THE ROLE OF THYROID HORMONES IN PLACENTAL AND FETAL CENTRAL NERVOUS SYSTEM DEVELOPMENT.

BY

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Abstract.

Thyroid hormones are critical to growth and development of the human fetus. In particular, the fetal central nervous system is extremely sensitive to the actions of the active ligand, tri-iodothyronine (T3). The placenta is the organ during pregnancy that allows transport between the mother and her baby by close interaction of the maternal and fetal circulations. Endogenous fetal thyroid hormone production does not occur until the beginning of the second trimester. However, there appears to be transplacental transport of thyroid hormones to the fetus earlier in gestation and organs, such as the central nervous system, appear to be exquisitely sensitive to their actions. The content of this Thesis describes my work, published in peer reviewed papers over the last fifteen years. It outlines the molecular mechanisms controlling the delivery and actions of thyroid hormones to the fetus.

Dedication.

To my wife Julia and my children, Jack and Emma.

Thank you all for your support, help and tolerance over the years.

Acknowledgements

I would like to thank my mentors over the years that I have been a senior academic at the University of Birmingham. In particular Professor Jayne Franklyn, who I have worked with closely both on the actions of thyroid hormone metabolism within the fetoplacental unit and the molecular mechanisms controlling the delivery of the active ligand T3 to the fetus. Her encouragement, support and advice have been invaluable.

During this journey, I have been fortunate to work with and collaborate with those working in the laboratories of the Institute of Biomedical Research (in the Centre for Diabetes, Endocrinology and Metabolism) of the College of Medical and Dental Sciences. This has lead to other endocrine work focusing on the placental and fetal interactions with Professor Paul Stewart (with glucocorticoids and mineralocorticoid metabolism) and Professor Martin Hewison (now of UCLA) (with respect to research on vitamin D and the fetoplacental unit). I have collaborated with some excellent non-clinical (particularly Professor Chris McCabe) and clinical scientists (Dr Shiao Chan and Kristien Boelaert) and this work would not have been possible without their significant input into experiments and results.

Dr Chan began working with me when she was appointed as a MRC clinical training fellow in 2000 and after obtaining her PhD (under my supervision) has gone onto be a first class clinical scientist, working closely with me to date. I have also worked with

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1.1 Introduction.

The link between perinatal brain development and thyroid dysfunction through iodide deficiency has been known for over a hundred years (McCarrison, 1908).

Normal fetal development requires the presence of active thyroid hormone (both in the mother and fetus). Disruption of any of the processes regulating the bioavailability of thyroid hormone, or indeed its cellular interaction may contribute to abnormal fetal development (Kester *et al.* 2001).

This thesis will focus upon my work over the last fifteen years exploring the molecular apparatus for thyroid hormone action, the factors controlling bioavailability of the 'active' ligand to the human fetus and the effects of in-utero disease, particularly intrauterine growth restriction (IUGR).

The placenta is a highly specialised organ, the primary function of which is to facilitate the passage of nutrients, gases and waste products between the maternal and fetal blood. In human pregnancy, placentation is haemochorial providing direct contact, in an intimate fashion, between the maternal circulation and the chorionic (fetal) villi.

Malplacentation, which in human pregnancy is associated with partial or complete failure of extravillous trophoblast (EVT) to invade and remodel the maternal 'uteroplacental' spiral arteries by mid-pregnancy, is associated with relatively poor pregnancy outcome (Avagliano *et al.*2011). This ranges from IUGR, a condition where the fetus does not achieve its growth potential and is associated with significant perinatal morbidity (Avagliano *et al.*2011) to perinatal death (Cross 2006)

Thyroid hormones are synthesised in the thyroid gland. Iodide from the diet is actively taken up into follicular cells in the thyroid gland by the sodium iodide symporter (pump) and is converted to iodine. Tyrosine becomes coupled to form pre-thyroglobulin. Coupling of mono-iodothyronine (T1) and di-iodothyronine (T2) form tri-iodothyronine (T3) and thyroxine (T4), which are stored in the colloid. Thyroid hormones are secreted in response to the binding of thyrotrophin (TSH) to a transmembrane receptor linked to a G-protein second messenger system (Hennemann and Visser.1997).

Proportionally, more T4 than T3 is released into the circulation (in a ratio of 4:1). However T4 is converted to the active ligand T3 by peripheral tissues. In plasma, more than 99% of all T3 and T4 are bound to hormone binding proteins and only unbound ligand is available for cellular uptake (Friesema. 2001).

1.2. Thyroid receptors and their isoforms.

The majority of actions of thyroid hormone are mediated through the binding of the ligand T3, through its binding to nuclear receptors (thyroid receptors [TR]) (Evans *et al.* 1988).

Ligand binding studies have revealed the presence of high affinity nuclear binding sites for T3 in numerous human tissues (Oppenheimer *et al.* 1997). Two TR genes have been described and designated α and β (based on sequence similarities and chromosome localisation). The TRα1 and TRβ1 variants are known to bind T3, and through interaction with the T3 response elements, regulate transcription of specific genes (Lazar 1993; Yen 2001). The 'non-binding' α2 variant may inhibit the function of the other ligand binding isoforms. The two TRα and the TRβ are ubiquitously expressed but an alternate splicing product of the β-gene, termed β2, is expressed in significant amounts only in the anterior pituitary gland and hypothalamic areas of the CNS (Nakai *et al.* 1988).

1.3 Thyronine deiodinase subtypes.

The availability of the active ligand T3 for local tissue receptor binding is determined, at least in part, by the activity of three selenocysteine monodeiodinase enzymes: deiodinase subtypes 1 (D1 or type I), 2 (D2 or type II) and 3 (D3 or type III). The deiodinases are integral membrane proteins located on the endoplasmic reticulum (ER) or external plasma membrane where the active site is exposed to cytoplasm. T4 is converted by outer ring deiodination (ORD) to the active ligand T3 or by inner ring deiodination (IRD) to the inactive metabolite, reversed T3 (rT3) (Visser 1988). Although

concentrations of rT3 may be relatively significant in the fetus, it does not form a binding ligand for the thyroid hormone receptors. T3 and rT3 are further deiodinated by IRD and ORD respectively to 3,3ʹ-T2.

Deiodinase type 1 (D1) catalyses both ORD and IRD and is expressed in human adult liver, kidney and thyroid gland (Kester 2001). D1 is the main enzyme for plasma T3 production and plasma rT3 clearance in adults. Deiodinase type 2 (D2) has only ORD activity and thus converts T4 solely to active T3. In human adults it is mainly expressed in the CNS and pituitary gland. In addition, it is expressed in neonatal 'brown' adipose tissue. However mRNA encoding D2 has also been noted to be expressed in human cardiac and skeletal muscle (Kester 2001).

