolerated(1)
2	97808510	ANKRD36	G	A	280	G/D	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.85)
12	101368637	ANO4	A	C	191	Y/S	NON_SYNONYMOUS_CODING	probably_damaging(0.983)	tolerated(0.39)
12	101368625	ANO4	G	A	187	R/K	SPLICE_SITE:NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(1)
22	39498015	APOBEC3H	C	G	171	R/G	NON_SYNONYMOUS_CODING	benign(0.057)	deleterious(0.01)
22	39498005	APOBEC3H	T	A	167	D/E	NON_SYNONYMOUS_CODING	benign(0.18)	tolerated(0.35)
5	137503739	BRD8	G	A	224	S/F	NON_SYNONYMOUS_CODING	probably_damaging(0.996)	deleterious(0.01)
5	137503737	BRD8	C	A	225	G/C	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.06)
17	56386386	BZRAP1	G	T	1416	P/Q	NON_SYNONYMOUS_CODING	possibly_damaging(0.948)	deleterious(0)
17	56386402	BZRAP1	A	G	1411	S/P	NON_SYNONYMOUS_CODING	probably_damaging(0.995)	deleterious(0)
12	112622338	C12orf51	T	G	3056	T/P	NON_SYNONYMOUS_CODING	benign(0.187)	.
12	112688167	C12orf51	A	C	822	V/G	NON_SYNONYMOUS_CODING	probably_damaging(0.979)	.
20	18433273	C20orf12	T	G	179	T/P	NON_SYNONYMOUS_CODING	benign(0.005)	tolerated(0.15)
20	18433277	C20orf12	T	G	25	H/P	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0)
1	40535481	CAP1	T	C	310	S/P	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.29)
1	40535490	CAP1	C	G	313	R/G	NON_SYNONYMOUS_CODING	benign(0.048)	tolerated(0.34)
17	45234417	CDC27	A	G	235	I/T	NON_SYNONYMOUS_CODING	benign(0.004)	tolerated(0.19)
17	45234303	CDC27	G	C	273	A/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.783)	tolerated(0.07)
17	45214528	CDC27	A	T	635	Y/N	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
17	7796794	CHD3	A	C	293	I/L	NON_SYNONYMOUS_CODING	benign(0)	.
17	7796803	CHD3	T	C	296	S/P	NON_SYNONYMOUS_CODING	benign(0.001)	.
17	7796819	CHD3	T	C	301	L/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.88)	.
2	9599739	CPSF3	T	A	593	I/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.51)
2	9599742	CPSF3	G	A	594	R/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.93)
8	68062165	CSPP1	G	A	703	S/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.64)
8	68062160	CSPP1	T	A	701	N/K	NON_SYNONYMOUS_CODING	benign(0.153)	tolerated(0.98)
15	51766636	DMXL2	G	A	1736	A/V	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(1)
15	51766637	DMXL2	C	A	1736	A/S	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.57)
16	15457629	ENSG00000183793	T	A	314	T/S	NON_SYNONYMOUS_CODING	benign(0.158)	tolerated(0.82)
16	15457628	ENSG00000183793	G	T	314	T/N	NON_SYNONYMOUS_CODING	benign(0.348)	tolerated(0.39)
16	15474873	ENSG00000183793	C	T	11	R/Q	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(1)
19	56283297	ENSG00000229292	G	A	43	D/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.52)
19	56283289	ENSG00000229292	A	G	40	K/R	NON_SYNONYMOUS_CODING	benign(0.39)	tolerated(0.51)
10	135440122	FRG2B	T	C	42	E/G	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.44)
10	135440159	FRG2B	T	A	30	T/S	NON_SYNONYMOUS_CODING	possibly_damaging(0.94)	tolerated(0.45)

22	30952000	GAL3ST1	T	C	71	E/G	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.44)
22	30951998	GAL3ST1	A	C	72	C/G	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
15	82635194	GOLGA6L10	T	C	459	E/G	NON_SYNONYMOUS_CODING	benign(0.003)	tolerated(0.33)
15	82637069	GOLGA6L10	C	G	339	E/D	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.47)
15	82637079	GOLGA6L10	C	T	336	R/Q	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(1)
1	37319269	GRIK3	G	A	387	R/W	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
1	37319270	GRIK3	C	A	386	L/F	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.75)
14	106573358	IGHV3-11	T	A	76	T/S	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
14	106573357	IGHV3-11	G	T	76	T/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.62)
14	107034854	IGHV5-51	C	A	76	D/Y	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.24)
14	107034869	IGHV5-51	A	C	71	Y/D	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.32)
14	107034873	IGHV5-51	G	C	69	I/M	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.22)
14	107034874	IGHV5-51	A	C	69	I/S	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.41)
14	107034847	IGHV5-51	C	T	78	R/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.37)
14	107034846	IGHV5-51	T	G	78	R/S	NON_SYNONYMOUS_CODING	benign(0.136)	tolerated(0.44)
12	247865	IQSEC3	C	A	446	P/T	NON_SYNONYMOUS_CODING	possibly_damaging(0.925)	deleterious(0.05)
12	176089	IQSEC3	A	G	14	Y/C	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
7	151932991	MLL3	G	A	894	R/W	NON_SYNONYMOUS_CODING	probably_damaging(1)	.
7	151927025	MLL3	A	G	987	Y/H	NON_SYNONYMOUS_CODING	probably_damaging(1)	.
7	151932997	MLL3	C	T	892	G/R	NON_SYNONYMOUS_CODING	probably_damaging(1)	.
19	9002623	MUC16	C	T	39	R/H	NON_SYNONYMOUS_CODING	benign(0.011)	tolerated(0.24)
19	9002636	MUC16	C	T	35	V/I	NON_SYNONYMOUS_CODING	benign(0.051)	tolerated(0.37)
19	9002612	MUC16	T	G	43	K/Q	NON_SYNONYMOUS_CODING	benign(0.055)	tolerated(0.23)
19	9002659	MUC16	C	T	27	G/E	NON_SYNONYMOUS_CODING	possibly_damaging(0.582)	tolerated(0.19)
19	9002597	MUC16	C	T	48	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.866)	deleterious(0)
19	9002660	MUC16	C	T	27	G/R	NON_SYNONYMOUS_CODING	probably_damaging(0.998)	tolerated(0.5)
3	195513846	MUC4	C	G	1535	M/I	NON_SYNONYMOUS_CODING	benign(0.012)	.
3	195513847	MUC4	A	G	1535	M/T	NON_SYNONYMOUS_CODING	benign(0.042)	.
3	195506150	MUC4	T	C	4101	S/G	NON_SYNONYMOUS_CODING	benign(0.095)	.
3	195508790	MUC4	T	C	3221	S/G	NON_SYNONYMOUS_CODING	benign(0.095)	.
3	195506137	MUC4	A	G	4105	V/A	NON_SYNONYMOUS_CODING	benign(0.095)	.
3	195507673	MUC4	A	G	3593	V/A	NON_SYNONYMOUS_CODING	benign(0.095)	.
3	195505859	MUC4	T	C	4198	T/A	NON_SYNONYMOUS_CODING	benign(0.182)	.
3	195506099	MUC4	T	C	4118	T/A	NON_SYNONYMOUS_CODING	benign(0.182)	.
3	195508667	MUC4	T	C	3262	T/A	NON_SYNONYMOUS_CODING	benign(0.182)	.
3	195510228	MUC4	C	G	2741	E/D	NON_SYNONYMOUS_CODING	benign(0.182)	.
3	195505836	MUC4	G	C	4205	H/Q	NON_SYNONYMOUS_CODING	benign(0.204)	.
3	195507228	MUC4	G	C	3741	H/Q	NON_SYNONYMOUS_CODING	benign(0.204)	.
3	195506149	MUC4	C	T	4101	S/N	NON_SYNONYMOUS_CODING	benign(0.204)	.
3	195508789	MUC4	C	T	3221	S/N	NON_SYNONYMOUS_CODING	benign(0.204)	.
3	195515017	MUC4	A	G	1145	V/A	NON_SYNONYMOUS_CODING	benign(0.231)	.
3	195510194	MUC4	G	C	2753	L/V	NON_SYNONYMOUS_CODING	benign(0.352)	.
3	195510636	MUC4	C	G	2605	Q/H	NON_SYNONYMOUS_CODING	benign(0.391)	.
3	195508661	MUC4	A	G	3264	S/P	NON_SYNONYMOUS_CODING	benign(0.421)	.
3	195507193	MUC4	G	A	3753	A/V	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195507385	MUC4	G	A	3689	A/V	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.

3	195507434	MUC4	C	A	3673	A/S	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195508500	MUC4	G	C	3317	D/E	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195508668	MUC4	G	C	3261	D/E	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195507062	MUC4	C	T	3797	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195507446	MUC4	C	T	3669	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195506983	MUC4	G	A	3823	T/I	NON_SYNONYMOUS_CODING	possibly_damaging(0.607)	.
3	195507827	MUC4	G	A	3542	L/F	NON_SYNONYMOUS_CODING	possibly_damaging(0.607)	.
3	195507475	MUC4	G	C	3659	T/R	NON_SYNONYMOUS_CODING	possibly_damaging(0.607)	.
3	195507443	MUC4	T	G	3670	T/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.607)	.
3	195511285	MUC4	T	C	2389	D/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.748)	.
3	195511525	MUC4	T	C	2309	D/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.748)	.
3	195511286	MUC4	C	T	2389	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.748)	.
3	195511526	MUC4	C	T	2309	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.748)	.
3	195507604	MUC4	C	G	3616	R/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.811)	.
3	195507445	MUC4	T	A	3669	D/V	NON_SYNONYMOUS_CODING	possibly_damaging(0.862)	.
3	195507653	MUC4	G	A	3600	P/S	NON_SYNONYMOUS_CODING	possibly_damaging(0.864)	.
3	195506146	MUC4	A	G	4102	L/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.881)	.
3	195507694	MUC4	A	G	3586	L/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.881)	.
3	195506914	MUC4	G	A	3846	P/L	NON_SYNONYMOUS_CODING	possibly_damaging(0.931)	.
3	195506990	MUC4	C	G	3821	D/H	NON_SYNONYMOUS_CODING	possibly_damaging(0.934)	.
3	195508670	MUC4	C	G	3261	D/H	NON_SYNONYMOUS_CODING	possibly_damaging(0.934)	.
3	195515414	MUC4	T	C	1013	S/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.943)	.
3	195515008	MUC4	C	G	1148	G/A	NON_SYNONYMOUS_CODING	probably_damaging(0.972)	.
3	195514948	MUC4	G	A	1168	P/L	NON_SYNONYMOUS_CODING	probably_damaging(0.975)	.
3	195510622	MUC4	G	T	2610	P/H	NON_SYNONYMOUS_CODING	probably_damaging(0.991)	.
3	195508336	MUC4	C	T	3372	G/D	NON_SYNONYMOUS_CODING	probably_damaging(0.994)	.
3	195510347	MUC4	T	C	2702	T/A	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510373	MUC4	T	C	2693	D/G	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510396	MUC4	A	C	2685	H/Q	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510614	MUC4	T	C	2613	S/G	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510310	MUC4	T	G	2714	Y/S	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510341	MUC4	A	G	2704	S/P	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510350	MUC4	C	G	2701	D/H	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510389	MUC4	A	G	2688	S/P	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510466	MUC4	A	G	2662	L/P	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510367	MUC4	G	T	2695	S/Y	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510374	MUC4	C	T	2693	D/N	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510611	MUC4	G	T	2614	L/I	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510613	MUC4	C	T	2613	S/N	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195505742	MUC4	G	C	963	H/D	SPLICE_SITE:NON_SYNONYMOUS_CODING	possibly_damaging(0.662)	tolerated(0.09)
11	1018095	MUC6	G	A	1569	P/L	NON_SYNONYMOUS_CODING	benign(0.014)	deleterious(0.01)
11	1016959	MUC6	T	C	1948	R/G	NON_SYNONYMOUS_CODING	benign(0.034)	tolerated(0.6)
11	1016957	MUC6	T	G	1948	R/S	NON_SYNONYMOUS_CODING	benign(0.034)	tolerated(0.84)
11	1017466	MUC6	T	C	1779	R/G	NON_SYNONYMOUS_CODING	benign(0.142)	tolerated(0.57)
11	1017502	MUC6	T	C	1767	T/A	NON_SYNONYMOUS_CODING	possibly_damaging(0.857)	tolerated(0.44)
11	1017693	MUC6	T	A	1703	H/L	NON_SYNONYMOUS_CODING	probably_damaging(0.978)	tolerated(0.82)

11	1018042	MUC6	A	G	1587	S/P	NON_SYNONYMOUS_CODING	probably_damaging(0.979)	deleterious(0.01)
11	1016554	MUC6	C	T	2083	A/T	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.6)
11	1016565	MUC6	C	T	2079	S/N	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.09)
1	145367800	NBPF10	G	A	586	D/N	NON_SYNONYMOUS_CODING	benign(0.009)	deleterious(0.03)
1	145299838	NBPF10	G	A	221	R/H	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.27)
9	139413211	NOTCH1	T	G	311	T/P	NON_SYNONYMOUS_CODING	benign(0.004)	deleterious(0)
9	139413097	NOTCH1	T	G	349	T/P	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
1	228434292	OBSCN	C	G	1274	A/G	NON_SYNONYMOUS_CODING	benign(0.006)	.
1	228437749	OBSCN	G	A	1373	E/K	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	.
1	248436582	OR2T33	T	C	179	T/A	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.25)
1	248436638	OR2T33	A	G	160	V/A	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.4)
8	110455187	PKHD1L1	A	T	1469	Y/F	NON_SYNONYMOUS_CODING	possibly_damaging(0.799)	tolerated(0.33)
8	110455184	PKHD1L1	C	T	1468	S/F	NON_SYNONYMOUS_CODING	probably_damaging(0.982)	deleterious(0.01)
19	4511376	PLIN4	C	A	852	G/C	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.14)
19	4511379	PLIN4	G	C	851	L/V	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
9	33798574	PRSS3	G	A	182	S/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
9	33798075	PRSS3	T	C	150	F/S	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
2	85571228	RETSAT	G	C	265	A/G	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.43)
2	85571225	RETSAT	T	C	266	E/G	NON_SYNONYMOUS_CODING	benign(0.048)	tolerated(0.28)
12	109017672	SELPLG	G	C	154	P/A	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.4)
12	109017674	SELPLG	A	G	153	V/A	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.94)
7	142498738	TRBC2	C	G	5	N/K	NON_SYNONYMOUS_CODING	benign(0.002)	tolerated(0.34)
7	142498735	TRBC2	A	C	4	K/N	NON_SYNONYMOUS_CODING	benign(0.111)	tolerated(0.08)
7	142008783	TRBV3-1	A	G	86	K/E	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142008785	TRBV3-1	A	C	86	K/N	NON_SYNONYMOUS_CODING	benign(0.001)	deleterious(0.02)
7	142008727	TRBV3-1	G	T	67	S/I	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.4)
7	142008718	TRBV3-1	T	C	64	I/T	NON_SYNONYMOUS_CODING	benign(0.002)	deleterious(0.01)
7	142045680	TRBV4-2	T	C	70	F/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.67)
7	142045693	TRBV4-2	C	T	74	T/I	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.36)
7	142180596	TRBV6-5	G	A	88	S/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.31)
7	142180737	TRBV6-5	T	A	41	Q/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.16)
7	142180618	TRBV6-5	T	C	81	N/D	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.78)
7	142180635	TRBV6-5	T	G	75	D/A	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.22)
7	142180641	TRBV6-5	A	G	73	I/T	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.91)
7	142180585	TRBV6-5	C	T	92	D/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.79)
7	142180590	TRBV6-5	G	T	90	T/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142180593	TRBV6-5	G	T	89	T/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.44)
7	142180633	TRBV6-5	G	T	76	Q/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142180704	TRBV6-5	G	T	52	S/Y	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142180578	TRBV6-5	G	A	94	P/L	NON_SYNONYMOUS_CODING	benign(0.002)	tolerated(0.17)
7	142180588	TRBV6-5	C	G	91	E/Q	NON_SYNONYMOUS_CODING	benign(0.005)	tolerated(0.2)
7	142180647	TRBV6-5	G	T	71	A/D	NON_SYNONYMOUS_CODING	benign(0.08)	tolerated(0.18)
17	5036210	USP6	T	G	67	I/M	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0.04)
17	5036211	USP6	C	T	68	R/W	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0)

CHD6: Homozygous variants

CHR ID	NUCLEOTIDE POSITION	GENE NAME	REF SEQUENCE	ALTERNATE SEQUENCE	AMINO ACID NO.	RESIDUE CHANGE	FUNCTION OF VARIANT	POLYPHEN	SIFT
10	51748584	AGAP6	A	G	37	R/G	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.72)
2	97915896	ANKRD36	G	A	1940	E/K	NON_SYNONYMOUS_CODING	benign(0.043)	deleterious(0)
1	22315762	CELA3B	C	A	268	A/E	NON_SYNONYMOUS_CODING	benign(0.203)	tolerated(0.09)
8	7673126	DEFB107A	C	A	9	V/F	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
15	82932518	ENSG00000215749	T	C	502	E/G	NON_SYNONYMOUS_CODING	benign(0.003)	tolerated(0.24)
15	82932561	ENSG00000215749	T	C	488	S/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.737)	deleterious(0)
15	82934598	ENSG00000215749	A	G	314	C/R	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.4)
1	146215113	ENSG00000232637	G	A	84	H/Y	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.05)
1	146248970	ENSG00000232637	A	C	6	L/R	NON_SYNONYMOUS_CODING	benign(0.037)	tolerated(0.23)
15	32686392	ENSG00000249931	T	C	417	Q/R	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
10	47915891	FAM21B	C	A	270	S/Y	NON_SYNONYMOUS_CODING	benign(0.004)	tolerated(0.13)
10	47911590	FAM21B	G	A	193	G/D	NON_SYNONYMOUS_CODING	benign(0.282)	tolerated(0.14)
10	89120394	FAM22D	A	C	108	N/T	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.39)
9	97080827	FAM22F	C	G	565	G/R	NON_SYNONYMOUS_CODING	benign(0.424)	tolerated(0.07)
15	82637079	GOLGA6L10	C	T	336	R/Q	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(1)
15	34825132	GOLGA8B	C	G	67	R/P	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.21)
7	74212311	GTF2IRD2	G	T	61	H/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
6	32609236	HLA-DQA1	G	A	78	G/R	NON_SYNONYMOUS_CODING	benign(0.003)	tolerated(0.42)
22	17590582	IL17RA	G	C	773	A/P	NON_SYNONYMOUS_CODING	probably_damaging(0.959)	tolerated(0.08)
19	54725835	LILRB3	G	C	175	R/G	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.32)
4	151935787	LRBA	C	T	3	S/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.812)	deleterious(0)
17	44408066	LRRC37A	C	A	1141	F/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.11)
17	62892071	LRRC37A3	G	C	435	H/Q	NON_SYNONYMOUS_CODING	benign(0.003)	tolerated(0.66)
7	100647338	MUC12	C	A	4498	D/E	NON_SYNONYMOUS_CODING	benign(0.03)	.
7	100647339	MUC12	G	A	4499	A/T	NON_SYNONYMOUS_CODING	benign(0.104)	.

3	195512186	MUC4	T	C	2089	I/V	NON_SYNONYMOUS_CODING	benign(0.028)	.
3	195510389	MUC4	A	G	2688	S/P	NON_SYNONYMOUS_CODING	unknown(0)	.
1	145327548	NBPF10	A	G	1369	N/D	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
1	146409969	NBPF12	A	C	263	I/L	NON_SYNONYMOUS_CODING	benign(0.036)	deleterious(0)
9	102590845	NR4A3	A	C	185	D/A	NON_SYNONYMOUS_CODING	benign(0.381)	tolerated(0.31)
1	248737259	OR2T34	C	G	267	R/P	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
14	20010235	POTEM	A	G	393	V/A	NON_SYNONYMOUS_CODING	benign(0.006)	tolerated(1)
1	13695685	PRAMEF19	G	A	358	S/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.68)
1	13695787	PRAMEF19	C	G	324	G/A	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
1	13696022	PRAMEF19	A	G	246	W/R	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.25)
1	13329350	PRAMEF3	A	T	310	V/E	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.46)
1	13329354	PRAMEF3	T	C	309	K/E	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
2	108479432	RGPD4	G	A	805	A/T	NON_SYNONYMOUS_CODING	possibly_damaging(0.67)	tolerated(0.2)
2	179634421	TTN	T	G	2917	T/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.539)	.
17	46115072	COPZ2	C	CG	22	.	FRAMESHIFT_CODING	.	.
21	43298954	PRDM15	C	CG	88	.	FRAMESHIFT_CODING	.	.
4	152201016	PRSS48	T	TGGCAG	41	.	FRAMESHIFT_CODING	.	.

CHD6: Compound heterozygous variants

CHR ID	NUCLEOTIDE POSITION	GENE NAME	REF SEQUENCE	ALTERNATE SEQUENCE	AMINO ACID NO.	RESIDUE CHANGE	FUNCTION OF VARIANT	POLYPHEN	SIFT
19	58862835	A1BG	T	G	156	T/P	NON_SYNONYMOUS_CODING	probably_damaging(0.996)	deleterious(0.02)
19	58862796	A1BG	A	C	169	S/A	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0.03)
9	139911506	ABCA2	C	G	874	G/A	NON_SYNONYMOUS_CODING	probably_damaging(0.984)	deleterious(0.02)
9	139908380	ABCA2	C	G	1450	A/P	NON_SYNONYMOUS_CODING	probably_damaging(0.998)	deleterious(0.01)
10	101552060	ABCC2	C	A	93	Q/K	NON_SYNONYMOUS_CODING	benign(0.017)	tolerated(0.73)
10	101544396	ABCC2	A	C	22	D/A	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
10	101569915	ABCC2	G	A	614	E/K	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.48)
17	35631165	ACACA	A	T	214	N/K	NON_SYNONYMOUS_CODING	benign(0.016)	tolerated(0.94)
17	35445961	ACACA	G	T	976	Q/K	NON_SYNONYMOUS_CODING	benign(0.408)	tolerated(0.24)
17	35548174	ACACA	G	T	198	L/I	NON_SYNONYMOUS_CODING	possibly_damaging(0.919)	tolerated(0.23)
17	35445967	ACACA	C	T	974	E/K	NON_SYNONYMOUS_CODING	probably_damaging(0.973)	deleterious(0)
10	4879755	AKR1E2	C	A	188	F/L	NON_SYNONYMOUS_CODING	benign(0.055)	deleterious(0.03)
10	4875551	AKR1E2	A	C	73	T/P	NON_SYNONYMOUS_CODING	benign(0.426)	deleterious(0)
10	37482117	ANKRD30A	C	A	912	P/T	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
10	37482123	ANKRD30A	A	G	914	R/G	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.18)
10	37482118	ANKRD30A	C	A	912	P/H	NON_SYNONYMOUS_CODING	possibly_damaging(0.596)	deleterious(0.03)
5	94030820	ANKRD32	A	T	994	K/*	STOP_GAINED	.	.
5	94030836	ANKRD32	C	A	999	T/N	NON_SYNONYMOUS_CODING	benign(0.144)	deleterious(0.01)
5	94030832	ANKRD32	G	A	998	E/K	NON_SYNONYMOUS_CODING	probably_damaging(0.955)	deleterious(0)
2	97808524	ANKRD36	A	G	285	I/V	NON_SYNONYMOUS_CODING	benign(0.296)	tolerated(1)
2	97845632	ANKRD36	T	C	566	I/T	NON_SYNONYMOUS_CODING	possibly_damaging(0.767)	tolerated(0.23)
2	97808515	ANKRD36	G	A	282	E/K	NON_SYNONYMOUS_CODING	probably_damaging(0.959)	tolerated(1)
2	97808510	ANKRD36	G	A	280	G/D	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.85)
22	39498015	APOBEC3H	C	G	171	R/G	NON_SYNONYMOUS_CODING	benign(0.057)	deleterious(0.01)
22	39498014	APOBEC3H	T	G	170	S/R	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
1	155311840	ASH1L	C	A	2788	D/Y	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
1	155322556	ASH1L	C	G	2441	R/P	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
1	1423284	ATAD3B	A	G	419	K/R	NON_SYNONYMOUS_CODING	probably_damaging(0.966)	tolerated(0.25)
1	1423286	ATAD3B	C	G	420	R/G	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0.03)
1	1392550	ATAD3C	A	G	244	K/R	NON_SYNONYMOUS_CODING	benign(0.184)	tolerated(0.23)
1	1392552	ATAD3C	C	G	245	R/G	NON_SYNONYMOUS_CODING	probably_damaging(0.99)	deleterious(0)
12	14613915	ATF7IP	T	A	882	V/E	NON_SYNONYMOUS_CODING	possibly_damaging(0.837)	deleterious(0.01)
12	14613917	ATF7IP	C	A	883	Q/K	NON_SYNONYMOUS_CODING	possibly_damaging(0.882)	deleterious(0.04)
11	64666179	ATG2A	A	G	1338	S/P	NON_SYNONYMOUS_CODING	benign(0.403)	deleterious(0.02)
11	64666182	ATG2A	C	G	1337	A/P	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
11	63426568	ATL3	G	C	120	A/G	NON_SYNONYMOUS_CODING	probably_damaging(0.988)	deleterious(0.01)
11	63410966	ATL3	A	C	220	V/G	SPLICE_SITE:NON_SYNONYMOUS_CODING	benign(0.065)	deleterious(0.02)
11	108175544	ATM	C	G	1880	T/R	NON_SYNONYMOUS_CODING	benign(0.001)	deleterious(0.03)
11	108114752	ATM	T	A	190	I/K	NON_SYNONYMOUS_CODING	probably_damaging(0.991)	deleterious(0.03)
11	108114755	ATM	T	A	191	I/N	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
3	113524266	ATP6V1A	G	C	552	R/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.902)	tolerated(0.09)

3	113508633	ATP6V1A	G	T	312	V/L	NON_SYNONYMOUS_CODING	probably_damaging(0.995)	deleterious(0)
20	3565431	ATRN	C	A	956	Q/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.72)
20	3565356	ATRN	T	G	931	C/G	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
21	30969925	BACH1	A	G	184	M/V	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0.01)
21	30969926	BACH1	T	G	184	M/R	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0)
6	70048890	BAI3	A	C	55	T/P	NON_SYNONYMOUS_CODING	probably_damaging(0.998)	deleterious(0.03)
6	69758083	BAI3	G	A	705	S/N	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
15	73023727	BBS4	G	T	93	A/S	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.8)
15	73023725	BBS4	G	T	92	C/F	NON_SYNONYMOUS_CODING	benign(0.119)	tolerated(0.07)
1	147095726	BCL9	A	C	1083	T/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.899)	tolerated(0.1)
1	147092352	BCL9	G	T	797	L/F	NON_SYNONYMOUS_CODING	possibly_damaging(0.91)	tolerated(0.13)
1	147092353	BCL9	C	T	798	R/W	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.19)
5	137503739	BRD8	G	A	224	S/F	NON_SYNONYMOUS_CODING	probably_damaging(0.996)	deleterious(0.01)
5	137503737	BRD8	C	A	225	G/C	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.06)
3	9785283	BRPF1	T	G	772	V/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.916)	deleterious(0.01)
3	9786696	BRPF1	A	C	969	L/F	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	tolerated(0.09)
17	56385918	BZRAP1	T	C	1572	K/R	NON_SYNONYMOUS_CODING	possibly_damaging(0.485)	tolerated(0.19)
17	56386383	BZRAP1	C	CTT	1417	S/KS	FRAMESHIFT_CODING	.	.
12	112667600	C12orf51	T	C	1719	K/E	NON_SYNONYMOUS_CODING	possibly_damaging(0.662)	.
12	112667597	C12orf51	G	T	1720	L/I	NON_SYNONYMOUS_CODING	possibly_damaging(0.806)	.
12	112650430	C12orf51	T	C	242	E/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.875)	tolerated(0.17)
12	112688167	C12orf51	A	C	822	V/G	NON_SYNONYMOUS_CODING	probably_damaging(0.979)	.
12	112688164	C12orf51	T	C	823	E/G	NON_SYNONYMOUS_CODING	probably_damaging(0.98)	.
20	18433273	C20orf12	T	G	179	T/P	NON_SYNONYMOUS_CODING	benign(0.005)	tolerated(0.15)
20	18433277	C20orf12	T	G	25	H/P	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0)
11	73789554	C2CD3	C	G	211	E/D	NON_SYNONYMOUS_CODING	possibly_damaging(0.485)	tolerated(0.52)
11	73760510	C2CD3	A	C	553	Y/D	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
2	231911622	C2orf72	G	C	258	E/D	NON_SYNONYMOUS_CODING	possibly_damaging(0.741)	tolerated(0.42)
2	231911621	C2orf72	A	C	258	E/A	NON_SYNONYMOUS_CODING	possibly_damaging(0.875)	tolerated(0.6)
3	126915976	C3orf56	T	C	150	S/P	NON_SYNONYMOUS_CODING	benign(0.278)	.
3	126915973	C3orf56	A	C	149	T/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.449)	.
4	100434303	C4orf17	G	A	22	R/K	NON_SYNONYMOUS_CODING	possibly_damaging(0.942)	tolerated(0.25)
4	100434307	C4orf17	T	A	23	N/K	NON_SYNONYMOUS_CODING	probably_damaging(0.995)	deleterious(0)
3	53839025	CACNA1D	C	A	1887	Y/*	STOP_GAINED	.	.
3	53700459	CACNA1D	T	G	24	V/G	NON_SYNONYMOUS_CODING	benign(0.013)	tolerated(0.2)
3	53756421	CACNA1D	T	G	243	V/G	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
3	49897074	CAMKV	T	G	321	T/P	NON_SYNONYMOUS_CODING	probably_damaging(0.958)	tolerated(0.08)
3	49896952	CAMKV	T	G	367	H/P	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.24)
9	138713600	CAMSAP1	G	C	969	H/Q	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.52)
9	138714963	CAMSAP1	T	G	515	N/T	NON_SYNONYMOUS_CODING	benign(0.284)	tolerated(0.05)
19	38851455	CATSPERG	A	C	618	T/P	NON_SYNONYMOUS_CODING	benign(0.18)	tolerated(0.51)
19	38851193	CATSPERG	T	G	514	C/G	NON_SYNONYMOUS_CODING	unknown(0)	.
17	77808570	CBX4	A	G	291	S/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.727)	deleterious(0.04)
17	77808572	CBX4	C	G	290	R/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.94)	tolerated(0.14)
2	219893096	CCDC108	T	G	494	T/P	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0.01)
2	219883763	CCDC108	C	A	113	L/F	NON_SYNONYMOUS_CODING	unknown(0)	.

19	49898378	CCDC155	C	G	55	A/G	NON_SYNONYMOUS_CODING	benign(0.429)	tolerated(0.07)
19	49898375	CCDC155	T	G	54	V/G	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
17	78069197	CCDC40	G	A	990	D/N	NON_SYNONYMOUS_CODING	benign(0.027)	tolerated(0.41)
17	78073435	CCDC40	G	C	1097	R/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.936)	tolerated(0.3)
13	37012869	CCNA1	A	G	252	E/G	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
13	37012872	CCNA1	T	G	253	V/G	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
19	14507228	CD97	A	C	141	T/P	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.28)
19	14515315	CD97	A	C	475	T/P	NON_SYNONYMOUS_CODING	probably_damaging(0.996)	tolerated(0.08)
17	45234417	CDC27	A	G	235	I/T	NON_SYNONYMOUS_CODING	benign(0.004)	tolerated(0.19)
17	45234303	CDC27	G	C	273	A/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.783)	tolerated(0.07)
1	109801674	CELSR2	A	C	1311	T/P	NON_SYNONYMOUS_CODING	probably_damaging(0.994)	deleterious(0.01)
1	109794709	CELSR2	G	C	670	A/P	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
20	34082403	CEP250	T	G	973	V/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.805)	deleterious(0)
20	34090351	CEP250	G	C	1329	R/P	NON_SYNONYMOUS_CODING	probably_damaging(0.998)	tolerated(0.14)
17	7796794	CHD3	A	C	293	I/L	NON_SYNONYMOUS_CODING	benign(0)	.
17	7796803	CHD3	T	C	296	S/P	NON_SYNONYMOUS_CODING	benign(0.001)	.
1	6185909	CHD5	T	C	771	D/G	NON_SYNONYMOUS_CODING	benign(0.009)	deleterious(0.03)
1	6185908	CHD5	A	C	771	D/E	NON_SYNONYMOUS_CODING	benign(0.024)	tolerated(0.27)
15	101717654	CHSY1	T	C	783	E/G	NON_SYNONYMOUS_CODING	benign(0.177)	deleterious(0.03)
15	101717647	CHSY1	A	T	785	N/K	NON_SYNONYMOUS_CODING	possibly_damaging(0.452)	tolerated(0.18)
16	74446721	CLEC18B	C	G	165	S/T	NON_SYNONYMOUS_CODING	benign(0.08)	deleterious(0.04)
16	74446719	CLEC18B	T	G	166	T/P	NON_SYNONYMOUS_CODING	probably_damaging(0.996)	deleterious(0.03)
22	19213150	CLTCL1	C	A	652	V/F	NON_SYNONYMOUS_CODING	benign(0.419)	deleterious(0)
22	19213138	CLTCL1	C	A	656	G/C	NON_SYNONYMOUS_CODING	probably_damaging(0.996)	deleterious(0)
5	79025224	CMYA5	C	A	212	H/Q	NON_SYNONYMOUS_CODING	probably_damaging(0.959)	deleterious(0)
5	79034586	CMYA5	C	A	3333	T/N	NON_SYNONYMOUS_CODING	probably_damaging(0.988)	deleterious(0)
7	147926842	CNTNAP2	A	C	177	T/P	NON_SYNONYMOUS_CODING	probably_damaging(0.961)	deleterious(0.04)
7	147183121	CNTNAP2	A	C	589	T/P	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
17	48264043	COL1A1	T	G	1258	T/P	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0)
17	48264054	COL1A1	T	G	1254	N/T	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0)
17	14110449	COX10	G	C	417	W/C	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
17	14110451	COX10	A	C	418	H/P	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
10	93870839	CPEB3	G	T	522	D/E	NON_SYNONYMOUS_CODING	benign(0.111)	tolerated(0.06)
10	93870841	CPEB3	C	T	522	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.873)	deleterious(0.03)
9	99798872	CTSL2	C	T	185	R/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.46)
9	99797018	CTSL2	C	G	299	V/L	NON_SYNONYMOUS_CODING	benign(0.212)	deleterious(0)
6	43013806	CUL7	C	T	979	S/N	NON_SYNONYMOUS_CODING	benign(0.014)	deleterious(0.01)
6	43006603	CUL7	G	T	1557	L/M	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
11	6645186	DCHS1	G	C	2574	A/G	NON_SYNONYMOUS_CODING	probably_damaging(0.984)	tolerated(0.17)
11	6661865	DCHS1	A	C	327	V/G	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	tolerated(0.11)
1	155004214	DCST2	A	G	192	L/P	NON_SYNONYMOUS_CODING	probably_damaging(0.957)	tolerated(0.32)
1	155005949	DCST2	G	C	77	R/G	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0.01)
1	162745952	DDR2	C	A	692	T/N	NON_SYNONYMOUS_CODING	benign(0.027)	deleterious(0.02)
1	162745958	DDR2	T	A	694	I/N	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
14	94517736	DDX24	T	G	751	H/P	NON_SYNONYMOUS_CODING	benign(0.35)	tolerated(0.2)
14	94517728	DDX24	A	G	754	S/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.746)	deleterious(0.01)

14	94521529	DDX24	A	C	621	V/G	SPLICE_SITE:NON_SYNONYMOUS_CODING	probably_damaging(0.998)	deleterious(0)
13	42734171	DGKH	G	T	116	E/*	STOP_GAINED	.	.
13	42742929	DGKH	C	G	312	R/G	NON_SYNONYMOUS_CODING	probably_damaging(0.977)	deleterious(0.01)
3	38151765	DLEC1	A	C	1146	T/P	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.32)
3	38125677	DLEC1	C	T	401	T/M	NON_SYNONYMOUS_CODING	possibly_damaging(0.937)	deleterious(0.01)
3	38157998	DLEC1	T	G	1304	V/G	NON_SYNONYMOUS_CODING	probably_damaging(0.989)	deleterious(0)
17	76503840	DNAH17	G	C	1425	H/Q	NON_SYNONYMOUS_CODING	benign(0.01)	tolerated(0.17)
17	76421666	DNAH17	C	G	1509	V/L	NON_SYNONYMOUS_CODING	benign(0.047)	tolerated(0.19)
5	13866368	DNAH5	C	A	1359	L/F	NON_SYNONYMOUS_CODING	benign(0.407)	deleterious(0.01)
5	13754379	DNAH5	C	A	3496	L/F	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
6	38816506	DNAH8	G	C	1493	V/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.86)
6	38917321	DNAH8	A	C	3858	T/P	NON_SYNONYMOUS_CODING	benign(0.023)	tolerated(0.38)
12	56221271	DNAJC14	A	C	391	V/G	NON_SYNONYMOUS_CODING	probably_damaging(0.963)	deleterious(0)
12	56221154	DNAJC14	C	G	430	R/P	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
11	6567433	DNHD1	G	A	46	R/H	NON_SYNONYMOUS_CODING	possibly_damaging(0.449)	deleterious(0)
11	6566731	DNHD1	C	G	1521	A/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.616)	tolerated(0.45)
11	6588672	DNHD1	G	C	3978	R/P	NON_SYNONYMOUS_CODING	probably_damaging(0.996)	tolerated(0.14)
19	10935797	DNM2	T	G	260	V/G	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
19	10935800	DNM2	A	G	261	E/G	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
8	25216495	DOCK5	G	T	956	C/F	NON_SYNONYMOUS_CODING	probably_damaging(0.991)	deleterious(0.04)
8	25216524	DOCK5	G	C	966	D/H	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0.04)
8	25216525	DOCK5	A	C	966	D/A	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.36)
21	41648091	DSCAM	G	T	515	Y/*	STOP_GAINED	.	.
21	41514605	DSCAM	G	T	848	Q/K	NON_SYNONYMOUS_CODING	benign(0.042)	tolerated(0.91)
13	41515337	ELF1	T	G	326	T/P	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.08)
13	41556183	ELF1	G	C	3	A/G	NON_SYNONYMOUS_CODING	benign(0.046)	.
16	15457629	ENSG00000183793	T	A	314	T/S	NON_SYNONYMOUS_CODING	benign(0.158)	tolerated(0.82)
16	15457568	ENSG00000183793	C	A	334	C/F	NON_SYNONYMOUS_CODING	benign(0.347)	deleterious(0)
16	15457628	ENSG00000183793	G	T	314	T/N	NON_SYNONYMOUS_CODING	benign(0.348)	tolerated(0.39)
16	15474873	ENSG00000183793	C	T	11	R/Q	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(1)
18	11619510	ENSG00000257513	A	C	351	*/E	STOP_LOST	.	.
18	11619549	ENSG00000257513	T	G	338	K/Q	NON_SYNONYMOUS_CODING	probably_damaging(0.998)	deleterious(0.03)
1	38227650	EPHA10	T	C	93	I/V	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.21)
1	38227136	EPHA10	A	C	264	V/G	NON_SYNONYMOUS_CODING	probably_damaging(0.991)	deleterious(0)
1	16474932	EPHA2	A	C	255	V/G	NON_SYNONYMOUS_CODING	benign(0.006)	deleterious(0)
1	16474930	EPHA2	G	C	256	P/A	NON_SYNONYMOUS_CODING	probably_damaging(0.994)	deleterious(0.02)
4	66467416	EPHA5	G	C	285	P/A	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0.03)
4	66467418	EPHA5	A	C	284	V/G	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
1	6504703	ESPN	T	C	385	S/P	NON_SYNONYMOUS_CODING	probably_damaging(0.998)	deleterious(0)
1	6488413	ESPN	C	T	141	P/L	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0.01)
16	74761211	FA2H	T	G	146	H/P	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0.01)
16	74761247	FA2H	A	C	134	V/G	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0.02)
6	71187018	FAM135A	A	C	131	T/P	NON_SYNONYMOUS_CODING	benign(0.013)	deleterious(0)
6	71187020	FAM135A	A	C	133	H/P	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0.01)
15	41043735	FAM82A2	C	G	138	R/P	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.15)
15	41029923	FAM82A2	A	C	376	V/G	SPLICE_SITE:NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)

2	207651481	FASTKD2	T	A	484	S/R	NON_SYNONYMOUS_CODING	benign(0.291)	deleterious(0.05)
2	207651480	FASTKD2	G	A	484	S/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.712)	tolerated(0.34)
11	92616488	FAT3	T	C	624	L/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.542)	tolerated(0.12)
11	92616485	FAT3	A	C	623	N/T	NON_SYNONYMOUS_CODING	probably_damaging(0.978)	tolerated(1)
3	13679659	FBLN2	T	G	106	V/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.811)	deleterious(0)
3	13677992	FBLN2	A	C	60	T/P	NON_SYNONYMOUS_CODING	probably_damaging(0.961)	deleterious(0.01)
7	100187851	FBXO24	A	C	65	T/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.462)	deleterious(0)
7	100192051	FBXO24	A	C	266	D/A	NON_SYNONYMOUS_CODING	possibly_damaging(0.907)	deleterious(0.03)
19	40367831	FCGBP	T	G	4377	T/P	NON_SYNONYMOUS_CODING	benign(0.097)	tolerated(0.21)
19	40384149	FCGBP	T	G	3154	N/T	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.4)
6	167417226	FGFR1OP	G	A	76	S/N	NON_SYNONYMOUS_CODING	benign(0.064)	tolerated(0.41)
6	167417227	FGFR1OP	T	A	76	S/R	NON_SYNONYMOUS_CODING	probably_damaging(0.972)	tolerated(0.15)
9	117995	FOXD4	G	C	42	A/G	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.06)
9	118001	FOXD4	T	C	40	E/G	NON_SYNONYMOUS_CODING	benign(0)	deleterious(0)
4	48559517	FRYL	G	T	231	Q/K	NON_SYNONYMOUS_CODING	benign(0.042)	deleterious(0.02)
4	48559519	FRYL	G	T	230	P/Q	NON_SYNONYMOUS_CODING	probably_damaging(0.97)	tolerated(0.05)
4	48583513	FRYL	C	G	405	R/P	NON_SYNONYMOUS_CODING	probably_damaging(0.991)	deleterious(0.01)
4	22737710	GBA3	C	G	55	S/R	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.12)
4	22694669	GBA3	G	C	11	A/P	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0.01)
14	39591707	GEMIN2	G	C	160	G/A	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.78)
14	39601192	GEMIN2	C	T	222	L/F	NON_SYNONYMOUS_CODING	probably_damaging(0.958)	deleterious(0.01)
14	39601187	GEMIN2	C	CT	220	.	FRAMESHIFT_CODING	.	.
1	231411911	GNPAT	A	C	595	E/D	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.19)
1	231413267	GNPAT	A	C	613	K/N	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
5	89986671	GPR98	A	C	2255	N/T	NON_SYNONYMOUS_CODING	benign(0.361)	tolerated(0.57)
5	89949754	GPR98	G	A	1455	E/K	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
1	186094789	HMCN1	C	A	4185	Q/K	NON_SYNONYMOUS_CODING	benign(0.059)	tolerated(0.7)
1	186062678	HMCN1	C	A	3358	T/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.909)	tolerated(0.35)
1	186052030	HMCN1	T	G	2941	Y/D	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
1	22175151	HSPG2	T	G	2574	L/F	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0.01)
1	22199879	HSPG2	T	TG	116	.	FRAMESHIFT_CODING	.	.
4	3136225	HTT	T	G	864	V/G	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.12)
4	3230343	HTT	T	G	2617	V/G	SPLICE_SITE:NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
14	106573357	IGHV3-11	G	T	76	T/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.62)
14	106573358	IGHV3-11	T	A	76	T/S	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
14	106993935	IGHV3-48	A	G	77	I/T	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
14	106994010	IGHV3-48	C	T	52	S/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.29)
14	106993933	IGHV3-48	A	T	78	Y/N	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.23)
14	107113742	IGHV3-64	C	T	118	R/K	NON_SYNONYMOUS_CODING	benign(0.002)	tolerated(0.18)
14	107113745	IGHV3-64	G	A	117	A/V	NON_SYNONYMOUS_CODING	benign(0.003)	tolerated(0.06)
14	106478321	IGHV4-4	C	T	46	G/D	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.06)
14	106478298	IGHV4-4	T	C	54	S/G	NON_SYNONYMOUS_CODING	benign(0.004)	tolerated(0.51)
14	106478322	IGHV4-4	C	A	46	G/C	NON_SYNONYMOUS_CODING	benign(0.096)	deleterious(0)
14	106478310	IGHV4-4	T	C	50	S/G	NON_SYNONYMOUS_CODING	benign(0.1)	tolerated(0.08)
14	107034869	IGHV5-51	A	C	71	Y/D	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.32)
14	107034873	IGHV5-51	G	C	69	I/M	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.22)

