AN AUDIT OF THYROID FUNCTION TESTS IN A COHORT OF SOUTH AFRICAN CHILDREN WITH DOWN SYNDROME

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DECLARATION

I, Shahida Moosa, declare that this research report is my own work. It is being submitted for the degree of Master of Medicine (Medical Genetics) at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

20 July 2012

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Shahida Moosa Date

DEDICATION

All praise and thanks is due to the One, by whose Grace all good things can be accomplished.

ABSTRACT

Down syndrome (DS) (OMIM #190685), the most common viable chromosome abnormality, is associated with an increased risk of medical complications. The most frequent endocrine abnormalities observed in children with DS involve the thyroid gland, and the risk of thyroid dysfunction increases with age. Global studies have documented a wide spectrum of thyroid dysfunction in children with DS.

Due to the paucity of data from sub-Saharan Africa regarding thyroid function in African children with DS, this study was conceived. The main aim of the study was to document the range of thyroid function in a cohort of 391 South African children with DS, seen at the Genetic Clinics from 2003 to 2008. Referral and treatment practices at two tertiary hospitals in Johannesburg were also documented. The majority (84%) of children had at least one thyroid function test (TFT) performed, and the most common form of thyroid dysfunction encountered was subclinical hypothyroidism (25.3%). Notably, up to one third of patients with abnormal TFT results were not referred to the Endocrine Clinics for evaluation, and were thus not receiving the necessary treatment. There were 13 neonates with congenital hypothyroidism; at least two of them were not referred, and thus not treated during the sensitive neonatal period.

A significant difference was noted between the results from Chris Hani Baragwanath Hospital and those from the other two hospitals. The difficulties in interpretation of results obtained from different biochemical machines and different populations, as compared to those used to derive the reference ranges, were raised. Problems with regular follow-up of patients and annual thyroid surveillance were also highlighted.

The clinical features of hypothyroidism may be difficult to distinguish from the phenotypic features of DS. Thus, regular biochemical screening, even in the absence of physical signs and symptoms, is warranted in this group of children to ensure that hypothyroidism is treated, and further, irreversible neurological and physical impairment prevented.

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ABBREVIATIONS

AACE - American Association of Clinical Endocrinologists

AAP - American Academy of Pediatrics

ACTH - Adrenocorticotropic Hormone

AMA - Advanced maternal age

ANOVA - Analysis of variance

ATA - American Thyroid Association

CHBH - Chris Hani Baragwanath Hospital

CHT - Congenital hypothyroidism

CMJAH - Charlotte Maxeke Johannesburg Academic Hospital

DS - Down syndrome

FSH – Follicle Stimulating Hormone

FT3 - Free triiodothyronine

FT4 - Free thyroxine

GC - Genetic Counselling

HJH - Helen Joseph Hospital

IGF1 – Insulin Growth Factor 1

LDL - Low-density lipoprotein

LH – Luteinising Hormone

NBS – Newborn Screening

NHLS - National Health Laboratory Service

OHT - Overt hypothyroidism

OMIM - Online Mendelian Inheritance in Man

RMH - Rahima Moosa Hospital

SCH - Subclinical hypothyroidism

T3 - Triiodothyronine

T4 - Thyroxine

TOP - Termination of pregnancy

TRH - Thyrotropin releasing hormone

TSH - Thyroid stimulating hormone

UK – United Kingdom

USA – United States of America

1. INTRODUCTION

1.1 DOWN SYNDROME

Down syndrome (DS) (OMIM #190685) is the most common chromosomal abnormality observed in live-born infants, and the most frequent genetic cause of mental retardation. DS is a congenital disorder caused by the presence of a third copy of the whole or a critical part of chromosome 21 (Trisomy 21). In most affected individuals (94%), the trisomy of chromosome 21 involves the whole chromosome through maternal or paternal non-disjunction, whereas in others (3.3%) DS is the result of an unbalanced translocation or of mosaicism of trisomy 21 (2.4%) (Thuline and Pueschel, 1982) .

DS is named after Dr John Langdon Down, who identified and described the typical phenotypic features in 1866. Live-birth rates for DS from around the globe range between 1.5 per 1000 (1 in 660 infants) (Penrose and Smith, 1966) and 1.2 per 1000 (1 in 826 infants) (Hook, 1992), with no predilection for race or socio-economic group. Studies conducted in South Africa show a DS prevalence of 1.8 and 2.09 per 1000 live births in hospital-based studies in urban and rural populations, respectively (Venter, Christianson, Gericke *et al.*, 1995; Christianson, 1996).

The birth prevalence of DS increases with advancing maternal age (Hook, 1992). In some populations the overall birth prevalence of DS has decreased due to increased use of contraception, prenatal screening programmes and the availability of termination of pregnancy. These programmes offer prenatal invasive testing to women of advanced maternal age, and others who are at high risk of carrying a fetus with a chromosomal abnormality (Carothers, Boyd, Lowther *et al*, 1999; Benn, Egan, Fang *et al*, 2004). There

is a paucity of data from South Africa reflecting changing population demographics and the influences of prenatal testing and termination of pregnancy.

DS is characterised by typical dysmorphic features (see Figure 1.1 below and Table 1.4 on page 13), and is associated with an increased incidence of certain medical complications.

Figure 1.1: Pictures of an infant with DS, showing typical facial dysmorphic features (Reproduced with the written permission of the parents)

Some degree of mental, developmental and growth retardation is seen in all people with DS. About 40% to 50% of individuals with DS have a congenital heart defect, most commonly endocardial cushion defects and ventricular septal defects (Freeman, Torfs, Romitti *et al*, 2009). Gastrointestinal abnormalities, such as oesophageal atresia, duodenal atresia, pyloric stenosis and Hirschsprung's disease, are found in more than 10% of individuals with DS. All the children tend to be hypotonic and have delayed milestones. Acute lymphocytic leukaemia is more common in affected children, as are visual, urogenital and endocrine abnormalities (Jones, 2006). The most common endocrine disorder associated with DS involves the thyroid gland (Pueschel, Jackson, Giesswein *et al.*, 1991).

1.2 THYROID HORMONES AND THEIR FUNCTION

Under the regulatory control of the hypothalamus and the pituitary gland, the thyroid gland secretes hormones, principally thyroxine (T4) and triiodothyronine (T3), which regulate the rate of metabolism and affect the growth and rate of function of many other systems in the body. Free T3 and free T4 (hereafter abbreviated as FT3 and FT4) are the biologically active components of the total T3 or total T4, respectively. The production of T4 and T3 is regulated by thyroid-stimulating hormone (TSH), released by the thyrotrope cells in the anterior pituitary. The thyroid and thyrotropes form a negative feedback loop: TSH production is suppressed when the T4 levels are high, and vice versa. The TSH production itself is modulated by thyrotropin-releasing hormone (TRH), which is produced by the hypothalamus and secreted at an increased rate in situations of accelerated metabolism (see Figure 1.2) (Rossi, Caplin and Alter, 2005).

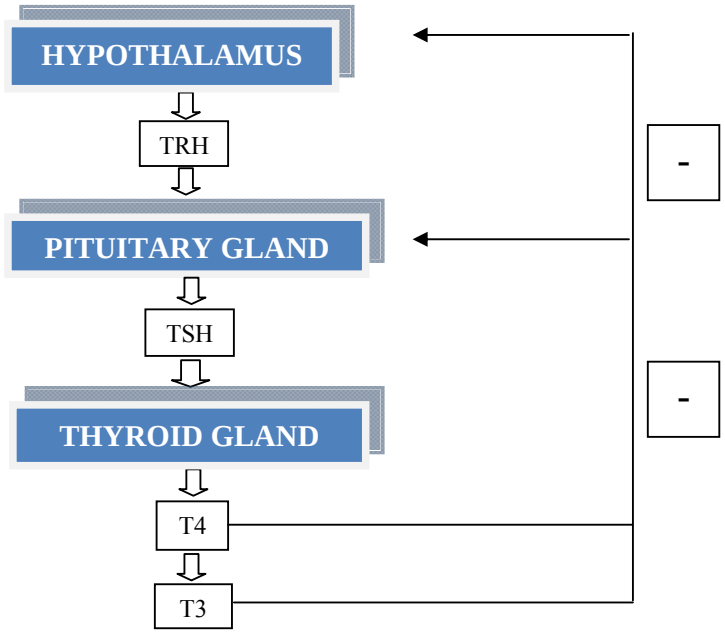

Figure 1.2: Hypothalamic-pituitary-thyroid feedback.

Normal thyroid hormone levels are necessary for optimal growth and cognitive development and functioning. Thyroid hormones play a crucial role as regulators of growth and skeletal development, puberty and metabolism (Rossi, Caplin and Alter, 2005). Specifically, in the central nervous system, thyroid hormones are important in neuronal migration and differentiation, activation of the sympathetic nervous system, myelination and regulation of gene expression of neuronal cells (Bernal and Nunez, 1995).

1.3 THYROID DYSFUNCTION

When thyroid function is normal, meaning that both the TSH and the FT4 are within normal limits, the patient is termed "euthyroid". Thyroid dysfunction can broadly be divided into two categories: hypothyroidism (underactive thyroid) and hyperthyroidism (overactive thyroid). The diagnosis of thyroid dysfunction may be complicated by a few issues. Firstly, the clinical symptoms may be very mild. Secondly, on a biochemical level, one may encounter TFT results, which demonstrate seemingly paradoxical results, such as a low TSH level with normal FT4 and FT3 results, or an elevated TSH level with normal FT4 and FT3 levels (discussed further below). Thyroid function can be labile, with hormone level shifts occurring over weeks or months. Thyroid function tests may also be influenced by concomitant illness or drug administration (Rossi, Caplin and Alter, 2005). It is therefore important to keep all these elements in mind when interpreting TFT results.

1.3.1 HYPOTHYROIDISM

Hypothyroidism results from underproduction of the thyroid hormones FT4 and FT3. There are different forms of hypothyroidism, which differ by age of onset, biological mechanism and degree of abnormality of the other thyroid hormones in addition to the TSH (Rossi, Caplin and Alter, 2005). Hypothyroidism in the newborn is termed congenital

hypothyroidism (CHT) and is discussed in more detail in section 1.3.1.3. Hypothyroidism in older children and adolescents can have many causes (see table 1.1) (Hueston, 2001).

Most cases (95%) of hypothyroidism in children are due to primary hypothyroidism or failure of the thyroid gland to produce thyroid hormones. These are typically associated with elevated levels of TSH and low levels of FT4 and FT3. The remaining 5% of cases are due to central causes, also known as secondary and tertiary hypothyroidism, caused by pituitary gland or hypothalamic dysfunction, respectively (Hueston, 2001). In these cases, the TSH can be low, normal or inadequately elevated for the degree of FT4 and FT3 deficiency (Hueston, 2001). Subclinical hypothyroidism (SCH) is a state of usually asymptomatic, mild thyroid failure, with normal levels of FT4 and FT3, and minimal elevation of TSH (Rossi, Caplin and Alter, 2005).

Table 1.1: Causes of hypothyroidism in older children and adolescents*

Autoimmune	Hashimoto's thyroiditis
Iodine-related	Deficient intake of iodine Excessive intake iodine/related substances
Thyroid gland damage	Infiltration into thyroid Radiation therapy Radioactive iodine therapy Thyroidectomy
Congenital	Late onset congenital hypothyroidism Thyroid dysgenesis Inborn errors of thyroxine synthesis
General	Hypothalamic-pituitary disorder Generalised thyroid hormone deficiency
Drugs	Several including thionamides, lithium, anti-convulsants, interferon alfa, tyrosine kinase inhibitors
Dietary	Goitrogen ingestion (eg cassava beans)
Other/rare	Histiocytosis X Cystinosis

*****Adapted from Hueston 2001*

1.3.1.1 Primary Hypothyroidism

Children with primary hypothyroidism have high serum TSH concentrations and low serum free T4 values. This biochemical presentation may be associated with clinical signs, such as lethargy, weight gain, dry skin, cold intolerance, constipation, delayed puberty, goitre, poor growth and delayed bone and dental age. Short stature and intellectual deficiency are other invariable long-term sequelae, if the hypothyroidism is not treated (Rossi, Caplin and Alter, 2005). For the purposes of this study, hypothyroidism as demonstrated by an elevated TSH with a corresponding decrease in FT4 will be referred to as overt hypothyroidism (OHT).

1.3.1.2 Central Hypothyroidism

Central hypothyroidism is caused by pituitary and/or hypothalamic dysfunction. Of note, TSH levels are low or normal. If the TSH is elevated, it is usually not to the degree one would expect relative to the FT4 and FT3 levels (Rose, Brown, Foley *et al*., 2006). This may occur as a result of decreased stimulation of the anterior pituitary by TRH, or by deficient synthesis or secretion of TSH by the anterior pituitary. In some cases, the lack of active TSH is a consequence of biologically ineffective TSH being secreted (Braverman and Utiger, 2005). The clinical manifestations of central hypothyroidism are related to a deficiency of FT3 and FT4 and therefore are similar to primary hypothyroidism (Brown, 2001).

Patients with central hypothyroidism may have concomitant derangements in one or more pituitary hormones, including ACTH, gonadotrophin hormones (LH and FSH), growth hormone, and prolactin deficiencies. If central hypothyroidism is caused by a functioning pituitary adenoma, other hormonal dysfunction may be present (Braverman and Utiger, 2005).

1.3.1.3 Congenital hypothyroidism

Congenital hypothyroidism (CHT) refers to hypothyroidism which is present from birth. It occurs in 1 in 2000 to 1 in 4000 live births. It is most commonly caused by dysgenesis (abnormal development) of the thyroid gland (85%). Other causes include abnormalities of thyroid hormone synthesis, also known as dyshormonogenesis, central abnormalities (hypothalamic-pituitary axis defects) and peripheral defects (due to abnormalities in thyroid hormone metabolism, action or transport) (Rastogi and LaFranchi, 2010). Furthermore, CHT may be classified as transient (CHT which resolves) or persistent. There are several genetic syndromes, including Down syndrome, which are associated with increased prevalence of CHT (Rastogi and LaFranchi, 2010). Table 1.2 summarises the main causes of CHT.

Table 1.2: Causes of congenital hypothyroidism

PRIMARY CHT	
Thyroid dysgenesis	Ectopic gland, hypoplasia or hemiagenesis
Thyroid dyshormonogenesis	Impaired hormone production
CENTRAL CHT	
Isolated TSH deficiency	TSH β subunit gene mutation
TRH abnormality	Deficiency of TRH, resistance to TRH, TRH receptor gene mutation
Structural abnormality	Pituitary or hypothalamic lesion
Other	Rare genetic mutations resulting in abnormalities of the hypothalamic-pituitary axis
PERIPHERAL CHT	Resistance to thyroid hormone Hormone receptor or transporter abnormalities
TRANSIENT CHT	Maternal antithyroid medication ingestion Transplacental passage of TSH receptor blocking antibodies Iodine deficiency (maternal/neonatal) Congenital hepatic haemangioma
OTHER	Genetic syndromes

**Adapted from Rastogi and LaFranchi, 2010*

Normally, within the first half hour after birth, newborns experience a surge in TSH levels in response to the change in environmental temperature. This rise in TSH can be accompanied by increased levels of FT4, especially in the first 48 hours. Thus, testing for thyroid dysfunction in the first two days of life may not be routinely done (Rossi, Caplin and Alter, 2005). However, in settings where newborns are discharged within 48 hours of birth, thyroid function is still tested but compared with cord blood TFT reference ranges (Dr George van der Watt – personal communication).

Clinical features of hypothyroidism in the newborn are often non-specific, especially in the newborn, and may include decreased activity, increased sleep, difficulty feeding, constipation, prolonged jaundice, large fontanelles, macroglossia and hypotonia (Rossi, Caplin and Alter, 2005). Congenital hypothyroidism (CHT) can be diagnosed within the first few days of life (usually between three and seven days) as is usually the case in countries where a Newborn Screening (NBS) protocol exists.

1.3.1.4 Subclinical hypothyroidism

Subclinical hypothyroidism (SCH) is defined by persistent elevation of TSH, while FT4 remains normal (Rossi, Caplin and Alter, 2005). It is called "subclinical" as there are often no clinically discernable features of hypothyroidism. The diagnosis relies on the biochemical markers.

1.3.2 HYPERTHYROIDISM

Hyperthyroidism is diagnosed in the presence of a decreased TSH level. The FT4 and FT3 levels are typically elevated. There may be associated clinical signs, such as inattention,

decreased sleep, heat intolerance, increased heart rate, frequent stools, poor weight gain, decreased school performance and personality changes (Rossi, Caplin and Alter, 2005).

1.3.3 INTERPRETATION OF ABNORMAL THYROID HORMONE RESULTS

When TFTs are performed, it is important to consider the entire hormonal profile, i.e. TSH and FT4 (with or without FT3), together with the clinical presentation. The results should be compared to age-specific normal values. Table 1.3 summarises the main differential diagnoses, which are considered to be associated with different abnormal TFT results (Wallach, 2000; Surks, Ortiz, Daniels et al., 2004; The Association for Clinical Biochemistry, British Thyroid Association, British Thyroid Foundation, 2006; Marshall and Bangert, 2008).

