

**ENHANCED DIABETES CARE TO PEOPLE OF
SOUTH ASIAN ETHNICITY
THE UNITED KINGDOM ASIAN DIABETES STUDY
(UKADS)**

A thesis submitted to
The University of Birmingham
for the degree of

DOCTOR OF MEDICINE

by

Dr. SRIKANTH BELLARY

Department of Medicine
The University of Birmingham
January 2010

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This work is dedicated to
Ambika and Saurav
my grandparents and parents
my teachers

Acknowledgements

I am greatly indebted to Prof. Anthony Barnett for his constant encouragement, guidance and inspiration without which this work would not have been possible.

I am grateful to Dr. M.A.Kelly for her guidance, support and patience in teaching me the challenging aspects of genetics of Diabetes and the help in preparing this thesis.

I would like to thank Dr. Neil Raymond, Warwick University for the statistical support, guidance and the many hours of discussions spent during the interpretation of the data.

To Shanaz Mughal and Kam Johal who worked tirelessly for the success of the UKAD study and assisting me with the collection of data.

To Dr Simon Rees, Dr Abigail Britten and Karen Jones for teaching me the laboratory techniques and helping me with the analysis of the genetics data.

To Prof.Sudhesh Kumar, Dr Paul O'Hare, Dr.Jayadave Shakher, Dr. Cathy Lloyd, Safia Begum, Tahmina Kauser ,Naheed Akhtar, and rest of the UKADS Team for their support from time to time.

To all the General Practitioners and the patients who participated in the UKADS trial.

To all the pharmaceutical industries and the Assurance Medical Society who generously supported this work.

Abstract

The United Kingdom Asian Diabetes Study (UKADS) is a large community based cluster randomised controlled trial designed to evaluate a culturally sensitive intervention to reduce cardiovascular risk in south Asians with type 2 diabetes. The study was conducted over a 2 year period and involved 21 General Practices in Coventry and Birmingham. Two major components of the UKADS trial –the clinical intervention (chapters 2,3 and 4) and the genetic characterization for type 2 diabetes susceptibility genes (chapters 5 to 8) are presented in this thesis.

Over a 2 year period there were significant improvements in mean arterial and diastolic blood pressures in the intervention group that included additional practice nurse time, asian link workers and specialist diabetes nurse input. The intervention, however, had no effect on total cholesterol or glycaemic control. Prescription of statins and anti-hypertensives increased significantly during the study period with a greater proportion of subjects in both groups achieving General Practice targets for blood pressure and cholesterol.

Genetics studies for association with type 2 diabetes showed a strong association with the common polymorphisms of the *TCF7L2* gene. Studies for associations with other susceptibility genes with small effect sizes (*PPARG*, *PPARG1A*, *CALPAIN10*) were not adequately powered to detect possible associations.

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Abbreviations

ACCORD	Action for Control of Cardiovascular Risk in Diabetes
ACE	Angiotensin Converting Enzyme
ADA	American Diabetes Association
ADVANCE	Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation
ANOVA	Analysis Of Variance
ARB	Angiotensin Receptor Blocker
ATP	Adult Treatment Panel
BMI	Body Mass Index
CARDS	Collaborative Atorvastatin Diabetes Study
CHD	Coronary Heart Disease
CVD	Cardio Vascular Disease
DBP	Diastolic Blood Pressure
DCCT	Diabetes Control and Complications Trial
DKA	Diabetic Keto Acidosis
DNA	Deoxyribose Nucleic Acid
EDTA	Ethylene Diamene Tetra Acetate
EQ5D	Euro Quality of Life 5 D
FPG	Fasting Plasma Glucose
GCK	Glucokinase
GDM	Gestational Diabetes Mellitus

GLP	Glucagon Like Peptide
GLUT	Glucose Transporter
GWA	Genome Wide Association
HDL	High Density Lipoprotein
HLA	Human Leukocyte Antigen
HNF	Hepatocyte Nuclear Factor
HONK	Hyperosmolar Non Ketotic Coma
HTA	Health Technology Assessment
ICC	Intra Class Correlation
IDDM	Insulin Dependent Diabetes Mellitus
IDF	International Diabetes Federation
IFG	Impaired Fasting Glucose
IGT	Impaired Glucose Tolerance
IPF	Insulin Promoter Factor
LDL	Low Density Lipoprotein
LOCF	Last Observation Carried Forward
MAP	Mean Arterial Pressure
MODY	Maturity Onset Diabetes in the Young
MRDM	Malnutrition Related Diabetes Mellitus
NEUROD	Neurogenic Differentiation
NHS	National Health Service
NIDDM	Non Insulin Dependent Diabetes Mellitus
OGTT	Oral Glucose Tolerance Test
OR	Odds Ratio
PCOS	Polycystic Ovary Syndrome

PCR	Polymerase Chain Reaction
PCT	Primary Care Trust
PEPCK	Phosphoenol Pyruvate Carboxykinase
PGC	Peroxisome Proliferator Gamma Co-activator
QALY	Quality Adjusted Life Years
QOF	Quality Outcomes Framework
REML	Restricted Maximum Likelihood
RFLP	Restriction Fragment Length Polymorphism
RR	Relative Risk
RXR	Retinoid X Receptor
SA	South Asians
SAS	Statistical Analysis System
SBP	Systolic Blood Pressure
SD	Standard Deviation
SNP	Single Nucleotide Polymorphism
UKPDS	United Kingdom Prospective Diabetes Study
VADT	Veterans Affairs Diabetes Trial
VEGF	Vascular Endothelial Growth Factor
WE	White European
WHO	World Health Organisation

Chapter 1: Introduction to Diabetes

1.1 History of Diabetes

The earliest recorded descriptions of ‘Diabetes’ as a metabolic disorder date back to 1550 BC(1). A polyuric condition that fits the modern day description of diabetes was recorded in *Ebers Papyrus* and the sweet taste of urine in patients with diabetes was described in the work of two Indian physicians, Charak and Susrut, around 500BC(1). Charak and Susrut also described two main types of diabetes- one that was ‘prevalent in obese and the indolent’ and the other ‘more serious condition that was difficult to treat’ and needed “nourishing diet”. The name ‘Diabetes’ itself is derived from the Greek word for ‘Siphon’ and the word ‘Mellitus’(meaning ‘sweet’) was added subsequently to distinguish it from another polyuric condition, ‘Diabetes Insipidus’, in which the urine was tasteless. Much of our current understanding of diabetes, however, has progressed with various observations made since the beginning of the 17th century(1).

Several periods could be identified as major landmarks in our understanding of diabetes. These include the description of hyperglycaemia by Matthew Dobson in 1776 (2), glucose metabolism by Claude Bernard(3) and linking diabetes to the internal secretion of the pancreas by Oskar Minkowski and Josef von Mering (4). A significant development was the discovery of islet cells by Paul Langerhans (5). This was followed by an intensive search for insulin, culminating in its discovery by Banting and Best in 1921(6) and the eventual identification of insulin sequence

by Frederick Sanger in 1955 (7). Over the last 50 years, there has been a better understanding of the epidemiology of diabetes, its classification and the role of hyperglycaemia in the causation of micro- and macrovascular complications of diabetes (8;9).

Simultaneously, there have been major advances in the development of new oral agents, insulin analogues and drug delivery techniques for diabetes. Despite these developments, management of diabetes still remains a formidable challenge.

1.2 Diagnosis of Diabetes

The original criteria for diagnosis of diabetes were recommended by the World Health Organisation (WHO) in 1965 and a cut-off value of fasting blood glucose greater than 7.2mmol/L was used for diagnosis(10). This criterion was revised subsequently in 1980, 1985 and in 1999(11). In addition to the criteria proposed by WHO, a separate diagnostic criterion was proposed by the National Diabetes Data Group in 1979 (12), which also introduced the terms “non-insulin dependent diabetes mellitus” (NIDDM) and “insulin dependent diabetes mellitus” (IDDM) to distinguish the two main types of diabetes, along with the category “impaired glucose tolerance”. In 1997, the American Diabetes Association (ADA) revised the criteria for diagnosis of diabetes and lowered the cut-off for fasting blood glucose to 7mmol/L from previous 7.8mmol/L and also introduced a new category called “impaired fasting glucose” (IFG) for those with fasting glucose between 6 and 6.9mmol/L(11). In 2003, the ADA lowered the cut-off value of IFG to

5.6mmol/L based on Pima Indian, San Antonio, Mauritius and Hoorn study data (13). This recommendation was, however, not accepted by the WHO, which kept the cut-off level for IFG at ≥ 6.1 mmol/L. On the whole, the current criteria for diagnosis of diabetes as recommended by the WHO and the ADA are similar, although ADA appears to place greater emphasis on the fasting blood sugar (**Table1.1**).

The current WHO recommendations for the diagnosis of diabetes are:

Fasting glucose > 7 mmol/L (126mg/dL) OR

2 Hour glucose > 11 mmol/L (200mg/dL) with symptoms or confirmed on a repeat measurement (on OGTT) in the absence of symptoms.

The intermediate groups at risk of diabetes were defined as

a) Impaired Glucose Tolerance (IGT) when the 2 hour glucose value is >7.8 mmol/L and <11 mmol/L AND

b) Impaired Fasting Glucose (IFG) when fasting glucose is between 6.1 mmol/L and 6.9mmol/L

Table 1.1: Diagnostic criteria for Diabetes, IFG and IGT as per WHO and ADA.

	WHO 1999	ADA 2003
Diabetes		
Fasting glucose	≥ 7.0 mmol/L or	≥ 7.0 mmol/L or
2 hr glucose	≥ 11.1 mmol/L	≥ 11.1 mmol/L
IGT		
Fasting glucose	< 7 mmol/L (if measured)	Not required
2 hr glucose	and ≥ 7.8 and < 11.1 mmol/L	≥ 7.8 and < 11.1 mmol/L
IFG		
Fasting glucose	6.1 to 6.9mmol/L	5.6 to 6.9mmol/L
2 hr glucose	---	-----

1.3 Diabetes Classification

In 1980, the WHO published the first classification of diabetes mellitus. This was subsequently modified in 1985(14). Both the 1980 and 1985 classifications included clinical classes and two statistical risk classes. In the 1980 classification, two major classes of diabetes were introduced and named “Type 1 diabetes” or “IDDM” and “Type 2 diabetes” or “NIDDM”. The terms “Type 1” and “Type 2” were omitted in the classification in 1985 and a new class of diabetes- malnutrition related diabetes mellitus (MRDM)- was introduced. Both

classifications included gestational diabetes and impaired glucose tolerance. The new classification of diabetes was proposed in 1998 following collaboration between the WHO and ADA expert groups(13;15). Diabetes is classified into four major groups – Type 1 diabetes, Type 2 Diabetes, other specific types and gestational diabetes (Table 1.2). Of these Type 1 diabetes accounts for nearly 5% of the cases while a greater proportion of the remainder (85-95%) is Type 2 diabetes(13).

Type 1 diabetes mellitus (IDDM or juvenile diabetes) is characterized by autoimmune beta cell destruction resulting in absolute insulin deficiency. The onset is usually acute, with symptoms progressing over a period of a few days to weeks(13). Over 95 percent of persons with type 1 diabetes mellitus develop the disease before the age of 25, with an equal incidence in both sexes and proportionately more common in the white population.

Type 2 diabetes mellitus (NIDDM, type II or adult-onset diabetes) is characterized by beta cell dysfunction and insulin resistance. This is the most common form of diabetes mellitus and is highly associated with a family history of diabetes, older age, obesity and lack of exercise(13). The aetiology of type 2 diabetes mellitus is complex and involves both genetic and environmental factors.

“Other specific types” includes diabetes mellitus of various known etiologies grouped together(14). This group includes persons with genetic defects of beta-cell function (MODY or maturity-onset diabetes in youth) or with defects of insulin action; persons with diseases of the exocrine pancreas, such as pancreatitis

or cystic fibrosis; persons with dysfunction associated with other endocrinopathies (e.g., acromegaly); and persons with pancreatic dysfunction caused by drugs, chemicals or infections.

Table 1.2 Aetiological classification of diabetes mellitus

I. Type 1 diabetes (β-cell destruction, usually leading to absolute insulin deficiency)
<i>A. Immune mediated</i>
<i>B. Idiopathic</i>
II. Type 2 diabetes (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly insulin secretory defect with insulin resistance)
III. Other specific types
<i>A. Genetic defects of β-cell function</i>
<i>B. Genetic defects in insulin action</i>
<i>C. Diseases of the exocrine pancreas</i>
<i>D. Endocrinopathies</i>
<i>E. Drug- or chemical-induced</i>
<i>F. Infections</i>
<i>G. Uncommon forms of immune-mediated diabetes</i>
<i>H. Other genetic syndromes sometimes associated with diabetes</i>
IV. Gestational diabetes mellitus (GDM)

Gestational diabetes includes a spectrum of conditions ‘with any degree of glucose intolerance with onset or first recognition during pregnancy’. Individuals with type 1 diabetes or type 2 diabetes detected first during pregnancy are therefore also classified as gestational diabetes. Other important changes were the

deletion of terms NIDDM and IDDM. The new classification encompasses both clinical and aetiological classifications and is widely accepted(14).

Figure 1.1

Types \ Stages	Normoglycemia	Hyperglycemia		
	Normal glucose regulation	Impaired glucose Tolerance or Impaired Fasting Glucose	Diabetes Mellitus	
			Not insulin requiring	Insulin requiring for control
Type 1*				
Type 2*				
Other Specific Types**				
Gestational Diabetes**				

Disorders of glycemia: etiologic types and stages. *Even after presenting in ketoacidosis, these patients can briefly return to normoglycemia without requiring continuous therapy (i.e., "honeymoon" remission); **in rare instances, patients in these categories (e.g., Vacor toxicity, type 1 diabetes presenting in pregnancy) may require insulin for survival.

Adapted from reference 14.

1.4 Global prevalence of diabetes

Epidemiological trends indicate that the world prevalence of diabetes has increased steadily over the last few decades(16;17). Although increases in both type 1 and type 2 diabetes have been observed, much of this increase is attributable to type 2 diabetes. In 1998, King *et al* predicted global prevalence (in adults over 20 years of age) to increase to 154 million by the year 2000. Subsequent data have shown that these figures were an underestimate(17-19). In

the year 2000, the global prevalence was 171million. Recently published projections suggest that diabetes prevalence is set to double over the next 2 decades and reach an alarming 366 million by 2030(17). The greatest relative increases are expected to occur in the Middle Eastern crescent, sub-Saharan Africa and India while the greatest absolute increase will be in India. India, China and the United States retain the top three spots for the greatest number of people with diabetes.

Currently, there are an estimated 31.7 million people with diabetes in India and this is expected to increase to 79.4 million by 2030(17). The age-related prevalence of diabetes varies between developing and developed countries, with the majority of those with diabetes in developing countries being in the age group 45 to 64 years and those in developed countries being >64 years. Gender-specific prevalence shows similar estimates for men and women overall but the prevalence is greater in men in the age group less than 60 years and in women over 60 years. The increasing prevalence of diabetes can be attributed to rising rates of obesity, sedentary lifestyles and improved survival rates across the globe. Rapid changes in lifestyles following globalisation have contributed to the increased prevalence observed in urban populations. Increased life expectancy and the proportion of the elderly population is thought to be contributing to the rise in prevalence in the developed nations(18). While the estimated increase in prevalence is likely to occur even if obesity rates remain constant, the observed increase in the prevalence of obesity may suggest that the true prevalence may far exceed predictions. From a public health perspective, the impact of diabetes and its

complications is likely to place a huge demand on health care resources and a greater emphasis on preventative measures is required to tackle this problem(17).

1.5 *Complications of diabetes*

1.5.1 Acute complications

Disturbances in glucose metabolism as a result of relative or absolute insulin deficiency can be associated with acute complications(20). These can be broadly differentiated into those associated with hyperglycemia and hypoglycaemia. Acute complications associated with hyperglycemia include diabetic ketoacidosis (DKA) and non-ketotic hyperosmolar coma (HONK). DKA often occurs in subjects with type 1 diabetes and can also be the initial presentation. It occurs rarely in type 2 diabetes. It is characterized by normal or elevated blood sugars, dehydration and ketosis, which results in severe metabolic acidosis. HONK, on the other hand, occurs in type 2 diabetes and is characterized by very high blood sugars, dehydration and shock. Both these are potentially serious and require aggressive management with fluids and insulin replacement(20).

Although hypoglycaemia is regarded as an acute complication of diabetes, it is frequently iatrogenic and occurs in subjects treated with glucose lowering agents(21). Milder degrees of hypoglycaemia are often associated with increased autonomic symptoms and are easily correctable. Severe degrees of hypoglycaemia, however, may require assistance and can be associated with severe morbidity particularly in children and the elderly(21-23).

1.5.2 Chronic complications of diabetes

Although there are epidemiological differences between the two major types of diabetes, the long term exposure to hyperglycemic states results in significant microvascular and macrovascular damage. Diabetes is now the leading cause of blindness in the working population (retinopathy), end-stage renal disease and non-traumatic amputations(24). The pathogenesis of these microvascular complications (retinopathy, nephropathy and neuropathy) is complex and involves several pathways leading to accumulation of advanced glycation end products and eventual end organ damage (25). These complications are often progressive and irreversible. Increased awareness and advances in understanding of the pathogenesis and screening techniques have allowed early detection and the development of interventions to slow the progression of these complications.

1.1.5.1. Nephropathy

One of the major microvascular complications is diabetic nephropathy. This is the most common cause of renal failure in the developed world (26). It is estimated that approximately 25-30% of patients with diabetes develop some degree of nephropathy (27). Certain ethnic groups, for example, South Asians, appear to be at an even higher risk (27). Diabetic nephropathy progresses through a series of stages, which can be defined by measuring the excretion of albumin in the urine as an indicator of microvascular and endothelial cell damage. Sub-clinical disease progresses to a state of microalbuminuria (defined as a urinary albumin excretion rate of greater than 30mg per day but less than 300mg per day), which is the earliest point of detection of renal disease. In some individuals this may progress

to overt nephropathy (macroalbuminuria, defined as a urinary albumin excretion rate greater than 300mg per day), with accompanying renal dysfunction, and may eventually progress to end-stage renal disease (28).

1.1.5.2. Retinopathy

Diabetic retinopathy is the most common cause of acquired blindness in the western world (27;29;30). Retinopathy also progresses through recognizable stages. Initially there are early non-proliferative changes, which appear in almost all individuals with type 1 diabetes within about 20 years of disease onset, as well as a significant percentage of those with type 2 diabetes. This may develop into pre-proliferative retinopathy, followed by proliferative retinopathy and macular oedema. At this stage, there is a risk of retinal detachment and vitreous haemorrhage. The later stages of retinopathy can be sight-threatening. Approximately 20% of patients with diabetes have some evidence of retinopathy at diagnosis of the disease, but the prevalence increases with age (27). There is a close link between diabetic retinopathy and nephropathy (31).

1.1.5.3. Neuropathy

This is the most common chronic complication of diabetes (27). Neurological disturbances in diabetes are caused by changes in the blood supply to the nerves or degeneration of peripheral nerves due to an overproduction of neurotrophic factors in response to hyperglycaemia. Neuropathy refers to a complex group of conditions that can take many different forms. These fall into two major

categories; focal and generalised (32). Examples of focal neuropathies include femoral neuropathy and peroneal nerve and cranial nerve palsies (33).

The most common generalised neuropathy is sensorimotor polyneuropathy, which often first presents as peripheral neuropathy. This can lead to foot ulceration, poor healing and gangrene. As a result patients with diabetes have an approximately 15-fold higher risk of non-traumatic lower limb amputation compared with the non-diabetic population (27). The autonomic nervous system is also commonly involved, resulting in cardiac dysfunction, gastroparesis, bladder dysfunction and erectile dysfunction (34). Neuropathy can result in poor blood flow to the feet, causing foot problems through a number of mechanisms; a) diabetic patients with neuropathy find it difficult to know if they have a foot injury, causing a delay in treatment, b) neuropathy reduces the rate of healing of foot injuries due to poor blood flow, and c) neuropathy is associated with an increased risk of foot ulcers.

In the UK, foot problems are the most common reason for hospitalisation of patients with diabetes and good foot care can greatly reduce the risk of amputation (27;35). Patients with diabetes are actively screened for signs of neuropathy, as a means of prevention of foot ulcers and other complications (36).

1.1.5.4 Macrovascular complications

Both type 1 and type 2 diabetes are associated with increased risk of macrovascular complications. The co-existence of other risk factors for macrovascular disease often such as obesity, hypertension, dyslipidaemia and

hypercoagulable state with type 2 diabetes is frequently responsible for the excess cardiovascular risk associated with type 2 diabetes(37). The risk of coronary heart disease, peripheral vascular disease and stroke are all increased in individuals with diabetes. Epidemiological studies have shown that the risk of cardiovascular disease is three times greater in individuals with type 2 diabetes and is proportionate to that observed in those who have already had a cardiovascular event(38). Diabetes increases the rate of atheroma progression and in pre - menopausal women in particular may offset the cardiovascular protection derived from estrogens. Epidemiological studies have shown a 'J' shaped relationship with glycaemia with risk of cardiovascular disease increasing with blood sugars above the diabetes range. Despite this, there is inconsistent evidence to suggest that lowering blood glucose actually reduces cardiovascular risk (39;40). Recent evidence, however, suggests that tight glycaemic control in early years of type 2 diabetes can indeed be associated with significant risk reduction(41). Effective management of cardiovascular disease in diabetes therefore requires both tight glycaemic control as well as control of other risk factors.

1.6 Management of diabetes- risk factor control

1.6.1 Effects of glycaemic control

The fact that tight glycaemic control is essential for minimising the consequences of diabetes was recognized early in the post-insulin era. E.P Joslin in his book stated the importance of glycaemic control and urged for this to be pursued with "missionary zeal"(42). Despite this recognition, it was only in the late 1990's that

the importance of tight glycaemic control in the management of type 1 and type 2 diabetes was firmly established(8;9).

1.6.1.1 Microvascular disease

The first definitive evidence that tight glycaemic control reduced the risk of microvascular complications came from the results of the Diabetes Complications and Control Trial (DCCT) in 1994(8). The DCCT trial was in patients with type 1 diabetes and compared intensive treatment with conventional management. Significant reductions in microvascular complications were observed in the intensively treated group with a relative risk reduction of 60% in retinopathy and neuropathy and 39% reduction in microalbuminuria. Given that the epidemiology and pathogenesis of type 2 diabetes is considerably different to type 1 diabetes, it was important to verify the benefits of intensive glycaemic control in patients with type 2 diabetes. The United Kingdom Prospective Diabetes Study (UKPDS) was designed to answer this specific question. The UKPDS was a large prospective trial in newly-diagnosed patients with type 2 diabetes(9). Patients were randomised to intensive glucose control or conventional therapy. After a median follow up of 10 years, the intensive group was shown to have significant reductions in diabetes-related end points, all-cause mortality and microvascular end points. Reduction in HbA1c (glycated haemoglobin) of 0.9 % was associated with 12% relative risk reduction for any diabetes-related end point, 6% reduction in all- cause mortality and 25% reduction in microvascular end points. Interestingly, patients allocated to intensive control with metformin had better reductions in all these end points than other intensive therapies. Patients on

metformin had a 32 % risk reduction in diabetes-related end points, 42% reduction in diabetes-related death and 36% reduction in all-cause mortality. The results of the UKPDS had a major impact on management of patients with type 2 diabetes and led to the setting of targets for glycaemic control. Following the success of the UKPDS there has been much debate about the lowest possible target for glycaemic control. Recent studies suggest that targets as low as HbA1c of 6.5% can be associated with significant reductions in microvascular complications but may be hampered by the risk of hypoglycaemia(43).

1.6.1.2 Macrovascular disease

There is strong epidemiological evidence to suggest a link between the risk of cardiovascular disease and hyperglycaemia. However, unlike microvascular complications, where the risk increases beyond a certain level of blood sugar, there is no threshold below which the risk of macrovascular disease becomes negligible(44;45). The relationship between blood glucose and cardiovascular risk therefore appears to be linear. Type 2 diabetes increases the risk of cardiovascular disease and mortality from cardiovascular disease is nearly 3 times more common in diabetic subjects than in individuals without diabetes(38). Despite this association between blood glucose and cardiovascular disease, there is some degree of uncertainty about the benefits of intensive glycaemic control to reduce cardiovascular disease. In the UKPDS, the risk of myocardial infarction in the intensive group was 16% less compared to the conventional treatment group. This result was, however, not statistically significant. Recently, three major trials – the ADVANCE, VADT and the ACCORD trials - reported that intensive glycaemic control is associated with no cardiovascular benefit (VADT)(43) and may in fact

be associated with increased cardiovascular mortality (ACCORD) (40). At the same time, a 10 year post-study follow-up of patients in the UKPDS study suggested that the benefits of early intensive treatment offered significant cardiovascular protection even after the intensive control worsened(41). While the uncertainty about the benefits of intensive glycaemic control for cardiovascular risk reduction continues, it is now widely accepted that, in newly-diagnosed patients at least, intensive treatment is useful.

1.6.2 Effects of blood pressure and lipid control

There is now substantial evidence to show that blood pressure and lipid control is associated with significant reductions in cardiovascular risk. Although most of these trials have been in the general population, many have included subjects with diabetes. In addition to the reduction in cardiovascular events, blood pressure control is associated with a decreased risk of microvascular events in patients with diabetes. In the UKPDS, intensive control of blood pressure, resulting in a reduction of 10 mmHg systolic blood pressure, was associated with 24% relative risk reduction in diabetes-related end points, 32% reduction in diabetes-related death and 44% reduction in microvascular end points(46). The benefits were attributed to the reductions in blood pressure rather than the agents used. Subsequent trials have shown that certain agents, such as ACE inhibitors and angiotensin receptor blockers, may have additional benefits over and above that expected from blood pressure lowering alone, particularly in the context of diabetic nephropathy. Unlike glycaemic control, however, the benefits of good blood pressure control can be lost unless the control is sustained(47).

Dyslipidaemia is another frequent cardiovascular risk factor associated with diabetes and is characterised by high or normal LDL cholesterol, low HDL cholesterol and elevated triglycerides. Lowering of cholesterol (LDL) has been shown to be associated with major reduction in cardiovascular events(48). While initial statin trials established the benefits of cholesterol lowering on cardiovascular outcomes, subsequent trials (some diabetes-specific) have shown that relative risk reduction in diabetes patients may be substantial. In the CARDS study, a 1 mmol/L reduction in LDL cholesterol was associated with 37% relative risk reduction in cardiovascular events(49). Given the high risk of cardiovascular disease in diabetes patients, lower treatment targets of <4 mmol/L of total cholesterol or < 2mmol/L of LDL cholesterol are recommended. The role of therapies targeted at elevating HDL cholesterol or reducing triglycerides are not well established and is at present considered in those with severe dyslipidemia not managed on statins alone(50).

1.6.3 Multiple risk factor intervention

Although there is now sufficient evidence to suggest that control of single risk factors reduces long term complications, few trials have examined the cumulative benefits of multiple risk factor intervention in type 2 diabetes. In the STENO 2 trial, involving 160 patients with type 2 diabetes, the additive benefits of intensive multiple risk factor treatment was compared against conventional treatment(51;52). Over a follow-up period of 7.8 years, there were significantly fewer cardiovascular events and microvascular complications such as nephropathy and retinopathy. A post-study follow-up of these patients for a further 5.5 years

showed that these benefits persisted with significantly decreased rates of death from any cause, cardiovascular deaths and microvascular disease in the group who were originally intensively treated(53). These results support a role for targeted and intensive multiple risk factor intervention in patients with diabetes.

1.6.4 Secular changes in diabetes management-the Quality and Outcomes Framework (QOF)

The realization that intensive risk factor control can reduce the burden of long term complications has led to a greater emphasis on treating to lower targets. Despite minor differences, there is general agreement between the different international bodies regarding these targets. In general, it is recommended that the targets for glycaemic control are HbA1c of 6.5 % or 7%, blood pressures of 130/80 mmHg systolic and LDL cholesterol levels of less than 2 mmol/L(54;55).

An important secular change in the UK in recent years has been the introduction of the Quality and Outcomes Framework (QOF) initiative by the UK Government in 2004(56). This links physician pay to performance against 146 quality indicators relating to 10 chronic diseases. The QOF program incurred an additional government expenditure of £1.8 billion over a three year period. A key feature of this has been the linking of physician performance to targets achieved both in clinical and organisational areas. Management of diabetes as a chronic disease is linked to 99 points and includes specific targets for blood pressure ($\leq 145/85$ mmHg), HbA1c ($\leq 7\%$ in $>50\%$ of patients) and total cholesterol (≤ 5 mmol/L), amongst many other parameters. Although evidence-based targets for

these risk factors are lower than those recommended in the QOF, initial data suggest that in general there has been an improvement in the standards of care received by patients(57).

In addition to the QOF initiative, the general increase in awareness of the risk factors, evidence from clinical trials and availability of effective therapeutic agents have led to a greater involvement of the primary care physicians in the management of diabetes patients. While these changes have no doubt been encouraging, there is still need for intensification of treatments. Moreover, current treatment targets are based on studies in white European populations and do not take into account ethnic differences. Given that the risk of disease and its complications varies between ethnic groups, there is an argument for ethnic-specific targets. At present, however, there is no evidence to support such an approach.