14	107034874	IGHV5-51	A	C	69	I/S	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.41)
1	18618361	IGSF21	T	G	62	V/G	SPLICE_SITE:NON_SYNONYMOUS_CODING	probably_damaging(0.998)	deleterious(0)
1	18691718	IGSF21	T	G	181	V/G	SPLICE_SITE:NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0.01)
7	50467818	IKZF1	C	A	351	H/Q	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.41)
7	50455118	IKZF1	G	C	222	R/P	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
14	105174274	INF2	T	G	25	V/G	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.09)
14	105180963	INF2	C	T	1155	A/V	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0)
7	41729523	INHBA	T	C	336	N/D	NON_SYNONYMOUS_CODING	benign(0.074)	deleterious(0.02)
7	41729465	INHBA	G	C	355	P/R	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
3	4725969	ITPR1	G	A	1159	G/E	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.22)
3	4725973	ITPR1	T	A	1160	N/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.94)
3	4725976	ITPR1	C	A	1161	N/K	NON_SYNONYMOUS_CODING	benign(0.004)	tolerated(0.94)
3	124114087	KALRN	A	C	688	T/P	NON_SYNONYMOUS_CODING	benign(0.119)	deleterious(0.03)
3	124044867	KALRN	C	T	376	S/F	NON_SYNONYMOUS_CODING	possibly_damaging(0.893)	deleterious(0.05)
17	61611421	KCNH6	G	A	284	D/N	NON_SYNONYMOUS_CODING	benign(0.003)	tolerated(0.08)
17	61611569	KCNH6	A	C	333	H/P	NON_SYNONYMOUS_CODING	probably_damaging(0.979)	deleterious(0.02)
17	61613079	KCNH6	T	G	384	V/G	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	tolerated(0.2)
3	183396927	KLHL24	AC	A	553	.	FRAMESHIFT_CODING	.	.
3	183396938	KLHL24	G	GA	556	.	FRAMESHIFT_CODING	.	.
3	183226121	KLHL6	T	G	212	D/A	NON_SYNONYMOUS_CODING	benign(0)	deleterious(0.01)
3	183210456	KLHL6	T	G	464	T/P	NON_SYNONYMOUS_CODING	benign(0.013)	deleterious(0.02)
4	88106903	KLHL8	T	A	89	I/F	NON_SYNONYMOUS_CODING	benign(0.139)	deleterious(0.02)
4	88106900	KLHL8	G	A	90	P/S	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	tolerated(0.15)
12	53044334	KRT2	G	C	197	R/G	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
12	53044336	KRT2	A	C	196	V/G	SPLICE_SITE:NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
14	50750696	L2HGDH	G	C	199	A/G	NON_SYNONYMOUS_CODING	benign(0.325)	deleterious(0.04)
14	50750699	L2HGDH	A	C	198	V/G	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
6	129828682	LAMA2	G	C	936	A/P	NON_SYNONYMOUS_CODING	benign(0.305)	tolerated(0.25)
6	129513846	LAMA2	T	G	544	Y/D	NON_SYNONYMOUS_CODING	possibly_damaging(0.893)	deleterious(0.01)
18	21364082	LAMA3	A	C	520	T/P	NON_SYNONYMOUS_CODING	benign(0.003)	tolerated(0.27)
18	21519304	LAMA3	A	C	2994	T/P	NON_SYNONYMOUS_CODING	probably_damaging(0.996)	tolerated(0.18)
1	209796950	LAMB3	A	C	753	V/G	NON_SYNONYMOUS_CODING	benign(0.001)	deleterious(0.04)
1	209791293	LAMB3	T	G	1004	T/P	NON_SYNONYMOUS_CODING	probably_damaging(0.954)	deleterious(0.01)
2	141259399	LRP1B	A	C	2841	C/G	NON_SYNONYMOUS_CODING	probably_damaging(0.995)	deleterious(0)
2	141625828	LRP1B	G	A	1330	L/F	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0.01)
1	160783621	LY9	A	C	217	D/A	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.22)
1	160784275	LY9	A	C	266	T/P	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
1	235914653	LYST	A	C	60	V/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.856)	deleterious(0)
1	235955172	LYST	T	G	1457	H/P	NON_SYNONYMOUS_CODING	probably_damaging(1)	.
7	20198700	MACC1	G	T	428	D/E	NON_SYNONYMOUS_CODING	benign(0.003)	tolerated(0.57)
7	20198702	MACC1	C	T	428	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.604)	tolerated(0.18)
1	39945661	MACF1	A	C	284	T/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.564)	tolerated(0.16)
1	39934315	MACF1	T	G	190	V/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.839)	deleterious(0)
1	117944954	MAN1A2	T	A	150	I/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.96)
1	117944964	MAN1A2	C	A	153	N/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.96)
15	43816035	MAP1A	A	C	788	Q/H	NON_SYNONYMOUS_CODING	probably_damaging(0.998)	deleterious(0)

15	43821989	MAP1A	A	C	2726	D/A	NON_SYNONYMOUS_CODING	probably_damaging(0.998)	deleterious(0)
5	71494945	MAP1B	C	A	1921	Y/*	STOP_GAINED	.	.
5	71491052	MAP1B	C	A	641	Q/K	NON_SYNONYMOUS_CODING	benign(0.007)	tolerated(1)
5	71490953	MAP1B	G	A	608	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.792)	tolerated(0.27)
5	71490955	MAP1B	C	A	608	D/E	NON_SYNONYMOUS_CODING	possibly_damaging(0.792)	tolerated(0.67)
21	47666659	MCM3AP	A	C	1478	S/A	NON_SYNONYMOUS_CODING	probably_damaging(0.973)	deleterious(0)
21	47665086	MCM3AP	A	C	1558	V/G	NON_SYNONYMOUS_CODING	probably_damaging(0.975)	tolerated(0.63)
15	41961151	MGA	C	G	20	A/G	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.3)
15	42040862	MGA	C	A	1747	T/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.812)	deleterious(0)
15	42000043	MGA	A	C	769	N/T	NON_SYNONYMOUS_CODING	possibly_damaging(0.911)	deleterious(0)
11	12315177	MICALCL	A	G	67	R/G	NON_SYNONYMOUS_CODING	benign(0.09)	deleterious(0)
11	12315180	MICALCL	C	G	68	R/G	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
10	129901721	MKI67	G	T	2794	Q/K	NON_SYNONYMOUS_CODING	possibly_damaging(0.806)	tolerated(0.13)
10	129901067	MKI67	G	C	3012	R/G	NON_SYNONYMOUS_CODING	probably_damaging(0.986)	tolerated(0.38)
7	151927025	MLL3	A	G	987	Y/H	NON_SYNONYMOUS_CODING	probably_damaging(1)	.
7	151932997	MLL3	C	T	892	G/R	NON_SYNONYMOUS_CODING	probably_damaging(1)	.
11	19077079	MRGPRX2	G	C	291	L/V	NON_SYNONYMOUS_CODING	benign(0.005)	tolerated(0.08)
11	19077074	MRGPRX2	C	G	292	Q/H	NON_SYNONYMOUS_CODING	benign(0.115)	deleterious(0.01)
7	100636338	MUC12	T	C	832	S/P	NON_SYNONYMOUS_CODING	probably_damaging(0.979)	.
7	100634922	MUC12	G	A	360	A/T	NON_SYNONYMOUS_CODING	unknown(0)	.
19	9002623	MUC16	C	T	39	R/H	NON_SYNONYMOUS_CODING	benign(0.011)	tolerated(0.24)
19	9002636	MUC16	C	T	35	V/I	NON_SYNONYMOUS_CODING	benign(0.051)	tolerated(0.37)
19	9002612	MUC16	T	G	43	K/Q	NON_SYNONYMOUS_CODING	benign(0.055)	tolerated(0.23)
19	8961997	MUC16	C	A	1101	L/F	NON_SYNONYMOUS_CODING	possibly_damaging(0.448)	deleterious(0)
19	9002659	MUC16	C	T	27	G/E	NON_SYNONYMOUS_CODING	possibly_damaging(0.582)	tolerated(0.19)
19	9002597	MUC16	C	T	48	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.866)	deleterious(0)
19	9002660	MUC16	C	T	27	G/R	NON_SYNONYMOUS_CODING	probably_damaging(0.998)	tolerated(0.5)
19	9059082	MUC16	C	A	9455	S/I	NON_SYNONYMOUS_CODING	unknown(0)	.
19	9063517	MUC16	A	G	7977	S/P	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195346803	MUC20	T	G	369	S/A	NON_SYNONYMOUS_CODING	possibly_damaging(0.891)	tolerated(0.14)
3	195346337	MUC20	G	C	213	W/C	NON_SYNONYMOUS_CODING	possibly_damaging(0.952)	tolerated(0.06)
3	195513846	MUC4	C	G	1535	M/I	NON_SYNONYMOUS_CODING	benign(0.012)	.
3	195513847	MUC4	A	G	1535	M/T	NON_SYNONYMOUS_CODING	benign(0.042)	.
3	195506549	MUC4	A	G	3968	S/P	NON_SYNONYMOUS_CODING	benign(0.044)	.
3	195507226	MUC4	A	G	3742	V/A	NON_SYNONYMOUS_CODING	benign(0.095)	.
3	195507673	MUC4	A	G	3593	V/A	NON_SYNONYMOUS_CODING	benign(0.095)	.
3	195508790	MUC4	T	C	3221	S/G	NON_SYNONYMOUS_CODING	benign(0.095)	.
3	195505859	MUC4	T	C	4198	T/A	NON_SYNONYMOUS_CODING	benign(0.182)	.
3	195506099	MUC4	T	C	4118	T/A	NON_SYNONYMOUS_CODING	benign(0.182)	.
3	195506987	MUC4	T	C	3822	T/A	NON_SYNONYMOUS_CODING	benign(0.182)	.
3	195508667	MUC4	T	C	3262	T/A	NON_SYNONYMOUS_CODING	benign(0.182)	.
3	195506582	MUC4	C	T	3957	D/N	NON_SYNONYMOUS_CODING	benign(0.183)	.
3	195508789	MUC4	C	T	3221	S/N	NON_SYNONYMOUS_CODING	benign(0.204)	.
3	195515017	MUC4	A	G	1145	V/A	NON_SYNONYMOUS_CODING	benign(0.231)	.
3	195506569	MUC4	A	G	3961	V/A	NON_SYNONYMOUS_CODING	benign(0.243)	.
3	195508787	MUC4	G	T	3222	L/I	NON_SYNONYMOUS_CODING	benign(0.352)	.

3	195510194	MUC4	G	C	2753	L/V	NON_SYNONYMOUS_CODING	benign(0.352)	.
3	195510636	MUC4	C	A	2605	Q/H	NON_SYNONYMOUS_CODING	benign(0.391)	.
3	195508661	MUC4	A	G	3264	S/P	NON_SYNONYMOUS_CODING	benign(0.421)	.
3	195508709	MUC4	A	G	3248	S/P	NON_SYNONYMOUS_CODING	benign(0.421)	.
3	195506533	MUC4	C	A	3973	S/I	NON_SYNONYMOUS_CODING	possibly_damaging(0.473)	.
3	195505849	MUC4	G	A	4201	A/V	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195507062	MUC4	C	T	3797	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195507193	MUC4	G	A	3753	A/V	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195507385	MUC4	G	A	3689	A/V	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195507434	MUC4	C	A	3673	A/S	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195507446	MUC4	C	T	3669	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195508500	MUC4	G	C	3317	D/E	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195508668	MUC4	G	C	3261	D/E	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195510649	MUC4	G	A	2601	A/V	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195506983	MUC4	G	A	3823	T/I	NON_SYNONYMOUS_CODING	possibly_damaging(0.607)	.
3	195507443	MUC4	T	G	3670	T/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.607)	.
3	195507475	MUC4	G	C	3659	T/R	NON_SYNONYMOUS_CODING	possibly_damaging(0.607)	.
3	195507827	MUC4	G	A	3542	L/F	NON_SYNONYMOUS_CODING	possibly_damaging(0.607)	.
3	195511525	MUC4	T	C	2309	D/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.748)	.
3	195511526	MUC4	C	T	2309	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.748)	.
3	195507604	MUC4	C	G	3616	R/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.811)	.
3	195506510	MUC4	G	C	3981	H/D	NON_SYNONYMOUS_CODING	possibly_damaging(0.817)	.
3	195507445	MUC4	T	A	3669	D/V	NON_SYNONYMOUS_CODING	possibly_damaging(0.862)	.
3	195507694	MUC4	A	G	3586	L/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.881)	.
3	195508786	MUC4	A	G	3222	L/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.881)	.
3	195506294	MUC4	T	C	4053	N/D	NON_SYNONYMOUS_CODING	possibly_damaging(0.9)	.
3	195506522	MUC4	C	A	3977	A/S	NON_SYNONYMOUS_CODING	possibly_damaging(0.916)	.
3	195505870	MUC4	G	A	4194	P/L	NON_SYNONYMOUS_CODING	possibly_damaging(0.931)	.
3	195506990	MUC4	C	G	3821	D/H	NON_SYNONYMOUS_CODING	possibly_damaging(0.934)	.
3	195508670	MUC4	C	G	3261	D/H	NON_SYNONYMOUS_CODING	possibly_damaging(0.934)	.
3	195515414	MUC4	T	C	1013	S/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.943)	.
3	195506554	MUC4	G	A	3966	A/V	NON_SYNONYMOUS_CODING	probably_damaging(0.959)	.
3	195515449	MUC4	A	T	1001	V/E	NON_SYNONYMOUS_CODING	probably_damaging(0.979)	.
3	195510622	MUC4	G	T	2610	P/H	NON_SYNONYMOUS_CODING	probably_damaging(0.991)	.
3	195510910	MUC4	G	T	2514	P/H	NON_SYNONYMOUS_CODING	probably_damaging(0.997)	.
3	195510310	MUC4	T	G	2714	Y/S	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510341	MUC4	A	G	2704	S/P	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510347	MUC4	T	C	2702	T/A	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510350	MUC4	C	G	2701	D/H	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510373	MUC4	T	C	2693	D/G	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510374	MUC4	C	T	2693	D/N	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510396	MUC4	A	C	2685	H/Q	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510415	MUC4	G	T	2679	S/Y	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510430	MUC4	G	T	2674	P/H	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510437	MUC4	G	A	2672	P/S	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510466	MUC4	A	G	2662	L/P	NON_SYNONYMOUS_CODING	unknown(0)	.

3	195510515	MUC4	C	T	2646	A/T	NON_SYNONYMOUS_CODING	unknown(0)	.
11	1263706	MUC5B	T	G	1866	F/V	NON_SYNONYMOUS_CODING	benign(0.014)	.
11	1263847	MUC5B	A	C	1913	T/P	NON_SYNONYMOUS_CODING	benign(0.049)	.
11	1262540	MUC5B	C	T	1477	T/M	NON_SYNONYMOUS_CODING	probably_damaging(0.985)	.
11	1269643	MUC5B	A	G	3845	R/G	NON_SYNONYMOUS_CODING	unknown(0)	.
11	1270876	MUC5B	A	C	4256	T/P	NON_SYNONYMOUS_CODING	unknown(0)	.
11	1283520	MUC5B	A	C	5468	D/A	NON_SYNONYMOUS_CODING	unknown(0)	.
11	1283527	MUC5B	G	C	5470	Q/H	NON_SYNONYMOUS_CODING	unknown(0)	.
11	1016957	MUC6	T	G	1948	R/S	NON_SYNONYMOUS_CODING	benign(0.034)	tolerated(0.84)
11	1017466	MUC6	T	C	1779	R/G	NON_SYNONYMOUS_CODING	benign(0.142)	tolerated(0.57)
11	1016919	MUC6	C	T	1961	R/K	NON_SYNONYMOUS_CODING	possibly_damaging(0.496)	tolerated(0.33)
11	1018042	MUC6	A	G	1587	S/P	NON_SYNONYMOUS_CODING	probably_damaging(0.979)	deleterious(0.01)
11	1017655	MUC6	G	A	1716	P/S	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.14)
11	1016554	MUC6	C	T	2083	A/T	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.6)
11	1016559	MUC6	G	C	2081	A/G	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.59)
11	1016565	MUC6	C	T	2079	S/N	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.09)
11	1017596	MUC6	T	G	1735	Q/H	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.18)
11	1017604	MUC6	T	G	1733	T/P	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.36)
11	1018552	MUC6	G	A	1417	P/S	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.73)
11	1018559	MUC6	A	C	1414	S/R	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.05)
11	1018561	MUC6	T	C	1414	S/G	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.07)
11	1018566	MUC6	G	C	1412	A/G	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.29)
11	1019308	MUC6	T	G	1333	T/P	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0)
11	1024039	MUC6	T	C	1097	D/G	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0.01)
13	77673117	MYCBP2	T	G	2686	K/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.46)
13	77672809	MYCBP2	A	G	2789	L/P	NON_SYNONYMOUS_CODING	benign(0.017)	deleterious(0.03)
13	77672228	MYCBP2	G	T	2983	Q/K	NON_SYNONYMOUS_CODING	benign(0.021)	tolerated(0.45)
13	77745669	MYCBP2	G	C	1880	R/G	NON_SYNONYMOUS_CODING	probably_damaging(0.98)	deleterious(0.02)
15	72192270	MYO9A	A	T	1057	S/R	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.63)
15	72192271	MYO9A	C	T	1057	S/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.55)
18	3129302	MYOM1	T	G	908	T/P	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.1)
18	3112368	MYOM1	G	C	1116	R/G	NON_SYNONYMOUS_CODING	probably_damaging(0.997)	deleterious(0.01)
1	145367800	NBPF10	G	A	586	D/N	NON_SYNONYMOUS_CODING	benign(0.009)	deleterious(0.03)
1	145304625	NBPF10	T	G	445	W/G	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.09)
1	145304635	NBPF10	C	T	448	A/V	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0)
22	37260160	NCF4	A	C	36	T/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.775)	tolerated(0.37)
22	37260158	NCF4	T	C	35	F/S	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.06)
22	37261010	NCF4	A	C	56	Y/S	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
10	51579171	NCOA4	C	G	10	S/R	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.16)
10	51585072	NCOA4	G	T	391	V/F	NON_SYNONYMOUS_CODING	possibly_damaging(0.785)	deleterious(0.03)
12	8242854	NECAP1	A	T	87	D/V	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
12	8242857	NECAP1	C	T	88	S/F	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
18	77208908	NFATC1	A	C	505	T/P	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.39)
18	77211052	NFATC1	T	G	563	V/G	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
11	129756238	NFRKB	G	C	147	L/V	NON_SYNONYMOUS_CODING	benign(0.351)	tolerated(0.97)
11	129756228	NFRKB	A	T	150	I/N	NON_SYNONYMOUS_CODING	probably_damaging(0.996)	deleterious(0)

3	25777564	NGLY1	G	T	360	D/E	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
3	25773841	NGLY1	C	A	465	W/L	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
3	25777566	NGLY1	C	T	360	D/N	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
5	156895736	NIPAL4	C	G	176	A/G	NON_SYNONYMOUS_CODING	benign(0.008)	tolerated(0.17)
5	156890324	NIPAL4	C	G	120	R/G	NON_SYNONYMOUS_CODING	unknown(0)	.
10	103918982	NOLC1	G	C	214	A/P	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.17)
10	103918981	NOLC1	A	AC	213-214	.	FRAMESHIFT_CODING	.	.
9	139413211	NOTCH1	T	G	311	T/P	NON_SYNONYMOUS_CODING	benign(0.004)	deleterious(0)
9	139413097	NOTCH1	T	G	349	T/P	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
7	44579029	NPC1L1	T	G	323	T/P	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.45)
7	44579031	NPC1L1	C	G	322	G/A	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.85)
9	134003052	NUP214	C	T	63	P/S	NON_SYNONYMOUS_CODING	benign(0.02)	tolerated(0.63)
9	134003042	NUP214	G	T	59	L/F	NON_SYNONYMOUS_CODING	benign(0.123)	deleterious(0.01)
11	3765778	NUP98	T	G	457	T/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.909)	deleterious(0.02)
11	3752738	NUP98	C	G	538	R/P	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0.04)
1	228430947	OBSCN	C	G	998	A/G	NON_SYNONYMOUS_CODING	benign(0.003)	.
1	228464168	OBSCN	G	C	2080	V/L	NON_SYNONYMOUS_CODING	probably_damaging(0.998)	.
11	78525384	ODZ4	T	G	580	T/P	NON_SYNONYMOUS_CODING	benign(0.103)	tolerated(0.29)
11	78380763	ODZ4	G	T	2209	D/E	NON_SYNONYMOUS_CODING	benign(0.119)	tolerated(0.63)
3	193332725	OPA1	C	A	82	Y/*	STOP_GAINED	.	.
3	193386379	OPA1	A	C	121	L/F	NON_SYNONYMOUS_CODING	unknown(0)	.
3	193386395	OPA1	G	C	127	A/P	NON_SYNONYMOUS_CODING	unknown(0)	.
1	248436582	OR2T33	T	C	179	T/A	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.25)
1	248436638	OR2T33	A	G	160	V/A	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.4)
2	60995630	PAPOLG	C	A	47	T/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.7)
2	60988881	PAPOLG	T	G	18	V/G	NON_SYNONYMOUS_CODING	benign(0.025)	tolerated(0.09)
7	82595154	PCLO	A	T	1256	I/K	NON_SYNONYMOUS_CODING	benign(0.009)	.
7	82578977	PCLO	A	G	3574	Y/H	NON_SYNONYMOUS_CODING	probably_damaging(0.998)	.
9	35095103	PIGO	C	G	154	A/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.756)	deleterious(0)
9	35093527	PIGO	T	C	277	D/G	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
9	35090633	PIGO	C	CA	895	.	FRAMESHIFT_CODING	.	.
5	108698639	PJA2	T	G	518	E/D	NON_SYNONYMOUS_CODING	probably_damaging(0.971)	tolerated(0.66)
5	108698646	PJA2	C	T	516	G/E	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.27)
6	51920399	PKHD1	C	T	608	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.574)	deleterious(0.03)
6	51920465	PKHD1	A	C	586	F/V	NON_SYNONYMOUS_CODING	possibly_damaging(0.903)	deleterious(0.04)
8	110463218	PKHD1L1	C	A	2064	Q/K	NON_SYNONYMOUS_CODING	benign(0.023)	tolerated(1)
8	110455184	PKHD1L1	C	T	1468	S/F	NON_SYNONYMOUS_CODING	probably_damaging(0.982)	deleterious(0.01)
8	144992368	PLEC	A	G	4011	L/P	NON_SYNONYMOUS_CODING	probably_damaging(1)	.
8	145049468	PLEC	T	G	24	S/R	NON_SYNONYMOUS_CODING	unknown(0)	.
1	208272311	PLXNA2	G	C	537	C/W	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
1	208272313	PLXNA2	A	C	537	C/G	SPLICE_SITE:NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
14	74179763	PNMA1	A	C	194	S/A	NON_SYNONYMOUS_CODING	benign(0.121)	tolerated(0.17)
14	74179765	PNMA1	A	C	193	V/G	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
7	108155497	PNPLA8	G	T	47	Q/K	NON_SYNONYMOUS_CODING	benign(0.032)	tolerated(0.4)
7	108155503	PNPLA8	A	C	45	L/V	NON_SYNONYMOUS_CODING	benign(0.271)	tolerated(0.17)
12	81655761	PPFIA2	T	G	1157	*/C	STOP_LOST	.	.

12	81741484	PPFIA2	C	T	669	S/N	NON_SYNONYMOUS_CODING	probably_damaging(0.998)	deleterious(0)
12	81769573	PPFIA2	A	C	196	V/G	ESSENTIAL_SPLICE_SITE:NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0)
16	68349938	PRMT7	A	G	19	E/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.922)	deleterious(0.04)
16	68379609	PRMT7	T	C	270	F/S	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
9	134366847	PRRC2B	G	C	94	Q/H	NON_SYNONYMOUS_CODING	benign(0.088)	deleterious(0)
9	134357909	PRRC2B	A	C	1712	D/A	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0.02)
9	134357908	PRRC2B	G	C	1712	D/H	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0.01)
3	184019670	PSMD2	A	G	172	E/G	NON_SYNONYMOUS_CODING	benign(0.046)	tolerated(0.1)
3	184019400	PSMD2	G	A	145	V/M	NON_SYNONYMOUS_CODING	possibly_damaging(0.915)	tolerated(0.07)
3	184024549	PSMD2	T	G	654	V/G	NON_SYNONYMOUS_CODING	probably_damaging(0.998)	deleterious(0)
10	27702786	PTCHD3	A	C	132	W/G	NON_SYNONYMOUS_CODING	benign(0.003)	tolerated(0.36)
10	27700857	PTCHD3	A	C	364	V/G	SPLICE_SITE:NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
12	70934671	PTPRB	A	C	1546	V/G	NON_SYNONYMOUS_CODING	benign(0.341)	tolerated(0.2)
12	71003065	PTPRB	A	G	37	S/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.909)	deleterious(0.02)
3	191179127	PYDC2	T	C	59	F/S	NON_SYNONYMOUS_CODING	benign(0.29)	deleterious(0)
3	191179129	PYDC2	A	C	60	T/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.817)	tolerated(0.06)
14	36140611	RALGAPA1	G	A	1223	S/F	NON_SYNONYMOUS_CODING	benign(0.229)	deleterious(0.01)
14	36153095	RALGAPA1	C	T	958	S/N	NON_SYNONYMOUS_CODING	probably_damaging(0.99)	deleterious(0.03)
13	49033835	RB1	G	A	658	A/T	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
13	49033836	RB1	C	A	658	A/D	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
12	114392983	RBM19	T	G	292	T/P	NON_SYNONYMOUS_CODING	benign(0.021)	tolerated(0.16)
12	114356207	RBM19	G	C	811	R/G	NON_SYNONYMOUS_CODING	benign(0.37)	deleterious(0)
9	3293246	RFX3	A	C	188	L/V	NON_SYNONYMOUS_CODING	benign(0.004)	tolerated(0.11)
9	3395524	RFX3	C	T	22	S/N	NON_SYNONYMOUS_CODING	benign(0.026)	deleterious(0.04)
12	130892351	RIMBP2	G	C	86	R/G	NON_SYNONYMOUS_CODING	benign(0.13)	tolerated(0.41)
12	130892349	RIMBP2	A	C	10	V/G	NON_SYNONYMOUS_CODING	unknown(0)	.
1	25233788	RUNX3	G	T	129	T/K	NON_SYNONYMOUS_CODING	benign(0.086)	tolerated(0.06)
1	25228963	RUNX3	T	G	247	T/P	NON_SYNONYMOUS_CODING	benign(0.102)	tolerated(0.21)
7	4007044	SDK1	A	C	508	K/N	NON_SYNONYMOUS_CODING	benign(0.245)	tolerated(0.36)
7	4259872	SDK1	C	A	139	Q/K	NON_SYNONYMOUS_CODING	benign(0.253)	tolerated(0.31)
7	4247821	SDK1	A	C	17	T/P	NON_SYNONYMOUS_CODING	probably_damaging(0.975)	tolerated(0.11)
4	119718894	SEC24D	C	T	330	A/T	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.87)
4	119718897	SEC24D	G	T	329	Q/K	NON_SYNONYMOUS_CODING	benign(0.291)	tolerated(0.09)
1	53158524	SELRC1	A	C	41	V/G	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.86)
1	53158521	SELRC1	T	C	42	D/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.951)	deleterious(0.01)
22	26773662	SEZ6L	G	C	978	R/P	NON_SYNONYMOUS_CODING	probably_damaging(0.996)	tolerated(0.21)
22	26709766	SEZ6L	T	G	638	V/G	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
6	144416537	SF3B5	A	C	33	V/G	NON_SYNONYMOUS_CODING	benign(0.103)	deleterious(0.01)
6	144416535	SF3B5	T	C	34	N/D	NON_SYNONYMOUS_CODING	probably_damaging(0.997)	deleterious(0.01)
2	230914604	SLC16A14	C	A	92	L/F	NON_SYNONYMOUS_CODING	benign(0.045)	tolerated(0.31)
2	230914600	SLC16A14	T	A	94	I/F	NON_SYNONYMOUS_CODING	probably_damaging(0.996)	deleterious(0.01)
6	74351582	SLC17A5	G	T	119	Y/*	STOP_GAINED	.	.
6	74351580	SLC17A5	A	T	120	I/N	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
1	108681800	SLC25A24	C	A	358	A/S	NON_SYNONYMOUS_CODING	benign(0.002)	tolerated(0.79)
1	108681808	SLC25A24	T	C	355	D/G	NON_SYNONYMOUS_CODING	benign(0.023)	tolerated(0.28)
10	98770775	SLIT1	A	G	1106	S/P	NON_SYNONYMOUS_CODING	benign(0.104)	deleterious(0.04)

10	98778796	SLIT1	T	G	939	T/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.707)	tolerated(0.09)
4	20598044	SLIT2	G	T	1105	L/F	NON_SYNONYMOUS_CODING	possibly_damaging(0.823)	deleterious(0.03)
4	20525686	SLIT2	G	A	446	A/T	NON_SYNONYMOUS_CODING	probably_damaging(0.99)	deleterious(0.01)
7	127484411	SND1	G	C	426	S/T	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.71)
7	127343337	SND1	G	C	267	S/T	NON_SYNONYMOUS_CODING	probably_damaging(0.989)	tolerated(0.11)
4	7735055	SORCS2	G	C	867	A/P	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.28)
4	7735058	SORCS2	G	C	868	A/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.848)	tolerated(0.28)
4	88416177	SPARCL1	C	T	53	E/K	NON_SYNONYMOUS_CODING	benign(0.263)	deleterious(0.02)
4	88416171	SPARCL1	C	T	55	E/K	NON_SYNONYMOUS_CODING	probably_damaging(0.991)	tolerated(0.06)
1	16258001	SPEN	A	C	1756	T/P	NON_SYNONYMOUS_CODING	benign(0.053)	tolerated(0.41)
1	16258610	SPEN	C	G	1959	R/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.536)	tolerated(0.05)
1	16203071	SPEN	G	A	260	S/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.767)	tolerated(0.1)
1	16203072	SPEN	C	A	260	S/R	NON_SYNONYMOUS_CODING	possibly_damaging(0.903)	tolerated(0.13)
22	42276900	SREBF2	A	C	648	T/P	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.34)
22	42273997	SREBF2	T	G	544	V/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.501)	deleterious(0)
22	42276902	SREBF2	G	C	682	A/P	NON_SYNONYMOUS_CODING	unknown(0)	.
2	120003090	STEAP3	C	A	6	D/E	NON_SYNONYMOUS_CODING	benign(0.427)	tolerated(0.09)
2	120003088	STEAP3	G	A	6	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.513)	tolerated(0.08)
6	36489582	STK38	G	A	107	Q/*	STOP_GAINED	.	.
6	36489585	STK38	C	A	106	V/F	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
9	113169203	SVEP1	T	G	2893	T/P	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.28)
9	113265476	SVEP1	C	G	442	R/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.927)	tolerated(0.19)
10	29779847	SVIL	A	C	1374	L/R	NON_SYNONYMOUS_CODING	possibly_damaging(0.952)	deleterious(0)
10	29813511	SVIL	A	G	826	S/P	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
6	152470619	SYNE1	C	G	374	R/P	NON_SYNONYMOUS_CODING	benign(0.029)	deleterious(0.02)
6	152786454	SYNE1	C	T	624	S/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.649)	tolerated(0.27)
7	35288322	TBX20	A	C	171	V/G	NON_SYNONYMOUS_CODING	probably_damaging(0.991)	deleterious(0)
7	35288307	TBX20	T	G	176	D/A	NON_SYNONYMOUS_CODING	probably_damaging(0.993)	deleterious(0)
7	35242076	TBX20	C	T	437	R/H	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
8	56737244	TGS1	A	C	848	R/S	NON_SYNONYMOUS_CODING	benign(0.021)	deleterious(0.02)
8	56708572	TGS1	T	G	468	V/G	NON_SYNONYMOUS_CODING	benign(0.189)	deleterious(0)
16	426329	TMEM8A	A	C	151	V/G	NON_SYNONYMOUS_CODING	benign(0.055)	tolerated(0.56)
16	422045	TMEM8A	A	G	560	F/S	NON_SYNONYMOUS_CODING	benign(0.282)	tolerated(0.13)
12	83251115	TMTC2	T	G	137	V/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.926)	deleterious(0)
12	83251120	TMTC2	C	G	139	R/G	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
7	142498738	TRBC2	C	G	5	N/K	NON_SYNONYMOUS_CODING	benign(0.002)	tolerated(0.34)
7	142498735	TRBC2	A	C	4	K/N	NON_SYNONYMOUS_CODING	benign(0.111)	tolerated(0.08)
7	142008783	TRBV3-1	A	G	86	K/E	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142008785	TRBV3-1	A	C	86	K/N	NON_SYNONYMOUS_CODING	benign(0.001)	deleterious(0.02)
7	142045680	TRBV4-2	T	C	70	F/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.67)
7	142045693	TRBV4-2	C	T	74	T/I	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.36)
7	142149029	TRBV5-5	T	G	81	D/A	NON_SYNONYMOUS_CODING	benign(0.001)	deleterious(0.01)
7	142149030	TRBV5-5	C	G	81	D/H	NON_SYNONYMOUS_CODING	benign(0.002)	deleterious(0.01)
7	142180566	TRBV6-5	A	T	98	L/Q	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.22)
7	142180567	TRBV6-5	G	C	98	L/V	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.06)
7	142180573	TRBV6-5	T	C	96	R/G	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.12)

7	142180585	TRBV6-5	C	T	92	D/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.79)
7	142180590	TRBV6-5	G	T	90	T/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142180593	TRBV6-5	G	T	89	T/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.44)
7	142180596	TRBV6-5	G	A	88	S/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.31)
7	142180618	TRBV6-5	T	C	81	N/D	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.78)
7	142180633	TRBV6-5	G	T	76	Q/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142180635	TRBV6-5	T	G	75	D/A	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.22)
7	142180641	TRBV6-5	A	G	73	I/T	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.91)
7	142180704	TRBV6-5	G	T	52	S/Y	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142180737	TRBV6-5	T	A	41	Q/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.16)
7	142180578	TRBV6-5	G	A	94	P/L	NON_SYNONYMOUS_CODING	benign(0.002)	tolerated(0.17)
7	142180588	TRBV6-5	C	G	91	E/Q	NON_SYNONYMOUS_CODING	benign(0.005)	tolerated(0.2)
7	142180647	TRBV6-5	G	T	71	A/D	NON_SYNONYMOUS_CODING	benign(0.08)	tolerated(0.18)
7	142099548	TRBV7-8	A	G	85	F/S	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142099581	TRBV7-8	A	G	74	L/P	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.35)
7	142099512	TRBV7-8	T	G	97	K/T	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.14)
21	38498381	TTC3	T	G	394	V/G	SPLICE_SITE:NON_SYNONYMOUS_CODING	benign(0.032)	deleterious(0)
21	38560797	TTC3	T	G	1642	V/G	SPLICE_SITE:NON_SYNONYMOUS_CODING	probably_damaging(0.995)	deleterious(0)
2	179634424	TTN	C	G	2916	A/P	NON_SYNONYMOUS_CODING	benign(0.02)	.
2	179472353	TTN	T	C	8748	K/E	NON_SYNONYMOUS_CODING	benign(0.099)	.
2	179419226	TTN	A	C	20676	I/M	NON_SYNONYMOUS_CODING	possibly_damaging(0.789)	.
2	179413028	TTN	C	A	22169	V/L	NON_SYNONYMOUS_CODING	possibly_damaging(0.891)	.
2	179468803	TTN	T	C	9264	E/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.936)	.
2	179595427	TTN	A	C	4701	S/A	NON_SYNONYMOUS_CODING	unknown(0)	.
2	179597615	TTN	G	C	4186	R/G	NON_SYNONYMOUS_CODING	unknown(0)	.
9	140137382	TUBB2C	A	C	238	T/P	NON_SYNONYMOUS_CODING	benign(0.081)	.
9	140137500	TUBB2C	G	C	277	G/A	NON_SYNONYMOUS_CODING	possibly_damaging(0.914)	.
1	19524272	UBR4	T	G	262	N/T	NON_SYNONYMOUS_CODING	possibly_damaging(0.9)	tolerated(0.27)
1	19442066	UBR4	T	G	45	D/A	NON_SYNONYMOUS_CODING	possibly_damaging(0.932)	tolerated(0.26)
1	19415470	UBR4	A	C	402	V/G	SPLICE_SITE:NON_SYNONYMOUS_CODING	benign(0.004)	deleterious(0.01)
2	128896359	UGGT1	T	G	551	V/G	NON_SYNONYMOUS_CODING	benign(0.052)	deleterious(0)
2	128945090	UGGT1	A	G	91	D/G	NON_SYNONYMOUS_CODING	probably_damaging(0.996)	tolerated(0.34)
1	216251520	USH2A	T	G	1828	D/A	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
1	215960138	USH2A	T	G	3421	T/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.832)	deleterious(0.05)
1	215960153	USH2A	A	C	3416	C/G	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
4	76708327	USO1	C	A	325	T/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.778)	tolerated(0.08)
4	76708333	USO1	T	G	327	V/G	NON_SYNONYMOUS_CODING	probably_damaging(0.988)	deleterious(0)
12	62798066	USP15	G	C	924	A/P	NON_SYNONYMOUS_CODING	benign(0.063)	tolerated(0.05)
12	62786861	USP15	C	A	788	Q/K	NON_SYNONYMOUS_CODING	possibly_damaging(0.581)	tolerated(0.28)
2	61456113	USP34	G	T	2254	T/N	NON_SYNONYMOUS_CODING	benign(0)	.
2	61575497	USP34	G	T	446	A/E	NON_SYNONYMOUS_CODING	probably_damaging(0.98)	.
17	5036210	USP6	T	G	67	I/M	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0.04)
17	5036211	USP6	C	T	68	R/W	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0)
12	101685769	UTP20	G	T	354	V/L	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.26)
12	101764315	UTP20	C	A	2221	Q/K	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
1	12317102	VPS13D	T	G	300	V/G	NON_SYNONYMOUS_CODING	benign(0.004)	tolerated(0.1)

1	12387776	VPS13D	A	C	2688	T/P	NON_SYNONYMOUS_CODING	benign(0.007)	tolerated(0.26)
7	38781702	VPS41	T	A	714	L/F	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0.02)
7	38781705	VPS41	C	A	713	L/F	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0.01)
12	118511659	VSIG10	ATGATCACCTCGGGCTGGG	A	349-355	TQPEVII/I	NON_SYNONYMOUS_CODING	.	.
12	118519961	VSIG10	C	T	212	R/Q	NON_SYNONYMOUS_CODING	probably_damaging(0.974)	tolerated(0.41)
2	98779398	VWA3B	T	G	208	V/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.485)	tolerated(0.06)
2	98736120	VWA3B	C	G	146	L/V	NON_SYNONYMOUS_CODING	probably_damaging(0.996)	tolerated(0.14)
1	20671955	VWA5B1	A	C	878	N/T	NON_SYNONYMOUS_CODING	benign(0.003)	tolerated(0.31)
1	20678602	VWA5B1	C	A	1031	S/R	NON_SYNONYMOUS_CODING	probably_damaging(0.976)	deleterious(0.05)
3	49051528	WDR6	T	G	884	V/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.517)	deleterious(0.01)
3	49049503	WDR6	C	G	209	A/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.649)	deleterious(0.03)
1	22446566	WNT4	C	G	345	V/L	NON_SYNONYMOUS_CODING	possibly_damaging(0.798)	deleterious(0.05)
1	22446565	WNT4	A	G	345	V/A	NON_SYNONYMOUS_CODING	probably_damaging(0.984)	deleterious(0.02)
16	69921959	WWP2	A	C	241	T/P	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.36)
16	69833118	WWP2	G	C	87	S/T	NON_SYNONYMOUS_CODING	probably_damaging(0.996)	deleterious(0.03)
3	39230842	XIRP1	T	G	32	D/A	NON_SYNONYMOUS_CODING	benign(0.235)	tolerated(0.21)
3	39230866	XIRP1	A	G	24	L/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.808)	tolerated(0.08)
3	39230843	XIRP1	C	G	32	D/H	NON_SYNONYMOUS_CODING	probably_damaging(0.967)	tolerated(0.07)
16	87448068	ZCCHC14	T	G	382	T/P	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.15)
16	87448079	ZCCHC14	C	G	378	R/P	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.13)
20	39832697	ZHX3	T	G	287	H/P	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.14)
20	39831419	ZHX3	C	G	713	S/T	NON_SYNONYMOUS_CODING	benign(0.004)	tolerated(0.68)
7	148951025	ZNF212	G	C	336	R/P	NON_SYNONYMOUS_CODING	benign(0.004)	tolerated(0.32)
7	148950905	ZNF212	T	G	296	V/G	NON_SYNONYMOUS_CODING	benign(0.159)	deleterious(0.01)
19	42584233	ZNF574	A	G	582	E/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.615)	deleterious(0)
19	42583125	ZNF574	A	C	213	T/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.819)	tolerated(0.24)
15	85326799	ZNF592	G	A	298	S/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.3)
15	85326800	ZNF592	T	A	298	S/R	NON_SYNONYMOUS_CODING	benign(0.073)	tolerated(0.11)
1	227842961	ZNF678	G	T	392	S/I	NON_SYNONYMOUS_CODING	benign(0.011)	tolerated(0.4)
1	227843213	ZNF678	C	T	476	T/I	NON_SYNONYMOUS_CODING	benign(0.102)	tolerated(0.36)
8	102213947	ZNF706	A	T	8	I/N	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0)
8	102213962	ZNF706	C	G	3	R/P	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0)
22	29445795	ZNRF3	C	A	249	Y/*	STOP_GAINED	.	.
22	29383125	ZNRF3	T	G	21	V/G	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)

CHD13: Homozygous variants

CHR ID	NUCLEOTIDE POSITION	GENE NAME	REF SEQUENCE	ALTERNATE SEQUENCE	AMINO ACID NO.	RESIDUE CHANGE	FUNCTION OF VARIANT	POLYPHEN	SIFT
10	46321904	AGAP4	C	T	260	R/H	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0.02)
22	16157622	ENSG00000206252	G	C	106	P/R	NON_SYNONYMOUS_CODING	probably_damaging(0.989)	.
19	56283297	ENSG00000229292	G	A	43	D/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.52)
19	56284090	ENSG00000229292	G	A	137	A/T	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.41)
19	56283289	ENSG00000229292	A	G	40	K/R	NON_SYNONYMOUS_CODING	benign(0.39)	tolerated(0.51)
1	146215113	ENSG00000232637	G	A	84	H/Y	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.05)
10	47911590	FAM21B	G	A	193	G/D	NON_SYNONYMOUS_CODING	benign(0.282)	tolerated(0.14)
19	47259734	FKRP	G	C	343	E/Q	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
10	49388901	FRMPD2	C	T	912	G/E	NON_SYNONYMOUS_CODING	benign(0.026)	tolerated(0.06)
16	30016636	INO80E	T	C	203	L/P	NON_SYNONYMOUS_CODING	probably_damaging(0.965)	tolerated(0.15)
19	50832152	KCNC3	T	C	63	D/G	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(1)
19	54725835	LILRB3	G	C	175	R/G	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.32)
3	195512186	MUC4	T	C	2089	I/V	NON_SYNONYMOUS_CODING	benign(0.028)	.
3	195510563	MUC4	G	C	2630	P/A	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510565	MUC4	C	T	2629	S/N	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510566	MUC4	T	C	2629	S/G	NON_SYNONYMOUS_CODING	unknown(0)	.
1	145327548	NBPF10	A	G	1369	N/D	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
1	145359049	NBPF10	A	G	2997	K/E	NON_SYNONYMOUS_CODING	benign(0.049)	tolerated(1)
1	248737259	OR2T34	C	G	267	R/P	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
18	14533105	POTEC	G	C	337	S/C	NON_SYNONYMOUS_CODING	probably_damaging(0.97)	tolerated(0.19)
2	130832358	POTEF	T	C	896	H/R	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
14	20010235	POTEM	A	G	393	V/A	NON_SYNONYMOUS_CODING	benign(0.006)	tolerated(1)
1	13695685	PRAMEF19	G	A	358	S/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.68)
1	13695787	PRAMEF19	C	G	324	G/A	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
1	13696022	PRAMEF19	A	G	246	W/R	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.25)
1	13696047	PRAMEF19	A	T	237	D/E	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.24)
1	13696054	PRAMEF19	G	A	235	S/F	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.69)
1	13696090	PRAMEF19	T	C	223	K/R	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.47)
22	18901004	PRODH	C	T	166	R/Q	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.6)
2	108479432	RGPD4	G	A	805	A/T	NON_SYNONYMOUS_CODING	possibly_damaging(0.67)	tolerated(0.2)
7	72436652	TRIM74	A	G	13	W/R	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.29)
15	90320134	MESP2	AGGGCAGGGGCAG	A	183-186	GQGQ/-	NON_SYNONYMOUS_CODING	.	.
17	46115072	COPZ2	C	CG	22	.	FRAMESHIFT_CODING	.	.
17	46115084	COPZ2	T	TGG	18	.	FRAMESHIFT_CODING	.	.
21	43298954	PRDM15	C	CG	88	.	FRAMESHIFT_CODING	.	.
3	133969437	RYK	A	AG	19-20	.	SPLICE_SITE:FRAMESHIFT_CODING	.	.