Table 1.3: Differential diagnosis with abnormal thyroid function test results

TSH Level	FT4 Level	Differential Diagnosis
LOW	LOW	**Suggests central hypothyroidism** - TSH may be decreased, normal, or slightly elevated. Investigation for deficiencies in other pituitary hormones should be undertaken and may include: ACTH with cortisol, FSH, LH, oestradiol (female), testosterone (male), prolactin, growth hormone, and insulin-like growth factor 1 (IGF1). **Other non-thyroidal illness**. TSH can be normal or low followed by rebound elevation during recovery from acute illness. FT4 can be normal, low, or high.
LOW	NORMAL	**May suggest mild hyperthyroidism**. **In non-thyroid illness**, TSH can be normal or low followed by rebound elevation during recovery from acute illness. FT4 can be normal, low, or high. **Drugs** which inhibit pituitary TSH secretion, including dopamine, dopaminergic agonists, glucocorticoids, cytokines, among others. **Recent treatment** of hyperthyroidism with anti-thyroid medication.

TSH Level	**FT4 Level**	**Differential Diagnosis**
HIGH	HIGH	**TSH-secreting pituitary tumour** or a syndrome of resistance to thyroid hormone. Further endocrine investigation is indicated. **Thyroxine replacement therapy** (for possible hypothyroidism) taken shortly before test
HIGH	LOW	**Suggests primary hypothyroidism**. Other causes include **damage to the thyroid** (autoimmune destruction/thyroidectomy/ radioactive iodine treatment).
HIGH	NORMAL	**Suggests subclinical hypothyroidism** **Other diagnoses** include recovery from non-thyroid illness, poor adherence to thyroxine replacement therapy or its malabsorption. Drugs should also be considered.
NORMAL	LOW	**May be suggestive of central hypothyroidism** **Drugs** (including phenytoin, rifampicin, carbamazepine, barbiturates)
LOW	HIGH	**Suggestive of hyperthyroidism**. Other diagnoses: subacute thyroiditis during the initial stages of the disease; factitious thyrotoxicosis (from excessive intake of thyroxine) or iodine-related.

1.3.4 THE SPECTRUM OF THYROID DYSFUNCTION IN DOWN SYNDROME

Individuals with DS exhibit a wide range of thyroid dysfunction. Clinical forms of hypothyroidism found in individuals with DS include congenital hypothyroidism (CHT), subclinical hypothyroidism (SCH), transient and primary hypothyroidism, thyroxin-binding globulin deficiency and chronic lymphocytic thyroiditis (Coleman, 1994). There is no evidence to suggest that children with Down syndrome are at greater risk of developing central hypothyroidism than the general population. However, the diagnosis of thyroid dysfunction in DS is complicated by the overlap between thyroid-associated symptoms and clinical features of the syndrome, as well as the difficulty in interpreting TFT results from children with DS (Coleman, 1994).

Subclinical or "compensated" hypothyroidism (SCH) is the most common form of thyroid dysfunction in DS patients, being reported in 25-32% of patients (Rubello, Pozzan, Casara *et al.*, 1995; Tüysüz and Beker, 2001).

Hyperthyroidism, although rarer, also occurs more frequently in DS individuals than in the general population (Takahashi, Bordy, Sharma *et al.*, 1979). A study on the prevalence of hyperthyroidism in Catalonia, Spain revealed a figure of 6.5 cases per 1000 patients with DS. This too, is higher than expected for the general population (Goday-Arno, Cerda-Esteva, Flores-Le-Roux *et al.*, 2009).

1.3.5 PROPOSED CAUSES OF THYROID DYSFUNCTION IN DOWN SYNDROME

The underlying pathological mechanisms responsible for thyroid dysfunction in individuals with DS are not well understood. Several hypotheses have been proposed:

1.3.5.1 Abnormal Thyroid Gland Development

It has been postulated that defective migration and differentiation of thyroid cells during embryonic development and abnormal growth of the thyroid gland in fetal life, results in agenesis or hypogenesis of the gland (Gruneiro de Papendick, Chiesa, Bastida *et al*., 2002). However, the cause of congenital hypothyroidism may not be entirely due to thyroid agenesis, as most patients have normal thyroid scans (Kennedy, Jones and Cuckle, 1992).

1.3.5.2 An Auto-Immune Process

Auto-immune thyroiditis may cause both SCH and OHT (Fort, Lifschitz, Bellisario *et al.*, 1984; Ivarsson, Ericsson, Gustafsson *et al.*, 1997). Children with DS exhibit

an increased risk of autoimmune thyroiditis (Pueschel and Pezullo 1985; Sharav, Collins, Baab *et al.*, 1988; Ivarsson *et al.,* 1997). Autoimmune thyroiditis has also been implicated in the pathogenesis of SCH in children with DS. (Rubello *et al.*, 1995; Konings, Van Trotsenburg, Ris-Stalpers *et al.*, 2001). The anti-thyroid antibodies implicated are directed toward thyroglobulin and thyroid peroxidise. Rubello *et al* (1995) observed a higher prevalence of SCH and anti-thyroid antibodies in DS patients compared with controls. Patients with SCH, who had thyroid antibodies, tended to have a higher risk of developing overt hypothyroidism compared to thyroid-antibody negative individuals (Rubello *et al.,* 1995). Shawky, Elsedfy and Amer *et al* (2005) studied 40 Egyptian children with DS and compared their thyroid function results and thyroid antibody results to 40 children from the general population. They found that the most common form of thyroid dysfunction in their DS study group was SCH (32.5%) compared to 12.5% in the control group. Also, 65% of DS children had thyroid antibodies, compared to 15% among controls. Zori, Schatz and Ostrer *et al* (1990) studied 61 individuals with DS, aged 5 months to 48 years, and sought to clarify the role of antibodies in DS thyroid dysfunction. They, however, suggested that thyroid dysfunction in patients with DS occurs at all ages and is a "common heterogeneous disorder which cannot be solely explained on the basis of autoimmunity".

1.3.5.3 Delayed Maturation of the Hypothalamic-Pituitary Axis

Delayed maturation of the hypothalamic-pituitary axis has been hypothesised as the probable cause of hypothyroidism (particularly SCH) in DS patients. Sharav, Landau and Zadik *et al* (1991) demonstrated more exaggerated TSH responses to TRH in their patients with DS compared to controls. Oliveira, Longui and Calliari *et al* (2002) conducted a study on 14 children with DS, aged between 1.6 to 5.2

years (mean 3.2 years). They compared the thyroid function in these patients to 16 controls, and surmised that basal TSH levels were higher in the DS group, and that they displayed a more striking response after stimulation with TRH. They concluded that "an abnormal pattern of TSH secretion occurred in patients with Down syndrome, possibly due to hypothalamic dysfunction".

1.3.5.4 Primary Thyroid Gland Abnormality in Neonates with Down Syndrome

Van Trotsenburg, Kempers and Endert *et al* (2006) showed that neonates with DS can be mildly hypothyroid. They proposed that this mild but persistent hypothyroid state could be primarily thyroidal in origin, although the exact mechanism is still unclear.

1.3.5.5 Mutations in the TSH-Receptor Gene

Mutations in the TSH-receptor gene have been postulated to cause thyroid dysfunction in individuals with DS. However, Agretti, De Marco and Collecchi *et al* (2003) did not find inactivating mutations of the TSH-receptor gene in 12 patients with DS who had elevated TSH levels.

1.3.5.6 Genomic Imbalance due to Trisomy of Chromosome 21

A DS-specific thyroid regulation disorder due to a genomic dosage imbalance caused by the extra chromosome 21, has also been suggested (Antonarakis, Lyle, Dermitzakis *et al*., 2004; Van Trotsenburg *et al*., 2006). If this is the case, further studies are needed to delineate the loci and genes involved.

1.3.5.7 Decreased Bioactivity of TSH

The bioactivity of TSH in DS individuals was questioned. Konings *et al* (2001) found no evidence that children with DS have reduced bioactivity of serum TSH compared to euthyroid controls.

1.3.5.8 Zinc Deficiency

Zinc deficiency appears to be more frequent in DS patients, and can affect endocrine and immunological function. Normalisation of serum zinc caused normalisation of TSH in a randomised control study in 25 children with DS. After 4 months of oral zinc supplementation, children with DS who had initially had higher levels of TSH compared to the 14 healthy controls, showed normalised TSH levels (Licastro, Mocchegiani, Zannotti *et al.*, 1992). This was later replicated in another study in children with DS (Bucci, Napolitano, Giuliani *et al*., 1999). However, Romano, Pettinato and Ragusa *et al* (2002) found no difference in the thyroid function of patients with DS who were zinc deficient, compared to those who were not (Romano *et al.*, 2002).

1.4 CLINICAL OVERLAP BETWEEN DOWN SYNDROME AND HYPOTHYROIDISM

The diagnosis of hypothyroidism in individuals with DS can be difficult to establish clinically, as hypothyroid features can be masked by the clinical phenotype of the syndrome. Indeed, many of the symptoms of hypothyroidism overlap with common features of DS (see Table 1.4).

Table 1.4: Comparison of Physical Features in children with DS and Hypothyroidism*

	Down syndrome	**Hypothyroidism**
Head	Brachycephaly Intermediate fontanelle	Normal
Tongue	Protruding	Large
Nasal Bridge	Underdeveloped	Underdeveloped
Eyes	Upslanted palpebral fissures Epicanthic folds Brushfield spots (in blue eyes)	Normal
Neck	Short	Short
Heart	Murmur (mainly atrio-ventricular canal)	Murmur (often non-specific)
Abdomen	Umbilical hernia	Umbilical hernia
Neuromuscular	Hypotonia	Hypotonia
Skin	Dry	Dry
Extremities	Short hands and feet, transverse palmar crease, clinodactyly, wide sandal gaps, hyperextensible joints	Short hands and feet

***Adapted from Coleman (1994)*

One American study showed that 20% of community-based patients with DS, aged two to 59 years, had previously undiagnosed overt hypothyroidism. These patients had not complained of hypothyroid symptoms, nor had their doctors suspected hypothyroidism clinically (Friedman, Kastner and Pond, 1989). Other studies confirm the incidence of undiagnosed overt hypothyroidism and SCH in newborns, children and adults with DS (Loudon, Day and Duke, 1985; Pozzan, Rigon, Girelli *et al.*, 1990). These findings occur in the presence of a national NBS programme in European countries, which includes thyroid testing for all children at birth (see Appendix A).

Fifty Saudi Arabian children with DS (ages seven months to nine years) were studied by Abdullah and colleagues in 1994. They found that 6% of the DS children had hypothyroidism, including two individuals who were symptomatic, but had not been previously evaluated for thyroid dysfunction or thyroid antibody status. Thus, even though

these children displayed "typical" signs of hypothyroidism, the diagnosis of hypothyroidism was not entertained as the signs were thought to be part of the DS phenotype (Abdullah, Salman, Al-Habib *et al*., 1994).

1.5 EPIDEMIOLOGY OF THYROID DYSFUNCTION IN DOWN SYNDROME

Since the early 1960's, studies have been published documenting thyroid status in individuals with DS (Prasher, 1999). Study design and populations tested have varied. For example, Prasher (1995), Toledo *et al* (1997) and Noble *et al* (2000) used a cross-sectional study design, whereas the studies of Rubello *et al* (1995), Ivarsson *et al* (1997), Karlsson *et al* (1998) and Tüysüz and Beker (2001) were all longitudinal.

A number of studies of thyroid function in DS have shown an increased prevalence of both congenital and acquired forms of hypothyroidism, as well as hyperthyroidism, albeit the latter to a lesser extent. However, prevalence figures for thyroid dysfunction in DS vary according to the age ranges of patients and the population tested.
Thyroid disorders have been reported to have a prevalence of 3 to 54% in people with DS, with the frequency of thyroid dysfunction increasing with age (Karlsson *et al*., 1998). However, it is estimated that the lifetime prevalence of hypothyroidism in DS is 30 to 50% (Prasher, 2006).

Shaw and colleagues (2006) described a prevalence of hypothyroidism in the zero to one year age group as being 16.7% compared to 20% in the nine to 12 year group, reflecting an increase with age (Shaw *et al.*, 2006). Around 15% of adolescents with DS have hypothyroidism, with thyroid function decreasing with age (Pueschel *et al*., 1991).

Moreover, in a longitudinal study of 85 patients with DS (aged zero to 25 years), who did not have congenital hypothyroidism, Karlsson *et al* (1998) found that up to 15 years later 30/85 (35%) had developed thyroid dysfunction: 28/30 (93%) had some form of hypothyroidism and 2/30 (7%) had hyperthyroidism.

The prevalence of hypothyroidism in the newborn population of DS has been reported as 0.7%, which is 28 times higher than the general population (Fort *et al.,* 1984). Reporting on statistics from the New York State Newborn Screening program, Fort *et al* (1984) described an incidence of congenital hypothyroidism in neonates with DS of 1 in 141 live births, compared to an incidence of 1 in 3000 to 1 in 4000 among healthy newborns.

Summarised in table 1.5 are the findings from the significant studies conducted in both developed and developing countries over the past 30 years, which have investigated thyroid function in populations of infants and children with DS. All studies have included a proportion of children with DS, although several have extended the studies to include adults with DS as well.

The studies in table 1.5 show significantly increased frequency rates of the entire thyroid dysfunction spectrum associated with DS. Most of the authors recommend regular (six monthly to annual) screening for thyroid dysfunction (with or without thyroid antibody testing) in their respective populations with DS. Estimated frequency values vary. Differences between the studies can also be explained due to operational definitions employed and whether SCH was included under hypothyroidism, or given as a separate value. However, evaluation of the studies presented in table 1.4 suggests that SCH accounts for the majority of thyroid dysfunction (frequency range of 5-88%).

Table 1.5: Summary of studies on thyroid dysfunction, which included children with DS over the last 30 years

Author	Year	Country	No. of patients	Age ranges	% of patients with thyroid dysfunction
Fort *et al*	1984	USA	1130	3-16D	1.1% CHT (transient and persistent primary)
Abassi and Coleman	1984	USA	206	<18Y	8% OHT
Pueschel and Pezzullo	1985	USA	151	3-21Y	**14% SCH** 6.6% OHT
Loudon *et al*	1985	UK	116	9M-19Y	3.4% OHT
Cutler *et al*	1986	USA	49	4M-3Y	**27% SCH** 2% OHT 6% CHT 2% hyperthyroidism
Sharav *et al*	1988	Israel	147	4M-27Y	**60% SCH**
Tirosh *et al*	1989	Israel	44	2-51Y	**16% SCH** 7% OHT
Zori *et al*	1990	USA	61	5M-48Y	**39% SCH** 23% OHT 3% hyperthyroidism
Pozzan *et al*	1990	Italy	108	3M-38Y	**31% SCH** 5% OHT 2% hyperthyroidism
Pueschel *et al*	1991	USA	181	0-30Y	**8% SCH** 6% OHT
Selikowitz *et al*	1992	Australia	132	4.58-17.08Y	**9.1% SCH** 3% OHT
Abdullah *et al*	1994	Saudi Arabia	50	7M-9Y	6% hypothyroidism
Rubello *et al*	1995	Italy	344	N/S	**32.5% SCH**
Toledo *et al*	1997	France	105	3M-20Y	51.4% hypothyroidism (including SCH and OHT)
Jaruratansirikul *et al*	1998	Thailand	112	<1Y	15.2% some form CHT (overt and SCH)
Karlsson *et al*	1998	Sweden	85	1-25Y	33% hypothyroidism 2.4% hyperthyroidism
Tüysüz and Beker	2001	Turkey	320	5D-10Y	**5% SCH** (25.3% of those who had some form of thyroid dysfunction) 1.8% CHT

Author	Year	Country	No. of patients	Age ranges	% of patients with thyroid dysfunction
Gruneiro de Papendick *et al*	2002	Argentina	137	0.04-16Y	**88% SCH** 6% OHT 2.9% CHT 3% hyperthyroidism
Van Trotsenburg *et al*	2003	The Netherlands	284	4-7D	3.5% CHT
Shawky *et al*	2005	Egypt	40	N/S	**32.5% SCH**
Dias *et al*	2005	Brazil	169	1-16Y	**39.6% SCH**
Mak *et al*	2006	China	351	0-19Y	**22.5% SCH** 2% OHT 2% CHT 2.3% hyperthyroidism
Shaw *et al*	2006	Nepal	32	1-12Y	**12.5% SCH** 3.1% OHT
Unachak *et al*	2008	Thailand	140	3D-14Y	**32.9% SCH** 7.1% OHT 2.1% hyperthyroidism
Pascanu *et al*	2009	Romania	63	5D-18Y	**27% SCH** 9.5% OHT 1.6% CHT

D:day; M:month; Y:year; CHT: congenital hypothyroidism; OHT: overt hypothyroidism; SCH: subclinical hypothyroidism; N/S: not stated

1.6 THE SUBCLINICAL HYPOTHYROIDISM CONTROVERSY

There is no disagreement among doctors about the management of overt hypothyroidism (increased TSH with low levels of FT4). It is universally recognised that congenital hypothyroidism, especially, carries a high risk of severe developmental delay if not treated promptly (Rossi, Caplin and Alter, 2005). The controversy arises with regard to SCH. This controversy is not confined to SCH in children with DS; it extends to normal children, pregnant women and even adults (Mc Dermott and Ridgway, 2001; Surks, Ortiz, Daniels *et al*., 2004; Papi, Uberti, Betterle *et al.*, 2005; Surks, 2005; Gharib, Tuttle, Baskin *et al.*, 2005).