1.7 Diabetes in the south Asian Diaspora

Effects of migration

Over the past 50 years, there have been significant changes to the world population. Political turmoil and economic changes and demand for skilled labour with increasing industrialization have resulted in migration of people from poorer and developing countries to developed countries. Migration of south Asians to Europe occurred in two phases(58). The first wave of migration from the Indian sub-continent occurred in the post-Second World War era, following increased

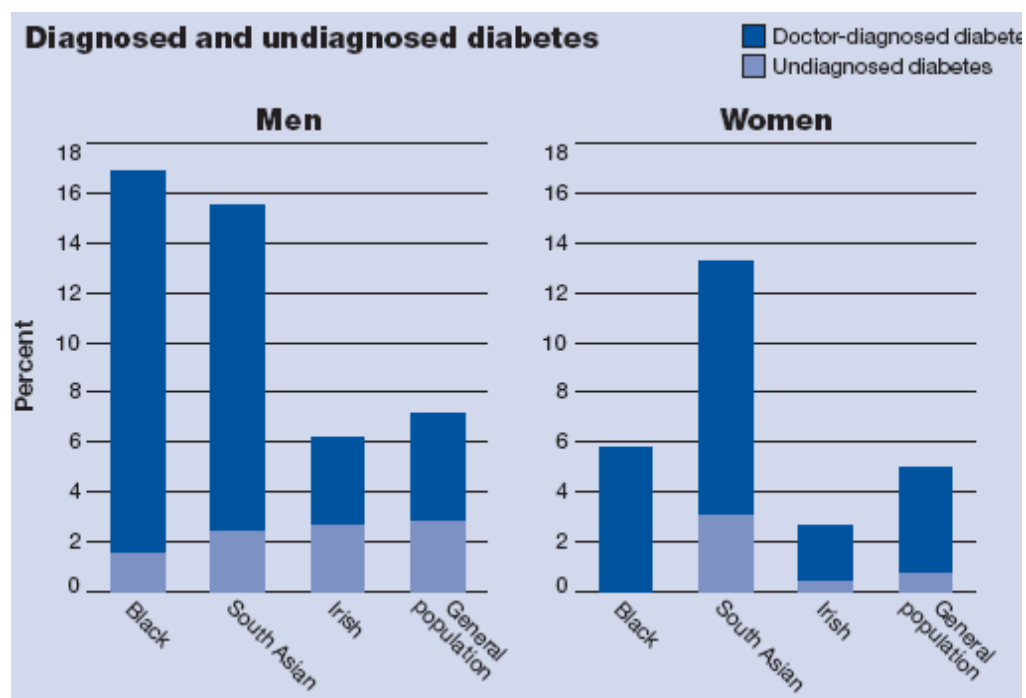
labour demands. A further wave of migration occurred in the 1960s and 70's because of the political turmoil in East Africa. More recently, technological progress and the need for skilled labour has resulted in migration to different parts of the world, including the United States Canada and Australia. The term 'South Asian' broadly refers to people of Indian, Pakistani and Bangladeshi origin, but those from Sri Lanka and Nepal are also commonly included. Although there is considerable heterogeneity between these sub groups, they share many socio-cultural factors and characteristically high susceptibility to type 2 diabetes and cardiovascular disease(58).

1.7.1 Diabetes prevalence and risk factors

It is estimated there are over 25 million people of south Asian origin living outside the Indian subcontinent. Prevalence of diabetes in this community is particularly high and is rising at a faster rate than in any other ethnic group (16). In the UK, south Asians constitute about 4% of the total population and are the largest ethnic minority group. The majority live in inner city areas and have a very high prevalence of diabetes. One of the first indications for the high prevalence of type 2 diabetes in south Asians was the publication of the Southall diabetes survey in 1985 (59). This revealed that prevalence of type 2 diabetes in south Asians was three times that in white Europeans and five times more in those aged between 40 and 69 years. According to recent estimates, diabetes prevalence in south Asians living in the UK is around 20% (60) compared to 9% in urban India and 4% in rural India (61;62) (Figure 1.2). This figure is much higher than in native countries suggesting migration to affluent societies has been a key factor in the

rise of type 2 diabetes in the population. High rates of prevalence amongst south Asians have also been reported in other countries in Europe, the United States and Canada(18). Diabetes also occurs around 10 years earlier compared with white Caucasians and is often associated with established complications at diagnosis. Prevalence rates also vary within the different sub groups of south Asians, with figures being particularly high amongst those of Bangladeshi and Pakistani origin(60).

Figure 1.2: Prevalence of type 2 diabetes amongst UK ethnic groups.



Adapted from reference:(60)

1.7.2 Genes or Environment?

The complex aetiology of type 2 diabetes is influenced both by genetic and environmental factors(63). There is little doubt that environmental changes have been the primary reason for the increase in the levels of type 2 diabetes seen globally. Moreover, the fact that type 2 diabetes is disproportionately high in migrant south Asians suggests a vital role for environment. Cultural and social values and differing lifestyles invariably affect the susceptibility to diseases such as type 2 diabetes and there is evidence to suggest that these factors predispose to increased susceptibility to type 2 diabetes(64).

Diets rich in saturated fat and reduced levels of physical activity have been noted in migrant south Asians and contribute to the increased levels of obesity, insulin resistance and diabetes(58). Increased BMI, central adiposity and higher prevalence of diabetes in migrant south Asians has been reported in comparative studies involving natives and migrants. The susceptibility to diabetes therefore appears to be proportionate to the degree of environmental exposure.

Environmental factors alone, however, cannot fully explain the increased susceptibility to type 2 diabetes in South Asians compared with other ethnic groups. Even amongst south Asians who share the same environment, there are considerable differences in disease risk. There is also familial clustering of diabetes, suggesting that susceptibility to type 2 diabetes may be partly genetically determined. Until recently, genetic studies in south Asians have been relatively few and the considerable heterogeneity within this group adds to the difficulties in

characterising this population(65).Studies published so far have merely replicated the findings in other ethnic groups and, although subtle differences have been reported, as yet no gene specific to this ethnic group has been identified(66). The increased prevalence of diabetes may therefore be due to more individuals sharing susceptibility genotypes rather than a novel gene itself. The genetic basis of type 2 diabetes and the studies in south Asians are discussed in Chapter 6 in the latter part of this thesis.

1.7.3 Macrovascular disease

People of south Asian ethnicity have the highest rates of cardiovascular disease worldwide and are expected to account for nearly 40% of the global burden by 2020. Standardised mortality rates from cardiovascular disease in UK south Asians are 150 % of those observed in the local white European population(67). Diabetes is thought to contribute significantly to this excess risk, with death rates in UK south Asian diabetic individuals being 3 times greater than in the white diabetic population(68). Similarly, higher rates of cardiovascular disease have been reported in immigrant south Asians in other countries(69). This increased propensity to cardiovascular disease is thought to be due to a higher burden of known risk factors and a greater predisposition to insulin resistance and diabetes(70). South Asian ethnicity itself has been suggested as a possible independent risk factor but the role of ethnicity remains unclear.

The relationship between diabetes and CHD can be further explored through studies of the 'Metabolic Syndrome'. Originally described by Reaven in 1988

(71), this syndrome comprises the co-existence of insulin resistance, hypertension, raised triglycerides and low HDL in a single individual. Several alternative definitions of the metabolic syndrome have since been proposed(72;73)(Table1). Subtle differences exist between the definitions but increased waist circumference/BMI, hypertension, dyslipidaemia and glucose intolerance are common to most of them. The prevalence of metabolic syndrome is considerably higher amongst South Asians than in Europeans using both the WHO and the ATPIII criteria(74). In another study using the ATPIII criteria, the prevalence of metabolic syndrome was reported to be as high as 41% in urban Indians with the figures rising to over 70% in those with diabetes(72). This potentially ominous combination of diabetes and the metabolic risk factors in an individual thus predisposes to the high risk of CHD observed in this population.

Table 1.3. The IDF and ATPIII definitions of Metabolic Syndrome.

IDF Definiton of Metabolic Syndrome using ethnic specific values for South Asians
<p>The diagnosis of Metabolic Syndrome in a person using IDF criterion requires</p> <p>The presence of central obesity defined as waist circumference > 90cm in males and > 80 cm in females</p> <p><i>plus</i> any two of the following</p> <p>Raised Triglyceride level : >150mg/dl (1.7 mmol/L) or specific treatment for this lipid abnormality</p> <p>Reduced HDL cholesterol : <40mg /dl (1.03mmol/L) in males and <50mg/dl(1.29mmol/L) in females or specific treatment for this lipid abnormality</p> <p>Raised blood pressure: systolic blood pressure >130 or diastolic blood pressure >85mmHg, or treatment of previously diagnosed hypertension</p> <p>Raised fasting plasma glucose (FPG) >100mg/dl (5.6mmol/L), or previously diagnosed type 2 diabetes</p>
ATP III criterion for the diagnosis of Metabolic Syndrome
<p>The diagnosis of Metabolic Syndrome using the ATP III (2001) criterion in a person requires at least three of the following</p> <p>Central Obesity : Waist circumference >102cm (male) ,>88cm (female)</p> <p>Dyslipidaemia : Triglycerides >1.695mmol/L (150mg/dl)</p> <p>Dyslipidaemia : HDL cholesterol <40mg/dl (male) ,<50mg/dl (female)</p> <p>Blood pressure : >130/85 mmHg</p> <p>Fasting plasma glucose : >6.1mmol/L (110mg/dl)</p>

1.7.4 Role of traditional risk factors

The contribution of conventional risk factors to the excess cardiovascular risk in South Asians has been investigated in several epidemiological studies. Most of these have compared migrant South Asians with local white Caucasians. Other studies have included comparisons between the migrant and indigenous South Asian populations. These have highlighted important differences between the ethnic groups.

1.7.4.1 Blood pressure

Studies comparing the prevalence of hypertension in white Caucasians and South Asians have shown varying results(75-78). While some studies have reported higher blood pressures in South Asians, others have reported lower values. A review of the cross-sectional data on blood pressure in South Asians found that there was considerable variation in the blood pressure values reported(77). In men, seven of twelve studies reported a lower mean systolic blood pressure, while six of nine studies reported a lower systolic blood pressure in women. Relatively higher diastolic pressures were reported in both South Asian men and women compared with white Caucasians. A multiethnic comparison of risk factors amongst patients recruited to the United Kingdom Prospective Diabetes Study (UKPDS) showed that South Asians had lower blood pressures compared with Afro-Caribbeans, but similar values to those observed in white Caucasians(78). In general, however, it is accepted that the blood pressure values in South Asians are slightly lower or similar to those observed in white Caucasians. Despite this,

South Asians have a much higher prevalence of microalbuminuria and nephropathy (79).

The relationship between blood pressure and cardiovascular risk is continuous and a higher blood pressure in south Asians already known to have a high burden of other risk factors may further increase the risk of cardiovascular events(80). Moreover, there is some evidence to suggest that markers of cardiovascular risk such as microalbuminuria may be detected in south Asians even with blood pressures considered to be within the 'normal' range (81). This may be an argument to have lower thresholds for intervention and to aim for lower blood pressure targets in South Asians.

1.7.4.2 Dyslipidaemia

Despite the reportedly lower or similar levels of total cholesterol, South Asians have a more atherogenic lipid profile than white Caucasians. Higher levels of triglycerides and lower HDL levels have been observed amongst South Asians-features frequently associated with insulin resistance and the metabolic syndrome(82). This pattern of dyslipidemia appears to be further exaggerated in migrants, suggesting a strong environmental influence (83). South Asians also have smaller and denser LDL particles and altered apoprotein B /apoprotein A1 ratio, which may help explain their propensity for early atherosclerosis (84).

1.7.4.3 Smoking

Overall rates of smoking in South Asian migrants are comparable to those seen in white Europeans. Smoking in south Asians, however, appears to be more common in men than in women(70;85;86). Smoking rates as high as 82% have been reported in Bangladeshi men living in East London, while smoking rates are lower in Indian and Pakistani men(87). Although the rates of smoking are lower, under reporting of smoking status in south Asian women may be common. In addition tobacco chewing has also been reported to be common in south Asians and may contribute to excess cardiovascular risk(88).

1.7.4.4 Physical activity

Moderate physical activity has been shown to be associated with significant benefits in cardiovascular risk reduction, improvement in glycaemic control and prevention of diabetes(89;90). Several studies that have compared physical activity levels in different ethnic groups have shown lower levels of activity in south Asians(91). Within the south Asian population, there is considerable heterogeneity with Bangladeshi women in particular having the lowest level of physical activity(60). In the Health Survey of England 2004, physical activity levels in south Asians was lowest compared to other ethnic groups. Only 11% of the Bangladeshi women and 14% of Pakistani women met the criterion for at least 30 minutes of moderate physical activity for five days a week. Low levels of physical activity were also seen in 51% of the Pakistani and Bangladeshi men and 68% Bangladeshi women and 52% Pakistani women(60). Similarly low levels of physical activity were reported in south Asians by Fischbacher et al and Hayes et

al(91;92). Although physical activity in south Asians was generally lower than in white Europeans, these differences were more apparent in the older age groups(92). There is limited data on the physical activity levels in native south Asians but results from the INTERHEART study suggest that low physical activity levels are comparably lower amongst native south Asians than in other ethnic groups(70).

Until now, very little research has been undertaken to understand the barriers to physical activity in south Asians. Available evidence suggests that there is a complex interaction of social, cultural and economic reasons contributing to this(88;91;93). Lack of time, commitment to family needs, language barriers and the weather has all been quoted as reasons for lack of exercise. Cultural and religious factors, such as using a unisex swimming pool, may dissuade south Asian women from using such facilities(94). There is also lack of awareness of the benefits of exercise and a fatalistic attitude towards life that makes south Asians not attach much importance to exercise. Despite these barriers, it may be possible to promote physical activity in south Asians. A HTA report suggested more south Asian women were willing to undertake exercise if offered on prescription (95). Such initiatives have, however, not been evaluated on a bigger scale.

Methodological differences in studies reporting physical activity levels and the fact that not all studies have taken into account both the leisure time activities and household work may have underestimated true activity levels in south Asians(91;92). Very few studies have used objective measures of physical activity

but even these studies have reported low levels of activity in south Asians(92;96). Nevertheless, the common message from all studies is that lower physical activity levels in south Asians is of concern and needs to be addressed if the risk of diabetes and cardiovascular disease in this population is to be reduced.

1.7.4.5 Dietary factors

It is not entirely clear what aspects of the south Asian diet predispose to increased risk of type 2 diabetes(88). There is considerable dietary heterogeneity within the south Asian groups making it difficult to make generalisations. Moreover, vegetarianism is also common in some south Asian sub groups. In an uncontrolled trial, traditional south Asian vegetarian diet induced higher and prolonged rises in plasma glucose than a traditional European meal(97). South Asian diet is also considered to have more carbohydrates, less protein and more fibre than indigenous European diets. Cooking practices also vary considerably and notably, involves the use of Ghee or clarified butter particularly in the Northern Indian diets(98).

There is some variation in the reports concerning fruit and vegetable intake. In the Health Survey of England, the average consumption of fruit and vegetables was greater amongst south Asians compared to other ethnic groups with more than a third of south Asians meeting the 'five a day' recommendation(60). Other studies, however, report that the average consumption of fruit and vegetables to be lower in south Asians. It is also common practice in south Asian cooking to over cook the vegetables thus depriving of the essential vitamins. Cultural practices may also

influence dietary habits. South Asian men are less likely to take part in meal preparation and in general south Asians consume traditional sweets rich in fat and sugar(88).

1.7.4.6 Obesity

The rise in prevalence of diabetes is closely linked to the rising rates of obesity. South Asians have a lower average BMI than their European counterparts. Even at lower BMI, however, South Asians have been found to have a higher percentage of visceral fat and features of insulin resistance(99) . The cut-off values for obesity in South Asians have therefore been revised by the WHO, with a BMI greater than 27.5 kg/m^2 considered obese in this group and greater than 23 kg/m^2 as overweight (100). Recent data from India, however, suggest that the appropriate cut off for defining obesity in those from the Indian sub continent would be a BMI > 23 for overweight and BMI > 25 for obesity (101). In the Health Survey of England (2004) (60) the prevalence of obesity ($\text{BMI} > 30 \text{ kg/m}^2$) in South Asian men was around 14% and in women was 20%, with considerable heterogeneity within the sub groups. Interestingly, in the same report the proportion of South Asians aged over 35 years and with BMI > 25 was 53% for men and 55% for women (60). Thus if the lower cut off values for obesity were to be applied for South Asians, the prevalence could be much higher. As the risk of type 2 diabetes increases with increasing degrees of obesity there is a need for interventions targeted towards reducing obesity in this ethnic group.

1.7.4.7 Novel risk factors

The fact that the excess cardiovascular risk observed in South Asians may not be fully explained by traditional risk factors has led to increased attention on novel risk factors. These include adipocytokines, prothrombotic factors and inflammatory markers and some of these are of particular interest. Adiponectin is an adipocytokine known to have insulin-sensitising, anti-atherogenic and anti-inflammatory properties. Low levels of adiponectin are associated with insulin resistant states and strongly correlate with visceral adiposity. Studies comparing adiponectin levels in white Caucasians and South Asians have shown consistently low levels of adiponectin in the latter(102). There also appears to be a correlation between low HDL levels and adiponectin(103). These features are present even in patients with impaired glucose tolerance and may explain the propensity of South Asians to early cardiovascular disease(103). Similarly, higher levels of C reactive protein, homocysteine, plasminogen activator inhibitor and fibrinogen and impaired endothelium-dependent vasodilatation have been reported in South Asians(104;105). While there is considerable interest in these novel risk factors, they have not been evaluated in prospective studies and their potential role in the pathogenesis of cardiovascular disease is not fully established.

1.7.4.8 Socio-cultural factors

‘Culture’ can be defined in several ways. It is a set of ‘distinct features that includes amongst other things lifestyles, value systems, traditions, and beliefs of a society or social group(106). There is considerable evidence to suggest that South

Asians in the UK have distinct dietary and lifestyle habits. Some of these influence cultural practices and may act as barriers to healthy lifestyle. Diet and exercise are an important part of diabetes management and cultural practices therefore have a huge effect on the life of an individual with diabetes.

The health needs of the migrant population are unique and differ significantly from those of the native country and the host country. Migration is associated with major changes in the socio-cultural environment which often influence health status(64). The conflict of adherence to old cultural values and the process of integrating with the society in the new country can be stressful and challenging. Differences in lifestyle habits of south Asians and white Europeans are well recognised(88). Traditional cooking practices, attitudes and barriers to exercise, large families, strictly defined gender roles and communication difficulties are all known to contribute directly and indirectly to increased risk of diabetes(58;88).

Lack of effective communication is another major barrier in delivering quality health care. A significant proportion of south Asians who have migrated to the UK have poor knowledge of the English language. This often leads to communication problems and difficulties for both the individual and the health professionals(94). Physicians may have difficulty in understanding patient symptoms and making an accurate diagnosis. Often advice given in English is not well understood by the patients. As a result there is misunderstanding of the instructions, poor adherence to treatments and missed appointments. There is also a tendency to involve other family members in decision-making, even when it is about one's own health. This results in making shared treatment decisions difficult. In addition to the above, South Asians have a different attitude to health.

They often have a fatalistic attitude to illness and seek help much later, leading to delay in diagnosis. There is also a reliance on alternative or complimentary therapies, frequently leading to poor compliance with medication.

1.8 Summary

1.8.1 Why do we need an intervention in the south Asian population with diabetes?

Despite the high prevalence of diabetes and cardiovascular disease, there have been very few randomised controlled trials in the South Asian population (107;108). Representation of South Asians in clinical trials has also been poor(108). Consequently, many of the treatments are based on evidence from other ethnic groups. While ethnic differences may be subtle, risk between ethnic groups varies and it is important to have a strong evidence base that is ethnic-specific. This is particularly important as some of the targets for treatment based on studies in white Europeans may underestimate the risk in south Asians. Further there are likely to be differences within an ethnic group as well as between groups and identifying these differences is essential to develop more effective treatments. The precise reasons for poor representation of South Asians in clinical trials are not clear. Studies looking at recruitment of south Asians in clinical trials suggest that they have the same attitudes towards research as other ethnic groups(109). There are, however, identified barriers to participation in research(110;111). These include fears and misconceptions about research, but also relate to the attitudes of researchers (example: difficulties in consenting and interpretation)

(112;113). Others factors include language barriers, poor understanding of the research processes and discriminatory practices(112).

The impact of culturally sensitive care models has previously been evaluated in other ethnic groups with some success. Studies in Mexican Americans showed that by using an integrated care package that met the needs of the immigrant population, substantial benefits could be achieved(114). Significant improvements in HbA1c and blood pressure were seen using culturally sensitive methods. A similar approach in south Asians in the UK using community link workers was shown to be beneficial(115). None of these methods have been evaluated in a randomised controlled trial, however, and the cost effectiveness of such models of health care has not been established.

Type 2 diabetes is a complex disease where there is a strong interaction between genes and environment(63). Although until recently, success in genetic studies has largely been confined to monogenic forms of the disease, recent advances in genetics have allowed characterization of the more 'common' form of type 2 diabetes(116). Most of these studies have been in white Caucasians and, despite the high prevalence of diabetes, there have been very few attempts to characterize genetic susceptibility in south Asians. Understanding of the pathogenic mechanisms is essential to identify any potential differences between the ethnic groups and could have wider implications for disease management.

1.9 Aims and objectives of this thesis

The United Kingdom Asian Diabetes Study (UKADS) is designed to evaluate the effectiveness of a culturally sensitive health care delivery model in south Asians with type 2 diabetes. Consistent with the design of complex interventions, a pilot trial (described in chapter 2) was completed and results published in 2004. The definitive trial, involving a larger cohort of general practices, was commenced to evaluate the intervention over a 2 year period. The study had several components including clinical, economic evaluation, genetic characterisation and psychosocial aspects involving south Asians with type 2 diabetes. At the time of commencement of my research, the recruitment for the trial was underway and the intervention had commenced in some of the practices recruited to the trial. Recruitment of white Europeans and the control samples for the genetics arm of the study was in progress. With both the baseline and the two year data likely to be available during the period of my study, the objectives for my thesis included:

- Description and comparison of the baseline characteristics of both the ethnic groups
- Assessment of clinical outcomes after the two year culturally sensitive intervention that included a brief economic evaluation
- Genetic characterisation of the south Asian cohort for known type 2 diabetes susceptibility genes

1.9.1 Ethnic comparison of clinical characteristics at baseline

Although the UKADS intervention was in south Asians with type 2 diabetes, the plan was to recruit white Europeans from the same practices to be able to compare the two ethnic groups and identify any disparities. I was involved in the strategy and planning for recruitment of white Europeans to the study. I was also involved in the collection of data, running data queries and checking the data for errors for both south Asians and white Europeans. I also performed the data analysis for the comparison of baseline characteristics, with the help from the group statistician, and interpretation of the data.

1.9.2 Clinical outcomes after 2 year culturally sensitive intervention in south Asians with type 2 diabetes

This constituted the main focus of my work. At the time of commencement of my research, recruitment of south Asians was in progress and I assisted in the recruitment process by ensuring an adequate number of participants for each group and adherence to the inclusion/exclusion criteria. Patients were recruited from practices in Coventry and Birmingham and part of my responsibility was to co-ordinate with project managers at both sites on a weekly basis to ensure progress of the trial and adherence to timelines. During the course of the study, I provided clinical support, supervised the conduct of the research clinics, co-ordinated timely data collection, checked for data errors and reviewed progress of the trial at research group meetings. I was responsible for the collection of the data at the end of two years and was involved in the data analysis and interpretation with the help of the group statistician.

1.9.3 Genetic characterisation of the south Asian cohort for known type 2 diabetes susceptibility genes

The genetics work constituted the main part of my laboratory work. All subjects who participated in the clinical arm of the UKADS trial were also invited to be a part of the genetics arm of the trial. Their participation was, however, voluntary and involved a separate consenting process. The overall aim was to create a DNA resource from the UK Mirpuri/Punjabi south Asian population to facilitate genetics studies in this ethnic group. The goal is to recruit 3000 south Asians with type 2 diabetes and the same number of control subjects. I was involved in the initial stages of DNA collection and recruitment of control subjects. This was done through GP practices, community clinics and organising Diabetes Awareness Days in the community. My laboratory work involved looking for associations between common polymorphisms of the genes previously associated with type 2 diabetes in white Caucasian populations and the disease in the South Asian cohort. All of my laboratory work was performed at Prof. Barnett's laboratory at the University of Birmingham Medical School, under the supervision of Dr. Ann Kelly. I performed the genotyping for all the genes included in my thesis as well as the data collection, interpretation and statistical analysis.

Chapter 2

The United Kingdom Asian Diabetes Study: UKADS

2.1 Background

The task of translating the successes of clinical trials in practice is a major challenge. Patients in primary care often present with complex problems. Understanding the health risks and needs of the population is essential for successful delivery of health care. Health care delivery to various groups may be further complicated by the differing social and cultural needs and requires an integrated approach(58). In the UK, traditionally, diabetes specialist nurses have been hospital based and have concentrated their efforts on insulin-dependent or -treated patients. Practice nurses with an interest and training in diabetes, and working with primary care physicians, play a major role in the care of patients with type 2 diabetes. Application of the findings of major clinical trials, such as the UKPDS, to type 2 diabetes patients is therefore likely to require a structured, protocol-driven, nurse educator-led multi-professional approach. Such a multi-professional approach is likely to be successful if the language, religious and cultural needs are incorporated. The constraints in professional manpower and the language and cultural needs of the south Asian population have given rise to the use of diabetes link workers as a key resource in improving education, screening and compliance. The United Kingdom Asian Diabetes Study was designed to evaluate the effectiveness of a health care delivery model that addressed some of these needs of the south Asian community living in the UK(58).

2.2 Hypotheses

The UKADS hypotheses were a) that the introduction of structured care for diabetes, tailored to the needs of the Asian community, can improve surrogate endpoints in a cost-effective manner for diabetic complications (and by extrapolation, morbidity and mortality) in Asian patients with type 2 diabetes; b) that the optimal intervention will include input from South Asian link workers, community-based Diabetes Specialist Nurses (with a focus on care for South Asian patients working across the primary/secondary care interface) and practice nurses, with protected time and an enhanced role, working to a joint protocol with clearly defined targets.

2.3 The UKADS pilot

2.3.1 Study design and methods

Keeping in with the framework for complex interventions, the first phase of the UKADS included a pilot study, involving 401 south Asian patients with type 2 diabetes recruited from 6 general practices in Coventry and Birmingham, UK(117). For the purposes of the study, South Asian ethnicity was defined as all patients in UK census ethnic origin categories of Indian, Pakistani and Bangaldeshi. Patients eligible were of South Asian ethnicity, with type 2 diabetes plus at least one of three defined risk factors: elevated blood pressure, systolic>140 mmHg or diastolic >80 mmHg, HbA_{1c} > 7%, total cholesterol > 5.0 mmol/l. Practices were randomised to intervention (enhanced care- comprising

additional practice nurse time, link worker assistance and specialist nurse input) and control (standard care). Both groups received the same treatment protocols for blood pressure, cholesterol and HbA_{1c}. Patients in intervention practices were seen at weekly research clinics. The practice team was encouraged to follow protocols for glycaemia, lipid and blood pressure control, to achieve targets of HbA_{1c} < 7.0%, cholesterol < 5.0 mmol/l and blood pressure < 140/80 mmHg if no microvascular complications and < 130/80 if microalbuminuria or proteinuria was present. Control practices received the same guidelines to achieve targets, but used existing practice resources for managing their patients with diabetes. Microalbuminuria for this study was defined based on values at the reference laboratory of Birmingham Heartlands Hospital; albumin/creatinine ratio (ACR) of > 3 mg/mmol and < 15 mg/mmol. Albuminuria was defined as ACR of >15 mg/mmol (values are mg of albumin per mmol of creatinine). The primary study outcomes were blood pressure, HbA_{1c} and lipid control after one year of follow-up. Baseline data were collected during February to April 2001 in Coventry, and May to June 2001 in Birmingham, with follow-up data collected during the same months one year later.

Whilst the unit for randomisation in this study was the cluster (general practice), the statistical analyses were planned at the individual level. The rationale for this approach was that only six clusters were to be used as a proof of concept, with insufficient power to detect differences if adjusted for cluster effects. The intention was to design a full study if there was evidence to suggest that the intervention was effective.

2.3.2 Outcomes after 1 year intervention

361 of the 401 patients eligible on the basis of the presence of risk factors consented to take part and were included in the study. All six practices randomised completed one year of follow-up and analyses were on the basis of intention to treat. The study showed high retention rates, with 90% of the eligible participants completing one year follow-up. At baseline, between 50 and 60% of intervention and control patients had elevated blood pressure (systolic > 140mmHg or diastolic >80 mmHg) or total cholesterol > 5 mmol/L, and over 60% had HbA_{1c} above 7.0%, suggesting a major proportion of these patients had poorly controlled risk factors.

After one year intervention, there was a significant difference between both groups for blood pressure and cholesterol(117). Systolic blood pressure decreased by a mean 6.7 mm Hg in the intervention group against a reduction of 2.1 mm Hg in the control group (difference 4.6 mmHg) (Table 2.1). After adjusting for confounding factors, intervention status remained statistically significant for follow-up diastolic blood pressure and was of borderline significance for systolic blood pressure. The difference in total cholesterol reduction was no longer significant. Interestingly, there was no overall reduction in HbA_{1c} and no significant difference between the intervention and control groups.

Table 2.1: Risk factor change over one year of follow-up in the pilot study

	Intervention Mean change	Control Mean change	Difference between intervention and control	95% CI	p value
Systolic BP (mmHg)	-6.7	-2.1	-4.6	-8.8 to - 0.3	0.035
Diastolic BP (mmHg)	-3.1	+0.3	-3.4	-5.7 to - 1.2	0.003
Total Cholesterol (mmol/l)	-0.51	-0.12	-0.38	-0.65 to -0.12	0.005
HbA1c (%)	-0.23	-0.20	-0.03	-0.36 to + 0.30	0.866

Note: All values presented are after adjusting for confounders between groups.

Over the one year study period, albumin:creatinine ratios deteriorated for 32 patients (13%) and improved for 47 patients (19%). Deterioration of albumin:creatinine ratio was associated with those with less (or no) improvement in mean systolic blood pressure over the year.

There were substantial and statistically significant differences between intervention and control groups with respect to numbers of patients progressing through treatment protocols. More intervention than control patients started insulin (6% vs. 3%) or increased their dosages or number of oral antidiabetes agents used (50% vs. 32). Similarly, more patients started or increased doses of anti-hypertensives (35% vs. 25%), angiotensin converting enzyme (ACE)

inhibitors (41% vs. 28%), angiotensin receptor blockers (22% vs. 11%) and statins (50% vs. 27%) in the intervention than control group.

2.4 Relevance of the pilot study outcomes

The success of the pilot study was essential for the commencement of the larger study to evaluate the effectiveness of the culturally sensitive enhanced care intervention. Results of the pilot study confirmed two important things. Firstly, it showed that the intervention strategy was feasible and could be implemented in UK primary care. Secondly, it proved that by engaging and employing link workers from the same community, it was possible to recruit and retain an ethnic group in a clinical trial that was hitherto considered difficult. This pilot phase confirmed the feasibility of applying the enhanced care intervention strategy in primary care and showed significant reductions in risk factors. The estimated additional reduction in 10 year absolute risk for CHD due to the intervention in the UKADS pilot study was 5%. The baseline data also produced important insights into the nature of the prevalence of complications, cardiovascular risk and obesity in this group, confirming their high susceptibility to cardiovascular disease. However, although the reductions in the risk factors were encouraging, lack of improvement in HbA1c was still disappointing. It was also not clear if the results achieved could be reproduced in a larger cohort and whether the benefits could be sustained. It was also important to establish if the intervention was cost effective if it were to be implemented in UK general practice. A larger trial involving a greater number of practices and a longer follow-up period was therefore felt necessary.