Deiodinase type 3 (D3) has only IRD activity. In humans, it has been found to be expressed in the CNS and placenta. It is important in the inactivation of both tissue and plasma T3 as well as the production of rT3.

Besides deiodination, conjugation reactions of glucouronidation and sulfation facilitate urinary and biliary excretion of thyroid hormones (Kester 2001).

1.4. Thyroid hormone transporters.

Historically, it was believed that thyroid hormones, lypophilic molecules, pass through the lipid bilayer of the plasma membranes by passive diffusion. However, it is now

known that specific transport mechanisms exist for both uptake and export of thyroid hormones in cells.

In-vitro evidence from cultured human hepatocytes has demonstrated an 'energy-dependency' of thyroid transport (James *et al.* 2007). In 1986, a monochromal antibody raised against rat hepatocytes was noted to inhibit the uptake of both T4 and T3. This action was identified as a putative 52KD thyroid hormone transporter protein (Mol *et al.* 1986). Since this time, several proteins from a wide variety of tissues have been characterised as specific thyroid hormone transporters. These proteins belong to several 'families', each of which has its own tissue specificity and is briefly outlined below.

The Na+/tautochlorate co-transporting polypeptide (NTCP), a major transporter of conjugated bile acids across biliary duct cells, has also been found to be associated with Na+-dependent transport of iodothyronines (Friesema *et al.* 1999). Iodothyronines are organic anions and transported by the organic anion transporting polypeptide (OATP) family. This comprises a large group of multispecific proteins that have been demonstrated to be responsible for the transport of several amphipathic organic compounds in multiple tissues. Several members of this OATP 'family' have been demonstrated to transport thyroid hormone (Friesema *et al.*2005).

As the structure of the iodothyronines is based upon the 'linkage' of two tyrosine residues, amino-acid transporters have been examined for their potential also to transport thyroid hormones

The L-type or system L amino-acid transporters have been demonstrated to transport large, neutral, branched chain amino-acids as well as thyroid hormones (Blondeau *et al.* 1993; Zhou *et al*. 1990).

In addition, specific transports for the amino acids tyrosine (Tyr), phenylalanine (Phe) and tryptophan (Trp), the so called 'T-type' transporters have also been noted to be involved in the transport of thyroid hormones (Zhou *et al.* 1992).

One such transporter, human T-type amino acid transporter 1 (TAT1) has been cloned and expression studies have demonstrated that it transports Phe, Tyr and Trp but not iodothyronines (Kim *et al.* 2001). More recent specific characterisation of this transporter has subsequently noted that this protein, largely expressed in placental tissue, does in fact play a role in thyroid hormone into and across such tissue (Friesema *et al*. 2006).

TAT1 demonstrates homology with members of the monocarboxylate transporter (MCT) family. This aforementioned protein has recently been classified and re-named MCT10. It is closely related to the MCT8 transporter. The MCT8 transporter has been

characterised and found to be a specific and potent thyroid hormone transporter, which again potentially facilitates the direct action of thyroid hormones on tissues that play a vital role in fetal brain development. The role of these two isoforms of the MCT transporter family, MCT8 and MCT10 has formed the basis of much of my group's work over the last five years.

1.5. Transplacental passage of maternal thyroid hormones to the developing fetus.

The importance of thyroid hormone in brain maturation and development has been demonstrated in clinical studies in iodine deficient areas of the world (such as China) (Delange and Ermans. 1996), as well as in elegant animal models (Morreale de Escobar *et al*. 1994).

In the human fetus, TRH may be detected in the hypothalamus by the end of the first trimester (13 weeks), at a time when the thyroid gland begins to concentrate iodine. It is possible to measure TSH in the fetal pituitary and serum in early pregnancy, with concentrations rising towards term and often exceeding adult levels (Fisher *et al*. 1997a; Thorpe-Beeston *et al.* 1991a). Concentrations of total T4 and T3 in fetal serum have been described as rising between the 10th to 30th weeks of gestation in human pregnancy, paralleled by rising thyroxine binding globulin (TBG) levels.

The use of fetal blood sampling by cordocentesis from the second trimester has noted an increase in free T4 and free T3 concentrations with increasing gestational age,

although these levels remain lower than corresponding thyroid hormone concentrations reported in the maternal circulation (Thorpe-Beeston *et al*. 1991a; Kilby *et al.* 1998).

Classically it has been believed that the placenta allows little maternal thyroid hormones to cross to the developing fetus. However, investigations of the human fetus (prior to surgical termination of pregnancy) in the first trimester has indicated that coelomic puncture under ultrasound-guidance to obtain embryonic fluid, demonstrated the presence of human thyroid hormones at in the early first trimester (5-8 weeks) before endogenous fetal thyroid hormone production occurs (Calvo *et al.* 1992; Calvo *et al.* 2002).

The clinical association of maternal hypothyroxaemia during the first trimester and neurological and intellectual impairment (including neuromotor anomalies) in the endemic cretin is well described (Halpern *et al.* 1991. However in the 1990's, attention was focussed upon relatively mild (perhaps subclinical) neuropsychomotor deficits in babies born to women who in pregnancy had only mild iodine deficiency (Hetzel and Dunn. 1989).

Pop and his group (1995) described an association between mothers who at 32 weeks gestation were known to have circulatory thyroperoxidise (TPO) antibodies and lower intelligence quotient (IQ) assessments in offspring (assessed by the general cognitive scale) than in offspring of mothers who had no circulatory TPO antibodies in

pregnancy. At this time the pathophysiological mechanism underlying this observation was that the presence of TPO antibodies led to a reduction in maternal thyroid hormones at this gestation (Lazarus. 1999).

Thyroperoxidase antibodies are found relatively frequently in women of reproductive age and thus in early pregnancy. A recent systematic review of the literature noted that in 18 studies performed over the last 30 years (in 10 countries), the prevalence of TPO antibodies ranges from 5 – 19.6% (Glinoer. 2008). These may have an action in terms of lowering circulating free T4 and T3 concentrations but may also indicate a generalised increase in 'autoimmune' activity. Certainly, the presence of these antibodies appears to be associated with worsening perinatal outcome (Glinoer. 2008; Thangaratinam *et al.* 2011). The mechanism of this morbidity is presently unknown.

Two papers published in the late 1990s led to an explosion of interest in the perinatal influence of maternal thyroid hormone metabolism and subsequent neurodevelopmental outcome in offspring. This interest remains influential today.