SCH is frequently encountered in general paediatric practice, but its clinical significance is widely debated. There is unfortunately no consensus with regards to: (i) the morbidity and clinical significance of SCH; (ii) whether to further investigate individuals with SCH, (iii) whether patients with SCH should be treated or not; and (iv) if treated, at which TSH levels treatment should be instituted.

There are a few reasons for the lack of consensus surrounding the management of SCH. Firstly, there is a paucity of data on the natural history of SCH in children, and some clinicians are concerned that if left untreated, SCH would progress to overt hypothyroidism. Individuals with SCH in the general population have a yearly risk of two to five percent of developing overt hypothyroidism. This risk may be even higher in the presence of thyroid antibodies (Surks *et al*., 2004). The clinicians caring for patients with DS are also faced with a paucity of data derived from patients with DS. They too are required to extrapolate data from studies on normal children and normal adults, which may not be applicable to children with DS.

Secondly, there are no large paediatric randomised controlled studies comparing outcomes in patients with SCH who were treated with thyroxine and those left untreated. Thus, most clinicians have to rely on data from adult studies, which may also produce conflicting recommendations.

Several recent studies have attempted to clarify the natural history of thyroid function in healthy children (Moore, 1996; Biondi and Cooper, 2008; Lazar, Frumkin, Battat *et al.*, 2009). Lazar *et al* (2009) studied over 120 000 non-DS children aged between six months and 16 years, 3.3% of whom initially had an abnormally elevated TSH result (>5.5mIU/l).

They followed these children over a five year period. Children with lower initial TSH levels (<7.5mIU/l), tended to experience spontaneous recovery, or their TSH levels remained constant. This was in agreement with previous studies, which suggested that SCH was a self-remitting process in childhood (Moore, 1996; Biondi and Cooper, 2008). Lazar *et al* (2009) identified female gender and higher TSH levels (>7.5mIU/l) as risk factors for sustained thyroid dysfunction, which warranted further investigation and monitoring.

A similar study conducted on 122 children with DS was done by Gibson, Newton and Selby *et al* in 2005. Their findings also suggest that SCH is a transient, self-limiting phenomenon (Gibson *et al*., 2005). Gibson and colleagues, however, were only able to retest 20 children of the original 24 who had SCH and they found 14 (70%) had normal TSH levels. Researchers would argue that this small sample size cannot be used to draw definitive conclusions regarding the natural history of SCH in the paediatric population with DS.

Country-specific DS guidelines, such as those from the United Kingdom (UK), do not support the treatment of SCH (with a TSH level less than 10mIU/L), based on lack of evidence that treatment is beneficial in the paediatric population (Down Syndrome Medical Interest Group, 2005).

Proponents of treatment of sub-clinical hypothyroidism offer as justification for their decision, the fact that decreased thyroid levels might be a confounding contributor to the poor growth and mental retardation in DS. Increased TSH levels reflect the sensitivity of the hypothalamic-pituitary axis to small decreases in circulating thyroid hormone. A FT4

level, while still mainly within the normal reference range, may actually be low for that particular patient, in the light of the increased TSH. Some authors believe that SCH represents mild thyroid failure and is a clinically important disorder that has adverse clinical consequences and therefore should be treated in most, if not all, cases (McDermott and Ridgway, 2001). Papi *et al* (2007) recommended that treatment should be instituted in all newborns with SCH to prevent possible detrimental sequelae: mental and growth retardation.

Because some authors believe that SCH will eventually lead to overt hypothyroidism, Karlsson *et al* (1998) suggested that thyroxine replacement be encouraged even in cases of marginal hypothyroidism to prevent the development of a more severe hypothyroid state. Cutler and colleagues (1986) suggested that the isolated elevation of TSH in children with DS, even in the absence of thyroid antibodies, might be an early sign of primary autoimmune hypothyroidism.

In 1998, Karlsson *et al* conducted a longitudinal study of 85 individuals with DS under the age of 25 years. They treated 30 patients with DS, who were either diagnosed with SCH or OHT, and reported an increased growth velocity in seven patients with DS. Other authors have recommended treatment for normal children and adolescents with SCH, and have linked treatment to significant improvements in height and development (Fatourechi, 2002; Cetinkaya, Aslan, Vidinlisan *et al.,* 2003).

Van Trotsenburg and colleagues (2003) described "mild" plasma TSH elevation as being extremely prevalent in individuals with DS: 80-90% in early infancy and 30-50% thereafter. They studied 294 newborn samples from neonates with DS and found that the

decreased FT4 (normally distributed, but shifted to the left of the normal curve) and increased TSH levels pointed to a mild hypothyroid state in newborns with DS, and supported the existence of a DS-specific thyroid regulation disorder. This lower thyroid hormone tissue availability might be disadvantageous for the already compromised brain development and somatic growth found in children with DS. On the basis of the data from their study, suggesting that commencing treatment in young DS children may improve growth and development, they recommend that all DS newborns receive thyroxine treatment.

A randomised control study on 196 patients was performed to test the benefit of thyroxine treatment in children with DS, and it showed that the patients receiving thyroxine showed less developmental delay and better growth than the patients who received placebos. These improvements in those receiving thyroxine were statistically significant ($p<0.001$) (Van Trotsenburg, Vulsma, Van Santen *et al*., 2005). Although patients in this study were only followed-up for 24 months, and some exhibited some form of central nervous system derangement at re-examination, these findings were in contrast to an earlier study, which showed no significant difference between the DS group compared to a group of matched euthyroid controls with mental retardation of unknown cause, when treated with thyroxine supplements (Tirosh, Taub, Scher *et al*., 1989). However, Tirosh *et al* only reported on seven patients, and their study evaluated patients shortly (eight to 24 weeks) after commencement of treatment. They did not do any long-term follow-up on their original patients. Longer-term studies are needed to evaluate the effects of thyroxine treatment on growth and development.

Other researchers view SCH as a distinct and relevant entity to be treated. A study by Tüysüz and Beker (2001), recommends that every infant with DS be treated with thyroxine until the age of three years. They felt that the advantage of this approach would be the prevention of OHT for those children whose thyroid function was not stable, and whose increasing TSH levels would eventually lead to a depressed FT4.

1.7 ADVERSE EFFECTS OF HYPOTHYROIDISM

One of the functions of thyroid hormone is to ensure optimal cardiac function. In adult studies, neurobehavioural disturbances, cardiac dysfunction and dyslipidaemias have been reported in individuals with SCH (Mc Dermott and Ridgway, 2001). Subclinical hypothyroidism (SCH) is proposed to have adverse effects on the cardiovascular system: directly by altering systolic and diastolic function and indirectly by negatively altering the lipid profile, by increasing both the total cholesterol and low-density lipoprotein (LDL) levels (Papi *et al.,* 2007).

Mao, Wang and Jiang (2008) showed that non-DS neonates with CHT have impaired cardiac function, which reverses with appropriate thyroxine supplementation. They studied 50 Chinese neonates with CHT, aged 17-28 days, and provided evidence of significant left ventricular systolic dysfunction in neonates with CHT compared with age-matched euthyroid controls (Mao *et al.,* 2008). No studies have been done to test the effect of CHT on the cardiac function in neonates with DS.

The relationship between thyroid function and lipid status in a group of children was investigated by Nader and colleagues in 2010. They found that children with TSH levels

between 2.5 and 5.0 mIU/L had higher triglyceride and insulin levels than those with lower TSH values (between 0.3 and 2.4 mIU/L). Their results suggest that further study is needed to delineate the optimal thyroid hormone levels necessary to decrease the future risk of cardiovascular disease in children who have additional risk factors.

Subclinical hypothyroidism (SCH) was suggested to decrease myocardial contractility and cardiac output (Biondi, Fazio, Palmieri e*t al*., 1999; Di Bello, Monzani, Giorgi *et al*., 2000; Kahaly 2000). Some studies in adults with SCH have shown that thyroxine replacement causes improved myocardial function (Biondi *et al*., 1999; Monzani, Di Bello, Caraccio *et al*., 2001). Toscano, Pacileo and Limongelli *et al* (2003) performed a study on children with DS to ascertain the effects of SCH on myocardial structure and function. They found that children with DS who have SCH are not at higher risk of having cardiac disease. However, their sample, which comprised 16 affected children with SCH and 25 matched euthyroid controls, was too small to draw any definitive conclusions.

1.8 INTERNATIONAL SCREENING AND TREATMENT POLICIES

As early as 1995, a consensus statement regarding the optimal medical care for individuals with DS was drawn up by experts from several countries (Pueschel, Annerén, Durlach *et al*., 1995). As it is important to monitor potential determinants of morbidity and mortality to proactively improve the overall health and quality of life for individuals with DS, health surveillance protocols have been drawn up in several countries.

Because hypothyroidism is treatable, early detection and treatment are essential in order to maximise the growth and cognitive abilities in this already impaired population. Several

countries, including the UK and the USA, have protocols which describe the medical vulnerabilities and treatment of children with DS.

The guidelines recommend a programme for regular screening, in order to identify treatable causes for a variety of symptoms, including hypothyroidism, which might otherwise be overlooked as being part of the DS presentation (UK - Down Syndrome Medical Interest Group, 2005); USA - Cunniff , Frias, Kaye *et al*., 2001; Bull, 2011). Appendix A is a summary of the main international guidelines as regards thyroid function testing in individuals with DS.

Blood for TFT testing is traditionally drawn by routine venepuncture. As children with DS have higher haematocrit levels compared to other children, a larger quantity of blood is often needed to carry out the TFT effectively (Stark, 1992). Murphy, Philip and Macken *et al* (2008) explored the use of finger-prick blood spot TSH testing in pre-school children, and were able to verify the accuracy of the blood spot testing previously described by Noble, Leyland and Findlay *et al* (2000). They propose finger-prick testing to be a viable alternative to routine venepuncture, and site several advantages of this approach, which include less trauma to the child and increased parental compliance with testing. For the diagnosis of hyperthyroidism, however, Murphy *et al* (2008) advise not relying on bloodspot TSH alone.

Hypothyroidism is one of the conditions included in the Newborn Screening (NBS) programmes in many parts of the world. Blood specimens (usually heel-prick blood spots for TSH with or without FT4) are collected between two and seven days of age (optimally after 48 hours and before four days). If abnormal, further confirmatory testing is

performed and thyroid hormone therapy is started if hypothyroidism is confirmed. If therapy is started within the first two weeks after birth, cognitive development can be normalised in non-DS patients (Rossi, Caplin and Alter, 2005).

While there are several DS thyroid screening recommendation protocols from around the world, standardised biochemical treatment thresholds for DS do not exist. Clinicians are thus left to judge clinically when to institute treatment, which based on the DS phenotype, can prove problematic. Reference ranges for TFT differ from laboratory to laboratory. No DS-specific TFT reference ranges exist. Instead, values are extrapolated from studies conducted on healthy children. Many of the published guidelines only support treatment with thyroid hormone for DS patients where the TSH level is greater than 10mIU/L (UK - Down Syndrome Medical Interest Group, 2005). Other authors have similarly noted that a TSH level greater than 10mIU/L after two weeks of age would be considered abnormal, and treatment should be instituted (Fisher, 2002; Rose *et al*., 2006). However, the evidence for using this value in children is poor, and is extrapolated from adult studies.

In addition, the Consensus Development Conferences sponsored by the Endocrine Society, the American Association of Clinical Endocrinologists (AACE) and the American Thyroid Association (ATA) recommend beginning thyroxine treatment when TSH values exceed 10 mIU/L, based on the adverse effects on serum lipids and the risk of progression to overt hypothyroidism. Therapy for lower levels of TSH remains controversial (Hollowell *et al*., 2002; Surks *et al*., 2004).

1.9 SCREENING AND TREATMENT IN SOUTH AFRICA

No standardised policy exists in South Africa as regards thyroid function screening, testing or treatment in individuals with DS. None of the standard paediatric textbooks, like Nelson Textbook of Pediatrics or the South African Coovadia Textbook of Paediatrics mentions standard guidelines for the care of children with DS (Kliegman, Behrman, Jenson *et al*., 2007; Wittenberg, D.F., 2009), despite the fact that the American Academy of Pediatrics (AAP) first issued their DS guidelines in 2001 (Cunniff *et al.*, 2001).
South Africa, unlike other newly industrialised countries like Thailand, has not adopted the AAP guidelines as best practice for the care of the DS population. The lack of a standardised protocol means that even children who are born in a tertiary hospital and have access to tertiary medical care, may never have their thyroid function tested. Even less is known about thyroid screening in the non-academic, peripheral state hospital sector, where the majority of children with DS access medical care.

In line with international practice, the clinicians in the Division of Human Genetics decided, in 2003, to test the thyroid function of all children with DS seen at the Genetic Counselling (GC) clinics. Initial testing would take place at the first visit to the GC clinics, and annual testing would be performed thereafter, for as long as the children were seen at the GC clinics. If the results were found to be abnormal, the patients were either retested or referred to a paediatric endocrinologist for further monitoring and treatment. Upon discharge from the GC clinic, all patients with DS with normal TFTs are referred to their nearest hospital for further follow-up, including annual thyroid testing.

South Africa does not have a national NBS programme. Thus, congenital hypothyroidism is not diagnosed in the critical, treatment-sensitive period of the newborn's life.

Furthermore, neonates with DS children will not be diagnosed with hypothyroidism, due to the lack of discriminating physical features to suggest that diagnosis.

This makes the thyroid blood test screening of DS children even more important. However, even if the DS patients are tested for thyroid dysfunction, there are still no standardised South African guidelines as to when to treat, and whether or not to treat the controversial SCH.

Outlined below are the treatment practices at the hospitals included in this study.

1.9.1 CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL

The consultants at CMJAH Paediatric Endocrine Clinic would like to assess all children with DS at their clinic as soon as the diagnosis of DS is made. Almost all patients with DS are placed on thyroid hormone supplementation, unless they are overtly hyperthyroid. They note that thyroxine replacement is easy to prescribe, easy for the patients to ingest and easy to monitor by performing regular TFTs. The justification for this blanket approach to supplementation is that it has been shown that increased levels of thyroid hormone may provide better cognitive and developmental outcomes, as seen in the short term study by Van Trotsenburg *et al* (2005). The results of the study by Karlsson *et al* (1998) also found some evidence for improved growth velocity with treatment. In seven out of eight children with DS, who had hypothyroidism and were treated with thyroxine, growth velocity in the year prior to commencing treatment was slower than after commencing treatment. The growth velocity of these children was also better than that of age-matched euthyroid controls with DS. Tüysüz and Beker (2001) recommend treating all infants with DS with thyroxine until the age of three years, to prevent progression to OHT. In addition, no adverse effects have been noted in children with SCH, when treated

with thyroxine (Dr D. Segal- personal communication). Children with DS are placed on the lowest dose of thyroxine necessary to keep their TSH levels close to 2 mIU/L, with a normal to high-normal FT4 level. The patients are followed-up six to twelve monthly. The patients are monitored clinically for side-effects, and biochemically, in the form of repeat TFTs, for response to treatment (Dr D. Segal, personal communication).

1.9.2 RAHIMA MOOSA HOSPITAL

The consultants at the Paediatric Endocrine Clinic at RMH prefer to see all children with DS at their clinic, regardless of the initial TFT results. Only patients with a TSH>10 mIU/L are placed on thyroxine treatment, unless there is significant clinical evidence to suggest hypothyroidism at lower TSH levels, in keeping with the guidelines from the UK and USA. The protocol is based on the premise that there is a lack of long-term benefit of thyroxine replacement in patients with DS who have TSH levels less than 10mIU/L (UK - Down Syndrome Medical Interest Group, 2005).The patients are followed-up at six to twelve month intervals and their response to treatment is monitored with repeat TFTs (Dr A Chiba, personal communication).

1.9.3 CHRIS HANI BARAGWANATH HOSPITAL

Unfortunately, the researcher was unable to obtain access to the data at the Endocrine Clinic at CHBH. Therefore, sufficient information about the referral and treatment practices at that hospital is lacking.

1.10 AIMS AND OBJECTIVES OF THE STUDY

1.10.1 AIM:

- To investigate thyroid function testing in a cohort of South African paediatric patients with DS

1.10.2 OBJECTIVES:

- To document thyroid function test results in patients with DS attending the GC clinics between 2003 and 2008
- To document how thyroid function in this cohort compares with results from international studies
- To document referral and treatment patterns in this cohort

1.11 LIMITATIONS OF THE STUDY

Due to the differences in the biochemical analyses of the thyroid tests using different machines at the various laboratories, the results could not be pooled. Statistical tests to prove variance were employed. Thus the results from CHBH had to be analysed independently. Additionally, a direct comparison between the TFT results in our study and the reference ranges was not directly possible for the following reasons. Firstly, the reference ranges were derived using a German group of healthy children, and secondly, there was not enough information in the published study to draw the necessary distribution curves. The referral and treatment practices from CHBH are not comprehensive, due to the lack of data made available from that hospital.

1.12 UNIQUENESS OF THE STUDY

This is the first study to document the frequency and range of thyroid dysfunction in African children with DS. It is also the first attempt at describing the testing, referral and treatment policies at the Endocrine Clinics at two academic hospitals in South Africa.

2. PATIENTS and METHODS

The study was a retrospective, file-based audit of all the thyroid function tests performed on children with DS at the three selected academic hospitals. These hospitals, attached to the University of the Witwatersrand Medical School within the Greater Johannesburg area, were Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), Chris Hani Baragwanath Hospital (CHBH) and Rahima Moosa Hospital (RMH).