2.5 UKADS- Main study

2.5.1 Project outline and Aims

The feasibility of the novel approach both in the project's ability to work with general practices in deprived areas and to recruit and retain a large number of type 2 South Asian diabetic patients was demonstrated in the pilot study. It was therefore proposed to extend the study to provide definitive testing of this healthcare intervention in a wider practice population, using surrogate outcomes of cardiovascular and microvascular risk reduction over a two year period. The West Midlands is an ideal place for such a large scale study to evaluate this new approach, with its high concentrations of South Asian patients. In the longer term, if the delivery of health care can be improved for this important group of patients, this innovation should make a significant impact on the morbidity and mortality associated with this disease.

The intervention strategy used in the pilot study was retained but a few additional components were included, keeping in view the larger cohort size. Accordingly, the definitive UKADS trial had four major components- Clinical, Economic, Genetic and Qualitative.

The aim of the UKADS is to provide reliable evidence on the clinical- and cost-effectiveness of a novel delivery strategy to improve the management of South Asian patients with type 2 diabetes, and to provide evidence that this intervention can be delivered on a wider scale.

To support this overall aim, the study has a number of specific objectives:

- i) to test the feasibility of implementing a strategy of risk reduction to reduce blood pressure, lipids, microvascular progression and improve glycaemic control in an Asian population with type 2 diabetes;
- ii) to measure changes in clinical endpoints associated with the introduction of this strategy for patients with type 2 diabetes;
- iii) to measure the costs of the strategy, including healthcare and patient costs and to compare costs and short-term outcomes to current practice.
- iv) genetic characterisation of South Asians with type 2 diabetes
- v) to provide qualitative data on the processes of care

2.5.2 Methods

2.5.2.1 Patient selection

To achieve these aims, the study was extended to involve 18 practices from Coventry and Birmingham, each recruiting about 100 South Asian patients (total 1800). The extension phase included all adult patients of South Asian ethnicity with type 2 diabetes. In order to make ethnic comparisons in respect to known cardiovascular risk factors an additional 500 White Caucasian patients with diabetes were recruited from the same practices. Patients were excluded if they had previously diagnosed type 1 diabetes or impaired glucose tolerance. The pilot study included patients with at least one known modifiable cardiovascular risk factor other than diabetes.

The results from the pilot study indicated that the benefits seen in the intervention group were offset in part by progression of risk factors in those initially not recorded as hypertensive or dyslipidaemic. Therefore, in the definitive study, all adult South Asian patients with type 2 diabetes were eligible. Eligible participants from each practice were invited to participate in the study. The response rate for the study for both the sites was just over 60%.

2.5.3 Intervention

The intervention in the UKADS comprised three essential components -enhanced practice nurse time, link worker support and specialist nurse supervision. These roles are described below:

- (i) Practices were randomised to provide either the 'active' UKADS package of care or 'conventional care'. Practice nurses in the active group received additional training to be able to deliver the UKADS care package. Each practice nurse had protected time (4 hours a week) to provide an additional intensive control clinic for diabetes care. Each patient randomised to active intervention was seen monthly in the intensive control clinic until the targets were met and two to three monthly thereafter for maintenance. Practice nurses worked with primary care physicians to implement the protocol and encourage appropriate prescribing, provide face-to-face patient education in a clinic setting and achieve targets for blood pressure, lipid and glycaemic control.

- (ii) The Asian link workers were recruited from the same community and spoke the same languages (Urdu, Mirpuri and Punjabi) as the patients. All link workers attended a workshop for diabetes and received training from the nurse co-ordinator. Link workers contacted each patient before and in between appointments to ensure clinic attendance. The link workers attended the research clinics along with the practice nurses and provided interpretation and face to face education and lifestyle advice.
- (iii) The Diabetes Specialist Nurse attended some of the research clinics in the active group and provided additional educational support as well as clinical advice to patients. In addition the Diabetes Specialist Nurse supervised the practice nurses and link workers.

Both the intervention and control practices were provided with common treatment algorithms. The control practices did not receive any additional support and managed with their own resources.

2.5.4 Sample Size estimation

The results of the pilot study suggest that improvements in blood pressure and cholesterol can be achieved over one year. Over a longer follow-up period, and with patients from the whole spectrum of diabetes, more substantial gains may be possible. In our original design we allowed for 25% drop-outs and refusals. The actual drop-out rate in the pilot study was only around 10%. We observed a mean

decrease in systolic blood pressure of 6.7 mmHg, and 0.5 mmol/l total cholesterol.

No significant difference was observed in HbA1c levels. However, given that the main trial would be for a longer duration and to reflect the changes in targets in UK primary care, expected a 0.75% change in HbA1c over the two year period. Using these differences, plus standard deviations and estimates of intra-cluster correlation from our pilot data (0.0352 (SD 21.25) for systolic blood pressure and 0.0597 (SD 1.1) for total cholesterol and 0.75 (SD 2.1) for HbA1c) we estimated that we will need 18-20 clusters of 70-90 patients each. Allowing for 10% drop-outs, we estimated that 18 clusters of 80-100 patients would be sufficient to give us power of 80% (at a significance level of 0.05).

Data from the UKPDS baseline suggest that 40% of patients may have elevated blood pressure. In our sample of South Asians this may be closer to 50%. In the pilot study we were able to achieve a 12% change, from 55% to 43%. Over a longer period a change of 15% would seem reasonable and to achieve this would require 152 patients per group, which allowing for the cluster design effect and 10% drop-outs, translates to 16 clusters of 80-100 patients.

2.5.5 Outcome measurements

A number of clinical outcomes were identified for the main study.

Primary outcomes were:

Changes in blood pressure, lipids and HbA1c at 2 years

Secondary outcomes were changes in:

- i) cardiovascular risk (Framingham)
- ii) waist circumference
- iii) diabetes knowledge scale, quality of life measures
- iv) albumin/creatinine ratio
- v) plasma creatinine + urea

2.5.6 Evaluation of the process of care

The effectiveness of the new process of care will be assessed by comparing the active and conventional care practices with respect to the proportion of patients achieving target values for the cardiovascular risk factors as well as absolute reductions in blood pressure, total cholesterol and HbA1c. Comparisons will also be made with risk factors in White European patients recruited from the conventional care practices. Patient satisfaction with care will be assessed by Health Related Quality of Life (HRQOL) questionnaire. Assessments of the above parameters will be made at baseline and then annually. The research team in each area will travel to general practices to ensure high patient compliance and assist each practice with the annual assessment process.

Preliminary data from the pilot study suggests that the relationship between hypertension and microalbuminuria in this population is different to that seen in White Europeans. Further follow-up of this population will therefore, also provide novel data on the efficacy of risk factor control and of the relationship between risk factors and development of complications in this population.

2.5.7 Economic evaluation

a) Costing study:

The study measured intervention and treatment costs in both patient groups. All costings were subject to sensitivity analysis. The costing study involved a broad societal perspective and sought to estimate the difference in the cost of the resources used by patients in the two arms of the study. This enabled costs and consequences to be compared from a societal perspective (including patient costs) as well as from a health care perspective. The latter is likely to be of more interest to NHS decision makers if such structured interventions were to be introduced in primary care and delivered by PCTs.

Intervention costs: The cost of providing the intervention included staff time, capital (including accommodation and any equipment necessary), overheads and consumables. The use of services will be costed from a variety of sources, including detailed local rates of pay, rent etc provided by the finance departments of the hospitals and services concerned and national sources. Intervention costs will be calculated to exclude research protocol costs (e.g. annual outcome measurement) unless these normally occurred as part of the intervention. Costs to patients in terms of personal, out-of-pocket expenses will be obtained from a questionnaire administered to a sample of participants.

Treatment costs: An initial overview will be used to identify areas of treatment cost difference for patients in the two groups. The resource consequences of each area will be estimated using costing approaches similar to those outlined above.

Where appropriate, costs will be discounted to adjust for the distribution of costs incurred over time using annual discount rates.

b) Comparison of costs and consequences:

An initial economic evaluation will be performed based on a comparative assessment of the marginal costs and outcomes of the intervention introduced. This will be performed both from a societal perspective (including patient costs) and from a health care perspective. An initial cost consequence analysis will be conducted in order to map any resource consequences and measurable benefits. In addition, if significant differences in clinical outcomes are observed in the two groups a cost-effectiveness analysis using the EQ5D (EuroQol) index will be performed. A standard EQ-5D questionnaire will be administered to all trial participants to facilitate this. A careful analysis of the sensitivity of any findings will also be undertaken.

c) Decision-modelling analysis

A decision-modelling approach will be used to model costs and benefits. Short term outcomes measured in this study will be coupled with research evidence on the distribution of treatment modality effectiveness and disease progression in specific groups. The study findings will provide an analysis for PCTs, strategic health authorities and clinicians to indicate whether, and in what way, such a protocol-driven, multi-disciplinary intervention could be worthwhile for South Asian patients with type 2 diabetes.

Chapter 3

Comparison of cardiovascular risk factors between south Asians and white Europeans with type 2 diabetes

3.1 Introduction

Cardiovascular disease is a major cause of morbidity and mortality worldwide. Like diabetes, however, the prevalence and susceptibility to cardiovascular disease varies significantly between ethnic groups. Reported mortality from cardiovascular causes is disproportionately higher in south Asians compared to other ethnic groups and contributes to nearly 40% of the global burden of cardiovascular disease. On its own, diabetes produces a two- to threefold increase in cardiovascular events and mortality(38) and, for South Asians there is a further 50% increase (68). A higher burden of known risk factors has been suggested as a reason for the increased susceptibility to cardiovascular disease seen in south Asians. The known major risk factors, however, do not completely explain this increased risk. South Asian ethnicity may be associated with other risk factors or potentiate the effect of known risk factors.

Many studies have compared the risk profiles of South Asians (SA) with white Europeans (WE) - (78;87;118), but only a few have examined the effects of ethnicity on these risk factors in people with type 2 diabetes(119). South Asians typically have more visceral fat at a given BMI(99), lower HDL cholesterol and higher triglyceride levels and commonly develop type 2 diabetes at a younger age than white Europeans(78). Rates of smoking, particularly in south Asian women,

on the other hand, are significantly lower. The relative contribution of individual risk factors to the overall cardiovascular risk is different for South Asians(70), but it is not clear if these differences are sufficient to explain the risk or are amenable to change. Risk factors operate in a continuum(120) and while a risk factor such as blood pressure recorded in south Asians resident in the UK may be within the “normal range” based on studies in Western urbanised populations, it may not be normal when compared to the indigenous blood pressures in rural South Asia (83). Therefore, thresholds for intervention based on studies in other populations may not be relevant to the South Asian population.

The aim of this study is to describe the cardiovascular risk factors in a south Asian population with type 2 diabetes and draw comparisons with a white European (WE) control population, examining the effect of ethnicity on individual risk factors, and explore differences using the Framingham(121) and United Kingdom Prospective Diabetes Study (UKPDS)(122) risk engines.

3.2 Methods

The UKADS study design and details of patient selection have been described in the earlier chapter (Section 2.5). Briefly, 1486 South Asian (SA) subjects were recruited from 21 general practices in Coventry and Birmingham, UK, as a part of the main UKADS intervention study between May 2004 and April 2005. An additional 492 white European subjects were recruited from 4 general practices within the same area during the same period. The white European patients were

not part of the intervention and were recruited only for comparison of baseline characteristics.

Detailed clinical history, diabetes onset, duration and current medication, plus anthropometric data, were collected for all patients at the time of entry to the study. Anthropometric measures included height, weight (from which BMI was estimated), and waist circumference. Ethnicity-specific BMI categories were used; for SA, normal $<23 \text{ kgm}^{-2}$, overweight $23\text{--}<25 \text{ kgm}^{-2}$, obese $\geq 25 \text{ kgm}^{-2}$, and for WE, normal $<25 \text{ kgm}^{-2}$, overweight $25\text{--}<30 \text{ kgm}^{-2}$ and obese $\geq 30 \text{ kgm}^{-2}$ (100;101). Blood pressure was measured using an 'OMRON' electronic blood pressure measuring device and the average of 3 consecutive readings recorded.

Laboratory: Serum electrolytes, HbA1c, total cholesterol, HDL cholesterol and triglycerides were measured in all patients. Lipid profile, Electrolytes and urea were measured by colorimetric method (Roche diagnostics) and HbA1c was measured using high performance liquid chromatography (HPLC-Tosoh inc.) method. Urinary albumin excretion (microalbuminuria) was estimated from one single measurement using an early morning urine sample. The albumin:creatinine ratio (ACR) was estimated in chemical pathology laboratories following measurement of urinary albumin by an immunoturbidimetric assay and urinary creatinine using a kinetic Jaffe assay. Proteinuria and microalbuminuria were defined as $\text{ACR} \geq 30.0 \text{ mg/mmol}$ for both males and females and $\text{ACR } 2.5 - 30.0 \text{ mg/mmol}$ for males and $3.5 - 30.0 \text{ mg/mmol}$ for females respectively, with values below these thresholds indicating normal albuminuria.

10 year CHD risk was estimated for patients using both the Framingham and the UKPDS risk engines. In analysing CHD risk scores the Framingham age limits were applied, including patients aged 30 to 74 years and with no previous history of cardiovascular disease (CVD-including CHD, stroke, peripheral vascular disease) only. Risk scores were stratified for men and women separately into 10 year age bands.

3.3 Statistical methods

Data measured on a continuous scale were compared between groups using Students t-test and Wilcoxon non-parametric tests as appropriate, while categorical variables were compared using the chi squared test. To investigate individual risk factors,(blood pressures, total and HDL cholesterol, triglycerides and HbA1c), taking confounding and the potential clustering effects of General Practice into account, multi-level mixed models were used. In all models, terms for age at diagnosis, duration of diabetes and gender were included, with further terms for other risk factors and their treatment assessed with respect to changes in the ethnicity effect observed. Smoking was coded “current” vs. “non smoking” for logistic regression analyses and odds ratio (OR) and 95% confidence intervals (95% CI) for SA vs. the WE ethnicity group adjusted for age, gender and other factors estimated. To allow comparison of Framingham and UKPDS 10 years CHD risk scores between ethnicity- defined groups, stratified data analyses were performed for women and men separately, using age bands 30-44, 45-54, 55-64 and 65-74 years.

3.4 Results

Risk factor data were collected for all patients in both ethnicity-defined groups (Table 3.1). South Asians were significantly younger than white Europeans (57 vs. 65 years) and had a longer duration of diabetes (7.8 vs. 6.3 years). There were significantly more women in south Asian cohort compared to white Europeans ($p=0.0150$). The mean waist circumference and BMI were significantly lower in south Asians (Table 3.1). When ethnicity-specific cut-offs for obesity were applied, however, 92% of SA vs. 82% WE were either obese or overweight.

Unadjusted comparisons showed significant differences between the groups in blood pressure, HbA1c and lipid profiles (Table 3.1). Mean systolic blood pressure (SBP) was 4mmHg lower in South Asians but diastolic blood pressure (DBP) was similar in both groups. Total cholesterol and triglyceride levels were significantly higher, and HDL cholesterol levels lower, in SA compared to WE (Table 3.1). HbA1c was significantly higher amongst SA than in WE (8.2% v 7.2%) and these differences were observed across both genders.

Table 3.1: Comparison of risk profiles in South Asian (SA) and white European (WE) patients.,

	SA N=1486	WE N=492	Statistical test result p
Age	57.0 (11.9)	64.8 (11.6)	<0.0001
Duration	7.8 (6.7)	6.3 (7.0)	<0.0001
BMI (Kg/m ²)	28.6 (5.5)	31.0 (7.2)	<0.0001*
Waist Circumference (cm)	101.7 (11.8)	105.5 (14.2)	<0.0001*
SBP (mmHg)	140.1 (20.8)	143.9 (19.7)	0.0004
DBP (mmHg)	83.3 (11.0)	82.4 (10.7)	0.1400
Total Cholesterol (mmol/l)	4.7 (1.1)	4.3 (0.9)	<0.0001*
Triglycerides (mmol/l)	2.5 (1.9)	2.0 (1.2)	<0.0001*
HDL (mmol/l)	1.3 (0.5)	1.5 (0.5)	<0.0001
HbA1c (%)	8.2 (1.9)	7.2 (1.4)	<0.0001
BMI (Kg/m2)categories #			
Normal	131 (9)	89 (18)	<0.0001
Overweight	231 (16)	150 (31)	
Obese	1123 (76)	252 (51)	
Smoking status			
Current	221 (15)	75 (15)	<0.0001
Ex-	128 (9)	186 (38)	
Never	1135 (76)	231 (47)	
Gender			
Females	709 (48)	203 (41)	0.0150
Males	776 (52)	287 (59)	
CVD history	268 (18)	105 (21)	0.1041
Diabetes treatment			
Insulin	290 (20)	65 (13)	<0.0001
Tablets	1020 (69)	285 (58)	
Diet only	176 (12)	142 (29)	
Prescribing:			
Statins	711 (48)	350 (71)	<0.0001
Anti-hypertensives	817 (55)	336 (68)	<0.0001
ACE/ARB	567 (38)	258 (52)	<0.0001

Values shown are Mean (Standard Deviation) and N (%) for categorical values. Missing data: SA duration diabetes (n=7), BMI (n=1), waist (n=39), systolic and diastolic BP (n=2), total cholesterol (n= 28), triglycerides (n=55), HDL cholesterol (n=100), HbA1c (n=68)

WE age (n=3), duration diabetes (n=2), BMI (n=1), waist (n=6), total cholesterol (n=3), triglycerides (n=12), HDL cholesterol (n= 8), HbA1c (n=17)

Notes:* statistical test is non-parametric (Wilcoxon)# BMI (Kg/m2) categories are ethnicity-specific, SA normal <23, overweight 23-<25, obese >=25, WE normal <25, overweight 25-<30, obese >=30

After adjusting for confounding factors and clustering effects, there were no differences between the ethnic groups for SBP or DBP (Table 3.2). Whilst there was no significant difference in total cholesterol or triglycerides after adjustment for clustering and confounding, HDL cholesterol was significantly lower and HbA1c significantly higher in the SA (Table 3.2).

Table 3.2: Ethnicity effect (SA vs. WE) after adjustment for confounding plus clustering:

Risk factor:	Effect size: SA – WE	P value
Systolic <u>BP</u>	-1.34 (-4.19 to 5.12)	P=0.3587
Diastolic <u>BP</u>	-0.30 (-1.97 to 1.36)	P=0.7221
HbA1c	0.50 (0.22 to 0.77)	P=0.0004
Total cholesterol	0.13 (-0.03 to 0.28)	P=0.1039
HDL cholesterol	-0.09 (-0.17 to -0.01)	P=0.0266
Triglycerides	0.14 (-0.10 to 0.39)	P=0.2488

Missing data for SA and WE inclusive:

Systolic BP (n=21), diastolic BP (n=21), total cholesterol (n=49), HDL cholesterol (n=124), triglycerides (n=85), HbA1c (n=102)

Confounding factors – all estimates are adjusted for age at diagnosis, duration of diabetes, gender, any diabetes treatment, statin treatment, obesity status (ethnicity-specific), history of CHD, hypertension, area and smoking status.

Current smoking prevalence was similar comparing SA to WE (Table 3.1). However, whilst there were 349 (24%) current or ex-smokers in the SA group, vs. 261 (53%) WE, a greater proportion of WE were ex rather than current smokers (Table 3.1). In logistic regression analysis, adjusting for potentially confounding factors, including area (Birmingham vs. Coventry), diabetes treatment (insulin vs. diet, tablets vs. diet), treatment with statins and anti-hypertensives (No vs. Yes), CVD history (No vs. Yes), ethnicity-specific obesity (Normal vs. obese, overweight vs. obese) and age, a significant interaction between ethnicity and gender was identified. Repeating the adjusted multiple logistic regression for women and men separately, showed SA women were significantly less likely to be current smokers (OR= 0.35 [0.20 to 0.62], $p<0.0003$) while no significant difference was observed in men.

There was a significant difference in albuminuric status comparing ethnicity groups; the prevalence of microalbuminuria was higher in the WE group than in SA (24% vs. 20%; $P=0.354$) but the combined prevalence of microalbuminuria and proteinuria was greater in SA (Table 3.3). SA had a significantly higher prevalence of proteinuria, (7% vs. 2%, $P<0.0001$). In both ethnic groups there was a significant association between albuminuric status and increasing duration of diabetes, systolic blood pressure and HbA1c (Table 3.3) .

Table 3.3 Association of albuminuria status with ethnic background and other factors

Albuminuria status:					
	Normal	Microalb- uminuria	Proteinuria	Total	P-value
	(1350)	(390)	(112)	(1852)	
Ethnicity:					
SA	1007 (73)	281 (20)	101 (7)	1389	P<0.0001
White	343 (74)	109 (24)	11 (2)	463	
DM Duration:					
< 5 years	618 (77)	152 (19)	30 (4)	800	P<0.0001
5-9	373 (74)	102 (19)	26 (6)	501	
10-19	282 (68)	104 (25)	31 (7)	417	
20 +	72 (56)	33 (24)	23 (20)	128	
Systolic BP:					P<0.0001
<120	246 (83)	40 (14)	10 (3)	296	
120-139	505 (75)	136 (20)	30 (5)	671	
140-159	436 (73)	126 (21)	35 (6)	597	
160-179	125 (60)	65 (31)	19 (10)	209	
180+	37 (49)	23 (30)	16 (21)	76	P<0.0001
HbA1c bands:					
<7.0%	502 (80)	107 (17)	18 (3)	627	
7.0-<8.0	314 (76)	79 (19)	22 (5)	415	
8.0% +	481 (65)	189 (24)	65 (10)	735	

* Missing data: age (n=3), duration DM (n=6), systolic BP (n=3), HbA1c (n=75).

1140 (77 %) SA and 317 (64%) WE fulfilled the Framingham age criterion and had no previous CVD history. Framingham 10 years CHD risk scores were estimated for 1064 (93%) SA and 308 (97%) WE. UKPDS 10 years CHD risk scores were estimated for 1022 (90%) SA and 296 (93%) WE. Mean 10 year CHD risk (Framingham) in SA was 11.7% in males and 7.3% in females, vs. 11.7% for males and 6.5% for females in the WE group. The estimated mean 10 years CHD risk using the UKPDS risk engine was 21.9% and 10.8% for SA males and females respectively and 22.6% and 10.1% for WE males and females respectively. In age band stratified analysis of 10 year CHD risk, statistically significantly raised risk scores for SA vs. WE were observed for age bands 55-64 and 65-74 years. (Table 3.4). A history of CVD was recorded for 371 (19%) patients; 18% of the SA group and 21% of the WE group.

Major differences in treatments were observed between the groups (Table 3.1). Compared with WE, SA were less likely to be prescribed statins (48% v 71%) and ACE inhibitors/ Angiotensin Receptor Blockers (38% v 52%) than WE. On the other hand, SA were more likely to be receiving oral anti-diabetes agents or insulin treatment (89 % v 71%) compared to WE (Table 3.1).

Table 3.4: All, plus age band-specific, Framingham equation and UKPDS 10 years CHD risk scores divided by ethnicity and gender:

	South Asian	White European	Test result
	Mean (sd)	Mean (sd)	p
Framingham 10 years CHD risk scores:			
Females (30-74)			
All	7.3 (6.2)	6.5 (4.2)	0.8648*
30-44	1.8 (2.1)	1.3 (2.0)	0.5335
45-54	5.3 (3.9)	5.3 (4.9)	0.9533
55-64	9.4 (5.7)	7.0 (3.6)	0.0195*
65-74	11.9 (7.4)	7.4 (4.0)	<0.0001*
Males (30-74)			
All	11.7 (8.8)	11.7 (9.7)	0.9631
30-44	4.7 (4.5)	2.9 (2.9)	0.1451
45-54	9.1 (6.6)	8.6 (7.4)	0.6891
55-64	13.5 (7.5)	11.1 (8.0)	0.0468
65-74	19.7 (7.4)	15.4 (11.0)	0.0039
UKPDS 10 years CHD risk scores:			
Females (30-74)			
All	10.8 (8.8)	10.1 (5.7)	0.2657*
30-44	4.0 (2.3)	3.7 (2.1)	0.7533
45-54	7.2 (4.2)	6.0 (2.7)	0.3059*
55-64	12.0 (6.4)	8.8 (3.2)	0.0010*
65-74	21.1 (10.9)	13.7 (6.1)	<0.0001
Males (30-74)			
All	21.9 (14.3)	22.6 (13.8)	0.5970
30-44	10.7 (5.9)	10.0 (15.6)	0.0815*
45-54	16.2 (9.5)	14.7 (9.0)	0.4220
55-64	25.1 (11.4)	19.4 (8.4)	0.0006*
65-74	37.0 (14.7)	30.9 (14.7)	0.0063

Test results are from t-test, except:

* variances unequal, P-values from non-parametric Wilcoxon test.

3.5 Discussion

Strength of this large study is that the SA and WE subjects were recruited from the same community from inner city practices and both groups shared a common health care system and socio-economic environment. The cohort reflects current UK experience and compared to the United Kingdom Prospective Diabetes Study (UKPDS) cohort, subjects in our study were older, had higher blood pressure, but lower HbA1c and total cholesterol. These differences in cholesterol and HbA1c reflect the secular trends in the management of patients with diabetes and dyslipidaemia over the last decade and the fact that in the UKPDS, subjects were selected at diagnosis.

Comparison of the risk factors between the two ethnic groups showed significant differences in cardiovascular risk profiles. Typically, south Asians were younger and had lower BMI, waist circumferences and lower overall smoking rates (in women) than white Europeans. Systolic blood pressure and HDL cholesterol levels were lower, but triglycerides and HbA1c were higher in South Asians. These differences are consistent with those observed in previous (69;78;123) studies. Despite these major differences in risk profiles of SA and WE, after adjusting for confounding variables a significant ethnicity effect remained only for lower HDL cholesterol, much lower smoking and much higher HbA1c in the South Asians. These results suggest that the effect of South Asian ethnicity itself on the overall cardiovascular risk is modest and is probably mediated through increased predisposition to insulin resistance.

While SA in our study were younger, with a longer duration of diabetes compared to WE, as seen in other studies (78;123), the situation with regard to body composition is more complicated. BMI and waist circumference were both significantly lower in SA than in WE. The majority of SA individuals would be classified as non-obese by using standard cut-offs for BMI. Studies suggest that for a given BMI, SA have more visceral fat than WE(99) and that it is not the absolute BMI value but fat distribution (particularly excess visceral fat) that best defines risk associated with obesity(124). To account for body build differences, ethnic-specific cut-offs should be used to define obesity(125) . Applying these cut-offs showed overweight/obesity to be higher in SA than WE.

Many earlier studies have reported similar or lower levels of cholesterol in South Asians compared to WE. The mean total cholesterol levels in South Asians in this study were interestingly higher than in WE. This is more likely to be due to the fact that fewer SA were on statins or lipid-lowering treatment compared to WE rather than a true difference. Epidemiological studies have consistently reported lower levels of HDL cholesterol in South Asians(70;126) and while we confirm this, the absolute difference in HDL is small and its clinical relevance (when total cholesterol is not different) is questionable. Studies have shown that SA have much more adverse lipid profiles and measuring total cholesterol alone may in fact be misleading. Instead, measuring the ApoB/ApoA ratio has been suggested as a better marker of dyslipidemia in south Asians(70). The utility of measuring ApoB/ApoA in routine clinical practice, however, is still not established.

SA in this study had poorer glycemic control than WE. The difference in HbA1c remained significant even after adjusting for duration of diabetes. The fact that this was so, despite the fact that a significantly greater proportion of SA were on glucose-lowering therapies (including insulin) than WE, is of great concern. While the poorer glycaemic control in SA in comparison to WE is likely to be a major factor in the high prevalence of microvascular complications, its relationship to cardiovascular disease may be more complicated(41;127). There is a clear epidemiological link between HbA1c and cardiovascular morbidity and mortality and the assumption is that this is causal and the current strategy for type 2 diabetes is to try to normalise blood sugars and reduce HbA1c(54). Recent studies suggest that this is difficult to achieve in SA(123;128;129). Furthermore the cardiovascular protection offered by current treatment strategies to tighten glycaemic control remains uncertain and may indeed do harm in older, long duration patients(39;40).

Overall, rates of smoking in SA have been reported to be lower than in WE(58;60). While this was true in SA women, the rate of smoking in SA males in our cohort was comparable to that in WE. Further, compared to WE, the proportion of ex-smokers was significantly less in SA. Significant decline in the rates of smoking and CHD has been observed in most western populations in recent years following public health measures(130-132). The finding from this study raises concerns about the effectiveness of these measures in the SA population.

An interesting observation in this study is that the overall prevalence of microalbuminuria was similar in both ethnic groups. No difference in the prevalence of microalbuminuria between ethnic groups was observed in the UKPDS and our results support this, even though the subjects in our study had a longer duration of diabetes than in UKPDS. In contrast, significantly higher rates of microalbuminuria in south Asians have been reported in many earlier studies, including one from our own group(133;134). This variance may have arisen due to methodological differences and the fact that some of these studies were in smaller cohorts. On the other hand, the prevalence of overt proteinuria was significantly higher in south Asians. The reasons for this are not known. Given these contrasting results, more work studying microalbuminuria and proteinuria in south Asians needs to be done.

To compare the CHD risk in both groups we used a general (Framingham) and a diabetes-specific (UKPDS) risk engine. The predicted risk scores for both groups were considerably lower than previously reported(135) and may reflect the widespread use of statins in recent years. Both the Framingham and the UKPDS risk engine predicted equal risk for South Asians and WE. This appears to be at variance with the prognosis we and others document(136;137). However, age is the most important determinant of CHD risk and, as the mean age of SA was lower than WE, the reality of our finding is that SA have the same risk as WE who were seven years older. As a rule of thumb, adjusting existing risk engines by ten years appears justifiable(138) but, if risk scores are to be used in routine practice, to provide valid estimates of risk, ethnicity-specific risk engines, based

on longer follow up of cardiovascular morbidity and mortality, are urgently needed.

It may be particularly relevant here that SAs were less likely to be prescribed statins and inhibitors of the rennin-angiotensin system than WE. Despite the reported increase in statin prescriptions in recent years, the overall rates of statin prescriptions in SA are still lower than recommended(128). Even a small reduction in cholesterol is associated with significant cardiovascular protection(139)and it is difficult to justify denying statin treatment to a population that is at high risk.