CHD13: Compound heterozygous variants

CHR ID	NUCLEOTIDE POSITION	GENE NAME	REF SEQUENCE	ALTERNATE SEQUENCE	AMINO ACID NO.	RESIDUE CHANGE	FUNCTION OF VARIANT	POLYPHEN	SIFT
2	97808524	ANKRD36	A	G	285	I/V	NON_SYNONYMOUS_CODING	benign(0.296)	tolerated(1)
2	97845632	ANKRD36	T	C	566	I/T	NON_SYNONYMOUS_CODING	possibly_damaging(0.767)	tolerated(0.23)
2	97808515	ANKRD36	G	A	282	E/K	NON_SYNONYMOUS_CODING	probably_damaging(0.959)	tolerated(1)
2	97845616	ANKRD36	G	T	561	D/Y	NON_SYNONYMOUS_CODING	probably_damaging(0.995)	deleterious(0)
2	97808510	ANKRD36	G	A	280	G/D	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.85)
12	101368637	ANO4	A	C	191	Y/S	NON_SYNONYMOUS_CODING	probably_damaging(0.983)	tolerated(0.39)
12	101368625	ANO4	G	A	187	R/K	SPLICE_SITE:NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(1)
22	39498015	APOBEC3H	C	G	171	R/G	NON_SYNONYMOUS_CODING	benign(0.057)	deleterious(0.01)
22	39498005	APOBEC3H	T	A	167	D/E	NON_SYNONYMOUS_CODING	benign(0.18)	tolerated(0.35)
11	64666179	ATG2A	A	G	1338	S/P	NON_SYNONYMOUS_CODING	benign(0.403)	deleterious(0.02)
11	64666182	ATG2A	C	G	1337	A/P	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
5	137503739	BRD8	G	A	224	S/F	NON_SYNONYMOUS_CODING	probably_damaging(0.996)	deleterious(0.01)
5	137503737	BRD8	C	A	225	G/C	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.06)
12	112622338	C12orf51	T	G	3056	T/P	NON_SYNONYMOUS_CODING	benign(0.187)	.
12	112688167	C12orf51	A	C	822	V/G	NON_SYNONYMOUS_CODING	probably_damaging(0.979)	.
1	40535481	CAP1	T	C	310	S/P	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.29)
1	40535490	CAP1	C	G	313	R/G	NON_SYNONYMOUS_CODING	benign(0.048)	tolerated(0.34)
19	14507228	CD97	A	C	141	T/P	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.28)
19	14507213	CD97	A	C	136	T/P	NON_SYNONYMOUS_CODING	benign(0.001)	deleterious(0.01)
17	45234417	CDC27	A	G	235	I/T	NON_SYNONYMOUS_CODING	benign(0.004)	tolerated(0.19)
17	45234303	CDC27	G	C	273	A/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.783)	tolerated(0.07)
17	45214528	CDC27	A	T	635	Y/N	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
17	7796794	CHD3	A	C	293	I/L	NON_SYNONYMOUS_CODING	benign(0)	.
17	7796803	CHD3	T	C	296	S/P	NON_SYNONYMOUS_CODING	benign(0.001)	.
8	68062165	CSPP1	G	A	703	S/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.64)
8	68062160	CSPP1	T	A	701	N/K	NON_SYNONYMOUS_CODING	benign(0.153)	tolerated(0.98)
15	51766636	DMXL2	G	A	1736	A/V	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(1)
15	51766637	DMXL2	C	A	1736	A/S	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.57)
10	47769356	ENSG00000215033	C	A	59	D/E	NON_SYNONYMOUS_CODING	unknown(0)	.
10	47769358	ENSG00000215033	G	A	60	G/D	NON_SYNONYMOUS_CODING	unknown(0)	.
9	46386995	ENSG00000237198	C	A	3	R/M	NON_SYNONYMOUS_CODING	benign(0.013)	tolerated(0.36)
9	46386902	ENSG00000237198	G	A	34	T/I	NON_SYNONYMOUS_CODING	probably_damaging(0.998)	deleterious(0)
9	46386956	ENSG00000237198	C	G	16	R/P	NON_SYNONYMOUS_CODING	probably_damaging(0.998)	deleterious(0)
9	46386806	ENSG00000237198	C	T	66	R/Q	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.75)
12	132445256	EP400	A	C	31	H/P	NON_SYNONYMOUS_CODING	unknown(0)	.
12	132445273	EP400	T	C	37	S/P	NON_SYNONYMOUS_CODING	unknown(0)	.
8	12285102	FAM86B2	T	C	91	E/G	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0)
8	12285103	FAM86B2	C	T	91	E/K	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0)
4	187518120	FAT1	T	C	4194	I/V	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.34)
4	187540958	FAT1	G	A	2263	T/M	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0.01)
20	29628328	FRG1B	C	G	110	I/M	NON_SYNONYMOUS_CODING	benign(0.191)	deleterious(0.03)

20	29623254	FRG1B	G	A	24	D/N	SPLICE_SITE:NON_SYNONYMOUS_CODING	possibly_damaging(0.607)	tolerated(0.6)
10	135440123	FRG2B	C	T	42	E/K	NON_SYNONYMOUS_CODING	benign(0.009)	tolerated(0.33)
10	135440159	FRG2B	T	A	30	T/S	NON_SYNONYMOUS_CODING	possibly_damaging(0.94)	tolerated(0.45)
1	37319269	GRIK3	G	A	387	R/W	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
1	37319270	GRIK3	C	A	386	L/F	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.75)
6	32489937	HLA-DRB5	G	A	39	Q/*	STOP_GAINED	.	.
6	32489935	HLA-DRB5	C	G	39	Q/H	NON_SYNONYMOUS_CODING	benign(0.004)	tolerated(0.12)
6	32489852	HLA-DRB5	A	G	67	L/S	NON_SYNONYMOUS_CODING	possibly_damaging(0.558)	deleterious(0)
14	106471410	IGHV1-3	T	C	63	R/G	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
14	106471515	IGHV1-3	C	A	28	A/S	NON_SYNONYMOUS_CODING	probably_damaging(0.975)	tolerated(0.17)
14	106573354	IGHV3-11	A	G	77	I/T	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
14	106573357	IGHV3-11	G	T	76	T/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.62)
14	106573358	IGHV3-11	T	A	76	T/S	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
14	106573378	IGHV3-11	T	G	69	Y/S	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.39)
14	106573418	IGHV3-11	T	C	56	I/V	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
14	106573423	IGHV3-11	C	T	54	S/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.65)
14	106573352	IGHV3-11	A	T	78	Y/N	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.23)
14	107034847	IGHV5-51	C	T	78	R/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.37)
14	107034854	IGHV5-51	C	A	76	D/Y	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.24)
14	107034846	IGHV5-51	T	G	78	R/S	NON_SYNONYMOUS_CODING	benign(0.136)	tolerated(0.44)
17	39346592	KRTAP9-1	ACCT	A	152-153	TC/S	NON_SYNONYMOUS_CODING	.	.
17	39346433	KRTAP9-1	A	C	99	T/P	SPLICE_SITE:NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.08)
1	152777625	LCE1C	A	C	110	S/R	NON_SYNONYMOUS_CODING	unknown(0)	.
1	152777627	LCE1C	T	C	110	S/G	NON_SYNONYMOUS_CODING	unknown(0)	.
12	122685179	LRRC43	C	A	402	T/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.92)
12	122685176	LRRC43	G	A	401	R/K	NON_SYNONYMOUS_CODING	benign(0.005)	tolerated(1)
12	122685163	LRRC43	A	G	397	K/E	NON_SYNONYMOUS_CODING	probably_damaging(0.99)	tolerated(0.89)
12	122685164	LRRC43	A	G	397	K/R	NON_SYNONYMOUS_CODING	probably_damaging(0.99)	tolerated(0.23)
7	151927025	MLL3	A	G	987	Y/H	NON_SYNONYMOUS_CODING	probably_damaging(1)	.
7	151932997	MLL3	C	T	892	G/R	NON_SYNONYMOUS_CODING	probably_damaging(1)	.
19	9002623	MUC16	C	T	39	R/H	NON_SYNONYMOUS_CODING	benign(0.011)	tolerated(0.24)
19	9002636	MUC16	C	T	35	V/I	NON_SYNONYMOUS_CODING	benign(0.051)	tolerated(0.37)
19	9002612	MUC16	T	G	43	K/Q	NON_SYNONYMOUS_CODING	benign(0.055)	tolerated(0.23)
19	9002659	MUC16	C	T	27	G/E	NON_SYNONYMOUS_CODING	possibly_damaging(0.582)	tolerated(0.19)
19	9002597	MUC16	C	T	48	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.866)	deleterious(0)
19	9002660	MUC16	C	T	27	G/R	NON_SYNONYMOUS_CODING	probably_damaging(0.998)	tolerated(0.5)
3	195510653	MUC4	A	C	2600	S/A	NON_SYNONYMOUS_CODING	benign(0.035)	.
3	195506549	MUC4	A	G	3968	S/P	NON_SYNONYMOUS_CODING	benign(0.044)	.
3	195508796	MUC4	C	G	3219	V/L	NON_SYNONYMOUS_CODING	benign(0.095)	.
3	195510228	MUC4	C	G	2741	E/D	NON_SYNONYMOUS_CODING	benign(0.182)	.
3	195509212	MUC4	G	A	3080	S/L	NON_SYNONYMOUS_CODING	benign(0.204)	.
3	195506569	MUC4	A	G	3961	V/A	NON_SYNONYMOUS_CODING	benign(0.243)	.
3	195510649	MUC4	G	A	2601	A/V	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195511076	MUC4	T	A	2459	T/S	NON_SYNONYMOUS_CODING	possibly_damaging(0.609)	.
3	195511102	MUC4	G	A	2450	P/L	NON_SYNONYMOUS_CODING	probably_damaging(0.975)	.
3	195510622	MUC4	G	T	2610	P/H	NON_SYNONYMOUS_CODING	probably_damaging(0.991)	.

3	195506542	MUC4	G	T	3970	P/H	NON_SYNONYMOUS_CODING	probably_damaging(1)	.
3	195510347	MUC4	T	C	2702	T/A	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510350	MUC4	C	G	2701	D/H	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510509	MUC4	A	C	2648	S/A	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510601	MUC4	A	G	2617	V/A	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510610	MUC4	A	G	2614	L/P	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510611	MUC4	G	T	2614	L/I	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510613	MUC4	C	T	2613	S/N	NON_SYNONYMOUS_CODING	unknown(0)	.
11	1018095	MUC6	G	A	1569	P/L	NON_SYNONYMOUS_CODING	benign(0.014)	deleterious(0.01)
11	1016957	MUC6	T	G	1948	R/S	NON_SYNONYMOUS_CODING	benign(0.034)	tolerated(0.84)
11	1017466	MUC6	T	C	1779	R/G	NON_SYNONYMOUS_CODING	benign(0.142)	tolerated(0.57)
11	1017307	MUC6	G	A	1832	P/S	NON_SYNONYMOUS_CODING	benign(0.34)	tolerated(0.21)
11	1016919	MUC6	C	T	1961	R/K	NON_SYNONYMOUS_CODING	possibly_damaging(0.496)	tolerated(0.33)
11	1018042	MUC6	A	G	1587	S/P	NON_SYNONYMOUS_CODING	probably_damaging(0.979)	deleterious(0.01)
11	1016554	MUC6	C	T	2083	A/T	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.6)
11	1016565	MUC6	C	T	2079	S/N	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.09)
11	1016583	MUC6	T	G	2073	Q/P	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0.03)
11	1018207	MUC6	T	C	1532	T/A	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.28)
11	1018552	MUC6	G	A	1417	P/S	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.73)
11	1018555	MUC6	C	T	1416	V/I	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.39)
9	139413211	NOTCH1	T	G	311	T/P	NON_SYNONYMOUS_CODING	benign(0.004)	deleterious(0)
9	139417464	NOTCH1	T	G	194	T/P	NON_SYNONYMOUS_CODING	benign(0.045)	tolerated(0.07)
9	139413097	NOTCH1	T	G	349	T/P	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
1	228430947	OBSCN	C	G	998	A/G	NON_SYNONYMOUS_CODING	benign(0.003)	.
1	228434292	OBSCN	C	G	1274	A/G	NON_SYNONYMOUS_CODING	benign(0.006)	.
1	248436582	OR2T33	T	C	179	T/A	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.25)
1	248436638	OR2T33	A	G	160	V/A	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.4)
6	106546559	PRDM1	A	G	4	E/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.878)	.
6	106546576	PRDM1	A	C	10	T/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.901)	deleterious(0)
8	48817516	PRKDC	C	G	985	E/D	NON_SYNONYMOUS_CODING	probably_damaging(0.976)	tolerated(0.13)
8	48732067	PRKDC	T	A	3113	Y/F	NON_SYNONYMOUS_CODING	probably_damaging(0.992)	tolerated(0.18)
9	33794812	PRSS3	G	T	8	G/V	NON_SYNONYMOUS_CODING	benign(0)	deleterious(0)
9	33798075	PRSS3	T	C	150	F/S	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
9	33798574	PRSS3	G	A	182	S/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
2	85571228	RETSAT	G	C	265	A/G	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.43)
2	85571225	RETSAT	T	C	266	E/G	NON_SYNONYMOUS_CODING	benign(0.048)	tolerated(0.28)
2	87205020	RGPD1	G	T	810	R/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
2	87204998	RGPD1	G	A	803	A/T	NON_SYNONYMOUS_CODING	benign(0.15)	tolerated(0.19)
2	87205031	RGPD1	T	C	814	C/R	NON_SYNONYMOUS_CODING	possibly_damaging(0.85)	tolerated(0.78)
18	76752544	SALL3	G	T	185	A/S	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.14)
18	76752545	SALL3	C	T	185	A/V	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.06)
12	109017672	SELPLG	G	C	154	P/A	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.4)
12	109017674	SELPLG	A	G	153	V/A	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.94)
5	131705926	SLC22A5	A	C	88	T/P	NON_SYNONYMOUS_CODING	benign(0.241)	tolerated(0.16)
5	131705923	SLC22A5	G	C	87	A/P	NON_SYNONYMOUS_CODING	benign(0.388)	tolerated(0.12)
1	16262465	SPEN	A	C	3244	T/P	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.05)

1	16262471	SPEN	A	C	3246	T/P	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0.02)
7	142498738	TRBC2	C	G	5	N/K	NON_SYNONYMOUS_CODING	benign(0.002)	tolerated(0.34)
7	142498735	TRBC2	A	C	4	K/N	NON_SYNONYMOUS_CODING	benign(0.111)	tolerated(0.08)
7	142008783	TRBV3-1	A	G	86	K/E	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142008675	TRBV3-1	A	G	50	T/A	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(1)
7	142008785	TRBV3-1	A	C	86	K/N	NON_SYNONYMOUS_CODING	benign(0.001)	deleterious(0.02)
7	142008718	TRBV3-1	T	C	64	I/T	NON_SYNONYMOUS_CODING	benign(0.002)	deleterious(0.01)
7	142008672	TRBV3-1	G	A	49	D/N	NON_SYNONYMOUS_CODING	benign(0.006)	tolerated(0.61)
7	142045680	TRBV4-2	T	C	70	F/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.67)
7	142045693	TRBV4-2	C	T	74	T/I	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.36)
7	142168414	TRBV5-4	G	C	103	D/E	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142168466	TRBV5-4	A	C	86	L/R	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.24)
7	142180585	TRBV6-5	C	T	92	D/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.79)
7	142180590	TRBV6-5	G	T	90	T/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142180593	TRBV6-5	G	T	89	T/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.44)
7	142180596	TRBV6-5	G	A	88	S/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.31)
7	142180618	TRBV6-5	T	C	81	N/D	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.78)
7	142180633	TRBV6-5	G	T	76	Q/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142180635	TRBV6-5	T	G	75	D/A	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.22)
7	142180641	TRBV6-5	A	G	73	I/T	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.91)
7	142180704	TRBV6-5	G	T	52	S/Y	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142180737	TRBV6-5	T	A	41	Q/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.16)
7	142180578	TRBV6-5	G	A	94	P/L	NON_SYNONYMOUS_CODING	benign(0.002)	tolerated(0.17)
7	142180588	TRBV6-5	C	G	91	E/Q	NON_SYNONYMOUS_CODING	benign(0.005)	tolerated(0.2)
7	142180647	TRBV6-5	G	T	71	A/D	NON_SYNONYMOUS_CODING	benign(0.08)	tolerated(0.18)
7	142099548	TRBV7-8	A	G	85	F/S	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142099581	TRBV7-8	A	G	74	L/P	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.35)
7	142099512	TRBV7-8	T	G	97	K/T	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.14)
7	142099533	TRBV7-8	T	C	90	E/G	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.36)
7	142099537	TRBV7-8	G	T	89	P/T	NON_SYNONYMOUS_CODING	benign(0.003)	tolerated(0.39)
17	5036210	USP6	T	G	67	I/M	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0.04)
17	5036211	USP6	C	T	68	R/W	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0)
6	33423203	ZBTB9	G	C	109	R/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.936)	tolerated(0.38)
6	33423200	ZBTB9	T	C	108	L/P	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
19	53269628	ZNF600	T	A	461	K/*	STOP_GAINED	.	.
19	53269621	ZNF600	A	G	463	I/T	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.29)
19	53269622	ZNF600	T	A	463	I/F	NON_SYNONYMOUS_CODING	benign(0.204)	deleterious(0.01)
19	53269623	ZNF600	T	C	462	I/M	NON_SYNONYMOUS_CODING	benign(0.381)	deleterious(0.01)
19	53269627	ZNF600	T	A	461	K/M	NON_SYNONYMOUS_CODING	probably_damaging(0.96)	tolerated(0.06)
19	52887918	ZNF880	A	G	362	N/S	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
19	52887929	ZNF880	C	T	366	H/Y	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
19	52887924	ZNF880	A	T	364	N/I	NON_SYNONYMOUS_CODING	benign(0.005)	tolerated(0.24)
19	52887930	ZNF880	A	G	366	H/R	NON_SYNONYMOUS_CODING	benign(0.01)	tolerated(0.48)
19	52887923	ZNF880	A	C	364	N/H	NON_SYNONYMOUS_CODING	benign(0.012)	tolerated(0.19)
19	52887926	ZNF880	G	C	365	A/P	NON_SYNONYMOUS_CODING	benign(0.303)	deleterious(0.01)

CHD16: Homozygous variants

CHR ID	NUCLEOTIDE POSITION	GENE NAME	REF SEQUENCE	ALTERNATE SEQUENCE	AMINO ACID NO.	RESIDUE CHANGE	FUNCTION OF VARIANT	POLYPHEN	SIFT
10	46321880	AGAP4	C	T	268	R/H	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
10	46321904	AGAP4	C	T	260	R/H	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0.02)
10	51748584	AGAP6	A	G	37	R/G	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.72)
10	51464656	AGAP7	G	C	600	D/E	NON_SYNONYMOUS_CODING	probably_damaging(0.995)	tolerated(0.06)
10	51465650	AGAP7	C	T	269	R/Q	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0.03)
14	105415361	AHNAK2	T	C	2143	N/D	NON_SYNONYMOUS_CODING	benign(0.004)	tolerated(1)
14	105417646	AHNAK2	G	A	1381	P/L	NON_SYNONYMOUS_CODING	probably_damaging(0.99)	deleterious(0.02)
1	22315762	CELA3B	C	A	268	A/E	NON_SYNONYMOUS_CODING	benign(0.203)	tolerated(0.09)
10	89124859	FAM22D	G	A	473	G/S	NON_SYNONYMOUS_CODING	benign(0.183)	tolerated(0.22)
9	97080827	FAM22F	C	G	565	G/R	NON_SYNONYMOUS_CODING	benign(0.424)	tolerated(0.07)
8	12285064	FAM86B2	A	G	104	S/P	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0)
8	12285132	FAM86B2	C	G	81	C/S	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0)
7	74212311	GTF2IRD2	G	T	61	H/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
6	31324931	HLA-B	A	C	3	L/R	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
3	195512186	MUC4	T	C	2089	I/V	NON_SYNONYMOUS_CODING	benign(0.028)	.
3	195510610	MUC4	A	G	2614	L/P	NON_SYNONYMOUS_CODING	unknown(0)	.
1	145327548	NBPF10	A	G	1369	N/D	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
9	102590845	NR4A3	A	C	185	D/A	NON_SYNONYMOUS_CODING	benign(0.381)	tolerated(0.31)
1	248737259	OR2T34	C	G	267	R/P	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
2	130832358	POTEF	T	C	896	H/R	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
1	13695685	PRAMEF19	G	A	358	S/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.68)
1	13695787	PRAMEF19	C	G	324	G/A	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
1	13696022	PRAMEF19	A	G	246	W/R	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.25)
1	13696047	PRAMEF19	A	T	237	D/E	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.24)
1	13696054	PRAMEF19	G	A	235	S/F	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.69)
2	108479432	RGPD4	G	A	805	A/T	NON_SYNONYMOUS_CODING	possibly_damaging(0.67)	tolerated(0.2)
7	72436652	TRIM74	A	G	13	W/R	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.29)
21	43298954	PRDM15	C	CG	88	.	FRAMESHIFT_CODING	.	.
7	36552787	AOAH	A	AG	639	.	SPLICE_SITE:FRAMESHIFT_CODING	.	.

CHD16: Compound heterozygous variants

CHR ID	NUCLEOTIDE POSITION	GENE NAME	REF SEQUENCE	ALTERNATE SEQUENCE	AMINO ACID NO.	RESIDUE CHANGE	FUNCTION OF VARIANT	POLYPHEN	SIFT
17	35631165	ACACA	A	T	214	N/K	NON_SYNONYMOUS_CODING	benign(0.016)	tolerated(0.94)
17	35445961	ACACA	G	T	976	Q/K	NON_SYNONYMOUS_CODING	benign(0.408)	tolerated(0.24)
17	35548174	ACACA	G	T	198	L/I	NON_SYNONYMOUS_CODING	possibly_damaging(0.919)	tolerated(0.23)
17	35445967	ACACA	C	T	974	E/K	NON_SYNONYMOUS_CODING	probably_damaging(0.973)	deleterious(0)
14	23559259	ACIN1	G	A	181	S/F	NON_SYNONYMOUS_CODING	probably_damaging(0.958)	deleterious(0)
14	23531731	ACIN1	A	C	1020	V/G	SPLICE_SITE:NON_SYNONYMOUS_CODING	probably_damaging(0.988)	deleterious(0)
10	4879755	AKR1E2	C	A	188	F/L	NON_SYNONYMOUS_CODING	benign(0.055)	deleterious(0.03)
10	4875551	AKR1E2	A	C	73	T/P	NON_SYNONYMOUS_CODING	benign(0.426)	deleterious(0)
2	29543655	ALK	A	C	503	V/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.496)	deleterious(0.05)
2	29416173	ALK	C	T	1594	E/K	NON_SYNONYMOUS_CODING	probably_damaging(0.991)	deleterious(0.01)
4	114280246	ANK2	G	A	3458	R/K	NON_SYNONYMOUS_CODING	probably_damaging(0.987)	.
4	114275541	ANK2	G	A	1890	E/K	NON_SYNONYMOUS_CODING	probably_damaging(0.991)	.
4	114275548	ANK2	A	C	1892	H/P	NON_SYNONYMOUS_CODING	probably_damaging(0.996)	.
10	61836157	ANK3	G	T	1494	Y/*	STOP_GAINED	.	.
10	61829520	ANK3	C	G	3707	A/P	NON_SYNONYMOUS_CODING	probably_damaging(0.962)	.
2	97808524	ANKRD36	A	G	285	I/V	NON_SYNONYMOUS_CODING	benign(0.296)	tolerated(1)
2	97845632	ANKRD36	T	C	566	I/T	NON_SYNONYMOUS_CODING	possibly_damaging(0.767)	tolerated(0.23)
2	97808515	ANKRD36	G	A	282	E/K	NON_SYNONYMOUS_CODING	probably_damaging(0.959)	tolerated(1)
2	97808510	ANKRD36	G	A	280	G/D	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.85)
22	39498015	APOBEC3H	C	G	171	R/G	NON_SYNONYMOUS_CODING	benign(0.057)	deleterious(0.01)
22	39498005	APOBEC3H	T	A	167	D/E	NON_SYNONYMOUS_CODING	benign(0.18)	tolerated(0.35)
1	155311840	ASH1L	C	A	2788	D/Y	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
1	155322556	ASH1L	C	G	2441	R/P	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
1	1423284	ATAD3B	A	G	419	K/R	NON_SYNONYMOUS_CODING	probably_damaging(0.966)	tolerated(0.25)
1	1423286	ATAD3B	C	G	420	R/G	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0.03)
1	1392550	ATAD3C	A	G	244	K/R	NON_SYNONYMOUS_CODING	benign(0.184)	tolerated(0.23)
1	1392552	ATAD3C	C	G	245	R/G	NON_SYNONYMOUS_CODING	probably_damaging(0.99)	deleterious(0)
11	64666179	ATG2A	A	G	1338	S/P	NON_SYNONYMOUS_CODING	benign(0.403)	deleterious(0.02)
11	64666182	ATG2A	C	G	1337	A/P	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
11	63426568	ATL3	G	C	120	A/G	NON_SYNONYMOUS_CODING	probably_damaging(0.988)	deleterious(0.01)
11	63410966	ATL3	A	C	220	V/G	SPLICE_SITE:NON_SYNONYMOUS_CODING	benign(0.065)	deleterious(0.02)
11	108175544	ATM	C	G	1880	T/R	NON_SYNONYMOUS_CODING	benign(0.001)	deleterious(0.03)
11	108114752	ATM	T	A	190	I/K	NON_SYNONYMOUS_CODING	probably_damaging(0.991)	deleterious(0.03)
11	108114755	ATM	T	A	191	I/N	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
3	113524266	ATP6V1A	G	C	552	R/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.902)	tolerated(0.09)
3	113508633	ATP6V1A	G	T	312	V/L	NON_SYNONYMOUS_CODING	probably_damaging(0.995)	deleterious(0)
20	3565431	ATRN	C	A	956	Q/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.72)
20	3565356	ATRN	T	G	931	C/G	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
21	30969925	BACH1	A	G	184	M/V	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0.01)

21	30969926	BACH1	T	G	184	M/R	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0)
6	70048890	BAI3	A	C	55	T/P	NON_SYNONYMOUS_CODING	probably_damaging(0.998)	deleterious(0.03)
6	69758083	BAI3	G	A	705	S/N	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
5	137503739	BRD8	G	A	224	S/F	NON_SYNONYMOUS_CODING	probably_damaging(0.996)	deleterious(0.01)
5	137503737	BRD8	C	A	225	G/C	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.06)
3	9785283	BRPF1	T	G	772	V/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.916)	deleterious(0.01)
3	9786696	BRPF1	A	C	969	L/F	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	tolerated(0.09)
3	49689227	BSN	C	A	746	S/R	NON_SYNONYMOUS_CODING	benign(0.009)	.
3	49699596	BSN	A	G	3440	S/G	NON_SYNONYMOUS_CODING	unknown(0)	.
12	112688161	C12orf51	G	C	824	A/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.806)	.
12	112650430	C12orf51	T	C	242	E/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.875)	tolerated(0.17)
12	112688167	C12orf51	A	C	822	V/G	NON_SYNONYMOUS_CODING	probably_damaging(0.979)	.
12	112688164	C12orf51	T	C	823	E/G	NON_SYNONYMOUS_CODING	probably_damaging(0.98)	.
1	172414269	C1orf105	C	A	26	N/K	NON_SYNONYMOUS_CODING	benign(0.081)	deleterious(0)
1	172414265	C1orf105	T	A	25	V/E	NON_SYNONYMOUS_CODING	benign(0.106)	deleterious(0.01)
11	73789554	C2CD3	C	G	211	E/D	NON_SYNONYMOUS_CODING	possibly_damaging(0.485)	tolerated(0.52)
11	73760510	C2CD3	A	C	553	Y/D	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
3	126915976	C3orf56	T	C	150	S/P	NON_SYNONYMOUS_CODING	benign(0.278)	.
3	126915973	C3orf56	A	C	149	T/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.449)	.
4	100434303	C4orf17	G	A	22	R/K	NON_SYNONYMOUS_CODING	possibly_damaging(0.942)	tolerated(0.25)
4	100434307	C4orf17	T	A	23	N/K	NON_SYNONYMOUS_CODING	probably_damaging(0.995)	deleterious(0)
1	9011491	CA6	C	G	90	A/G	NON_SYNONYMOUS_CODING	benign(0.424)	deleterious(0.03)
1	9030968	CA6	A	C	258	T/P	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	tolerated(0.15)
3	53839025	CACNA1D	C	A	1887	Y/*	STOP_GAINED	.	.
3	53700459	CACNA1D	T	G	24	V/G	NON_SYNONYMOUS_CODING	benign(0.013)	tolerated(0.2)
3	53756421	CACNA1D	T	G	243	V/G	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
17	77808570	CBX4	A	G	291	S/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.727)	deleterious(0.04)
17	77808572	CBX4	C	G	290	R/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.94)	tolerated(0.14)
13	37012869	CCNA1	A	G	252	E/G	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
13	37012872	CCNA1	T	G	253	V/G	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
17	45234417	CDC27	A	G	235	I/T	NON_SYNONYMOUS_CODING	benign(0.004)	tolerated(0.19)
17	45234303	CDC27	G	C	273	A/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.783)	tolerated(0.07)
20	34082403	CEP250	T	G	973	V/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.805)	deleterious(0)
20	34090351	CEP250	G	C	1329	R/P	NON_SYNONYMOUS_CODING	probably_damaging(0.998)	tolerated(0.14)
17	7796794	CHD3	A	C	293	I/L	NON_SYNONYMOUS_CODING	benign(0)	.
17	7796803	CHD3	T	C	296	S/P	NON_SYNONYMOUS_CODING	benign(0.001)	.
1	6185909	CHD5	T	C	771	D/G	NON_SYNONYMOUS_CODING	benign(0.009)	deleterious(0.03)
1	6185908	CHD5	A	C	771	D/E	NON_SYNONYMOUS_CODING	benign(0.024)	tolerated(0.27)
19	42795827	CIC	G	C	939	G/A	NON_SYNONYMOUS_CODING	benign(0.106)	tolerated(0.44)
19	42795826	CIC	G	C	939	G/R	NON_SYNONYMOUS_CODING	possibly_damaging(0.765)	deleterious(0.03)
16	74446721	CLEC18B	C	G	165	S/T	NON_SYNONYMOUS_CODING	benign(0.08)	deleterious(0.04)
16	74446719	CLEC18B	T	G	166	T/P	NON_SYNONYMOUS_CODING	probably_damaging(0.996)	deleterious(0.03)
19	7833803	CLEC4M	A	G	310	N/D	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
19	7830154	CLEC4M	G	A	72	V/M	SPLICE_SITE:NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0.01)

17	71193206	COG1	A	C	243	N/T	NON_SYNONYMOUS_CODING	benign(0.011)	tolerated(1)
17	71192877	COG1	G	C	183	A/P	NON_SYNONYMOUS_CODING	probably_damaging(0.98)	tolerated(0.1)
6	56044835	COL21A1	C	A	61	V/F	NON_SYNONYMOUS_CODING	possibly_damaging(0.775)	deleterious(0.02)
6	56044517	COL21A1	C	G	167	A/P	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
10	126678128	CTBP2	T	A	433	T/S	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
10	126678177	CTBP2	G	T	416	N/K	NON_SYNONYMOUS_CODING	benign(0.01)	tolerated(0.46)
6	43013806	CUL7	C	T	979	S/N	NON_SYNONYMOUS_CODING	benign(0.014)	deleterious(0.01)
6	43006603	CUL7	G	T	1557	L/M	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
11	6645186	DCHS1	G	C	2574	A/G	NON_SYNONYMOUS_CODING	probably_damaging(0.984)	tolerated(0.17)
11	6661865	DCHS1	A	C	327	V/G	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	tolerated(0.11)
14	94517736	DDX24	T	G	751	H/P	NON_SYNONYMOUS_CODING	benign(0.35)	tolerated(0.2)
14	94517728	DDX24	A	G	754	S/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.746)	deleterious(0.01)
14	94521529	DDX24	A	C	621	V/G	SPLICE_SITE:NON_SYNONYMOUS_CODING	probably_damaging(0.998)	deleterious(0)
13	42734171	DGKH	G	T	116	E/*	STOP_GAINED	.	.
13	42742929	DGKH	C	G	312	R/G	NON_SYNONYMOUS_CODING	probably_damaging(0.977)	deleterious(0.01)
3	38151765	DLEC1	A	C	1146	T/P	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.32)
3	38157998	DLEC1	T	G	1304	V/G	NON_SYNONYMOUS_CODING	probably_damaging(0.989)	deleterious(0)
15	51766636	DMXL2	G	A	1736	A/V	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(1)
15	51766637	DMXL2	C	A	1736	A/S	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.57)
5	13866368	DNAH5	C	A	1359	L/F	NON_SYNONYMOUS_CODING	benign(0.407)	deleterious(0.01)
5	13754379	DNAH5	C	A	3496	L/F	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
6	38816506	DNAH8	G	C	1493	V/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.86)
6	38917321	DNAH8	A	C	3858	T/P	NON_SYNONYMOUS_CODING	benign(0.023)	tolerated(0.38)
12	56221271	DNAJC14	A	C	391	V/G	NON_SYNONYMOUS_CODING	probably_damaging(0.963)	deleterious(0)
12	56221154	DNAJC14	C	G	430	R/P	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
8	25216495	DOCK5	G	T	956	C/F	NON_SYNONYMOUS_CODING	probably_damaging(0.991)	deleterious(0.04)
8	25216525	DOCK5	A	C	966	D/A	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.36)
21	41648091	DSCAM	G	T	515	Y/*	STOP_GAINED	.	.
21	41514605	DSCAM	G	T	848	Q/K	NON_SYNONYMOUS_CODING	benign(0.042)	tolerated(0.91)
13	41515337	ELF1	T	G	326	T/P	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.08)
13	41556183	ELF1	G	C	3	A/G	NON_SYNONYMOUS_CODING	benign(0.046)	.
10	93577	ENSG00000173876	T	A	180	K/M	NON_SYNONYMOUS_CODING	benign(0.366)	deleterious(0)
10	93576	ENSG00000173876	C	A	180	K/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.479)	deleterious(0.01)
19	56283297	ENSG00000229292	G	A	43	D/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.52)
19	56284090	ENSG00000229292	G	A	137	A/T	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.41)
19	56283289	ENSG00000229292	A	G	40	K/R	NON_SYNONYMOUS_CODING	benign(0.39)	tolerated(0.51)
2	46609200	EPAS1	C	A	753	D/E	NON_SYNONYMOUS_CODING	benign(0.003)	tolerated(1)
2	46609198	EPAS1	G	A	753	D/N	NON_SYNONYMOUS_CODING	benign(0.418)	tolerated(0.07)
7	143096891	EPHA1	T	C	230	T/A	NON_SYNONYMOUS_CODING	benign(0.124)	tolerated(0.15)
7	143096809	EPHA1	A	C	257	V/G	NON_SYNONYMOUS_CODING	probably_damaging(0.996)	deleterious(0)
1	38227650	EPHA10	T	C	93	I/V	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.21)
1	38227136	EPHA10	A	C	264	V/G	NON_SYNONYMOUS_CODING	probably_damaging(0.991)	deleterious(0)
4	66467416	EPHA5	G	C	285	P/A	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0.03)
4	66467418	EPHA5	A	C	284	V/G	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)

6	93967945	EPHA7	G	A	661	A/V	NON_SYNONYMOUS_CODING	benign(0.012)	tolerated(0.49)
6	93967943	EPHA7	C	A	662	V/L	NON_SYNONYMOUS_CODING	probably_damaging(0.994)	deleterious(0)
1	6504703	ESPN	T	C	385	S/P	NON_SYNONYMOUS_CODING	probably_damaging(0.998)	deleterious(0)
1	6488413	ESPN	C	T	141	P/L	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0.01)
3	185797863	ETV5	G	T	131	F/L	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.1)
3	185802064	ETV5	G	A	80	L/F	NON_SYNONYMOUS_CODING	benign(0.01)	tolerated(0.51)
16	74761211	FA2H	T	G	146	H/P	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0.01)
16	74761247	FA2H	A	C	134	V/G	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0.02)
6	71187018	FAM135A	A	C	131	T/P	NON_SYNONYMOUS_CODING	benign(0.013)	deleterious(0)
6	71187020	FAM135A	A	C	133	H/P	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0.01)
15	41043735	FAM82A2	C	G	138	R/P	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.15)
15	41029923	FAM82A2	A	C	376	V/G	SPLICE_SITE:NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
2	207651481	FASTKD2	T	A	484	S/R	NON_SYNONYMOUS_CODING	benign(0.291)	deleterious(0.05)
2	207651480	FASTKD2	G	A	484	S/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.712)	tolerated(0.34)
7	100187851	FBXO24	A	C	65	T/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.462)	deleterious(0)
7	100192051	FBXO24	A	C	266	D/A	NON_SYNONYMOUS_CODING	possibly_damaging(0.907)	deleterious(0.03)
3	121340505	FBXO40	T	C	77	S/P	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.31)
3	121340335	FBXO40	A	C	20	N/T	NON_SYNONYMOUS_CODING	benign(0.052)	tolerated(0.06)
9	117371	FOXD4	C	G	250	G/A	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
9	117995	FOXD4	G	C	42	A/G	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.06)
11	126146290	FOXRED1	T	G	311	Y/D	SPLICE_SITE:NON_SYNONYMOUS_CODING	possibly_damaging(0.804)	deleterious(0.01)
11	126146291	FOXRED1	A	G	311	Y/C	SPLICE_SITE:NON_SYNONYMOUS_CODING	possibly_damaging(0.938)	deleterious(0)
10	135440122	FRG2B	T	C	42	E/G	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.44)
10	135440123	FRG2B	C	T	42	E/K	NON_SYNONYMOUS_CODING	benign(0.009)	tolerated(0.33)
10	135440159	FRG2B	T	A	30	T/S	NON_SYNONYMOUS_CODING	possibly_damaging(0.94)	tolerated(0.45)
17	7507359	FXR2	A	C	90	W/G	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
17	7507352	FXR2	G	GC	92	.	FRAMESHIFT_CODING	.	.
14	39591707	GEMIN2	G	C	160	G/A	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.78)
14	39601192	GEMIN2	C	T	222	L/F	NON_SYNONYMOUS_CODING	probably_damaging(0.958)	deleterious(0.01)
14	39601187	GEMIN2	C	CT	220	.	FRAMESHIFT_CODING	.	.
1	231411911	GNPAT	A	C	595	E/D	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.19)
1	231413267	GNPAT	A	C	613	K/N	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
5	89986671	GPR98	A	C	2255	N/T	NON_SYNONYMOUS_CODING	benign(0.361)	tolerated(0.57)
5	89949754	GPR98	G	A	1455	E/K	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
5	176868780	GRK6	A	C	574	T/P	NON_SYNONYMOUS_CODING	benign(0.006)	deleterious(0.04)
5	176863218	GRK6	T	G	401	V/G	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
6	43589395	GTPBP2	G	T	231	Q/K	NON_SYNONYMOUS_CODING	benign(0.143)	tolerated(0.29)
6	43589397	GTPBP2	A	T	230	V/E	NON_SYNONYMOUS_CODING	possibly_damaging(0.88)	deleterious(0)
7	106836338	HBP1	A	C	376	Q/P	NON_SYNONYMOUS_CODING	probably_damaging(0.963)	deleterious(0.02)
7	106836335	HBP1	G	C	375	R/P	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
2	37215874	HEATR5B	T	G	43	R/S	NON_SYNONYMOUS_CODING	probably_damaging(0.982)	tolerated(0.18)
2	37215885	HEATR5B	C	T	40	E/K	NON_SYNONYMOUS_CODING	probably_damaging(0.998)	tolerated(0.15)
15	28566562	HERC2	G	C	6	F/L	NON_SYNONYMOUS_CODING	possibly_damaging(0.794)	tolerated(0.37)
15	28501298	HERC2	A	C	895	S/A	NON_SYNONYMOUS_CODING	possibly_damaging(0.812)	deleterious(0.03)

15	28566548	HERC2	T	C	11	Q/R	NON_SYNONYMOUS_CODING	possibly_damaging(0.916)	tolerated(0.79)
6	32548544	HLA-DRB1	T	G	248	I/L	NON_SYNONYMOUS_CODING	benign(0)	deleterious(0)
6	32557449	HLA-DRB1	G	A	24	S/F	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.06)
6	32549357	HLA-DRB1	G	A	210	T/I	NON_SYNONYMOUS_CODING	possibly_damaging(0.481)	deleterious(0)
8	28908545	HMBOX1	C	G	402	T/R	NON_SYNONYMOUS_CODING	possibly_damaging(0.537)	tolerated(0.57)
8	28866621	HMBOX1	T	G	207	L/V	NON_SYNONYMOUS_CODING	possibly_damaging(0.653)	tolerated(0.08)
1	186094789	HMCN1	C	A	4185	Q/K	NON_SYNONYMOUS_CODING	benign(0.059)	tolerated(0.7)
1	186062678	HMCN1	C	A	3358	T/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.909)	tolerated(0.35)
1	186052030	HMCN1	T	G	2941	Y/D	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
14	23745525	HOMEZ	T	G	306	K/N	NON_SYNONYMOUS_CODING	benign(0.406)	deleterious(0.02)
14	23745520	HOMEZ	A	G	308	L/P	NON_SYNONYMOUS_CODING	probably_damaging(0.996)	deleterious(0.02)
4	3109121	HTT	G	T	240	G/C	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
4	3136225	HTT	T	G	864	V/G	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.12)
4	3230343	HTT	T	G	2617	V/G	SPLICE_SITE:NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
1	18618361	IGSF21	T	G	62	V/G	SPLICE_SITE:NON_SYNONYMOUS_CODING	probably_damaging(0.998)	deleterious(0)
1	18691718	IGSF21	T	G	181	V/G	SPLICE_SITE:NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0.01)
7	50467818	IKZF1	C	A	351	H/Q	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.41)
7	50455118	IKZF1	G	C	222	R/P	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
14	105180962	INF2	G	T	1155	A/S	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0)
14	105180963	INF2	C	T	1155	A/V	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0)
7	41729523	INHBA	T	C	336	N/D	NON_SYNONYMOUS_CODING	benign(0.074)	deleterious(0.02)
7	41729465	INHBA	G	C	355	P/R	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
3	4725969	ITPR1	G	A	1159	G/E	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.22)
3	4725973	ITPR1	T	A	1160	N/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.94)
3	4725976	ITPR1	C	A	1161	N/K	NON_SYNONYMOUS_CODING	benign(0.004)	tolerated(0.94)
14	58934505	KIAA0586	C	A	629	S/R	NON_SYNONYMOUS_CODING	benign(0.128)	tolerated(0.16)
14	58954767	KIAA0586	A	C	1016	D/A	NON_SYNONYMOUS_CODING	possibly_damaging(0.902)	deleterious(0.02)
17	44109576	KIAA1267	C	A	270	S/I	NON_SYNONYMOUS_CODING	possibly_damaging(0.527)	deleterious(0.03)
17	44109607	KIAA1267	T	G	260	T/P	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0.01)
17	39346592	KRTAP9-1	ACCT	A	152-153	TC/S	NON_SYNONYMOUS_CODING	.	.
17	39346433	KRTAP9-1	A	C	99	T/P	SPLICE_SITE:NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.08)
13	76287349	LMO7	A	T	86	D/V	NON_SYNONYMOUS_CODING	probably_damaging(0.991)	deleterious(0.01)
13	76287354	LMO7	C	G	88	R/G	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0.01)
4	151236754	LRBA	T	G	2551	D/A	NON_SYNONYMOUS_CODING	probably_damaging(0.996)	tolerated(0.3)
4	151837565	LRBA	G	T	294	F/L	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	tolerated(0.19)
2	141259399	LRP1B	A	C	2841	C/G	NON_SYNONYMOUS_CODING	probably_damaging(0.995)	deleterious(0)
2	141625828	LRP1B	G	A	1330	L/F	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0.01)
1	53746346	LRP8	T	G	137	T/P	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.25)
1	53724069	LRP8	A	G	264	S/P	NON_SYNONYMOUS_CODING	probably_damaging(0.966)	deleterious(0.01)
11	65315445	LTBP3	A	G	598	S/P	NON_SYNONYMOUS_CODING	probably_damaging(0.969)	tolerated(0.1)
11	65310596	LTBP3	A	C	859	V/G	NON_SYNONYMOUS_CODING	probably_damaging(0.985)	tolerated(0.39)
1	160783621	LY9	A	C	217	D/A	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.22)
1	160784275	LY9	A	C	266	T/P	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
1	235914653	LYST	A	C	60	V/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.856)	deleterious(0)

1	235955172	LYST	T	G	1457	H/P	NON_SYNONYMOUS_CODING	probably_damaging(1)	.
7	20198700	MACC1	G	T	428	D/E	NON_SYNONYMOUS_CODING	benign(0.003)	tolerated(0.57)
7	20198702	MACC1	C	T	428	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.604)	tolerated(0.18)
1	39945661	MACF1	A	C	284	T/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.564)	tolerated(0.16)
1	39934315	MACF1	T	G	190	V/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.839)	deleterious(0)
1	117944954	MAN1A2	T	A	150	I/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.96)
1	117944964	MAN1A2	C	A	153	N/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.96)
5	71491052	MAP1B	C	A	641	Q/K	NON_SYNONYMOUS_CODING	benign(0.007)	tolerated(1)
5	71490953	MAP1B	G	A	608	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.792)	tolerated(0.27)
5	71490955	MAP1B	C	A	608	D/E	NON_SYNONYMOUS_CODING	possibly_damaging(0.792)	tolerated(0.67)
5	71490718	MAP1B	G	C	529	K/N	NON_SYNONYMOUS_CODING	probably_damaging(0.995)	deleterious(0)
19	45783923	MARK4	A	C	403	T/P	NON_SYNONYMOUS_CODING	benign(0.003)	tolerated(1)
19	45805775	MARK4	G	C	16	R/P	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0.01)
15	41961151	MGA	C	G	20	A/G	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.3)
15	42040862	MGA	C	A	1747	T/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.812)	deleterious(0)
15	42000043	MGA	A	C	769	N/T	NON_SYNONYMOUS_CODING	possibly_damaging(0.911)	deleterious(0)
11	12315177	MICALCL	A	G	67	R/G	NON_SYNONYMOUS_CODING	benign(0.09)	deleterious(0)
11	12315180	MICALCL	C	G	68	R/G	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
10	129901721	MKI67	G	T	2794	Q/K	NON_SYNONYMOUS_CODING	possibly_damaging(0.806)	tolerated(0.13)
10	129901067	MKI67	G	C	3012	R/G	NON_SYNONYMOUS_CODING	probably_damaging(0.986)	tolerated(0.38)
7	151932961	MLL3	G	A	904	R/*	STOP_GAINED	.	.
7	151927025	MLL3	A	G	987	Y/H	NON_SYNONYMOUS_CODING	probably_damaging(1)	.
7	151932997	MLL3	C	T	892	G/R	NON_SYNONYMOUS_CODING	probably_damaging(1)	.
7	100636383	MUC12	G	A	847	V/I	NON_SYNONYMOUS_CODING	possibly_damaging(0.871)	.
7	100645731	MUC12	G	A	3963	E/K	NON_SYNONYMOUS_CODING	possibly_damaging(0.952)	.
7	100634922	MUC12	G	A	360	A/T	NON_SYNONYMOUS_CODING	unknown(0)	.
19	9002623	MUC16	C	T	39	R/H	NON_SYNONYMOUS_CODING	benign(0.011)	tolerated(0.24)
19	9002636	MUC16	C	T	35	V/I	NON_SYNONYMOUS_CODING	benign(0.051)	tolerated(0.37)
19	9002612	MUC16	T	G	43	K/Q	NON_SYNONYMOUS_CODING	benign(0.055)	tolerated(0.23)
19	8961997	MUC16	C	A	1101	L/F	NON_SYNONYMOUS_CODING	possibly_damaging(0.448)	deleterious(0)
19	9002597	MUC16	C	T	48	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.866)	deleterious(0)
19	9002660	MUC16	C	T	27	G/R	NON_SYNONYMOUS_CODING	probably_damaging(0.998)	tolerated(0.5)
19	9059082	MUC16	C	A	9455	S/I	NON_SYNONYMOUS_CODING	unknown(0)	.
19	9063517	MUC16	A	G	7977	S/P	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195513846	MUC4	C	G	1535	M/I	NON_SYNONYMOUS_CODING	benign(0.012)	.
3	195513847	MUC4	A	G	1535	M/T	NON_SYNONYMOUS_CODING	benign(0.042)	.
3	195507226	MUC4	A	G	3742	V/A	NON_SYNONYMOUS_CODING	benign(0.095)	.
3	195505859	MUC4	T	C	4198	T/A	NON_SYNONYMOUS_CODING	benign(0.182)	.
3	195510228	MUC4	C	G	2741	E/D	NON_SYNONYMOUS_CODING	benign(0.182)	.
3	195507228	MUC4	G	C	3741	H/Q	NON_SYNONYMOUS_CODING	benign(0.204)	.
3	195508716	MUC4	A	C	3245	H/Q	NON_SYNONYMOUS_CODING	benign(0.204)	.
3	195515017	MUC4	A	G	1145	V/A	NON_SYNONYMOUS_CODING	benign(0.231)	.
3	195510194	MUC4	G	C	2753	L/V	NON_SYNONYMOUS_CODING	benign(0.352)	.
3	195508709	MUC4	A	G	3248	S/P	NON_SYNONYMOUS_CODING	benign(0.421)	.