2.1 ASCERTAINMENT OF PATIENTS WITH DOWN SYNDROME

The subjects in the study were all patients with DS seen at the Division of Human Genetics' three main Genetic Counselling (GC) Clinics in the Johannesburg area. The time period under investigation was a six year period from the beginning of 2003 until the end of 2008. Patients seen within the Division were recorded in two separate databases. The first was a manual card system which was kept by the departmental secretary. She added the names of individuals with a diagnosis of DS (indicated on the GC file facesheets), when she registered the files. The second was an electronic Microsoft Access database which was updated with patient information, including diagnosis, at the time of patient billing.

There were 468 children with a diagnosis of "Down syndrome", "Down's syndrome", "Trisomy 21" and "T21" in the electronic Microsoft Access database, and 487 in the manual card database. Reconciling the two databases yielded 446 patients with a registered diagnosis of DS. Further investigation through examination of the Genetic Counselling (GC) files excluded 55 patients. Patients were excluded for the several reasons. Some were mothers of advanced maternal age (AMA), who were pregnant with a

fetus with DS, and were incorrectly entered into the databases as DS patients. Some had another syndrome, or "dysmorphic features", incorrectly entered as DS on the databases. Others were from hospitals other than the three included in this study. One was a case of a termination of pregnancy (TOP) for a fetus with DS.

A sample of 391 patients was therefore used in the final analysis. Figure 2.1 shows the individual number of patients obtained from each of the hospitals: CHBH, CMJAH and RMH, respectively, as well as the reasons for excluding patients, and the final number of patients which comprised the study sample.

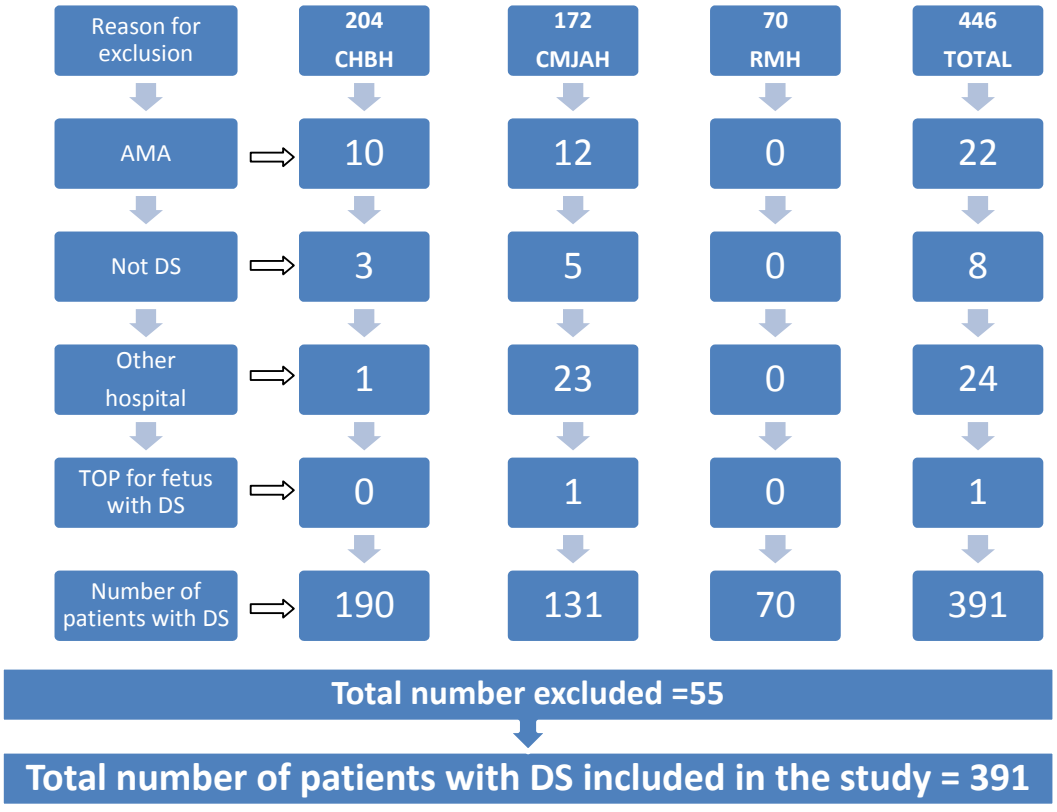

Figure 2.1: Composition of the sample (N=391)

2.2 METHODS

2.2.1 CONSTRUCTION OF THE DATA COLLECTION SHEET

Information relevant to this study was gathered from five sources: Genetic Counselling (GC) Clinic files, Paediatric Endocrine Clinic files, the National Health Laboratory Service (NHLS) Disa computer system at all three hospitals, the consultant paediatricians at the Paediatric Endocrine Clinics and the parents of the children with DS in certain cases.

A data collection sheet was drawn up so that data relevant to the aims of the project could be systematically gathered. The sheet (see Appendix C) consisted of 12 items, which included the following: demographic data (hospital, date of birth, age, race and gender), chromosome results, thyroid function test results (TSH and FT4 values and who requested the test) and referral and treatment information.

2.2.2 DEMOGRAPHIC DATA

Patient names, dates of birth, gender and ethnic group were gathered from the face sheets of the GC patient files. To maintain confidentiality, all patients were then assigned a numerical code. Only the researcher and her supervisors had access to the original patient name.

2.2.3 CHROMOSOME RESULTS

Chromosome results were generally available in the GC files. Results were verified on the NHLS Disa computer system within the Division of Human Genetics.

2.2.4 THYROID FUNCTION TEST ANALYSIS, REFERENCE RANGES AND RESULTS

Thyroid function testing at the NHLS laboratories at the teaching hospitals of the University of the Witwatersrand is undertaken using three different machines, although the biochemical assays are said to be similar. At the CMJAH a Bayer Advia Centaur® Immunoassay System (Siemens Medical Solutions Diagnostics) is used. Prior to 2005, all TFT assays from CHBH were sent to the CMJAH laboratory. Subsequently, tests at CHBH were run on their own Roche cobas® 6000 analyser. All TFT samples from RMH are referred to Helen Joseph Hospital (HJH) and run on an Abbott Architect i1000SR machine.

As of 2004, the NHLS follows the paediatric reference range recommendations of the Chemical Pathology Department at the University of Cape Town. The recommended paediatric values were adopted from results obtained in a German study to determine TFT reference values in paediatric age groups (Hübner *et al*., 2002). These recommendations were forwarded to and accepted by the Chemical Pathology Expert Committee (Dr George Van der Watt – personal communication). The current NHLS TFT reference ranges are shown in Appendix B (Dr George Van der Watt – personal communication).

For the purposes of the present study, only TSH and FT4 results were collected, as FT3 is rarely performed in routine TFT studies. The evaluation of FT3 is mostly useful in determining and monitoring hyperthyroidism; it is less useful in cases of suspected hypothyroidism (Rossi, Caplin & Alter, 2004).

The TFT results were sourced directly from the NHLS Disa computer systems at CHBH, CMJAH and RMH. Hardcopy printouts were available in most cases. In addition, some

TFT results were available in the GC files: either as printouts or written in the GC clinical reports or letters. In cases where the results were written in the file, results were verified and a hardcopy was printed from the NHLS system, where possible.

Whether the patients were referred to a paediatric endocrinologist was recorded by the clinical geneticist or counsellor, in the GC file.

If the TFT was found to be abnormal, the patient files at the Endocrine Clinics at CMJAH and RMH were reviewed to assess whether the affected patients were examined by the paediatric endocrinologist, and whether or not they were on treatment. Unfortunately, the Endocrine Clinic files from CHBH could not be similarly accessed.

In cases where patients with abnormal TFTs were not referred, or no record existed of them at the CMJAH and RMH Endocrine Clinics, attempts were made to contact them. This was undertaken by either phoning or writing to parents requesting that they bring the child to the hospital for a TFT and referral if necessary. If patients were found to have no TFT result, attempts were made to contact the parents in the same way.

2.2.5 INCLUSION AND EXCLUSION CRITERIA

All TFTs performed on the patients with DS included in the study were considered as valid for inclusion, unless they were performed at times of admission to hospital, during concurrent illness or within the first 48 hours after birth. Alterations in thyroid function test results are common in these situations, and can be misleading (Rossi, Caplin & Alter, 2004). In addition, TFT results were excluded if the patient was found to be on thyroid hormone replacement therapy at the time that the test was performed.

2.2.6 TREATMENT PRACTICES

The consultant paediatricians in charge of the paediatric endocrine clinics at CMJAH and RMH were interviewed to assess the treatment methodologies at their respective hospitals. Information gathered directly from them included:

- When they would prefer children with DS to be referred to their clinic
- Whether they believe that SCH is a clinically significant entity
- At which TSH levels children with DS are generally treated with thyroid hormone
- What the justification for their approach was
- The follow-up protocol at their clinics

2.3 PROCEDURE

Following the initial patient ascertainment, a comprehensive review of the GC files was undertaken. Most of the demographic data was then added to the data collection sheet. Subsequently, TFT results were obtained from the three hospitals. Upon review of the TFT results, the GC files of patients with abnormal results were again reviewed to verify referral information. Referral practices were inferred from test results found on the hospital computer system, which were requested by doctors in the respective Endocrine clinics. These test results were initially not added to the data set, as verification of treatment status needed to be undertaken first. This was done by consulting the Endocrine Clinic files at CMJAH and RMH. Information regarding treatment was collected simultaneously. If patients who had abnormal results were not seen at the Endocrine Clinics, an attempt was made to contact the parents and retest or refer the patients as indicated. Missing data included specific referral and treatment practices at CHBH, and not all CHBH patient data could be verified.

2.4 ANALYSIS

2.4.1 OPERATIONAL DEFINITIONS

For the purposes of this study, we defined the various forms of thyroid dysfunction as follows (all using the NHLS Paediatric reference ranges – see Appendix B):

- *Congenital Hypothyroidism*: any patient with a high TSH level, which was demonstrated within the newborn period (first two to 28 days of life)
- *Subclinical Hypothyroidism*: A high TSH in the presence of a normal FT4
- *Overt Hypothyroidism*: A high TSH and a correspondingly low FT4
- *Hyperthyroidism*: A low TSH and a high FT4

2.4.2 STATISTICS

Data from the completed data sheets were transferred into a Microsoft Excel spreadsheet for initial analysis. After coding the responses, basic statistics including frequencies and the TFT distribution graphs were generated using Statistica (Version 10; StatSoft Inc, Tulsa, OK). A statistician was consulted for assistance regarding the analysis of variation between the TFT distribution graphs from the three hospitals, so that a decision could be made whether to pool the results, or whether to analyse each hospital individually. Logistic regression was used to verify whether any of the demographic data had a significant influence on the TSH results. Throughout, a p-value of less than or equal to 0.05 was accepted as indicating statistical significance, unless otherwise stated. Where relevant, the 95% confidence interval was used.

2.5 ETHICS APPROVAL

Ethics approval for this study was obtained from the Human Research Ethics Committee (Medical) of the University of the Witwatersrand: Certificate number M090710 (see Appendix D).

3. RESULTS

The results from the main analyses performed on the collected data are presented in this chapter. Collective data from all three hospitals were pooled for the initial analysis. Regarding the TFTs, an initial analysis of variance was performed to test whether there was a significant difference between the three different hospital groups. Thus the TFT data from CHBH were analysed independently, while the data from CMJAH and RMH were analysed jointly.

3.1 PATIENTS WITH DS INCLUDED IN THE STUDY

A diagnosis of DS was confirmed in 391 patients. All were included in the study. Figure 3.1 shows the proportion of patients obtained from each hospital.

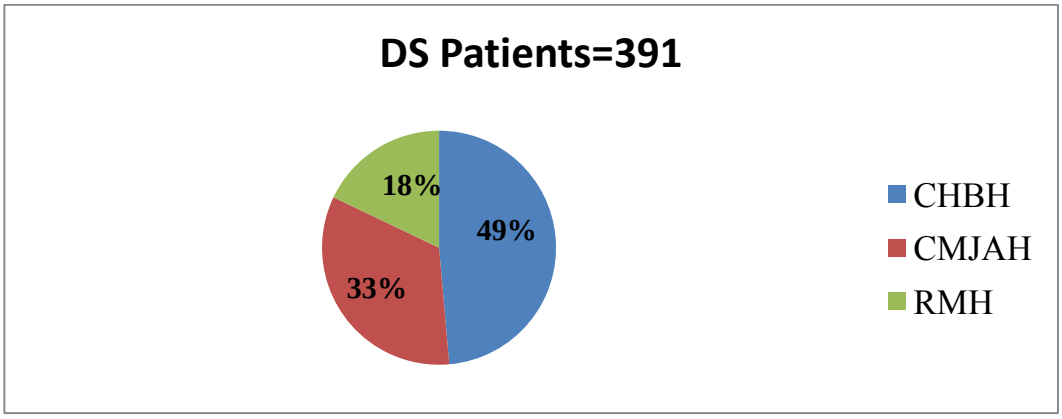

Figure 3.1: Proportion of patients from each hospital (N=391)

3.2 CHARACTERISTICS OF THE SAMPLE

The GC files of all 391 patients (100%) were located and reviewed.

3.2.1 DEMOGRAPHIC DATA

3.2.1.1 Gender distribution

The sample of patients comprised approximately equal numbers of males and females, as shown in table 3.1.

Table 3.1: Gender distribution by hospital (N=391)

Hospital	Male	Female
CHBH	100 (53%)	90 (47%)
CMJAH	59 (45%)	72 (55%)
RMH	34 (49%)	36 (51%)
TOTAL	**193 (49%)**	**198 (51%)**

3.2.1.2 Race distribution

Black patients accounted for the majority of patients in the study (360/391; 92%). The rest of the sample was made up of 3% white patients (11/391) and 4% coloured patients (16/391), and 1% of patients were of Indian descent (3/391).

3.2.1.3 Age distribution of patients at first test

The age distribution of the patients with DS at the time of their first TFT is shown in figure 3.2. All categories were chosen to correspond with those of the NHLS reference ranges (see Appendix B).

The majority (327/391; 84%) of patients had a TFT result available. The age range of the patients at the time when the first TFT was performed was from one day to 6.8 years (average age 9 months). Most (161/327; 49.2%) of the TFTs were performed between the ages of two and 12 months, with very few being done during the newborn period or after the age of five years. These results are further clarified in figures 3.2 and 3.3.

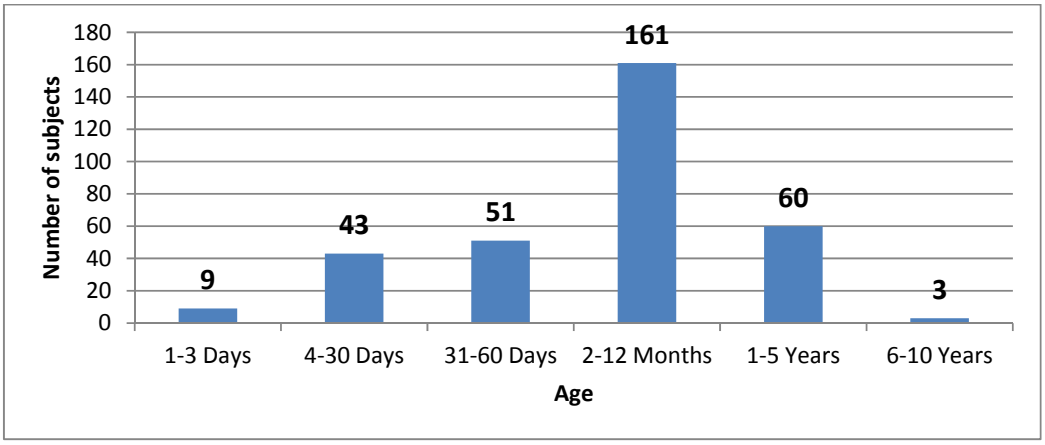

Figure 3.2: Age stratification of sample at initial TFT test (N=327)

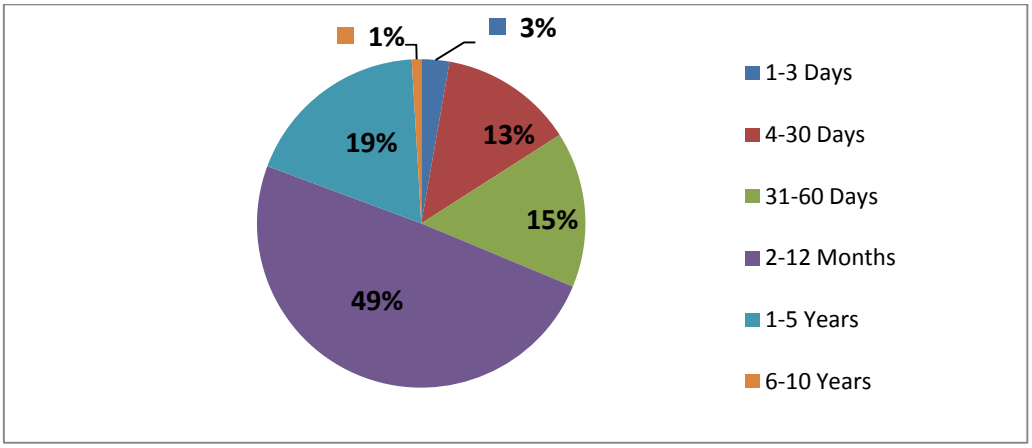

Figure 3.3: Age stratification in percentages of sample at first TFT test (N=327)

3.2.1.4 Age distribution of entire sample

Although 391 patients were included in the study, several patients had more than one TFT result, which fulfilled criteria for inclusion in the study (i.e. tests performed at times of good health and patient not on any treatment). The age distribution of the entire sample of 516 TFTs is shown in Figure 3.4.