3.6 Conclusion

Cardiovascular disease and mortality risk are both potentially modifiable(140), but earlier detection of diabetes, impaired glycaemia and cardiovascular disease are the essential prerequisites if health inequalities are to be rectified. The practicality and potential benefits of systematic screening for diabetes and cardiovascular disease in South Asians at high risk remains a challenge for future study. Furthermore, even in those where the condition is detected, there remains much work to be done to define and implement targets of risk factor reduction for blood pressure, lipids and glycaemic control. Enhanced and ethnic-specific targets for obesity, hypertension, lipids and glycaemia, combined with more sensitive and focused healthcare delivery, have great potential to reduce these inequalities.

Chapter 4

The United Kingdom Asian Diabetes Study (UKADS): outcomes after 2 year culturally sensitive intervention.

4.1 Introduction

Many clinical trials have shown that effective risk factor control reduces both microvascular and macrovascular complications in patients with type 2 diabetes. Implementing the findings of these trials in clinical practice, however, is a major challenge. Prevalence of diabetes and the risk of its complications vary significantly between ethnic groups and are also influenced by social, economic and cultural factors. An integrated approach that addresses the specific needs of a community is therefore essential to achieve better health outcomes. Culturally sensitive models of health care in some ethnic groups have been shown to improve outcomes. Such models of health care delivery, however, have not been evaluated in randomised controlled trials and in particular in the South Asian population.

The United Kingdom Asian Diabetes Study (UKADS) is a cluster randomised controlled trial to evaluate a culturally sensitive intervention tailored to the needs of the South Asian population with type 2 diabetes. Following the success of the pilot trial (described in chapter 2) a definitive trial was undertaken between 2004 and 2007.

The timing of the trial coincided with the introduction of the Quality and Outcomes Framework (QOF), a new initiative by the U.K Government to improve standards in primary care, and provided an opportunity to evaluate the impact of this initiative on diabetes care in our study population. In this chapter, the results of the two year culturally sensitive intervention are described and the clinical and economic implications discussed.

4.2 Methods

Twenty-one General Practices (seven in Coventry and fourteen in Birmingham, UK) with a very high proportion (>80%) of South Asians were included in the study. All the practices were approached individually with an aim to recruit 18 to 20 clusters. Response rate from the practices was 100% with all the practices that were approached agreeing to participate in the study. 10 of the 21 practices were group practices while remaining 11 were managed by a single practitioner. Number of patients registered with these practices ranged from 3600 to 5000 for single GP practices and 10,000 to 14000 for the group practices. Nine practices were randomised to enhanced (intervention) and twelve to conventional (control) care; a common treatment algorithm was provided (Appendix 1 to 3). All adult patients with type 2 diabetes were eligible for inclusion in the study (Figure 4.1).

4.2.1 Protocol and targets

Enhanced care included an additional practice nurse session per week supported by link workers and a community diabetes specialist nurse. Patients in the intervention group were followed up on average every two months in weekly clinics run by the practice nurses.

Practice nurses worked with primary care physicians to implement the protocol and encourage appropriate prescribing, provide face-to-face patient education in a clinic setting and achieve targets for blood pressure, lipid and glycaemic control. Each patient was contacted by a link worker before and between appointments to encourage clinic attendance. In addition, link workers provided interpretation and additional educational input to the patients in the community setting in local languages to improve compliance and understanding and to encourage dietary and lifestyle change. The community diabetes specialist nurse attended some of the research clinics and provided additional educational and clinical support, including insulin initiation, to the practice teams. All staff had formal training in diabetes care and were experienced in delivering diabetes care in the practice setting.

Practices were encouraged to adhere to treatment protocols and to achieve targets. The study targets followed internationally accepted norms and were HbA_{1c} 7.0%, total Cholesterol 4.0 mmol/l (160mg/dl) and blood pressure 130/80 mmHg if no microvascular complications (as recommended by the Joint British Societies and international bodies)(48;141;142) and 125/75 mmHg if microalbuminuria or

proteinuria were present. Control practices received the same treatment protocols and the practices managed patients with their existing resources.

The study protocol was approved by East Birmingham and Coventry Primary Care Trust Ethics Committees. Graphical representation of components and timings of the complex intervention are outlined in figure 4.1(143).

Primary outcomes were follow-up measurements for blood pressure, total cholesterol and HbA_{1c}, with secondary outcomes of waist circumference, body mass index (BMI) and Framingham 10 years Coronary Heart Disease (CHD) risk score(121), microalbuminuria and plasma creatinine.

Figure 4.1: Graphic representation of the complex intervention trial.

Timeline	Intervention	Control
Month 0 Randomisation of practices	F C	C,F
Months 1-8 Recruitment of patients and baseline data collection	B, C, D E,G	B,F,E G
2 monthly research clinic appointments	B, C, D A F	A, F
Research monitoring meetings- (2-3 months apart)	G, C, D	G, C
Months-12-32 Outcomes assessed	B, C, D E	B, C E

- A Prescribing algorithms - algorithms for blood pressure, blood glucose and lipid control (Appendix 1-3)
- B Practice Nurse - protected time to run research diabetes clinic in intervention practices. Dietary advice and implementation of protocols. Practice nurses were formally trained in diabetes and had 1:1 observed sessions in DSN run clinics. For control practices, data collection only.
- C Specialist Nurse - clinical input including insulin initiation and educational role. Attendance at some, but not necessarily all, research clinics. Two specialist nurses were responsible for all 21 practices in the trial, one based in Coventry and one in Birmingham. For control practices, data collection only.
- D Link Workers - educational, communication and facilitation role, promoting patients' understanding and concordance. Link workers attended research clinics in intervention practices. A total of 5 link workers were employed, 3 in Birmingham (14 practices) and 2 in Coventry (7 Practices), with each responsible for 3 or more practices. All link workers attended a foundation course in diabetes management and also passed a long distance course for diabetes care technicians.
- E Questionnaires for patients – Quality of Life (EQ5D), and economic analysis data collection.
- F General Practitioners - overall responsibility for implementation of the study protocol within their practice. This was mainly devolved to the responsible Practice Nurse. GPs were involved in changing prescribing processes.
- G Research team - oversaw study processes. Meetings to monitor recruitment and data collection, to discuss and address issues of study conduct and management.

4.2.2 Sample size estimation and power

This was a cluster randomized trial with General Practice the unit of randomization. Sample size estimations were made based on the observed differences and intra-class correlations (ICC-defined as between groups/ within groups variance) from the pilot study or an ICC = 0.05, which is derived from published estimates for primary care studies(144;145). In all estimations, power was set to 80%, and the probability value to P=0.05. Estimates were made for differences in changes in systolic blood pressure (7mmHg, with standard deviation (s.d.) 21.25 and ICC=0.035), total cholesterol (0.45mmol/l, s.d.=1.1, ICC=0.05) and HbA_{1c} (0.75, s.d=2.1, ICC= 0.05). All estimates from above data values resulted in 16-18 clusters of 80-100 patients being needed, allowing for 10% drop-out rate, as observed in the pilot study. The rationale for these effect sizes was that they were similar to those observed in the pilot study, changes of this magnitude would be clinically significant and also they reflected prescribing algorithm targets.

4.2.3 Statistical methods

Data were analysed using the SAS software package. Baseline variables were compared between groups using χ^2 tests of independence, with t-tests for continuous variables, which were first assessed for normality. Primary and secondary outcomes were continuous. In the main intervention evaluation, final measured outcomes were modelled, with grand mean centred baseline measures included as covariates. To adjust for clustering and potential confounding effects,

the SAS PROC MIXED procedure was used to fit hierarchical, combined fixed and random effects models(146;147). In all cases, mixed models included fixed effects for area (Birmingham vs. Coventry), gender, age at diagnosis of diabetes, duration of diabetes and corresponding grand mean centred baseline measurement. For HbA1c, treatment with insulin at baseline was included in final models. Terms for anti-hypertensive treatments, angiotensin-converting enzyme inhibitors (ACE) or angiotensin receptor blockers (ARB) at baseline were included in blood pressure models. For total cholesterol, statins and fibrates were included. Random effects were fitted, within a subject term for General Practice, allowing for different intercepts and regression slopes for each individual practice (random coefficients models).

Restricted maximum likelihood models (REML) were used to analyse data. The correlation structure used in reported results was “unstructured” in all cases; “variance components” structures were considered. SAS graphics options were used to plot and evaluate residuals and influential data points, which were then removed and models re-run; results presented do not exclude outliers. For the main intention to treat analysis comparing outcomes, all patients were included, with the exception of those who had died (n=48). For patients included in analyses but whose follow-up data were not available (Fig.4.2), data imputation using last observation carried forward (LOCF) was used; this was an interim value measured after one year for around 50% of cases, or the baseline value. Analyses using the same final models were performed using only subjects with complete data; whilst estimates of effect differed slightly, results and their interpretation were essentially the same.

Economic analysis and Quality of life data

Detailed data on staff salaries, travel and subsistence, equipment costs, payment to practices, and prescribing were collected to estimate the net intervention cost over a 2 year period. Changes in quality adjusted life years between intervention and control groups were measured using the EQ5D questionnaire(148). *An interim within trial cost effectiveness analysis was done using the above measures with a plan for long term decision modelling in due course. All the economic analysis and cost effectiveness evaluation was done by Prof. Ala Szczepura and her team from the Warwick Medical School.* The original study plan intended to collect Quality of Life measures in trial participants. However, due to logistics reasons and lack of resources, this data was not collected and a decision to not include these measures was made during the research group meeting at the start of the trial.

4.2.4 Sub group analysis

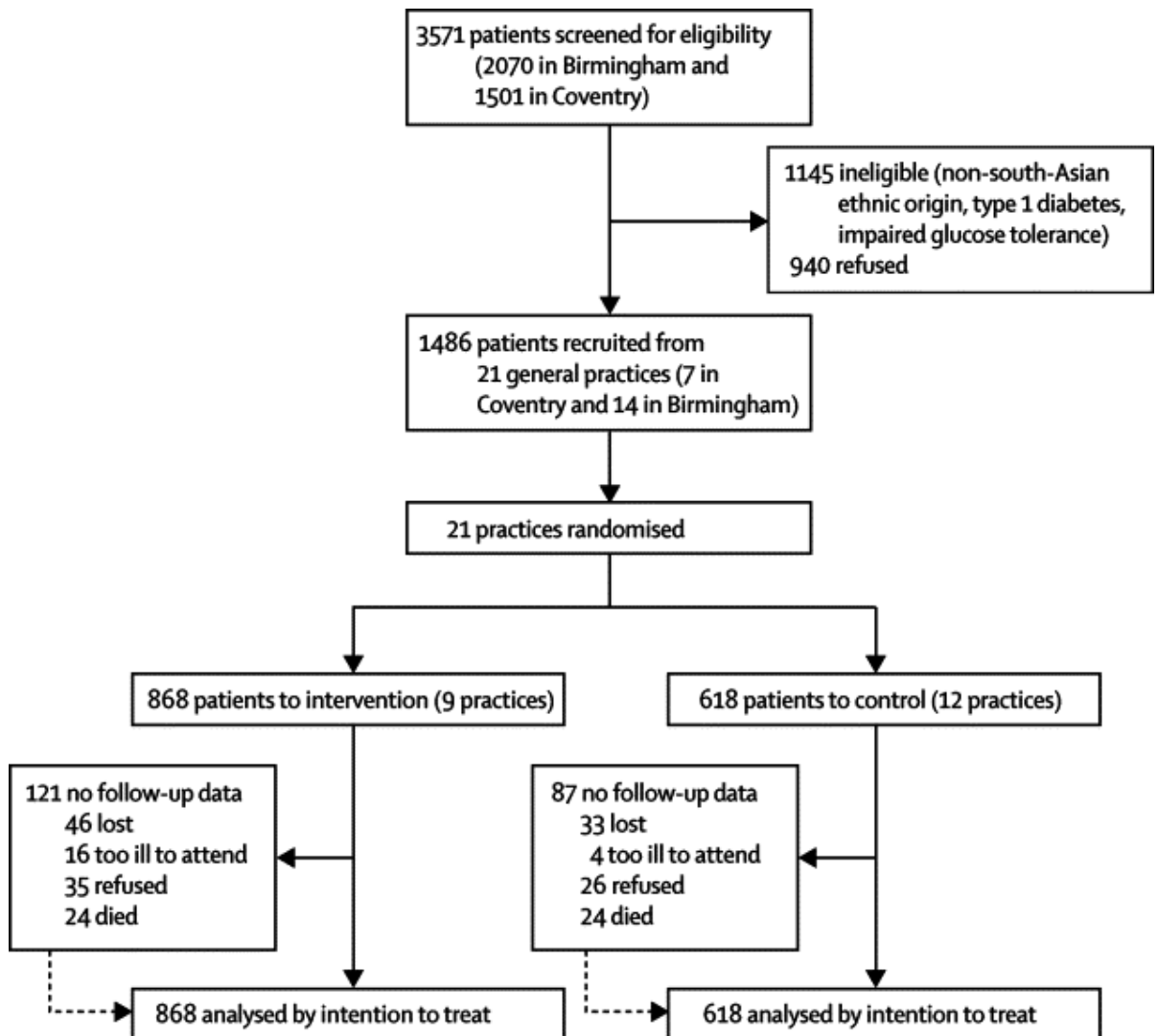
In order to evaluate the improvements in risk factors in patients whose risk factors at baseline were above the targets recommended by QOF (blood pressure \geq 145/85 mmHg, total cholesterol \geq 5mmol/L and HbA1c \geq 7.5%), a *post hoc* analysis was carried out. Differences between the treatment groups were compared using similar methods and statistical techniques described above. A separate analysis was carried out to determine the proportion of patients achieving the UKAD study and the QOF targets.

4.3 Results

4.3.1 Patient demographics and baseline risk factors

1486 patients of South Asian ethnicity, with established type 2 diabetes, consented to take part and were included in the study; 500 (33.7%) from Coventry and 986 (66.3%) from Birmingham. Of these patients, final intention to treat analyses included 1438 (97%), excluding 48 who died during 2 years follow-up (Figure 4.2).

Figure 4.2 – Practice and patient recruitment and progress through the trial



Baseline risk factor profile, comparing the intervention and control groups, is shown in Table 4.1. Mean age for the whole group was 57.0 years, standard deviation (SD) 11.9 years. Differences observed between groups for gender, age, duration of diabetes and diabetes treatment modalities were not statistically significant. Current smoking prevalence (15%) was similar in both groups, but more control patients were ex-smokers. There were no differences in weight, body mass index (BMI) or waist circumference measurements. Significantly more intervention than control patients were treated with statins (Table 4.1)

At baseline, 268 (18%) patients had evidence of existing CHD or prior cardiovascular events; angina, myocardial infarction, cardiovascular accident, coronary artery bypass graft or other heart problems; this group was comprised of 150 (17%) intervention and 118 (19 %) control patients.

Urinary albumin:creatinine ratio was measured for 1295 (87%) patients and microalbuminuria (defined as a ratio $>2.5\text{mg}/\text{mmol}$ in males and $>3.5\text{ mg}/\text{mmol}$ in females) was present in 268 (19%) patients. Significant proteinuria, defined as albumin:creatinine ratio of $>30\text{mg}/\text{mmol}$ was detected in 114 patients (8%). The prevalence of combined microalbuminuria or proteinuria was 28%, with no difference between intervention and control groups. Using the Framingham equation, mean (S.D) 10 year CHD risk score was 10.6 (8.8) with no difference between treatment groups (Table 4.1).

Table 4.1: Baseline characteristics of intervention and control subjects.

	Intervention (N=868)	Control (N=618)	Total
Sex			
Women	396 (46%)	313 (51%)	709 (48%)
Men	472 (54%)	304 (49%)	776 (52%)
Age (years)			
<45	131(15%)	84 (14%)	215 (14%)
45-64	467 (54%)	363 (59%)	830 (56%)
≥ 65	270 (31%)	171 (28%)	441 (30%)
Duration of Diabetes (years)			
0-4	367 (42%)	222 (36%)	589 (40%)
5-9	230 (27%)	189 (31%)	419 (28%)
10-19	197 (23%)	161 (26%)	358 (24%)
≥ 20	72 (8%)	41 (7%)	113 (8%)
Treatment			
Insulin	161 (19%)	129 (21%)	290 (20%)
Oral	591 (68%)	429 (69%)	1020 (69%)
Diet only	116 (13%)	60 (10%)	176 (12%)
Smoking status			
Current Smoker	135 (16%)	86 (14%)	221 (15%)
Ex-Smoker	59 (7%)	69 (11%)	128 (9%)
Non-Smoker	673 (78%)	462 (75%)	1135 (76%)
Weight (kg)	76.2 (14.6)	75.2 (14.6)	75.8 (14.5)
Waist (cm)	102.0 (11.5)	101.3 (12.3)	101.7(11.8)
BMI (kg/m²)	28.5 (4.8)	28.6 (4.9)	28.5(4.9)
Framingham 10 years CHD risk score	10.5(8.8)	10.6(8.8)	10.6(8.8)
Risk Factors			
Mean arterial pressure (mmHg)	101.7 (12.9)	102.9 (12.9)	102.2 (12.9)
Systolic Blood Pressure (mmHg)	139.4(21.1)	141.1(20.3)	140.1(20.8)
Diastolic Blood Pressure (mmHg)	82.9(11.0)	83.8 (11.1)	83.3 (11.0)
Total Cholesterol (mmol/L)	4.7 (1.1)	4.7 (1.1)	4.7 (1.1)
HbA1c (%)	8.2 (1.9)	8.2 (1.8)	8.2 (1.9)
Prescribed drugs			
All antihypertensives	475(55%)	342 (55%)	817 (55%)
ACE/ARB	321(37%)	246 (40%)	567 (38%)
Statins	438(50%)	273 (44%)	711 (48%)

Data are number (%) or mean (SD). Missing data: duration of diabetes (n=7), smoking status (2), weight (2), waist circumference (5),BMI (10),total cholesterol (2) and HbA1c (13). Significant difference between groups for smoking (p=0.01) and statins (p=0.02).

Effect of intensive control for 2 years

During 2 years of follow-up, 48 (3%) patients died 24 (3%) intervention and 24 (4%) control ($P=0.972$). New cardiovascular events were recorded for 97 (7%) patients, 62 (7%) intervention and 35 (6%) controls. None of these small differences between intervention and control groups were significant. Patients with CHD at baseline were more likely to die (18 [7%] vs. 30 [2%] of those without CHD), or to experience CHD events during follow-up (34 [13%] vs. 63 [5%] of those without CHD), irrespective of treatment group.

Results for comparison of outcomes between intervention and control groups are presented in Table 4.2; unadjusted differences compared with a t-test, plus results from multiple linear and mixed regression modelling are shown. Comparing treatment groups, after two years there was a reduction of 5.1 mmHg in systolic blood pressure and 4.6 mmHg in diastolic blood pressure for the intervention group vs. 4.6 mmHg and 2.9 mmHg respectively in the control group. T-tests showed significant differences in favour of the intervention group for diastolic BP and HbA1c. After adjustment for potentially confounding effects, diastolic BP, mean arterial pressure (MAP) and HbA1c showed significant advantages for the intervention group. In final models taking clustering effects into account, significant effects persisted for the intervention group for both MAP and diastolic BP (Table 4.2). BMI was significantly increased in the intervention group. Differences between the groups in HbA1c, total cholesterol, waist circumference and CHD risk scores were small and not statistically significant after adjustment for confounding and clustering (Table 4.2).

Table 4.2. Outcomes, adjusted for potential confounding and for clustering:

Outcomes:	(A) Difference between means (P value)	(B) Differences least squares means (P value)	(C) Differences least squares means (P value)
Primary:			
MAP	-1.28 (-2.60 to 0.03) (0.0560)	-2.21 (-3.30 to -1.11) (0.0001)	-1.58 (-2.73 to -0.43) (0.0072)
Systolic BP	-0.48 (-2.43 to 1.47) (0.6280)	-1.67 (-3.30 to 0.05) (0.0437)	-0.56 (-2.69 to 1.56) (0.6027)
Diastolic BP	-1.68 (-2.89 to -0.48) (0.0060)	-2.46 (-3.48 to -1.46) (<0.0001)	-2.12 (-3.12 to -1.12) (0.0001)
Total cholesterol	-0.02 (-0.09 to 0.14) (0.7001)	0.03 (-0.07 to 0.13) (0.5595)	0.02 (-0.07 to 0.12) (0.6537)
HbA1c	-0.18 (-0.34 to -0.01) (0.0040)	-0.13 (-0.28 to -0.02) (0.0825)	-0.16 (-0.34 to 0.01) (0.0680)
Secondary:			
CHD risk (Fram) (n=1342)*	0.08 (-0.55 to 0.71) (0.8030)	-0.11 (-0.64 to 0.43) (0.6899)	-0.002 (-0.58 to 0.58) (0.9944)
Waist (cm)	-0.28 (-1.04 to 0.49) (0.4780)	-0.20 (-0.82, 0.43) (0.5396)	-0.30 (-1.41, 0.82) (0.6017)
BMI	0.37 (0.19 to 0.55) (<0.0001)	0.39 (0.21, 0.56) (<0.0001)	0.40 (0.20, 0.60) (0.0001)

* Note:- Framingham CHD risk only estimated for patients aged 30 to 74 yrs at baseline.

Notes

A - crude differences based on t-test comparison, no adjustment.

B - differences based on fixed effects model, adjusted for confounding.

C - differences based on mixed model, adjusted for confounding and clustering.

Intervals presented in parentheses are 95% confidence interval (95%CI)

The percentage of patients with microalbuminuria or proteinuria increased from 27% at baseline to 32% after 2 years, with no significant difference between the intervention and control groups. Patients at high renal risk, defined by plasma creatinine >120mmol for females and >150 mmol for males, increased from 3% at baseline to 4% after 2 years, with no difference between treatment groups.

Combining all patients from intervention and control groups after two years, there was an overall decrease of 4.9 mmHg (4.0-5.9) in systolic blood pressure ($P<0.001$), 3.9 mmHg (3.3-4.4) in diastolic blood pressure ($P<0.001$) and 4.2 mmHg (3.6-4.9) in MAP ($P<0.001$). Total cholesterol decreased by 0.46 mmol/L (0.40-0.52) ($P<0.001$). A very small and statistically non-significant increase was observed for HbA1c; 0.06% (-0.02 to 0.15), $P=0.135$.

4.3.2 Prescribing changes at 2 years

After two years follow-up, the proportion of patients treated with anti-hypertensives had increased to 75% overall, with no difference between groups. Treatment with statins had increased and the difference between treatment groups disappeared, with 540 (64%) intervention vs. 389 (65%) controls treated. The use of ACE inhibitors or Angiotensin Receptor Blockers increased substantially from 37% to 65% in the intervention group and 40% to 62% in the control group (no significant difference between groups). Similar proportions of patients were treated with insulin at baseline; 161 (19%) intervention and 129 (21%) control.

After 2 years, more intervention than control patients had started insulin therapy (47 [8%] vs. 23 [5%]), but this was not statistically significant (relative risk [RR] 1.44 [0.89 to 2.34]).

4.3.3 Sub-group analysis: patients at high risk

In patients with blood pressures greater than 145/85 mmHg at presentation, the mean reduction in SBP was 12.8 mmHg in the intervention group and 10.4 mmHg in the control group. Mean difference in SBP in these patients was 2.5mmHg (P=0.084). After adjustment for confounding and clustering factors the difference was only borderline significance. For DBP the difference was 2.8mmHg (t=-3.41, P=0.001) and the difference between treatment groups remained significant after adjusting for confounders and clustering (Table 4.3). No significant differences between groups were observed for serum total cholesterol and HbA1c.

In post-hoc analyses, the number of patients achieving the study targets for blood pressure was 310(36%) of the 868 in the intervention group versus 191(31%) of 618 in the control group; for cholesterol, 411(47%) of 867 in the intervention group versus 311(50%) of 617 in the control group; and for HbA1c, 275(32%) of 858 in the intervention group versus 165(27%) of 615 in the control group. For QOF targets, the corresponding numbers for blood pressure less than 145/85mmHg was 575(66%) versus 346(56%); for cholesterol less than 5mmol/l, 700(81%) versus 509(83%); and for HbA1c less than 7.5%, 377(44%) versus 240 (39%). These are summarised in Table 4.4.

The proportion of patients in the intervention group achieving the study targets for blood pressure and HbA1c was greater in the intervention group but these differences were not statistically significant.

Table 4.3: High risk groups outcomes, adjusted for potential confounding and for clustering:

Systolic BP analyses include only patients with systolic BP>145 mmHg at

baseline

Diastolic BP analyses include only patients with diastolic BP>85 mmHg at

baseline

	(A)	(B)	(C)
	Difference between means	Differences least squares mean*	Differences least squares mean**
	<i>T-Test P value</i>	<i>Fixed effects P value</i>	<i>Cluster adjusted P value</i>
Systolic BP	-4.72 (-7.96 to -1.48) <i>0.0044</i>	-4.57 (-7.52 to -1.62) <i>0.0024</i>	-2.87 (-5.78 to -0.04) <i>0.0527</i>
Diastolic BP	-1.73 (-3.44 to -0.01) <i>0.0494</i>	-2.21 (-3.83 to -0.59) <i>0.0075</i>	-3.41 (-5.27 to -1.51) <i>0.0014</i>

Notes :

A - differences based on t-test comparison, no adjustment for confounding or clustering

.B - differences based on fixed effects model, adjusted for confounding.

C - differences based on mixed model, adjusted for confounding and clustering.

Table 4.4: The proportion of patients in intervention and control groups achieving UKADS and QOF targets at 2 years.

	Intervention	Control
	N (%)	N(%)
<u>UKADS Targets</u>		
Blood Pressure ≤ 130/80 mmHg	310 (36)	191(31)
Total Cholesterol ≤ 4mmol/L	411 (47)	311(50)
HbA1c ≤ 7%	275 (32)	165(27)
<u>QOF Targets</u>		
Blood Pressure ≤ 145/85 mmHg	575(66)	346(56)
Total Cholesterol ≤ 5mmol/L	700 (81)	509(83)
HbA1c ≤ 7.5%	377 (44)	240 (39)

Missing data after 2 years: blood pressure (n=128), total cholesterol (n=134), HbA1c (n=135).

4.3.4 Cost of intervention and quality of life

A detailed cost breakdown is presented in Table 4.5. The total cost of enhanced diabetes care over two years was £ 303,554 with over two thirds of this cost accounted towards the salaries of practice nurses, link workers and clinical time of specialist nurses. Over two years, the cost of intervention per patient was £434 (£406 net service and £28 net prescribing costs). The net prescribing cost for non diabetic drugs was £16 and £12 for diabetes drugs. Overall quality of life in the studied subjects deteriorated over 2 years. In spite of that, the resultant net change in quality of life in the intervention over control group was positive, although small. The incremental cost-effectiveness ratio is calculated to be £28,933 per Quality of Life Year (QALY) gained.

Table 5. Intervention costs and incremental cost-effectiveness over 2 years*Intervention costs (incremental cost between intervention & control)*

1. Staff salaries*	£224,774
2. Payment to practices(<i>incremental cost between intervention and control practices</i>)**	£50,000
3. Travel and subsistence	£17,720
4. Clinical equipment	£11,060
Total enhanced diabetes care service over 2 years	£303,554
Per patient enhanced service cost over 2 years	£406
<i>Prescribing cost (incremental cost between intervention & control)</i>	
Per patient net prescribing cost for non-diabetic drugs over 2 years	£16
Per patient net prescribing cost for diabetic drugs over 2 years	£12
Per patient net prescribing cost over 2 years	£28
Total per patient incremental net cost of intervention over 2 years	£434
Per patient quality adjusted life year (QALY) gain over 2 years	0.015
Incremental cost per QALY gained	£28,933

* Staff salaries covered two specialist nurses' and five link workers' clinical time.

The salaries included national insurance and pension contributions.

** It is the net amount paid to nine intervention practices to implement the intervention over two years.

4.4 Discussion

The achievement of targets set by national and international advisory bodies(48;55;141;142) in general practices in inner city areas with a high prevalence of socially diverse ethnic groups poses a major challenge. The twenty-one practices included in our study represent those in areas with a large number of patients of South Asian origin. These areas generally have a higher prevalence of diabetes mellitus, impaired glucose tolerance and are socially deprived with high Townsend scores(141;149). In keeping with previous reports, the South Asian patients with type 2 diabetes described in our study were younger than the average age described in general practice and had a shorter duration of diabetes(78).

At baseline, a large majority of our patients had HbA_{1c} >7% (70%), BP >130/80 mmHg (76%) and total cholesterol >4 mmol/l (70%); above targets recommended by international standards for diabetes care. After two years in which secular changes included the pay for performance initiative, there were significant improvements in blood pressure and total cholesterol across the whole study population, but no change in HbA_{1c}. Systolic blood pressure decreased by 4.9 mmHg, diastolic by 3.9mmHg and mean cholesterol by 0.46 mmol/l. These reductions are both statistically and (if sustained) clinically highly significant given the population considered. Many studies have shown a direct relationship between blood pressure and cardiovascular risk with a reduction in blood pressure associated with rapid reduction in such risk(150-152). The relationship between blood pressure and cardiovascular risk is such that a reduction of 5 mmHg, if sustained would confer substantial protection from cardiovascular events(153).

The improvements in blood pressure and cholesterol in our study were associated with increased prescribing of anti-hypertensive agents and statins and are consistent with improvements reported by several others following introduction of the QOF initiatives (154). Overall cardiovascular risk estimated by the Framingham formula showed a small but significant improvement overall, but no difference between groups.

The mortality observed during the study (3%), together with the baseline (18%) and follow-up (7%) frequency of cardiovascular events, confirm that the South Asian group we studied has a high cardiovascular risk and that substantial benefits could be obtained by aggressive risk factor reduction. The failure to prevent an increase in microalbuminuria, despite a 5mmHg reduction in blood pressure, is surprising and suggests that lower targets may be needed for this group.

Comparing intervention and control groups after two years, significant differences were observed between groups for diastolic blood pressure and mean arterial pressure after adjustment for confounding factors and clustering. Systolic blood pressure was lower in the intervention group but this was not statistically significant. The reductions seen in diastolic pressure were comparable to those observed in our pilot study but the reduction in systolic pressure was less than previously achieved. It is possible that the relatively young age of onset and ethnicity may be a factor in this observation and it is interesting that a more pronounced diastolic effect has been reported in some other studies(155) .

In the control group HbA1c tended to rise, while in the intervention group it remained stable over two years. The lack of improvement in HbA1c may be due, at least in part, to the natural disease progression commonly seen in type 2 diabetes(156). It is disappointing, given the healthcare resources provided, that neither the QOF incentives nor our culturally sensitive enhanced care package impacted significantly on glycaemic control in these patients of South Asian ethnicity.