Haddow et al (1999) described a retrospective case-controlled study of sixty two singleton offspring identified on the basis of their mothers' increased TSH level in the early second trimester. Although it was described that the pregnant women had subclinical hypothyroidism (SCH), it was subsequently noted that a proportion (18 of the 62), with the mildest elevations in TSH, also had low total thyroxine concentrations,

making biochemical overt hypothyroidism (OH) a possibility. This aside, the infants/children subsequently underwent neurological/psychological evaluation between ages 7-9 years. The mean IQ of children born to untreated women in pregnancy (n=48) was 7 points lower than controls. A much larger proportion of offspring in the untreated hypothyroid cohort had IQs < 85 compared to treated controls (19% versus 5%). The offspring of the treated but still biochemically hypothyroid women (n=14) demonstrated no difference in IQ from controls. This led to researchers in the USA to postulate that these women were actually euthyroid in early pregnancy at a gestation when maternally-derived thyroid hormone (thyroxine) might be important. A subsequent follow up to this 'sentinel' paper demonstrated an inverse association between severity of maternal total T4 concentration and IQ of offspring (Klein *et al*. 2001).

Almost simultaneously in Europe (the Netherlands), Pop and colleagues (1999) assessed infant neurodevelopment at 10 months of age in 220 apparently healthy children from 'uncomplicated' pregnancies (Pop *et al*. 1999). Significantly lower psychomotor development indices were noted in 22 children born to women with free T4 concentrations below the 5th percentiles (as measured at 12 weeks gestation). In those with FT4 concentrations >10th centile significantly higher psychomotor developmental indices were noted at 10 months. In a proportion of the women with overt biochemical hypothyroxaemia (with normal TSH), TPO antibodies were also often detected (Pop *et al.*1995).

It was these publications that led to the significant body of work summarised in this thesis and describing the biochemical and molecular thyroid hormone 'apparatus' in human placenta and the developing human fetal brain. Subsequent observational studies addressing subclinical hypothyroidism in pregnancy have reported mixed results; although many of these studies have small sample sizes, retrospective design and relatively short follow up periods of study (Radetti *et al. 2000*; Smit *et al*. 2000; Okon *et al.* 2009; Li *et al*. 2010).

1.6. Intrauterine growth restriction.

As has been indicated, IUGR is associated with significant perinatal morbidity and indeed mortality. In the prospective study described by my group, we utilised ultrasound to biometrically define IUGR (as outlined by other authors: Chang *et al*. 1993). IUGR was prospectively defined as an abdominal circumference (AC) of < 10th centile for gestation and a reduction in fetal growth velocity as defined as Δ AC of $\leq$ one standard deviation in 14 days. In addition there was oligohydramnios (reduced amniotic fluid as defined as maximal pool depth < 10th centile) and increased impedance or pulsatility index (PI) in the umbilical artery circulation (a significant proportion having absent end diastolic velocity). This cohort of babies may have significant long term morbidity with up to 10% having neurodevelopmental morbidity and neurologic sequlae (Gaffney *et al*. 1994). Prospective follow up of surviving infants has demonstrated that up to 5% have significant neurodevelopmental delay at 9 years of age (Kok *et al.* 1998). Thyroid

hormone status is one of several factors that have been postulated to play a critical role in the pathogenesis of such morbidity, especially with respect to the growth and development of the fetal and childhood CNS (Fisher and Klein. 1981).

1.7. Thyroid hormone metabolism and intrauterine growth restriction.

When fetuses noted to be 'small for gestational age' were investigated by fetal blood sampling, hypoxaemia and acidosis were noted. In addition, low concentrations of free T4 and T3 were also noted in conjunction with modest elevations in TSH concentration (Thorpe-Beeston *et al*. 1991b). Our own group investigated a cohort of severely growth restricted fetuses (with up to 76% mortality in the perinatal period) in the late second and early third trimesters of human pregnancy. Again, fetal serum free T3 and free T4 concentrations were significantly lower (than appropriately grown and gestationally matched fetuses), although the serum TSH was not significantly different (Kilby *et al*. 1998).

Likewise, collection of umbilical cord blood from very low birth weight babies (VLBW) at delivery has revealed significant reductions in total T3 and T4 concentrations. This has been postulated as being secondary to reductions in TBG concentrations; as the free thyroid hormone concentration in umbilical cord blood was not significantly reduced (compared to appropriately grown 'term' babies) (Klein *et al.* 1997). In addition, 'transient' hypothyroxaemia, characterised as low total and free thyroxine concentrations, with or without an elevation in TSH, is frequently associated with small

for gestation, premature neonates in the first month of postnatal life (Fisher. 1997a; Fisher. 1997b). Whilst treatment of permanent and 'transient' primarily hypothyroidism (associated with an elevated TSH) is considered important in terms of long-term neurological outcome, the significance of hypothyroxaemia of prematurity (with no rise in TSH) remains controversial (Oppenheimer and Schwartz. 1997), as does thyroxine replacement in this situation.

These data have led my group of clinical and basic scientists to investigate the mechanism of thyroid hormone action and metabolism in the human placenta and fetal CNS in uncomplicated and IUGR pregnancies.

Summary of work presented.

2. Thyroid hormones and the placenta

2.1. Thyroid hormone receptor expression in the human placenta and changes associated with IUGR.

The expression of nuclear T3 binding to TRs was first described in placental homogenates using radio labelled techniques (Banovac *et al*. 1986). Such experiments were subsequently extended using cultured cytotrophoblasts demonstrating that the nuclear binding of radio labelled T3 was specific to this cell type (Nishii *et al.* 1989).

Our relatively contemporary studies have refined and further added to data in the literature on this topic. We studied the expression of all TR isoforms in human placentas across gestation. Using semi-quantitative RT-PCR and increase in expression of TRα1, α2

and β2 transcripts were noted from the first to the third trimester of human pregnancy in whole placental homogenates. This was associated with a decline in mRNA encoding TRβ1 (Kilby *et al*. 1998). In addition, semi-quantitative immunohistochemistry defined nuclear expression of the isoforms TRα1, α2 and β1 (but not β2) being localised within the villous trophoblast and mesenchymal stromal core of the placental villi, which again increased with gestational age (Kilby *et al.* 1998). Subsequently we went on to use laser capture microdissection (LCM) and quantitative Taqman RT-PCR to further define TR isoform expression in human 'term' placenta. These data again confirmed the expression of TRα1, α2 and β1 in syncytiotrophoblast and cytotrophoblasts layers (but not β2 isoform). However, the expression of these TRα and β isoforms appeared to increase in the heterogeneous stromal cells of the mesenchymal villous core' compared to the trophoblast layer (Chan *et al*. 2004).

These data in human pregnancy were in contrast to data relating to rat placenta. In these tissues between 16 and 22 days of gestation no change in mRNA encoding TRα1, TR β1, c-erbA α2 and c-erbA α3 was noted with advancing gestation (Leonard *et al*. 2001). In addition, the maximal T3 binding capacity (B_{max}) in nuclei extracted from placental homogenates doubled in the final half of gestation, indication that in rodents, TR binding activity is post-transcriptionally regulated, in contrast to human pregnancy.