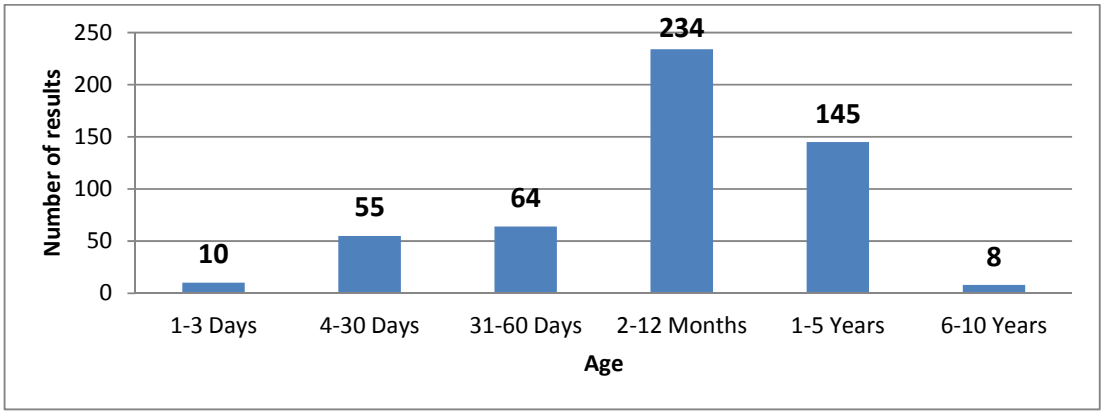

Figure 3.4: Age distribution of entire sample of TFT results (N=516)

The average age of the entire sample was 12.3 months (range one day to 7.67 years). Once again, the majority of TFT samples were taken when the patients were between the ages of two months and one year. The age distribution of patients from each of the three hospitals is shown in figure 3.5.

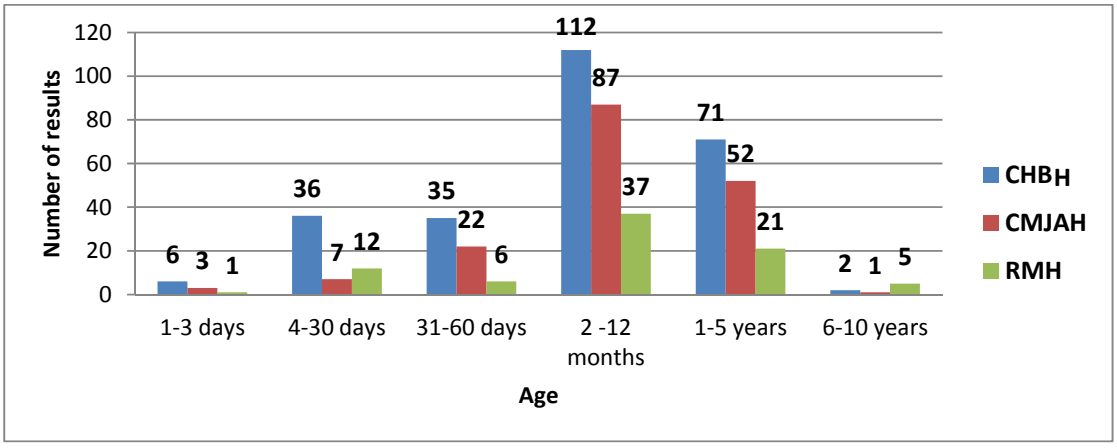

Figure 3.5: Age distribution by hospital (N=516)

3.2.1.5 Chromosome results

Most of the patients (356/391; 91%) had a full karyotype, and were thus sub-classified as having non-disjunction, translocation or mosaic DS. The minority (28/391; 7%) had a PCR aneuploidy test only. Seven patients (7/391; 2%) had no chromosome results. In these cases the lack of a result was due to technical error, where the analysis was unsuccessful. The tests were not repeated, and the diagnosis of DS remained clinical.

Details of the chromosome results are shown in table 3.2. Patients with non-disjunction made up the majority of those who had a full karyotype analysis, comprising 92.4% (329/356). DS due to a translocation was found in 5.34% (19/356) of patients, and 2.25% (8/256) had mosaic DS.

Table 3.2: Chromosome result (N=391) - distribution according to hospital

	Non-disjunction	Translocation	Mosaic	PCR aneuploidy	No result	Total
CHBH	165	12	4	7	2	190
CMJAH	112	3	4	10	2	131
RMH	52	4	0	11	3	70
TOTAL	**329 (84%)**	**19 (5%)**	**8 (2%)**	**28 (7%)**	**7 (2%)**	**391**

3.3 NUMBER OF PATIENTS TESTED

Of the 391 patients in the study, 327 (84%) had at least one TFT performed. The remaining 64 (16%) patients had no TFT result. Of those who had no result, 83% (53/64) had never had their thyroid function tested, and 17% (11/64) either had a failed phlebotomy attempt, or there was insufficient blood submitted for analysis. Details of all patients, by individual hospital, are shown in table 3.3.

Table 3.3: Number (N) of patients tested

HOSPITAL (N)	TFT result available	No TFT/No result available
CHBH (N=190)	165	25
CMJAH (N=131)	106	25
RMH (N=70)	56	14
TOTAL (N=391)	**327 (83.6%)**	**64 (16.4%)**

3.3.1 REPEAT TFTs PERFORMED

Repeat TFTs are performed for a number of reasons: initial abnormal results, to monitor treatment outcomes or as a tool in thyroid function surveillance. Altogether 113/327 (35%) patients had at least one repeat TFT, which could be included in the study. Twenty of these results were included after patients were contacted during the course of the study.

Most of the repeat blood samples (60/113; 53%) were taken following an abnormal result. The repeat samples showed no uniform trend. Several patients (19/113; 17%) initially had an abnormal result, but upon retesting the levels were found to be within the normal ranges. Others (9/113; 8%) had variable results upon retesting, with more than one follow-up result being either normal or abnormal.

A proportion of patients (32/113; 28%) had consistently abnormal results. Of these 32 patients, six were tested during the neonatal period. They all had repeat testing later in infancy, and their results were still abnormal. In some cases, up to nine repeat TFTs were done on the same patient, all of which were abnormal. There is a record of only two of these patients being started on treatment at CMJAH. The other CMJAH patients were not

referred to the Endocrine Clinic. The rest of the patients were from CHBH, and there was no way to confirm their treatment status during the course of this study.

There were 40 patients (40/113; 35%) who had more than one result, which remained consistently within the normal ranges.

Very few patients (11/327; 3.4%) had a regular, yearly TFT performed. Regular annual tests were performed by the doctors in the GC clinics (6/11; 54.5%), at the Endocrine or Developmental Clinic (3/11; 27.3%), or at a peripheral hospital (2/11; 18.2%). Some patients (10/327; 3.1%) however, had ad hoc TFTs performed, presumably when they accessed healthcare.

3.3.2 CONTACTING PATIENTS WITH NO TFT OR AN ABNORMAL RESULT

In addition to the 64 patients who had never had a TFT (see table 3.3), 152 patients had TFT results outside of the reference ranges. An attempt was made to contact these patients telephonically or in writing. Contact could not be achieved with 60% of patients (116/194): the telephone contact numbers were no longer correct, they had moved house, there was no postal address available, or a combination of these factors applied. They were thus lost to follow-up.

Of the 40% (78/194) of patients who could be contacted: 20/78 (26%) patients came for a repeat TFT (they were included with the other patients, who had repeat results in 3.3.1); 11/78 (14%) parents informed us of the deaths of their infants/children; eight parents (10%) informed us that their children had regular TFT tests at the hospital closest to their homes; nine parents (11.5%) confirmed a follow-up appointment but did not arrive and 30 patients (38.5%) did not respond to the letters sent to them.

Thus, of the 194 patients who had never been tested or had an abnormal TFT result requiring follow-up, a total of 155 (80%) were unable to be tested/re-tested.

3.4 NUMBER OF THYROID FUNCTION TEST RESULTS COLLECTED

In total, 536 results were collected from the 327 patients who had their thyroid function tested from each of the three hospitals. Data available on the test results included the laboratory reference number, the name of the doctor who requested the test and the date the test was performed.

Forty results were excluded from the study: 24/40 (60%) because they were performed at times of admission to hospital or concurrent illness, 13/40 (32.5%) as the patients were on thyroid hormone replacement therapy at the time the TFT was performed and 3/40 (7.5%) because they were performed within the first 48 hours after birth.

As mentioned in 3.3.2, of the 78 patients who were contacted during the course of the study, 20/78 (26%) responded and subsequently had a TFT performed during the course of this study: 17 patients with no previous result and three patients with abnormal results had a repeat TFT.

Therefore 516 results were included in the study: 485 full TFTs (TSH and FT4), 12 individual TSH results and 19 individual FT4 results.

3.5 THYROID FUNCTION TEST REQUESTS

The majority of tests were requested by the doctors at the Genetic Counselling (GC) Clinics (282/522; 54%), followed by the general paediatricians (183/522; 35%) and other paediatric specialists (57/522; 11%). Figure 3.6 shows the proportion of tests requested by the main health professionals involved in the care of children with DS.

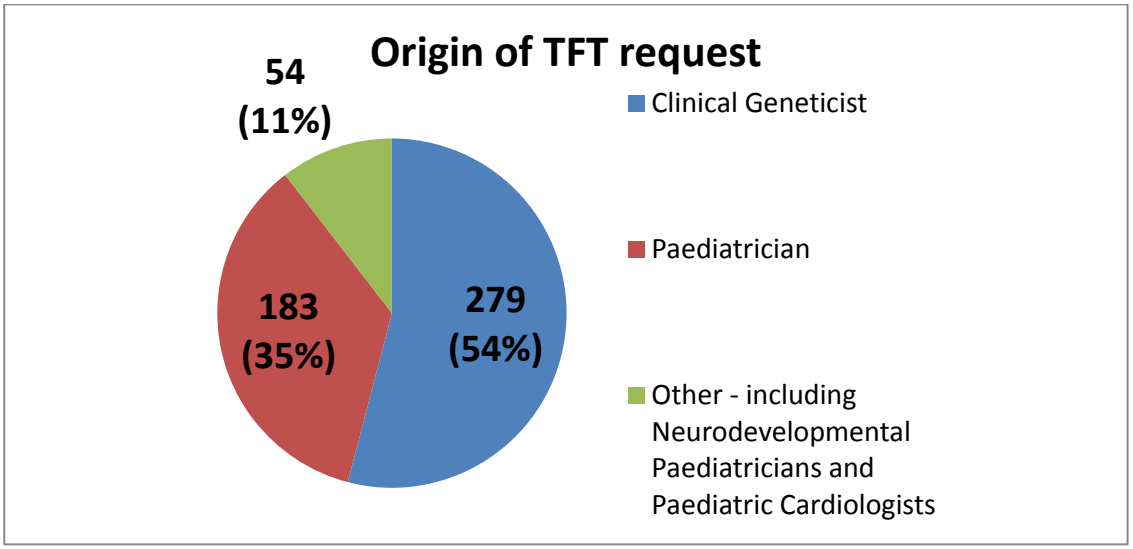

Figure 3.6: Number and percentage of TFTs requested - by health care professional

3.6 LABORATORY ANALYSIS OF THYROID FUNCTION TESTS

All TFT results were checked using the laboratory number, which indicates the laboratory at which the test was analysed. Several of the blood samples from CHBH patients were referred to the CMJAH laboratory for analysis. Of the 262 tests performed on patients from CHBH, 159 were analysed at CMJAH's laboratory. In addition, six results from RMH patients were analysed at CMJAH. All other RMH TFT blood specimens are referred to the HJH laboratory for analysis. The total number of TFTs analysed by each hospital laboratory is presented in figure 3.7.

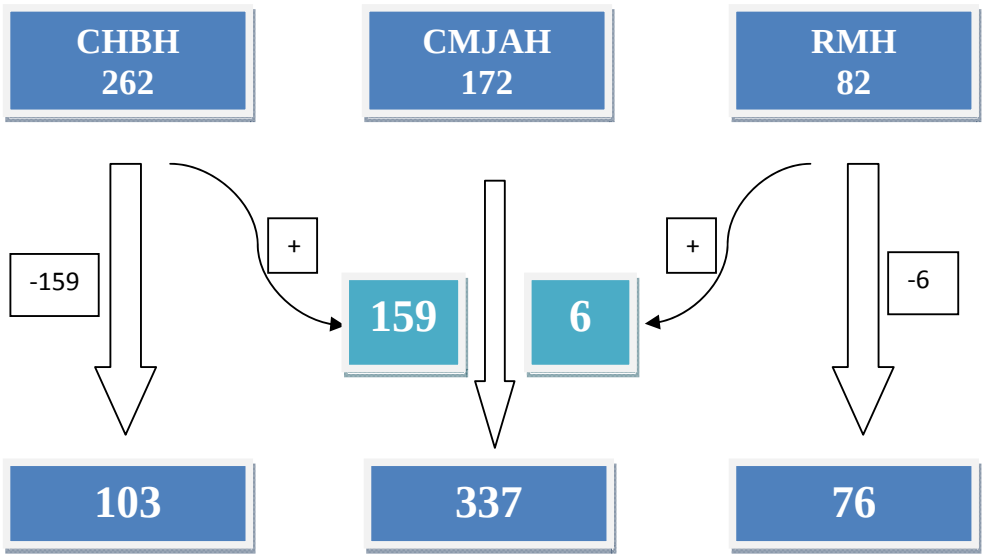

Figure 3.7: Number of TFTs analysed by each hospital laboratory (N=516)

For the purposes of the statistical analysis, one patient from RMH with a clearly outlying result, was excluded from further analysis and will be discussed separately (see section 3.6.2.3 on page 49). The following analysis was thus performed on 515 TFT results, which included 496 TSH results and 503 FT4 results.

Many of the TFTs from patients at CHBH were analysed at CMJAH because doctors at the GC clinics suspected a discrepancy between results at CHBH compared to those from CMJAH. This occurred in 2005, when a patient with DS had a TFT analysed at CHBH and a repeat TFT at CMJAH a week later. The child had not had any illness at the time of initial testing at CHBH and no treatment was initiated between the two tests. The initial CHBH result showed a TSH of 8.4 mIU/L (normal values: 0.4-5.25) and a FT4 of 14.8. (normal values: 10.8-19.0), compared to the repeat sample which was analysed at CMJAH, which showed a TSH= 5.0 (normal values: 0.4-5.25) and FT4= 16.2 (normal values: 10.8-19.0).

The medians of both the TSH and FT4 results from each of the hospitals were generated independently. They were then compared using an analysis of variance statistical test. As both the TSH and FT4 groups were not normally distributed (see sections 3.6.2 and 3.6.3 below), a non-parametric Kruskal-Wallis test was used.

Initial investigation found that at least one of the hospitals was significantly different to the others ($p<0.001$). Further study of these findings revealed that the TSH and FT4 results from CHBH were very significantly different to the results obtained from CMJAH ($p<0.001$) and RMH ($p<0.00001$). See Section 3.6.1 for more details. For this reason the TSH and FT4 results from CHBH were analysed separately.

3.6.1 ANALYSIS OF VARIANCE BETWEEN THE HOSPITALS

3.6.1.1 Thyroid Stimulating Hormone (TSH) Results

The medians and means of the TSH results for the three hospitals were generated (presented in table 3.4). Medians were preferred due to the non-normal distribution of the data, although, due to the high N-values, means were also calculated.

Table 3.4: TSH valid number, means, standard deviations, medians, minimum and maximum values by hospital

HOSPITAL	TSH Number (N)	TSH Means (mIU/L)	TSH Standard Deviations	TSH Medians (mIU/L)	TSH Minimum (mIU/L)	TSH Maximum (mIU/L)
CHBH	99	8.26	6.59	6.30	0.95	41.05
RMH	74	4.60	3.22	4.01	0.79	19.31
CMJAH	323	5.80	4.21	4.64	0.38	27.83
Combined	496	6.11	4.79	4.89	0.38	41.05

The initial non-parametric analysis of variance of the medians showed a significant variance ($p<0.05$), indicating that the means of the TSH values from at least one of the

hospitals was statistically different from the other hospitals. Tables 3.5 and 3.6 present a detailed description of the analysis.

Table 3.5: TSH: Kruskal-Wallis analysis of variance (results in red are significant at <0.005)

TSH	Kruskal-Wallis ANOVA by Ranks; TSH Independent (grouping) variable: HOSPITAL Kruskal-Wallis test: H (2, N= 496) =23.66211 **p =0.00001**			
	Code	Valid N	Sum of Ranks	Mean Rank
CHBH	1	99	29994.00	302.9697
RMH	2	74	14706.00	198.7297
CMJAH	3	323	78556.00	243.2074

However, further analysis using a paired-wise Mann-Whitney non-parametric test proved that the difference between the TSH results from CHBH and the other two hospitals was statistically significant (p<0.0005) (see Table 3.6). Thus the TSH results from RMH and CMJAH could be pooled, but the data from CHBH had to be analysed separately.