Despite clear evidence of failure to reach target levels of HbA1c via diet and oral anti-diabetes therapy, only a small increase in the percentage of patients treated with insulin was observed in both groups (8% vs. 5% for intervention and control groups respectively). Even though the intervention included support from specialist diabetes nurses with experience of insulin initiation and enhanced time for patient education, this appears to have had only a limited effect in terms of behavioural change or patient acceptance of insulin.

Initiating insulin in primary care in the UK is relatively new and building up confidence of both the health care team and South Asian patients may be as important as any financial incentives paid to the former. Changing patient behaviour through motivation and patient education might take longer than the two years follow-up in this study. Alternative methods of motivation, including structured patient education(157) and more aggressive insulin initiation, may be needed. To fully optimise adherence to treatment protocols, extending the role of the nurse to prescribe in primary care may improve patient outcomes.

A small but statistically significant increase in BMI was noted in the intervention group. One likely reason for this could be the increased use of insulin in the intervention group but other factors such as poor adherence to lifestyle advice may have also contributed. Clearly, research is required into the barriers to therapeutic use in patients of South Asian origin with type 2 diabetes, as well as new methods to incentivise patients to accept sustained lifestyle change, anti-diabetes drugs, and insulin treatment.

One limitation of our study is the inability to assess the relative contributions of individual components of the intervention; such difficulties are inherent to evaluation of complex interventions. Interestingly, only a small proportion of patients achieved the study targets for cholesterol, blood pressure and HbA1c compared to those achieving the QOF targets. Neither the tight treatment algorithms, additional time input from the practice nurses and link workers nor the QOF initiatives had any impact on these outcomes suggesting that overall there was poor adherence to treatment protocols. The failure to meet the study targets suggests that there was reluctance on the part of health professionals to intensify treatments and aim for evidence-based targets rather than the arbitrary targets set by the QOF initiative. Other factors such as reduced patient motivation, reluctance of primary care staff to initiate insulin and resistance from patients might have impeded the achievement of better metabolic control. Such factors will not be exclusive to South Asians in UK primary care and may be relevant to other racial groups and healthcare settings.

The economic analysis showed that the financial investment required over two years (£434 per patient) did not produce sufficient health related quality of life gain to make such a nurse-led intervention clearly cost-effective. At £28,933 per QALY gained, compared to an indicative norm of £30,000 per QALY(158), wide-scale implementation is not indicated without improvement in effectiveness.

4.5 Conclusion

Considerable difficulties in recruiting and retaining individuals of South Asian ethnicity have been reported previously by several investigators(109;110), which may account for the lack of large scale studies in this area(159). However, our experience indicates that recruitment and retention is possible in this hard to reach group. Our results suggest that small but sustained improvements in blood pressure can be achieved through the introduction of a culturally sensitive enhanced care package for South Asian patients in addition to improvements from the QOF financial incentives. Improvement in glycaemic control remains a major challenge and further work to enhance effectiveness of healthcare delivery in general practice and to improve motivation is clearly needed for this group if healthcare inequalities are to be reduced. Thus while progress has been made, there remains a substantial challenge in achieving the more stringent targets required by national and international expert advisory bodies.

Chapter 5

Genetics of type 2 diabetes

5.1 Introduction

The prevalence and severity of diabetes varies significantly between populations(16). While environmental factors are known to greatly influence disease susceptibility they alone do not fully explain the differences between ethnic groups. Genetic factors often determine an individual's response to the environment and recognising the role of these factors is essential to our understanding of the molecular mechanisms involved in the causation of the disease.

5.2 Role of Genetics in Diabetes

The role of genes in the pathogenesis of type 2 diabetes is supported by studies involving monozygotic twins and the familial clustering of diabetes. Studies in monozygotic twins have reported concordance rates for the disease of up to 70 % (160) compared to rates of 30% in dizygotic twins(160;161) . The genetic basis of type 2 diabetes is further supported by familial aggregation. The life time risk of developing type 2 diabetes is about 40% if one parent is affected and this risk increases to almost 70% if both parents are affected(160;162). Diabetes prevalence also varies significantly between ethnic groups and certain ethnic groups, such as Nauru, Pima Indians and the South Asians, have much higher

rates of type 2 diabetes than white Europeans(16). Although environmental factors play a major role in these differences, genetic factors also appear to be important. Admixture studies involving different ethnic groups have shown an inverse relationship between the extent of interbreeding and diabetes prevalence, suggesting differences in genetic susceptibility determine the risk of disease(163). Type 2 diabetes, however, includes a wide spectrum of disorders with variable degrees of gene –environment interaction, including those with single gene defects and minimal environmental influence and those that involve many genes and a greater environmental influence(63;164). This complex interaction between genes and the environment makes understanding of the pathogenesis of type 2 diabetes challenging.

5.2.1 Monogenic diabetes

Monogenic forms of type 2 diabetes are predominantly due to single gene defects and account for less than 5% of all diabetes(63;165). They manifest early, have high phenotypic penetrance and are less influenced by environmental factors. Several sub groups of monogenic forms of type 2 diabetes are now recognized and include insulin resistance syndromes, mitochondrial diabetes and Maturity Onset Diabetes in the Young (MODY)(165).

First described in 1965, MODY comprises a distinct group of monogenic forms of diabetes characterized by early onset, autosomal dominant inheritance, insulin independence, distinct extra- pancreatic features and a lack of association with

obesity. MODY can be differentiated from other forms of early onset diabetes based on genetic, clinical and associated features (Table 5.1)(165)

	Type 1 diabetes	Early Type 2 diabetes	MODY	Genetic syndromes associated with diabetes
Inheritance	Polygenic Strong HLA association	Polygenic	Monogenic, Autosomal dominant	Variable Commonly-recessive
Number of parents affected	0	1 or 2	1	0
Obesity	Rare	Common	Rare	Common
Associated features	Positive anti GAD and IAA antibodies	Dyslipidaemia, acanthosis nigricans, Polycystic ovaries	Dyslipidaemia Renal cysts Short stature Pancreatic agenesis	Variable extra-pancreatic features

Table 5.1: Differentiation of MODY from other forms of early onset diabetes

Adapted from: Textbook of Diabetes. Pickup and Williams. Third edition. Chapter 24 (166). GAD : Glutamic acid decarboxylase. IAA: Insulin auto antibody

Early onset and high penetrance allowing detailed study of family pedigrees are the primary reasons for the success of genetic characterization of MODY. Unlike the common type 2 diabetes, MODY results from distinct mutations in genes associated with beta cell function. At least 6 different subtypes of MODY have been described thus far. MODY 2 is due to a mutation in the glucokinase gene while MODY 1(*HNF4α*), MODY 3 (*HNF1α*), MODY 4(*IPF-1*), MODY 5

(*HNF1 β*) and MODY 6 (*NEUROD1*) are due to mutations in transcription factors(165;167). Each subtype of MODY is associated with distinct phenotypic features that are helpful in clinical characterisation (Table 5.2). Allelic heterogeneity is a characteristic feature of MODY and explains the phenotypic variation between MODY families.

Subtype	Gene	Prevalence of individual subtypes within MODY in UK (%)	Protein encoded	Extra pancreatic features
MODY1	<i>HNF4 α</i>	2	Transcription factor	
MODY2	<i>GCK</i>	20	Glucokinase	Reduced birth weight
MODY3	<i>HNF1α</i>	64	Transcription factor	Low renal threshold Sensitivity to sulfonylureas
MODY4	<i>IPF-1</i>	1	Transcription factor	Pancreatic agenesis in homozygotes
MODY5	<i>HNF1β</i>	1	Transcription factor	Renal cysts, proteinuria Uterine and genital abnormalities
MODY6	<i>NEUROD1</i>	0	Transcription factor	

Table 5.2: The prevalence, protein encoded and extrapancreatic features of different MODY subtypes in the UK population.

5.2.2 Polygenic type 2 diabetes

In contrast to the monogenic forms of diabetes, susceptibility to the more common form of type 2 diabetes is thought to be determined by several genes and a greater environmental influence(63). Diabetes can occur in individuals exposed to a diabetogenic environment even in the absence of genetic susceptibility and conversely, can be delayed substantially in those with genetic susceptibility in the absence of environmental influences. This complex relationship between genes and environment has been a major problem in identification of the causative genes.

The genes associated with polygenic type 2 diabetes may be predominantly related to beta cell function, insulin resistance or a combination of both. To date, research has focused on identifying common variants of genes that are present in those with diabetes and in healthy individuals. The presence of these variants confers only a small risk to the individual but the effects could be much greater at a population level. Frequencies of these variants vary in different ethnic groups and may explain the overall differences in disease susceptibility(168).

5.3 *Techniques/approaches for genetic studies in type 2 diabetes*

Two major approaches have commonly been used in the search for type 2 diabetes genes-the candidate gene and the genome wide studies(164;169). In the candidate

gene approach, a gene whose physiological function is known and is thought to have a role in the pathogenesis of diabetes is selected and then systematically searched for sequence variations(164;168). Once the variations have been identified, the next step is to establish disease association. Currently, the preferred approach for establishing such an association is through population based case-control studies, in which the frequency of the variant is compared between diabetic and non-diabetic groups. Population based case-control studies offer the advantage of large analytical power but on the other hand are disadvantaged by poor reproducibility, problems of population stratification and relatively large numbers needed to detect small effects(164). The *PPARG*, *KCNJ11*, *TCF2* and *WFS1* genes are some of those identified using the candidate gene approach.

An alternative approach to search for type 2 diabetes genes is the genome scan in which no prior assumptions are made (hypothesis free)(63;164). Genome scans have involved two basic approaches-the traditional linkage and genome-wide association (GWA) studies(116;170). Traditional linkage analysis involves looking for co-segregation between the disease and microsatellite markers in affected pedigrees or looking for linkage with microsatellite markers in affected sib pairs. Evidence of linkage with a particular microsatellite marker suggests that a disease susceptibility gene is likely to lie in close proximity to the marker. Further fine mapping studies are then required to define the exact location and identity of the gene. The first three MODY genes, *CAPN10* gene and the *TCF7L2* gene are some of the successes using linkage studies(162). Completion of the Human Genome Project and the creation of SNP databases such as HapMap, listing all the sequence variants across the genome in different populations, now

allow us to directly search for polymorphisms associated with disease using case-control studies. Between 2006 and 2008, large scale association and genome-wide association studies identified at least 16 loci with strong associations with susceptibility to type 2 diabetes(171-182), as well as confirming associations with *PPARG* and *KCNJ11* (Table 5.3). Some of these loci are major susceptibility markers and associated with greater effects on disease risk than previously identified candidate genes. Identification of susceptibility variants, however, represents the initial step in establishing disease association. As the associated variants may be in linkage disequilibrium with true disease risk variants, further fine mapping and functional studies are needed to understand the role of genetic variants in the causation of type 2 diabetes.

Gene/Locus	Polymorphism	Approach	Effect (odds ratio)	Risk allele Frequency
<i>PPARG</i>	rs 1801282	Candidate	1.14	0.87
<i>KCNJ11</i>	rs 5215	Candidate	1.14	0.35
<i>TCF7L2</i>	rs 7901695	Large scale association	1.37	0.31
<i>FTO</i>	rs 8050136	Genome wide association	1.17	0.40
<i>HHEX/IDE</i>	rs 1111875	Genome wide association	1.15	0.65
<i>SLC30A8</i>	rs13266634	Genome wide association	1.15	0.69
<i>CDKAL1</i>	rs 10946398	Genome wide association	1.14	0.32
<i>CDKN2A/2B</i>	rs 10811661	Genome wide association	1.20	0.83
<i>IGF2BP2</i>	rs 4402960	Genome wide association	1.14	0.32
<i>HNF1β</i>	rs 4430796	Candidate/Large scale association	1.10	0.47
<i>WFS1</i>	rs 10010131	Candidate/Large scale association	1.12	0.60
<i>JAZF1</i>	rs 864745	Genome wide association	1.10	0.50
<i>CDC123/CAMK1D</i>	rs 12779790	Genome wide association	1.11	0.18
<i>TSPAN8/LGR5</i>	rs 7961581	Genome wide association	1.09	0.27
<i>THADA</i>	rs 7578597	Genome wide association	1.15	0.90
<i>ADAMTS9</i>	rs 4607103	Genome wide association	1.09	0.76
<i>NOTCH2</i>	rs 10923931	Genome wide association	1.13	0.10
<i>KCNQ1</i>	rs 2237892	Genome wide association	1.29	0.93

Table 5.3: Type 2 diabetes susceptibility genes/loci identified to date using the candidate gene, large scale association and genome wide association approaches.

5.4 Genetic studies in South Asians

Epidemiological studies have shown that the prevalence of type 2 diabetes amongst South Asians (people of Indian, Pakistani, Bangladeshi and Sri Lankan origin) is significantly greater than in many other ethnic groups and is up to four times greater than in white European populations(60). This increased susceptibility to diabetes is largely determined by environmental factors. Rising obesity, sedentary lifestyles, urbanisation and increased life expectancy have all been thought to contribute to the excess risk of diabetes in South Asians(18). On the other hand there are factors that suggest a possible genetic influence. Diabetes in South Asians occurs at a much younger age (5 to 10 years earlier than in white Europeans)(59). Distinct phenotypic characteristics such as visceral obesity and elevated triglycerides are more common in South Asians, as is familial clustering of diabetes(99). Studies comparing South Asians with white Europeans have shown that features of insulin resistance are manifest in South Asians as early as in infancy(183;184). More recently, studies in South Asian pregnant women have shown that nutritional deficiencies in the mother and the intrauterine environment influence insulin resistance in new born babies(185). These studies suggest a strong gene-environment interaction that indeed begins very early in South Asians.

5.4.1 Monogenic type2 diabetes

Much of our understanding of these disorders is from studies in white Europeans. Studies involving south Asians alone are rare and there is a considerable overlap

between MODY and ‘common’ type 2 diabetes (which has a very high prevalence and high familial clustering in this ethnic group), making it difficult to estimate the true prevalence of MODY in this population. It is, however, reasonable to assume that the prevalence of these disorders in South Asians is not significantly different to that seen in white Europeans. In a South Indian study, the prevalence of MODY amongst those with type 2 diabetes was estimated to be around 4.8%(186) .

In another study the same group reported 7 novel mutations in the *HNF1 α* (MODY 3) gene in 9.6% of all south Indian subjects with MODY(187). These studies, however, have not been replicated in any other sub group of south Asians. Considering that many of the genes involved in MODY are also candidate genes for polygenic type 2 diabetes, there is a need for more studies in south Asians.

5.4.2 Polygenic type 2 diabetes

In comparison with the number of studies in the European populations, genetic studies involving south Asians have been few. Most of these have been in the south Indian population. Studies involving south Asians have predominantly looked at type 2 diabetes susceptibility genes shown to have a strong association in other ethnic groups. These include the *PPARG* gene, *PPARGC1A* , *KCNJ11*, *CAPN10* and *TCF7L2* genes(186). Peroxisome proliferator activator receptor gamma (*PPARG*) gene is an important regulator of glucose and lipid metabolism. The common Pro12Ala polymorphism of this gene has been shown to be

protective against type 2 diabetes in white European populations(188). Studies in a south Indian population showed that this polymorphism was present at the same frequency in both diabetic and non-diabetic individuals and the presence of this polymorphism was not associated with either improved insulin sensitivity or decreased risk of type 2 diabetes(189). The *Calpain10* gene was the first important gene associated with type 2 diabetes to be identified using the linkage mapping technique. A haplotype of three important polymorphisms (UCSNP 43, -19 and -63) of this gene was originally shown to be associated with increased risk of type 2 diabetes in a Mexican –American population(190). Replication of this study in other populations, has, however, shown mixed results with much lower frequencies of the risk haplotype in other populations(191-193). Studies in a south Indian population have shown that, while the haplotype does increase the risk of type 2 diabetes, its frequency in the background population is very low and its contribution to the risk of type 2 diabetes in this ethnic group is therefore likely to be small(194).

The identification of the common variants of the *TCF7L2* gene was by far the most significant discovery in the genetics of type 2 diabetes(195). First reported in Icelandic subjects, intronic variants of this gene were associated with the highest risk of susceptibility to diabetes reported to date(196). These associations have been replicated in several other populations including south Asians. In the two south Asian cohort studies, strong associations were reported with two SNPs (rs 7903146, rs12255372)(197;198). In another study involving north Indian Sikhs, 9 of the putative susceptibility variants identified in white Europeans by GWA were investigated)(199). A strong association with type 2 diabetes was

found with 4 of these; *PPARG2* (rs 1801282), *IGF2BP2* (rs 4402960), *FTO* (rs 9939609) and *TCF7L2* (rs 10885409). The cohort sizes in this study were small, however, and were not adequately powered to detect small effects. It was not therefore possible to rule out an effect of the other 5 loci on disease risk. Other type 2 diabetes susceptibility genes identified in south Asians include the PGC1 alpha gene, adiponectin gene, UCP Ectoenzyme Nucleotide Polypeptide (*ENPP1*) gene, uncoupling protein genes (*UCP2* and *UCP3*) and insulin receptor substrate (*IRS-2*) gene which were shown to have modest associations with type 2 diabetes(198;200;201).

5.4.2.1 Genetics of obesity

Genome-wide association studies have also been useful in the identification of genes associated with obesity-a major risk factor for type 2 diabetes. Common variants of the fat mass and obesity associated gene (*FTO*) were shown to be associated with obesity in white European populations(202). Individuals with these variants were on average 3 kilograms heavier than those who did not possess them. Individuals were also at an increased risk of type 2 diabetes but this was secondary to the effects on obesity rather than a direct effect of the variants on the pathogenesis of T2D. In a recent study in south Asians, however, one polymorphism (*FTO* rs9939609) was associated with an increased risk of type 2 diabetes but this risk was independent of the effect on body mass index (203).

Another important gene identified in a study in south Asians and white Europeans living in the UK was the Melanocortin 4 Receptor (*MC4R*) gene(66). The study found that a variant of *MC4R* (rs12970134) was associated with increased risk of adiposity and insulin resistance. Individuals with the variant of this gene had a waist circumference ~2cm greater and insulin resistance (HOMA-IR) ~10% greater than those who did not have it. As obesity is an independent risk factor for type 2 diabetes it is suggested that the *MC4R* gene may indirectly influence the risk of diabetes in individuals possessing the risk allele. These findings have also been replicated recently in Khatri Sikhs(204). The increased frequency of the risk allele in south Asians has been proposed as an explanation for the increased levels of type 2 diabetes in this population.

5.4.2.2 Diabetic complications

The predisposition to diabetic complications also varies significantly between ethnic groups. In general, the prevalence of diabetic nephropathy and retinopathy is higher in south Asians than in Caucasians(79;205). Risk of these complications, particularly nephropathy, is thought to be genetically determined. As such a search for genetic variants that predispose to these complications is an attractive proposition. Several potential genes associated with diabetes complications have been identified in other populations but studies in south Asians are few. Polymorphisms of the *ACE1* gene and aldosterone synthase gene (for nephropathy) and the Vascular Endothelial Growth Factor (*VEGF*) gene (for retinopathy) (206-208)are some of the genes studied in south Asians but the

findings of these studies have been inconsistent and need verification in larger cohorts.

5.5 Clinical significance of genetic studies

Although our knowledge of type 2 diabetes has improved considerably over the last few decades, many of the processes involved in the pathogenesis remain unknown. Understanding the genetic basis is essential to define the molecular pathways involved in disease causation, prediction of disease susceptibility, disease prevention, and development of new therapeutic agents. South Asians have a disproportionately higher prevalence and earlier onset of diabetes (compared with many other ethnic groups) and a strong family history. Phenotypic features such as visceral obesity and insulin resistance are also more common in this ethnic group and are manifest very early in life thus pointing to a genetic predisposition. Despite such obvious differences there is generally a poor understanding of the genetics of diabetes in this population. Until now, most of the studies involving south Asians are replications of studies in other populations. Although these studies have improved our understanding to some extent, there are still many limitations. The term ‘South Asians’ includes several different sub groups and although they share many cultural and environmental factors, there are subtle phenotypic differences and marked genetic heterogeneity between the sub groups. The prevalence of diabetes between these sub groups is also different(60). Given these differences it would be difficult to generalise the results from one sub group to others. Most of the studies in south Asians have been in the south Indian population and north Indian Sikhs. Further, the cohort

sizes in most of these studies have been small, limiting our ability to detect any true associations. Experience from genetic studies in other populations has shown that larger cohorts are needed to demonstrate associations with risk variants that have the small effect sizes expected for T2D and exclude false positives and false negatives. Genetic characterisation of the different sub groups is therefore essential to understand these differences and verify true disease associations.

Chapter 6

Common polymorphisms of the *PPARG* and the *PPARGC1A* genes and the risk of type 2 diabetes in a UK resident south Asian population

6.1 Introduction

The pathogenesis of type 2 diabetes involves defects in insulin secretion and insulin action. Although several candidate genes involved in both these aspects have been studied, to date few have been reproducibly associated with type 2 diabetes. Two of these genes-the *KCNJ11* gene (primarily involved in regulation of insulin secretion) and the *PPARG* gene (associated with insulin sensitivity)- have generally been accepted to have an effect on the risk of type 2 diabetes.

6.1.1 The PPAR Gamma gene

The Peroxisome Proliferator Activator Receptors (PPARs) belong to a family of nuclear receptors involved in lipid and glucose metabolism(209). There are three known members of the PPAR family – PPAR α , PPAR γ and PPAR δ . PPAR α was the first member of the family to be cloned and is expressed in liver, kidney, heart and skeletal muscle(210). PPAR α is thought to have an important role in the regulation of fatty acid oxidation and is a target for the fibrate class of drugs(211). PPAR δ is expressed mainly in the brain, adipose tissue and skin(212).

The functional role of PPAR δ is less well understood although there is some evidence to suggest that it may be involved in cholesterol transport in macrophages(213;214). PPAR γ is expressed predominantly in adipose tissue and plays a critical role in adipogenesis and adipocyte differentiation, factors that influence insulin sensitivity (215)(figure 6.1).

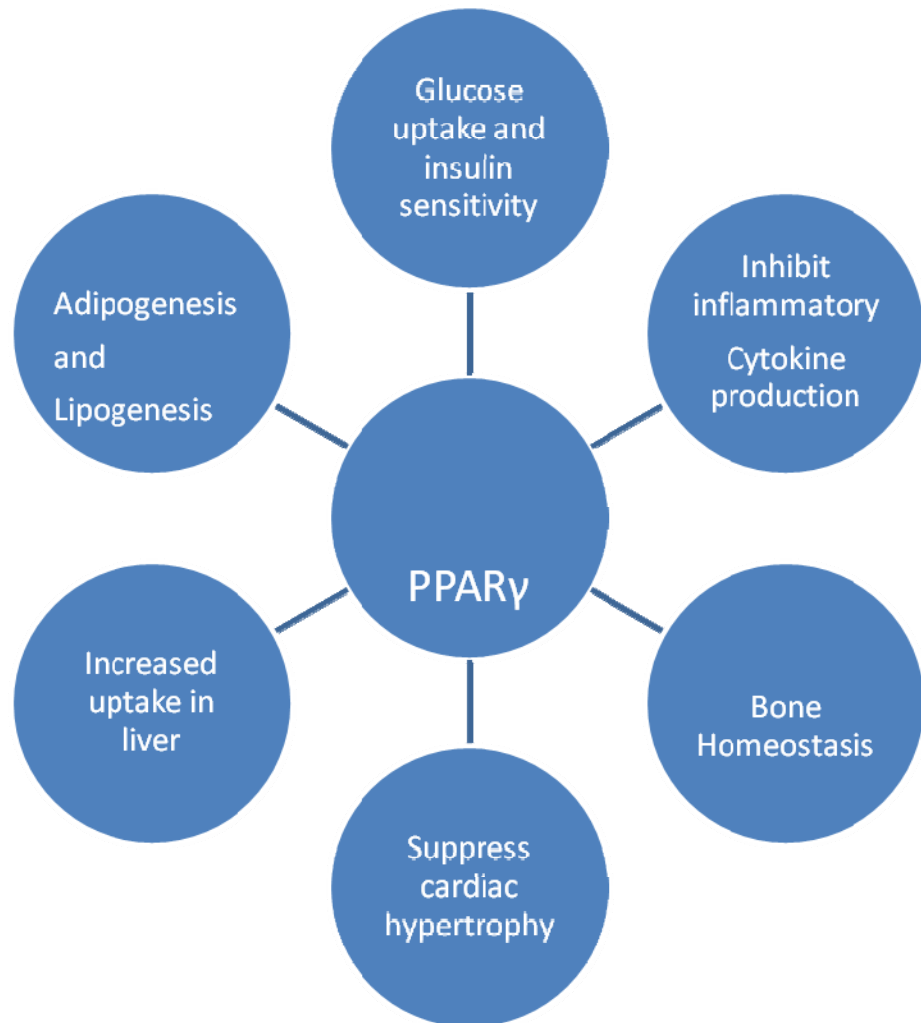


Figure 6.1: Various metabolic functions of PPAR gamma.

The PPAR γ gene (*PPARG*) is localised to chromosome 3 and encodes two distinct proteins, PPAR γ 1 and PPAR γ 2. The PPAR γ 2 protein has 28 additional amino acids and is expressed highly in the adipose tissue(212). Transcriptional activity of PPAR γ is dependent on its binding to another transcription factor, RXR. The PPAR γ -RXR heterodimer complex then binds to a co-activator complex on the DNA binding site and in turn initiates the expression of target genes(216). Dominant negative mutations of the PPAR γ gene result in the heterodimer complex binding to a co-repressor complex resulting in inactivation of gene expression(217;218). PPAR γ is activated by both natural and synthetic ligands. Natural ligands for PPAR γ include polyunsaturated fatty acids and eicosanoids(219;220) while the common synthetic ligands include the thiazolidenedione class of drugs(209).

6.1.2 PGC 1 alpha

Peroxisome proliferators activator receptor γ co-activator alpha (PGC-1 α) is an important member of a family of transcription co-activators involved in various biological processes(221). It plays a central role in cellular energy metabolism and is also thought to regulate the activity of PPAR gamma(222). PGC-1 α is widely expressed in brown adipose tissue, skeletal muscle, and the heart and in low levels in the liver(223). In skeletal muscle, PGC-1 α expression is associated with an increase in type 1 muscle fibre and increased energy expenditure(224). In the liver, PGC-1 α expression is increased in response to fasting and is thought to promote gluconeogenesis through the activation of the phosphoenol pyruvate carboxykinase (PEPCK)

pathway(225). In the heart, PGC-1 α facilitates oxidative phosphorylation in response to fasting(226). PGC-1 α is also known to increase the expression of insulin-sensitive GLUT4 and indirectly facilitates insulin action(227). The role of PGC-1 α in energy metabolism makes it an important target for therapies related to diabetes and obesity.

6.1.3 Polymorphisms of the PPAR γ (*PPARG*) and the PGC-1 α (*PPARGC1A*) genes

Given the important role for the PPAR in insulin resistance and the pathogenesis of type 2 diabetes, both *PPARG* and *PPARGC1A* have been candidate genes of interest. Both of these have been extensively studied in many populations and while there is reasonable evidence to support an association between variants of *PPARG* and diabetes, the evidence is much less convincing for *PPARGC1A*.

Common polymorphism within the amino- terminus of the PPAR γ 2 protein, replacing alanine for proline at codon 12 (Pro12Ala), has been associated with protection against the risk of type 2 diabetes(188). The prevalence of this polymorphism in white Caucasian populations is thought to be approximately 12% but prevalence in other populations may vary(228). Although there has been some inconsistency about the role of this polymorphism in initial studies it is now widely accepted that the proline

allele of the Pro12Ala polymorphism is associated with risk of type 2 diabetes(229;230)

Two common polymorphisms of *PPARGC1A* – rs 8192678 (Gly482Ser) and rs 3736265 (Thr612Met) - have previously been studied in various ethnic groups(231-235). Increased risk of type 2 diabetes with the Ser482 polymorphism has been reported in some but not all populations. Polymorphisms of *PPARGC1A* have been investigated in the south Indian population but not in any other south Asian groups (235). Given the role of this gene in energy metabolism and insulin resistance (perceived to be more common in south Asians) and its potential influence on the risk of type 2 diabetes, it merits further investigation in the south Asian population.

Although several candidate genes have been examined for association with type 2 diabetes, a majority of these have been linked to beta cell dysfunction. Genes associated with insulin resistance/ sensitivity on the other hand are rare. Both *PPARG* and *PPARGC1A* genes have been shown to have an important role in energy metabolism and insulin sensitivity and polymorphisms of these genes have been shown to be associated with type 2 diabetes in many different populations. Considering that insulin resistance is common in south Asians the possible role these two genes may have in the pathogenesis of type 2 diabetes is truly exciting. If such associations did exist it would also offer greater opportunities for treatment in this ethnic group especially as there are now therapeutic agents that specifically target are available.

The aim of this study was therefore to investigate the association between type 2 diabetes and common variants of the *PPARG* and *PPARGC1A* genes in the UK resident Punjabi/Mirpuri population. As both these genes share a common pathway they have been included in the same chapter.

6.2 Methods

6.2.1 Patient selection

Type 2 diabetic subjects were recruited as part of the United Kingdom Asian Diabetes Study (UKADS) The UKADS cohort included subjects from a number of genetically diverse South Asian subgroups, the largest of which was of Punjabi origin. Subjects of Punjabi ancestry (N=831) were therefore selected to create a genetically homogeneous cohort for analysis. Ancestry was confirmed over three generations using data on family origin and self-reported ethnicity. The majority of the selected individuals originated from the Mirpur area of Azad Kashmir, Pakistan. Diabetes was defined using the WHO criteria(15). Ethnically-matched normoglycaemic control subjects (N = 436) were recruited from the Punjabi/Mirpuri populations of Birmingham and Coventry through community screening. Normal glucose tolerance was defined as fasting glucose <6mmol/l and 2 hr glucose <7.8mmol/l on a 75g OGTT. Where OGTT was not feasible, normal glucose tolerance was defined as random blood glucose <7mmol/l. Venous blood was collected from each subject after obtaining informed consent and genomic DNA extracted using an adaptation of the Nucleon[®] protocol

(Nucleon Biosciences, Coatbridge, UK)(see section 6.2.2).The study was approved by the East Birmingham Research and Ethics Committee.

Control subjects were recruited over a period of two years between 2005 and 2007. As recruitment for control subjects and DNA extraction for diabetes subjects was still in progress, Genotyping for *PPARGC1A* was performed in only 516 diabetes and 134 control subjects recruited until the end of 2006. A further 315 diabetes (total 831) and 302 control subjects (total 436) were included in the analysis for the Pro12Ala polymorphism of *PPARG*.