The demonstration (in human placental tissues) by my group, to describe a parallel increase in circulating fetal free thyroid hormone concentrations and placental TRα1,

α2 and β1 expression apparently supported the role of thyroid hormones in 'target' fetal tissues during development. Our work then focused on the effects of thyroid hormones on trophoblast (both cyto- and extravillous) function and differentiation (Kilby *et al.* 2005).

In-vitro experiments of cultured first trimester cytotrophoblasts cells has previously been described, where increasing concentrations of T3 (10^{-6} – 10^{-8} mol/l) increased the expression of immunoreactive epidermal growth factor (EGF), a cytokine reported to be important in trophoblast differentiation (Matsuo *et al*. 1993). These effects were described as being via the TRB, an effect attenuated by cycloheximide (5 X 10^{-5} mol/l), indicating that these effects were transcriptionally mediated (Maruo *et al.* 1991; Matsuo *et al*. 1993); Maruo *et al*. 1995). This group also noted that 'markers' of cytotrophoblast differentiation- human chorionic gonadotrophin, human placental lactogen and 17β oestradiol, were increased with increasing concentrations of T3.

My group also described endovascular, extravillous trophoblast, remodelling maternal spiral arteries in the first and second trimester placental bed, also expressed TR α and β isoforms on immunohistochemistry (Barber *et al*. 2005).

These 'expression studies' in human placental tissues went on to provide a basis for our attempts to define the mechanisms by which T3 acts on human trophoblast and is summarised later in this thesis.

Comparisons of TR isoform expression between those in apparent uncomplicated human pregnancies and those complicated by severe prenatally-detected IUGR (as previously described) were made. Comparison of IUGR placental samples with those of gestationally-matched uncomplicated pregnancies revealed greater expression of TR α1, α2 and β1 variants in IUGR using semiquantitative immunohistochemistry. In contrast the mRNA encoding the TR isoforms were not differentially expressed when comparing IUGR and uncomplicated placentas (Kilby *et al*. 1998). It is postulated that the significantly lowered free T3 (and T4) in the circulations of fetuses with severe IUGR leads to an increased expression of TR α and β proteins in trophoblast nuclei.

2.2. Placental iodothyronine deiodinase subtype expression and changes associated with IUGR.

The iodothyronine deiodinases are pre-receptor selenocysteine-containing enzymes that regulate local delivery of T3 (as described). Activities of all three iodothyronine deiodinase subtypes (D1, D2 and D3) have been demonstrated in rat placenta (Bates JM *et al*. 1999). However, in contrast to human pregnancy, rodent total serum T3 and T4 increase with gestation and the predominant subtype expressed appears to be D3.

My group, using placental biopsies from human pregnancy across gestation utilised quantitative Taqman RT-RCR, iodothyronine deiodinase activity assays and immunohistochemistry to describe the ontogeny of deiodinase subtypes in this human

tissue. The predominant deiodinase expressed in human placenta was type III (D3) (Chan *et al.* 2003), but type 2 (D2) was also expressed. In general terms, the activities of the enzymes D2 and D3 (and the mRNA encoding the genes to these enzymes) were higher earlier in gestation than at term; displaying an inverse relationship with advancing gestational age of the pregnancy. Our data were in agreement with Professor Theo Visser's group which noted that although placental D2 and D3 activities (fmol/min/mg of placental tissue and thus corrected for protein) decreased with gestation (Koopdonk-Kool et *al.* 1996; Chan *et al.* 2003), the total activity (especially for D3) increases as the placental mass increases. There has been debate as to whether there is a functional significance to D2 activity in human placenta. Again, Visser's group concluded that placental D2 activity was 'hardly detectable', being present 200 times lower than activity of D3 (Koopdonk-Kool *et al.* 1996). This was indeed the case in our group's study (Chan *et al*. 2003). One may speculate that villous trophoblast D2 is less likely therefore to play a pivotal role in the homoeostatic modulation of fetal plasma T3/T4 but may play an autocrine/paracrine role, in that local generation of T3 within placenta and decidua may allow interaction with placental TRα and β isoforms) affecting trophoblast differentiation (Maruo *et al.* 1995). Such an effect is likely to be greatest at earliest gestations (Barber *et al*. 2005).

As indicated, it is D3 that appears to have the greatest total activity in the human placenta. Teleologically, a hypothesis has been derived postulating that placental D3 activity 'protects' the fetus from the relatively high concentrations of T4.

Our data are in accord with this theory, as in the first trimester, when the total placental surface area is relatively small, specific D3 activity is highest. In the third trimester (when total surface area is at its greatest), although specific D3 activity is relatively low (activity per mg placental weight), the total activity is increased (due to increasing placental size) and therefore this may increase T4 (in-)activation overall. This may increase physiologically the amounts of iodide passing through the human placenta to both the fetus and mother. It is of interest that there have been reports that placenta from babies born with thyroid agenesis, and thus hypothyroidism, do not have significant alterations in placental D3 activity (Koopdonk-Kool *et al*. 1996). This therefore implies a constitutive modulation of these enzyme activities.

In severe early onset IUGR, comparison of the relative expressions of mRNA encoding D2 and D3, in human placenta, as well as the activity of these enzyme subtypes, revealed no significant differences as compared to placenta of uncomplicated, gestationally-matched pregnancies (Chan *et al*. 2003).

Interestingly, treatment of primary cultures of human 'term' cytotrophoblasts cells in-vitro with increasing concentrations of T3 (1, 10 and 100nM) resulted in increased expression of mRNA encoding both D2 and D3; but only at a 100nM dose of T3, that is supraphysiological (Chan *et al*. 2003).

2.3. Expression of thyroid hormone transporters in human placenta and changes associated with IUGR.

As previously indicated, plasma membrane transport of thyroid hormones has now been shown to require specific transporter proteins. This work has been supported by several grant giving bodies, notably the Medical Research Council and I have been a co-principal investigator on these grants. I freely acknowledge the collaborative nature of this portion of the work presented in this thesis with my colleague, the clinical scientist, Dr Shiao Chan (who I have worked with and mentored since she was a clinical PhD student appointed in 2000).