Table 3.6: TSH: Mann-Whitney test by hospital (results in red are significant at <0.05)

variable	Mann-Whitney U Test - by Hospital Marked tests are significant at **p <0.05**								
	Rank Sum CHBH	Rank Sum RMH	U	Z	p-value	Z adjusted	p-value	Valid N CHBH	Valid N RMH
TSH	10100.0	4951.0	2176.00	4.5608	0.000005	4.56092	**0.000005**	99	74
	Rank Sum CHBH	Rank Sum CMJAH	U	Z	p-value	Z adjusted	p-value	Valid N CHBH	Valid N CMJAH
TSH	24844.0	64409.0	12083.0	3.6781	0.000235	3.67811	**0.000235**	99	323

Additionally, when comparing only the TSH values greater than 10mIU/L, there was a significant statistical difference between the CMJAH and RMH group and the CHBH results (p<0.05).

3.6.1.2 Thyroid Hormone (FT4) Results

A similar analysis was done for the FT4 medians, and they too were found to be statistically significantly different ($p<0.05$). These results are presented in tables 3.7 and 3.8.

Table 3.7: FT4 valid number, means, standard deviations, medians, minimum and maximum values by hospital

HOSP	FT4 N	FT4 Means (pmol/l)	FT4 Standard Deviation	FT4 Medians (pmol/l)	FT4 Minimum (pmol/l)	FT4 Maximum (pmol/l)
CHBH	100	17.30	4.397	17.15	3.00	31.20
RMH	72	15.98	4.661	15.05	3.00	33.50
CMJAH	331	16.23	3.355	16.00	3.00	30.10
Combined	503	16.41	3.806	16.00	3.00	33.50

Table 3.8: FT4: Kruskal-Wallis test (effects marked in red are significant at $p <0.05$)

FT4	Kruskal-Wallis ANOVA by Ranks; FT4 Independent (grouping) variable: HOSP Kruskal-Wallis test: H (2, N= 503) =8.158379 **p =0.0169**			
	Code	Valid N	Sum of Ranks	Mean Rank
CHBH	1	100	28587.00	285.8700
RMH	2	72	16226.00	225.3611
CMJAH	3	331	81943.00	247.5619

Once again, more detailed analysis illustrates that there is a significant difference between FT4 results from CHBH compared to the other two hospitals ($p<0.05$) (see Table 3.9). As with the TSH results, CHBH FT4 data were analysed separately.

Table 3.9: FT4: Mann-Whitney test (effects marked in red are significant at p <0.05)

Var	Mann-Whitney U Test – by Hospital Marked tests are significant at **p <0.05**								
	Rank Sum CHBH	Rank Sum RMH	U	Z	p-value	Z adjusted	p-value	Valid N CHBH	Valid N RMH
FT4	9429.5	5448.5	2820.5	2.418	0.015611	2.41821	**0.015598**	100	72
	Rank Sum CHBH	Rank Sum CMJAH	U	Z	p-value	Z adjusted	p-value	Valid N CHBH	Valid N CMJAH
FT4	24207.5	68888.5	13942.5	2.388	0.016930	2.38840	**0.016922**	100	331

3.6.2 COMBINED CMJAH AND RMH TFT RESULTS

A total of 336 thyroid function tests were analysed at the CMJAH laboratory and 76 thyroid function tests were analysed at the Helen Joseph Hospital (HJH) laboratory, where all RMH samples are referred. The combined total number of TFT results (N=412) included 397 TSH and 403 FT4 results.

3.6.2.1 Combined CMJAH and RMH: TSH Results

The results of the combined analysis of the TSH results from CMJAH and RMH are detailed in table 3.10.

Table 3.10: TSH median, mode, valid N, minimum, maximum and standard deviations for CMJAH and RMH combined

CMJAH & RMH	Valid N	Median (mIU/L)	Mode (mIU/L)	Frequency of Mode	Minimum (mIU/L)	Maximum (mIU/L)	Standard Deviation
TSH	397	4.38	4.18	4	0.38	27.83	4.06

The distribution of TSH results of the two hospitals combined is shown in the histogram in figure 3.8, which demonstrates that the TSH values were not normally distributed (Shapiro-Wilk p<0.2). The values are skewed to the left of the expected, calculated normal curve (red line).

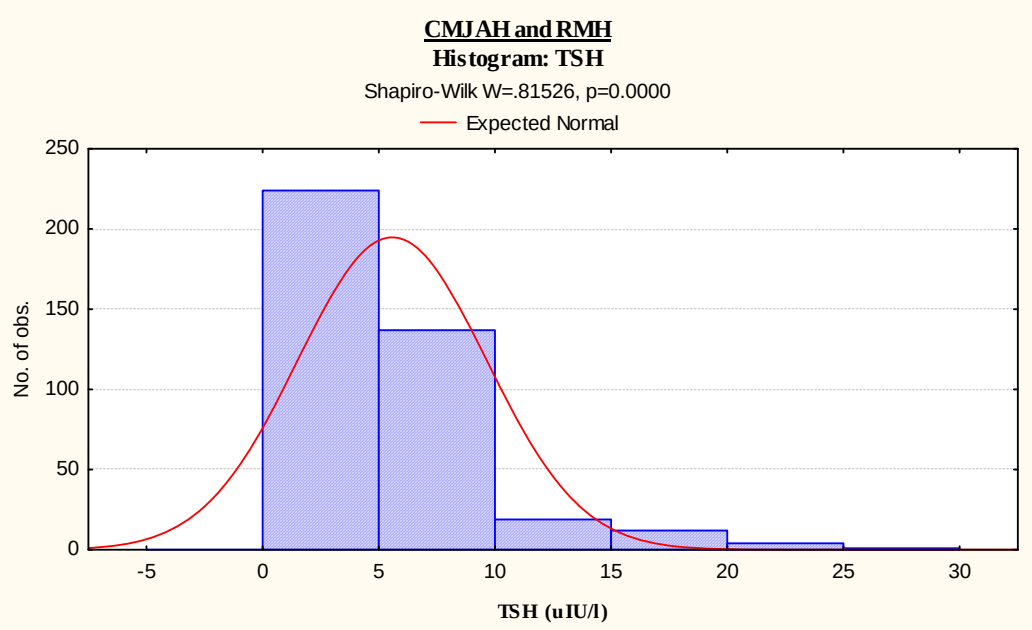

Figure 3.8: Distribution graph for patient TSH results (combined CMJAH and RMH) (N=397)

3.6.2.2 Combined CMJAH and RMH: FT4 Results

The results for the combined analysis of the FT4 results from CMJAH and RMH are detailed in table 3.11.

Table 3.11: FT4 results for patients from CMJAH and RMH combined

CMJAH & RMH	Valid N	Median (mIU/L)	Mode (mIU/L)	Frequency of Mode	Minimum (mIU/L)	Maximum (mIU/L)	Standard Deviation
FT4	403	15.90	14.80	10	3.00	33.50	3.62

The FT4 distribution of the entire sample is also not a normal Gaussian curve (Shapiro-Wilk $p<0.2$). The histogram in figure 3.9 demonstrates this non-normal distribution, with skewing of the FT4 values to the left of the expected, calculated normal distribution (red line).

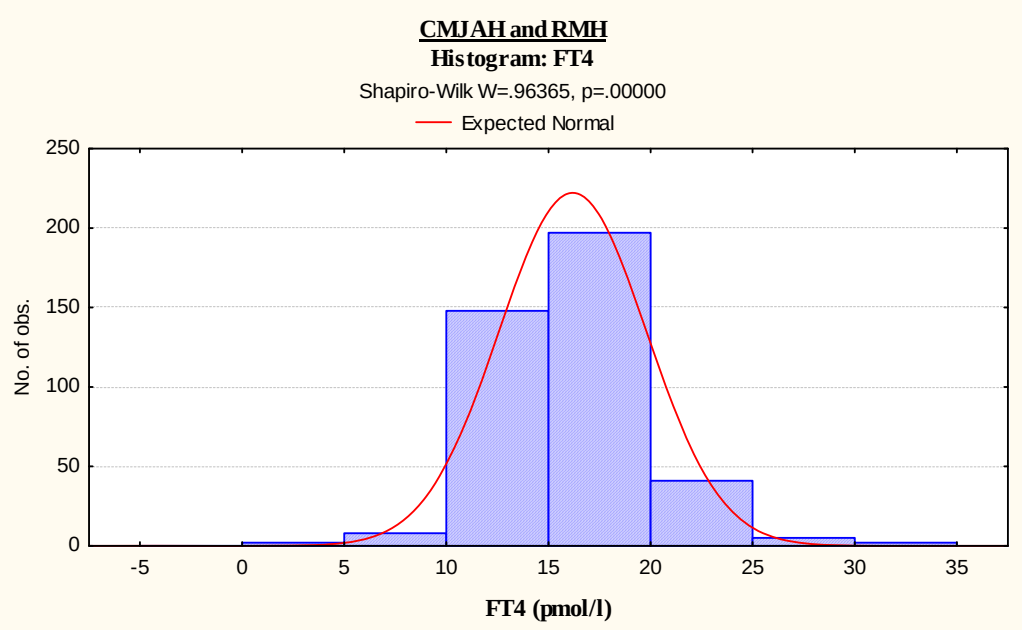

Figure 3.9: Distribution graph patient FT4 results (combined CMJAH and RMH) (N=403)

3.6.2.3 A patient from RMH with an outlying result

A 30 day old black female infant with DS, seen at RMH, had a TSH=100mIU/L (normal range: 0.16-8.48mIU/L) and a FT4=5pmol/l (normal range: 10.9-25.5pmol/l). She was thus overtly hypothyroid and was started on thyroxine replacement after a follow-up blood test revealed a similarly abnormal result. Due to her clearly outlying result, she was not included in the initial data analysis.

3.6.2.4 Percentage of patients in each thyroid dysfunction category

Table 3.12 summarises the range of thyroid dysfunction in the 412 results collected from 201 patients with DS from CMJAH and RMH, combined. The majority of the results indicated a normal or euthyroid state (60.9%). The results indicate SCH in 110/412 (26.7%) of results.

Table 3.12*:* Range of thyroid dysfunction in CMJAH and RMH results (N=412)

Category	Number of results CMJAH	Number of results RMH	Total	(%)
Euthyroid	197	54	251	60.9%
Congenital hypo-thyroidism (CHT)	7 †TSH>10= 6 †TSH>ref= 1	0	7	1.7%
Subclinical hypo-thyroidism (SCH)	99 †TSH>10= 23 †TSH>ref = 76	11 †TSH>10= 2 †TSH>ref= 9	110 †TSH>10= 25 †TSH>ref= 85	26.7%
Overt hypo-thyroidism (OHT)	7 †TSH>10= 2 †TSH>ref= 5	1 †TSH>10= 1	8 †TSH>10= 2 †TSH>ref= 6	1.9%
Both TSH and FT4 increased	5 †TSH>10= 1	4 †TSH>10= 2	9 †TSH>10= 3	2.2%
Isolated decreased FT4	9	4	13	3.2%
Isolated increased FT4	12	2	14	3.4%
TOTALS	**336**	**76**	**412**	**100%**
Total number abnormal¥	139	22	161	39.1%

† ">10": refers to a TSH value greater than 10mIU/L; ">ref" refers to a TSH greater than the upper limit of the reference range but less than 10mIU/L

¥ "Abnormal" refers to all results which did not reflect a euthyroid state

3.6.3 CHRIS HANI BARAGWANATH HOSPITAL

A total number of 103 thyroid function tests were analysed at the CHBH laboratory: 96 full TFT's (TSH and FT4), 3 isolated TSH and 4 isolated FT4. Thus, in total, 99 TSH results and 100 FT4 results were available for further analysis.

3.6.3.1 CHBH TSH results

The median, mode, maximum, minimum and standard deviation values for the sample of 99 TSH results from CHBH are shown in table 3.13.

Table 3.13: TSH valid N, median, mode, minimum, maximum and standard deviations for CHBH

CHBH	Valid N	Median (mIU/L)	Mode (mIU/L)	Frequency of Mode	Minimum (mIU/L)	Maximum (mIU/L)	Standard Deviation
TSH	99	8.26	multiple	2	0.95	41.05	6.59

The distribution curve for the CHBH TSH results is shown in figure 3.10. The histogram illustrates that the distribution is not normally distributed (Shapiro-Wilk p<0.2), but rather shifted to the left of the calculated normal curve (red line).

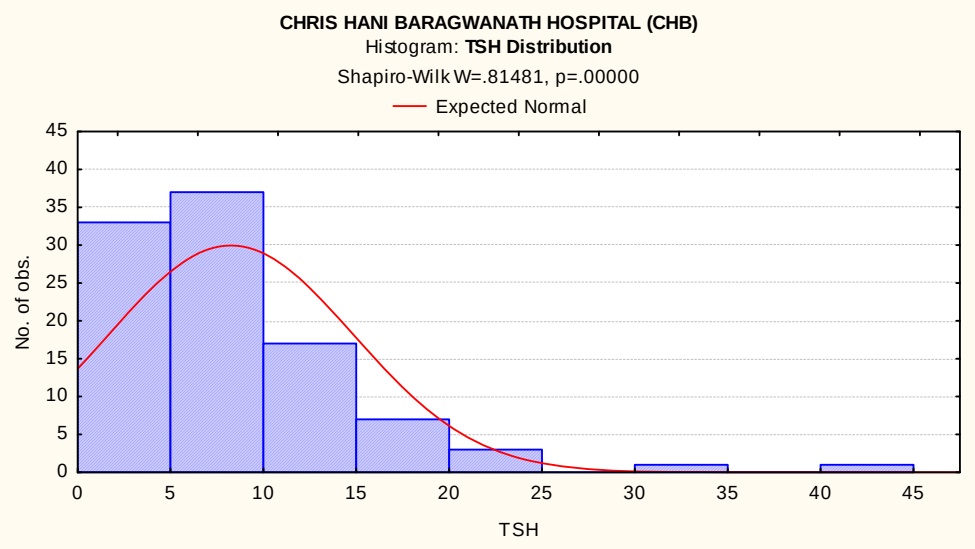

Figure 3.10: Distribution graph for patient TSH results from CHBH (N=99)

3.6.3.2 CHBH FT4 results

Details regarding the median, mode, minimum, maximum and standard deviations of the sample of 100 FT4 results from CHBH are illustrated in table 3.14.

Table 3.14: FT4 valid N, median, mode, minimum, maximum and standard deviations for CHBH

CHBH	Valid N	Median (mIU/L)	Mode (mIU/L)	Frequency of Mode	Minimum (mIU/L)	Maximum (mIU/L)	Standard Deviation
FT4	100	17.15	multiple	4	3.00	31.20	4.39

The distribution curve for the CHBH FT4 results is shown in figure 3.11. The histogram illustrates that the distribution is not normally distributed (Shapiro-Wilk p<0.2), but rather shifted to the left of the calculated normal curve (red line).

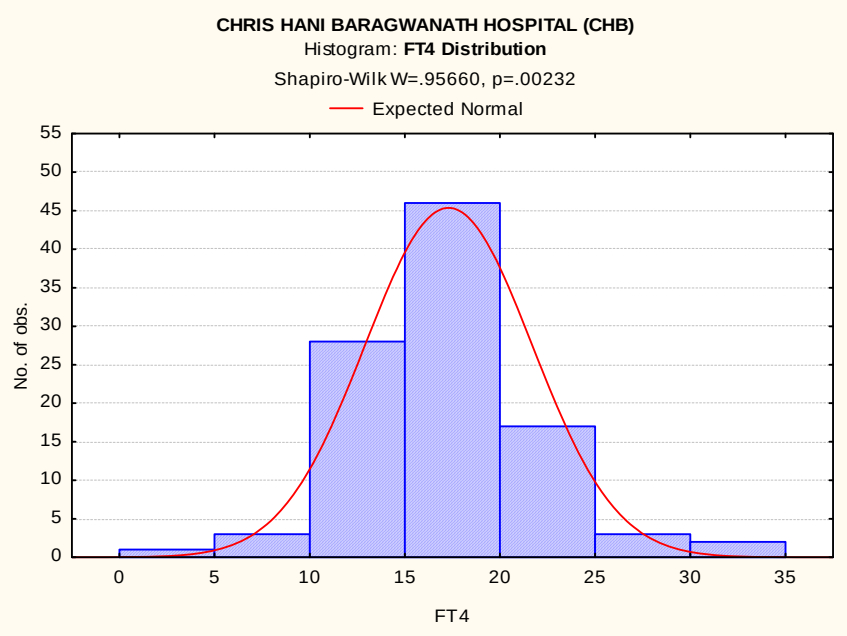

Figure 3.11: Distribution graph for patient FT4 results from CHBH (N=99)

3.6.3.3 Box and Whisker Plots for TSH and FT4 by hospital and gender

The box and whisker plots for the TSH and FT4 values from the 2 hospital groups (CHBH and the combined CMJAH and RMH group) are shown in figures 3.12 and 3.13. They graphically reflect the distribution of the TFT values according to gender and hospital. For both TSH and FT4, the medians from CHBH are significantly higher.