3	195507062	MUC4	C	T	3797	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195507193	MUC4	G	A	3753	A/V	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195507385	MUC4	G	A	3689	A/V	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195507446	MUC4	C	T	3669	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195508500	MUC4	G	C	3317	D/E	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195510649	MUC4	G	A	2601	A/V	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195506983	MUC4	G	A	3823	T/I	NON_SYNONYMOUS_CODING	possibly_damaging(0.607)	.
3	195507443	MUC4	T	G	3670	T/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.607)	.
3	195507827	MUC4	G	A	3542	L/F	NON_SYNONYMOUS_CODING	possibly_damaging(0.607)	.
3	195511285	MUC4	T	C	2389	D/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.748)	.
3	195511286	MUC4	C	T	2389	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.748)	.
3	195511525	MUC4	T	C	2309	D/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.748)	.
3	195511526	MUC4	C	T	2309	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.748)	.
3	195507445	MUC4	T	A	3669	D/V	NON_SYNONYMOUS_CODING	possibly_damaging(0.862)	.
3	195507461	MUC4	G	A	3664	P/S	NON_SYNONYMOUS_CODING	possibly_damaging(0.864)	.
3	195507694	MUC4	A	G	3586	L/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.881)	.
3	195505870	MUC4	G	A	4194	P/L	NON_SYNONYMOUS_CODING	possibly_damaging(0.931)	.
3	195515449	MUC4	A	T	1001	V/E	NON_SYNONYMOUS_CODING	probably_damaging(0.979)	.
3	195510622	MUC4	G	T	2610	P/H	NON_SYNONYMOUS_CODING	probably_damaging(0.991)	.
3	195510310	MUC4	T	G	2714	Y/S	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510341	MUC4	A	G	2704	S/P	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510373	MUC4	T	C	2693	D/G	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510374	MUC4	C	T	2693	D/N	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510396	MUC4	A	C	2685	H/Q	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510415	MUC4	G	T	2679	S/Y	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510466	MUC4	A	G	2662	L/P	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510492	MUC4	C	G	2653	Q/H	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510601	MUC4	A	G	2617	V/A	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510611	MUC4	G	T	2614	L/I	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510613	MUC4	C	T	2613	S/N	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510614	MUC4	T	C	2613	S/G	NON_SYNONYMOUS_CODING	unknown(0)	.
11	1263706	MUC5B	T	G	1866	F/V	NON_SYNONYMOUS_CODING	benign(0.014)	.
11	1263847	MUC5B	A	C	1913	T/P	NON_SYNONYMOUS_CODING	benign(0.049)	.
11	1283520	MUC5B	A	C	5468	D/A	NON_SYNONYMOUS_CODING	unknown(0)	.
11	1283527	MUC5B	G	C	5470	Q/H	NON_SYNONYMOUS_CODING	unknown(0)	.
11	1017466	MUC6	T	C	1779	R/G	NON_SYNONYMOUS_CODING	benign(0.142)	tolerated(0.57)
11	1017307	MUC6	G	A	1832	P/S	NON_SYNONYMOUS_CODING	benign(0.34)	tolerated(0.21)
11	1018042	MUC6	A	G	1587	S/P	NON_SYNONYMOUS_CODING	probably_damaging(0.979)	deleterious(0.01)
11	1016554	MUC6	C	T	2083	A/T	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.6)
11	1016559	MUC6	G	C	2081	A/G	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.59)
11	1016565	MUC6	C	T	2079	S/N	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.09)
11	1017604	MUC6	T	G	1733	T/P	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.36)
11	1018552	MUC6	G	A	1417	P/S	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.73)
11	1018561	MUC6	T	C	1414	S/G	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.07)

13	77673117	MYCBP2	T	G	2686	K/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.46)
13	77672809	MYCBP2	A	G	2789	L/P	NON_SYNONYMOUS_CODING	benign(0.017)	deleterious(0.03)
13	77672228	MYCBP2	G	T	2983	Q/K	NON_SYNONYMOUS_CODING	benign(0.021)	tolerated(0.45)
15	72192270	MYO9A	A	T	1057	S/R	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.63)
15	72192271	MYO9A	C	T	1057	S/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.55)
18	3129302	MYOM1	T	G	908	T/P	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.1)
18	3112368	MYOM1	G	C	1116	R/G	NON_SYNONYMOUS_CODING	probably_damaging(0.997)	deleterious(0.01)
18	3174178	MYOM1	G	A	351	R/W	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
1	145367800	NBPF10	G	A	586	D/N	NON_SYNONYMOUS_CODING	benign(0.009)	deleterious(0.03)
1	145302775	NBPF10	T	G	330	Y/D	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(1)
1	145304625	NBPF10	T	G	445	W/G	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.09)
1	145304635	NBPF10	C	T	448	A/V	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0)
22	37260160	NCF4	A	C	36	T/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.775)	tolerated(0.37)
22	37260158	NCF4	T	C	35	F/S	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.06)
12	8242854	NECAP1	A	T	87	D/V	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
12	8242857	NECAP1	C	T	88	S/F	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
18	77208908	NFATC1	A	C	505	T/P	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.39)
18	77211052	NFATC1	T	G	563	V/G	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
11	129756238	NFRKB	G	C	147	L/V	NON_SYNONYMOUS_CODING	benign(0.351)	tolerated(0.97)
11	129739483	NFRKB	G	A	1171	S/F	NON_SYNONYMOUS_CODING	probably_damaging(0.991)	deleterious(0.01)
3	25777564	NGLY1	G	T	360	D/E	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
3	25773841	NGLY1	C	A	465	W/L	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
5	156895736	NIPAL4	C	G	176	A/G	NON_SYNONYMOUS_CODING	benign(0.008)	tolerated(0.17)
5	156890324	NIPAL4	C	G	120	R/G	NON_SYNONYMOUS_CODING	unknown(0)	.
3	48337624	NME6	A	C	73	V/G	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
3	48340012	NME6	T	C	7	S/G	SPLICE_SITE:NON_SYNONYMOUS_CODING	benign(0.053)	tolerated(0.29)
9	139413211	NOTCH1	T	G	311	T/P	NON_SYNONYMOUS_CODING	benign(0.004)	deleterious(0)
9	139413097	NOTCH1	T	G	349	T/P	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
16	15224274	NPIPP1	A	G	143	S/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.911)	deleterious(0.01)
16	15222129	NPIPP1	G	A	319	P/S	NON_SYNONYMOUS_CODING	unknown(0)	.
3	193332725	OPA1	C	A	82	Y/*	STOP_GAINED	.	.
3	193386395	OPA1	G	C	127	A/P	NON_SYNONYMOUS_CODING	unknown(0)	.
4	146063400	OTUD4	T	G	590	L/F	NON_SYNONYMOUS_CODING	possibly_damaging(0.915)	deleterious(0.02)
4	146063397	OTUD4	T	TG	591	.	FRAMESHIFT_CODING	.	.
2	60995630	PAPOLG	C	A	47	T/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.7)
2	60988881	PAPOLG	T	G	18	V/G	NON_SYNONYMOUS_CODING	benign(0.025)	tolerated(0.09)
16	66919665	PDP2	C	G	493	A/G	NON_SYNONYMOUS_CODING	benign(0.211)	deleterious(0.04)
16	66918831	PDP2	A	G	215	E/G	NON_SYNONYMOUS_CODING	probably_damaging(0.994)	deleterious(0.01)
5	108698639	PJA2	T	G	518	E/D	NON_SYNONYMOUS_CODING	probably_damaging(0.971)	tolerated(0.66)
5	108698646	PJA2	C	T	516	G/E	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.27)
6	51920399	PKHD1	C	T	608	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.574)	deleterious(0.03)
6	51920465	PKHD1	A	C	586	F/V	NON_SYNONYMOUS_CODING	possibly_damaging(0.903)	deleterious(0.04)
8	110463218	PKHD1L1	C	A	2064	Q/K	NON_SYNONYMOUS_CODING	benign(0.023)	tolerated(1)
8	110455184	PKHD1L1	C	T	1468	S/F	NON_SYNONYMOUS_CODING	probably_damaging(0.982)	deleterious(0.01)

8	110523026	PKHD1L1	T	G	734	C/G	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0.02)
12	19475590	PLEKHA5	C	G	47	R/G	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0.01)
12	19459391	PLEKHA5	C	A	635	T/N	NON_SYNONYMOUS_CODING	unknown(0)	.
1	208252715	PLXNA2	A	C	826	C/G	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
1	208272313	PLXNA2	A	C	537	C/G	SPLICE_SITE:NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
14	74179763	PNMA1	A	C	194	S/A	NON_SYNONYMOUS_CODING	benign(0.121)	tolerated(0.17)
14	74179765	PNMA1	A	C	193	V/G	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
7	108155497	PNPLA8	G	T	47	Q/K	NON_SYNONYMOUS_CODING	benign(0.032)	tolerated(0.4)
7	108155503	PNPLA8	A	C	45	L/V	NON_SYNONYMOUS_CODING	benign(0.271)	tolerated(0.17)
15	89862549	POLG	A	C	1005	V/G	NON_SYNONYMOUS_CODING	probably_damaging(0.991)	tolerated(0.34)
15	89864175	POLG	T	G	935	T/P	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
2	86272763	POLR1A	A	C	955	F/V	NON_SYNONYMOUS_CODING	probably_damaging(0.997)	deleterious(0)
2	86272426	POLR1A	T	G	982	T/P	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
12	81741484	PPFIA2	C	T	669	S/N	NON_SYNONYMOUS_CODING	probably_damaging(0.998)	deleterious(0)
12	81769573	PPFIA2	A	C	196	V/G	ESSENTIAL_SPLICE_SITE:NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0)
9	134366847	PRRC2B	G	C	94	Q/H	NON_SYNONYMOUS_CODING	benign(0.088)	deleterious(0)
9	134357909	PRRC2B	A	C	1712	D/A	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0.02)
9	134357908	PRRC2B	G	C	1712	D/H	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0.01)
3	184019670	PSMD2	A	G	172	E/G	NON_SYNONYMOUS_CODING	benign(0.046)	tolerated(0.1)
3	184019400	PSMD2	G	A	145	V/M	NON_SYNONYMOUS_CODING	possibly_damaging(0.915)	tolerated(0.07)
3	184024549	PSMD2	T	G	654	V/G	NON_SYNONYMOUS_CODING	probably_damaging(0.998)	deleterious(0)
10	27702786	PTCHD3	A	C	132	W/G	NON_SYNONYMOUS_CODING	benign(0.003)	tolerated(0.36)
10	27700857	PTCHD3	A	C	364	V/G	SPLICE_SITE:NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
12	70934671	PTPRB	A	C	1546	V/G	NON_SYNONYMOUS_CODING	benign(0.341)	tolerated(0.2)
12	71003065	PTPRB	A	G	37	S/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.909)	deleterious(0.02)
3	191179127	PYDC2	T	C	59	F/S	NON_SYNONYMOUS_CODING	benign(0.29)	deleterious(0)
3	191179129	PYDC2	A	C	60	T/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.817)	tolerated(0.06)
13	49033835	RB1	G	A	658	A/T	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
13	49033836	RB1	C	A	658	A/D	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
9	3293246	RFX3	A	C	188	L/V	NON_SYNONYMOUS_CODING	benign(0.004)	tolerated(0.11)
9	3395524	RFX3	C	T	22	S/N	NON_SYNONYMOUS_CODING	benign(0.026)	deleterious(0.04)
2	87205020	RGPD1	G	T	810	R/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
2	87205031	RGPD1	T	C	814	C/R	NON_SYNONYMOUS_CODING	possibly_damaging(0.85)	tolerated(0.78)
12	130892351	RIMBP2	G	C	86	R/G	NON_SYNONYMOUS_CODING	benign(0.13)	tolerated(0.41)
12	130892349	RIMBP2	A	C	10	V/G	NON_SYNONYMOUS_CODING	unknown(0)	.
7	4259872	SDK1	C	A	139	Q/K	NON_SYNONYMOUS_CODING	benign(0.253)	tolerated(0.31)
7	4247821	SDK1	A	C	17	T/P	NON_SYNONYMOUS_CODING	probably_damaging(0.975)	tolerated(0.11)
22	26773659	SEZ6L	T	C	977	L/P	NON_SYNONYMOUS_CODING	probably_damaging(0.991)	deleterious(0)
22	26773662	SEZ6L	G	C	978	R/P	NON_SYNONYMOUS_CODING	probably_damaging(0.996)	tolerated(0.21)
22	26709766	SEZ6L	T	G	638	V/G	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
6	74351582	SLC17A5	G	T	119	Y/*	STOP_GAINED	.	.
6	74351580	SLC17A5	A	T	120	I/N	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
17	79249833	SLC38A10	A	C	283	V/G	NON_SYNONYMOUS_CODING	probably_damaging(0.998)	deleterious(0)
17	79254419	SLC38A10	C	G	206	A/P	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0.01)

4	20598044	SLIT2	G	T	1105	L/F	NON_SYNONYMOUS_CODING	possibly_damaging(0.823)	deleterious(0.03)
4	20525686	SLIT2	G	A	446	A/T	NON_SYNONYMOUS_CODING	probably_damaging(0.99)	deleterious(0.01)
4	20525687	SLIT2	C	A	446	A/E	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
4	88416177	SPARCL1	C	T	53	E/K	NON_SYNONYMOUS_CODING	benign(0.263)	deleterious(0.02)
4	88416183	SPARCL1	C	T	51	E/K	NON_SYNONYMOUS_CODING	probably_damaging(0.959)	deleterious(0.01)
4	88416171	SPARCL1	C	T	55	E/K	NON_SYNONYMOUS_CODING	probably_damaging(0.991)	tolerated(0.06)
1	16258610	SPEN	C	G	1959	R/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.536)	tolerated(0.05)
1	16203071	SPEN	G	A	260	S/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.767)	tolerated(0.1)
1	16203072	SPEN	C	A	260	S/R	NON_SYNONYMOUS_CODING	possibly_damaging(0.903)	tolerated(0.13)
6	36489582	STK38	G	A	107	Q/*	STOP_GAINED	.	.
6	36489585	STK38	C	A	106	V/F	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
22	24584027	SUSD2	C	A	755	Y/*	STOP_GAINED	.	.
22	24579157	SUSD2	G	T	70	G/V	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
6	152665230	SYNE1	C	T	4000	E/K	NON_SYNONYMOUS_CODING	benign(0.42)	.
6	152786454	SYNE1	C	T	624	S/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.649)	tolerated(0.27)
7	35288322	TBX20	A	C	171	V/G	NON_SYNONYMOUS_CODING	probably_damaging(0.991)	deleterious(0)
7	35288307	TBX20	T	G	176	D/A	NON_SYNONYMOUS_CODING	probably_damaging(0.993)	deleterious(0)
1	152084213	TCHH	C	G	494	E/Q	NON_SYNONYMOUS_CODING	unknown(0)	.
1	152084216	TCHH	C	G	493	E/Q	NON_SYNONYMOUS_CODING	unknown(0)	.
8	56737244	TGS1	A	C	848	R/S	NON_SYNONYMOUS_CODING	benign(0.021)	deleterious(0.02)
8	56708572	TGS1	T	G	468	V/G	NON_SYNONYMOUS_CODING	benign(0.189)	deleterious(0)
4	113199252	TIFA	A	T	107	N/K	NON_SYNONYMOUS_CODING	possibly_damaging(0.586)	tolerated(0.22)
4	113199244	TIFA	T	G	110	D/A	NON_SYNONYMOUS_CODING	possibly_damaging(0.907)	tolerated(0.1)
17	32956084	TMEM132E	T	C	310	F/S	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	tolerated(0.5)
17	32956086	TMEM132E	A	C	311	T/P	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0.03)
16	426329	TMEM8A	A	C	151	V/G	NON_SYNONYMOUS_CODING	benign(0.055)	tolerated(0.56)
16	422045	TMEM8A	A	G	560	F/S	NON_SYNONYMOUS_CODING	benign(0.282)	tolerated(0.13)
12	83251115	TMTC2	T	G	137	V/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.926)	deleterious(0)
12	83251120	TMTC2	C	G	139	R/G	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
17	26671484	TNFAIP1	C	A	166	T/K	NON_SYNONYMOUS_CODING	possibly_damaging(0.917)	deleterious(0)
17	26666686	TNFAIP1	A	C	47	T/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.952)	deleterious(0.01)
1	12198361	TNFRSF8	A	C	359	T/P	NON_SYNONYMOUS_CODING	benign(0.004)	deleterious(0)
1	12144552	TNFRSF8	A	C	32	N/T	NON_SYNONYMOUS_CODING	benign(0.025)	tolerated(0.61)
16	1279714	TPSB2	A	G	29	V/A	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.33)
16	1279732	TPSB2	C	A	23	G/V	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.17)
6	52369436	TRAM2	A	G	331	V/A	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.83)
6	52370454	TRAM2	C	G	273	R/P	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	tolerated(0.19)
7	142498738	TRBC2	C	G	5	N/K	NON_SYNONYMOUS_CODING	benign(0.002)	tolerated(0.34)
7	142498735	TRBC2	A	C	4	K/N	NON_SYNONYMOUS_CODING	benign(0.111)	tolerated(0.08)
7	142008645	TRBV3-1	A	C	40	I/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142008750	TRBV3-1	A	T	75	I/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.24)
7	142008783	TRBV3-1	A	G	86	K/E	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142008675	TRBV3-1	A	G	50	T/A	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(1)
7	142008727	TRBV3-1	G	T	67	S/I	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.4)

7	142008785	TRBV3-1	A	C	86	K/N	NON_SYNONYMOUS_CODING	benign(0.001)	deleterious(0.02)
7	142008718	TRBV3-1	T	C	64	I/T	NON_SYNONYMOUS_CODING	benign(0.002)	deleterious(0.01)
7	142008672	TRBV3-1	G	A	49	D/N	NON_SYNONYMOUS_CODING	benign(0.006)	tolerated(0.61)
7	142008745	TRBV3-1	T	C	73	L/P	NON_SYNONYMOUS_CODING	probably_damaging(0.993)	tolerated(0.27)
7	142045680	TRBV4-2	T	C	70	F/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.67)
7	142045693	TRBV4-2	C	T	74	T/I	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.36)
7	142045677	TRBV4-2	A	AG	69	.	FRAMESHIFT_CODING	.	.
7	142180566	TRBV6-5	A	T	98	L/Q	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.22)
7	142180567	TRBV6-5	G	C	98	L/V	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.06)
7	142180573	TRBV6-5	T	C	96	R/G	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.12)
7	142180585	TRBV6-5	C	T	92	D/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.79)
7	142180590	TRBV6-5	G	T	90	T/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142180593	TRBV6-5	G	T	89	T/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.44)
7	142180596	TRBV6-5	G	A	88	S/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.31)
7	142180618	TRBV6-5	T	C	81	N/D	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.78)
7	142180633	TRBV6-5	G	T	76	Q/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142180635	TRBV6-5	T	G	75	D/A	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.22)
7	142180641	TRBV6-5	A	G	73	I/T	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.91)
7	142180704	TRBV6-5	G	T	52	S/Y	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142180737	TRBV6-5	T	A	41	Q/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.16)
7	142180578	TRBV6-5	G	A	94	P/L	NON_SYNONYMOUS_CODING	benign(0.002)	tolerated(0.17)
7	142180588	TRBV6-5	C	G	91	E/Q	NON_SYNONYMOUS_CODING	benign(0.005)	tolerated(0.2)
7	142180647	TRBV6-5	G	T	71	A/D	NON_SYNONYMOUS_CODING	benign(0.08)	tolerated(0.18)
7	142099548	TRBV7-8	A	G	85	F/S	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142099581	TRBV7-8	A	G	74	L/P	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.35)
7	142099512	TRBV7-8	T	G	97	K/T	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.14)
7	142099533	TRBV7-8	T	C	90	E/G	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.36)
7	142099537	TRBV7-8	G	T	89	P/T	NON_SYNONYMOUS_CODING	benign(0.003)	tolerated(0.39)
21	38498381	TTC3	T	G	394	V/G	SPLICE_SITE:NON_SYNONYMOUS_CODING	benign(0.032)	deleterious(0)
21	38560797	TTC3	T	G	1642	V/G	SPLICE_SITE:NON_SYNONYMOUS_CODING	probably_damaging(0.995)	deleterious(0)
2	179472353	TTN	T	C	8748	K/E	NON_SYNONYMOUS_CODING	benign(0.099)	.
2	179419226	TTN	A	C	20676	I/M	NON_SYNONYMOUS_CODING	possibly_damaging(0.789)	.
2	179413028	TTN	C	A	22169	V/L	NON_SYNONYMOUS_CODING	possibly_damaging(0.891)	.
2	179468803	TTN	T	C	9264	E/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.936)	.
2	179592426	TTN	A	C	5383	S/A	NON_SYNONYMOUS_CODING	unknown(0)	.
2	179595427	TTN	A	C	4701	S/A	NON_SYNONYMOUS_CODING	unknown(0)	.
2	179597615	TTN	G	C	4186	R/G	NON_SYNONYMOUS_CODING	unknown(0)	.
10	60121265	UBE2D1	G	A	33	W/*	STOP_GAINED	.	.
10	60121266	UBE2D1	C	A	34	Q/K	NON_SYNONYMOUS_CODING	possibly_damaging(0.836)	deleterious(0.04)
1	19524272	UBR4	T	G	262	N/T	NON_SYNONYMOUS_CODING	possibly_damaging(0.9)	tolerated(0.27)
1	19442066	UBR4	T	G	45	D/A	NON_SYNONYMOUS_CODING	possibly_damaging(0.932)	tolerated(0.26)
2	128896359	UGGT1	T	G	551	V/G	NON_SYNONYMOUS_CODING	benign(0.052)	deleterious(0)
2	128945090	UGGT1	A	G	91	D/G	NON_SYNONYMOUS_CODING	probably_damaging(0.996)	tolerated(0.34)
1	216251520	USH2A	T	G	1828	D/A	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)

1	215960138	USH2A	T	G	3421	T/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.832)	deleterious(0.05)
1	215960151	USH2A	A	C	3416	C/W	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
1	215960153	USH2A	A	C	3416	C/G	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
12	62798066	USP15	G	C	924	A/P	NON_SYNONYMOUS_CODING	benign(0.063)	tolerated(0.05)
12	62786861	USP15	C	A	788	Q/K	NON_SYNONYMOUS_CODING	possibly_damaging(0.581)	tolerated(0.28)
17	5036210	USP6	T	G	67	I/M	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0.04)
17	5036211	USP6	C	T	68	R/W	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0)
12	101685769	UTP20	G	T	354	V/L	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.26)
12	101764315	UTP20	C	A	2221	Q/K	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
2	98779398	VWA3B	T	G	208	V/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.485)	tolerated(0.06)
2	98853082	VWA3B	G	T	854	L/F	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0.02)
1	20671955	VWA5B1	A	C	878	N/T	NON_SYNONYMOUS_CODING	benign(0.003)	tolerated(0.31)
1	20678602	VWA5B1	C	A	1031	S/R	NON_SYNONYMOUS_CODING	probably_damaging(0.976)	deleterious(0.05)
15	85191135	WDR73	A	C	113	V/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.948)	deleterious(0)
15	85197480	WDR73	A	C	9	V/G	NON_SYNONYMOUS_CODING	probably_damaging(0.998)	deleterious(0.01)
4	6296897	WFS1	A	C	281	D/A	NON_SYNONYMOUS_CODING	benign(0.019)	tolerated(0.08)
4	6303810	WFS1	A	C	141	H/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.625)	tolerated(0.1)
16	87448068	ZCCHC14	T	G	382	T/P	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.15)
16	87448079	ZCCHC14	C	G	378	R/P	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.13)
8	77761278	ZFHX4	A	C	1142	T/P	NON_SYNONYMOUS_CODING	benign(0.189)	.
8	77765378	ZFHX4	A	C	2029	D/A	NON_SYNONYMOUS_CODING	probably_damaging(0.978)	.
5	121488461	ZNF474	A	C	259	H/P	NON_SYNONYMOUS_CODING	benign(0.144)	deleterious(0.02)
5	121488458	ZNF474	T	C	258	L/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.924)	tolerated(0.29)
15	85326799	ZNF592	G	A	298	S/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.3)
15	85326800	ZNF592	T	A	298	S/R	NON_SYNONYMOUS_CODING	benign(0.073)	tolerated(0.11)
8	102213947	ZNF706	A	T	8	I/N	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0)
8	102213962	ZNF706	C	G	3	R/P	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0)

CHD20: Homozygous variants

CHR ID	NUCLEOTIDE POSITION	GENE NAME	REF SEQUENCE	ALTERNATE SEQUENCE	AMINO ACID NO.	RESIDUE CHANGE	FUNCTION OF VARIANT	POLYPHEN	SIFT
2	241631603	AQP12A	C	T	79	S/L	NON_SYNONYMOUS_CODING	benign(0.005)	tolerated(0.26)
19	43860251	CD177	G	A	204	D/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
19	43860255	CD177	T	G	205	M/R	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.38)
16	29395010	ENSG00000254206	G	T	415	P/T	NON_SYNONYMOUS_CODING	probably_damaging(0.992)	tolerated(0.07)
15	82637079	GOLGA6L10	C	T	336	R/Q	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(1)
7	74212311	GTF2IRD2	G	T	61	H/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
6	31324931	HLA-B	A	C	3	L/R	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
19	54725835	LILRB3	G	C	175	R/G	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.32)
17	44408066	LRRC37A	C	A	1141	F/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.11)
7	100636446	MUC12	G	A	868	D/N	NON_SYNONYMOUS_CODING	unknown(0)	.
7	100636447	MUC12	A	G	868	D/G	NON_SYNONYMOUS_CODING	unknown(0)	.
7	100636459	MUC12	C	T	872	T/M	NON_SYNONYMOUS_CODING	unknown(0)	.
7	100636467	MUC12	C	G	875	L/V	NON_SYNONYMOUS_CODING	unknown(0)	.
7	100639147	MUC12	A	G	1768	E/G	NON_SYNONYMOUS_CODING	unknown(0)	.
7	100639177	MUC12	C	T	1778	S/L	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195512186	MUC4	T	C	2089	I/V	NON_SYNONYMOUS_CODING	benign(0.028)	.
3	195510389	MUC4	A	G	2688	S/P	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510526	MUC4	A	G	2642	L/P	NON_SYNONYMOUS_CODING	unknown(0)	.
1	145327548	NBPF10	A	G	1369	N/D	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
1	145359049	NBPF10	A	G	2997	K/E	NON_SYNONYMOUS_CODING	benign(0.049)	tolerated(1)
1	248737259	OR2T34	C	G	267	R/P	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
1	13695685	PRAMEF19	G	A	358	S/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.68)
1	13695787	PRAMEF19	C	G	324	G/A	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
1	13696090	PRAMEF19	T	C	223	K/R	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.47)
22	18901004	PRODH	C	T	166	R/Q	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.6)
7	72436652	TRIM74	A	G	13	W/R	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.29)
18	30352057	ENSG00000228835	GCGCCGGCC	G	119-121	.	FRAMESHIFT_CODING	.	.
21	43298954	PRDM15	C	CG	88	.	FRAMESHIFT_CODING	.	.
18	44549025	TCEB3CL	T	TGC	425	.	FRAMESHIFT_CODING	.	.
3	133969437	RYK	A	AG	19-20	.	SPLICE_SITE:FRAMESHIFT_CODING	.	.

CHD20: Compound heterozygous variants

CHR ID	NUCLEOTIDE POSITION	GENE NAME	REF SEQUENCE	ALTERNATE SEQUENCE	AMINO ACID NO.	RESIDUE CHANGE	FUNCTION OF VARIANT	POLYPHEN	SIFT
5	434045	AHRR	C	G	401	T/R	NON_SYNONYMOUS_CODING	benign(0.284)	deleterious(0)
5	433020	AHRR	G	A	361	R/Q	NON_SYNONYMOUS_CODING	possibly_damaging(0.883)	deleterious(0.04)
2	97808524	ANKRD36	A	G	285	I/V	NON_SYNONYMOUS_CODING	benign(0.296)	tolerated(1)
2	97845632	ANKRD36	T	C	566	I/T	NON_SYNONYMOUS_CODING	possibly_damaging(0.767)	tolerated(0.23)
2	97808515	ANKRD36	G	A	282	E/K	NON_SYNONYMOUS_CODING	probably_damaging(0.959)	tolerated(1)
2	97808510	ANKRD36	G	A	280	G/D	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.85)
2	97833340	ANKRD36	A	G	520	T/A	SPLICE_SITE:NON_SYNONYMOUS_CODING	possibly_damaging(0.741)	tolerated(1)
20	18433273	C20orf12	T	G	179	T/P	NON_SYNONYMOUS_CODING	benign(0.005)	tolerated(0.15)
20	18433277	C20orf12	T	G	25	H/P	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0)
17	20243114	CCDC144C	G	T	304	V/F	NON_SYNONYMOUS_CODING	benign(0.127)	deleterious(0)
17	20243376	CCDC144C	A	G	391	K/R	NON_SYNONYMOUS_CODING	benign(0.29)	deleterious(0)
17	20269243	CCDC144C	A	G	901	Q/R	NON_SYNONYMOUS_CODING	benign(0.378)	tolerated(0.37)
17	45234417	CDC27	A	G	235	I/T	NON_SYNONYMOUS_CODING	benign(0.004)	tolerated(0.19)
17	45234303	CDC27	G	C	273	A/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.783)	tolerated(0.07)
17	7796794	CHD3	A	C	293	I/L	NON_SYNONYMOUS_CODING	benign(0)	.
17	7796803	CHD3	T	C	296	S/P	NON_SYNONYMOUS_CODING	benign(0.001)	.
8	68062165	CSPP1	G	A	703	S/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.64)
8	68062160	CSPP1	T	A	701	N/K	NON_SYNONYMOUS_CODING	benign(0.153)	tolerated(0.98)
6	132030560	CTAGE9	G	A	533	T/M	NON_SYNONYMOUS_CODING	benign(0.062)	tolerated(0.07)
6	132030966	CTAGE9	G	C	398	L/V	NON_SYNONYMOUS_CODING	probably_damaging(0.973)	deleterious(0.01)
16	15457629	ENSG00000183793	T	A	314	T/S	NON_SYNONYMOUS_CODING	benign(0.158)	tolerated(0.82)
16	15457628	ENSG00000183793	G	T	314	T/N	NON_SYNONYMOUS_CODING	benign(0.348)	tolerated(0.39)
19	56283297	ENSG00000229292	G	A	43	D/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.52)
19	56284090	ENSG00000229292	G	A	137	A/T	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.41)
9	46386995	ENSG00000237198	C	A	3	R/M	NON_SYNONYMOUS_CODING	benign(0.013)	tolerated(0.36)
9	46386902	ENSG00000237198	G	A	34	T/I	NON_SYNONYMOUS_CODING	probably_damaging(0.998)	deleterious(0)
9	46386956	ENSG00000237198	C	G	16	R/P	NON_SYNONYMOUS_CODING	probably_damaging(0.998)	deleterious(0)
9	46386785	ENSG00000237198	G	C	73	T/R	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0)
9	46386806	ENSG00000237198	C	T	66	R/Q	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.75)
18	11619510	ENSG00000257513	A	C	351	*/E	STOP_LOST	.	.
18	11619549	ENSG00000257513	T	G	338	K/Q	NON_SYNONYMOUS_CODING	probably_damaging(0.998)	deleterious(0.03)
10	88988115	FAM22A	G	A	160	A/T	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.32)
10	88992687	FAM22A	C	T	37	P/L	NON_SYNONYMOUS_CODING	probably_damaging(0.967)	deleterious(0)
20	29628328	FRG1B	C	G	110	I/M	NON_SYNONYMOUS_CODING	benign(0.191)	deleterious(0.03)

20	29632672	FRG1B	C	T	163	L/F	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0.01)
10	135440122	FRG2B	T	C	42	E/G	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.44)
10	135440123	FRG2B	C	T	42	E/K	NON_SYNONYMOUS_CODING	benign(0.009)	tolerated(0.33)
10	135440203	FRG2B	A	T	15	I/N	NON_SYNONYMOUS_CODING	benign(0.097)	deleterious(0.02)
10	135440159	FRG2B	T	A	30	T/S	NON_SYNONYMOUS_CODING	possibly_damaging(0.94)	tolerated(0.45)
10	135440108	FRG2B	C	T	47	A/T	NON_SYNONYMOUS_CODING	probably_damaging(0.997)	tolerated(0.31)
15	23264768	GOLGA8IP	A	G	458	S/G	NON_SYNONYMOUS_CODING	benign(0.002)	tolerated(0.85)
15	23262005	GOLGA8IP	G	A	373	V/I	NON_SYNONYMOUS_CODING	benign(0.188)	tolerated(0.18)
6	31324036	HLA-B	T	A	177	E/V	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
6	31324547	HLA-B	G	C	88	N/K	NON_SYNONYMOUS_CODING	benign(0.192)	deleterious(0.02)
9	35906556	HRCT1	A	C	91	H/P	NON_SYNONYMOUS_CODING	unknown(0)	.
9	35906559	HRCT1	A	C	92	H/P	NON_SYNONYMOUS_CODING	unknown(0)	.
14	106573354	IGHV3-11	A	G	77	I/T	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
14	106573357	IGHV3-11	G	T	76	T/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.62)
14	106573358	IGHV3-11	T	A	76	T/S	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
14	106573352	IGHV3-11	A	T	78	Y/N	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.23)
14	106993935	IGHV3-48	A	G	77	I/T	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
14	106994010	IGHV3-48	C	T	52	S/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.29)
14	107113742	IGHV3-64	C	T	118	R/K	NON_SYNONYMOUS_CODING	benign(0.002)	tolerated(0.18)
14	107113745	IGHV3-64	G	A	117	A/V	NON_SYNONYMOUS_CODING	benign(0.003)	tolerated(0.06)
14	107114170	IGHV3-64	A	G	10	F/L	NON_SYNONYMOUS_CODING	benign(0.012)	tolerated(0.06)
14	107034847	IGHV5-51	C	T	78	R/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.37)
14	107034854	IGHV5-51	C	A	76	D/Y	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.24)
14	107034869	IGHV5-51	A	C	71	Y/D	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.32)
14	107034873	IGHV5-51	G	C	69	I/M	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.22)
14	107034874	IGHV5-51	A	C	69	I/S	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.41)
14	107034846	IGHV5-51	T	G	78	R/S	NON_SYNONYMOUS_CODING	benign(0.136)	tolerated(0.44)
7	151927025	MLL3	A	G	987	Y/H	NON_SYNONYMOUS_CODING	probably_damaging(1)	.
7	151932991	MLL3	G	A	894	R/W	NON_SYNONYMOUS_CODING	probably_damaging(1)	.
7	151932997	MLL3	C	T	892	G/R	NON_SYNONYMOUS_CODING	probably_damaging(1)	.
7	100645731	MUC12	G	A	3963	E/K	NON_SYNONYMOUS_CODING	possibly_damaging(0.952)	.
7	100637073	MUC12	C	T	1077	R/C	NON_SYNONYMOUS_CODING	unknown(0)	.
19	9002623	MUC16	C	T	39	R/H	NON_SYNONYMOUS_CODING	benign(0.011)	tolerated(0.24)
19	9002612	MUC16	T	G	43	K/Q	NON_SYNONYMOUS_CODING	benign(0.055)	tolerated(0.23)
19	9002659	MUC16	C	T	27	G/E	NON_SYNONYMOUS_CODING	possibly_damaging(0.582)	tolerated(0.19)
19	9002597	MUC16	C	T	48	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.866)	deleterious(0)
19	9002660	MUC16	C	T	27	G/R	NON_SYNONYMOUS_CODING	probably_damaging(0.998)	tolerated(0.5)
3	195507053	MUC4	A	C	3800	S/A	NON_SYNONYMOUS_CODING	benign(0.035)	.

3	195513847	MUC4	A	G	1535	M/T	NON_SYNONYMOUS_CODING	benign(0.042)	.
3	195506549	MUC4	A	G	3968	S/P	NON_SYNONYMOUS_CODING	benign(0.044)	.
3	195507226	MUC4	A	G	3742	V/A	NON_SYNONYMOUS_CODING	benign(0.095)	.
3	195506099	MUC4	T	C	4118	T/A	NON_SYNONYMOUS_CODING	benign(0.182)	.
3	195510228	MUC4	C	G	2741	E/D	NON_SYNONYMOUS_CODING	benign(0.182)	.
3	195505836	MUC4	G	C	4205	H/Q	NON_SYNONYMOUS_CODING	benign(0.204)	.
3	195509212	MUC4	G	A	3080	S/L	NON_SYNONYMOUS_CODING	benign(0.204)	.
3	195515017	MUC4	A	G	1145	V/A	NON_SYNONYMOUS_CODING	benign(0.231)	.
3	195506569	MUC4	A	G	3961	V/A	NON_SYNONYMOUS_CODING	benign(0.243)	.
3	195506293	MUC4	T	C	4053	N/S	NON_SYNONYMOUS_CODING	benign(0.258)	.
3	195510194	MUC4	G	C	2753	L/V	NON_SYNONYMOUS_CODING	benign(0.352)	.
3	195508661	MUC4	A	G	3264	S/P	NON_SYNONYMOUS_CODING	benign(0.421)	.
3	195506533	MUC4	C	A	3973	S/I	NON_SYNONYMOUS_CODING	possibly_damaging(0.473)	.
3	195506746	MUC4	G	A	3902	A/V	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195507062	MUC4	C	T	3797	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195507193	MUC4	G	A	3753	A/V	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195507385	MUC4	G	A	3689	A/V	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195507494	MUC4	C	T	3653	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195507948	MUC4	G	T	3501	D/E	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195510649	MUC4	G	A	2601	A/V	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195506983	MUC4	G	A	3823	T/I	NON_SYNONYMOUS_CODING	possibly_damaging(0.607)	.
3	195507475	MUC4	G	C	3659	T/R	NON_SYNONYMOUS_CODING	possibly_damaging(0.607)	.
3	195507827	MUC4	G	A	3542	L/F	NON_SYNONYMOUS_CODING	possibly_damaging(0.607)	.
3	195511076	MUC4	T	A	2459	T/S	NON_SYNONYMOUS_CODING	possibly_damaging(0.609)	.
3	195511525	MUC4	T	C	2309	D/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.748)	.
3	195511526	MUC4	C	T	2309	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.748)	.
3	195506501	MUC4	G	A	3984	P/S	NON_SYNONYMOUS_CODING	possibly_damaging(0.768)	.
3	195507604	MUC4	C	G	3616	R/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.811)	.
3	195507461	MUC4	G	A	3664	P/S	NON_SYNONYMOUS_CODING	possibly_damaging(0.864)	.
3	195507694	MUC4	A	G	3586	L/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.881)	.
3	195506294	MUC4	T	C	4053	N/D	NON_SYNONYMOUS_CODING	possibly_damaging(0.9)	.
3	195513622	MUC4	G	C	1610	S/C	NON_SYNONYMOUS_CODING	possibly_damaging(0.909)	.
3	195506291	MUC4	C	T	4054	A/T	NON_SYNONYMOUS_CODING	possibly_damaging(0.922)	.
3	195506752	MUC4	C	T	3900	R/H	NON_SYNONYMOUS_CODING	possibly_damaging(0.924)	.
3	195506990	MUC4	C	G	3821	D/H	NON_SYNONYMOUS_CODING	possibly_damaging(0.934)	.
3	195511102	MUC4	G	A	2450	P/L	NON_SYNONYMOUS_CODING	probably_damaging(0.975)	.
3	195514948	MUC4	G	A	1168	P/L	NON_SYNONYMOUS_CODING	probably_damaging(0.975)	.
3	195510622	MUC4	G	T	2610	P/H	NON_SYNONYMOUS_CODING	probably_damaging(0.991)	.

3	195506302	MUC4	G	T	4050	P/H	NON_SYNONYMOUS_CODING	probably_damaging(0.998)	.
3	195506542	MUC4	G	T	3970	P/H	NON_SYNONYMOUS_CODING	probably_damaging(1)	.
3	195510310	MUC4	T	G	2714	Y/S	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510341	MUC4	A	G	2704	S/P	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510347	MUC4	T	C	2702	T/A	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510350	MUC4	C	G	2701	D/H	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510373	MUC4	T	C	2693	D/G	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510374	MUC4	C	T	2693	D/N	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510415	MUC4	G	T	2679	S/Y	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510505	MUC4	G	A	2649	A/V	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510509	MUC4	A	C	2648	S/A	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510515	MUC4	C	T	2646	A/T	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510518	MUC4	C	T	2645	D/N	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510553	MUC4	G	A	2633	A/V	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510565	MUC4	C	T	2629	S/N	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510610	MUC4	A	G	2614	L/P	NON_SYNONYMOUS_CODING	unknown(0)	.
11	1018095	MUC6	G	A	1569	P/L	NON_SYNONYMOUS_CODING	benign(0.014)	deleterious(0.01)
11	1017466	MUC6	T	C	1779	R/G	NON_SYNONYMOUS_CODING	benign(0.142)	tolerated(0.57)
11	1018042	MUC6	A	G	1587	S/P	NON_SYNONYMOUS_CODING	probably_damaging(0.979)	deleterious(0.01)
11	1016554	MUC6	C	T	2083	A/T	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.6)
11	1016565	MUC6	C	T	2079	S/N	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.09)
11	1018552	MUC6	G	A	1417	P/S	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.73)
17	16097792	NCOR1	T	C	31	N/S	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
17	16097786	NCOR1	C	T	33	R/H	NON_SYNONYMOUS_CODING	possibly_damaging(0.806)	tolerated(0.66)
11	78383335	ODZ4	C	T	1846	V/I	NON_SYNONYMOUS_CODING	benign(0.004)	tolerated(0.2)
11	78413365	ODZ4	T	G	1431	Q/H	NON_SYNONYMOUS_CODING	probably_damaging(0.998)	deleterious(0.01)
1	248436582	OR2T33	T	C	179	T/A	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.25)
1	248436638	OR2T33	A	G	160	V/A	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.4)
19	4511376	PLIN4	C	A	852	G/C	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.14)
19	4511379	PLIN4	G	C	851	L/V	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
9	33795579	PRSS3	C	T	3	P/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.73)
9	33795581	PRSS3	T	C	4	F/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
9	33795593	PRSS3	G	A	8	A/T	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.44)
9	33798075	PRSS3	T	C	150	F/S	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
12	11183406	TAS2R31	C	T	177	A/T	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.42)
12	11183475	TAS2R31	G	A	154	R/W	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.87)
12	11183496	TAS2R31	T	C	147	I/V	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.47)
12	11183793	TAS2R31	G	C	48	L/V	NON_SYNONYMOUS_CODING	benign(0.162)	tolerated(0.06)

7	142498738	TRBC2	C	G	5	N/K	NON_SYNONYMOUS_CODING	benign(0.002)	tolerated(0.34)
7	142498735	TRBC2	A	C	4	K/N	NON_SYNONYMOUS_CODING	benign(0.111)	tolerated(0.08)
7	142045680	TRBV4-2	T	C	70	F/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.67)
7	142045693	TRBV4-2	C	T	74	T/I	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.36)
7	142149029	TRBV5-5	T	G	81	D/A	NON_SYNONYMOUS_CODING	benign(0.001)	deleterious(0.01)
7	142149030	TRBV5-5	C	G	81	D/H	NON_SYNONYMOUS_CODING	benign(0.002)	deleterious(0.01)
7	142180566	TRBV6-5	A	T	98	L/Q	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.22)
7	142180573	TRBV6-5	T	C	96	R/G	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.12)
7	142180585	TRBV6-5	C	T	92	D/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.79)
7	142180590	TRBV6-5	G	T	90	T/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142180593	TRBV6-5	G	T	89	T/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.44)
7	142180596	TRBV6-5	G	A	88	S/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.31)
7	142180618	TRBV6-5	T	C	81	N/D	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.78)
7	142180633	TRBV6-5	G	T	76	Q/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142180635	TRBV6-5	T	G	75	D/A	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.22)
7	142180641	TRBV6-5	A	G	73	I/T	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.91)
7	142180704	TRBV6-5	G	T	52	S/Y	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142180737	TRBV6-5	T	A	41	Q/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.16)
7	142180578	TRBV6-5	G	A	94	P/L	NON_SYNONYMOUS_CODING	benign(0.002)	tolerated(0.17)
7	142180588	TRBV6-5	C	G	91	E/Q	NON_SYNONYMOUS_CODING	benign(0.005)	tolerated(0.2)
7	142180647	TRBV6-5	G	T	71	A/D	NON_SYNONYMOUS_CODING	benign(0.08)	tolerated(0.18)
7	142247271	TRBV7-3	G	A	62	P/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142247380	TRBV7-3	T	A	26	T/S	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.87)
7	142099548	TRBV7-8	A	G	85	F/S	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142099512	TRBV7-8	T	G	97	K/T	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.14)
7	142099533	TRBV7-8	T	C	90	E/G	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.36)
2	179465893	TTN	G	A	9640	P/S	NON_SYNONYMOUS_CODING	benign(0.014)	.
2	179641088	TTN	G	C	1789	Q/E	NON_SYNONYMOUS_CODING	benign(0.014)	.
2	179634421	TTN	T	G	2917	T/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.539)	.
8	11995122	USP17L2	T	C	383	E/G	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.2)
8	11995939	USP17L2	G	A	111	R/W	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0.01)
19	21948513	ZNF100	G	A	27	Q/*	STOP_GAINED	.	.
19	21948524	ZNF100	C	T	23	S/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.89)
19	21948511	ZNF100	C	G	27	Q/H	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.58)

CHD22: Homozygous variants

CHR ID	NUCLEOTIDE POSITION	GENE NAME	REF SEQUENCE	ALTERNATE SEQUENCE	AMINO ACID NO.	RESIDUE CHANGE	FUNCTION OF VARIANT	POLYPHEN	SIFT
7	150783917	AGAP3	T	G	30	L/R	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.06)
7	150783920	AGAP3	T	G	31	V/G	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.74)
7	150783922	AGAP3	T	G	32	C/G	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.42)
10	46321904	AGAP4	C	T	260	R/H	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0.02)
19	43860251	CD177	G	A	204	D/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
9	66553727	ENSG00000170161	T	C	60	V/A	NON_SYNONYMOUS_CODING	benign(0)	.
10	47911590	FAM21B	G	A	193	G/D	NON_SYNONYMOUS_CODING	benign(0.282)	tolerated(0.14)
8	12285102	FAM86B2	T	C	91	E/G	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0)
8	12285103	FAM86B2	C	T	91	E/K	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0)
15	82637079	GOLGA6L10	C	T	336	R/Q	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(1)
15	34673973	GOLGA8A	C	T	513	R/Q	NON_SYNONYMOUS_CODING	benign(0.003)	tolerated(1)
7	74212311	GTF2IRD2	G	T	61	H/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
6	32549357	HLA-DRB1	G	A	210	T/I	NON_SYNONYMOUS_CODING	possibly_damaging(0.481)	deleterious(0)
1	22215199	HSPG2	G	C	6	P/R	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195512186	MUC4	T	C	2089	I/V	NON_SYNONYMOUS_CODING	benign(0.028)	.
3	195514768	MUC4	T	C	1228	D/G	NON_SYNONYMOUS_CODING	benign(0.144)	.
3	195507494	MUC4	C	T	3653	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195514733	MUC4	C	A	1240	A/S	NON_SYNONYMOUS_CODING	possibly_damaging(0.533)	.
3	195514948	MUC4	G	A	1168	P/L	NON_SYNONYMOUS_CODING	probably_damaging(0.975)	.
3	195510341	MUC4	A	G	2704	S/P	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510389	MUC4	A	G	2688	S/P	NON_SYNONYMOUS_CODING	unknown(0)	.
1	145327548	NBPF10	A	G	1369	N/D	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
1	248737259	OR2T34	C	G	267	R/P	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
1	248802469	OR2T35	C	T	31	V/I	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.2)
2	131245713	POTEI	C	G	434	S/T	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)

2	131221622	POTEI	C	T	665	M/I	NON_SYNONYMOUS_CODING	possibly_damaging(0.539)	deleterious(0)
14	20010235	POTEM	A	G	393	V/A	NON_SYNONYMOUS_CODING	benign(0.006)	tolerated(1)
1	13695685	PRAMEF19	G	A	358	S/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.68)
1	13695787	PRAMEF19	C	G	324	G/A	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
1	13696022	PRAMEF19	A	G	246	W/R	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.25)
1	13696047	PRAMEF19	A	T	237	D/E	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.24)
1	13696054	PRAMEF19	G	A	235	S/F	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.69)
1	13696090	PRAMEF19	T	C	223	K/R	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.47)
22	18901004	PRODH	C	T	166	R/Q	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.6)
7	72436652	TRIM74	A	G	13	W/R	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.29)
1	117122285	IGSF3	G	GTCC	1041	D/ED	NON_SYNONYMOUS_CODING	.	.
15	90320134	MESP2	AGGGCAGGGGCAG	A	183-186	GQGQ/-	NON_SYNONYMOUS_CODING	.	.
3	12046123	SYN2	AAGC	A	33-34	QA/Q	NON_SYNONYMOUS_CODING	.	.
20	60640305	TAF4	TGCCAGG	T	186-187	PG/-	NON_SYNONYMOUS_CODING	.	.
17	46115072	COPZ2	C	CG	22	.	FRAMESHIFT_CODING	.	.
21	43298954	PRDM15	C	CG	88	.	FRAMESHIFT_CODING	.	.
17	46115122	COPZ2	A	AG	05-Jun	.	SPLICE_SITE:FRAMESHIFT_CODING	.	.