We described the gestational autogeny of thyroid hormone transporters MCT8, MCT10, LAT1, LAT2, OATP1A2 and OATP4A1 in a relatively large series (n=110) of human placentas across gestation in uncomplicated pregnancies and their expression changes with IUGR (n=22). Quantitative, Taqman RT-PCR revealed that all the mRNAs encoding the aforementioned thyroid hormone transporters were expressed in human placenta from six weeks gestation onwards and throughout gestation. The transporters OATP4A1 and CD98 (LATs obligatory associated protein) mRNA expression reached a nadir in mid-gestation, before increasing towards 'term'. LAT2 mRNA expression did not alter significantly with gestation. Our group also performed immunohistochemistry to localise MCT10 and OATP1A2 proteins to villous cytotrophoblasts and syncytiotrophoblasts (in villous placenta) and extravillous trophoblasts in the placental bed. In contrast, OATP4A1 appeared preferentially expressed in villous syncytiotrophoblasts (Loubiere *et*

al. 2010). Previously, we had noted the expression of MCT8 protein in human placenta (Chan *et al*. 2006). In first trimester placentas there was 'focal' immunoreactivity of MCT8 in villous cytotrophoblasts and syncytiotrophoblast. Particularly low immuno reactivity was noted in villous placenta in contact with the maternal circulation. MCT8 protein expression appeared to increase in immunoreactivity towards the third trimester (Chan *et al*. 2006). Second trimester placental bed biopsies (from the Newcastle collection) demonstrated strong MCT8 protein localisation to decidual stromal cells (with no expression in decidual lymphocytes). Mononuclear and multinucleated interstitial trophoblast demonstrated variable MCT8 protein expression, whilst endovascular and intramural trophoblast cells demonstrated strong expression.

In placentas from pregnancies complicated by severe IUGR (delivered in the early third trimester), MCT8 protein was increased (on Western immunoblots) (Loubiere *et al*. 2010) a finding mirrored in mRNA encoding MCT8 (Chan *et al.* 2006), compared to placenta from gestationally-matched uncomplicated pregnancies. In contrast, mRNA encoding MCT10 transporter decreased in placental tissue of pregnancies complicated by IUGR. However, no change in mRNA or protein expression was noted in any other thyroid hormone transporters with IUGR.

Thus, several thyroid hormone transporters are expressed in human placenta from the early first trimester. Their coordinated effects may aid local regulation of transplacental thyroid hormone passage and supply to trophoblast cells. Such action may be critically

important for the normal development of the human placenta and fetus (Chan *et al.* 2009).

2.4. The in-vitro effects of triiodothyronine on trophoblast function: differential responsiveness in normal and growth restricted pregnancies.

I have already outlined in this synopsis some preliminary evidence that human trophoblast may be thyroid-responsive (Matsuo *et al.* 1991; Maruo *et al.* 1995). The relatively descriptive work performed and described to date has outlined that both villous and extravillous trophoblast have the 'molecular apparatus' to be thyroid-responsive and potentially to regulate local T3 delivery allowing paracrine/autocrine responsiveness.

In-vitro studies of human trophoblast derived cell lines and primary cultures of cytotrophoblasts demonstrated that T3 epidermal growth factor (EGF) acted to exert antiproliferative effects on the extravillous-like cell line, SGHPL-4 (with the largest effects observed at low concentrations of both EGF [1 and 10ng/ml] and T3 [1 and 10nm]). However, in contrast EGF and T3 exerted a proliferative response in JEG-3 choriocarcinoma cells (increasing with increasing doses of EGF and T3) (Barber *et al.* 2005). The proliferative response was increased using both uptake of tritiated thymidine and a methyltetrazoleum assay (MTT).

EGF enhanced survival of non-proliferative 'term' cytotrophoblasts cells was also noted. However, T3 alone had no effect on MTT assay. The addition of T3 (up to 10nM) had no additional effects on EGF action on these cultured term cytotrophoblasts. We were also interested to evaluate whether 'in-vitro' models simulating trophoblast invasion were affected by T3. EGF appeared to enhanced invasion significantly (as assessed by both length of and total number of invasive processes) of SGHPL-4 cells (EVT-like cells) into fibrin gel (maximal at EGF dose of 10ng/ml). This effect appeared to be attenuated by T3 (at 1nM) (Barber KJ et al, 2005). Both T3 and EGF also significantly enhanced EVT-like cell motility, both independently and in combination (Barber *et al*. 2005).

Recently, we have compared the T3 responsiveness and transport of primary cytotrophoblasts cells isolated from placentas of uncomplicated pregnancies (normal cytotrophoblasts) and those complicated by IUGR (gestationally matched). Compared with normal cytotrophoblasts, the viability of IUGR cytotrophoblasts (assessed using the MT assay) were significantly reduced, whereas apoptosis (assessed using cleared caspase 3/7 activity and M30 immunoreactivity) was significantly increased after T3 culture for 48hours (in trophoblast from both types of placenta). The reaction of human chorionic gonadotrophin was significantly increased by IUGR cytotrophoblasts compared to 'normal' trophoblast. Net transport of [125I]T3 was 20% greater by IUGR cytotrophoblasts compared to normal cytotrophoblasts and this was accompanied by a two fold increase in expression of MCT8 protein (as assessed by Western immunoblotting) (Vasilopoulou *et al*. 2010). These data indicate an altered

responsiveness of cytotrophoblasts cultured from IUGR placenta to T3 with significant effects upon cell survival and apoptosis. Increased expression of MCT8 and T3 (intracellular) accumulation may contribute to these observations (Vasilopoulou *et al.* 2010).

Finally, we investigated the effects of wild type (WT) MCT8 and a pathological mutant L471P on the growth and function of MCT8; null MCT8 expressing JEG cells. Transfection of WT MCT8 into JEG-3 cells increased T3 uptake but over expression of MCT8 in these cells attenuated cell proliferation (in the presence and absence of T3) in a dose-dependant manner (James *et al*. 2009). The data presented to date are focussed upon the potential action of T3 on trophoblast function and the mechanisms that potentially control transplacental delivery of thyroid hormones to the developing fetus.

Epidemiological data indicates that the developing CNS in the human fetus may be exquisitely sensitive to the actions of thyroid hormone.

The next part of this thesis outlines the data amassed by my group to define the molecular apparatus present in the human fetal brain that affect T3 sensitivity.

3. Human fetal CNS.

3.1. Thyroid hormone receptor isoforms: Expression in the fetal CNS and changes with IUGR.

In the late 1990's, I established a relatively large collection of first and second trimester (dated by ultrasound) fetal cerebral cortex samples obtained from surgical termination samples between 7-20 weeks gestation. This collection was established with approval of the local Research Ethics Committee (requiring written consent) and within the terms of the Polkinghorne recommendations and then more recently within the terms of the Human Tissue Act. In addition, fetal cerebral cortex and cerebellar samples were collected from stillborn babies, in the late second and third trimesters, both when the fetus was appropriately grown (n=21; gestational age [GA] 26-40 weeks) and complicated by IUGR (n=18; GA 23-39 weeks). Again appropriate research governance was in place for the collection of these human fetal tissues.