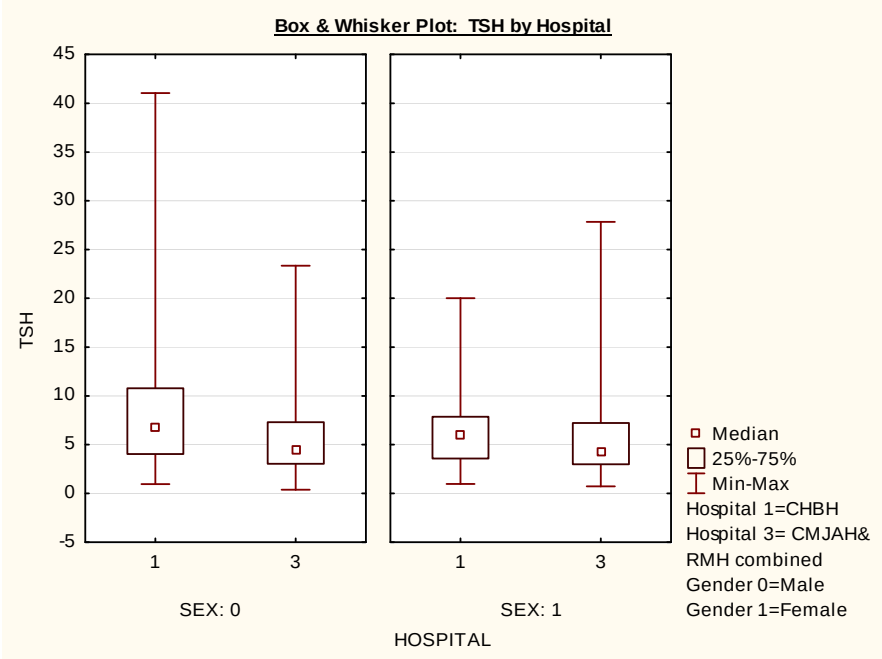

Figure 3.12: Box and Whisker plot – TSH by hospital and gender

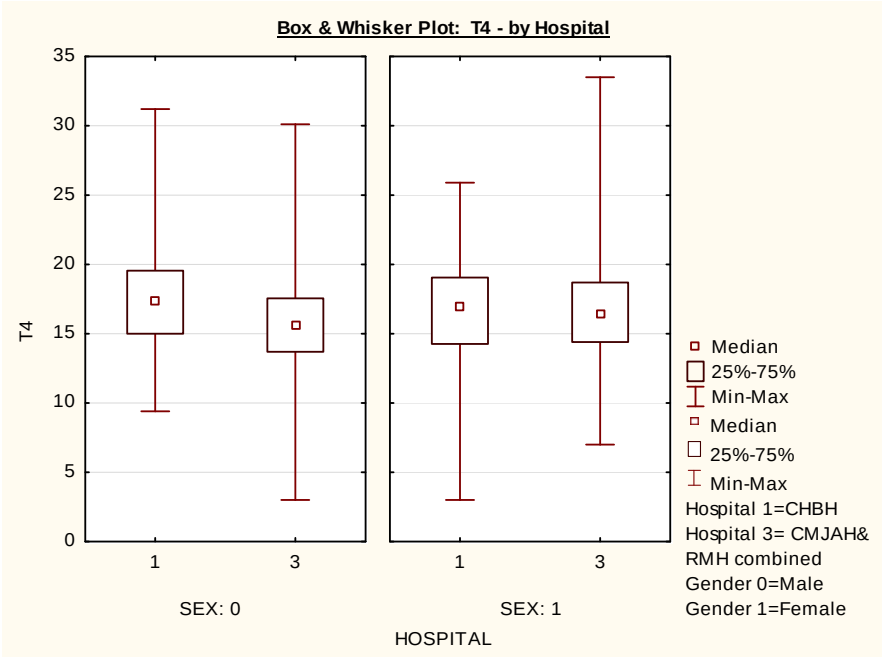

Figure 3.13: Box and Whisker plot – FT4 by hospital and gender

3.6.3.4 Percentage of CHBH patients in each thyroid function category

Table 3.15 summarises the range of thyroid dysfunction of patients with DS from CHBH. Of the results which reflected some form of thyroid dysfunction, SCH was the most frequent, accounting for 36.9% of all results.

Table 3.15: Range of thyroid dysfunction in CHBH results (N = 103)

Category	Number of results	%
Euthyroid	44	42.7%
Congenital hypothyroidism (CHT)	9 †TSH>10 = 6 (67%) †TSH>ref = 3 (33%)	8.7%
Subclinical hypothyroidism (SCH)	38 †TSH>10 = 19 (50%) †TSH>ref = 19 (50%)	36.9%
Overt hypothyroidism (OHT)	3 †TSH>10 = 3 (100%)	2.9%
Both TSH and FT4 elevated	3 †TSH>10 = 1 (33%) †TSH>ref = 2 (67%)	2.9%
Isolated decreased FT4	1	1%
Isolated increased FT4	5	4.9%
Hyperthyroidism	0	0
TOTALS	**103**	**100%**
Total number abnormal[¥]	59	57.3%

† ">10": refers to a TSH value greater than 10mIU/L; ">ref" refers to a TSH greater than the upper limit of the reference range but less than 10mIU/L

[¥] "Abnormal" refers to all results which did not reflect a euthyroid state

3.6.3.5 Frequencies of thyroid dysfunction in this cohort

The frequencies of thyroid dysfunction noted in this cohort are outlined below. Subclinical hypothyroidism (SCH) accounted for the majority of patients with abnormal TFT results. There were no patients with hyperthyroidism.

SCH: Results compatible with a diagnosis of SCH formed 28.7% (148/516) of the sample; these were collected from 99 patients, giving an overall frequency of SCH of 25.3% (99/391). A TSH greater than 10mIU/L was found in 46 results collected from 39 patients with SCH from all three hospitals. Thus 39.4% of patients (39/99) with SCH had a TSH value greater than 10mIU/L.

CHT: Of the 62 tests performed in the newborn period, 16 showed a high TSH value, compatible with CHT (25.8%). In total 51 children were tested during the newborn period: 12 had CHT, giving a frequency of 23.5% (12/51).

OHT: OHT was found in 14/516 of the results. These results were obtained from 14 patients (14/391), giving an overall frequency of OHT in this cohort of 3.6%.

3.7 EFFECT OF DEMOGRAPHIC VARIABLES ON THE TSH VALUES

In order to evaluate whether any of the demographic variables had an effect on the TSH values, logistic regression was used. The data from all three hospitals were categorised into two categories: TSH higher than 10mIU/L; and TSH lower than 10mIU/L but above the reference range's upper limit. The cut-off of 10mIU/L was used because it is a common treatment-initiating threshold. Only the results from the age category analysis

were significant. Neither race nor chromosome results seems to have an effect on the TSH level ($p>0.05$). Gender, although also not significant, is discussed in more detail in 3.7.2 below.

3.7.1 THE INFLUENCE OF AGE CATEGORY

The age categories chosen were according to those used in the reference ranges (see Appendix B). Hence, the age categories used for the analysis were as shown in Table 3.16:

Table 3.16: Age categories used in the analysis

Reference range age	Age category used for analysis
1- 3 days	1
4 - 30 days	2
31 - 60 days	3
2 months-1 year	4
1 - 5 years	5
6 -10 years	6

The results of the logistic regression show that age category 5 (one to five years) was significantly different from the other categories. Calculating the exponents of the estimates generated, allowed the calculation of odds ratios. A child in age category 5 (one to five years) was 0.61 times less likely to have a TSH greater than 10mIU/L than a child in age category 1 (one to three days) (estimate = 0.93423; odds ratio=0.39; 95% confidence intervals 0.781 to 0.198) ($p= 0.0077$)). Therefore, a very young child is more likely to have a raised TSH compared to older children.

3.7.2 THE INFLUENCE OF GENDER

Lazar *et al* (2009) identified female gender as a risk factor for thyroid dysfunction in the general paediatric population. Table 3.16 reflects the number of abnormally high TSH values, according to age group and gender for all three hospitals. The results show that

more results were available from male patients, and that proportionally more males had a TSH level greater than the upper limit of the reference ranges (39.6% versus the female 30.9%) and a larger proportion of males had TSH values above 10mIU/L (13.9% versus 10.7% in the female group).

Table 3.17: Number of abnormal TSH results by gender and age category

AGE CATEG.	GENDER						
	Total number of results from males	MALES WITH INCREASED TSH		Total number of results from females	FEMALE WITH INCREASED TSH		
		TSH>ref range	TSH>10 mIU/L		TSH>ref range	TSH>10 mIU/L	Total TSH>10 mIU/L
1	8	4	4	2	1	1	5
2	31	12	7	24	2	2	9
3	26	11	4	37	10	5	9
4	122	40	16	114	34	12	28
5	81	39	7	63	26	5	12
6	5	2	0	3	2	1	1
TOTAL	**273**	**108 (39.6%)**	**38 (13.9%)**	**243**	**75 (30.9%)**	**26 (10.7%)**	**64 (12.4%)**

Logistic regression also revealed no significant difference between males and females. The analysis revealed an upper and lower confidence interval (-0.09978 to 0.43113) which included one, and a p-value of 0.22.

3.8 REFERRAL INFORMATION

Some patients had repeat tests, and if the repeat tests fell into the same category of thyroid dysfunction, the patient was counted only once. The number of patients reflects those who were ever categorised into the various classes of thyroid dysfunction.

3.8.1 CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL

In summary, 139 TFT results analysed at CMJAH fell outside of the reference ranges. A total of 99 of these results were collected from 56 CMJAH patients. The rest of the results were collected from CHBH and RMH patients, and will be discussed later.

SCH: Of the 99 results classified as SCH, which were analysed at CMJAH (see Table 3.12), 59 were collected from 35 CMJAH patients.

- A proportion of the results (15/59; 25.4%) reflected a TSH value greater than 10mIU/L. These results were collected from 10 patients, six of whom (6/10; 60%) were subsequently started on thyroxine replacements therapy, either at the Endocrine Clinic at CMJAH or at another clinic/hospital (Developmental Clinic at CMJAH, Tembisa Hospital or Edenvale Hospital). The other four patients (4/10; 40%) were not referred.
- The majority of results (44/59; 74.6%) indicated abnormally elevated TSH levels (above reference range but <10mIU/L). These were collected from 25 patients, nine of whom (9/25; 36%) were on treatment at the Endocrine Clinic at CMJAH or the Developmental Clinic. There were two patients (2/25; 8%) who have their thyroid function checked annually at the Cardiac Clinic. They have not been referred to the Endocrinologists or started on treatment. A single patient (1/25; 4%) was referred to the Endocrine Clinic but did not attend the appointment and 13 others (13/25; 52%) were not referred to the Endocrine Clinic.

OHT: Of the seven results classified as OHT, which were analysed at CMJAH (see Table 3.12), five were collected from five CMJAH patients. The rest of the results were collected from CHBH and RMH patients, and will be discussed later.

- TSH values greater than 10mIU/L were found in two patients (2/5; 40%). Both patients (100%) are on thyroxine replacements therapy at the Endocrine Clinic at CMJAH.
- The other three patients (3/5; 60%) had abnormally elevated TSH levels (above reference range but <10mIU/L). Of these, only one (1/5; 20%) was on treatment at the Endocrine Clinic at CMJAH. The other two (2/5; 40%) were not referred to the Endocrine Clinic.

CHT: Of the seven results classified as CHT, which were analysed at CMJAH (see Table 3.12), five were collected from five CMJAH patients. The rest of the results were collected from CHBH patients, and will be discussed later.

- TSH values greater than 10mIU/L were found in three patients (3/5; 60%). All three patients (100%) are on thyroxine replacements therapy at the Endocrine Clinic at CMJAH
- The other two patients (2/5; 40%) had elevated TSH levels (above reference range but <10mIU/L). Both were not referred to the Endocrine Clinic during the neonatal period. These patients were retested at age four and seven months, respectively, and were still hypothyroid. They were subsequently referred to the Endocrine Clinic at CMJAH and are on thyroxine treatment.

Other abnormal results: There were further abnormal TFTs, which included 11 results from 11 patients were classified as having isolated elevated FT4, isolated decreased FT4 or elevated TSH with an elevated FT4. None of these patients were referred to the Endocrine Clinic.

3.8.2 RAHIMA MOOSA HOSPITAL

There were 22 abnormal results analysed at HJH, all from RMH patients. Additionally, two abnormal results from RMH patients were produced from the CMJAH laboratory. These abnormal results were collected from 18 RMH patients

SCH: While 12 results were classified as SCH (11 from RMH and 1 from CMJAH), these were collected from eight RMH patients.

- Of the 12 results, two had TSH values greater than 10mIU/L (2/12; 16.7%). These were collected from two patients: both patients are on thyroxine replacements therapy at the Endocrine Clinic at RMH.
- The other ten results (10/12; 83.3%) showed elevated TSH levels (above reference range but <10mIU/L). These were collected from six patients. A single patient (1/6; 16.7%) was on treatment at the Endocrine Clinic at RMH. The other two (2/6; 33.3%) have their thyroid function checked annually but have not been started on treatment. A further three patients (3/6; 50%) were not referred to the Endocrine Clinic.

OHT: The two results classified as OHT (one from RMH and one from CMJAH), were collected from two RMH patients. One patient (50%) had a TSH greater than 10mIU/L and is on treatment at the Endocrine Clinic at RMH. The other patient was not referred.

Other abnormal results: A further eight results from eight patients were classified as having isolated elevated FT4, isolated decreased FT4 or elevated TSH with an elevated FT4. Of those with both elevated values, two patients had TSH levels greater than 10mIU/L. None of these patients were referred to the Endocrine Clinic.

3.8.3 CHRIS HANI BARAGWANATH HOSPITAL

There were 59 abnormal results analysed at CHBH. Additionally, 44 abnormal results from CHBH patients were analysed at the CMJAH laboratory, making a total of 103 abnormal results. These abnormal results were collected from 78 CHBH patients.

SCH: It was possible to classify 77 results as SCH (38 from CHBH and 39 from CMJAH). These results were collected from 56 CHBH patients.

- TSH values greater than 10mIU/L were reflected in 37 results (37/77; 48.1%). These were collected from 27 patients: A few patients (4/27; 14.8%) were assessed at the Endocrine Clinic at CHBH and are on thyroxine replacement therapy. A further three patients (3/27; 11.1%) were referred to the Endocrine Clinic, but it is not clear whether they are on treatment. A single patient (1/27; 3.7%) was referred to Natalspruit Hospital but it is not clear whether this patient is on treatment. Additionally, three patients (3/27; 11.1%) had a repeat TSH, which were found to be within the normal range, and they were thus not referred. The rest of the patients (15/27; 55.6%) were not referred to the Endocrine Clinic, and 1/27 (3.7%) had no available referral information.
- The majority of the results (40/77; 51.9%) showed elevated TSH levels above reference range but <10mIU/L. These were collected from 29 patients. The majority of them (22/29; 75.86%) were not referred to the Endocrine Clinic. Genetic counselling (GC) clinic records from two patients (2/29; 6.9%) showed that they were on treatment at the Endocrine Clinic. A single patient (1/29; 3.45%) was referred to the Endocrine Clinic but didn't attend the appointment, according to the parents. A further four patients (4/29; 13.79%) were referred but it is not clear whether or not they are on treatment.

OHT: The six results classified as OHT (three from CHBH laboratory and three from CMJAH laboratory), were collected from six CHBH patients.

- Of the six patients, four (4/6; 66.7%) had a TSH greater than 10mIU/L. Of these, 3/4 (75%) patients are on treatment at the Endocrine Clinic at CHBH, and 1/4 (25%) was not referred.
- There is no referral or treatment information available on the other 2/6 (33.3%) patients who had TSH values less than 10mIU/L.

CHT: Of the 11 results classified as CHT, nine were analysed at CHBH and two at CMJAH. They were collected from 7 CHBH patients

- Those patients with TSH values greater than 10mIU/L formed the majority (5/7; 71.4%).
- The other two patients (2/7; 28.6%) had elevated TSH levels (above reference range but <10mIU/L):

Referral and treatment information on these patients remain unavailable, as the CHBH Endocrine Clinic files could not be accessed (see Limitations section 1.11)

Other abnormal results: Nine patients (nine results) were classified as having isolated elevated FT4, isolated decreased FT4 or elevated TSH with an elevated FT4. There is no referral or treatment information available on these patients.

3.8.4 SUMMARY OF PATIENT REFERRAL AND TREATMENT

Tables 3.18 and 3.19 provide summaries of the referral practices for patients with elevated TSH values. These include patients with SCH, CHT, OHT and those who had elevated levels of both TSH and FT4.

Table 3.18: Summary of referral practices by hospital for patients with TSH>10mIU/L

Hospital	TSH > 10mIU/L (N)	Number on treatment (N) (%)	Number not on treatment (N) (%)	Number not referred (N) (%)
CHBH	37	7* (18.9%)	Unknown	16* (43.2%)
CMJAH	15	11 (73.3%)	-	4 (26.7%)
RMH	5	3 (60%)	-	2 (40%)

(* indicates number of patients for which information is available. Total will not equal 100%)

Table 3.19: Summary of referral practices by hospital for patients with TSH results above reference range but <10mIU/L

Hospital	TSH above reference range but < 10mIU/L (N)	Number on treatment (N) (%)	Number not on treatment (N) (%)	Number not referred (N) (%)
CHBH	46	2 (4.35%)*	Unknown	22 (47.8%)*
CMJAH	30	10 (33.3%)	3 (10%)	17 (56.7%)
RMH	7	1 (14.29%)	2 (28.57%)	4 (57.14%)

(* indicates number of patients for which information is available. Total will not equal 100%)

3.8.5 SUMMARY OF SIGNIFICANT RESULTS

3.8.5.1 Demographics

- The sample comprised 391 children with DS, most of whom (49%) were seen at CHBH.
- The majority of the children were black (92%) and there were equal numbers of males and females.
- Most of the patients were aged between two and twelve months, with few falling into the neonatal and older age groups.