CHD22: Compound heterozygous variants

CHR ID	NUCLEOTIDE POSITION	GENE NAME	REF SEQUENCE	ALTERNATE SEQUENCE	AMINO ACID NO.	RESIDUE CHANGE	FUNCTION OF VARIANT	POLYPHEN	SIFT
2	97808524	ANKRD36	A	G	285	I/V	NON_SYNONYMOUS_CODING	benign(0.296)	tolerated(1)
2	97808515	ANKRD36	G	A	282	E/K	NON_SYNONYMOUS_CODING	probably_damaging(0.959)	tolerated(1)
2	97808510	ANKRD36	G	A	280	G/D	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.85)
12	101368637	ANO4	A	C	191	Y/S	NON_SYNONYMOUS_CODING	probably_damaging(0.983)	tolerated(0.39)
12	101368625	ANO4	G	A	187	R/K	SPLICE_SITE:NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(1)
22	39498015	APOBEC3H	C	G	171	R/G	NON_SYNONYMOUS_CODING	benign(0.057)	deleterious(0.01)
22	39498005	APOBEC3H	T	A	167	D/E	NON_SYNONYMOUS_CODING	benign(0.18)	tolerated(0.35)
5	137503739	BRD8	G	A	224	S/F	NON_SYNONYMOUS_CODING	probably_damaging(0.996)	deleterious(0.01)
5	137503737	BRD8	C	A	225	G/C	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.06)
17	56386386	BZRAP1	G	T	1416	P/Q	NON_SYNONYMOUS_CODING	possibly_damaging(0.948)	deleterious(0)
17	56386402	BZRAP1	A	G	1411	S/P	NON_SYNONYMOUS_CODING	probably_damaging(0.995)	deleterious(0)
12	112622338	C12orf51	T	G	3056	T/P	NON_SYNONYMOUS_CODING	benign(0.187)	.
12	112688167	C12orf51	A	C	822	V/G	NON_SYNONYMOUS_CODING	probably_damaging(0.979)	.
20	18433273	C20orf12	T	G	179	T/P	NON_SYNONYMOUS_CODING	benign(0.005)	tolerated(0.15)
20	18433277	C20orf12	T	G	25	H/P	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0)
12	121093651	CABP1	TGTGC	T	13-14	.	FRAMESHIFT_CODING	.	.
12	121093653	CABP1	TGC	T	14	.	FRAMESHIFT_CODING	.	.
17	45234417	CDC27	A	G	235	I/T	NON_SYNONYMOUS_CODING	benign(0.004)	tolerated(0.19)
17	45234303	CDC27	G	C	273	A/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.783)	tolerated(0.07)
17	7796794	CHD3	A	C	293	I/L	NON_SYNONYMOUS_CODING	benign(0)	.
17	7796803	CHD3	T	C	296	S/P	NON_SYNONYMOUS_CODING	benign(0.001)	.
2	9599739	CPSF3	T	A	593	I/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.51)
2	9599742	CPSF3	G	A	594	R/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.93)
10	126678128	CTBP2	T	A	433	T/S	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
10	126678177	CTBP2	G	T	416	N/K	NON_SYNONYMOUS_CODING	benign(0.01)	tolerated(0.46)
15	51766636	DMXL2	G	A	1736	A/V	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(1)
15	51766637	DMXL2	C	A	1736	A/S	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.57)
16	15457629	ENSG00000183793	T	A	314	T/S	NON_SYNONYMOUS_CODING	benign(0.158)	tolerated(0.82)
16	15457628	ENSG00000183793	G	T	314	T/N	NON_SYNONYMOUS_CODING	benign(0.348)	tolerated(0.39)
2	131985945	ENSG00000188219	A	G	349	H/R	NON_SYNONYMOUS_CODING	benign(0.429)	tolerated(0.2)
2	132021023	ENSG00000188219	G	A	665	M/I	NON_SYNONYMOUS_CODING	possibly_damaging(0.539)	deleterious(0)
12	132445256	EP400	A	C	31	H/P	NON_SYNONYMOUS_CODING	unknown(0)	.
12	132445261	EP400	A	C	33	N/H	NON_SYNONYMOUS_CODING	unknown(0)	.
12	132445273	EP400	T	C	37	S/P	NON_SYNONYMOUS_CODING	unknown(0)	.
2	97757290	FAHD2B	C	T	52	V/I	NON_SYNONYMOUS_CODING	benign(0.004)	tolerated(1)
2	97757296	FAHD2B	C	T	50	G/R	NON_SYNONYMOUS_CODING	probably_damaging(0.989)	tolerated(0.21)
8	12042969	FAM86B1	G	A	42	R/W	NON_SYNONYMOUS_CODING	benign(0.016)	deleterious(0.03)
8	12042783	FAM86B1	A	G	104	S/P	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0)
8	12042851	FAM86B1	C	G	81	C/S	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0)

15	84909434	GOLGA6L4	G	A	119	R/Q	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.5)
15	84908750	GOLGA6L4	T	A	27	L/Q	SPLICE_SITE:NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.23)
6	31324036	HLA-B	T	A	177	E/V	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
6	31324931	HLA-B	A	C	3	L/R	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
6	32548544	HLA-DRB1	T	G	248	I/L	NON_SYNONYMOUS_CODING	benign(0)	deleterious(0)
6	32549402	HLA-DRB1	C	T	195	R/Q	NON_SYNONYMOUS_CODING	benign(0.002)	tolerated(1)
6	32549582	HLA-DRB1	G	T	135	T/N	NON_SYNONYMOUS_CODING	benign(0.374)	deleterious(0)
6	32497985	HLA-DRB5	A	G	6	L/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.512)	deleterious(0)
6	32489852	HLA-DRB5	A	G	67	L/S	NON_SYNONYMOUS_CODING	possibly_damaging(0.558)	deleterious(0)
17	39346592	KRTAP9-1	ACCT	A	152-153	TC/S	NON_SYNONYMOUS_CODING	.	.
17	39346433	KRTAP9-1	A	C	99	T/P	SPLICE_SITE:NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.08)
11	12183916	MICAL2	C	T	72	R/C	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
11	12225829	MICAL2	G	T	99	L/F	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0.03)
11	12315350	MICALCL	C	G	124	I/M	NON_SYNONYMOUS_CODING	possibly_damaging(0.519)	tolerated(0.07)
11	12315162	MICALCL	A	C	62	T/P	NON_SYNONYMOUS_CODING	probably_damaging(0.988)	deleterious(0)
7	100634922	MUC12	G	A	360	A/T	NON_SYNONYMOUS_CODING	unknown(0)	.
7	100636446	MUC12	G	A	868	D/N	NON_SYNONYMOUS_CODING	unknown(0)	.
7	100636447	MUC12	A	G	868	D/G	NON_SYNONYMOUS_CODING	unknown(0)	.
7	100636459	MUC12	C	T	872	T/M	NON_SYNONYMOUS_CODING	unknown(0)	.
7	100639147	MUC12	A	G	1768	E/G	NON_SYNONYMOUS_CODING	unknown(0)	.
7	100642455	MUC12	G	A	2871	A/T	NON_SYNONYMOUS_CODING	unknown(0)	.
19	9002623	MUC16	C	T	39	R/H	NON_SYNONYMOUS_CODING	benign(0.011)	tolerated(0.24)
19	9005721	MUC16	C	G	69	D/H	NON_SYNONYMOUS_CODING	benign(0.02)	tolerated(0.14)
19	9002636	MUC16	C	T	35	V/I	NON_SYNONYMOUS_CODING	benign(0.051)	tolerated(0.37)
19	9002612	MUC16	T	G	43	K/Q	NON_SYNONYMOUS_CODING	benign(0.055)	tolerated(0.23)
19	9005697	MUC16	C	T	77	A/T	NON_SYNONYMOUS_CODING	benign(0.138)	tolerated(0.19)
19	9005706	MUC16	T	C	74	R/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.847)	tolerated(0.65)
19	9002597	MUC16	C	T	48	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.866)	deleterious(0)
3	195513846	MUC4	C	G	1535	M/I	NON_SYNONYMOUS_CODING	benign(0.012)	.
3	195506387	MUC4	G	T	4022	P/T	NON_SYNONYMOUS_CODING	benign(0.025)	.
3	195513847	MUC4	A	G	1535	M/T	NON_SYNONYMOUS_CODING	benign(0.042)	.
3	195506549	MUC4	A	G	3968	S/P	NON_SYNONYMOUS_CODING	benign(0.044)	.
3	195506590	MUC4	G	A	3954	P/L	NON_SYNONYMOUS_CODING	benign(0.057)	.
3	195505910	MUC4	T	C	4181	S/G	NON_SYNONYMOUS_CODING	benign(0.095)	.
3	195505945	MUC4	A	G	4169	V/A	NON_SYNONYMOUS_CODING	benign(0.095)	.
3	195506137	MUC4	A	G	4105	V/A	NON_SYNONYMOUS_CODING	benign(0.095)	.
3	195506150	MUC4	T	C	4101	S/G	NON_SYNONYMOUS_CODING	benign(0.095)	.
3	195506215	MUC4	C	G	4079	S/T	NON_SYNONYMOUS_CODING	benign(0.095)	.
3	195508022	MUC4	T	C	3477	S/G	NON_SYNONYMOUS_CODING	benign(0.095)	.
3	195508343	MUC4	A	T	3370	S/T	NON_SYNONYMOUS_CODING	benign(0.095)	.
3	195506364	MUC4	G	C	4029	H/Q	NON_SYNONYMOUS_CODING	benign(0.118)	.
3	195506473	MUC4	A	G	3993	V/A	NON_SYNONYMOUS_CODING	benign(0.137)	.
3	195505859	MUC4	T	C	4198	T/A	NON_SYNONYMOUS_CODING	benign(0.182)	.
3	195506099	MUC4	T	C	4118	T/A	NON_SYNONYMOUS_CODING	benign(0.182)	.

3	195506723	MUC4	T	C	3910	T/A	NON_SYNONYMOUS_CODING	benign(0.182)	.
3	195506987	MUC4	T	C	3822	T/A	NON_SYNONYMOUS_CODING	benign(0.182)	.
3	195508667	MUC4	T	C	3262	T/A	NON_SYNONYMOUS_CODING	benign(0.182)	.
3	195506582	MUC4	C	T	3957	D/N	NON_SYNONYMOUS_CODING	benign(0.183)	.
3	195505836	MUC4	G	C	4205	H/Q	NON_SYNONYMOUS_CODING	benign(0.204)	.
3	195505909	MUC4	C	T	4181	S/N	NON_SYNONYMOUS_CODING	benign(0.204)	.
3	195506149	MUC4	C	T	4101	S/N	NON_SYNONYMOUS_CODING	benign(0.204)	.
3	195507228	MUC4	G	C	3741	H/Q	NON_SYNONYMOUS_CODING	benign(0.204)	.
3	195508716	MUC4	A	C	3245	H/Q	NON_SYNONYMOUS_CODING	benign(0.204)	.
3	195508789	MUC4	C	T	3221	S/N	NON_SYNONYMOUS_CODING	benign(0.204)	.
3	195515017	MUC4	A	G	1145	V/A	NON_SYNONYMOUS_CODING	benign(0.231)	.
3	195506569	MUC4	A	G	3961	V/A	NON_SYNONYMOUS_CODING	benign(0.243)	.
3	195507428	MUC4	T	A	3675	T/S	NON_SYNONYMOUS_CODING	benign(0.352)	.
3	195508787	MUC4	G	T	3222	L/I	NON_SYNONYMOUS_CODING	benign(0.352)	.
3	195510194	MUC4	G	C	2753	L/V	NON_SYNONYMOUS_CODING	benign(0.352)	.
3	195506076	MUC4	C	G	4125	Q/H	NON_SYNONYMOUS_CODING	benign(0.391)	.
3	195510636	MUC4	C	G	2605	Q/H	NON_SYNONYMOUS_CODING	benign(0.391)	.
3	195508661	MUC4	A	G	3264	S/P	NON_SYNONYMOUS_CODING	benign(0.421)	.
3	195508709	MUC4	A	G	3248	S/P	NON_SYNONYMOUS_CODING	benign(0.421)	.
3	195506533	MUC4	C	A	3973	S/I	NON_SYNONYMOUS_CODING	possibly_damaging(0.473)	.
3	195505897	MUC4	G	A	4185	A/V	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195506650	MUC4	G	A	3934	A/V	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195506704	MUC4	T	C	3916	D/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195506746	MUC4	G	A	3902	A/V	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195506963	MUC4	C	T	3830	A/T	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195507062	MUC4	C	T	3797	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195507155	MUC4	C	T	3766	A/T	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195507193	MUC4	G	A	3753	A/V	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195507385	MUC4	G	A	3689	A/V	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195507434	MUC4	C	A	3673	A/S	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195507446	MUC4	C	T	3669	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195508009	MUC4	G	A	3481	A/V	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195508070	MUC4	C	T	3461	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195508500	MUC4	G	C	3317	D/E	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195508502	MUC4	C	T	3317	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195508668	MUC4	G	C	3261	D/E	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195505906	MUC4	G	A	4182	T/I	NON_SYNONYMOUS_CODING	possibly_damaging(0.607)	.
3	195506147	MUC4	G	A	4102	L/F	NON_SYNONYMOUS_CODING	possibly_damaging(0.607)	.
3	195506213	MUC4	T	G	4080	T/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.607)	.
3	195506983	MUC4	G	A	3823	T/I	NON_SYNONYMOUS_CODING	possibly_damaging(0.607)	.
3	195507379	MUC4	G	C	3691	T/R	NON_SYNONYMOUS_CODING	possibly_damaging(0.607)	.
3	195507443	MUC4	T	G	3670	T/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.607)	.
3	195507475	MUC4	G	C	3659	T/R	NON_SYNONYMOUS_CODING	possibly_damaging(0.607)	.
3	195507827	MUC4	G	A	3542	L/F	NON_SYNONYMOUS_CODING	possibly_damaging(0.607)	.

3	195508678	MUC4	G	T	3258	S/Y	NON_SYNONYMOUS_CODING	possibly_damaging(0.607)	.
3	195511412	MUC4	T	A	2347	T/S	NON_SYNONYMOUS_CODING	possibly_damaging(0.609)	.
3	195508127	MUC4	G	C	3442	P/A	NON_SYNONYMOUS_CODING	possibly_damaging(0.692)	.
3	195506602	MUC4	G	C	3950	T/S	NON_SYNONYMOUS_CODING	possibly_damaging(0.717)	.
3	195511285	MUC4	T	C	2389	D/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.748)	.
3	195511286	MUC4	C	T	2389	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.748)	.
3	195511525	MUC4	T	C	2309	D/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.748)	.
3	195511526	MUC4	C	T	2309	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.748)	.
3	195506501	MUC4	G	A	3984	P/S	NON_SYNONYMOUS_CODING	possibly_damaging(0.768)	.
3	195506282	MUC4	A	C	4057	L/V	NON_SYNONYMOUS_CODING	possibly_damaging(0.806)	.
3	195507412	MUC4	C	G	3680	R/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.811)	.
3	195507604	MUC4	C	G	3616	R/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.811)	.
3	195507445	MUC4	T	A	3669	D/V	NON_SYNONYMOUS_CODING	possibly_damaging(0.862)	.
3	195506146	MUC4	A	G	4102	L/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.881)	.
3	195507694	MUC4	A	G	3586	L/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.881)	.
3	195508510	MUC4	A	G	3314	L/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.881)	.
3	195508786	MUC4	A	G	3222	L/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.881)	.
3	195506341	MUC4	C	T	4037	S/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.893)	.
3	195506342	MUC4	T	C	4037	S/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.893)	.
3	195506626	MUC4	G	A	3942	T/I	NON_SYNONYMOUS_CODING	possibly_damaging(0.893)	.
3	195505870	MUC4	G	A	4194	P/L	NON_SYNONYMOUS_CODING	possibly_damaging(0.931)	.
3	195506914	MUC4	G	A	3846	P/L	NON_SYNONYMOUS_CODING	possibly_damaging(0.931)	.
3	195508018	MUC4	G	A	3478	P/L	NON_SYNONYMOUS_CODING	possibly_damaging(0.931)	.
3	195506990	MUC4	C	G	3821	D/H	NON_SYNONYMOUS_CODING	possibly_damaging(0.934)	.
3	195507422	MUC4	C	G	3677	D/H	NON_SYNONYMOUS_CODING	possibly_damaging(0.934)	.
3	195508238	MUC4	C	G	3405	D/H	NON_SYNONYMOUS_CODING	possibly_damaging(0.934)	.
3	195508670	MUC4	C	G	3261	D/H	NON_SYNONYMOUS_CODING	possibly_damaging(0.934)	.
3	195515414	MUC4	T	C	1013	S/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.943)	.
3	195506554	MUC4	G	A	3966	A/V	NON_SYNONYMOUS_CODING	probably_damaging(0.959)	.
3	195508228	MUC4	G	C	3408	P/R	NON_SYNONYMOUS_CODING	probably_damaging(0.968)	.
3	195515008	MUC4	C	G	1148	G/A	NON_SYNONYMOUS_CODING	probably_damaging(0.972)	.
3	195513826	MUC4	G	A	1542	P/L	NON_SYNONYMOUS_CODING	probably_damaging(0.975)	.
3	195514825	MUC4	G	A	1209	A/V	NON_SYNONYMOUS_CODING	probably_damaging(0.978)	.
3	195515449	MUC4	A	T	1001	V/E	NON_SYNONYMOUS_CODING	probably_damaging(0.979)	.
3	195515435	MUC4	C	T	1006	A/T	NON_SYNONYMOUS_CODING	probably_damaging(0.985)	.
3	195506734	MUC4	G	T	3906	P/H	NON_SYNONYMOUS_CODING	probably_damaging(0.991)	.
3	195515134	MUC4	G	T	1106	P/H	NON_SYNONYMOUS_CODING	probably_damaging(0.991)	.
3	195508336	MUC4	C	T	3372	G/D	NON_SYNONYMOUS_CODING	probably_damaging(0.994)	.
3	195510910	MUC4	G	T	2514	P/H	NON_SYNONYMOUS_CODING	probably_damaging(0.997)	.
3	195510310	MUC4	T	G	2714	Y/S	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510347	MUC4	T	C	2702	T/A	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510350	MUC4	C	G	2701	D/H	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510396	MUC4	A	C	2685	H/Q	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510415	MUC4	G	T	2679	S/Y	NON_SYNONYMOUS_CODING	unknown(0)	.

3	195510526	MUC4	A	G	2642	L/P	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510611	MUC4	G	T	2614	L/I	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510613	MUC4	C	T	2613	S/N	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510614	MUC4	T	C	2613	S/G	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195538657	MUC4	CA	C	11	.	FRAMESHIFT_CODING	.	.
3	195505742	MUC4	G	C	963	H/D	SPLICE_SITE:NON_SYNONYMOUS_CODING	possibly_damaging(0.662)	tolerated(0.09)
11	1264346	MUC5B	C	A	2079	T/K	NON_SYNONYMOUS_CODING	benign(0.29)	.
11	1265107	MUC5B	C	T	2333	P/S	NON_SYNONYMOUS_CODING	benign(0.409)	.
11	1264717	MUC5B	C	T	2203	P/S	NON_SYNONYMOUS_CODING	possibly_damaging(0.662)	.
11	1269557	MUC5B	C	T	3816	T/M	NON_SYNONYMOUS_CODING	unknown(0)	.
11	1269707	MUC5B	C	T	3866	P/L	NON_SYNONYMOUS_CODING	unknown(0)	.
11	1270647	MUC5B	G	C	4179	Q/H	NON_SYNONYMOUS_CODING	unknown(0)	.
11	1017744	MUC6	T	C	1686	N/S	NON_SYNONYMOUS_CODING	benign(0.014)	tolerated(0.97)
11	1017466	MUC6	T	C	1779	R/G	NON_SYNONYMOUS_CODING	benign(0.142)	tolerated(0.57)
11	1017307	MUC6	G	A	1832	P/S	NON_SYNONYMOUS_CODING	benign(0.34)	tolerated(0.21)
11	1016919	MUC6	C	T	1961	R/K	NON_SYNONYMOUS_CODING	possibly_damaging(0.496)	tolerated(0.33)
11	1018042	MUC6	A	G	1587	S/P	NON_SYNONYMOUS_CODING	probably_damaging(0.979)	deleterious(0.01)
11	1016554	MUC6	C	T	2083	A/T	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.6)
11	1016565	MUC6	C	T	2079	S/N	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.09)
11	1018207	MUC6	T	C	1532	T/A	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.28)
11	1018552	MUC6	G	A	1417	P/S	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.73)
1	145367800	NBPF10	G	A	586	D/N	NON_SYNONYMOUS_CODING	benign(0.009)	deleterious(0.03)
1	145359049	NBPF10	A	G	2997	K/E	NON_SYNONYMOUS_CODING	benign(0.049)	tolerated(1)
1	145302775	NBPF10	T	G	330	Y/D	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(1)
2	152436012	NEB	T	G	5515	K/T	NON_SYNONYMOUS_CODING	possibly_damaging(0.462)	.
2	152448640	NEB	T	C	4912	N/D	NON_SYNONYMOUS_CODING	unknown(0)	.
1	228430947	OBSCN	C	G	998	A/G	NON_SYNONYMOUS_CODING	benign(0.003)	.
1	228434292	OBSCN	C	G	1274	A/G	NON_SYNONYMOUS_CODING	benign(0.006)	.
1	248722611	OR2T29	C	G	61	S/T	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
1	248722624	OR2T29	C	T	57	A/T	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.67)
1	248722621	OR2T29	G	A	58	H/Y	NON_SYNONYMOUS_CODING	benign(0.001)	deleterious(0.03)
1	248436582	OR2T33	T	C	179	T/A	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.25)
1	248436638	OR2T33	A	G	160	V/A	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.4)
1	248737293	OR2T34	G	A	256	L/F	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
1	248737230	OR2T34	T	C	277	M/V	NON_SYNONYMOUS_CODING	benign(0.018)	deleterious(0.01)
9	33795581	PRSS3	T	C	4	F/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
9	33795593	PRSS3	G	A	8	A/T	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.44)
9	33798075	PRSS3	T	C	150	F/S	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
9	33798574	PRSS3	G	A	182	S/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
19	14104595	RFX1	C	G	21	A/P	NON_SYNONYMOUS_CODING	unknown(0)	.
19	14104610	RFX1	A	G	16	S/P	NON_SYNONYMOUS_CODING	unknown(0)	.
19	14104590	RFX1	C	CG	22	.	FRAMESHIFT_CODING	.	.
19	50154276	SCAF1	A	C	210	T/P	NON_SYNONYMOUS_CODING	unknown(0)	.
19	50154285	SCAF1	G	C	213	A/P	NON_SYNONYMOUS_CODING	unknown(0)	.

19	51920115	SIGLEC10	C	T	171	E/K	NON_SYNONYMOUS_CODING	benign(0.124)	tolerated(0.42)
19	51920112	SIGLEC10	C	T	172	E/K	NON_SYNONYMOUS_CODING	benign(0.16)	tolerated(0.77)
18	11610570	SLC35G4	C	T	287	R/W	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.19)
18	11610580	SLC35G4	T	G	290	I/S	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
19	49132198	SPHK2	A	C	351	Y/S	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
19	49132201	SPHK2	T	C	352	L/P	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
12	11244027	TAS2R43	T	C	268	R/G	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
12	11244036	TAS2R43	T	G	265	K/Q	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
12	11244015	TAS2R43	G	A	272	P/S	NON_SYNONYMOUS_CODING	benign(0.113)	deleterious(0.04)
12	11244017	TAS2R43	T	C	271	Y/C	NON_SYNONYMOUS_CODING	benign(0.256)	deleterious(0.04)
7	142498738	TRBC2	C	G	5	N/K	NON_SYNONYMOUS_CODING	benign(0.002)	tolerated(0.34)
7	142498735	TRBC2	A	C	4	K/N	NON_SYNONYMOUS_CODING	benign(0.111)	tolerated(0.08)
7	142008783	TRBV3-1	A	G	86	K/E	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142008785	TRBV3-1	A	C	86	K/N	NON_SYNONYMOUS_CODING	benign(0.001)	deleterious(0.02)
7	142045680	TRBV4-2	T	C	70	F/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.67)
7	142045693	TRBV4-2	C	T	74	T/I	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.36)
7	142168414	TRBV5-4	G	C	103	D/E	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142168466	TRBV5-4	A	C	86	L/R	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.24)
7	142180585	TRBV6-5	C	T	92	D/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.79)
7	142180590	TRBV6-5	G	T	90	T/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142180593	TRBV6-5	G	T	89	T/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.44)
7	142180596	TRBV6-5	G	A	88	S/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.31)
7	142180618	TRBV6-5	T	C	81	N/D	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.78)
7	142180633	TRBV6-5	G	T	76	Q/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142180635	TRBV6-5	T	G	75	D/A	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.22)
7	142180641	TRBV6-5	A	G	73	I/T	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.91)
7	142180704	TRBV6-5	G	T	52	S/Y	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142180737	TRBV6-5	T	A	41	Q/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.16)
7	142180578	TRBV6-5	G	A	94	P/L	NON_SYNONYMOUS_CODING	benign(0.002)	tolerated(0.17)
7	142180588	TRBV6-5	C	G	91	E/Q	NON_SYNONYMOUS_CODING	benign(0.005)	tolerated(0.2)
7	142180647	TRBV6-5	G	T	71	A/D	NON_SYNONYMOUS_CODING	benign(0.08)	tolerated(0.18)
7	142099548	TRBV7-8	A	G	85	F/S	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142099581	TRBV7-8	A	G	74	L/P	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.35)
7	142099512	TRBV7-8	T	G	97	K/T	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.14)
7	142099533	TRBV7-8	T	C	90	E/G	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.36)
7	142099537	TRBV7-8	G	T	89	P/T	NON_SYNONYMOUS_CODING	benign(0.003)	tolerated(0.39)

CHD23: Homozygous variants

CHR ID	NUCLEOTIDE POSITION	GENE NAME	REF SEQUENCE	ALTERNATE SEQUENCE	AMINO ACID NO.	RESIDUE CHANGE	FUNCTION OF VARIANT	POLYPHEN	SIFT
10	46321904	AGAP4	C	T	260	R/H	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0.02)
9	67968476	ANKRD20A1	C	T	679	R/C	NON_SYNONYMOUS_CODING	benign(0.382)	deleterious(0)
16	4748050	ANKS3	C	T	580	E/K	NON_SYNONYMOUS_CODING	possibly_damaging(0.937)	deleterious(0)
9	66553727	ENSG00000170161	T	C	60	V/A	NON_SYNONYMOUS_CODING	benign(0)	.
4	9237400	ENSG00000223569	G	C	430	Q/H	NON_SYNONYMOUS_CODING	benign(0.114)	tolerated(0.27)
4	9221944	ENSG00000227551	T	C	23	S/P	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.16)
4	9222968	ENSG00000227551	G	C	364	S/T	NON_SYNONYMOUS_CODING	possibly_damaging(0.598)	tolerated(0.13)
4	9337474	ENSG00000229579	G	C	364	S/T	NON_SYNONYMOUS_CODING	probably_damaging(0.969)	tolerated(0.13)
4	9351709	ENSG00000231051	G	C	364	S/T	NON_SYNONYMOUS_CODING	probably_damaging(0.969)	tolerated(0.13)
4	9213672	ENSG00000231396	G	C	430	Q/H	NON_SYNONYMOUS_CODING	benign(0.095)	tolerated(0.28)
4	9356454	ENSG00000231637	G	C	364	S/T	NON_SYNONYMOUS_CODING	probably_damaging(0.969)	tolerated(0.13)
4	9212449	ENSG00000232399	T	C	23	S/P	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.15)
4	9227712	ENSG00000232399	G	C	364	S/T	NON_SYNONYMOUS_CODING	possibly_damaging(0.935)	tolerated(0.12)
1	146215113	ENSG00000232637	G	A	84	H/Y	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.05)
4	9218420	ENSG00000233136	G	C	430	Q/H	NON_SYNONYMOUS_CODING	benign(0.154)	tolerated(0.29)
4	9217197	ENSG00000233136	T	C	23	S/P	NON_SYNONYMOUS_CODING	probably_damaging(0.992)	tolerated(0.18)
4	9346964	ENSG00000235780	G	C	364	S/T	NON_SYNONYMOUS_CODING	probably_damaging(0.969)	tolerated(0.13)
4	9256393	ENSG00000248920	G	C	430	Q/H	NON_SYNONYMOUS_CODING	benign(0.154)	tolerated(0.28)
4	9256194	ENSG00000248920	G	C	364	S/T	NON_SYNONYMOUS_CODING	possibly_damaging(0.935)	tolerated(0.13)
4	9270435	ENSG00000248933	G	C	364	S/T	NON_SYNONYMOUS_CODING	possibly_damaging(0.935)	tolerated(0.14)
4	9270634	ENSG00000248933	G	C	430	Q/H	NON_SYNONYMOUS_CODING	probably_damaging(0.961)	tolerated(0.29)
4	9246894	ENSG00000249104	G	C	430	Q/H	NON_SYNONYMOUS_CODING	benign(0.154)	tolerated(0.27)
4	9246695	ENSG00000249104	G	C	364	S/T	NON_SYNONYMOUS_CODING	possibly_damaging(0.935)	tolerated(0.13)
4	9264664	ENSG00000249811	T	C	23	S/P	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.16)
4	9265688	ENSG00000249811	G	C	364	S/T	NON_SYNONYMOUS_CODING	probably_damaging(0.969)	tolerated(0.13)

4	9260940	ENSG00000250745	G	C	364	S/T	NON_SYNONYMOUS_CODING	probably_damaging(0.969)	tolerated(0.14)
4	9250422	ENSG00000250844	T	C	23	S/P	NON_SYNONYMOUS_CODING	probably_damaging(0.992)	tolerated(0.16)
19	40382736	FCGBP	A	T	3384	S/T	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.24)
7	74212311	GTF2IRD2	G	T	61	H/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
19	54725156	LILRB3	T	C	252	N/D	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
17	44408066	LRRC37A	C	A	1141	F/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.11)
7	100636446	MUC12	G	A	868	D/N	NON_SYNONYMOUS_CODING	unknown(0)	.
7	100636447	MUC12	A	G	868	D/G	NON_SYNONYMOUS_CODING	unknown(0)	.
7	100636459	MUC12	C	T	872	T/M	NON_SYNONYMOUS_CODING	unknown(0)	.
7	100636467	MUC12	C	G	875	L/V	NON_SYNONYMOUS_CODING	unknown(0)	.
7	100639147	MUC12	A	G	1768	E/G	NON_SYNONYMOUS_CODING	unknown(0)	.
7	100639177	MUC12	C	T	1778	S/L	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195512186	MUC4	T	C	2089	I/V	NON_SYNONYMOUS_CODING	benign(0.028)	.
3	195506473	MUC4	A	G	3993	V/A	NON_SYNONYMOUS_CODING	benign(0.137)	.
1	145327548	NBPF10	A	G	1369	N/D	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
1	248737259	OR2T34	C	G	267	R/P	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
1	13695685	PRAMEF19	G	A	358	S/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.68)
1	13695787	PRAMEF19	C	G	324	G/A	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
1	13696022	PRAMEF19	A	G	246	W/R	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.25)
1	13696090	PRAMEF19	T	C	223	K/R	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.47)
2	113145814	RGPD8	C	T	1570	G/R	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
4	9342219	USP17L5	G	C	364	S/T	NON_SYNONYMOUS_CODING	probably_damaging(0.969)	tolerated(0.13)
15	30665280	CHRFAM7A	TCA	T	76	.	FRAMESHIFT_CODING	.	.
18	30352057	ENSG00000228835	GCGCCGGCC	G	119-121	.	FRAMESHIFT_CODING	.	.
21	43298954	PRDM15	C	CG	88	.	FRAMESHIFT_CODING	.	.
3	133969437	RYK	A	AG	19-20	.	SPLICE_SITE:FRAMESHIFT_CODING	.	.

CHD23: Compound heterozygous variants

CHR ID	NUCLEOTIDE POSITION	GENE NAME	REF SEQUENCE	ALTERNATE SEQUENCE	AMINO ACID NO.	RESIDUE CHANGE	FUNCTION OF VARIANT	POLYPHEN	SIFT
10	51464656	AGAP7	G	C	600	D/E	NON_SYNONYMOUS_CODING	probably_damaging(0.995)	tolerated(0.06)
10	51465650	AGAP7	C	T	269	R/Q	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0.03)
11	111741081	ALG9	G	T	281	N/K	NON_SYNONYMOUS_CODING	benign(0.111)	tolerated(0.05)
11	111741090	ALG9	T	A	278	L/F	NON_SYNONYMOUS_CODING	possibly_damaging(0.68)	tolerated(0.22)
2	97808524	ANKRD36	A	G	285	I/V	NON_SYNONYMOUS_CODING	benign(0.296)	tolerated(1)
2	97808515	ANKRD36	G	A	282	E/K	NON_SYNONYMOUS_CODING	probably_damaging(0.959)	tolerated(1)
2	97808510	ANKRD36	G	A	280	G/D	NON_SYNONYMOUS_CODING	probably_damaging(1)	tolerated(0.85)
12	101368637	ANO4	A	C	191	Y/S	NON_SYNONYMOUS_CODING	probably_damaging(0.983)	tolerated(0.39)
12	101368625	ANO4	G	A	187	R/K	SPLICE_SITE:NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(1)
17	56386386	BZRAP1	G	T	1416	P/Q	NON_SYNONYMOUS_CODING	possibly_damaging(0.948)	deleterious(0)
17	56386402	BZRAP1	A	G	1411	S/P	NON_SYNONYMOUS_CODING	probably_damaging(0.995)	deleterious(0)
3	54420749	CACNA2D3	T	G	110	V/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.887)	deleterious(0)
3	54420752	CACNA2D3	A	G	111	E/G	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0.01)
17	45234417	CDC27	A	G	235	I/T	NON_SYNONYMOUS_CODING	benign(0.004)	tolerated(0.19)
17	45234303	CDC27	G	C	273	A/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.783)	tolerated(0.07)
17	45214528	CDC27	A	T	635	Y/N	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
17	7788343	CHD3	C	CCCG	73-74	-/P	NON_SYNONYMOUS_CODING	.	.
17	7796794	CHD3	A	C	293	I/L	NON_SYNONYMOUS_CODING	benign(0)	.
17	7796803	CHD3	T	C	296	S/P	NON_SYNONYMOUS_CODING	benign(0.001)	.
17	7796819	CHD3	T	C	301	L/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.88)	.
19	42795827	CIC	G	C	939	G/A	NON_SYNONYMOUS_CODING	benign(0.106)	tolerated(0.44)
19	42795811	CIC	C	G	934	R/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.717)	deleterious(0.01)
2	9599739	CPSF3	T	A	593	I/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.51)
2	9599742	CPSF3	G	A	594	R/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.93)
8	68062165	CSPP1	G	A	703	S/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.64)
8	68062160	CSPP1	T	A	701	N/K	NON_SYNONYMOUS_CODING	benign(0.153)	tolerated(0.98)
5	13886235	DNAH5	G	A	861	L/F	NON_SYNONYMOUS_CODING	benign(0.004)	tolerated(0.7)
5	13830760	DNAH5	C	T	2003	V/I	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
19	18583686	ELL	C	A	12	V/F	NON_SYNONYMOUS_CODING	possibly_damaging(0.681)	.
19	18583688	ELL	G	A	11	S/F	NON_SYNONYMOUS_CODING	possibly_damaging(0.819)	.
19	18583691	ELL	T	A	10	D/V	SPLICE_SITE:NON_SYNONYMOUS_CODING	possibly_damaging(0.948)	.
2	96519492	ENSG00000174501	C	T	732	D/N	NON_SYNONYMOUS_CODING	probably_damaging(0.978)	tolerated(0.06)
2	96610395	ENSG00000174501	C	CA	490-491	.	FRAMESHIFT_CODING	.	.
16	15457629	ENSG00000183793	T	A	314	T/S	NON_SYNONYMOUS_CODING	benign(0.158)	tolerated(0.82)
16	15457628	ENSG00000183793	G	T	314	T/N	NON_SYNONYMOUS_CODING	benign(0.348)	tolerated(0.39)
9	46386995	ENSG00000237198	C	A	3	R/M	NON_SYNONYMOUS_CODING	benign(0.013)	tolerated(0.36)
9	46386956	ENSG00000237198	C	G	16	R/P	NON_SYNONYMOUS_CODING	probably_damaging(0.998)	deleterious(0)
9	46386806	ENSG00000237198	C	T	66	R/Q	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.75)
15	32693591	ENSG00000249931	T	G	45	K/T	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)

15	32693595	ENSG00000249931	C	G	44	E/Q	NON_SYNONYMOUS_CODING	benign(0.006)	tolerated(0.07)
14	104040459	ENSG00000256500	A	T	126	I/F	NON_SYNONYMOUS_CODING	benign(0.423)	deleterious(0)
14	104040450	ENSG00000256500	G	A	123	E/K	NON_SYNONYMOUS_CODING	possibly_damaging(0.609)	deleterious(0)
3	13679670	FBLN2	T	G	110	*/G	STOP_LOST	.	.
3	13679659	FBLN2	T	G	106	V/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.811)	deleterious(0)
1	240370946	FMN2	C	T	945	P/L	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.26)
1	240371426	FMN2	T	C	1105	V/A	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.09)
20	29625928	FRG1B	G	A	58	V/I	NON_SYNONYMOUS_CODING	benign(0.003)	tolerated(0.17)
20	29625947	FRG1B	T	C	64	I/T	NON_SYNONYMOUS_CODING	benign(0.017)	deleterious(0)
20	29628328	FRG1B	C	G	110	I/M	NON_SYNONYMOUS_CODING	benign(0.191)	deleterious(0.03)
7	150327209	GIMAP6	G	T	8	Q/K	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0)
7	150327226	GIMAP6	T	C	2	E/G	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0)
2	90249273	IGKV1D-43	C	G	77	Q/E	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.68)
2	90249269	IGKV1D-43	T	A	75	S/R	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.23)
17	38975147	KRT10	G	T	547	S/Y	NON_SYNONYMOUS_CODING	unknown(0)	.
17	38975149	KRT10	G	T	546	S/R	NON_SYNONYMOUS_CODING	unknown(0)	.
17	38975151	KRT10	T	C	546	S/G	NON_SYNONYMOUS_CODING	unknown(0)	.
12	122685179	LRRC43	C	A	402	T/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.92)
12	122685176	LRRC43	G	A	401	R/K	NON_SYNONYMOUS_CODING	benign(0.005)	tolerated(1)
12	122685163	LRRC43	A	G	397	K/E	NON_SYNONYMOUS_CODING	probably_damaging(0.99)	tolerated(0.89)
12	122685164	LRRC43	A	G	397	K/R	NON_SYNONYMOUS_CODING	probably_damaging(0.99)	tolerated(0.23)
7	151927025	MLL3	A	G	987	Y/H	NON_SYNONYMOUS_CODING	probably_damaging(1)	.
7	151932997	MLL3	C	T	892	G/R	NON_SYNONYMOUS_CODING	probably_damaging(1)	.
7	100634922	MUC12	G	A	360	A/T	NON_SYNONYMOUS_CODING	unknown(0)	.
7	100638484	MUC12	A	T	1547	K/I	NON_SYNONYMOUS_CODING	unknown(0)	.
19	9002623	MUC16	C	T	39	R/H	NON_SYNONYMOUS_CODING	benign(0.011)	tolerated(0.24)
19	9002636	MUC16	C	T	35	V/I	NON_SYNONYMOUS_CODING	benign(0.051)	tolerated(0.37)
19	9002612	MUC16	T	G	43	K/Q	NON_SYNONYMOUS_CODING	benign(0.055)	tolerated(0.23)
19	9002597	MUC16	C	T	48	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.866)	deleterious(0)
3	195507053	MUC4	A	C	3800	S/A	NON_SYNONYMOUS_CODING	benign(0.035)	.
3	195513847	MUC4	A	G	1535	M/T	NON_SYNONYMOUS_CODING	benign(0.042)	.
3	195506549	MUC4	A	G	3968	S/P	NON_SYNONYMOUS_CODING	benign(0.044)	.
3	195507226	MUC4	A	G	3742	V/A	NON_SYNONYMOUS_CODING	benign(0.095)	.
3	195508790	MUC4	T	C	3221	S/G	NON_SYNONYMOUS_CODING	benign(0.095)	.
3	195508796	MUC4	C	G	3219	V/L	NON_SYNONYMOUS_CODING	benign(0.095)	.
3	195514768	MUC4	T	C	1228	D/G	NON_SYNONYMOUS_CODING	benign(0.144)	.
3	195506099	MUC4	T	C	4118	T/A	NON_SYNONYMOUS_CODING	benign(0.182)	.
3	195508667	MUC4	T	C	3262	T/A	NON_SYNONYMOUS_CODING	benign(0.182)	.
3	195508789	MUC4	C	T	3221	S/N	NON_SYNONYMOUS_CODING	benign(0.204)	.
3	195509212	MUC4	G	A	3080	S/L	NON_SYNONYMOUS_CODING	benign(0.204)	.
3	195515017	MUC4	A	G	1145	V/A	NON_SYNONYMOUS_CODING	benign(0.231)	.
3	195506569	MUC4	A	G	3961	V/A	NON_SYNONYMOUS_CODING	benign(0.243)	.
3	195506293	MUC4	T	C	4053	N/S	NON_SYNONYMOUS_CODING	benign(0.258)	.
3	195508787	MUC4	G	T	3222	L/I	NON_SYNONYMOUS_CODING	benign(0.352)	.