RT-PCR revealed the presence of mRNAs encoding TRα1, β1 and β2 variants (although the latter was very week) and the non-functional TRα2 variant. Only the TRα1 mRNA expression was more abundantly expressed in fetal cerebral cortex than in adult cerebral cortical tissues (Kilby *et al.*2000; Chan *et al.* 2002). In contrast, TRα2 and TRβ1 variants were expressed less abundantly than in adult tissues (Chan S et al, 2002). Messenger RNA encoding TRβ2 was weakly expressed in only 26% of fetal cerebral cortex samples.

Immunohistochemistry using antibodies against the TR isoforms was also performed in these fetal cerebral cortical samples and compared to adult samples (provided by the Queen's Square neurological bank, London). Immunostaining of fetal cortex and cerebellum revealed significant TRα1 and TRα2 protein in nuclei from week 11 of human gestation (Kilby *et al*. 2000). Expression of all TR isoform proteins was largely confined to the nuclei of pyramidal neurons of the cerebral cortex and Purkinje cells of the cerebellum, with increasing receptor expression (percentage positive cells per high powered field) with advancing gestational age (Kilby *et al.* 2000). We performed a semi-quantitative observer (unaware of the sample gestations or type) scoring. This demonstrated that the proportion of 'immunopositive' cell types increased with advancing gestation and by the second trimester there was a marked increase in the proportion of Pyramidal and Purkinje cells expressing TRα and β isoforms. By the third trimester, almost all cortical and cerebellar cells were immunostained (Kilby *et al.* 2000). These data confirmed the presence of TRα and β receptors, potentially capable of interacting with the active ligand T3 from early human pregnancy.

In addition, comparison of TR immunostaining in human cerebral and cerebellar cortex from pregnancies complicated by IUGR (estimated fetal weight [customised by maternal BMI] of <10th centile for gestation) (n=18) noted significantly lowered intensity of immunostaining and the proportion of immunopositive cells in both cerebral cortex and cerebellum; as compared to gestationally-matched controls (Kilby *et al.* 2000). This was

confirmed using quantification using TR protein immunofluorescence and was so for all the TRα and β isoforms.

3.2. Iodothyronine deiodinase subtype expression: ontogeny and with changes in IUGR.

In the same human fetal cerebral cortex samples, my group used real time RT-PCR to quantify the expression of mRNA encoding iodothyronine deiodinase subtypes (D1, D2 and D3). In addition, in collaboration with Professor Theo Visser's group in Rotterdam, deiodinase subtype activities were also determined in these tissues and compared to adult cerebral cortex. Iodothyronine deiodinase mRNAs were expressed in human cerebral cortex from 7 weeks of gestation the expression of mRNA encoding D1 was variable in its expression with gestation but generally increased relative to adult cortex; whereas the D1 activity was below the level of assay detection. Subtype deiodinase mRNA and protein activity was detectable in fetal cortex from 7 weeks (but not different from adult expression), and at 15-16 weeks, when expression was higher than in adults. Fetal cortex D3 mRNA expression was again present from the first trimester. The D3 deiodinase enzyme activity appeared great at all gestations in fetal cortex compared to adults, reaching a zenith between 11-16 weeks (Chan *et al.* 2002).

These data described the expression of TRα and β isoforms and the deiodinase subtypes in human cerebral cortex. These data where some of the first to describe the expression of molecules controlling T3 action at a cellular level in the human fetus. However there

were limitations to this work, the most significant being that it was difficult to obtain sufficient intact human brain tissue to be able to describe the anatomical distribution of TR isoforms and deiodinase subtypes. Also in pathological human pregnancy there would be a variable direction between fetal death and post-mortem examination. This would lead to theoretical concerns relating to degradation in mRNA and protein. For this reason, I established a working collaboration with Professor Stephen Matthews at the University of Toronto to work with a guinea pig model to examine thyroid hormone receptor and deiodinase expression.

3.3. The effects of gestational maternal nutrient deprivation to induce in-utero fetal growth restriction in a guinea pig model.

In guinea pigs, a 48 hour period of maternal nutrient deprivation at gestational day 50 (term=70 days) resulted in fetal pups with hypothyroxaemia and increased brain/body weight ratios (indicative of IUGR). On gestational day 52 the guinea pigs were sacrificed and examination of the fetal brains using in-situ hybridization to localise and quantify mRNA encoding TRα1, α2 and β1, with neuroanatomical co-localisation of iodothyronine deiodinase D2 and D3 subtypes (Chan *et al.* 2005).

With maternal deprivation, by day 52, TRα1 and β1 mRNA expression was significantly increased in male fetuses, but decreased in corresponding female hippocampus and cerebellum that demonstrated high TR expression under euthyroid conditions. Maternal

nutrition deprivation also resulted in increased mRNA encoding D2 in many areas of the fetal cortex in both sexes of offspring (Chan *et al.* 2005).

Messenger RNA encoding D3 was only expressed in the fetal brains within the shell of the nucleus accumbens, the posterior amygdalohippocampal area, brain stem and cerebellum. This did not appear to be altered in maternal nutritionally-induced fetal growth restriction.

These animal experiments demonstrated that material nutrient deprivation, inducing apparent IUGR, results in sex-specific changes in TRα and β mRNA expression, with a corresponding generalised increase in mRNA encoding D2 within the fetal brain. These observed changes may represent a 'protective' mechanism to maintain appropriate thyroid hormone action and delivery to the fetal brain in the face of relative fetal hypothyroxaemia (Chan *et al*. 2005).

3.4. The expression of thyroid hormone transporters in the human fetal cerebral cortex during early development and their potential role in neuronal differentiation

Associations of neurological impairment have been made with mutations in the thyroid hormone transporter, MCT8 (Schwartz and Stevenson. 2007). MCT8 is highly expressed in brain and is located in chromosome Xq13. Several mutations have been described mainly by the Visser (Friesema *et al*. 2004) and Refetoff (Dumitrescu *et al.* 2004) groups in the MCT8 gene, being responsible for the X-linked condition, Allen-Herndom-Dudley

syndrome. This is characterised by congenital hypotonia, progressive spasticity and neuropsychomotor delays associated with elevated serum levels of free T3, low levels of free T4 and TSH within the normal range. The mechanisms by which MCT8 in particular, but the thyroid hormone transporters in general, influence neural development have remained poorly defined.

These data, produced by my group, go a small way to defining such roles. In many of the in-vitro experiments performed, we utilized NTERA-2 cl/D1 (NT2) cells. These are pluripotent embryonic cells from a line identified from a human teratocarcinoma and these cells exhibit similar biochemical and developmental characteristics of CNS precursor cells from the early human embryo (Chan *et al.* 2003).