6.2.2 DNA extraction

Genomic DNA was extracted from 9ml of venous blood collected in an EDTA-coated vacutainer. After carefully decanting the blood from the vacutainer into 50 ml pre-labelled centrifuge tubes, reagent A (Appendix 4) was added to bring up the volume to 40ml. Samples were mixed on a rotary mixer at 150rpm for 4 mins and then centrifuged at 840xg for 4mins to allow lymphocyte pelleting. The pellet was re-suspended in 15ml of reagent A after discarding the supernatant. The tubes were centrifuged for another 4 mins at 840xg and supernatant discarded. Lymphocyte pellets were stored at -80⁰ C for at least 2 hours before DNA was extracted.

After gentle thawing for 20-30 minutes, the lymphocyte pellets were fully suspended in 2ml of reagent B (described in Appendix 4) before being transferred to a pre-labelled 5ml cryovial. 500µl of sodium perchlorate (5M) (Sigma,

Poole,UK) was added and the tube was inverted 10 times to mix. Ice-cold chloroform was added up to a volume of 4.5ml and the tube was inverted to mix 10 times. The tube was centrifuged at 1310xg for 5 minutes, the upper layer was carefully transferred into a fresh 5ml cryovial and brought up to a final volume of 4.5ml with ice-cold ethanol (100%). The tube was gently inverted 10 times and then placed at -20°C for 30mins to facilitate DNA precipitation. The DNA was pelleted by centrifuging at 1310xg for 10mins, after which the supernatant was removed. The pellet was then washed with 1ml cold 70% ethanol and centrifuged for a further 5mins at 1310xg. The supernatant was removed and the pellet left to dry at room temperature overnight. The DNA was resuspended in 100µl Tris-EDTA (TE) buffer (10mM Tris.HCl, 0.1mM EDTA at pH 8.0, Appendix 4) at 4°C for 2-3 days, before being transferred to a 1.5ml tube for long-term storage at -80°C.

6.2.3 Genotyping methods

Broadly, two methods of genotyping were used to study the SNPs described in this and the following chapters. Genotyping for the *PPARGC1A* Gly482Ser variant was done using the polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) method. For all subsequent SNPs, Taqman genotyping assay supplied by Applied Biosystems (ABI, Warrington, UK.) was used. The rationale for this approach was that this would allow me sufficient exposure to the fundamental techniques involved in genotyping and familiarize myself with the laboratory processes involved in genetic analysis. A further

reason was that the PCR-RFLP method would be reasonable given the small sample sizes we had collected at the time of analysing the *PPARG1A* gene.

Genotyping method *PPARG1A*

Forward and reverse primer sequences for *PPARG1A* were 5'-TGC TAC CTG AGA GAG ACT TTG-3' and 5' – CTT TCA TCT TCG CTG TCA TC-3' respectively. PCR amplification was undertaken in a 25µl volume containing 100ng of DNA, 0.5µl of each primer (20mM) (SigmaGenosys, Suffolk ,UK), 1.25µl of dNTP mix (4mM) (Pharmacia Biotech, Herts,UK), 1µl magnesium chloride (50mM), 2.5µl of 10X buffer and 0.25µl of Taq Polymerase (5units/µL)(BioLine,London, UK). Following initial incubation at 94°C for 5 minutes, 30 amplification cycles were carried out with denaturation at 94°C for 30 secs, annealing at 52°C for 30 secs and extension at 72°C for 30secs, followed by a final extension phase at 72°C for 10minutes.

Digestion of the PCR product was done in a 10 µl reaction using 5 µl of the PCR product and 1 µl 10 X buffer, 0.5 µl Msp1 enzyme (20,000 units/ml, New England Biolabs, U.S.A), 3.5 µl water and incubated at 37 °C for 3 hours. Digestion products were subjected to electrophoresis on a 1% agarose gel (Geneflow Ltd. Staffordshire, UK) in 1x Tris-Borate EDTA buffer (89mM Tris-Borate, 2mM EDTA, pH8.3) and visualised under ultraviolet light after staining with 0.5 µg/ml ethidium bromide (Promega, Southampton, UK). The presence of the Msp1 restriction site (Glycine 482 allele) resulted in two fragments of

366bp and 245 bp and the absence of the restriction site (Serine 482 allele) resulted in a single fragment of 611bp.

Genotyping of the *PPARG*

Genotyping of the *PPARG* Pro12Ala polymorphism was performed using a pre-designed Taqman genotype assay supplied by Applied Biosystems (ABI, Warrington, UK.) A reaction mix of 775 µl of water, 750 µl of PCR master mix (ABI, Warrington UK) and 75 µl of SNPmix (ABI Warrington, UK) was made up and 4µl was aliquoted into each well of a 384 well PCR plate. The reaction was started by the addition of 1 microliter of DNA (5ng/µL) to each well. Fluorescence was detected on an ABI 7900 prism sequence detection system. Genotyping was repeated in approximately 15% of the subjects to estimate error rate which was less than 1%. Genotypes were scored using the allelic discrimination software supplied by ABI.

6.3 Statistical analysis

No sample size estimations were made prior to the commencement of these studies. Based on previous reports in other populations it was recognized that large cohort sizes would be necessary to detect weak associations (for effect sizes <1.6). However, the sample sizes of our cohort were comparable to those

previously reported in other south Asian studies and were therefore thought to provide a comparison/verification of the findings reported by others.

General characteristics of the diabetic and control groups were compared using the Mann-Whitney U test. For both diabetes and control subjects, conformity with Hardy-Weinberg equilibrium was verified using a chi square test (significance set at $p < 0.05$) and comparing the observed and expected genotype frequencies of homozygotes and heterozygotes. Genotype and allele frequencies between the groups were compared using binary logistic regression. Overall allele frequencies were used to calculate odds ratios (OR) after adjusting for confounders (age, waist circumference, family history of diabetes and gender). For each of the SNPs, odds ratios were calculated using additive, dominant and recessive models. The relationship between genotype and quantitative phenotypic characteristics was analysed using one way ANOVA and a 'p' value less than 0.05 was considered significant. All statistical analysis was performed using SPSS16 software (SPSS Inc., Chicago, U.S.A).

6.4 Results

Baseline characteristics of the diabetes and control subjects are summarized in tables 6.1 and 6.2. The control subjects genotyped for the *PPARGC1A* polymorphism were significantly older than the diabetes subjects (mean difference 1.98 years; $p = 0.005$). In the Pro12Ala cohorts, compared with diabetic subjects, control subjects were younger ($p = 0.005$) and had significantly lower

systolic blood pressure ($p = 0.001$) and waist circumference ($p = 0.002$). There were no significant differences in other phenotypic characteristics between the groups. Genotype frequencies for both the SNPs were in Hardy-Weinberg equilibrium. The minor allele frequencies for *PPARGC1A* Gly482Ser were similar (0.27 v 0.26) in both diabetic and non-diabetic controls. For *PPARG*, the common/minor allele frequencies of the Pro12Ala polymorphism in the diabetic and control subjects were $0.87/0.13$ v $0.85/0.16$ respectively.

	Diabetic subjects	Control subjects
n	516	134*
Gender (M/F)	272/244	79/55
Age at study (years)	55 ± 12	58 ± 10
BMI (kg/m^2)	28 ± 4	28 ± 4 .
Waist circumference (cm)	102 ± 10	99 ± 14
Systolic blood pressure (mm Hg)	138 ± 20	140 ± 20
Diastolic blood pressure (mm Hg)	83 ± 11	88 ± 12
Total cholesterol (mmol/l)	4.8 ± 1	ND
HbA1c (%)	8.3 ± 2	ND

Table 6.1: Clinical characteristics of diabetes and control subjects genotyped for the *PPARGC1A* Gly482Ser polymorphism * Data for BMI, waist circumference, blood pressure, cholesterol and HbA1c were available for only 42 of the 134 control subjects.

	Diabetic subjects	Control subjects
n	831	436
Gender (M/F)	450/381	220/216
Age at study (years)	56.9 ± 12.1	55.0 ± 11.8#
Age at diagnosis (years)	49.6 ± 11.9	N/A [†]
BMI (kg/m²)	28.3 ± 4.7	28.1 ± 4.9*
Waist circumference (cm)	102.4 ± 10.7	99.8 ± 13.1 *#
Systolic blood pressure (mm Hg)	140.5 ± 20.9	135.7 ± 20.4 *#
Diastolic blood pressure (mm Hg)	84.1 ± 11.5	85.1 ± 12.1
HDL cholesterol (mmol/l)	1.3 ± 0.5	ND
HbA1c (%)	8.3 ± 1.9	ND

Table 6.2: Clinical characteristics of diabetes and control subjects genotyped for *PPARG* Pro12Ala polymorphism. * BMI, waist circumference and blood pressure available in only 252 control subjects. # Significant difference between groups for age (p=0.005), waist circumference (p=0.002) and Systolic Blood pressure (p=0.001).

Table 6.3 shows the genotype distribution for both SNPs. This did not differ significantly between the diabetic and control groups for either SNP when analysed using dominant or recessive models.

Gene/ Polymorphism	Genotype	Diabetes N (%)	Control N (%)	Odds ratio (95% Confidence Intervals)
PPARGC1A <i>Gly482Ser</i>	GG	272 (52.7)	69 (51.5)	OR= 0.580 (0.322 to 1.046) ; p=0.07*
	GA	212 (41.1)	59 (44.0)	
	AA	32 (6.2)	6 (4.5)	
Allele frequency		0.73/0.27	0.74/0.26	
PPARG <i>Pro12Ala</i>	CC	626 (75.5)	316 (73.0)	OR= 0.774 (0.573 to 1.045) ; p=0.095**
	GC	190 (22.9)	107 (24.7)	
	GG	13 (1.6)	10 (2.3)	
Allele Frequency		0.87/0.13	0.86/0.14	

Table 6.3: Genotype frequencies and odd ratios for association with type 2 diabetes for both SNPs.

*adjusted for age, waist circumference, BMI and family history of diabetes

** adjusted for age, waist circumference, BMI, family history of diabetes and gender

The relationship between the quantitative traits (blood pressure, age at diagnosis, BMI, waist circumference and cholesterol levels) and genotype was examined for both SNPs. No significant association was found for any of these traits and the genotypes for either SNP studied (Table 6.4).

Genotype	Age at Diagnosis (yrs)	BMI (kg/m ²)	Waist circumference (cm)	HDL (mmol/L)	HbA1c (%)
Pro12Ala					
CC	51.2 (12.3)	28.0 (4.6)	101 (11.0)	1.30 (0.5)	8.3 (1.9)
CG	51.7 (11.8)	28.7 (4.9)	102 (11.1)	1.25 (0.3)	8.0 (1.9)
GG	54.7 (10.0)	27.9 (4.6)	99.7 (10.4)	1.30 (0.2)	7.9(1.6)
Gly482Ser					
GG	49.8 (12.4)	28.4 (4.7)	101.9 (10.7)	1.17 (0.2)	8.4 (2.0)
GA	50.6 (11.4)	28.2 (4.5)	102.1 (11.4)	1.21 (0.2)	8.2 (1.9)
AA	51.7 (11.1)	27.3 (3.7)	100.5 (9.9)	1.38 (0.9)	8.1 (2.0)

Table 6.4 Association between the genotypes of the Pro12Ala and Gly482Ser polymorphisms and the phenotypic characteristics.

All values shown are mean (SD) and adjusted for duration of diabetes, gender, family history.

6.5 Discussion

The role of *PPARG* in the causation of type 2 diabetes has been the subject of investigation given the role of the PPAR γ pathway in glucose metabolism(215). Initial studies looking for an association of the Pro12Ala polymorphism with type 2 diabetes were inconsistent and were undermined by small cohort sizes and population stratification. The Pro12Ala polymorphism was first reported to be associated with type 2 diabetes by Yen *et al* in 1997(228). Deeb *et al*, in a study in a Finnish population, showed an association between the Pro12Ala polymorphism and type 2 diabetes and reported that the Ala allele was associated with lower BMI and fasting insulin levels(236). Studies that followed these initial publications, however, were less supportive of its role in the causation of type 2 diabetes. No association was found in German(237), Italian and Polish populations(238;239). The inconsistency in the results of earlier studies reflects a common problem of lack of appreciation that large cohorts are needed to detect true associations with genes that have small effect sizes. It's therefore likely that the early studies that found an association with Pro12Ala did so simply by chance, while those that failed to show an association were probably underpowered to detect such a small effect. A significant association with type 2 diabetes and the Pro12Ala polymorphism was, however, confirmed in a study of family-based cohorts by Altshuler *et al*(188). The study reported that the more common proline allele was associated with a modest but significant risk of type 2 diabetes that translated into a population attributable risk of 25%. This association has now been confirmed in subsequent genome-wide association studies and by meta analysis and *PPARG* remains one of the important genes

associated with type 2 diabetes identified originally using the candidate gene approach(188;240).

The association between the Pro12Ala polymorphism and type 2 diabetes has been examined in at least two south Asian sub groups. In a study comparing south Indians in Chennai, India with south Indians and white Europeans resident in Dallas, U.S.A, Radha *et al* found no association with the Pro12Ala polymorphism and type 2 diabetes in either of the south Indian cohorts(189). The frequency of the ‘protective’ alanine allele was comparable in diabetes and non-diabetes subjects amongst south Indians while in white Europeans, the alanine allele was less frequent in the diabetics. The difference in prevalence of the alanine allele has been suggested as a reason for the high prevalence of type 2 diabetes in certain ethnic groups including south Asians. In a recent study in Khatri Sikhs, however, homozygosity for the ‘GG’ (alanine) genotype was associated with a significantly decreased risk of type 2 diabetes(199). The results from the present study therefore appear to be in agreement with those reported in the south Indian population but at variance with those in the Sikh population even though the allele frequencies in our study are similar to those seen in Khatri Sikhs, as expected from close genetic relationship. It’s most likely that these disparate findings are due to chance. In white populations, meta-analysis suggests Pro12Ala has an odds ratio of 1.14, which is a very modest effect size(188). The size of the study cohorts in our study and the study of Radha *et al* were too small to conclusively exclude an association of this size, while the findings in Khatri Sikhs may have been purely due to chance. Two recent studies by the same groups, however, have shown that the Pro12Ala polymorphism in combination

with a risk haplotype may indeed be associated with type 2 diabetes risk in South Asians. Vimalaswaran *et al* reported that three variants of the *PPARG* gene (-1279G/A, Pro12Ala and His478His) had no effect on diabetes risk when assessed individually(241). However, a specific 2 locus haplotype and 3 locus genotype that included Pro12Ala were associated with risk of type 2 diabetes. In a separate study, Sanghera *et al* examined the relationship between type 2 diabetes and 14 tagging SNPs of *PPARG* and found a strong disease association with Pro12Ala and a haplotype combination of two other SNPs (rs11715073 and rs 3892175) plus Pro12Ala but no association with any of the other SNPs(242). Only the Pro12Ala polymorphism was investigated in the present study, but in the light of recent evidence, it would be interesting to replicate the findings of Vimalaswaran *et al* and Sanghera *et al* and this would be a subject for future study.

Unlike the Pro12Ala polymorphism, the association between *PPARGC1A* polymorphisms and type 2 diabetes is less convincing. There are at least three variants of the *PPARGC1A* investigated for association with type 2 diabetes but the Gly482Ser variant has been the most frequently studied. A 1.32 fold increase in the risk of type 2 diabetes with the serine allele was reported in the Danish population(243). Similar association has been found in the Japanese population(232). These findings were supported by a meta-analysis of 9 published studies which showed a modest association (OR=1.1) between the risk of diabetes and the serine allele(231). The Gly482Ser variant was also found to be associated with a 1.6-fold increased risk of progression from impaired glucose tolerance to diabetes in the STOP-NIDDM trial(244). No association between the Gly482Ser polymorphism and type 2 diabetes was found in French, Chinese and

Pima Indians(245-247), however. In another study in non-diabetic Dutch and German populations, no association between diabetes-related traits and Gly482Ser was found(234). Only one previous study examined the role of this polymorphism in south Asians. Association between three variants of the *PPARGC1A* gene and diabetes was examined in a south Indian population(235). No association was found between type 2 diabetes and the Gly482Ser polymorphism but the group reported an association with another variant, Thr194Thr (OR= 1.68; p=0.0004). In the present study association between the Gly482Ser variant and risk of type 2 diabetes was examined. The lack of association found in this study is consistent with the findings of the south Indian study. Also, the allele frequencies reported in the south Indian study are similar to those found in this study (serine allele frequency: diabetes 0.28 v 0.27 and controls 0.28 v 0.26 in south Indian v Mirpuri/Punjabi populations respectively). These allele frequencies are lower compared to those reported in the white European populations (between 0.5 and 0.7) and may explain the differences in association reported between the ethnic groups(244). It must, however, be emphasized that even in the European populations, the effect on disease risk was weak (OR=1.1 as reported in the meta-analysis). The most likely reason for the lack of association in Asian cohorts is that they are too small and are underpowered to detect such a weak effect.

In the Mirpuri/Punjabi population studied, there was no association between measures of adiposity or any other phenotypic characteristics (blood pressure, HDL cholesterol, age at diagnosis) and either the Pro12Ala or the Gly482Ser polymorphism. Association between the Pro12Ala polymorphism and phenotypic

features, particularly adiposity, has been examined in many studies. Largely, the alanine allele of the Pro12Ala polymorphism was associated with lower BMI and increased insulin sensitivity in many studies (230;236) but other studies have found no such association(237-239) suggesting responses to this variant may vary in different ethnic groups. No significant association with phenotypic measures has been observed with the Gly482Ser polymorphism(231;234) but the study in the south Indian population reported increased adiposity with the *PPARGC1A* Thr394Thr (G>A) polymorphism(248).

The main limitation of this study is the small number of samples in both groups. Polymorphisms of both the *PPARG* and the *PPARGC1A* gene have been found to have small effect sizes (odds ratios of 1.1-1.14) in white populations. Given the allele frequencies observed in this study, our sample size would give only 23% power to detect an association of similar size for *PPARG* and only 11% for *PPARGC1A*. To be able to have 80% power to detect similar effects we would require over 3500 subjects with diabetes and an equal number of controls (calculated using the online Genetic Power Calculator)(249). In the absence of larger trials it is difficult to establish a role for these polymorphisms in south Asians and larger studies are therefore needed.

6.6 Conclusion

There is now substantial evidence to suggest that PPAR γ plays an important role in glucose and lipid metabolism and metabolic disorders such as polycystic ovary syndrome and metabolic syndrome(215;230). The role of PPAR γ in glucose metabolism is further supported by the fact that PPAR agonists are now an established part of type 2 diabetes treatment.

The precise mechanisms by which the *PPARG* polymorphisms influence susceptibility to diabetes, however, are still poorly understood. It is possible that these polymorphisms are in linkage disequilibrium with other polymorphisms that may control important functions in the insulin pathway. The results of these studies also illustrate the difficulties of identifying genes that are strongly associated with type 2 diabetes. Polygenic nature of type 2 diabetes and the interaction between several genes themselves and the environment make it even more difficult to establish a direct causal relationship between the polymorphisms and disease.

While the role for the polymorphisms of the *PPARGC1A* gene is less clear there is no doubt that this represents an area of great interest considering the role of PGC1 α in energy metabolism. Functional studies in this area will hopefully unravel some of the missing links in this pathway and enhance our understanding of how these loci contribute to disease risk in South Asians.

Chapter 7

Association between Calpain 10 polymorphisms and risk of type 2 diabetes in the UK south Asian population.

7.1 Introduction

Intensive search for type 2 diabetes genes using linkage mapping techniques led to the identification of a region on the long arm of chromosome 2 (*NIDDM 1*) that conferred disease risk in a Mexican American population(250). Subsequent positional cloning techniques resulted in the discovery of the Calpain 10 (*CAPN10*) gene(190). Calpains belong to the superfamily of cysteine proteases which are enzymes activated by calcium(251;252). The Calpain heterodimer is comprised of a large 80K catalytic subunit and a smaller 30K regulatory sub unit. The 80K sub unit contains domains I to IV and 30K contains domains V to VI. There are at least 15 members of the Calpain family identified so far. 8 of these are typical Calpains , 6 atypical and 1 calpain sub unit(252;253) . Typical Calpains are composed of domains I to IV while some of the domains are replaced or deleted in the atypical Calpains. Atypical Calpains lack domain IV and cannot interact with the regulatory 30K sub unit. In humans, Calpains are ubiquitous and are involved in a variety of biological functions including cell proliferation, differentiation and intracellular signalling. Disturbances in Calpain function have been associated with many disease states(254). Calpains 1 and 2 are associated with neurodegenerative disorders, Calpain 3 with limb girdle muscular dystrophy(255), Calpain 9 with gastric cancer (256) and Calpain 10 with type 2 diabetes(190). Calpain 10 is an atypical calpain and lacks domain IV

but instead has a tandem linking domain 3(252). The gene for Calpain 10 is located on chromosome 2 and consists of 15 exons. Calpain 10 is expressed in many human tissues including the heart, pancreas, liver and the skeletal muscle, suggesting it may be involved in both insulin secretion and action(252;257).

The association between type 2 diabetes and Calpain 10 was first reported in Mexican Americans by Horikawa *et al*(190). Individuals homozygous for the G allele at SNP43 who also possessed the specific haplotype 112/121(GRC/GRT) of SNP 43,-19 and -63 had a threefold increase in the risk of type 2 diabetes compared with individuals lacking this haplotype. These polymorphisms occur in the non-coding region of the Calpain 10 gene and are thought to affect calpain mRNA levels leading to lower calpain activity, up-regulation of protein kinase C and reduced insulin signalling. Subsequent studies in other populations have produced conflicting results with association reported in some but not all studies. Strong associations were reported by studies in Finnish/Botnians, Polish, African American and south Indian populations(194;258-260). No association between the original haplotype (112/121) and risk of type 2 diabetes was found in a British study(193). Similarly, no association was found in the Oji-Cree(261), Samoan(262), Japanese(191), Chinese(263), Finnish(264), Danish and Swedish populations(265). In a study in Pima Indians, no association was found between SNP43-GG genotype and diabetes but decreased rates of insulin- stimulated glucose turnover was reported in GG homozygotes with normal glucose tolerance(266). A possible association with another SNP (SNP44) was first described in a British population. SNP44 is only a few base pairs away from SNP43 and is in perfect linkage disequilibrium with a missense mutation

Thr504Ala (SNP110) (193). Independent association between SNP44 and diabetes was confirmed in a subsequent meta-analysis(267). In comparison with studies in those of European ancestry, the role of Calpain 10 polymorphisms in type 2 diabetes has not been extensively investigated in south Asians. In the only study involving south Asians, Cassel *et al* reported a fivefold increased risk of type 2 diabetes in individuals with the risk haplotype 112/121 in a south Indian population but the findings of this study were limited by the small cohort size(194). These findings, however, have not been verified in any other south Asian sub group. Given the genetic diversity of Calpain10 and the heterogeneity of the south Asian population, it is highly relevant to examine the role of these polymorphisms in other south Asian sub groups.

The aim of this study was to examine the association between the two most commonly associated Calpain 10 SNPs, SNP43 (rs3792267) and SNP44 (rs2975760), and type 2 diabetes in a UK-based south Asian population of Mirpuri ancestry.

7.2 Methods

7.2.1 Patient selection and Genotyping

Details of patient selection and techniques of DNA extraction are described in chapter 6. Genotyping for Calpain 10 SNP43 (rs3792267) and SNP44 (rs2975760) was performed using pre-designed Taqman genotyping assays using the protocol supplied by the manufacturer (Applied Biosystems [ABI], Warrington, UK.), using the same methods as described in section 6.2.3.

Genotyping was repeated in approximately 15% of the subjects to estimate error rate, which was less than 1%. Genotypes were scored using the allelic discrimination software supplied by ABI.

7.2.2 Statistical analysis

General characteristics of the diabetic and control groups were compared using the Mann-Whitney U test. Genotype and allele frequencies were compared between the groups using binary logistic regression and overall allele frequencies were used to calculate odds ratios (OR). For each polymorphism, association analysis was performed assuming additive, dominant and recessive models. The common 'G' allele was defined as the risk allele for SNP43. For SNP44, the minor 'C' allele was defined as the risk allele. Quantitative phenotypic differences between the genotypes were analysed using oneway ANOVA. A 'p' value less than 0.05 was considered statistically significant. All statistical analysis was done using SPSS 16 software (SPSS Inc.Chicago,U.S.A).

7.3 Results

The clinical characteristics of the diabetic and non-diabetic subjects are summarized in table 6.2,Chapter 6. Data were limited in control subjects and included only age, BMI, waist circumference and blood pressures. For diabetes subjects, age at diagnosis, HbA1c and lipid profiles were also available.

Allele and genotype frequencies for SNP43 and SNP44 are summarized in Table 7.1 Overall allele frequencies for both the SNPs were similar in the diabetic and control subjects. Allele frequencies (G/C) for SNP43 were 80%/20% in diabetic subjects and 77%/23% in control subjects and this was not statistically significant. Minor differences in genotype frequencies were observed between the groups with proportionately more 'GG' homozygotes in the control group than in the diabetic group (64% v 56% respectively; $p=0.001$). Genotype frequencies in the control group, however, did not conform to Hardy-Weinberg equilibrium ($\chi^2 11.1 p<0.0001$). For SNP 44, the allele frequencies (T/C) were 82%/18% in diabetic subjects and 80%/20 % in the control subjects respectively and this was not significantly different. Genotype frequencies for SNP44 were not in Hardy-Weinberg equilibrium in either diabetic ($\chi^2 16.4 p<0.0001$) or control groups ($\chi^2 14.9 p=0.02$).

SNP	Polymorphism	Genotype	
		Diabetes N(%)	Control N(%)
SNP43 (rs 3792267)	G/A	GG 458 (56)	278 (64)
		GA 311(38)	121(28)
		AA 49 (6)	35 (8)
		Allele Frequency 0.80/0.20	0.77/0.23
SNP 44 (rs2975760)	T/C	TT 561 (68)	281(66)
		TC 217(26)	122(28)
		CC 40 (5)	25(6)
		Allele Frequency 0.82/0.18	0.80/0.20

Table 7.1: Genotype and allele frequencies of SNP43 and SNP44 in subjects with diabetes and non diabetic controls.

To determine the association between the genotypes and diabetes risk, additive, dominant and recessive models were used in the analysis. A statistically significant association was found between ‘GG’ homozygosity at SNP43 and decreased risk of type 2 diabetes using the recessive model OR=0.71(0.562 to 0.907); p=0.006. This relationship persisted even after adjusting for age, gender and family history of diabetes OR=0.732 (0.567 to 0.945); p=0.017. No association was found with additive or dominant modelling. In contrast, no association was found between the risk allele ‘C’ at SNP44 and diabetes risk in any of the three models studied, either before OR=0.83 (0.49 to 1.38); p=0.474 or after adjustment for co-variables OR=0.836 (0.482 to 1.452); p=0.525. (Table 7.3).

Table 7.3: Association between the polymorphisms of the *CAPN 10* gene (SNP43 and SNP44) and the risk of Type 2 diabetes.

Model	SNP 43 (rs3792267) OR (95% CI)	SNP44 (rs2975760) OR (95% CI)
Additive	0.85 (0.705 to 1.033); p=0.104	0.893 (0.732 to 1.089); p=0.264
Dominant	1.37(0.878 to 2.160); p=0.164	0.87(0.68 to 1.12); p=0.295
Recessive	0.71(0.562 to 0.907); p=0.006	0.83(0.49 to 1.38); p=0.474

There was no relationship between the quantitative traits (age at diagnosis, blood pressure, BMI and waist circumference) and the genotypes for either SNP (Table 7.4).

SNP	Genotype	Age at diagnosis (years) Mean (SD)	BMI (kg/m ²) Mean (SD)	Waist Circumference (cm) Mean (SD)	HbA1c (%) Mean (SD)	Total Cholesterol (mmol/L) Mean (SD)	HDL (mmol/L) Mean (SD)
SNP43 rs3792267	GG	51.8 (12.2)	28.2 (4.8)	101.5 (11.8)	8.3 (1.9)	4.7 (1.1)	1.3 (0.4)
	GA	50.7 (11.9)	28.1 (4.5)	101.9 (10.2)	8.2 (1.8)	4.8 (1.1)	1.3 (0.5)
	AA	51.3 (12.6)	28.1 (4.3)	100.7 (13.2)	8.3 (2.0)	4.8 (1.1)	1.2 (0.4)
SNP44 rs2975760	TT	51.3 (11.9)	28.2(4.5)	101.6 (11.3)	8.3 (1.9)	4.7 (1.1)	1.3 (0.5)
	CT	51.7 (12.0)	28.2(5.0)	102.1 (11.0)	8.2 (1.8)	4.7 (1.0)	1.3 (0.5)
	CC	50.1 (11.6)	27.7(4.7)	98.9 (12.0)	8.2 (1.8)	4.9 (1.2)	1.3 (0.3)

Table 7.4: Relationship between the genotypes and the phenotypic characteristics for SNPs 43 and 44 of the Calpain10 gene.

7.4 Discussion

In this study I examined the association between two variants of the Calpain 10 gene –SNPs 43 and 44- and the risk of type 2 diabetes in a Punjabi/Mirpuri population. A significantly decreased risk of type 2 diabetes was observed with homozygosity for the ‘GG’ genotype at SNP43. There was no association between SNP44 and the risk of type 2 diabetes. These findings contrast with those of a previous study of South Asians which reported a strong association with SNP43 (with a specific haplotype combination 1112/1121) and the risk of type 2 diabetes in a group of South Indian ancestry. Association between increased diabetes risk and Calpain 10 polymorphisms has generally been attributed to specific haplotype combinations involving three or more SNPs(190;194;259;260;268). In contrast, the evidence for association between individual SNPs of the Calpain 10 gene and type 2 diabetes is much weaker. Significant association between the individual SNP43 and type 2 diabetes was reported in African-Americans(260). In a subsequent meta-analysis that included 21 population studies and 5 family studies, a significant association was found between SNP43 and the risk of type 2 diabetes (269). In the studies showing association with type 2 diabetes and SNP43 (either individually or in combination with the risk haplotype), the excess risk was attributed to the ‘GG’ genotype. A possible role for SNP43 is further supported by quantitative phenotypic studies in various populations. Homozygosity for the ‘G’ allele has been associated with decreased insulin sensitivity and preferential oxidation of fat, increased first phase insulin secretion and decreased skeletal muscle mRNA expression of Calpain10(266). Such support, however, is not consistent with several other studies reporting no association with disease risk,

although several of these may have been limited by small sample size (193;264;270).