3	195510194	MUC4	G	C	2753	L/V	NON_SYNONYMOUS_CODING	benign(0.352)	.
3	195510636	MUC4	C	G	2605	Q/H	NON_SYNONYMOUS_CODING	benign(0.391)	.
3	195508661	MUC4	A	G	3264	S/P	NON_SYNONYMOUS_CODING	benign(0.421)	.
3	195506533	MUC4	C	A	3973	S/I	NON_SYNONYMOUS_CODING	possibly_damaging(0.473)	.
3	195507062	MUC4	C	T	3797	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195507385	MUC4	G	A	3689	A/V	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195507446	MUC4	C	T	3669	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195507494	MUC4	C	T	3653	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195508500	MUC4	G	C	3317	D/E	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195508668	MUC4	G	C	3261	D/E	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195510649	MUC4	G	A	2601	A/V	NON_SYNONYMOUS_CODING	possibly_damaging(0.509)	.
3	195506983	MUC4	G	A	3823	T/I	NON_SYNONYMOUS_CODING	possibly_damaging(0.607)	.
3	195507443	MUC4	T	G	3670	T/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.607)	.
3	195507475	MUC4	G	C	3659	T/R	NON_SYNONYMOUS_CODING	possibly_damaging(0.607)	.
3	195507827	MUC4	G	A	3542	L/F	NON_SYNONYMOUS_CODING	possibly_damaging(0.607)	.
3	195511076	MUC4	T	A	2459	T/S	NON_SYNONYMOUS_CODING	possibly_damaging(0.609)	.
3	195511525	MUC4	T	C	2309	D/G	NON_SYNONYMOUS_CODING	possibly_damaging(0.748)	.
3	195511526	MUC4	C	T	2309	D/N	NON_SYNONYMOUS_CODING	possibly_damaging(0.748)	.
3	195506501	MUC4	G	A	3984	P/S	NON_SYNONYMOUS_CODING	possibly_damaging(0.768)	.
3	195506282	MUC4	A	C	4057	L/V	NON_SYNONYMOUS_CODING	possibly_damaging(0.806)	.
3	195507604	MUC4	C	G	3616	R/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.811)	.
3	195506510	MUC4	G	C	3981	H/D	NON_SYNONYMOUS_CODING	possibly_damaging(0.817)	.
3	195507445	MUC4	T	A	3669	D/V	NON_SYNONYMOUS_CODING	possibly_damaging(0.862)	.
3	195507461	MUC4	G	A	3664	P/S	NON_SYNONYMOUS_CODING	possibly_damaging(0.864)	.
3	195507694	MUC4	A	G	3586	L/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.881)	.
3	195508786	MUC4	A	G	3222	L/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.881)	.
3	195506291	MUC4	C	T	4054	A/T	NON_SYNONYMOUS_CODING	possibly_damaging(0.922)	.
3	195506914	MUC4	G	A	3846	P/L	NON_SYNONYMOUS_CODING	possibly_damaging(0.931)	.
3	195506990	MUC4	C	G	3821	D/H	NON_SYNONYMOUS_CODING	possibly_damaging(0.934)	.
3	195508670	MUC4	C	G	3261	D/H	NON_SYNONYMOUS_CODING	possibly_damaging(0.934)	.
3	195511102	MUC4	G	A	2450	P/L	NON_SYNONYMOUS_CODING	probably_damaging(0.975)	.
3	195514948	MUC4	G	A	1168	P/L	NON_SYNONYMOUS_CODING	probably_damaging(0.975)	.
3	195510622	MUC4	G	T	2610	P/H	NON_SYNONYMOUS_CODING	probably_damaging(0.991)	.
3	195506302	MUC4	G	T	4050	P/H	NON_SYNONYMOUS_CODING	probably_damaging(0.998)	.
3	195506542	MUC4	G	T	3970	P/H	NON_SYNONYMOUS_CODING	probably_damaging(1)	.
3	195510310	MUC4	T	G	2714	Y/S	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510341	MUC4	A	G	2704	S/P	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510347	MUC4	T	C	2702	T/A	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510350	MUC4	C	G	2701	D/H	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510373	MUC4	T	C	2693	D/G	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510374	MUC4	C	T	2693	D/N	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510389	MUC4	A	G	2688	S/P	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510396	MUC4	A	C	2685	H/Q	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510492	MUC4	C	G	2653	Q/H	NON_SYNONYMOUS_CODING	unknown(0)	.

3	195510509	MUC4	A	C	2648	S/A	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510518	MUC4	C	T	2645	D/N	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510563	MUC4	G	C	2630	P/A	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510565	MUC4	C	T	2629	S/N	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510566	MUC4	T	C	2629	S/G	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510601	MUC4	A	G	2617	V/A	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510610	MUC4	A	G	2614	L/P	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510611	MUC4	G	T	2614	L/I	NON_SYNONYMOUS_CODING	unknown(0)	.
3	195510613	MUC4	C	T	2613	S/N	NON_SYNONYMOUS_CODING	unknown(0)	.
11	1016957	MUC6	T	G	1948	R/S	NON_SYNONYMOUS_CODING	benign(0.034)	tolerated(0.84)
11	1017466	MUC6	T	C	1779	R/G	NON_SYNONYMOUS_CODING	benign(0.142)	tolerated(0.57)
11	1017307	MUC6	G	A	1832	P/S	NON_SYNONYMOUS_CODING	benign(0.34)	tolerated(0.21)
11	1016733	MUC6	G	T	2023	T/K	NON_SYNONYMOUS_CODING	probably_damaging(0.976)	tolerated(0.24)
11	1018042	MUC6	A	G	1587	S/P	NON_SYNONYMOUS_CODING	probably_damaging(0.979)	deleterious(0.01)
11	1016554	MUC6	C	T	2083	A/T	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.6)
11	1016559	MUC6	G	C	2081	A/G	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.59)
11	1016565	MUC6	C	T	2079	S/N	NON_SYNONYMOUS_CODING	unknown(0)	tolerated(0.09)
11	1016583	MUC6	T	G	2073	Q/P	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0.03)
16	74425734	NPIPL2	T	C	363	V/A	NON_SYNONYMOUS_CODING	benign(0.012)	tolerated(0.66)
16	74425727	NPIPL2	T	C	361	W/R	NON_SYNONYMOUS_CODING	possibly_damaging(0.919)	tolerated(0.08)
12	5603600	NTF3	C	G	74	R/G	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
12	5603598	NTF3	A	G	73	E/G	NON_SYNONYMOUS_CODING	benign(0.261)	deleterious(0.01)
11	45957282	PHF21A	C	T	39	E/K	NON_SYNONYMOUS_CODING	probably_damaging(0.992)	deleterious(0.01)
11	45957279	PHF21A	C	T	40	E/K	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
11	45957285	PHF21A	C	T	38	E/K	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)
12	106820987	POLR3B	C	T	314	L/F	NON_SYNONYMOUS_CODING	benign(0.088)	deleterious(0)
12	106820975	POLR3B	C	T	310	L/F	SPLICE_SITE:NON_SYNONYMOUS_CODING	benign(0.102)	deleterious(0)
18	14542791	POTEC	C	T	119	A/T	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.41)
18	14533105	POTEC	G	C	337	S/C	NON_SYNONYMOUS_CODING	probably_damaging(0.97)	tolerated(0.19)
22	18901004	PRODH	C	T	166	R/Q	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.6)
22	18905964	PRODH	C	T	76	R/H	NON_SYNONYMOUS_CODING	possibly_damaging(0.726)	tolerated(0.14)
2	85571228	RETSAT	G	C	265	A/G	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.43)
2	85571225	RETSAT	T	C	266	E/G	NON_SYNONYMOUS_CODING	benign(0.048)	tolerated(0.28)
19	14104595	RFX1	C	G	21	A/P	NON_SYNONYMOUS_CODING	unknown(0)	.
19	14104610	RFX1	A	G	16	S/P	NON_SYNONYMOUS_CODING	unknown(0)	.
6	108197860	SEC63	G	T	648	Q/K	NON_SYNONYMOUS_CODING	probably_damaging(0.987)	tolerated(0.15)
6	108197851	SEC63	A	C	651	W/G	NON_SYNONYMOUS_CODING	probably_damaging(1)	deleterious(0)
8	145095684	SPATC1	A	C	328	T/P	NON_SYNONYMOUS_CODING	benign(0.102)	tolerated(0.27)
8	145095681	SPATC1	A	C	327	T/P	NON_SYNONYMOUS_CODING	probably_damaging(0.995)	deleterious(0.03)
1	234601448	TARBP1	T	A	419	I/F	NON_SYNONYMOUS_CODING	benign(0.07)	tolerated(0.26)
1	234601451	TARBP1	T	A	418	I/F	NON_SYNONYMOUS_CODING	possibly_damaging(0.915)	tolerated(0.09)
7	142498738	TRBC2	C	G	5	N/K	NON_SYNONYMOUS_CODING	benign(0.002)	tolerated(0.34)
7	142498735	TRBC2	A	C	4	K/N	NON_SYNONYMOUS_CODING	benign(0.111)	tolerated(0.08)
7	142223959	TRBV11-1	C	T	70	D/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)

7	142224021	TRBV11-1	G	T	49	A/D	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.63)
7	142223962	TRBV11-1	G	C	69	Q/E	NON_SYNONYMOUS_CODING	benign(0.009)	tolerated(0.25)
7	142223964	TRBV11-1	A	T	68	F/Y	NON_SYNONYMOUS_CODING	benign(0.014)	tolerated(0.2)
7	142224003	TRBV11-1	C	A	55	R/L	NON_SYNONYMOUS_CODING	benign(0.428)	tolerated(0.05)
7	142180567	TRBV6-5	G	C	98	L/V	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.06)
7	142180573	TRBV6-5	T	C	96	R/G	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.12)
7	142180585	TRBV6-5	C	T	92	D/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.79)
7	142180590	TRBV6-5	G	T	90	T/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142180593	TRBV6-5	G	T	89	T/N	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.44)
7	142180596	TRBV6-5	G	A	88	S/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.31)
7	142180618	TRBV6-5	T	C	81	N/D	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.78)
7	142180633	TRBV6-5	G	T	76	Q/K	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142180635	TRBV6-5	T	G	75	D/A	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.22)
7	142180641	TRBV6-5	A	G	73	I/T	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.91)
7	142180704	TRBV6-5	G	T	52	S/Y	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142180737	TRBV6-5	T	A	41	Q/L	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.16)
7	142180578	TRBV6-5	G	A	94	P/L	NON_SYNONYMOUS_CODING	benign(0.002)	tolerated(0.17)
7	142180588	TRBV6-5	C	G	91	E/Q	NON_SYNONYMOUS_CODING	benign(0.005)	tolerated(0.2)
7	142180647	TRBV6-5	G	T	71	A/D	NON_SYNONYMOUS_CODING	benign(0.08)	tolerated(0.18)
7	142099548	TRBV7-8	A	G	85	F/S	NON_SYNONYMOUS_CODING	benign(0)	tolerated(1)
7	142099581	TRBV7-8	A	G	74	L/P	NON_SYNONYMOUS_CODING	benign(0)	tolerated(0.35)
7	142099512	TRBV7-8	T	G	97	K/T	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(0.14)
7	142099537	TRBV7-8	G	T	89	P/T	NON_SYNONYMOUS_CODING	benign(0.003)	tolerated(0.39)
2	179544685	TTN	C	CTCT	9928	E/EE	NON_SYNONYMOUS_CODING	.	.
2	179634421	TTN	T	G	2917	T/P	NON_SYNONYMOUS_CODING	possibly_damaging(0.539)	.
2	179483108	TTN	C	T	6753	V/I	NON_SYNONYMOUS_CODING	possibly_damaging(0.737)	.
2	179507024	TTN	C	A	4435	V/F	NON_SYNONYMOUS_CODING	possibly_damaging(0.741)	.
2	179400061	TTN	G	A	24693	R/W	NON_SYNONYMOUS_CODING	probably_damaging(1)	.
17	5036210	USP6	T	G	67	I/M	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0.04)
17	5036211	USP6	C	T	68	R/W	NON_SYNONYMOUS_CODING	unknown(0)	deleterious(0)
19	21992147	ZNF43	G	T	230	T/K	NON_SYNONYMOUS_CODING	benign(0.001)	tolerated(1)
19	21992138	ZNF43	T	A	233	E/V	NON_SYNONYMOUS_CODING	probably_damaging(0.966)	deleterious(0.04)
19	21991497	ZNF43	G	T	447	H/N	NON_SYNONYMOUS_CODING	probably_damaging(0.999)	deleterious(0)

APPENDIX H: Analysis of whole exome sequencing data from WTSI: Across the familial CHD cohort

Table 1: List of genes with functional homozygous variants in > 1 consanguineous families (i.e CHD1 and CHD4)

ENSG00000228835	MBD3L2	NBPF14	POTEM
ENSG00000232637	MESP2	NBPF16	PRAMEF19
GTF2IRD2	MUC4	OR2T34	PRDM15
HLA-B	NBPF10	POTEF	PRODH

Table 2: List of genes with functional homozygous variants in > 1 consanguineous or non-consanguineous family

AGAP4	ENSG00000228835	HLA-B	NBPF10	POTEI
AGAP6	ENSG00000232637	KCNC3	NBPF12	POTEM
AGAP7	FAM21B	LILRB3	NBPF14	PRAMEF19
CD177	FAM22D	LRRC37A	NBPF16	PRAMEF3
CELA3B	FAM22F	MBD3L2	NR4A3	PRDM15
COPZ2	FAM86B2	MESP2	OR2T34	PRODH
ENSG00000170161	GOLGA6L10	MUC12	OR2T35	RGPD4
ENSG00000215749	GTF2IRD2	MUC4	POTEF	RYK
				TRIM74

Table 3: List of genes with functional compound heterozygous variants in >1 non-consanguineous family (i.e. CHD5, 6, 13, 16, 20, 22, and 23)

AGAP7	CPSF3	GRIK3	MYOM1	SELPLG
ACACA	CSPP1	HLA-B	NBPF10	SEZ6L
AKR1E2	CTBP2	HLA-DRB1	NCF4	SLC17A5
ANKRD36	CUL7	HLA-DRB5	NECAP1	SLIT2
ANO4	DCHS1	HMCN1	NFATC1	SPARCL1
APOBEC3H	DDX24	HTT	NFRKB	SPEN
ASH1L	DGKH	IGHV3-11	NGLY1	STK38
ATAD3B	DLEC1	IGHV3-48	NIPAL4	SYNE1
ATAD3C	DMXL2	IGHV3-64	NOTCH1	TBX20
ATG2A	DNAH5	IGHV5-51	OBSCN	TGS1
ATL3	DNAH8	IGSF21	ODZ4	TMEM8A
ATM	DNAJC14	IKZF1	OPA1	TMTC2
ATP6V1A	DOCK5	INF2	OR2T33	TRBC2
ATRN	DSCAM	INHBA	PAPOLG	TRBV3-1
BACH1	ELF1	ITPR1	PJA2	TRBV4-2
BAI3	ENSG00000183793	KRTAP9-1	PKHD1	TRBV5-4
BRD8	ENSG00000229292	LRP1B	PKHD1L1	TRBV5-5
BRPF1	ENSG00000237198	LRRC43	PLIN4	TRBV6-5
BZRAP1	ENSG00000257513	LY9	PLXNA2	TRBV7-8
C12orf51	EP400	LYST	PNMA1	TTC3
C20orf12	EPHA10	MACC1	PNPLA8	TTN
C2CD3	EPHA5	MACF1	PPFIA2	UBR4
C3orf56	ESPN	MAN1A2	PRRC2B	UGGT1
C4orf17	FA2H	MAP1B	PRSS3	USH2A
CACNA1D	FAM135A	MGA	PSMD2	USP15
CAP1	FAM82A2	MICALCL	PTCHD3	USP6
CBX4	FASTKD2	MKI67	PTPRB	UTP20
CCNA1	FBLN2	MLL3	PYDC2	VWA3B
CD97	FBXO24	MUC12	RB1	VWA5B1
CDC27	FOXD4	MUC16	RETSAT	ZCCHC14
CEP250	FRG1B	MUC4	RFX1	ZNF592
CHD3	FRG2B	MUC5B	RFX3	ZNF706
CHD5	GEMIN2	MUC6	RGPD1	
CIC	GNPAT	MYCBP2	RIMBP2	
CLEC18B	GPR98	MYO9A	SDK1	

Table 4: List of genes with functional homozygous or compound heterozygous variants in > 1 consanguineous or non-consanguineous family

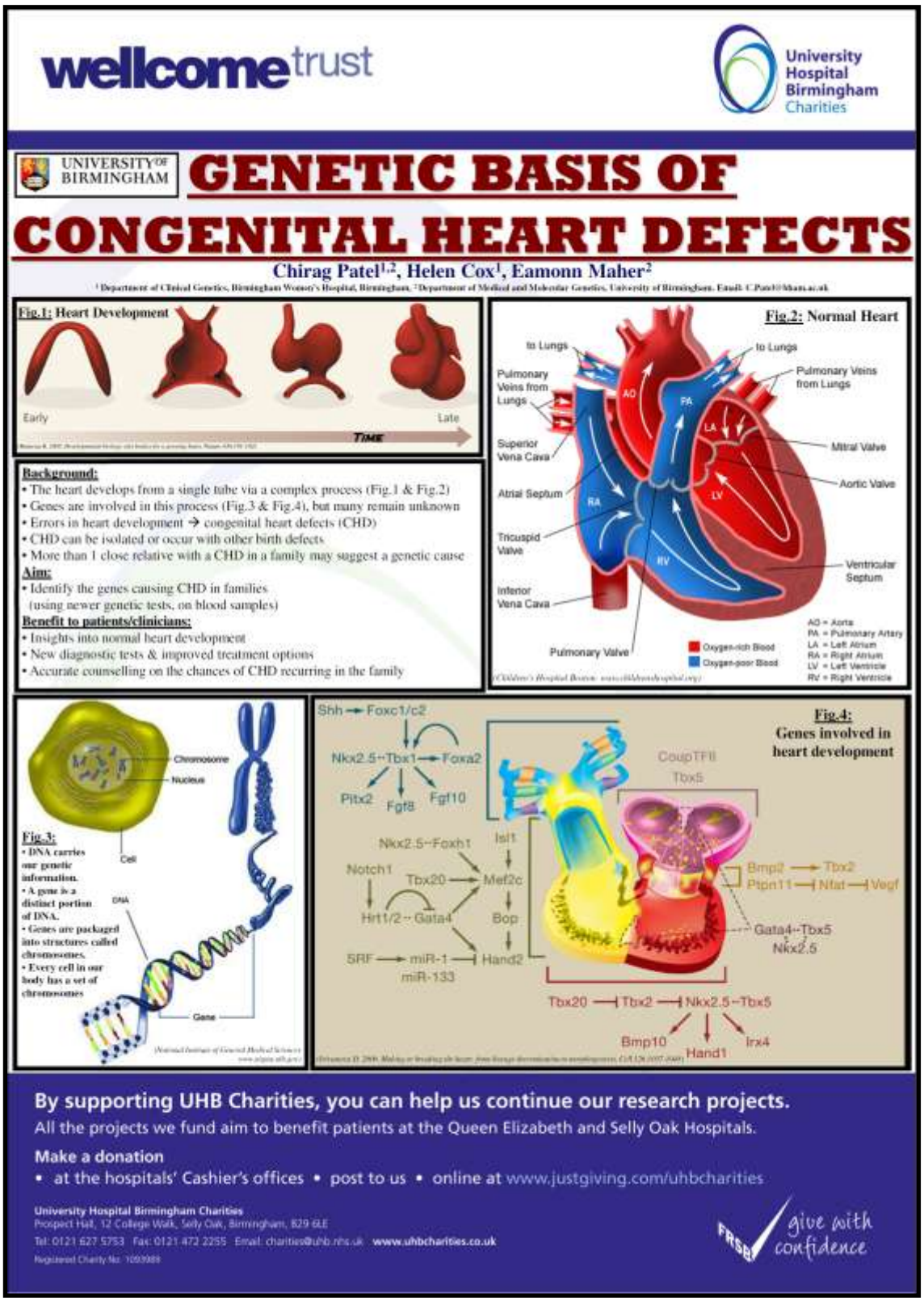
ACACA	CCNA1	ENSG00000215749	HLA-B
AGAP4	CD177	ENSG00000228835	HLA-DRB1
AGAP6	CD97	ENSG00000229292	HLA-DRB5
AGAP7	CDC27	ENSG00000232637	HMCN1
AHNAK2	CELA3B	ENSG00000237198	HSPG2
AKR1E2	CEP250	ENSG00000249931	HTT
ANK2	CHD3	ENSG00000257513	IGHV3-11
ANKRD32	CHD5	EP400	IGHV3-48
ANKRD36	CIC	EPHA10	IGHV3-64
ANO4	CLEC18B	EPHA2	IGHV5-51
APOBEC3H	CLIP1	EPHA5	IGSF21
ASH1L	CLTCL1	ESPN	IGSF3
ATAD3B	CNTNAP2	FA2H	IKZF1
ATAD3C	COL21A1	FAM135A	INF2
ATF7IP	COPZ2	FAM21B	INHBA
ATG2A	CPSF3	FAM22D	ITPR1
ATL3	CSPP1	FAM22F	KCNC3
ATM	CTAGE9	FAM82A2	KRTAP9-1
ATP6V1A	CTBP2	FAM86B2	LAMB3
ATRN	CUL7	FASTKD2	LCE1C
BACH1	DCHS1	FAT3	LILRB3
BAI3	DDX24	FBLN2	LMO7
BCL9	DGKH	FBXO24	LRBA
BRD8	DLEC1	FCGBP	LRP1B
BRPF1	DMXL2	FOXD4	LRRC37A
BZRAP1	DNAH5	FRG1B	LRRC37A3
C12orf51	DNAH8	FRG2B	LRRC43
C20orf12	DNAJC14	FRYL	LY9
C2CD3	DOCK5	GEMIN2	LYST
C3orf56	DSCAM	GNPAT	MACC1
C4orf17	ELF1	GOLGA6L10	MACF1
CACNA1D	ENSG00000170161	GOLGA8A	MAN1A2
CAP1	ENSG00000174501	GPR98	MAP1B
CBX4	ENSG00000183793	GRIK3	MBD3L2
CCDC108	ENSG00000188219	GTF2IRD2	MEGF9

MESP2	ODZ4	PSMD2	TRBC2
MGA	OPA1	PTCHD3	TRBV3-1
MICAL2	OR2T33	PTPRB	TRBV4-2
MICALCL	OR2T34	PYDC2	TRBV5-4
MKI67	OR2T35	RB1	TRBV5-5
MLL3	PAPOLG	RETSAT	TRBV6-5
MUC12	PDK4	RFX1	TRBV7-3
MUC16	PJA2	RFX3	TRBV7-8
MUC4	PKHD1	RGPD1	TRIM74
MUC5B	PKHD1L1	RGPD4	TTC3
MUC6	PLIN4	RIMBP2	TTN
MYCBP2	PLXNA2	RYK	UBR4
MYO9A	PNMA1	SDK1	UGGT1
MYOM1	PNPLA8	SELPLG	UTP20
NBPF10	POLR3B	SEZ6L	USH2A
NBPF12	POTEC	SIGLEC10	USP15
NBPF14	POTEF	SLC17A5	USP49
NBPF16	POTEI	SLIT2	USP6
NCF4	POTEM	SPARCL1	VWA3B
NECAP1	PPFIA2	SPEN	VWA5B1
NFATC1	PRAMEF19	STK38	ZBTB9
NFRKB	PRAMEF3	SYNE1	ZCCHC14
NGLY1	PRDM1	TBX20	ZNF592
NIPAL4	PRDM15	TCHH	ZNF706
NOTCH1	PRODH	TGS1	ZNF880
NR4A3	PRRC2B	TMEM8A	
OBSCN	PRSS3	TMTC2	

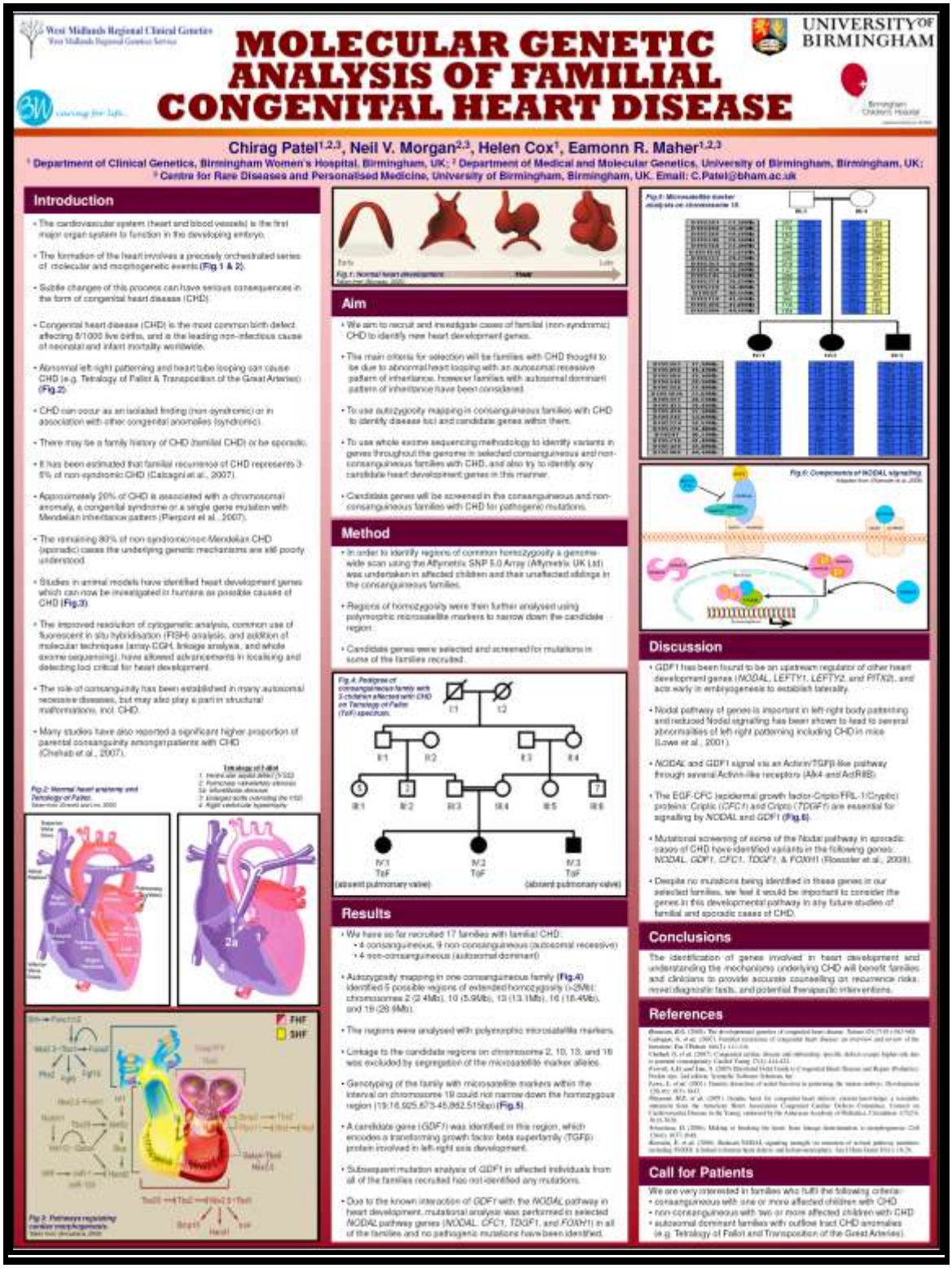
CHAPTER 8

PRESENTATIONS

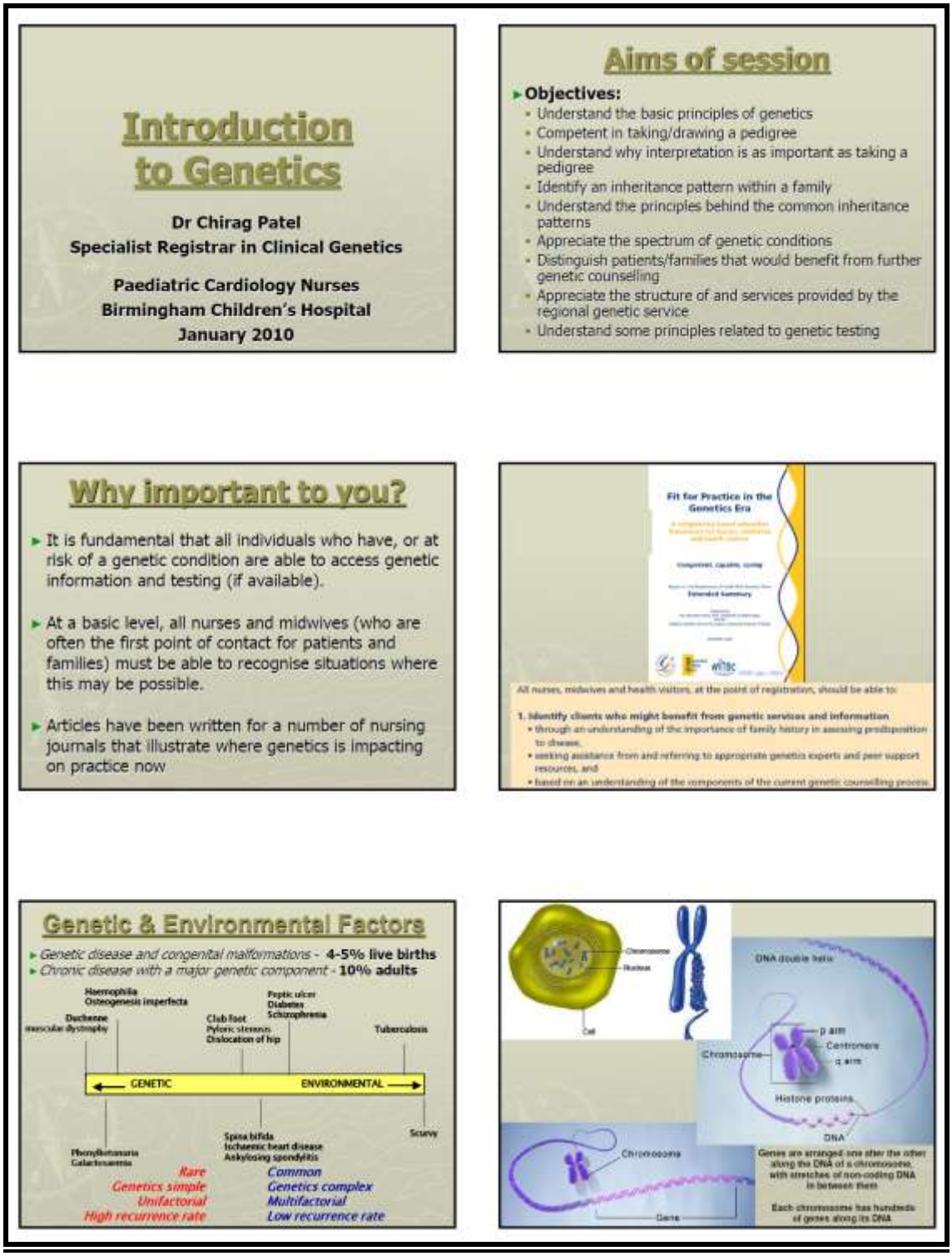
A) POSTER PRESENTATION:
Wellcome Trust Clinical Research Facility Open Day, University of Birmingham, February 2010

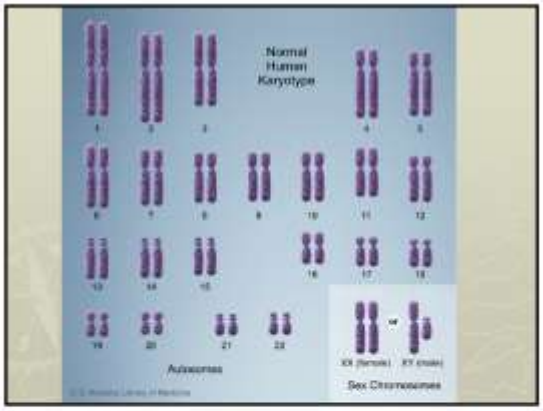


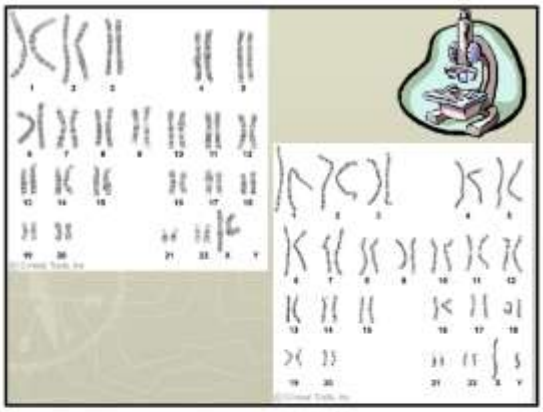
B) POSTER PRESENTATION:
British Human Genetics Conference, Warwick, September 2010



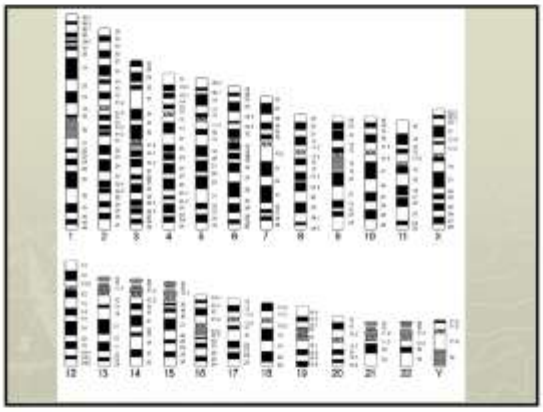
C) TEACHING PRESENTATION:
Paediatric Cardiology Specialist Nurses,
Birmingham Children's Hospital, January 2010

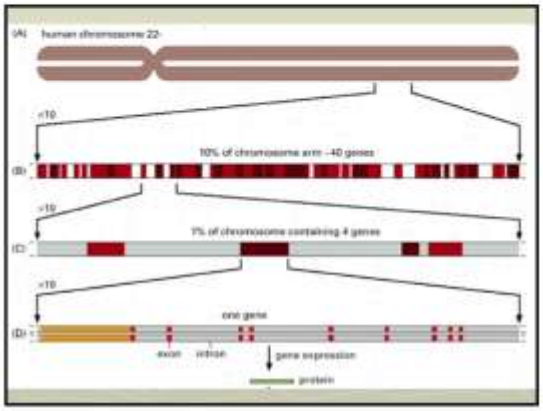



Normal
Human
Karyotype
Autosomes
Sex Chromosomes




GENES
The DNA in the chromosome codes for genes. These produce proteins that build and control the body. The human genome contains about 30 000 genes.



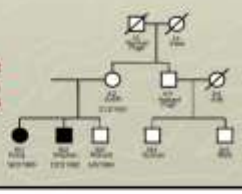

(A) human chromosome 22
~10
10% of chromosome arm ~40 genes
(B)
~10
1% of chromosome containing 4 genes
(C)
~10
one gene
(D)
exon
intron
gene expression
protein


8
146 million base pairs

Drawing a family pedigree

- Build up the tree from the "bottom" starting with affected child and siblings
  Record names, dates of birth

- Choose one parent
  Ask about sibs and their children, then parents

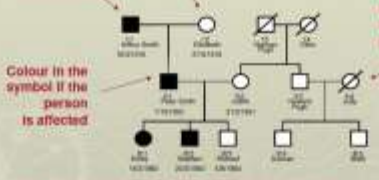


Add information on the other side of the family

Use clear symbols: circles for females squares for males

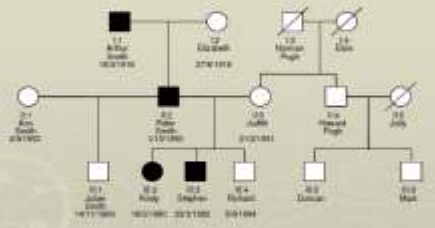
Put a sloping line through the symbol (from the bottom left hand corner) if the person has died

Colour in the symbol if the person is affected



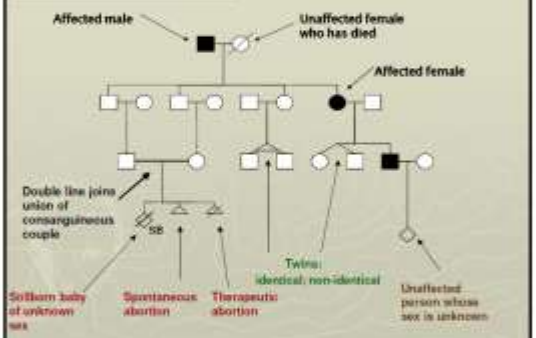
Record names, dates of birth and maiden names

Ask for miscarriages, stillbirths or deaths in each partnership



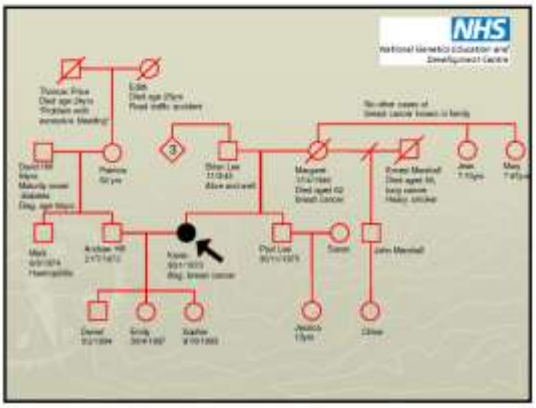
"May I ask: have you had any children with other partners?"

Other pedigree symbols



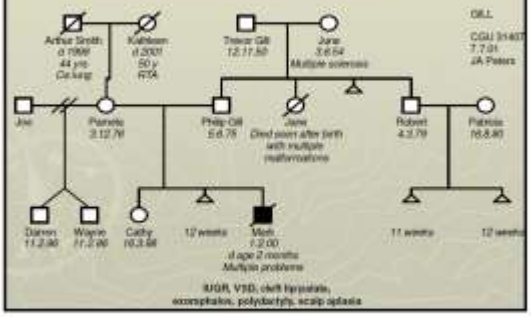
Drawing a family pedigree

- **Ask about consanguinity**
  "Are you and your partner related?
  Are there any surnames in common?"
- **Date and sign the pedigree**
- **Record at least basic information on both sides of the family even if a disorder is segregating on only one side**



Practice Pedigree

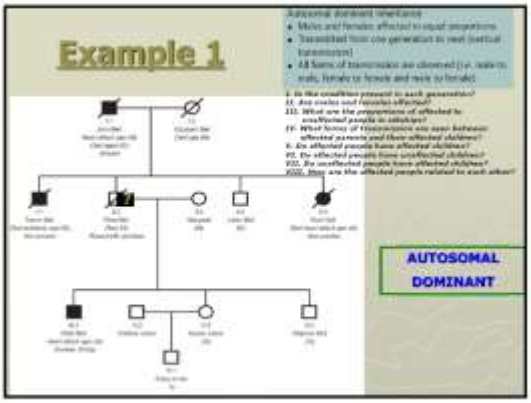
Practice Pedigree

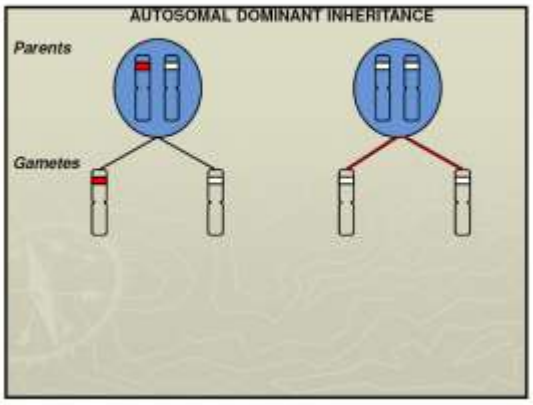


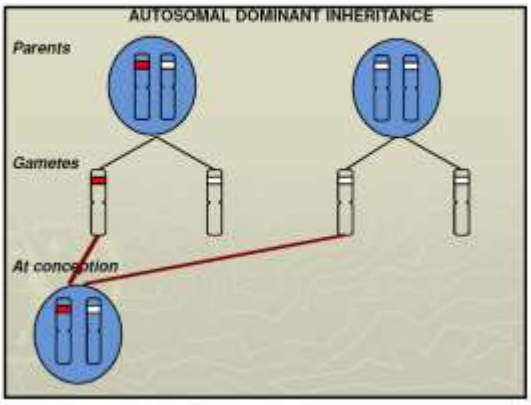
Why interpretation is important?