In the human fetal cerebral cortex (7-20 weeks gestation), compared to corresponding 'normal' adult cortex, mRNA encoding OATP1A2, OATP1C1, OATP3A1 variant2, OATP4A1, LAT2 and CD98 were significantly reduced. This was in contrast to mRNA encoding MCT8, MCT10, and OATP3A1 variant 1 and LAT1, which demonstrated similar expression (Chan *et al*. 2011). Using immunohistochemistry, MCT8 and MCT10 protein was localised to the cerebral microvasculature.

In-vitro culturing of NT2 cells (in T3 replete and depleted medium) and inducing neurodifferentation, demonstrated declining T3 uptake, accompanied by reduced expression of mRNA encoding MCT8, LAT1, CD98 and OATP4A1. In addition, T3

depletion of the culture medium significantly reduced MCT10 and LAT2 mRNA expression during neurodifferentiation (but not associated with change in T3 uptake) (Chan *et al*.2011).

NT2 retinoic acid induced-differentiation (either in T3 replete or deplete media) over 21 days had no significant effect upon markers of neurodifferentiation, neurone lengths or numbers of branches (Chan *et al.* 2011). We had previously noted that undifferentiated NT2 cells expressed mRNA encoding TRα and β isoforms and the deiodinase D2 and D3 subtypes (Chan *et al.* 2003). In addition these cells express the thyroid responsive genes, myelin basic protein (MBP) and neuroendocrine specific protein A (NSP-A). As indicated above, these undifferentiated NT2 cells could be terminally differentiated over 5-6 weeks. When terminally differentiated into post-mitotic neurons, TRα1 and β1 mRNA expression decreased by 74% and 95% respectively being associated with a 7-fold increase in NSP-A expression. Culturing the undifferentiated NT2 cells with increasing concentrations of T3 (at 10nM) led to a 2-fold increase in TRB expression and a 3-fold increase in D3 expression. In the terminally differentiated NT2 cells, T3 at 10nM led to a 20% reduction in D3 mRNA expression.

These data indicated that NT2 cells could be used as an in-vitro model of neuronal differentiation. These data demonstrated that undifferentiated NT2 are terminally differentiated cells demonstrating differing patterns of T3 responsiveness (Chan *et al.* 2003).

Finally, we investigated the role of MCT8 expression on NT2 cell proliferation. We investigated the effect of wild-type (WT) MCT8 and the previously reported L471P mutant (Friesema *et al.* 2004) on the growth and function of the human neuronal precursor NT2 cells and (as previously mentioned in the placental section of the thesis) on MCT8-null JEG-3 choriocarcinoma cells.

HA-tagged WTMCT8 correctly localised to the plasma membrane in NT2 cells and correspondingly increased T3 uptake (in NT2 and JEG-free cells). In contrast, L47IPMCT8 mutant (associated with human newborn neurologic morbidity) was retained in the intracellular endoplasmic reticulum and displayed significant attenuation of T3 uptake (James *et al*. 2009). Transient over expression of WTMCT8 and mutant MCT8 proteins failed to induce endoplasmic reticulum stress or apoptosis (James *et al.* 2009). However, MCT8 over expression significantly attenuated cell proliferation (as measured by MTT and thyrodine incorporation assays) in both the presence and absence of active T3. Finally, small interfering RNA depletion of endogenous MCT8 resulted in increased NT2 cell survival and decreased T3 uptake (James *et al.* 2009).

Given that T3 stimulated proliferation in undifferentiated NT2 cells, whereas MCT8 repressed cell growth, these data suggest a novel role for this transporter, in addition to T3 uptake, mediated through the modulation of cell proliferation in the developing brain.   These data indicate that thyroid action on the developing fetal brain and the

intricate modular mechanisms controlling T3 cellular delivery are complicated and their actions may not be mediated through classical endocrine mechanisms.

4. Future work: Beyond August 2011.

At the time of writing this thesis work continues on the areas relating to the action of MCT8 and MCT10 transporters (both directly and in relation to thyroid hormone delivery) in the placenta. This focuses on a series of in-vitro experiments using primary cytotrophoblasts and extravillous trophoblast (from first and third trimester placentas) and utilising transient transfection and small interfering RNA depletion to affect MCT8 (and MCT10) function in these cell types. These experimental designs are similar to but not identical to those described in our paper of 2009 (James *et al*. 2009) and will examine the effects of the thyroid hormone transporters in proliferation, differentiation, apoptosis and invasive potential. In addition, the primary cytotrophoblasts, extravillous trophoblast and explants are taken from both uncomplicated and complicated (by IUGR) pregnancies in and attempt to define aberrant physiology in these processes.

We are also investigating the role of trophoblast and decidua interaction and the effects of thyroid hormone on implantation and placentation. To help with this work we have collaborated with Dr Heike Hener in Jena, Germany to utilise both MCT8 null and heterozygous mice and to elucidate trophoblast function in these animals. My group continues to be interested in the potential role of exogenous thyroid hormone

replacement in potentially ameliorating adverse effects in human pregnancy associated with hypothyroxaemia and overt hypothyroidism.

To these ends, we have recently published a systematic review and critical appraisal of the literature investigating the effects of TPO antibodies in pregnancy when the women retain TSH within physiological range. Our systematic review includes data on 31 studies (12,126 women) relating to miscarriage and five studies (12,566 women) relating to preterm delivery. Meta-analysis of both the cohort (n=19) and case-controlled (n=12) studies demonstrated a positive association of TPO antibodies and miscarriage (OR 3.9; 95%CI 2.48-6.12; p<0.001) for cohort studies and 1.8; 1.25-2.6 (p<0.002) in the case controlled studies (Thangaratinam *et al*. 2011). In addition, a doubling of the odds of premature delivery was also noted (OR 2.07; 98%CI 1.17-3.68). It was notable that the mean TSH concentration was significantly higher in the TPO antibody positive group than those without antibodies but pregnancy loss. Furthermore, two relatively small randomised controlled trials have demonstrated in such circumstances that levothyroxine treatment leads to a significant reduction in miscarriage and preterm delivery (Thangaratinam *et al.* 2011).

The MRC EME board has funded our collaborative group from this year to perform a RCT of sufficient power to draw from conclusions relating to treatment of TPO activity positive women in human pregnancy and also to determine the mechanisms by which thyroid antibodies may exert their detrimental effects.

5. Conclusions

There is increasing evidence that transplacental passage of maternal thyroid hormones is important in the development of the human CNS. A recent unpublished RCT of maternal thyroxine replacement in maternal hypothyroxaemia failed to demonstrate any significant difference in outcome. However, limitation of treatment was up to 16 weeks of gestation, relatively late and after the time of endogenous fetal thyroid hormone production.