In the present study, I found that homozygosity for the 'G' allele at SNP43 was associated with decreased risk of type 2 diabetes in the Punjabi population. These findings are clearly at variance with those of earlier studies in different populations. Although the 121 haplotype in Japanese(271) and 111/221 haplotype combination in Europeans(268) have been associated with reduced risk of disease, no previous studies have reported a protective effect of the 'GG' genotype. These results therefore require careful interpretation. Although the results from this study suggest that the 'GG' genotype may have a protective effect on the risk of type 2 diabetes it is difficult to place too much emphasis on this. On the contrary, the statistical effect observed may be due to the departure from Hardy-Weinberg equilibrium seen in the control group. The excess of 'GG' homozygotes in the control group raises the possibility of genotype error. However, this is unlikely as genotyping in 15% of the samples was repeated with an error rate of less than 1%. Rather a more likely reason for the excess homozygotes would be consistent with the increased rates of consanguinity in the Mirpuri population(272), although why this is not seen in the diabetic subjects is unclear. Nevertheless this anomaly in genotype distribution makes it difficult to interpret our findings.

Unlike for SNP43, I did not find any association between SNP44 and diabetes in this population. As for SNP43, studies looking for association between SNP44 and type 2 diabetes have been inconsistent in their findings. Independent association between the minor 'C' allele at SNP 44 and the risk of type 2 diabetes was first reported in the British/ Irish population(193). Replication of these initial

findings have reported only modest associations with the 'CC' genotype in some populations and no association in other populations. Odds ratios reported in different studies have also varied significantly, with ratios as high as 2.13 in Mexican Americans(190) and only 0.83 in the Finnish population(258). A meta-analysis of 10 published studies, however, reported a strong association between SNP44 and diabetes risk(267). There is considerable variation in the frequency of the minor allele 'C', ranging from 6% in Mexican Americans, 16% in the British/Irish and up to 25% in Botnians(267). The minor allele frequency in the present study was 18%, which was similar to that observed in the British population (193) and the south Indian population(194). It is interesting that despite a reasonably high frequency of the minor allele neither of the South Asian studies found any association between this SNP and diabetes risk. The absence of disease association could be due to small sample sizes in both South Asian studies, making it difficult to detect weak associations. Additionally, the fact that the genotype frequencies in our cohort were not in Hardy-Weinberg equilibrium could have also contributed to our inability to demonstrate a disease association with SNP44.

Significant associations between *CAPN10* genotypes and BMI and waist/hip ratios have been reported in earlier studies. 'GG' homozygosity was associated with increased BMI and waist/hip ratios in Chinese and Pima Indians (266;273) but no association was observed in the Mexican American population(190). In the south Indian study, however, the GG genotype was associated with lower BMI and waist/hip ratios(194). SNP 44 on the other hand has been associated with measures of glucose intolerance (274) and PCOS(275). In this study I did not find any association between the phenotypic characteristics of the subjects with either

of the SNPs studied. This may be due to environmental differences specific to the population studied or may simply reflect the fact that the role of these SNPs is modest in influencing phenotypes and our dataset was underpowered to detect these small effects.

A distinct feature of Calpain10 studies has been the genetic heterogeneity between ethnic populations. Even when associations have been found, the greatest risk was associated with specific haplotype combinations rather than with individual SNPs. Most studies have compared associations between the original haplotype combinations of SNP43, -63 and -19 and diabetes and some have included SNP44 as well. Two of these four commonly studied SNPs were included in the present study as strongest associations were reported with these SNPs both in individual studies and in meta-analyses. SNP-63 was found to be strongly associated with type 2 diabetes in a south Indian study but the allele frequency for this SNP was too small to attribute much significance at a population level. An obvious limitation of my study therefore is the difficulty in ascertaining the disease association with the haplotype combinations specific to the Punjabi cohort. Replication of the remaining SNPs in a larger cohort would certainly help to define the susceptibility haplotype for this population.

The role of Calpain10 polymorphisms in the causation of type 2 diabetes remains debatable. The huge haplotype diversity between ethnic groups and the inconsistency in the results of association studies make it difficult to attribute causal effect. Despite evidence from animal and human studies suggesting a biological role for Calpains in various metabolic pathways, the exact mechanism

by which Calpain 10 influences the pathogenesis of type 2 diabetes remains unknown.

7.5 Conclusion

In the present study I found no association between SNP44 and the risk of diabetes while there was a suggestion that the ‘GG’ genotype at SNP43 may be associated with decreased risk. This relationship needs further verification in a larger independent cohort to determine whether the association is genuine or an artefact caused by the excess homozygosity for SNPs 43 and 44 observed in this population.

Chapter 8

Common variants of the *TCF7L2* gene are associated with increased risk of type 2 diabetes mellitus in a UK-based South Asian population

8.1 Introduction

The identification of the *TCF7L2* gene marked a significant advancement in the search for type 2 diabetes genes(195). Hitherto, success in the search for genes associated with type 2 diabetes was limited and with the exception of the *PPARG* and the *KCNJ11* genes, studies involving most other genes were characterized by either lack of association or poor reproducibility. Association between common polymorphisms within the transcription factor 7-like 2 (*TCF7L2*) gene and risk of type 2 diabetes was first reported by Grant *et al* in Icelandic, Danish and American cohorts (196). The microsatellite marker (*DG10S478*) in intron 3 within *TCF7L2* was associated with a 1.5 to 2 times increased risk of type 2 diabetes and a population attributable risk of 21%. The study also reported association with SNPs as proxy markers of the microsatellite. The association between the SNPs identified by Grant *et al* and type 2 diabetes was soon replicated in Amish (276), American (277), British (278) and Finnish (279) subjects and subsequently in many other populations with almost identical results(280). Two distinct features mark the discovery of the *TCF7L2* gene polymorphisms. Firstly, the effect sizes reported with this gene have been the strongest reported so far compared with any other type 2 diabetes susceptibility gene. Secondly, there is consistency of

association in almost all ethnic groups studied supporting a role for this gene in the causation of type 2 diabetes.

TCF7L2 (also known as TCF4) is a transcription factor and is an important part of the Wnt signalling pathway(281). Wnts are glycoproteins involved in the regulation of another protein, beta catenin. Their role was first described in relation to cancer of the colon(282). Activation of the Wnt receptor is followed by a series of reactions that prevent the degradation of beta catenin which then binds to a TCF protein in the nucleus to initiate transcription activity(281). The Wnt signalling pathway has been shown to regulate myogenesis and adipogenesis and plays a vital role in beta cell proliferation and embryogenesis. The exact mechanism by which TCF7L2 increases susceptibility to diabetes is not clear but it is thought it may influence insulin secretion through GLP-1 activity(283;284). Given that the common variants associated with type 2 diabetes occur in non-coding regions, it is possible that they may affect the expression of *TCF7L2*.

Variants of *TCF7L2* have been studied in three South Asian cohorts to date; these cohorts from western India(197), south India(198) and north Indian Khatri Sikhs(199) have all reported strong association with type 2 diabetes. The aim of this study was to replicate these findings and investigate the association between common polymorphisms within the *TCF7L2* gene and type 2 diabetes in a well-characterized UK-resident South Asian population of Punjabi ancestry, originating predominantly from the Mirpur area of Azad Kashmir, Pakistan.

8.2 Methods

8.2.1 Patient selection and DNA extraction

Selection of diabetes and non-diabetes control subjects has been described previously in chapter 6 (section 6.2.1) and the process of DNA extraction is the same as in chapter 6, section 6.2.2

8.2.2 SNP selection and genotyping

We genotyped four SNPs (rs7901695, rs7903146, rs11196205 and rs12255372) that showed association with type 2 diabetes in the study by Grant *et al*(196). Genotyping was carried out using TaqMan SNP Genotyping assays (as described in chapter 6, section 6.2.3) Approximately 20% of the samples were re-genotyped to estimate error rate, which was zero for all SNPs.

8.2.3 Statistical analyses

Genotype frequencies for each SNP were checked for Mendelian consistency using a X^2 goodness-of-fit test. Alleles and genotypes were tested for association with type 2 diabetes using logistic regression. Association between the risk alleles and disease susceptibility was estimated using a multiplicative model consistent with the modelling used by Grant *et al* in the original report. Association between genotypes and continuous variables was tested using one way ANOVA. All statistical analyses were done using statistical software SPSS version 16.0 (SPSS Inc, Chicago IL).

8.3 Results

The clinical characteristics of the diabetes and control subjects are the same as those shown in table 6.2, chapter 6.

Genotype frequencies for all SNPs were in Hardy –Weinberg equilibrium except rs 12255372 which had an excess of homozygotes for both the major and minor alleles in the control group ($p=0.02$). The minor allele of each SNP was significantly associated with type 2 diabetes (Table 8.1) and this association persisted even after adjusting for age, gender and family history of diabetes.

The strongest association was seen for the variants rs7903146 and rs11196205, with an allelic OR of 1.31 (95% CI 1.11 – 1.56, $p=1.96\times10^{-3}$) and 1.30 (1.11-1.54; $p=1.1\times10^{-3}$) respectively. For most variants, the risk of type 2 diabetes mellitus among minor allele homozygotes was greater than that among heterozygotes, consistent with previous suggestions by Grant *et al* for a multiplicative model of inheritance.

The relationship between phenotypic characteristics and risk variants is summarized in table 8.2. No significant association between the phenotypes and the risk allele was observed for any of the four SNPs. However, a trend towards lower BMI and age at diagnosis was observed with homozygosity for the minor alleles for SNPs rs7903146 (TT) and rs7901695 (CC).

SNP	Allele	Diabetic subjects F	Control subjects F	Genotype	Diabetic subjects n (%)	Control subjects n (%)	Allelic OR (95% CI)	Het OR (95% CI)	Hom OR (95% CI)
rs7901695	T	0.65	0.71	TT	362 (43.7)	226 (51.7)	1.29 (1.09 - 1.54)	1.31 (1.02 - 1.68)	1.65 (1.12 - 2.44)
				CT	355 (42.9)	169 (38.7)			
	C	0.35	0.29	CC	111 (13.4)	42 (9.6)	$p = 3.60 \times 10^{-3}$	$p = 0.032$	$p = 0.012$
rs7903146	C	0.64	0.71	CC	352 (42.5)	222 (51.4)	1.31 (1.11 - 1.56)	1.37 (1.07 - 1.76)	1.66 (1.13 - 2.44)
				CT	360 (43.5)	166 (38.4)			
	T	0.36	0.29	TT	116 (14.0)	44 (10.2)	$p = 1.96 \times 10^{-3}$	$p = 0.014$	$p = 9.74 \times 10^{-3}$
rs11196205	G	0.53	0.59	GG	229 (27.5)	159 (36.5)	1.30 (1.11 - 1.54)	1.46 (1.12 - 1.89)	1.65 (1.18 - 2.30)
				CG	417 (50.2)	199 (45.6)			
	C	0.47	0.41	CC	185 (22.3)	78 (17.9)	$p = 1.10 \times 10^{-3}$	$p = 5.29 \times 10^{-3}$	$p = 3.33 \times 10^{-3}$
rs12255372	G	0.68	0.73	GG	382 (46.8)	241 (55.4)	1.26 (1.05 - 1.51)	1.43 (1.11 - 1.83)	1.37 (0.92 - 2.05)
				GT	346 (42.3)	153 (35.2)			
	T	0.32	0.27	TT	89 (10.9)	41 (9.4)	$p = 3.60 \times 10^{-3}$	$p = 5.23 \times 10^{-3}$	$p = 0.127$

Table 8.1 : Allele frequencies (F) and genotype frequencies for all the four SNPs. Genotype odds ratio calculated compared with homozygotes for common allele; het OR= odds ratio for heterozygous genotype, hom OR= odds ratio for homozygous minor allele genotype.

SNP	Genotype	Age at diagnosis (years) Mean (SD)	BMI (kg/m ²) Mean (SD)	Waist Circumference (cm) Mean (SD)	HbA1c (%) Mean (SD)	Triglycerides (mmol/L) Mean(SD)	HDL (mmol/L) Mean (SD)
rs 7901695	TT	51.9 (12.0)	28.2 (4.6)	101.8 (11.6)	8.3 (1.9)	2.6 (2.3)	1.3 (0.5)
	TC	51.3 (12.2)	28.3 (4.8)	101.9 (11.0)	8.2 (1.7)	2.7 (1.8)	1.3 (0.5)
	CC	49.3 (12.6)	27.8 (4.5)	100.9(11.4)	8.3 (2.1)	2.6 (1.7)	1.2 (0.3)
rs 7903146	CC	51.8 (12.0)	28.3(4.6)	101.9 (11.0)	8.3 (1.9)	2.6 (2.4)	1.3 (0.5)
	CT	51.6 (12.1)	28.3(4.8)	101.6 (11.1)	8.3 (1.8)	2.6 (1.8)	1.3 (0.5)
	TT	49.4 (12.6)	27.8 (4.5)	101.0 (11.4)	8.4 (2.1)	2.7 (1.7)	1.2 (0.3)
rs 11196205	GG	51.6 (11.0)	28.4 (4.8)	101.8 (11.8)	8.2 (1.9)	2.7 (2.7)	1.3 (0.5)
	GC	51.6 (12.0)	28.2 (4.6)	101.8 (11.2)	8.3 (1.8)	2.5 (1.7)	1.3 (0.5)
	CC	50.6 (12.0)	28.8 (4.5)	101.2 (11.8)	8.4 (2.0)	2.7 (1.7)	1.2 (0.3)
rs 12255372	GG	51.9 (12.0)	28.5 (4.7)	102.2 (11.5)	8.3 (1.9)	2.6 (2.3)	1.3 (0.5)
	GT	51.2 (12.0)	28.0 (4.7)	101.4 (11.0)	8.3 (1.8)	2.6 (1.8)	1.3 (0.5)
	TT	50.4 (12.0)	28.7 (4.6)	100.6 (11.6)	8.3 (2.1)	2.6 (1.8)	1.3 (0.3)

Table 8.2: Relationship between the phenotypic characteristics and genotypes for all 4 *TCF7L2* SNPs

8.4 Discussion

The results from the present study provide important confirmation that variants of the *TCF7L2* gene are strongly associated with type 2 diabetes in populations of South Asian origin, with similar effect sizes to those seen in white Caucasian populations (196;277-279;285). In the study by Grant *et al*, two of the four variants included in this study (rs 7903146 and rs 12255372) were shown to have the strongest associations (OR 1.54 and 1.52 respectively). Studies in British (278), Amish (276), Finnish (279) and American populations (277) have also confirmed similar associations. Although the frequency of the risk allele 'T' for SNP rs7903146 was lower in the Finnish population, the overall effect size was comparable to other studies (279). Some of the only exceptions are in Pima Indians where the risk allele frequencies were surprisingly low (0.08 and 0.01 for rs 7903146 and rs 12255372 respectively)(286) and in the Saudi population where no association was found(287).The relationship between the variants of *TCF7L2* and type 2 diabetes was investigated previously in three South Asian cohorts. In a South Asian cohort from Western India, Chandak *et al* reported association with three variants, with the strongest association with the rs12255372 variant (197). Bodhini *et al* studied a south Indian population and reported similar results(198). Replication of four of the six original SNPs described by Grant *et al* in Khatri Sikhs by Sanghera *et al* found associations with all four variants, but the strongest association was with the variant rs10885409 (199). This is different to the findings of our study in which the strongest associations were seen with rs7903146 and rs11196205. The fact that different variants show strongest associations in different South Asian subgroups would suggest that the

contribution of these risk variants in different populations is different or, more likely, that none of the variants are true susceptibility determinants but are markers of risk alleles elsewhere in the region of the *TCF7L2* gene. Comparison of the odds ratios of the homozygous and heterozygous genotypes suggested our data were consistent with the multiplicative model proposed by Grant *et al.* Although our data also fits an additive model the small numbers of our cohort limit our ability to distinguish the true model of inheritance.

Allele frequencies for rs7903146 in our Punjabi population were similar to those reported in earlier South Asian studies, whereas the minor allele of rs12255372 appeared to be slightly more common in our cohort (32% in diabetes and 27% non-diabetes subjects) than in the south Indian and western Indian cohorts (23% and 19% respectively in the study by Bodhini *et al*, 30% and 22% respectively in the study by Chandak *et al*) but less than in Khatri Sikhs (37 % and 33% respectively). However, as the genotype frequencies for rs12255372 were not in Hardy-Weinberg equilibrium in our control group, it was not possible to accurately establish the effect size of this SNP. To establish if the minor departure from Hardy-Weinberg equilibrium was due to an error in genotyping, all samples were re-genotyped for rs12255372 using the same technique but with fresh reagents. Furthermore all minor allele homozygotes in the control group were genotyped by RFLP, using primers designed with the web-based tool SNP Cutter (288) and the enzyme Tsp509 (*Note: This part of the analysis was performed by Dr. S Rees, Research Scientist in our group*). Neither method revealed any inconsistencies with the initial genotyping. It seems likely, therefore, that the departure from Hardy-Weinberg equilibrium is purely due to chance sampling.

Phenotypic characteristics associated with the risk alleles have been examined in previous studies. Although there have been reported associations with BMI and waist circumference, these have been inconsistent (196-198;286). In this cohort, a trend towards lower BMI was observed with the homozygous genotype of the risk allele for rs7901695 and rs7903146 variants but this was not statistically significant. There was also a borderline association between age at diagnosis and homozygous genotypes of the risk allele for variants rs7901695 and rs7903146. These results are different to the findings of Chandak *et al* and Bodhini *et al* who did not find any association between BMI or waist hip ratios and the risk variants (197;198) but consistent with the findings of Grant *et al* (196) and indicate that the *TCF7L2* variants may increase the risk of diabetes independently of BMI and the carriers of the risk alleles may be more likely to develop diabetes early. Both Chandak *et al* and Bodhini *et al* reported associations with increased fasting and 2 hour blood glucose values and the risk allele 'T' at rs 12255372(197;198). As neither fasting nor 2 hour glucose values were measured in this cohort, this relationship could not be verified.

The identification of the *TCF7L2* variants represents a major milestone in the search for type 2 diabetes genes(289). The fact that these associations have been replicated in several ethnic groups with similar effect size suggests these polymorphisms play an important role in the pathogenesis of type 2 diabetes. This assumption is further supported by data from the Diabetes Prevention Program showing that individuals who possess the risk variants of *TCF7L2* were more likely to progress from impaired glucose tolerance to diabetes.(290) Over expression of *TCF7L2* has also been associated with decreased glucose-stimulated insulin secretion, suggesting it may play a role in pancreatic insulin response.

(283). There is some evidence to suggest that *TCF7L2* variants directly affect GLP-1 activity(284;291;292). However, other studies have shown that GLP-1 levels are unaffected in carriers of *TCF7L2* risk alleles(293). Rather it is more likely that these variants may affect GLP-1-stimulated insulin secretion. More functional studies are clearly needed to understand the pathways by which the risk variants of *TCF7L2* influence insulin secretion.

8.5 Conclusion

In conclusion, the results of this study add to the rapidly expanding body of evidence that implicates *TCF7L2* as an important risk factor for type 2 diabetes in multiple ethnic groups.

Chapter 9

Summary, criticisms and future research

9.1 Introduction

There is growing evidence from clinical trials to suggest that intensive risk factor management is essential to minimize the consequences of diabetes and its complications. Implementing the findings of these trials, especially in an ethnically diverse population with differing cultural, religious and economic needs, is particularly challenging. Integrated models of health care may be able to overcome some of these difficulties. The United Kingdom Asian Diabetes Study was set up to investigate if a culturally sensitive enhanced care package tailored to the needs of the South Asian population would improve the risk factors and eventually outcomes in individuals with diabetes. Although the main objective of the trial was to evaluate the effectiveness of the integrated care package, the scope of such a large study obviously extends beyond that. In this thesis I have presented a comparison of cardiovascular risk factors between South Asian and white European ethnic groups, the outcomes after 2 years of intervention in the former population and investigated the role of several common genetic polymorphisms in determining the risk of diabetes in a South Asian cohort of Punjabi ancestry.

9.2 *Ethnic comparison of risk factors at baseline*

The contribution of ethnicity to overall cardiovascular risk remains a contentious issue. Data from several groups suggest that traditional risk factors alone do not explain the excess cardiovascular morbidity and mortality in South Asians while others suggest that the increased susceptibility is due to the excess burden of known risk factors. The data presented in chapter 3 suggests that while there are significant differences in risk profiles, the effect of ethnicity itself appears to be small. Interestingly, the most notable effect was on HbA1c. As HbA1c can be modified by several factors, it is difficult to draw any firm conclusions from these results. Nevertheless, the fact that it remained significant despite adjustments for all confounders (including treatment and duration of diabetes) is interesting. Difficulties in achieving tight glycaemic control in South Asians has been reported in earlier studies and the precise reasons for this need further attention. Another important observation of this study is that for any age group above the age of 45, the predicted risk of cardiovascular disease is significantly higher in South Asians than in white Europeans. Other groups have also reported similar results, suggesting that South Asians have a greater propensity for cardiovascular disease at a younger age. Whether interventions in South Asians should therefore start at an earlier age is worth consideration. A major criticism of this study would be that it is observational and therefore would limit our ability to draw definite conclusions. Also, it could be argued that the white Europeans who chose to participate in the study were more motivated and may not be entirely representative of the background population. Despite these limitations the findings of this study are highly relevant and highlight the disparities in disease risk and its management between the ethnic groups.

9.3 *Enhanced care intervention*

A key feature of this thesis is the outcomes of the two year enhanced care intervention. The results of this study are presented in chapter 4. The definitive UKAD study involved a larger cohort and had a longer follow-up period than in the pilot study. Despite the additional resources, the intervention failed to demonstrate additional benefits in the enhanced care group. The obvious question is why did the intervention not have the expected effect? The lack of success of the UKADS intervention is likely to be due to several rather than a single factor. It is also likely that there was some degree of interaction between these factors. Firstly, the intervention focussed primarily on the improvement in major risk factors and consequently, relied on adherence to treatment protocols. Although strict treatment algorithms were provided, implementation of these algorithms and appropriate initiation and titration of treatments depended to a large extent on health professional involvement. There were no direct measures of protocol adherence but the fact that only a small percentage of patients achieved the study targets suggests that the adherence to treatment protocol was poor. Secondly, there were many other components of the intervention that were difficult to measure. All patients received educational input and access to link workers was supposed to increase compliance. However, these components were not structured and may therefore have failed to deliver the desired results. Further, in a complex intervention many of these factors interact with each other making it difficult to ascertain the benefits of these components individually. Thirdly, there was a significant impact of the secular changes in UK primary care on the outcomes of the study. The introduction of the QOF initiative changed the prescribing habits and the focus of general practitioners resulting in improved care to diabetes

patients. This was evident from the proportion of people achieving the targets for blood pressure and cholesterol as set out in the QOF initiative. As the patients in the intervention, as well as those in the control group, benefited from this initiative, the effect of intervention itself was diluted. Finally, it is likely that some of the components of the intervention, such as education and lifestyle changes, may take a longer time to have an effect. Perhaps a longer period of follow-up rather than the two years is required to detect the benefits from these interventions.

Despite the seemingly disappointing results there were some key messages from the study. Recruitment and participation of South Asians in clinical trials is generally considered difficult. The UKADS, however, showed that by engaging with the community using culturally sensitive measures it is possible to recruit and retain South Asians in clinical trials. During the course of the study, a greater proportion of patients in the intervention achieved the blood pressure and HbA1c targets and the prescriptions of ACE/ARBs and statins increased significantly. These findings suggest that even in this 'difficult to treat' population it is possible to achieve improvement in risk factors using strict treatment protocols and involvement of the health care professionals. The study also showed that there are huge variations in general practice and initiatives such as QOF are highly relevant in reducing some of these inequalities.

9.4 Genetic characterisation

Another important part of my thesis is the genetic characterisation of the South Asian population with regard to assessing the role of common polymorphisms of the type 2 diabetes susceptibility genes. Although South Asians have one of the highest rates of type 2 diabetes, genetic studies in this population have until now been sparse. Further, considerable genetic heterogeneity within this population makes it difficult to generalize the observations made in specific ethnic sub groups. In chapters 6, 7 and 8 of my thesis I have presented the results of the genetic replication studies in this UK South Asian population for common polymorphisms of some of the type 2 diabetes susceptibility genes previously identified in white European populations. These include the *PPARG*, *PPARGC1A*, *CAPN10* and *TCF7L2* genes. Interestingly, a positive association with susceptibility to type 2 diabetes was found only with the polymorphisms of the *TCF7L2* gene, while no clear conclusions could be drawn about the other genes.

Two important conclusions can be drawn from the results of these studies. Firstly, genetics of type 2 diabetes is complex and involves the interaction of several genes with environment. The prevalence of these genetic variants and the haplotype combinations varies significantly between people of different ethnicities and the risk of disease can be further modified by the degree of environmental exposure. Understanding the contribution of the individual gene to the overall risk of type 2 diabetes can therefore prove to be difficult. Secondly, most susceptibility genes have only a modest effect on disease risk. The ability to detect true associations is therefore greatly limited if the cohort size is small. Smaller cohorts have been shown to yield false positive results or fail to show an

association. A more successful approach would be to study large populations and/or identify a gene that has a very large effect size, but such genes are unlikely to be found for type 2 diabetes.

An obvious criticism of the studies presented in this thesis therefore is that the cohort sizes were too small to detect any true associations. The sample sizes included in the genetic analysis were small and in particular the number of non-diabetic control subjects was low. Small cohort sizes have been reported to yield false positive and false negative results and the sizes of our cohorts had to be taken into account while interpreting the results of these studies. Based on the results of all the studies reported in this thesis, we estimate that to detect a true association with our cohort sizes we will need to study polymorphisms with an effect size equal to greater than 1.6. At the time of writing this thesis the recruitment of South Asians with type 2 diabetes and also non-diabetic controls was in progress. Completion of this project should allow replication of the studies in larger cohorts. Another criticism is that the control samples used in my studies were recruited predominantly from the community and as such in some subjects only random and fasting blood sugars were used to define 'normal glucose tolerance'. Given the high prevalence of diabetes in this community, it is likely that a proportion of these subjects would have varying degrees of impaired glycaemia leading to inconclusive results. Non-availability of additional data (such as biochemistry and anthropometric data) in a proportion of subjects also limited the ability to detect associations between genetic variants and phenotypic characteristics. Similarly none of the studies had data on physical activity or diet and therefore it was not possible to adjust for these effects when examining the relationship between the SNPs and the genotypes. A further problem encountered

during the study was the deviation of genotype distribution from that expected under Hardy-Weinberg equilibrium. This had an impact on my ability to detect genotypic associations with disease by distorting the observed genotype frequencies in one or both study groups. It is unclear why these deviations occurred, although repeat genotyping showed that they were unlikely to be due to genotyping errors. It is possible that consanguinity in the Mirpuri population could explain the greater than expected degree of homozygosity at some loci, although we would not expect the rate of first-cousin marriages to differ between the diabetic and control groups. As such, this is not a satisfactory explanation for the loci that deviated from Mendelian consistency in the control group only. Despite these limitations the results of these studies provide initial insights into the genetic susceptibility of a population previously not well studied.

9.5 Recommendations for future work

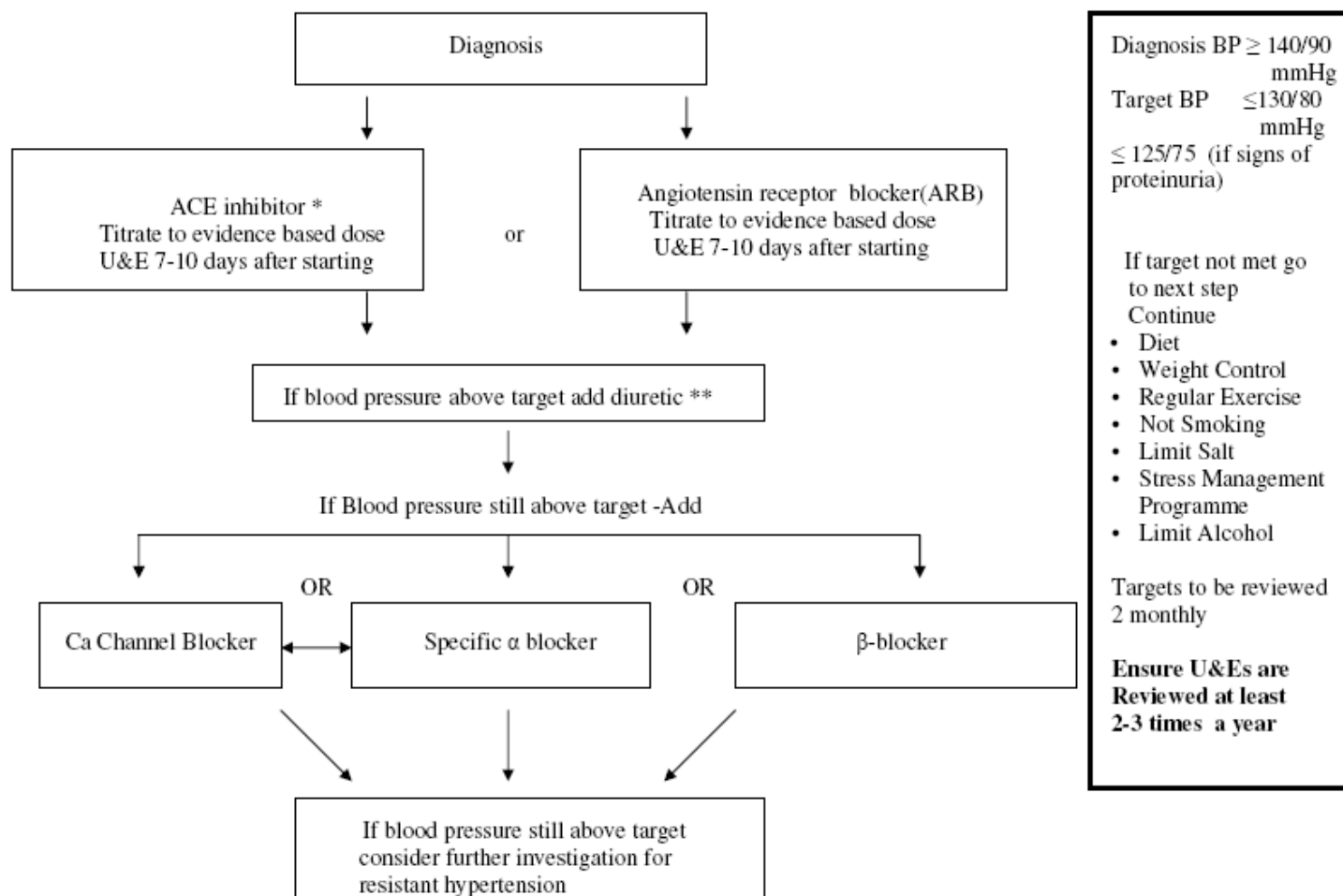
Health care delivery to people of South Asian ethnicity remains a considerable problem. The results from the UKAD study suggest that while some progress has been made in this area there are still considerable challenges. Like most studies there are several questions that remain to be answered. Both the pilot study and the definitive study highlighted the difficulties in achieving tight glycaemic control. The reasons for this need to be fully understood and barriers to insulin therapy need to be explored. A key factor of type 2 diabetes management is the health professional-patient interaction. The UKADS results suggest that better outcomes can be achieved with greater health professional involvement but

whether such results can be sustained in the long term needs to be investigated. Similarly other models of integrated health care with increased focus on patient education can be developed based on the UKADS experience.