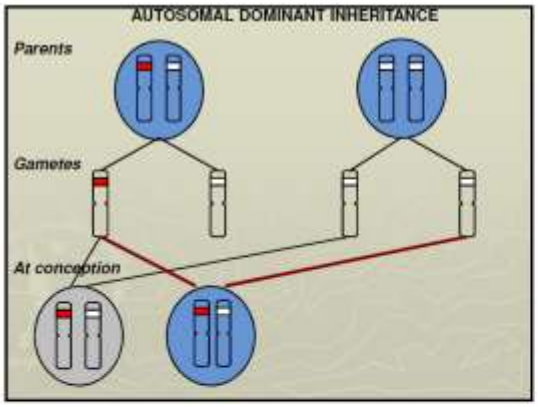
- Help to make/refine a diagnosis
- Reveal patterns of inheritance
- Assess likelihood of genetic disease in relatives
- Affect testing, treatment, and management strategies
- Highlight need for specialist referral
- Correct any family misconceptions

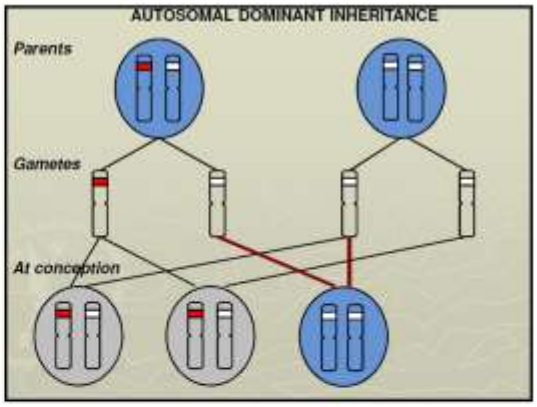
Example 1

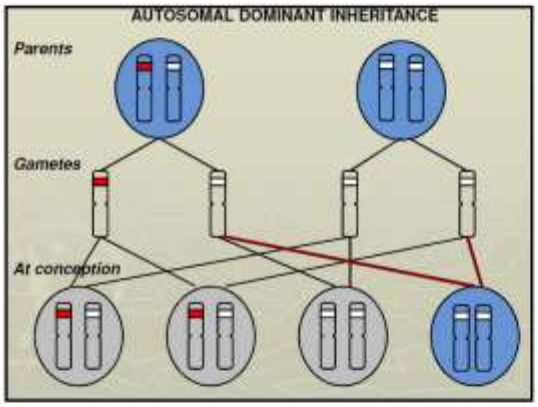


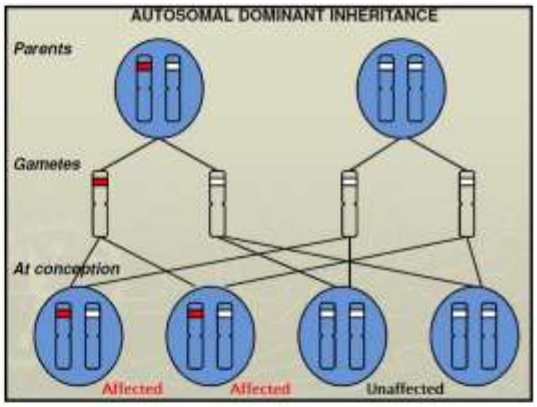


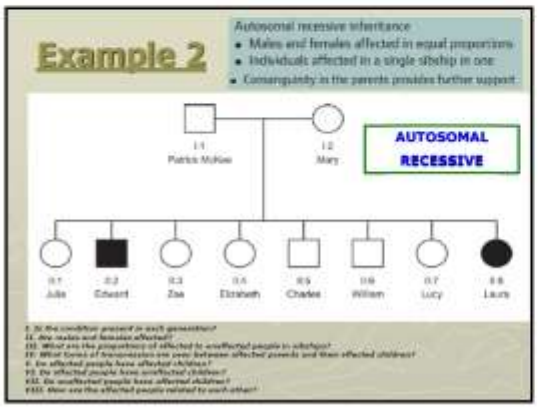


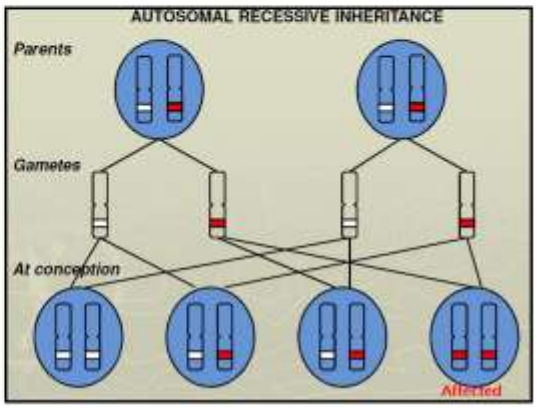

AUTOSOMAL DOMINANT INHERITANCE
Parents
Gametes
At conception


AUTOSOMAL DOMINANT INHERITANCE
Parents
Gametes
At conception

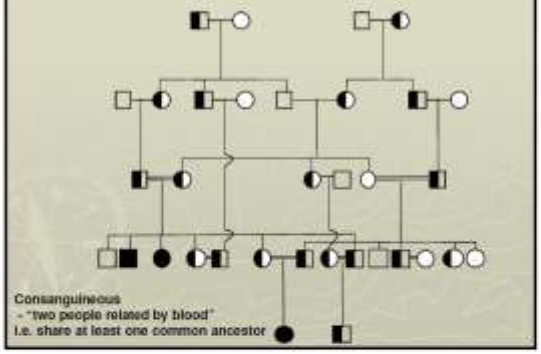

AUTOSOMAL DOMINANT INHERITANCE
Parents
Gametes
At conception


AUTOSOMAL DOMINANT INHERITANCE
Parents
Gametes
At conception
Affected
Affected
Unaffected


Example 2
Autosomal recessive inheritance
Males and females affected in equal proportions
Individuals affected in a single sibship in one
Consanguinity in the parents provides further support
AUTOSOMAL
RECESSIVE

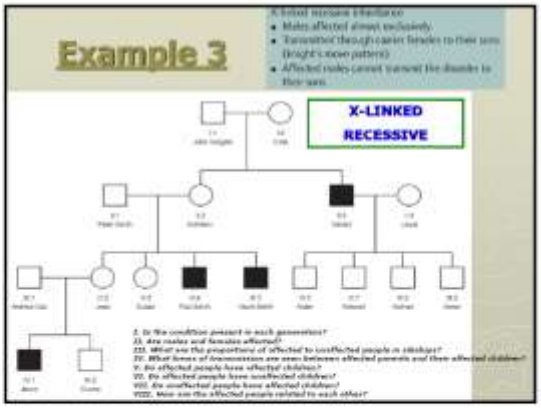

AUTOSOMAL RECESSIVE INHERITANCE
Parents
Gametes
At conception
Affected

Consanguinity & AR inheritance

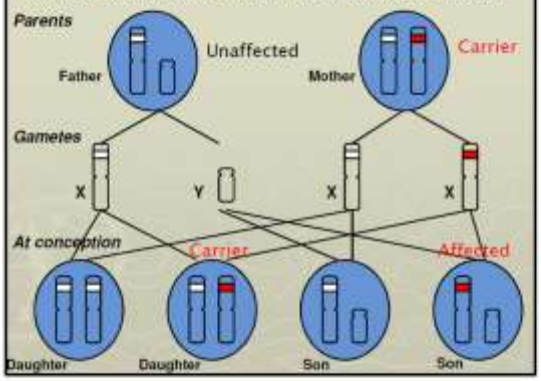


Consanguineous
- "two people related by blood"
i.e. share at least one common ancestor

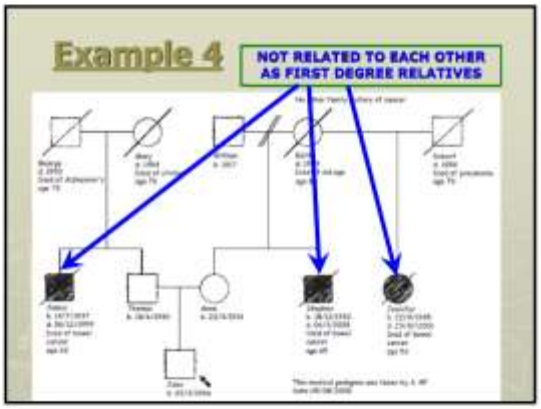
Example 3



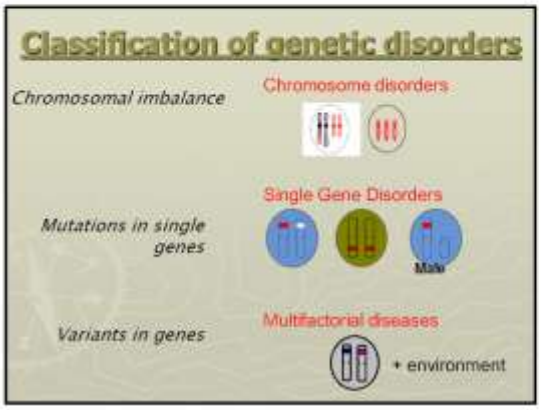
X-LINKED INHERITANCE WHERE THE MOTHER IS A CARRIER



Example 4



Classification of genetic disorders

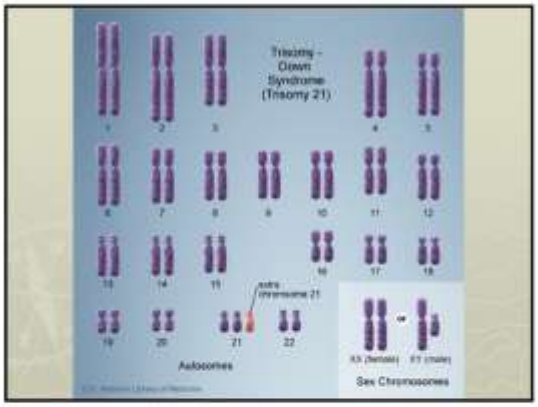


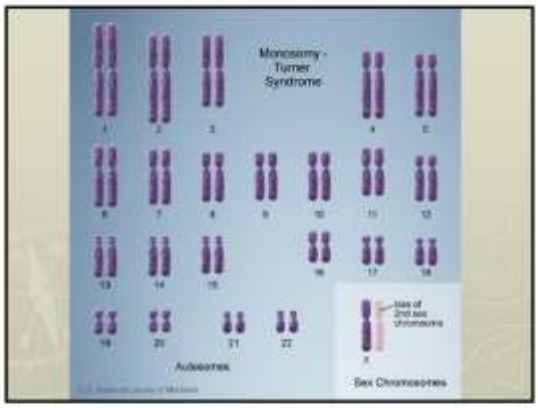
Chromosomal Disorders

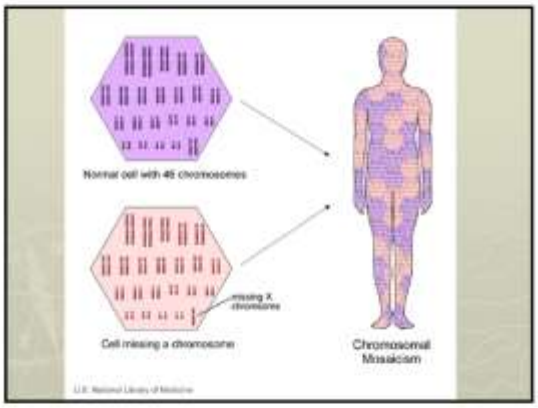
- IUGR
- Postnatal growth retardation
- Developmental delay
- Dysmorphic features
- Multiple organ system anomalies
- Hand flapping/writhing etc

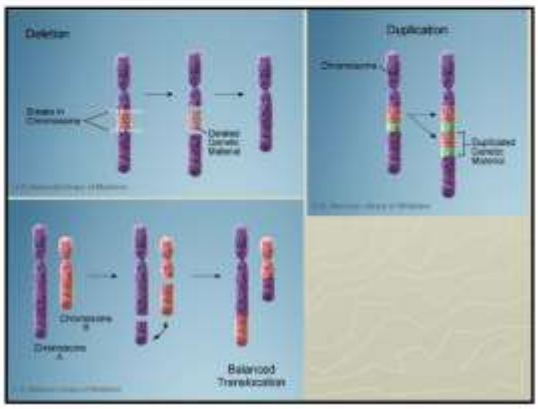
Chromosome Abnormalities

- Polyploidy (e.g. triploidy = 69,XXX)
- Autosomal trisomies 21, 18, 13
- Sex chromosome abnormalities
- Mosaicism
- Deletions / duplications / inversions / ring
- Translocations







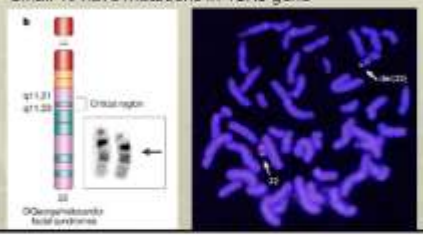


Microdeletion Syndromes

DiGeorge	22q11
Williams syndrome	7q11.2
Prader-Willi syndrome	15q11-q13
Angelman syndrome	15q11-q13

22q11.2 Deletion Syndrome

- **Genetics:**
  - Microdeletion (de novo - 94%, inherited - 6%)
  - Detected by FISH analysis
  - Encompasses ~30 genes
  - Small % have mutations in *TBX1* gene



22q11.2 Deletion Syndrome

- **Clinical Features:**
  - Growth retardation (83%)
  - Learning difficulties (68%) (esp. maths and reading)
  - Speech delay/communication problems (90%)
  - Psychiatric disorders (18%) (esp. schizophrenia, bipolar)
  - Congenital heart disease (75%)
  - Cleft palate (9%)/velopharyngeal insufficiency (32%)
  - Renal tract anomalies (36%)
  - Hypocalcaemia (60%)
  - Reduced T-cell number and function
  - Dysmorphic

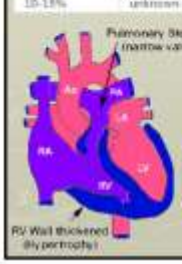
WILLIAMS syndrome

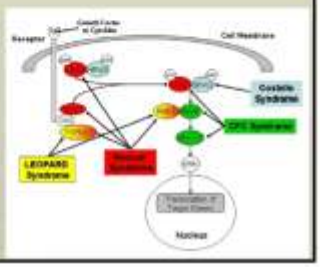
Single Gene Disorders

- **Dominant**
  - likely to be structural and variable in degree
- **Recessive**
  - often biochemical and tend to breed true
- **X-linked**
  - often detectable in female carriers

NOONAN syndrome

Noonan Syndrome Genetics	
Percent of Affected People	Gene
50%	PTPN11
20%	SOS1
10-15%	RAF1
5%	KRAS
10-15%	unknown





West Midlands Regional Genetic Service

CLINICAL DEPARTMENT
2 Professors
14 Consultants
6 SpRs
13 Generic GCs
8 Cancer GCs
2 GCs for Ethnic Minorities
2 Trainee GCs
5 Nurse Specialists in Genetic Disorders
(cardiac, renal, endocrine, haemoglobinopathy, NF1)

LABORATORIES
Molecular
Cytogenetic

ACADEMIC DEPARTMENT

Areas Covered - 5 million people

Referral Reasons

- Affected child/adult for investigation or diagnosis
- Family history of genetic disorder or condition with genetic component
- Fetal loss/abnormality
- Recurrent miscarriages
- Strong family history of cancer

Role of Clinical Geneticist

- Draw detailed pedigree
- Formulate +/- confirm diagnosis
- Information for patient
  - course of condition and treatment
- Establish risk to family members
  - cascade screening
- Discuss prenatal diagnosis options
  - CVS, amnio, PGD
- Educate professionals (hospital/community)

Genetic Testing

- Determine type and reason for test

a) *Diagnostic testing*
  - e.g. Noonan, Duchenne MD, Marfan, Down

b) *Predictive testing*
  - e.g. Huntington, Charcot-Marie-Tooth

c) *Carrier testing*
  - e.g. CF, Fragile X, DMD

d) *Prenatal testing*

Cost of Genetic Test (£)

Karyotype	140
FISH	140
Cystic Fibrosis	160
DMD	230
Achondroplasia	115
Fragile X	150-300
NF1	1200
Marfan	1000
FBC	<5

Blood Bottles

- **Chromosomal**
  - *Lithium Heparin*

- **DNA**
  - *EDTA*

Genetic Testing in Children

For single gene disorders-

- ***Only if:***
  - condition occurs in childhood
  - there is treatment that can be offered
- ***Not for:***
  - carrier testing (as no effect on health)
  - adult onset conditions

BUT always happy to DISCUSS with children

RESOURCES

NHS National Genetics Education and Development Centre — http://www.geneticseducation.nhs.uk

Telling Stories Understanding Real Life Genetics — http://www.geneticseducation.nhs.uk/tellingstories

OMIM Online Mendelian Inheritance in Man — http://www.ncbi.nlm.nih.gov/sites/entrez?db=OMIM

NHS — http://www.library.nhs.uk/genegoo/
http://www.library.nhs.uk/geneticconditions

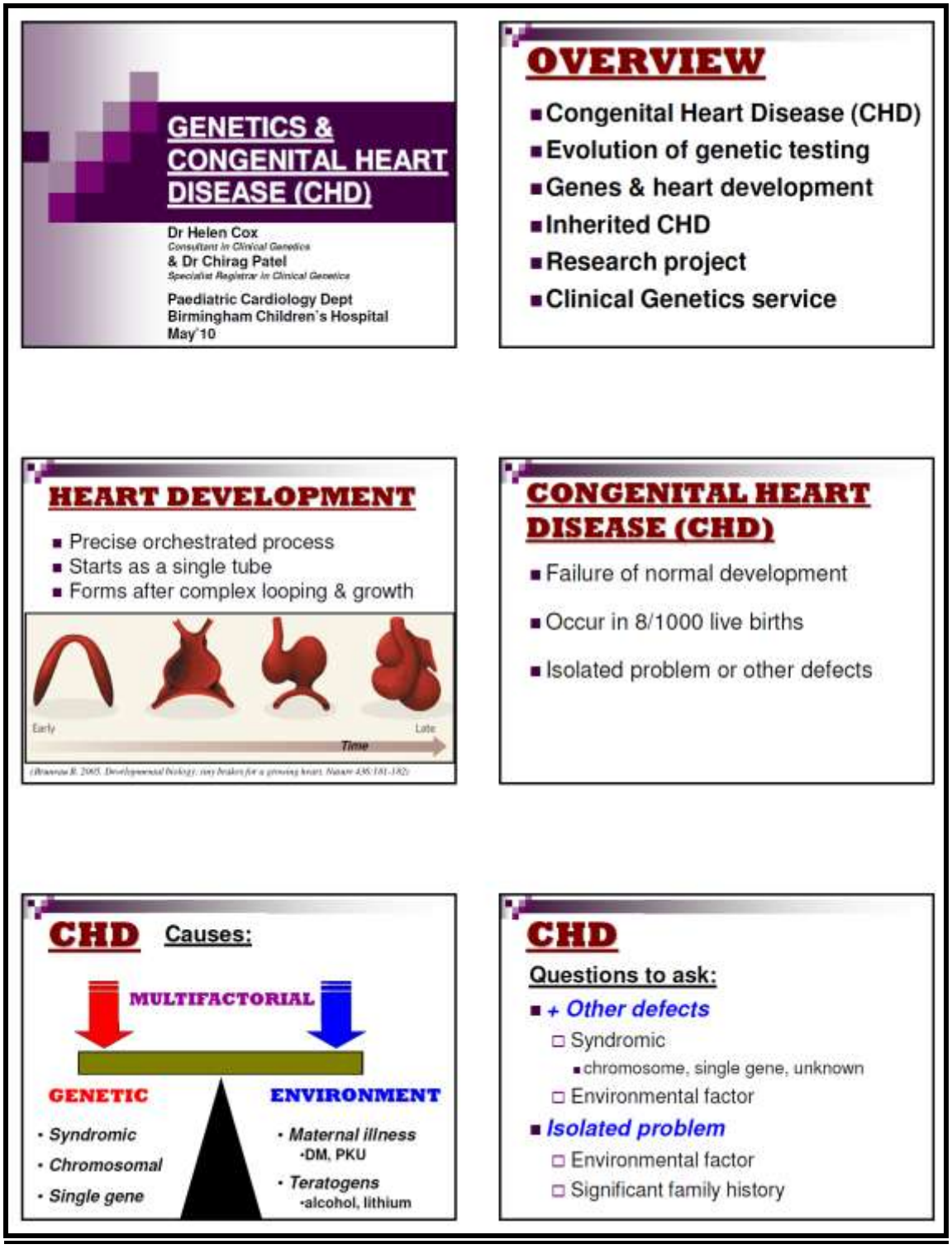
Unique ...but not alone — http://www.rarechromo.org

contact a family for families with disabled children — http://www.cafamily.org.uk

SUMMARY OF TOPICS

- **Basic genetics**
- **Pedigree drawing**
- **Inheritance patterns**
  - dominant, recessive, x-linked
- **Genetic conditions**
  - Chromosomal
  - Single gene
- **Clinical Genetics Service**
- **Genetic testing**

D) TEACHING PRESENTATION:
Paediatric Cardiology Department,
Birmingham Children's Hospital, May 2010



Making or Breaking the Heart: From Lineage Determination to Morphogenesis

Deepak Srivastava*

Cell 126, September 22, 2006 ©

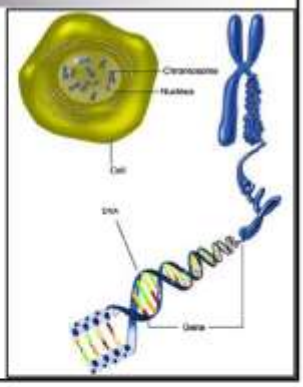
Table 1. Genetic Causes of Congenital Heart Disease

Genetic Mutation	Syndrome Name	Cardiac Disease
Nonsyndromic		
NKX2-5	—	Atrial septal defect, ventricular septal defect, electrical conduction defect
GATA4	—	Atrial septal defect, ventricular septal defect
MYH6	—	Atrial septal defect
NOTCH1	—	Aortic valve disease
Syndromic		
TBX5	Holt-Oram	Atrial septal defect, ventricular septal defect, electrical conduction defect
TBX1	DiGeorge	Cardiac outflow tract defect
TFAP2B	Char	Patent ductus arteriosus
JAG1	Alagille	Pulmonary artery stenosis, tetralogy of Fallot
PTPN11	Noonan	Pulmonary valve stenosis
Elastin	Williams	Supravalvar aortic stenosis
Fibrillin	Marfan	Aortic aneurysm

BASICS

Genes are arranged one after the other along the DNA of a chromosome, with stretches of non-coding DNA in between them.

Each chromosome has hundreds of genes along its DNA.

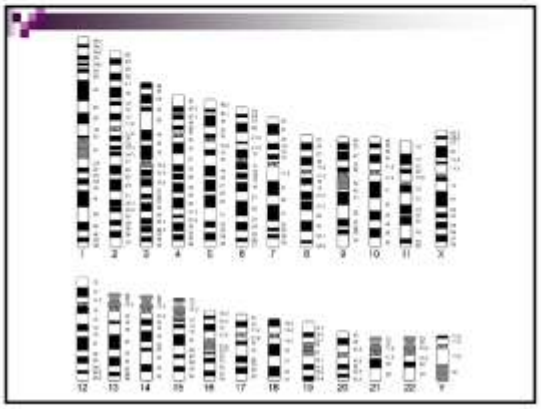


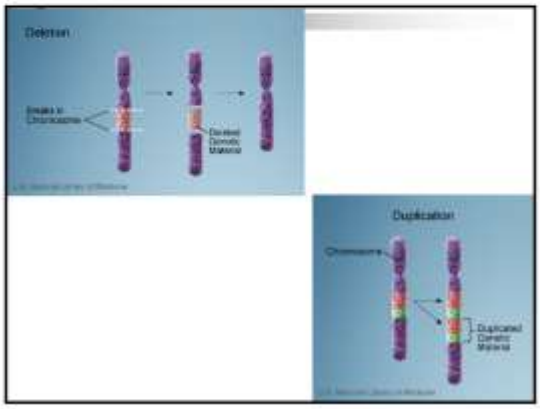
a) CHROMOSOMAL DISORDERS

- IUGR
- Postnatal growth retardation
- Developmental delay
- Dysmorphic features
- Multiple organ system anomalies
- Behaviour pattern (e.g. hand flapping/writhing)

CHROMOSOMAL ABNORMALITIES

- Polyploidy (e.g. triploidy = 69,XXX)
- Autosomal trisomies 21, 18, 13
- Sex chromosome abnormalities
- Mosaicism
- Deletions / duplications
- Inversions / ring
- Translocations



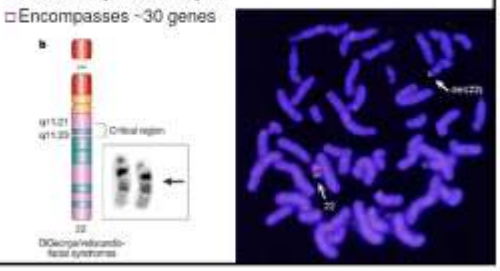


MICRODELETION SYNDROMES

- DiGeorge syndrome 22q11
- Williams syndrome 7q11.2
- 1p36 syndome 1p36

22q11.2 Deletion Syndrome

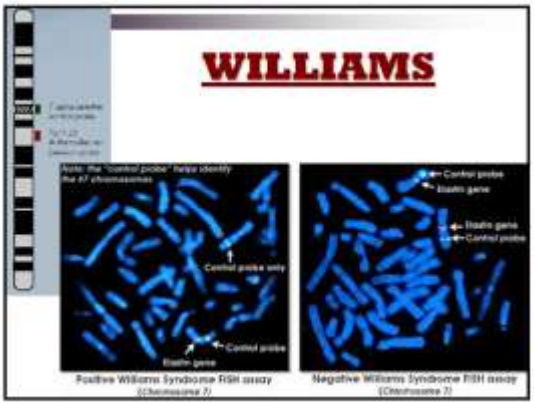
- Genetics:
  - Microdeletion (de novo - 94%, inherited - 6%)
  - Detected by FISH analysis
  - Encompasses ~30 genes



22q11.2 Deletion Syndrome

- Clinical Features:
  - Growth retardation (83%)
  - Learning difficulties (68%) (esp. maths and reading)
  - Speech delay/communication problems (90%)
  - Psychiatric disorders (18%) (esp. schizophrenia, bipolar)
  - Congenital heart disease (75%)
  - Cleft palate (9%)/velopharyngeal insufficiency (32%)
  - Renal tract anomalies (36%)
  - Hypocalcaemia (60%)
  - Reduced T-cell number and function
  - Dysmorphic

WILLIAMS

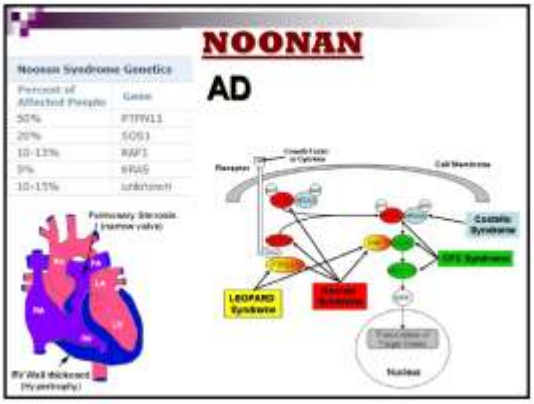


c) SINGLE GENE SYNDROMES

- *Dominant*
  - Noonan, Marfan, Holt-Oram, Alagille
- *Recessive*
  - Smith-Lemli-Opitz, Ivemark, Fanconi anamia
- *X-linked*
  - Opitz-G, X-linked laterality

NOONAN

AD



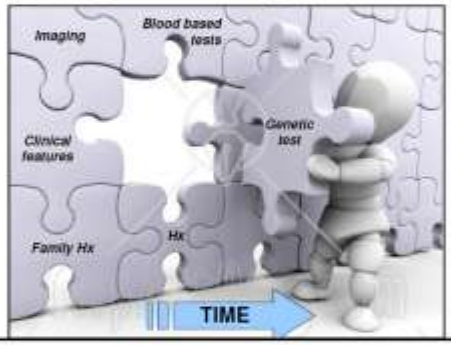
d) ENVIRONMENTAL DISORDERS

- Pregnancy history may give clues
- Maternal factors
  - Illnesses (e.g. diabetes)
  - Infections (e.g. rubella)
  - Medication (e.g. antiepileptics, lithium)
  - Alcohol
- Recurrence risk may be significant

e) ASSOCIATION DISORDERS

- **VATER/VACTERL**
  - Vertebral anomalies
  - Anal atresia
  - Cardiac defects
  - Tracheo-Esophageal fistula
  - Radial and Renal anomalies
  - Limb defects
- **CHARGE** (CHD7 gene)
  - Coloboma
  - Heart defect
  - Atresia of choanae
  - Retardation of growth
  - Genitourinary anomalies
  - Ear anomalies

Diagnosis of Syndromes



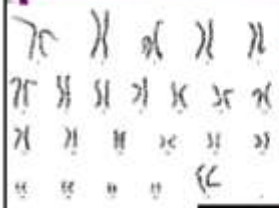
Type of Genetic Tests
past-present-future

a) ***Cytogenetic***
- e.g. karyotype, FISH

b) ***Molecular cytogenetic***
- e.g. array CGH

c) ***Molecular***
- e.g. sequencing, gene mapping

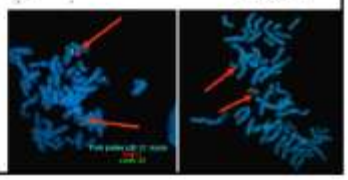
CYTOGENETIC



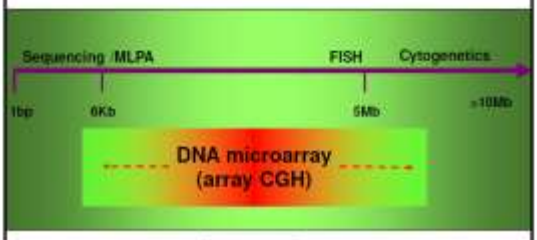
(pick up rate ~5-10%)

1980's FISH

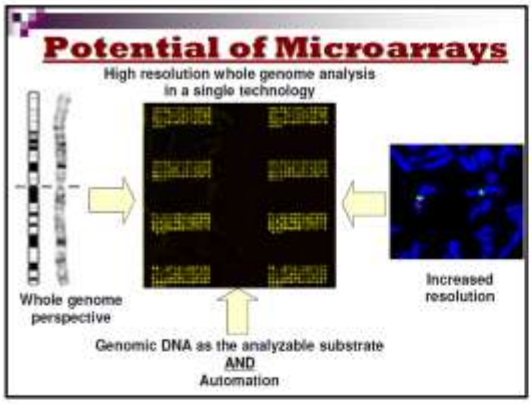
1960's banding techniques

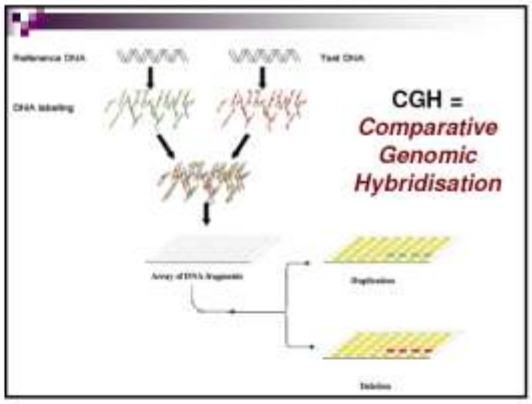


Microarrays and Resolution

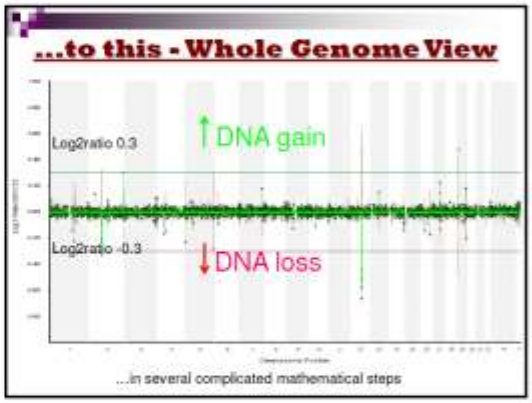


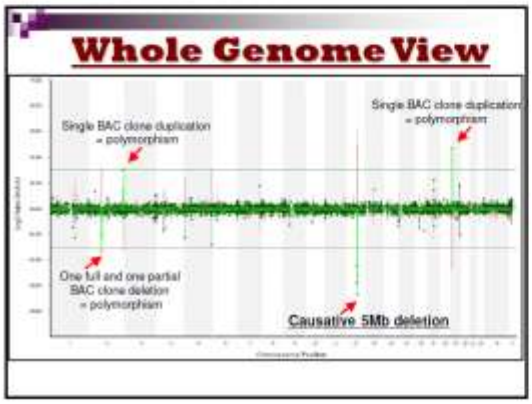
Late 1990's

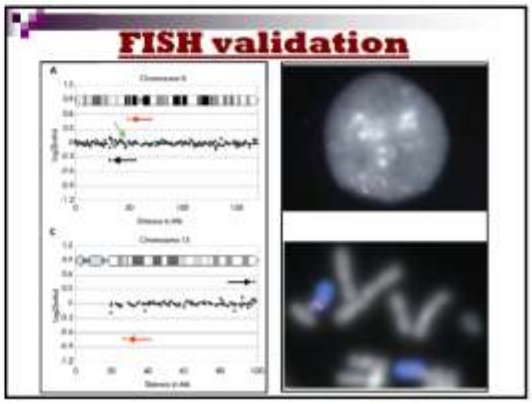

Potential of Microarrays
High resolution whole genome analysis
in a single technology
Whole genome
perspective
Increased
resolution
Genomic DNA as the analyzable substrate
AND
Automation


CGH =
Comparative
Genomic
Hybridisation

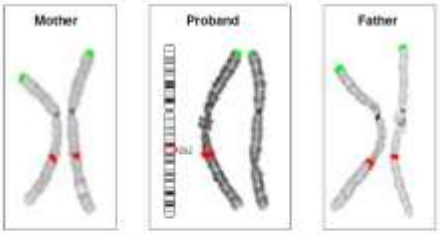

From this...
Raw image


...to this - Whole Genome View
Log2ratio 0.3
DNA gain
Log2ratio -0.3
DNA loss
...in several complicated mathematical steps


Whole Genome View
Single BAC clone duplication
= polymorphism
Single BAC clone duplication
= polymorphism
One full and one partial
BAC clone deletion
= polymorphism
Causative 5Mb deletion


FISH validation

FISH validation

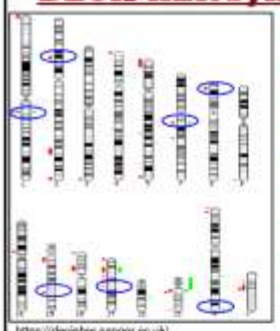


Deletion confirmed
Parents normal (position and copy number)
De novo pathogenic deletion

DATABASE validation

DECIPHER Compilation

DECIPHER Syndromes 2008

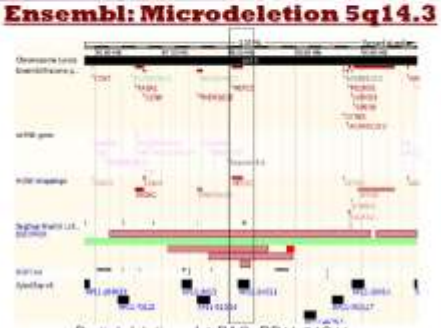


New DECIPHER syndromes:

- 1q21.1 del / dup
- 1q21.1 (TAR) del
- 2p15 del
- 7q11.23 (WBCR) dup
- 8p23.1 del
- 15q24 del
- 17q21.3 del/dup
- Xq28 (MECP2) dup

➡ 'REVERSE DYSMORPHOLOGY'

Ensembl: Microdeletion 5q14.3



Partial deletion of 1 BAC: RP11-24O11
High resolution array: 160kb deleting MEF2C

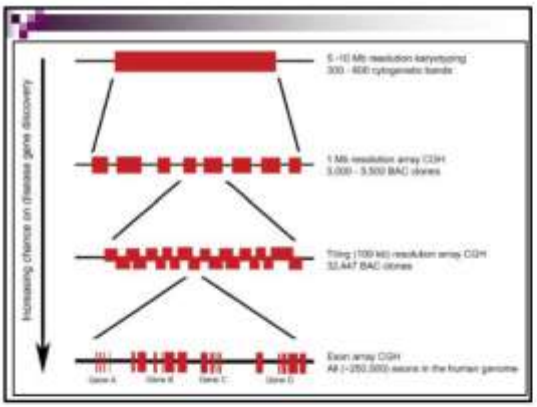
Microdeletion 5q14.3 & MEF2C

JMG

MEF2C haploinsufficiency caused either by microdeletion of the 5q14.3 region or mutation is responsible for severe mental retardation with stereotypic movements, epilepsy and/or cerebral malformations

Nathalie Le Meur, Muriel Holder-Espinasse, Sylvie Jaillard, et al.

J Med Genet published online July 9, 2009
doi: 10.1136/jmg.2009.069732



Array CGH: A Clinical Tool

ORIGINAL ARTICLE

Microarray based comparative genomic hybridisation (array-CGH) detects submicroscopic chromosomal deletions and duplications in patients with learning disability/mental retardation and dysmorphic features

C Shaw-Smith*, B Redon*, L Rickman*, M Rio, L Willatt, H Fiegler, H Firth, D Sanlaville, R Winter, L Colleaux, M Bobrow, N P Carter

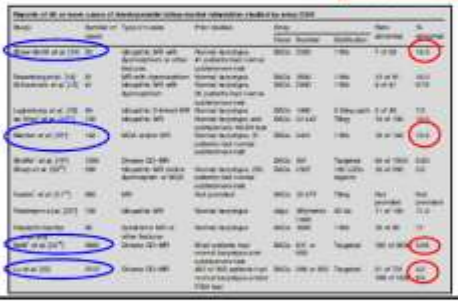
- Landmark report: array CGH as a clinically essential tool
- ~20% abnormality rate in ***select*** patients
- 2003: Genetics White Paper: 'Our Inheritance, Our Future'
- → funding for array CGH implementation in NHS

Use of array CGH in the evaluation of dysmorphology, malformations, developmental delay, and idiopathic mental retardation

Current Opinion in Genetics & Development 2007, 17:182–192

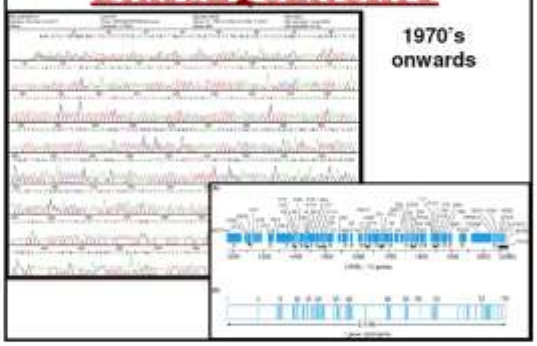
Pawel Stankiewicz[1,2] and Arthur L Beaudet[1]

(pick up rate ~15-25%)



DNA SEQUENCING

1970's onwards



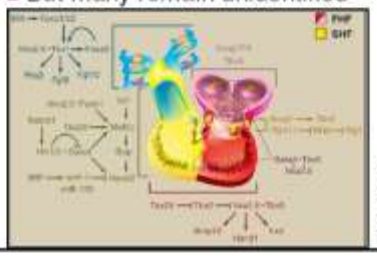
Chromosome → Single gene

- 22q11 → TBX1
- Williams → Elastin
- 9q34 → EHMT1
- Small % cases found to have mutations in genes instead of microdeletions!

HEART DEVELOPMENT

Genes:

- Many genes coordinate the process
- But many remain unidentified



(Srivastava D. 2006. Making or breaking the heart: from lineage determination to morphogenesis. Cell 126:1037-1048.)

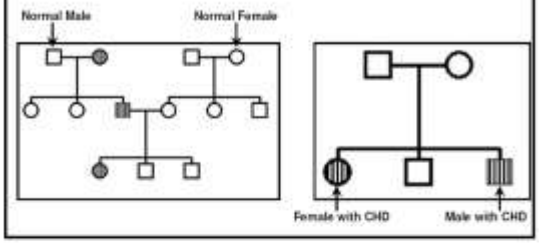
CHD

Inherited CHD:

- Improvements in the detection and management of CHD (clinical and surgical) → increased numbers of individuals surviving into adulthood
- Some of these individuals are now having children with CHD (parent-child)
- There are increasing numbers of families with multiple affected individuals with CHD of unknown cause (brothers/sisters).
- Having >1 close relative (with CHD) in a family may suggest a genetic cause

CHD

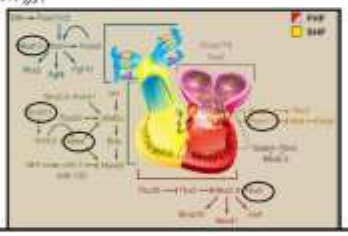
Inherited CHD examples:



CHD

Known genetic causes:

- Some genes found to be altered in some families with inherited CHD
- Currently difficult to test all genes in every family (limited technology)



Molecular mechanisms of congenital heart disease

Huang Jing-bin[a], Liu Ying-long[1,b], Sun Pei-wu[b], Lv Xiao-dong[c], Du Ming[a], Fan Xiang-ming[a]

Cardiovascular Pathology xx (2009)

Familial recurrence of congenital heart disease: an overview and review of the literature

Eur J Pediatr (2007) 166:111–116

GENETIC BASIS OF CHD
New Research Project

- Started November 2009
- Based at:
  - *University of Birmingham*
  - *Clinical Genetics department (BWH)*
- Funded by Birmingham Children's Hospital Charities (1 yr)

GENETIC BASIS OF CHD
New Research Project

Aim:

- Identify new genes causing CHD

Methods:

- Identify suitable families with CHD
- Obtain blood samples from individuals within the family
- Perform new genetic tests to identify genes and alterations within those genes

GENETIC BASIS OF CHD
New Research Project

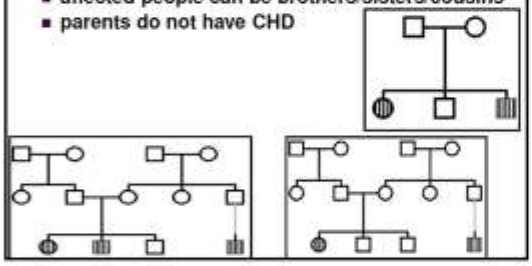
Benefit to patients/clinicians:

- Insights into normal heart development
- New diagnostic tests
- Improved management and outcome
- New treatment strategies in future
- Accurate recurrence risks for families

SUITABLE FAMILIES

A)

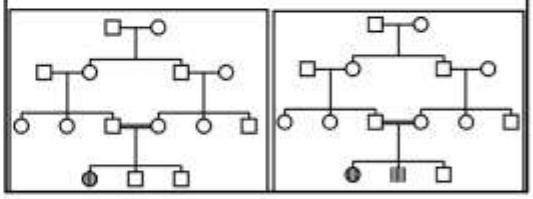
- 2 or more individuals in the family with CHD
- affected people can be brothers/sisters/cousins
- parents do not have CHD



SUITABLE FAMILIES

B)

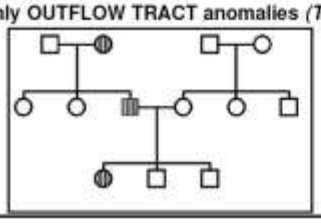
- 1 or more individuals in the family with CHD
- affected people can be brothers/sisters/cousins
- parents do not have CHD
- parents are blood relatives (e.g. cousins)



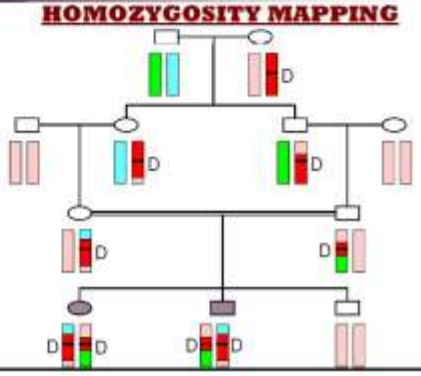
SUITABLE FAMILIES

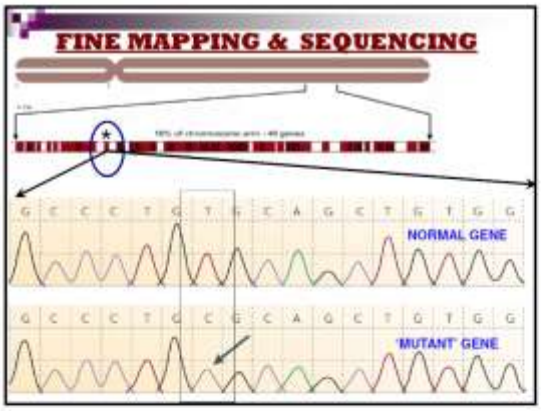
C)

- 2 or more individuals in the family with CHD
- Dominant inheritance pattern
- 1 parent has CHD
- Mainly OUTFLOW TRACT anomalies *(TOF, TGA)*



HOMOZYGOSITY MAPPING




FINE MAPPING & SEQUENCING
NORMAL GENE
'MUTANT' GENE


www.guch.org.uk

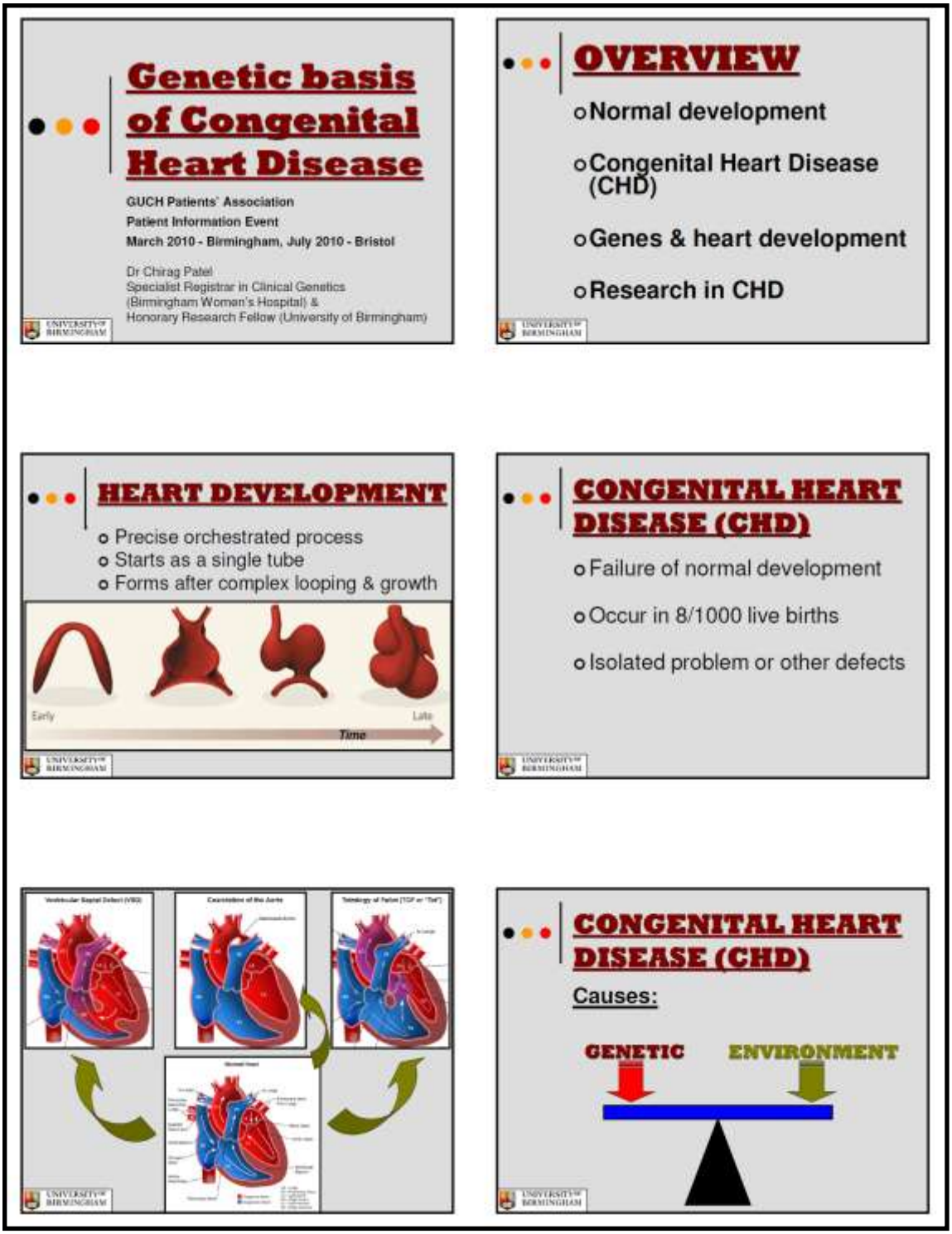

www.childrens-heart-fed.org.uk
Welcome


www.youngatheart.org.uk
YOUNG AT HE

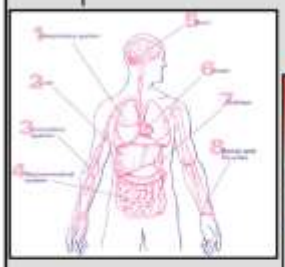

THANK YOU
Birmingham Children's Hospital Charities
Dr Helen Cox
Professor Eamonn Maher
Dr Sara Thorne (UHB)
Paediatric Cardiology Dept (BCH)
UNIVERSITY OF BIRMINGHAM
West Midlands Regional Clinical Genetics
West Midlands Regional Genetics Service
Birmingham Women's Health Care NHS
Birmingham Children's Hospital


QUESTIONS

E) SPOKEN PRESENTATION:
GUCH Patients' Association, Patient Information Event, Birmingham, March 2010 & Bristol, July 2010

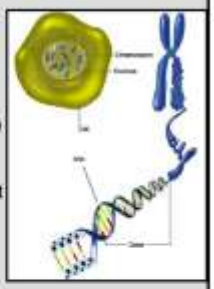


HUMAN BODY



DNA & GENES

- DNA carries our genetic information
- A gene is a distinct portion of DNA
- Genes (= 'instruction manuals') are packaged into structures called chromosomes
- Every cell in our body has a set of chromosomes
- There are ~23,000 genes in each cell of the body



HEART DEVELOPMENT

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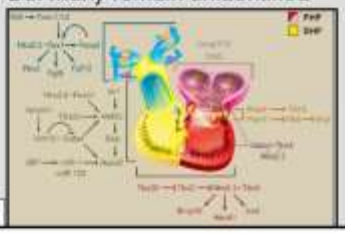

CONGENITAL HEART DISEASE (CHD)

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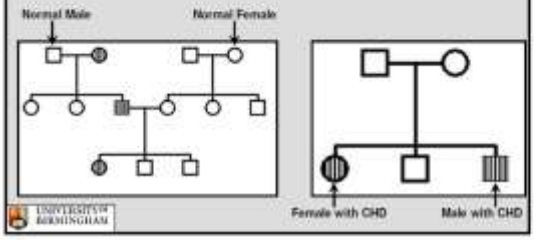
CONGENITAL HEART DISEASE (CHD)

Inherited CHD examples:



CONGENITAL HEART DISEASE (CHD)

Known genetic causes:

- Some genes found to be altered in some families with inherited CHD
- Currently difficult to test all genes in every family (limited technology)

GENETIC BASIS OF CHD

- New Research Project
- Started November 2009
- Based at University of Birmingham & Clinical Genetics department (Birmingham Women's Hospital)
- Funded by Birmingham Children's Hospital Charities

GENETIC BASIS OF CHD

Aim:

- Identify new genes causing CHD

Methods:

- Identify suitable families with CHD
- Obtain blood samples from individuals within the family
- Perform new genetic tests to identify genes and alterations within those genes

GENETIC BASIS OF CHD

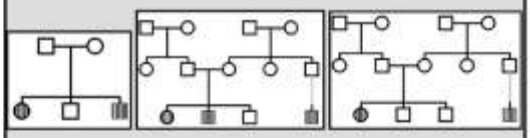
Benefit to patients/clinicians:

- Insights into normal heart development
- New diagnostic tests
- Improved management and outcome
- New treatment strategies in future
- Accurate recurrence risks for families

SUITABLE FAMILIES

A)

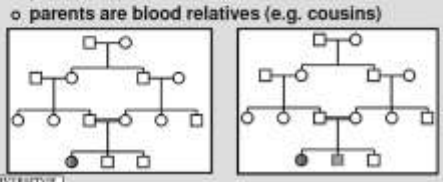
- 2 or more individuals in the family with CHD
- affected people can be brothers/sisters/cousins
- parents do not have CHD



SUITABLE FAMILIES

B)