Thyroid hormone deficiency or excess alters cell differentiation, migration and gene expression in the developing fetal brain and may have long term sequlae on neurodevelopmental morbidity. In addition, the placenta appears to have some thyroid hormone responsive properties. A well functioning placenta is essential for normal fetal development. IUGR is commonly associated with malplacentation and is a significant contributor to perinatal mortality and morbidity. It is postulated that the changes observed in the expression of thyroid hormone receptors, the pre-receptor deiodinase subtypes and transporters in human placenta and the developing fetal brain may be in response to the fetal hyperthyroxinemia present in this condition and affecting long term morbidity.

The full papers that have been peer reviewed and published between 1998-2011. These are enclosed in Appendix 1.

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7. Other works.

The main content of this DSc Thesis relates to my research focused upon thyroid hormone action on placental and fetal brain development. However, since being appointed as a Senior Lecturer to the University of Birmingham in 1996, I have published peer review papers relating to other aspects of clinical fetal medicine and therapy (listed in Appendix II). The broad subdivision of research work is divided into two areas:

7.1. Epidemiological, population based studies of outcomes on congenital anomalies.

In collaboration with the West Midland's Perinatal Institute, Congenital Anomaly Register (with Professor Jason Gardosi, Dr Michael Wyldes and Ms. Ann Tonks) I have been able to investigate and publish data relating to the epidemiology and fetal therapy /paediatric interventions on outcomes in congenital malformations. This work has used population data bases recording pregnancy outcome on all pregnancies, as well as those that have congenital malformations. Cross reference with our data base at the West Midlands Fetal Medicine Centre, regional cytogenetic laboratory, Birmingham Children's Hospital and the regional neonatal intensive acre units has allowed us to look at outcomes including accurate outcomes for spontaneous fetal loss, stillbirth, neonatal and infant loss rates as well as those pregnancies in whom termination of pregnancy is preformed. This has allowed the accurate 'mapping' of pregnancy outcomes for individual congenital malformations. This includes data as to whether the malformation is isolated or not, whether there is a coincidental chromosomal anomaly and the effects of fetal and neonatal therapy/intervention. All these data are corrected for accurate

population-based 'denominator' data. This has been performed for several of the major congenital malformations, including anterior abdominal wall defects, diaphragmatic hernia, severe cardiac malformations, including hypoplastic left heart syndrome and bladder neck obstruction. Trends in incidence with time have been noted and these data allow both healthcare professional and parents to have prognostic information relating to perinatal outcomes.

7.2. Assessment of diagnostic and therapeutic interventions in fetal medicine.

This work has focused upon several areas of diagnostic and therapeutic fetal medicine and therapy. I worked with Professor Khalid Khan and his group to critically appraise the literature, using systematic reviews and meta-analysis of outcomes relating to fetal diagnostic tests and fetal therapy. I have been fortunate, in that the West Midlands Fetal Medicine Centre is a supraregional referral centre (where I am clinical lead) and has a large throughput of babies with perinatal diseases that are potentially treatable. This has allowed me to publish data relating to relatively large cohort series on outcomes for fetal intervention and in many cases relate findings to population-based dominator data. This has allowed the publication of cohort series relating to diagnostic tests in fetal anaemia, treatment of Rhesus disease by in-utero, intravascular transfusion and comparisons with outcomes by different techniques and experience of operator. Similarly, we have critically appraised the literature relating to outcomes of babies of monochorionic twins with complications; such as twin to twin transfusion syndrome and single twin demise.

Perhaps most successfully, my group completed a series of systematic reviews examining outcomes in babies with congenital bladder neck obstruction (where perinatal mortality is high from pulmonary hypoplasia and early renal failure). Our systemic review evaluating in-utero vesicoamniotic shunting led to the case for a international randomised controlled trial to evaluate such treatment, which was funded by the HTA (the PLUTO trial). The results of this trial and being evaluated and will be published in early 2012.

These two other bodies of work have significantly contributed to the literature in the field of fetal medicine and therapy and form a significant proportion of peer reviewed and published data in my name (as listed in Appendix II).

Appendix

Peer reviewed publications and book chapters of

Mark D. Kilby until August 2011.

PUBLICATIONS
Original peer reviewed papers published.

2011.

Rasiah SV, Ewer AK, Miller P, Kilby MD. Prenatal Diagnosis, Management and Outcome of Fetal Dysrhythmia: A Tertiary Fetal Medicine Centre Experience over an Eight-Year Period. Fetal Diagn Ther. 2011 Jun 23.

Selman TJ, Morris RK, Kilby MD. Management of twin-to-twin transfusion syndrome. Arch Dis Child Fetal Neonatal Ed. 2011 Jun 15.

Thangaratinam S, Tan A, Knox E, Kilby MD, Franklyn J, Coomarasamy A. Association between thyroid auto-antibodies and miscarriage and preterm birth: meta-analysis of evidence. BMJ. 2011;342:d2616-2622.

Chan SY, Martín-Santos A, Loubière LS, González AM, Stieger B, Logan A, McCabe CJ, Franklyn JA, Kilby MD. The expression of thyroid hormone transporters in the human fetal cerebral cortex during early development and in N-Tera-2 neurodifferentiation. J Physiol. 2011 Jun 1;589(Pt 11):2827-45.

Kilby MD, Pretlove SJ, Bedford Russell AR. Multidisciplinary palliative care in unborn and newborn babies. BMJ. 2011 Apr 11;342:d1808.

 Kilby MD. Hypertension risk in the young. Pre-eclampsia is a risk marker. BMJ. 2011 Mar 15;342:d1631.

Morris RK, Ruano R, Kilby MD. Effectiveness of fetal cystoscopy as a diagnostic and therapeutic intervention for lower urinary tract obstruction: a systematic review. Ultrasound Obstet Gynecol. 2011 Jun;37(6):629-37.

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Morris R, Selman TJ, Harbidge A, Martin WL, Kilby MD. Fetoscopic laser coagulation for severe twin-to-twin transfusion syndrome: factors influencing perinatal outcome, learning curve of the procedure and lessons for new centres. BJOG. 2010;117(11):1350-7.

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Hillman SC, Morris RK, Kilby MD. Single twin demise: consequence for survivors. Semin Fetal Neonatal Med. 2010. 15(6):319-26.
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Morris RK, Malin GL, Khan KS, Kilby MD. Systematic review of the effectiveness of antenatal intervention for the treatment of congenital lower urinary tract obstruction. BJOG. 2010;117(4):382-90.

Loubière LS, Vasilopoulou E, Bulmer JN, Taylor PM, Stieger B, Verrey F, McCabe CJ, Franklyn JA, Kilby MD, Chan SY. Expression of thyroid hormone transporters in the human placenta and changes associated with intrauterine growth restriction. Placenta. 2010;31(4):295-304.

Malin GL, Kilby MD, Velangi M. Transient abnormal myelopoiesis associated with Down's syndrome presenting as severe hydrops fetalis: a case report. Fetal Diagn Ther. 2010;27(3):171-3.

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