Although our understanding of the role of genetics in type 2 diabetes has improved significantly in recent years, there are still major challenges to overcome in our understanding of the genetic basis of type 2 diabetes. Genetic studies looking for associations have largely used diabetes as a dichotomous variable. Such an approach may have limitations particularly as some of those classified as ‘normal’ may go on to develop diabetes at a future time. The genetics studies in subjects in the Diabetes Prevention Program and the Diabetes Epidemiology: collaborative analysis of diagnostic criterion in Europe (DECODE) study have looked at progressive degrees of dysglycaemia (Normal glucose tolerance, Impaired Glucose tolerance and Diabetes) and such an approach could be considered in future studies.

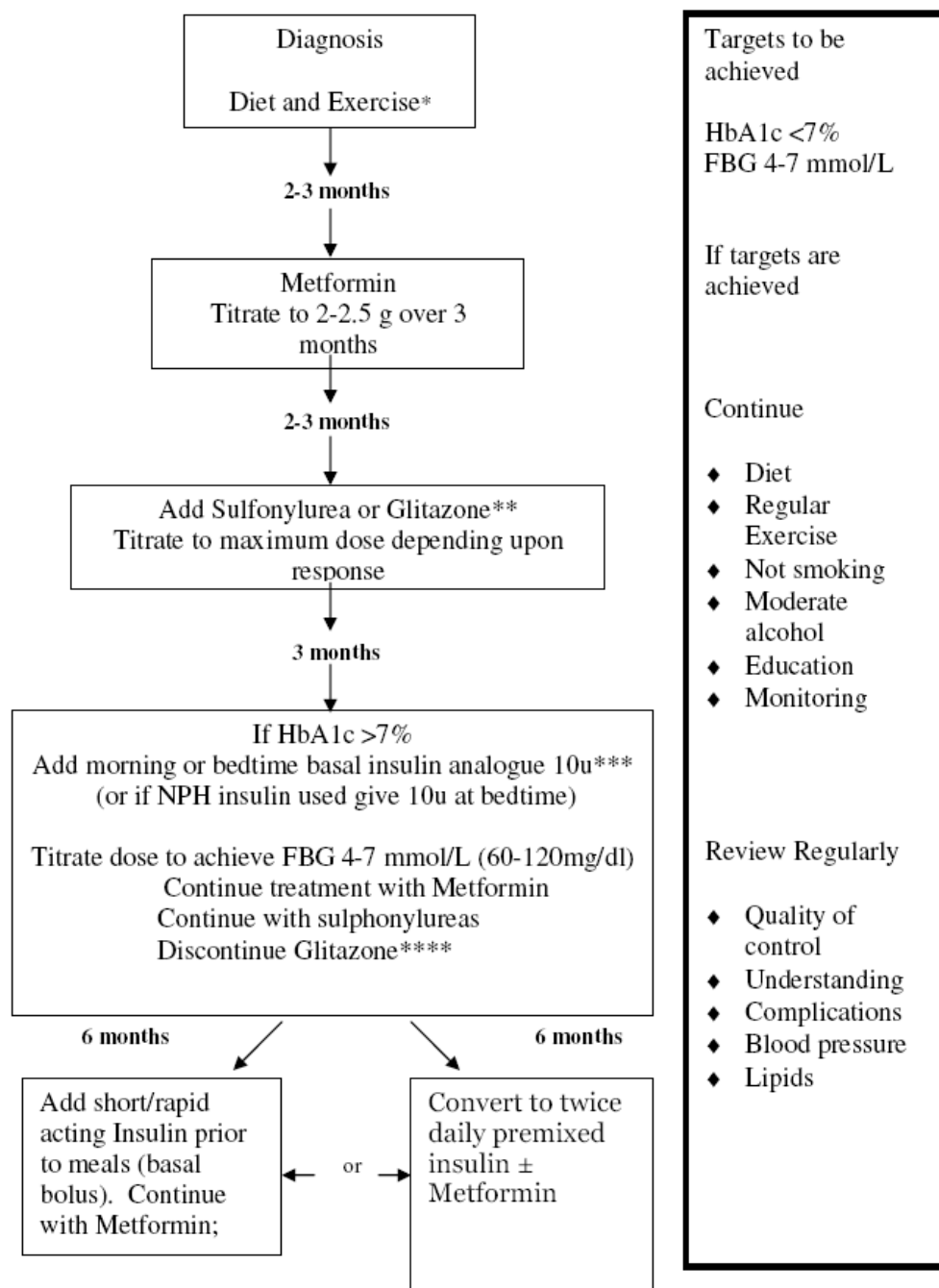
Despite the high prevalence of diabetes, the scarcity of genetic studies in South Asians is disappointing. Establishing genetic resources involving larger well-characterised South Asian cohorts is essential to understanding the differences between ethnic groups in pathogenesis and disease susceptibility. This could lead to the development of new therapies to tackle the disease more effectively in this ethnic group.

TREATMENT ALGORITHM FOR BP CONTROL



* If develops cough, substitute with ARB. ACE I and ARBs can also be used in combination particularly in the presence of proteinuria
 ** Thiazide or Thiazide like diuretic (eg: Indapamide) combining well with ACEi or ARB but with reduced risk of metabolic side effects

TREATMENT ALGORITHM FOR TYPE 2 DIABETES



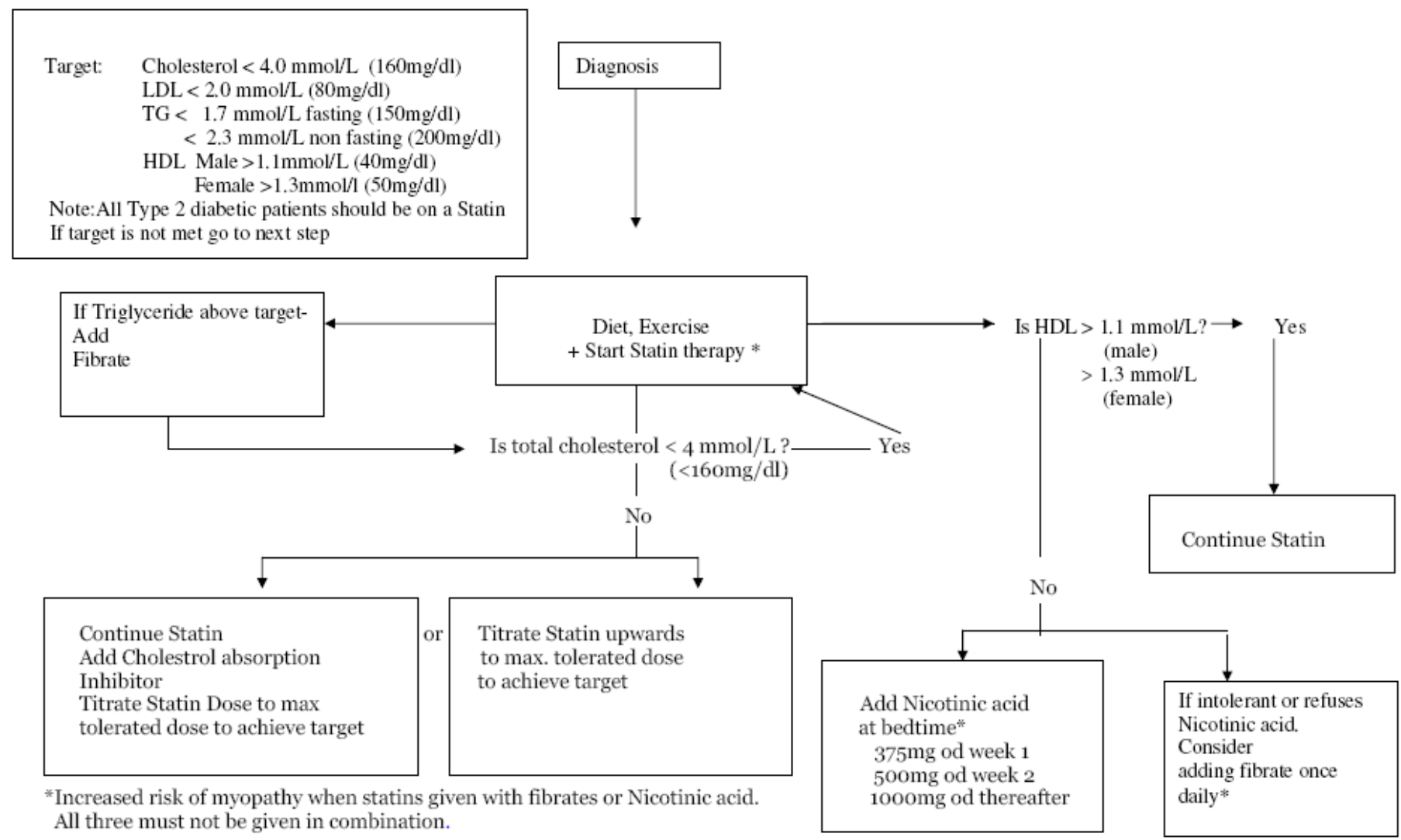
* Includes assessment of psychosocial issues and lifestyle modifications

** Consider Metformin and Glitazone if Metformin and SU contraindicated or not tolerated
Glitazone can also be prescribed as monotherapy if Metformin is not tolerated or contraindicated

Note: ***Because of its 24 hour profile basal insulin analogue can be given at anytime, but at the same time each day

**** Combination of Glitazone and insulin is contraindicated in Europe

Adapted from Barnett AH et al "Treating to target in Type 2 diabetes: from lifestyle changes to insulin therapy." Modern Diabetes Management 2003; 4:2-5



Appendix 4

All chemicals mentioned in this appendix come from Sigma Aldrich (Poole, UK), unless otherwise stated.

A1.1. DNA extraction reagents

Reagent A:

10mM Tris.HCl (pH 8.0)

320mM Sucrose

5mM MgCl₂

1% Triton X-100

Made up in distilled water and stored at 4°C.

Reagent B:

400mM Tris.HCl (pH 8.0)

60mM EDTA

150mM NaCl

1% sodium dodecyl sulphate (SDS)

The above solutions were diluted in distilled water and stored at room temperature.

TE buffer:

10mM Tris.HCl (pH 8.0)

0.1mM EDTA

The above solutions were diluted in distilled water, autoclaved and stored at room temperature.



BIRMINGHAM HEARTLANDS HOSPITAL
DARBOURNE GREEN, LEAVY
BIRMINGHAM B15 2TH

TEL: 0121 455 4401
FAX: 0121 455 5588

BIRMINGHAM HEARTLANDS HOSPITAL
EAST BIRMINGHAM LOCAL
RESEARCH & ETHICS COMMITTEE
UNDERGRADUATE CENTRE

Direct Line/Fax: 0121 455 3852

Administrator: 0121 455 4401

SJRLMH0553
22 June 2003

Dr S Kumar
Consultant Physician
BHH

Dear Sudhesh

UKADS - United Kingdom Asian Diabetes Study, a multiple risk intervention trial

Your project has been approved by the Ethics Committee, who have considered that this is quite acceptable as outlined in your submission. This approval is subject to the proviso that there is no significant deviation from the Protocol outlined and that any untoward reactions and complications are immediately reported to the Committee.

In order to comply with the Royal College of Physicians guidelines, I look forward to receiving a report on the outcome of this study.

Yours sincerely

Stephen J Rose
Chairman
Research & Ethics Committee

NB This Committee abides by the guidance of the Department of Health. The committee endorses the Royal College of Physicians Report on 'Fraud & Misconduct in Medical Research Practice 1991'. This states that all original data (such as questionnaires, lab books, and hard copies of any computer data), are kept for a minimum of ten years in a retrievable form. If storage is going to be outside Birmingham Heartlands Hospital, the submission should state the site of storage. It is a condition of ethics approval that such storage occurs.

Birmingham Heartlands and Solihull



Heart Team (Diabetes)

CASE RECORD BOOK

U.K.A.D.S

UNITED KINGDOM
ASIAN DIABETES STUDY

(New Phase)

Patient Study Number _____

Hospital Number _____

Patient Information Sheet

UK ASIAN DIABETES STUDY

Introduction

Please read this information sheet carefully. It gives details about a project being undertaken within your doctor's practice. This project is aimed at improving the care of Asian patients suffering with diabetes.

This is being undertaken as part of a research programme, which is described below. Before you decide to take part it is important for you to know why the research is being done and what it will involve. Please take time to read the information carefully, discuss it with friends and relatives. If there is anything that you do not understand, or if you require further information concerning the programme, please ask the medical or nursing staff.

Consumers for Ethics and Research (CERES) publish a leaflet entitled 'Medical Research and You'. This leaflet gives more information about medical research and looks at some questions you may want to ask. A copy may be obtained from CERES, PO Box 1365, London, N16 0VW, or maybe obtained through us.

Background

Diabetes is four times more common in people of Asian origin compared to people of white European origin. It is also thought that there is a high risk of heart trouble and kidney disease in people of Asian origin. There are many very effective treatments for treating diabetes and also to prevent complications of diabetes such as heart disease. We are keen to develop better means of providing diabetes care to people of Indo-Asian origin to try and overcome any cultural or language barriers that may exist.

What is the UK Asian Study?

In Birmingham and at Warwick Universities, hospital specialists are working together with colleagues in primary care and also other professionals such as nurse specialist and dieticians to find out how to deliver better care for our Asian patients. In order to do this, there will be a new Asian Link worker and also a nurse specialist who will work with your doctor in order to try and see if care can be improved.

What is the RESEACH part of the programme?

In order to find out whether this approach is of benefit, we will need to compare one general practice where patients receive the new method of providing treatment and one which is using currently existing methods. We will need to record information on your health problems. The results will be confidential but we require your permission to record these details. This information will be anonymous and there is no way that your details can be traced back to you.

What benefits can I expect?

If your practice is one that gets the new method you may benefit from extra support provided as part of this project. Even if you are not part of this practice, this project may help to establish the value of this particular type of approach to treating diabetes and you will benefit when this approach is made available to all patients.

What disadvantages might I have if I take part?

There are no disadvantages in taking part. Also as part of this project you will have annual assessments, which are detailed and the results of this will be made available to your General Practitioner or Practice Nurse so that they can manage your problems such as blood pressure, cholesterol etc. better. You will not be identified though any data recorded as part of this project.

Do I have to take part?

No, you do not have to take part.

It is up to you to decide to take part or not to take part. Should you decide to take part, we will ask you to sign a consent form indicating your willingness to participate. You are free to withdraw at any stage without giving any reasons. This will not affect in any way the standard of care you will receive.

You do not have to decide right now whether you want to take part or not. You can take away this copy of the information about the study with you and let us know later on, once you have given this matter some thought.

Confidentiality

Only the study doctors, your doctor, nurses and any other professionals authorised by your doctor will see your records. We intend to perform this project to the highest possible standards. Results from this project will however, be analysed and may be published in medical journals but this is based on anonymous data. Your identity will not be revealed at any stage. No information containing your name will be allowed off the practice premises, your personal records will only be identified by a number. To comply with the Data Protection Act, we would like to make you aware that the data obtained during the study will be held on computer, but you will not be identified by name in any of these records. You are assured that complete confidentiality will be maintained at all times.

Who can I talk to if I have questions about the study?

Your own General Practitioner is involved with the study and will be able to give you further information as will his/her practice nurse

General Practitioner Name: _____ Telephone Number: _____

Practice Nurse Name: _____ Telephone Number: _____

You can also discuss the study with the Diabetes Nurse Co-ordinator for this project:

Mrs Shanaz Mughal, Telephone Number: _____

She will be supported by Professor A H Barnett, and Dr S Kumar, Consultant Physicians.

If you decide not to take part this will not affect your medical care.

You are free to withdraw from the study at any time without giving a reason. This will not affect your medical care.

If you have any problems with the conduct of the study, you can telephone the secretary of the Ethics Committee who has considered this application, on 0121 424 0594, who will arrange for your worries to be investigated.

If you are harmed by taking part in this study, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for legal action.

Reference Number _____

Hospital Number _____

UK ASIAN DIABETES STUDY

FORM OF CONSENT

PATIENT

I, _____ (the patient) of _____

*have read the Explanation (overleaf) of the proposed Study/Trial of:

*have had the patient information sheet interpreted to me in a language I have fully understood of the proposed Study/Trial of:

which forms part of this Consent and the aims and purposes of the study/trial have been explained to me by: _____

I agree to take part in the study/trial and I understand that I am free to withdraw from the study/trial at any time without having to give a reason.

Signature:

Date:

DOCTOR

I confirm that the above named patient has read the Explanation (overleaf) and this Consent and that I have explained the study/trial to the patient and answered any questions arising therefrom.

Signature:

Date:

WITNESS

I confirm that I am satisfied that the patient has read and understood the Explanation and Consent.

Signature:

Date:

* Delete as appropriate

Name of Submitter: Dr. Ann Kelly

Title of Project: A DNA resource to facilitate the investigation of genetic susceptibility to microvascular and macrovascular complications in UK-resident Indo-Asians with type 2 diabetes.

EXPLANATION

For several years, the Diabetes Research Group at the University of Birmingham has studied genes that may be involved in the development of diabetes and its long-term complications. Type 2 diabetes is very common in the Indo-Asian population, affecting over 10% of adults. This ethnic group is also at a high risk of developing long-term complications, including heart disease and kidney disease. The United Kingdom Asian Diabetes Study aims to improve the medical care of Indo-Asians with type 2 diabetes and hence reduce the risk of complications. As part of this study, we are interested in studying the genes that influence the development of these conditions. This will help us to understand more about the causes of these complications and may help us to predict which individuals are prone to developing complications. The information may help in developing new treatments in the future.

To carry out the study, we need DNA samples from a large number of Indo-Asian subjects with type 2 diabetes and healthy control subjects from the same ethnic group. We also need similar samples from white Caucasian subjects, with and without type 2 diabetes. This will allow us to compare the racial groups and identify any population-specific differences that might have an impact on patient care.

We would be most grateful if you could donate approximately 20ml of blood from a vein in your arm, taken by a person experienced in doing this. This amount of blood is less than one tenth of what is normally taken from blood donors. The procedure may involve mild discomfort, as a small needle will need to be inserted into the vein in your arm for the blood sample to be taken. DNA will be isolated from your blood sample and analysed for a variety of genes in the laboratory.

If you decide not to take part this will not affect your medical care.

All the blood samples will be treated anonymously and information from the study will not be fed back to you or your GP. Results from the genetic analysis are confidential and will not be made available to any third party. Participation in the study will not affect your eligibility for health/life insurance. You are free to withdraw from the study at any time without giving a reason. This will not affect your medical care.

If you have any problems with the conduct of the study, you can telephone the secretary of the Ethics Committee who has considered this application, on 0121 424 0594, who will arrange for your worries to be investigated.

If you are harmed by taking part in this study, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for legal action.

This project has been considered by the Research & Ethics Committee

FORM OF CONSENT**PATIENT**

I, _____ (the patient) of _____

have read the Explanation (overleaf) of the proposed Study/Trial of: **A DNA resource to facilitate the investigation of genetic susceptibility to microvascular and macrovascular complications in UK-resident Indo-Asians with type 2 diabetes** which forms part of this Consent and the aims and purposes of the study/trial have been explained to me by:

☐ I agree to take part in the study/trial and I understand that I am free to withdraw from the study/trial at any time without having to give a reason.

Signature:

Date:

DOCTOR

I confirm that the above named patient has read the Explanation (overleaf) and this Consent and that I have explained the study/trial to the patient and answered any questions arising therefrom.

Signature:

Date:

WITNESS

I confirm that I am satisfied that the patient has read and understood the Explanation and Consent.

Signature:

Date:

U.K.A.D.S SCREENING DATA COLLECTION

Baseline ☐ 1 Year ☐ 2 Year ☐ 3 Year ☐ Date: _____

UKAD Number: _____
 Patient Name: _____
 D.O.B: _____
 Address: _____

Language Spoken: _____
 Ethnic Origin: _____
 Marital Status: _____
 Gender: Female ☐ Male ☐
 G.P Name: _____
 Address: _____

Tel No. _____

Year Diagnosis: _____

Tel No. _____

Smoking: Never Smoked: ☐ Ex Smoker: ☐
 Current Smoker: ☐ Amount per week: _____ Type: _____

Alcohol: Yes: ☐ Amount per week: _____
 No: ☐

Medication Diet ☐ Tablets ☐ Insulin ☐ Both ☐

Timing:

Breakfast	Midday	Evening Meal	Bedtime

☐ Only: ☐ Shared Care: ☐ Hospital/PID no. _____

Dietary Advice Given: Yes: ☐ No: ☐ Date: _____

Meter Given: Yes: ☐ No: ☐ Date: _____

Family History:

Father: _____

Mother: _____

Brother/s: _____

Sister/s: _____

Son/s: _____

Daughter/s: _____

Blood related: ☐ Fostered: ☐ Adopted: ☐

EXERCISE LEVEL:

No. of 30 minute sessions per week

Baseline ☐ 1 Year ☐ 2 Year ☐ 3 Year ☐

UKAD NO. _____

ALL MEDICATION

DATE _____

Medication	Strength	Dose	Indication of treatment	Date commenced

MEDICAL HISTORY

Condition	Date	Hospital admission

EYE COMPLICATIONS

	RIGHT	LEFT	YEAR
Partially Sighted	<input type="checkbox"/>	<input type="checkbox"/>	_____
Blind	<input type="checkbox"/>	<input type="checkbox"/>	_____
Cataract	<input type="checkbox"/>	<input type="checkbox"/>	_____
Retinopathy (Specify Type)	<input type="checkbox"/>	<input type="checkbox"/>	_____

Baseline ☐ 1 Year ☐ 2 Year ☐ 3 Year ☐

UKAD NO. _____

Physical Examination And Lab Results

	Date	Date
WEIGHT	Kg	Kg
HEIGHT	Cm	Cm
BMI		
WAIST CIRCUMFERENCE	Cm	Cm
BLOOD PRESSURE		
ECG	Date / /	Date / /
MONOFILAMENT		
LEFT		
RIGHT		
PERIPHERAL PULSES		
LEFT PT		
RIGHT PT		
LEFT DP		
RIGHT DP		
VISUAL ACUITY		
LEFT		
RIGHT		
ALB/CREATININE RATIO	mg/mmol	mg/mmol
PLASMA CREATININE	umol/L	umol/L
HBA1C	%	%
TOTAL CHOLESTEROL	mmol/L	mmol/L
HDL CHOLESTEROL	mmol/L	mmol/L
LDL CHOLESTEROL	mmol/L	mmol/L
TRIGLYCERIDES	mmol/L	mmol/L
CHD RISK	%	%

ADDITIONAL INFORMATION

UKADS

FOLLOW UP VISIT

UKADS NO: _____

DATE	WEIGHT	BP	REASON FOR VISIT	ACTION TAKEN

Reason for visit codes

- 1) Treatment change
- 2) Education
- 3) Review
- 4) Bloods

By placing a tick in one box in each group below, please indicate which statements best describe your own health state today.

Mobility

- | | |
|---------------------------------------|--------------------------|
| I have no problems in walking about | <input type="checkbox"/> |
| I have some problems in walking about | <input type="checkbox"/> |
| I am confined to bed | <input type="checkbox"/> |

Self-Care

- | | |
|---|--------------------------|
| I have no problems with self-care | <input type="checkbox"/> |
| I have some problems washing or dressing myself | <input type="checkbox"/> |
| I am unable to wash or dress myself | <input type="checkbox"/> |

Usual Activities (e.g. work, study, housework, family or leisure activities)

- | | |
|--|--------------------------|
| I have no problems with performing my usual activities | <input type="checkbox"/> |
| I have some problems with performing my usual activities | <input type="checkbox"/> |
| I am unable to perform my usual activities | <input type="checkbox"/> |

Pain/Discomfort

- | | |
|------------------------------------|--------------------------|
| I have no pain or discomfort | <input type="checkbox"/> |
| I have moderate pain or discomfort | <input type="checkbox"/> |
| I have extreme pain or discomfort | <input type="checkbox"/> |

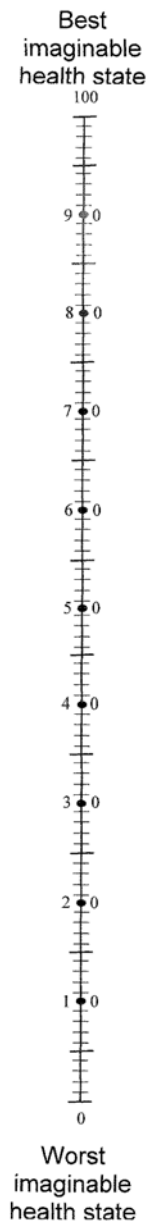
Anxiety/Depression

- | | |
|--------------------------------------|--------------------------|
| I am not anxious or depressed | <input type="checkbox"/> |
| I am moderately anxious or depressed | <input type="checkbox"/> |
| I am extremely anxious or depressed | <input type="checkbox"/> |

To help people say how good or bad a health state is, we have drawn a scale (rather like a thermometer) on which the best state you can imagine is marked 100 and the worst state you can imagine is marked 0.

We would like you to indicate on this scale how good or bad your own health is today, in your opinion. Please do this by drawing a line from the box below to whichever point on the scale indicates how good or bad your health state is today.

**Your own
health state
today**



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Publications/ Presentations

Book Chapters

1. Bellary S, Barnett AH. Genetics studies in South Asians. *Diabetes UK and South Asian Health Foundation recommendations on diabetes research priorities for British South Asians*.2009.21-26 .ISBN 978-1-899288-88-5.
2. Bellary S, Vora J, Barnett AH. Diabetic Nephropathy. *Diabetes UK and South Asian Health Foundation recommendations on diabetes research priorities for British South Asians*.2009.91-97 .ISBN 978-1-899288-88-5.
3. Bellary S. Should South Asians have lower targets? Clinical perspectives in Diabetes.2009 (In press).
4. Barnett AH, Dixon AN, Bellary S: Diabetes and Cardiovascular disease in South Asians: challenges and solutions. *Prevention, treatment and Rehabilitation of cardiovascular disease in South Asians*.2005 61-83

Recent papers

1. Raymond NT, Varadhan L, Reynold DR, Bush K, Sankaranarayanan S, Bellary S et al.
Higher prevalence of retinopathy in diabetic patients of South Asian ethnicity compared with white Europeans in the community: a cross-sectional study.*Diabetes Care* 2009 Mar;32(3):410-5
2. Bellary S, O'Hare JP, Raymond NT, Gumber A, Mughal S, Szczepura A, Kumar S, Barnett AH; UKADS study Group. Enhanced diabetes care to patients of south Asian ethnic origin (the United Kingdom Asian Diabetes Study): a cluster randomized controlled trial. *Lancet* 2008 May 24;371(9626):1769-76
3. Bellary S, Barnett AH. Diabetes and cardiovascular disease in South Asians.
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4. Rees SD, Bellary S, Britten AC, O'Hare JP, Kumar S, Barnett AH, Kelly MA.
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1. Raymond NT, Bellary S, O'Hare JP, Mughal S, Szczepura A, Kumar S, Barnett AH, on behalf of UKADS Study Group. Evaluation of a complex intervention: a cluster randomised controlled trial of enhanced diabetes care - the United Kingdom Asian Diabetes Study (UKADS). *Diabetologia* 2007;50(Suppl 1):S426(abstract)
2. Gumber A, Szczepura A, Raymond N, O'Hare J, Bellary S, Mughal S, Kumar S, Barnett A, on behalf of UKADS Study Group. Economic evaluation of improved diabetes care to patients of South Asian ethnicity: the United Kingdom Asian Diabetes Study (UKADS). *Diabetologia* 2007;50(Suppl 1):S429(abstract)
3. Bellary S, O'Hare JP, Raymond NT, Dixon AN, Mughal S, Johal K, Kumar S, Barnett AH. Impact of pay for performance initiative on care of South Asian patients with type 2 diabetes *Diabetes* 2007(Suppl.) OP116
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6. Varadhan L, Ullah Z, Reynold DR, Bondugulapathi LNR, Johal K, Tithecott D, Sankaranarayanan S, Barnett AH, Kumar S, O'Hare JP. Higher prevalence diabetic retinopathy in South Asians compared to Caucasians: a substudy from UKADS (United Kingdom Asian Diabetes Study). *Diabet Med* 2007;24(Suppl 1):13(abstract)

7. Bellary S, O'Hare JP, Raymond NT, Dixon AN, Mughal S, Johal K, Kumar S, Barnett AH. Evaluation of a culturally sensitive care package in South Asian patients with Type 2 diabetes in reducing cardiovascular risk – United Kingdom Asian Diabetes Study (UKADS): 1-year intervention results. *Diabet Med* 2007;24(Suppl 1):5(abstract)
8. Bellary S, Rees SD, Britten AC, Dixon AN, Mughal S, Johal K, O'Hare JP, Barnett AH, Kelly MA. Common variants of the TCF7L2 gene are associated with increased risk of Type 2 diabetes in South Asians. *Diabet Med* 2007;24(Suppl 1):36(abstract)
9. Bellary S, Raymond NT, Dixon AN, O'Hare JP, Mughal S, Johal K, Kumar S, Barnett AH. Ethnic differences in cardiovascular risk are not adequately addressed by current treatment strategies. *Diabetic Med.* 2006;23(Suppl).P477

Oral Presentations

- 1) Impact of pay for performance initiative on care of South Asian patients with type 2 diabetes
Presented at the American Diabetes Association Congress. June 2007
- 2) Evaluation of a culturally sensitive enhanced care package in South Asians with type 2 diabetes.
Presented at the Diabetes UK Annual Professional Conference- Glasgow , 2007. (This presentation was nominated for the Diabetes UK-Servier Award .)