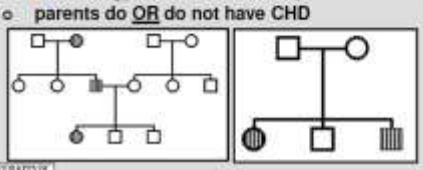
- 1 or more individuals in the family with CHD
- affected people can be brothers/sisters/cousins
- parents do not have CHD
- parents are blood relatives (e.g. cousins)



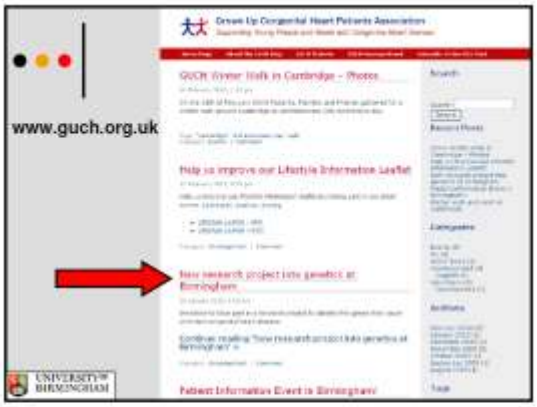
SUITABLE FAMILIES

C)

- 2 or more individuals in the family with outflow tract CHD anomalies
  - Transposition of the Great Arteries
  - Tetralogy of Fallot or on that spectrum
- parents do OR do not have CHD



www.guch.org.uk



THANK YOU

- GUCH Patients' Association
- Birmingham Children's Hospital Charities
- Professor Eamonn Maher
- Dr Helen Cox
- Dr Sara Thorne (UHB)
- Paediatric Cardiology Dept (BCH)

CONTACT DETAILS

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Email: c.patel@bham.ac.uk

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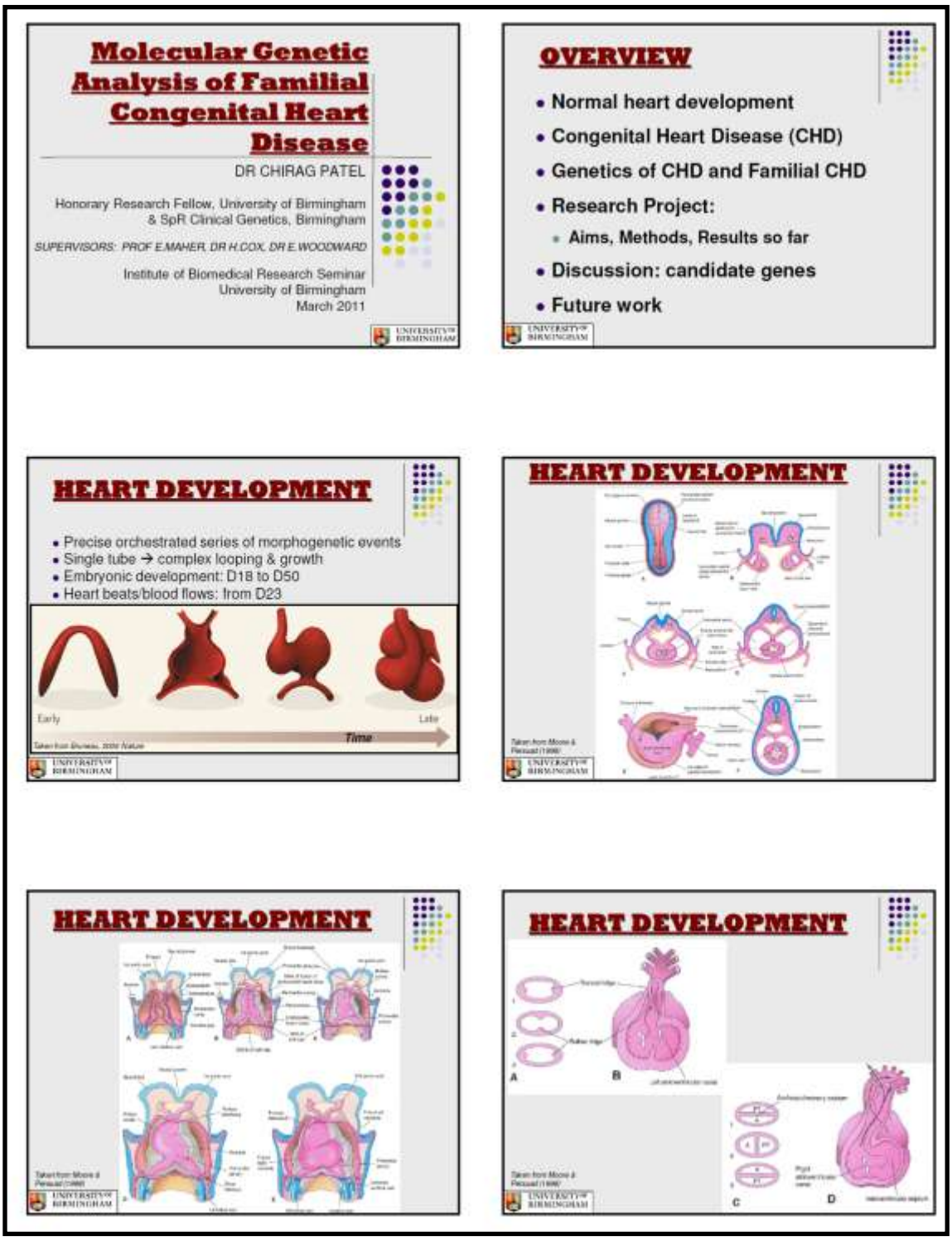
Norton Court
Birmingham Women's Hospital
Edgbaston
Birmingham
B15 2TG

F) SPOKEN PRESENTATION:
Institute of Biomedical Research Seminar, University of Birmingham, March 2011

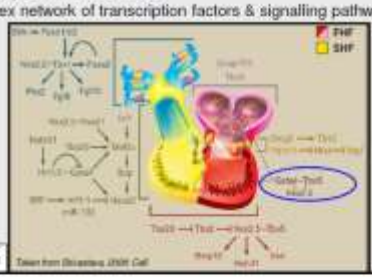


HEART DEVELOPMENT

- Cardiac progenitor cells contribute spatially (regulated pattern)
- FHF → heart tube, left ventricle
- SHF → outflow tracts, right ventricle, atria
- CNC → outflow tract septation, aortic arch arteries

HEART DEVELOPMENT GENES

- Molecular aspects still poorly understood
- Animal models have identified numerous genes,
- But many remain unidentified
- Complex network of transcription factors & signalling pathways



CONGENITAL HEART DISEASE (CHD)

- Gross structural abnormality of the heart of functional significance
- Occurs in 8/1000 live births
- Leading cause of neonatal/infant mortality worldwide
- Now >85% survive to adulthood (no. ↑by 5%/year)
- Classified by anatomy / physiology / both
- Sporadic / Familial
- Non-syndromic (isolated) / Syndromic (+ anomalies)

CHD: AETIOLOGY

MULTIFACTORIAL

GENETIC
- *Syndromic*
- *Chromosomal*
- *Single gene*

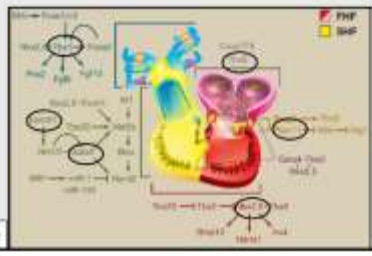
ENVIRONMENT
- *Maternal illness*
  - DM, PKU, infections
- *Teratogens*
  - alcohol, lithium, anticonvulsants

KNOWN GENETIC CAUSES HUMANS

Genetic Mutation	Syndrome Name	Cardiac Disease
Nonsyndromic		
NKX2-5	–	Atrial septal defect, ventricular septal defect, electrical conduction defect
GATA4	–	Atrial septal defect, ventricular septal defect
MYH6	–	Atrial septal defect
NOTCH1	–	Aortic valve disease
Syndromic		
TBX5	Holt-Oram	Atrial septal defect, ventricular septal defect, electrical conduction defect
TBX1	DiGeorge	Cardiac outflow tract defect
TFAP2B	Char	Patent ductus arteriosus
JAG1	Alagille	Pulmonary artery stenosis, tetralogy of Fallot
PTPN11	Noonan	Pulmonary valve stenosis
Elastin	Williams	Supravalvar aortic stenosis
Fibrillin	Marfan	Aortic aneurysm

KNOWN GENETIC CAUSES HUMANS

- Mutations in some genes found in some sporadic & familial cases
- Currently difficult to test all genes in every case (limited technology/funding)



FAMILIAL CHD

- Improvements in the detection and management of CHD (clinical and surgical)
  - → increased numbers of individuals surviving into adulthood
- Some CHD individuals are now having children with CHD (parent-child)
- ↑ No. families with multiple affected individuals with CHD of unknown cause (brothers/sisters/cousins)
- >1 close relative (with CHD) in a family
  - may suggest a genetic cause

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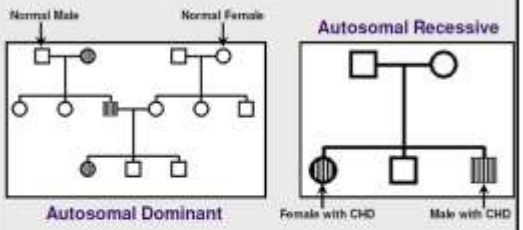
FAMILIAL CHD



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CONSANGUINITY & CHD

- Many population studies shown higher proportion of consanguinity in CHD cases

Congenital cardiac disease and inbreeding: specific defects escape higher risk due to parental consanguinity

Consanguinity and Congenital Heart Disease in Saudi Arabia

Parental Consanguinity and Congenital Heart Malformations in a Developing Country

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RESEARCH PROJECT

Aim:

- Identify new genes causing (familial) CHD (non-syndromic)

Benefit to patients/clinicians:

- Insights into normal heart development
- New diagnostic tests
- Improved management and outcome
- New treatment strategies in future
- Accurate recurrence risks for families

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RESEARCH PROJECT

Methods:

Identify & recruit cases of familial CHD

Identification:

- West Midlands Clinical Genetics Dept
- Birmingham Children's Hospital Cardiology Dept
- Patient support websites (UK)

Recruitment:

- Home visits, info re: research, consent
- Clinical evaluation and family history
- DNA samples (blood/saliva)
  - affected and unaffected individuals within the family

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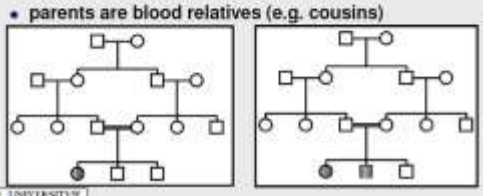
RESEARCH PROJECT

Methods:

Suitable Families

A)

- **1 or more individuals in the family with CHD**
- **affected people can be brothers/sisters/cousins**
- **parents do not have CHD**
- **parents are blood relatives (e.g. cousins)**



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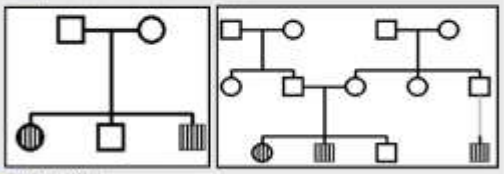
RESEARCH PROJECT

Methods:
Suitable Families

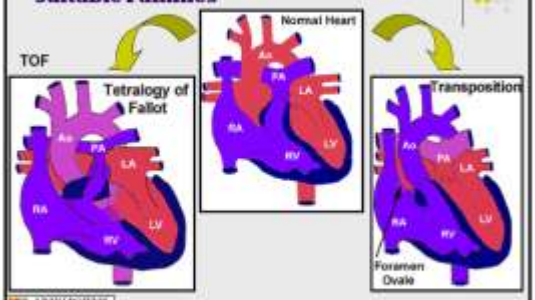
B)

- 2 or more individuals in the family with CHD
- affected people can be brothers/sisters/cousins
- parents do not have CHD



RESEARCH PROJECT

Methods:
Suitable Families

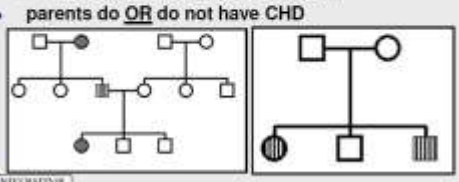


RESEARCH PROJECT

Methods:
Suitable Families

C)

- 2 or more individuals in the family with outflow tract CHD anomalies
  - Transposition of the Great Arteries
  - Tetralogy of Fallot or on that spectrum
- parents do OR do not have CHD



RESEARCH PROJECT

Results:
Families recruited

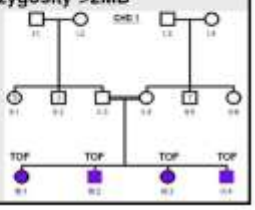
Overview:

- 22 families
- AR: 4 (consang) & 13 (non-consang), AD: 5
- Samples from 41 affected individuals (+ family members)
- Types of CHD:
  - Septal defects (24), PDA (1),
  - TOF (11), TGA (10), PS (2), AS (2),
  - CoA (7), BAV (6), HLHS (9), Tricuspid Atresia (1)
  - TAPVD (2), Ebstein's anomaly (1), RV hypoplasia (4),
  - Laterality defects (2), undetermined/complex CHD (4)
- Concordance: 15 families

RESEARCH PROJECT

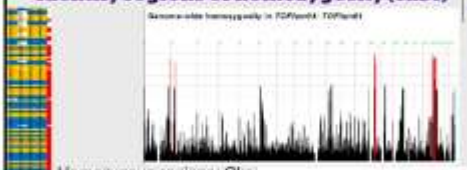
Methods:
Autozygosity Mapping in consanguineous families

- **Affymetrix SNP 5.0 array**
  - Genotyping for >500K single nucleotide polymorphisms
  - Performed in 2 consanguineous families (CHD1 & 4)
- **Identify regions of homozygosity >2MB**
  - Homozygosity mapper
  - Visually (excel)
- **Microsatellite markers**
  - Detailed genotyping
  - Exclude candidate regions
  - Narrow candidate regions



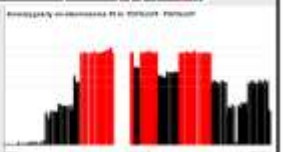
RESEARCH PROJECT

Results:
Identify regions of homozygosity (CHD1)



Homozygous regions: Chr

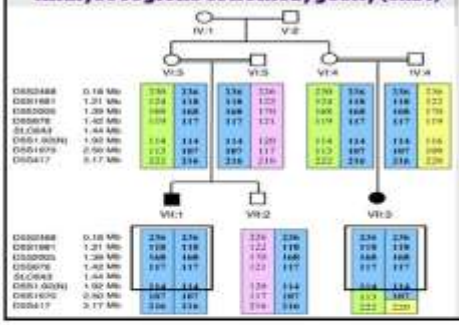
- 2 (2.4Mb)
- 10 (5.9Mb)
- 13 (13.1Mb)
- 16 (16.5Mb)
- 19 (28.9Mb)



RESEARCH PROJECT

Results:

Analyse regions of homozygosity (CHD1)



RESEARCH PROJECT

Methods:

Identify/analyse candidate genes (CHD1)

- 664 genes (Chr19, 28.9Mb)
- Database gene prioritisation:
  - *Ensembl*
  - *GeneDistiller*
  - *Suspects*
  - *UCSC Genome Bioinformatics*
  - *Endeavour*
  - *Mouse Genome Informatics*
- Sequenced candidate genes for mutations

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RESEARCH PROJECT

Results:

Candidate gene analysis (CHD1)

- ***GDF1* gene** (Growth differentiation factor 1)
  - Transforming growth factor beta superfamily (TGFβ)
  - *Pathway:* Nodal
  - *Function:* left-right axis development
  - *Mouse:* laterality defects & CHD
  - *Humans:* mutations in some sporadic cases CHD (TOF, TGA, DORV)

→**Sequencing: no pathogenic mutations found**

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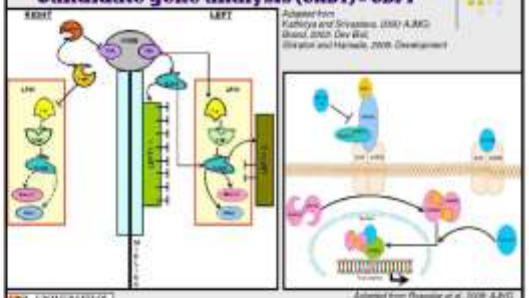
RESEARCH PROJECT

Results:

Candidate gene analysis (CHD1) – *GDF1*



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RESEARCH PROJECT

Additional Analyses:

- **Sequencing of select Nodal pathway genes**
  - *GDF1, NODAL, TDGF1, CFC1, FOXH1*
  - Families CHD: 1, 2, 5, 6, 7, 8, 9, 10, 12, 13
    - Outflow tract anomalies (TOF, TGA)

→ **No pathogenic mutations identified**

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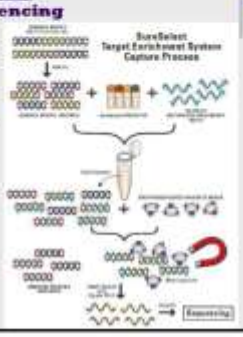
RESEARCH PROJECT

Additional Methods:

Whole exome sequencing

- 664 genes (Chr19, 28.9Mb)
- Collaboration with Beijing Genomics Institute (BGI)
- Families
  - CHD1 & CHD4



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RESEARCH PROJECT

Results:
Whole exome sequencing

Family CHD1:

- Whole Genome changes (61421):
  - *Novel changes* (10776)
    Nonsense (81), Splice site (124), Missense (2740)
    - *Homozygous*
      Nonsense (10), Splice site (8), Missense (223)
    - *Further database filters (1000 genomes/Guy's)*
      Nonsense (8), Splice site (2), Missense (93)
  - Candidate region Chr 19 (958):
    - *Novel/homozygous/DB filters*
      Nonsense (1), Missense (3)

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RESEARCH PROJECT

Results:
Whole exome sequencing

Family CHD1: novel homozygous changes

- **Nonsense (8), Splice site (2), Missense (93)**
  - *AMALUT*
    - Mutation diagnostics software, interprets mutations quickly/reliably, bringing together relevant molecular data and prediction methods.
  - *SIFT*
    - Predicts if AA substitution affects protein function based on sequence homology and the physical properties of AA.
  - *POLYPHEN*
    - Predicts impact of AA substitution on structure/function of protein using physical and comparative considerations.
  - *SPLICE SITE PREDICTION*
  - *GENE FUNCTION*
  - *MOUSE MODELS*

→ **Nonsense (8), Missense (12)**

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RESEARCH PROJECT

Results:
Whole exome sequencing

Family CHD1: novel homozygous pathogenic changes

- **Nonsense (8), Missense (12) → 2 selected for further analysis**
  - *GMFG (Nonsense)*
    - *Chr19 region from AZ mapping*
    - *Highly conserved nucleotide and amino acid*
    - *Not known in heart development ? vasculogenesis*
  - *WNT11 (Missense)*
    - *Predicted affect protein function (SIFT) & pathogenic (POLYPHEN)*
    - *Highly conserved nucleotide and amino acid*
    - *Linked to heart development in animal models*
- **Except: → 1 other selected for further analysis**
  - *DVL2 (Missense)*
    - *Tolerated protein function (SIFT) & not pathogenic (POLYPHEN)*
    - *Highly conserved nucleotide and amino acid*
    - *Linked to heart development in animal models*

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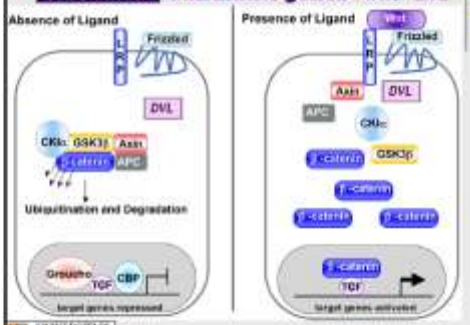
RESEARCH PROJECT

Discussion: Candidate genes – *WNT / DVL*



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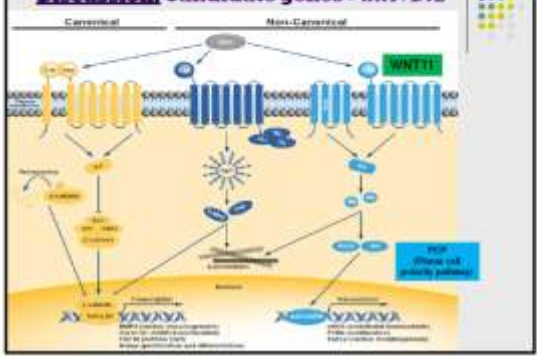
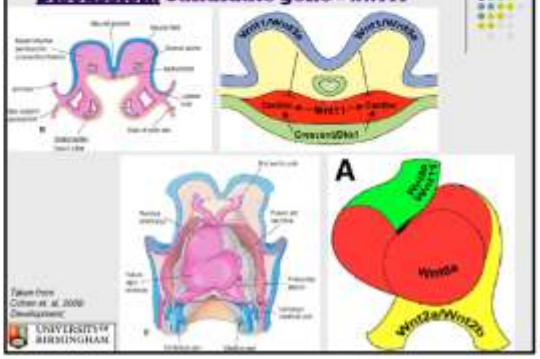
RESEARCH PROJECT

Discussion: Candidate genes – *WNT / DVL*



RESEARCH PROJECT

Discussion: Candidate gene – *WNT11*



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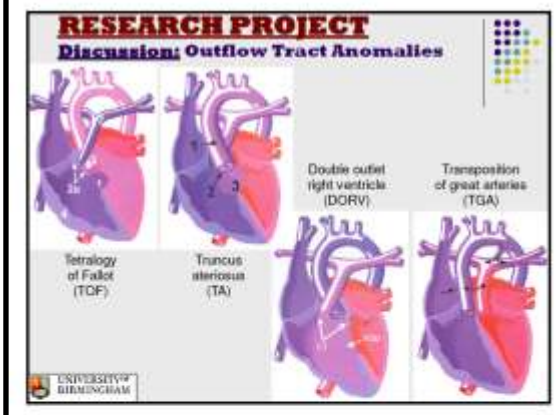
RESEARCH PROJECT
Discussion: Outflow Tract Anomalies
Double outlet right ventricle (DORV)
Transposition of great arteries (TGA)
Tetralogy of Fallot (TOF)
Truncus arteriosus (TA)
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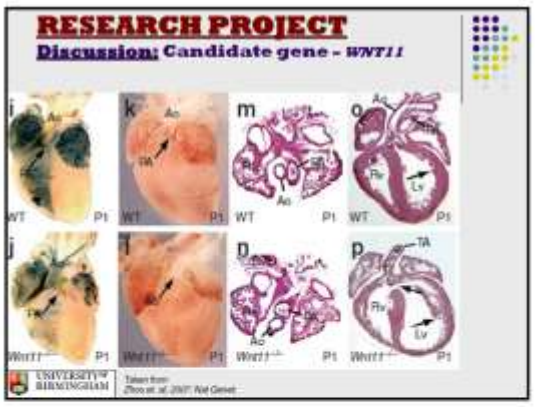
RESEARCH PROJECT
Discussion: Candidate gene - WNT11
WT
P1
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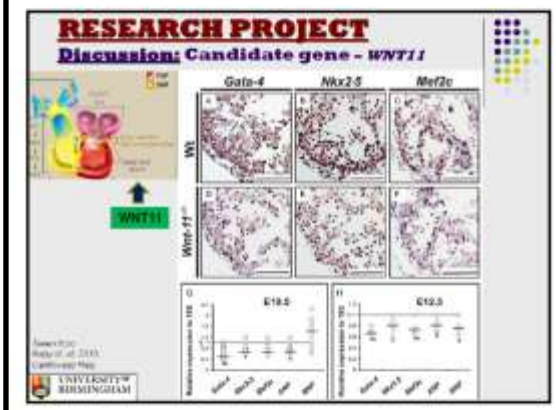
RESEARCH PROJECT
Discussion: Candidate gene - WNT11
Gata-4
Nkx2-5
Mef2c
WNT11
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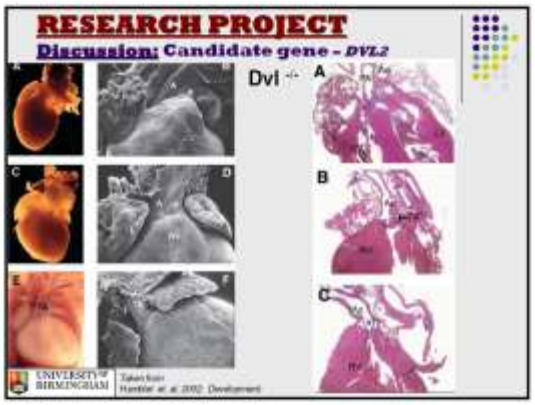
RESEARCH PROJECT
Discussion: Candidate gene - DVL2
Dvl -/-
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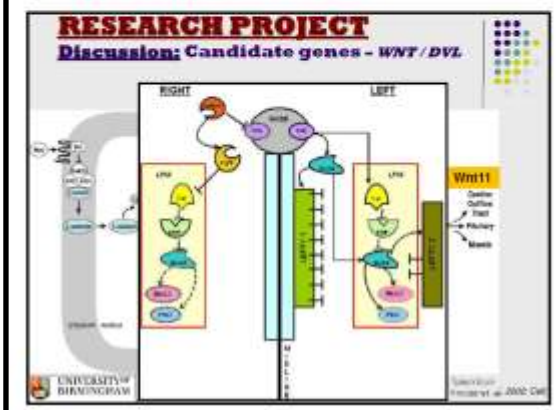
RESEARCH PROJECT
Discussion: Candidate genes - WNT / DVL
RIGHT
LEFT
Wnt11
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RESEARCH PROJECT
Discussion: Candidate gene - DVL2
WT
Pitx2(-/-)
Dvl2(-/-)
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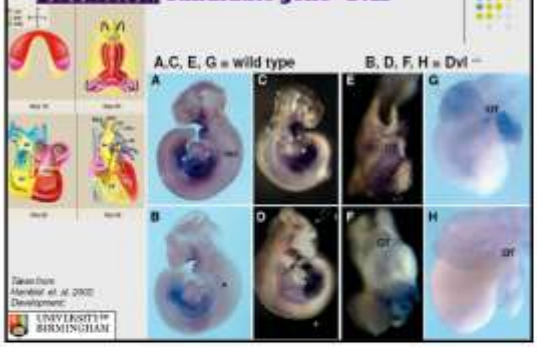
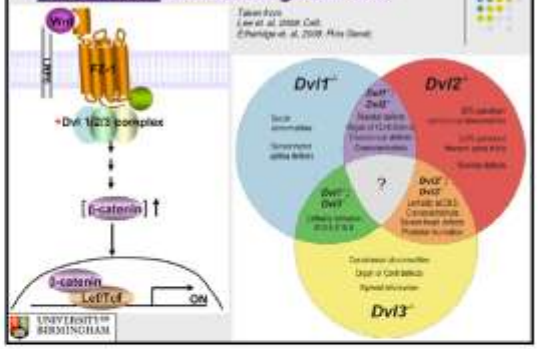
RESEARCH PROJECT

Discussion: Candidate gene – *DVL2*



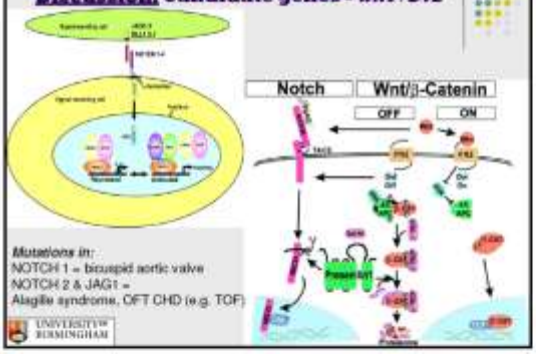
RESEARCH PROJECT

Discussion: Candidate gene – *DVL2*



RESEARCH PROJECT

Discussion: Candidate genes – *WNT / DVL*



Mutations in:
NOTCH 1 = bicuspid aortic valve
NOTCH 2 & JAG1 = Alagille syndrome, OFT CHD (e.g. TOF)

RESEARCH PROJECT

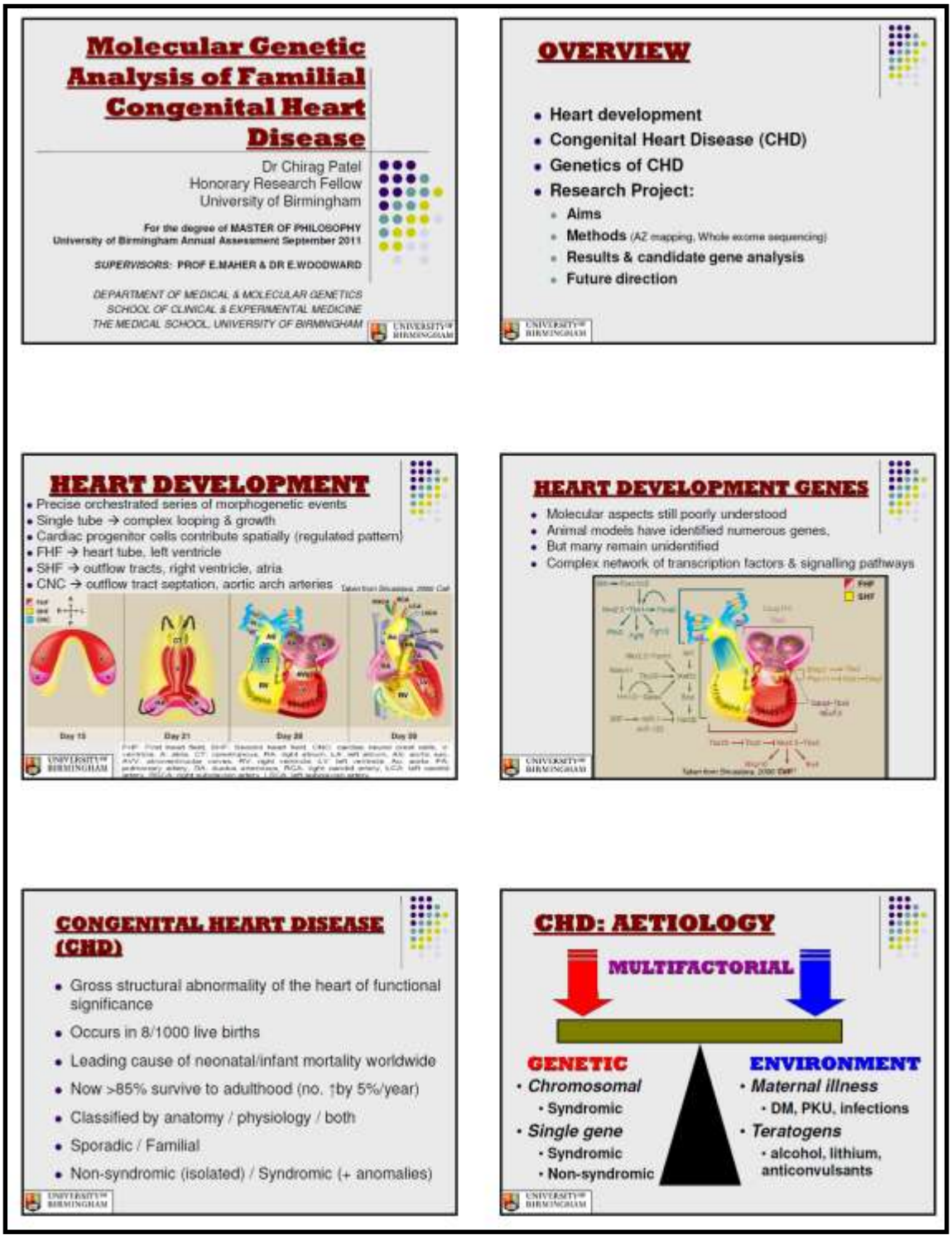
Future Work:

- Further analysis of WES results
  - Segregation analysis, ethnic matched controls
  - *GMFG, WNT11, DVL2*
- Whole exome sequencing of other AR families
  (in collaboration with Sanger Institute, Cambridge)
- Mutation screening of candidate genes
- Recruit more families into study
- Apply for more funding
- Functional analysis of variants

ACKNOWLEDGEMENTS

- Prof Eamonn Maher
- Dr Emma Woodward
- Dr Helen Cox
- Neil Morgan, Louise Tee
- Birmingham Children's Hospital Charity
- GUCH Patients' Association
- Children's Heart Federation
- Heartline
- Young at Heart
- All patients

G) SPOKEN PRESENTATION:
Annual Assessment Seminar,
University of Birmingham, September 2011



KNOWN GENETIC CAUSES HUMANS

Table 1. Genetic Causes of Congenital Heart Disease

Genetic Mutation	Syndrome Name	Cardiac Disease
Nonsyndromic		
MKK-6	–	Atrial septal defect, ventricular septal defect, electrical conduction defect
GATA4	–	Atrial septal defect, ventricular septal defect
MYH6	–	Atrial septal defect
NOTCH1	–	Aortic valve disease
Syndromic		
TBX5	Holt-Oram	Atrial septal defect, ventricular septal defect, electrical conduction defect
TBX1	DiGeorge	Cardiac outflow tract defect
TFAP2B	Char	Patent ductus arteriosus
JAG1	Alagille	Pulmonary artery stenosis, tetralogy of Fallot
PTPN11	Noonan	Pulmonary valve stenosis
Elastin	Williams	Supravalvar aortic stenosis
Fibrillin	Marfan	Aortic aneurysm

Taken from Srivastava, 2006 Cell

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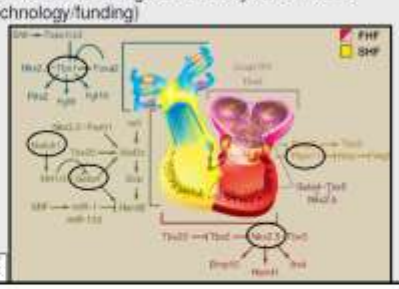
KNOWN GENETIC CAUSES HUMANS

- Mutations in some genes found in some sporadic & familial cases
- Currently difficult to test all genes in every case in NHS (limited technology/funding)



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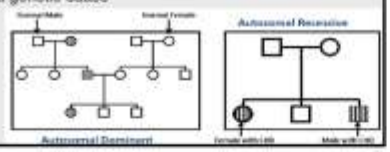
FAMILIAL CHD

- Improved detection /management (clinical/surgical)
  - → increased numbers of individuals surviving into adulthood
- Some CHD individuals now have children with CHD (parent-child)
- ↑ No. families with multiple affected individuals with CHD of unknown cause (brothers/sisters/cousins)
- >1 close relative (with CHD) in a family
  - may suggest a genetic cause



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CONSANGUINITY & CHD

- Many population studies have shown higher proportion of consanguinity in CHD cases

Congenital cardiac disease and inbreeding: specific defects escape higher risk due to parental consanguinity

Consanguinity and Congenital Heart Disease in Saudi Arabia

Parental Consanguinity and Congenital Heart Malformations in a Developing Country

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RESEARCH PROJECT

Aim:

- Identify new genes causing (familial) CHD (non-syndromic)

Benefit to patients/clinicians:

- Insights into normal heart development
- New diagnostic tests
- Improved management and outcome
- New treatment strategies in future
- Accurate recurrence risks for families

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RESEARCH PROJECT

Methods:
Identify & recruit cases of familial CHD

Identification:

- West Midlands Clinical Genetics Dept
- Birmingham Children's Hospital Cardiology Dept
- Patient support websites (UK)

Recruitment:

- Home visits, info re: research, consent
- Clinical evaluation and family history
- DNA samples (blood/saliva)
  - affected and unaffected individuals within the family

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RESEARCH PROJECT

Methods:
Suitable Families

- Consanguineous: AR inheritance
  - 1 or more individuals with CHD (sibs/cousins)
  - parents do not have CHD
- Non-Consanguineous: AR inheritance
  - 2 or more individuals with CHD (sibs)
  - parents do not have CHD
- AD inheritance:
  - 2 or more individuals with outflow tract CHD (e.g. TGA, TOF or on that spectrum)
  - parents do OR do not have CHD

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RESEARCH PROJECT

Results:
Families recruited

Overview:

- 23 families
- AR: 4 (consang) & 14 (non-consang), AD: 5
- Samples from 42 affected individuals (+ family members)
- Types of CHD:
  - Septal defects (21), PDA (1),
  - TOF (11), TGA (10), PS (5), AS (4),
  - CoA (7), BAV (6), HLHS (9),
  - TAPVD (2), Ebstein's anomaly (2), RV hypoplasia (4),
  - Laterality defects (2), undetermined/complex CHD (7)
- Concordance: 16 families (~70%)

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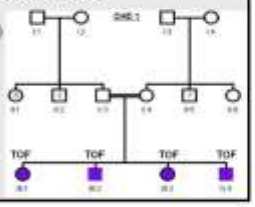
RESEARCH PROJECT

Methods/Results:
Autozygosity Mapping in consanguineous families

- **Affymetrix SNP 5.0 array**
  - Genotyping for >500K single nucleotide polymorphisms
  - Performed in 1 consanguineous families (CHD1)
- **Identify regions of homozygosity >2MB**
  - chr2 (2.4Mb), chr10 (5.9Mb)
  - chr13 (13.1Mb), chr16 (16.5Mb)
  - chr19 (28.9Mb)
- **Microsatellite markers**
  - Detailed genotyping
  - Exclude candidate regions
  - Narrow candidate regions



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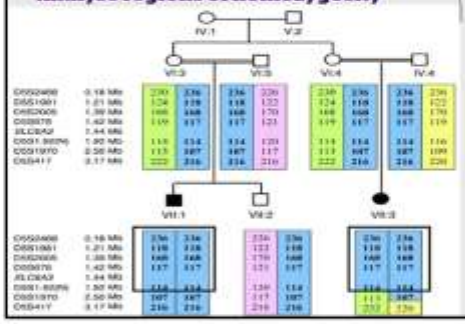
RESEARCH PROJECT

Results:
Analyse regions of homozygosity



RESEARCH PROJECT

Methods:
Identify/analyse candidate genes

- 664 genes (Chr19, 28.9Mb)
- Database gene prioritisation:
  - *Ensembl*
  - *GeneDistiller*
  - *Suspects*
  - *UCSC Genome Bioinformatics*
  - *Endeavour*
  - *Mouse Genome Informatics*
- Sequenced candidate genes for mutations

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RESEARCH PROJECT

Results:
Candidate gene analysis

- ***GDF1* gene** (Growth differentiation factor 1)
  - Transforming growth factor beta superfamily (TGFβ)
  - *Pathway:* Nodal
  - *Function:* left-right axis development
  - *Mouse:* laterality defects & CHD
  - *Humans:* mutations in some sporadic cases CHD (TOF, TGA, DORV)

→**Sequencing: no pathogenic mutations found**

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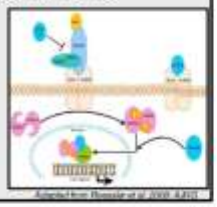
RESEARCH PROJECT

Results:
Additional Analyses

- **Sequencing of select Nodal pathway genes**
  - *GDF1, NODAL, TDGF1, CFC1, FOXH1*
  - Families CHD: 1, 2, 5, 6, 7, 8, 9, 10, 12, 13
    - Outflow tract anomalies (TOF, TGA)

→ **No pathogenic mutations identified**



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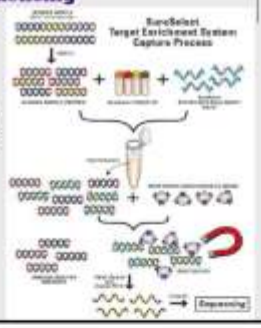
RESEARCH PROJECT

Additional Methods:
Whole exome sequencing

- CHD1: 664 genes (Chr19, 28.9Mb)
- Collaboration with Beijing Genomics Institute (BGI)
- 1 individual



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RESEARCH PROJECT

Results:
Whole exome sequencing

Family CHD1:

- Whole Genome changes (61421):
  - *Novel changes* (10776)
    Nonsense (81), Splice site (124), Missense (2740)
    - *Homozygous*
      Nonsense (10), Splice site (8), Missense (223)
    - *Further database filters (1000 genomes/Guy's)*
      Nonsense (8), Splice site (2), Missense (93) ←
  - Candidate region Chr 19 (959):
    - *Novel/homozygous/DB filters*
      Nonsense (1), Missense (3)

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RESEARCH PROJECT

Results:
Whole exome sequencing

Family CHD1: novel homozygous changes

- **Nonsense (8), Splice site (2), Missense (93)**
  - *AMALUT*
    - Mutation diagnostics software, interprets mutations quickly/reliably, bringing together relevant molecular data and prediction methods
  - *SIFT*
    - Predicts if AA substitution affects protein function based on sequence homology and the physical properties of AA
  - *POLYPHEN*
    - Predicts impact of AA substitution on structure/function of protein using physical and comparative considerations
  - *SPLICE SITE PREDICTION*
  - *GENE FUNCTION*
  - *MOUSE MODELS*

→ **Nonsense (8), Missense (12)** ← **3 genes selected**

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RESEARCH PROJECT

Results:
Whole exome sequencing

Family CHD1:

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RESEARCH PROJECT

Results:
Whole exome sequencing

Family CHD1: novel homozygous pathogenic changes

- **Nonsense (8), Missense (12) → 2 selected for further analysis**
  - *GMFG (Nonsense)*
    - *Chr19 region from AZ mapping*
    - *Highly conserved nucleotide and amino acid*
    - *Not known in heart development ? Vasculogenesis, no animal models*
  - *WNT11 (Missense)*
    - *Predicted affect protein function (SIFT) & pathogenic (POLYPHEN)*
    - *Highly conserved nucleotide and amino acid*
    - *Linked to heart development in animal models*
- **Except: → 1 other selected for further analysis**
  - *DVL2 (Missense)*
    - *Tolerated protein function (SIFT) & not pathogenic (POLYPHEN)*
    - *Highly conserved nucleotide and amino acid*
    - *Linked to heart development in animal models*

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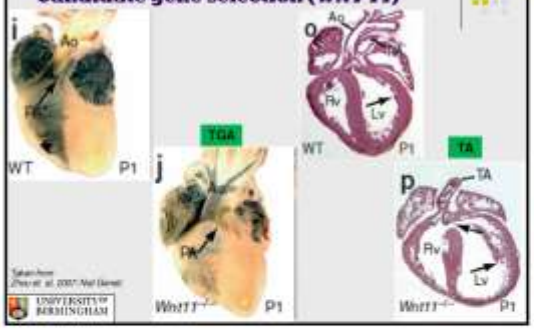
RESEARCH PROJECT

Results:

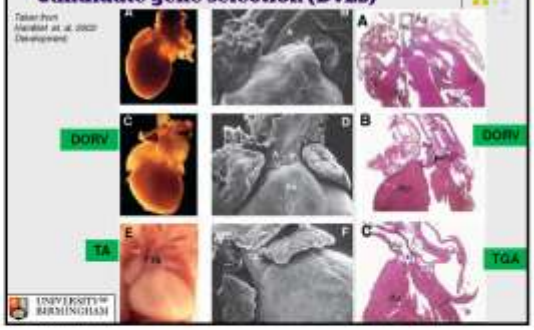
Candidate gene selection (*WNT 11*)



RESEARCH PROJECT

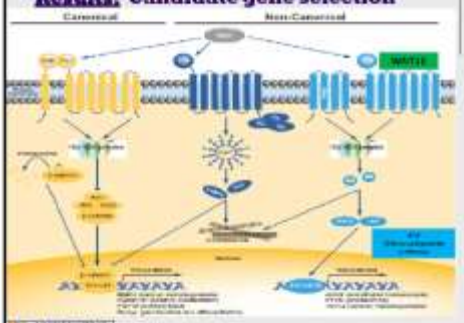
Results:

Candidate gene selection (*DVL2*)



RESEARCH PROJECT

Results: Candidate gene selection



RESEARCH PROJECT

Additional Analyses:

Sanger sequencing

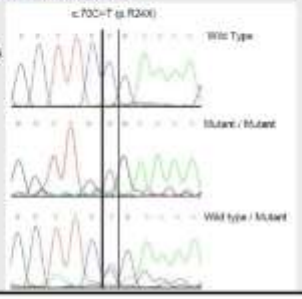
- Confirmation of variant in candidate genes by Sanger sequencing in affected
  - *DVL2* → *not confirmed (false +ve)*
    - c.152C>T, p.A51V
  - *WNT11* → *not confirmed (false +ve)*
    - c.519T>A, p.D173E
  - *GMFG* → *confirmed*
    - c.70C>T, p.R24X

RESEARCH PROJECT

Additional Analyses: *GMFG*

Segregation analysis

- variant segregates in family

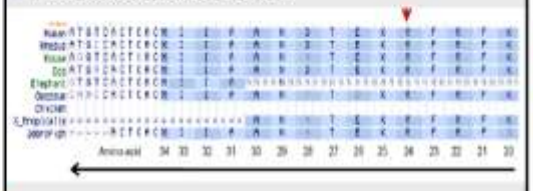


RESEARCH PROJECT

Additional Analyses: *GMFG*

Ethnic controls and conservation

- Variant absent >200 ethnic control chromosomes
- In highly conserved region



RESEARCH PROJECT
Ongoing Work:

WES of other AR CHD families

(in collaboration with Sanger Institute, Cambridge)

- Cons & non-cons (12 families)
  - CHD 1, 2, 4, 5, 6, 11, 13, 16, 17, 20, 22, 23
- Performed in all affecteds within a family (n=26)
- Results available for CHD 1, 2, 4, 5, 6, 11, 13, 16
- Currently analysing data
  - Identify variants shared between affecteds:
    - within a family
    - across families

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RESEARCH PROJECT
Ongoing Work:

FAMILY	no. AFFECTED	TOTAL VARIANTS SAME IN ALL AFFECTED NO FILTERS	TOTAL VARIANTS SAME IN ALL AFFECTED + FILTERS	HMZ VARIANTS	HTZ VARIANTS	HTZ VARIANTS >1 IN SAME GENE
1	3	26773	1845	82		
2	1	60357	6880	277		
4	2	28681	1435	116		
5	2	32666	1050	63	953	289
6	2	39020	3954	73	3863	1012
11	2	39416	3884	72	3751	844
13	2	34608	1115	73	997	260
16	3	31363	3230	78	3103	661

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RESEARCH PROJECT
Future Directions:

- New WES data analysis
- Further analysis of candidate genes
- *GMFG* further work
  - Analysis in familial and sporadic CHD
  - Functional studies
- Recruit more families into study
- Apply for more funding
- Functional analysis of variants

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ACKNOWLEDGEMENTS

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Birmingham Children's Hospital

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