3.8.5.2 TFTs Performed

- 84% of the 391 children with DS in this study had at least one TFT performed.
- Most of the TFTs (54%) were requested by the doctors in the GC clinics.
- A total of 516 TFT results were collected.
- The majority of TFTs (336/516; 65%) were analysed at CMJAH.
- There was a significant difference between results obtained from CHBH and the other two hospitals ($p<0.005$).

3.8.5.3 Thyroid Dysfunction Spectrum

- A wide spectrum of thyroid dysfunction was represented in this cohort, with the noticeable exception of hyperthyroidism.
- The results collected (295/516; 57.2%) mostly reflected a euthyroid state, but a large proportion (148/516; 28.7%) fell into the SCH category. These SCH results were collected from 99 patients, or 25.3% of the patients in the study.
- There were equal proportions of male and female patients with TSH levels above 10mIU/L.

3.8.5.4 Referral and Follow-Up

- A large proportion of patients (155/190; 84%) could not be contacted during the course of the study, and thus repeat TFTs could not be performed on those with abnormal initial TFTs.
- Several neonates with high TSH results, and therefore, CHT, were not referred to the Endocrine Clinics, and were therefore not started on treatment in the neonatal period.
- More than a third of all patients with high TSH results were not referred to the Endocrine Clinics for further management.
- All the patients from CMJAH and RMH with TSH levels greater then 10mIU/L, who were referred to the Endocrine Clinics, were receiving thyroxine treatment.

- Little information was available about the referral and treatment practices at CHBH.

4. DISCUSSION

DS is the most common viable chromosomal abnormality and children with DS form a substantial proportion of the patients seen in the GC clinics at the three academic hospitals in Johannesburg. All the records of the 391 children with DS, who were seen during the six year period between 2003 and 2008, were traced. Significant results obtained from the data analysis presented in Chapter 3 will be discussed.

4.1 DEMOGRAPHIC FACTORS

The majority (92%) of children with DS seen in the GC clinics are black, reflecting the demographics of the Greater Johannesburg area, where the study took place. An additional explanation for the fewer numbers of white, coloured and Indian children could be related to economic and logistic factors. Firstly, many children may be assessed and managed in the private health care system as opposed to the state system. This study did not include children seen at the private GC clinic in Johannesburg, but had it done so, the demographic profile of the cohort may have changed. Secondly, women of other ethnicities may have greater access to more sophisticated antenatal care, fetal screening, prenatal testing and termination of pregnancy, which may influence the birth rates of babies with DS among other ethnicities.

Most children with DS referred to our GC clinics were aged two to twelve months, corresponding to the TFT reference range age category three (see Table 3.16 and Appendix B). There was a noticeable paucity of newborns assessed and tested for thyroid dysfunction, despite many having had their diagnosis of DS confirmed within the neonatal period, before referral to the GC clinics. Also, children older than twelve months with DS

were under-represented, due to the fact that most children with DS are discharged from the GC clinics as soon as the genetic counselling of the family and the basic health supervision has taken place. Repeat TFTs then become the responsibility of non-genetic health care professionals.

During the period between 2003 and 2008, almost half (49%) of the patients with DS were seen at CHBH. This is consistent with general expectations, as the GC clinic at CHBH is the largest clinic run by the Division of Human Genetics.

4.2 CHROMOSOME RESULTS

There were significantly fewer PCR aneuploidy tests performed than anticipated. Most of the children with DS had a full karyotype. This figure may be a reflection of the postnatal DS testing protocol at the time (2003-2008). Prior to 2008, all children suspected of having DS had blood taken for a full karyotype analysis. After 2008, PCR aneuploidy was introduced as the investigation of choice for postnatal confirmation of DS (Professor A. Christianson- personal communication).

Altogether, 92.4% of the children had Trisomy 21 due to non-disjunction, as seen on the full karyotype analyses. A proportion of the 7% who had a PCR aneuploidy positive result, would also be expected to have the non-disjunction type of DS, although the PCR aneuploidy cannot discriminate between non-disjunction DS, translocation DS or mosaic DS. The proportion of patient with mosaic DS (2.25%) is similar to the quoted international figures (2.4%) (Thuline and Pueschel, 1982), but our frequency of

translocation DS is slightly higher: 5.34% compared to 3.3% internationally (Thuline and Pueschel, 1982).

However, this cohort comprised a relatively small sample size, and larger studies are needed to more accurately describe the frequency rates of each subtype of DS in the South African population.

4.3 NUMBER OF PATIENTS TESTED

The majority (327/391; 84%) of the patients with DS had their thyroid function tested, while 16% never had a TFT. More than half (279/516; 54%) of the TFTs were requested by the doctors in the GC clinics, compared to 35% (183/516) performed by the general paediatricians and 11% (54/516) by other health professionals, mainly the paediatric cardiologists and the neurodevelopmental paediatricians. This finding illustrates good compliance amongst the doctors within the Division of Human Genetics in following the departmental policy to screen each child with DS for thyroid dysfunction.

The Developmental Clinics at both RMH and CMJAH seem to be active in screening for thyroid dysfunction. The Developmental Clinic at CMJAH performs thyroid tests at least annually on all their patients with DS, and also initiates treatment and monitoring. Most of these children are not referred to the Endocrine Clinic, but are treated and monitored at the Developmental Clinic (Prof Lorna Jacklin - personal communication). The study did not include a review of the Developmental Clinic files, and there may be more patients treated and managed there than were recorded.

A number of TFT results were excluded from the study, either because they were performed when the children were ill, or during the first 48 hours after delivery. Testing thyroid function at these times yields results which are difficult to interpret, as they cannot be used to categorise a patient's thyroid status. It is known that non-thyroidal illness (and possibly administered drugs) can interfere with the TFT results, and may indicate abnormalities which are not necessarily truly reflective of thyroid dysfunction. Different TFT results may be obtained once the illness has resolved. Additionally, the newborn experiences a TSH surge post-delivery as part of the normal physiological processes which occur shortly after birth. This elevated TSH level usually returns to normal levels after the initial surge in healthy newborns (Brown, 2001; Rossi, Caplin & Alter, 2004). The fact that TFTs were performed shows that the awareness of thyroid dysfunction in DS exists, but that the implications of testing at the correct times are not fully understood. Increased awareness amongst health professionals is needed to ensure that the TFT testing takes place at the appropriate times.

4.4 TESTING STRATEGIES

Of the patients who had no TFT result, 11/64 (17%) had insufficient blood submitted for analysis. All TFTs are performed on venous blood, usually obtained from a peripheral vein. The reason too little blood was submitted may be that phlebotomy in children with DS may be more difficult due to their short necks and tendency to become overweight, requiring an experienced phlebotomist to perform the venepuncture.

There are currently no alternatives to routine venepuncture in Johannesburg. However, both Noble *et al* (2000) and Murphy *et al* (2008) concluded that a finger/heel prick blood

test was just as valid as routine venepuncture, at least in testing for hypothyroidism, which is the main form of thyroid dysfunction in our cohort. This less invasive method of obtaining a blood sample from the children with DS may overcome the reluctance by parents and doctors to have the children tested. It may also circumvent the technical difficulties involved in routine venepuncture, and lessen the trauma experienced by the child, thus promoting compliance with screening protocols.

The necessary technology is available in South Africa to undertake heel prick TSH testing. This could be a consideration for the future of thyroid testing and monitoring of children with DS, as it is easily performed by minimally trained staff and would be a good option for testing in the peripheral hospital and primary healthcare setting.

4.5 THYROID FUNCTION TEST RESULTS

4.5.1 TFT DISTRIBUTION CURVES

Both the TSH and FT4 distributions were non-normal curves (see figures 3.8, 3.9, 3.10 and 3.11 in Chapter 3). As Hübner *et al* (2003) also reported a non-normal distribution, we expected the results from our cohort to behave similarly. Van Trotsenburg *et al* (2006) noted that neonates with DS had a FT4 distribution curve which was normally distributed, but shifted to the left of the normal curve observed in non-DS neonates. Perhaps this finding is peculiar to neonates with DS. Unfortunately, in the present study, there were too few results from neonates with DS to produce a comparable distribution curve. There were also no non-DS control patients for comparison.

4.5.2 DIFFERENCE OBSERVED BETWEEN THE HOSPITALS

A difference between the medians of the TFTs from the three hospitals was anticipated, as they use different machines to analyse their thyroid function tests. There was no difference between the hospitals with regards to other variables (age ranges, race stratification, gender stratification or chromosome results). The analysis of variance confirmed a statistical difference between CHBH and the other two hospitals ($p<0.05$), but not between CMJAH and RMH ($p>0.05$). Thus the difference could only be accounted for on the basis of the laboratory at CHBH using a different biochemical analysis.

The child referred to in section 3.6 was tested at CHBH and CMJAH, a week apart. Using the result from CHBH, we may have classified this child as having SCH. However, the repeat test at CMJAH was within normal limits for his age. Thus, in an attempt to make the interpretation of results easier, all TFT specimens from the GC clinic at CHBH were referred to CMJAH for analysis from 2005.

The difference observed ($p<0.05$) between the CHBH laboratory and the other hospitals, with regards to all results and the group of TSH results greater than 10mIU/L, also highlights the possibility that there may be inter-laboratory differences when TFTs are analysed at other hospitals. This confounds the difficulty of interpretation of TFT results when patients are tested in peripheral hospitals.

4.6 COMPARISON WITH REFERENCE RANGES

The Advia® Centaur ™ was used to establish the TFT paediatric reference ranges (Hübner *et al*, 2003), which were adopted as the NHLS reference ranges (see Appendix B). These

reference ranges were derived from a study performed on healthy German children, and are specific age-dependent ranges calculated using that specific machine.

Manufacturers recommend that laboratories calculate their own reference ranges, based on their specific biochemical machine and population. However, this is often not feasible, as setting up reference ranges is time-consuming and costly, and there are ethical issues concerning venepuncture in healthy children for no clinically-indicated reason. Hübner *et al* (2003) found that their results differed from a previous study, conducted in the United States of America using a different machine for the analysis of the TFTs. They postulated that the difference arose due to the different populations tested, i.e. American rather than European children, rather than the machine involved. All biochemical assays should be standardised according to international standards.

As previously noted, the three hospitals in this study have three different machines, which run three different biochemical assays for the analysis of TFTs. The machine at CMJAH is the exact one used in the study by Hübner *et al* (2003). One could argue therefore, that using the reference ranges from the Advia® Centaur ™ would only be useful for TFTs analysed at CMJAH. However, it may not be possible for each laboratory to derive their own reference ranges, and clinicians may have no choice but to continue using reference ranges, which strictly speaking, may not be the most accurate ranges for their particular patients or hospital.

Moreover, doctors are faced with the challenge of trying to interpret results from African children, using reference ranges derived from a different population. The situation is even more complicated when those African children also have DS. There are no recommended

TFT reference ranges for children with DS; doctors the world over have had to continue treating their patients with DS based on local reference ranges, derived from studies on the general population.

4.7 SPECTRUM OF THYROID DYSFUNCTION

It was not possible to superimpose a distribution curve derived from the reference ranges onto the curve obtained from this sample, as insufficient information about the reference cohort was available and a different statistical analysis was used in the derivation of the normal range values. However, a comparison of the individual TFT results to the reference range values was possible. Tables 3.12 and 3.15 show the range of thyroid dysfunction in the patients from CMJAH/RMH and CHBH, respectively. The frequencies of SCH and OHT were comparable to those of other international studies. However, it was not possible to compare the present rates of CHT, as most of the patients in this study were not screened during the neonatal period.

4.7.1 CONGENITAL HYPOTHYROIDISM

Although 23.5% of newborns in this study were found to have CHT, this figure should be interpreted with caution. It cannot be compared with figures quoted in international studies (1.1% to 6% - see table 1.5), as all their newborns with DS are tested for thyroid dysfunction, as part of an NBS programme. Most of the children in this study were not. It is therefore not possible to state categorically how many of the present sample of children with DS were born with CHT. Additionally, there is no national NBS; hence there is a delay not only in the diagnosis of thyroid dysfunction in children with DS, but also in the general population.

We refer to the infant seen at RMH (section 3.6.2.3) who had a deranged TFT result at the age of 30 days. One can only speculate that she was probably born with abnormal thyroid function. Had all children in this cohort been tested within the newborn period, a more accurate CHT frequency rate could have been calculated.

4.7.2 SUBCLINICAL HYPOTHYROIDISM

The most common form of thyroid dysfunction in this cohort was SCH (see section 3.6.3.5 on page 55 for details). This figure (25.3%) falls within the broad range of SCH noted in the studies summarised in table 1.5.

There were 39 patients in the cohort with a TSH level greater than 10mIU/L. However, only 30.8% (12/39) of these patients were reportedly on thyroxine replacement treatment. As mentioned in the Introduction, the UK DS guideline, amongst others, supports treatment for patients with subclinical hypothyroidism with TSH levels greater than 10mIU/L (UK - Down Syndrome Medical Interest Group, 2005). Additionally, the AACE and ATA recommend beginning thyroxine treatment when TSH values exceed 10 mIU/L in the general population (Hollowell *et al*., 2002; Surks *et al*., 2004). The consensus statement of the Endocrinology and Diabetes Committee of the Royal College of Physicians of London and the Society for Endocrinology also supports treatment at the threshold of TSH greater than 10mIU/L (Vanderpump, Ahlquist, Franklyn *et al*., 1996).

The local result is largely due to non-referral and delays due to retesting. However, accurate information about the treatment practices at CHBH, the Developmental Clinics around Johannesburg or the peripheral hospitals, was not available. Thus more children with DS may be on treatment than have been documented in the present study.

4.7.3 OVERT HYPOTHYROIDISM

The frequency of OHT documented in this study was 3.6%, which falls at the lower end of the ranges quoted in the international studies (see table 1.5). All the patients from CMJAH and RMH, who had OHT with TSH levels greater than 10mIU/L were on treatment. However, not all with TSH values less then 10mIU/L were on treatment, due to the lack of referral to the Endocrine Clinic.

4.7.4 HYPERTHYROIDISM

No patients in this cohort had hyperthyroidism. This was in contrast to the published literature, where figures range between two to three percent. The patients with DS in the study by Cutler *et al* (1986) had a similar age distribution to this cohort, and two percent of their patients were hyperthyroid by age three (see table 1.5). The reasons for the discrepancy noted in this study may be two fold. Firstly, because congenital hyperthyroidism can present with normal, abnormally high or abnormally low thyroid hormone levels, the diagnosis remains complex (Brown, 2001; Braverman and Utiger, 2005). There is no dedicated screening protocol to diagnose at-risk newborns. Mothers at the antenatal clinics around Johannesburg are also not routinely screened for thyroid hormone abnormalities (Dr J. Jeebodh - personal communication). Secondly, few tests were performed in later childhood, while it is well known that thyroid dysfunction in DS increases with age (Pueschel and Pezullo, 1985; Pueschel *et al.*, 1991; Karlsson *et al.*, 1999). The mean age of diagnosis of hyperthyroidism in the study by Goday-Arno *et al* (2009) was 16.8 years. Fewer TFTs performed means fewer confirmed diagnoses of thyroid dysfunction.

4.7.5 FURTHER ABNORMAL RESULTS

Some FT4 results from the study proved challenging to interpret. Some results (14/516; 2.7%) showed isolated decreased FT4 in the presence of a normal TSH level. There may be several explanations for these results (refer to table 1.3). These results may be suggestive of central hypothyroidism. Further investigation is needed in these patients to exclude intracranial pathology. We have no evidence that these patients were ill or on any form of drug treatment, which may have caused similar results (Wallach, 2000).

An isolated increased FT4 was found in 19/516 (3.7%) results. This may indicate an inadequate central response. In the normal situation, an increased FT4 would form a negative feedback loop which would result in a decrease in the TSH level. If there is an inadequate response from the hypothalamic-pituitary-thyroid axis, the TSH will not respond, and would remain higher than expected, though still within the normal range. Several patients with DS at the CMJAH Endocrine Clinic have shown similar results (Dr D Segal - personal communication). Further endocrine investigation is indicated in these patients.

Further interesting results included 12/516 results (2.3%), in which both TSH and FT4 were elevated. This finding could be explained by any of the reasons given in table 1.3. There is no evidence that children with DS are at any greater risk than the general population to develop any of the mentioned underlying causes of elevated TSH and FT4. There is also no documentation that any of these patients were on thyroid hormone replacement therapy at the time of these tests. Further endocrine investigation of these patients is indicated.

4.7.6 CAUSE OF THYROID DYSFUNCTION

As detailed in the Introduction, many theories exist as to the cause of thyroid dysfunction in the DS population. Blood tests for anti-thyroid antibodies and serum zinc levels are available locally, although the genetic testing for thyroid receptors and other genes are not. No further investigation into the cause of the thyroid dysfunction was performed on this cohort; thus the main cause of their thyroid dysfunction remains unknown. Results discussed in section 4.7.5 show that there may be inadequate thyroid and central responses to increased FT4 and TSH, respectively. However, once again, the underlying mechanisms remain elusive. This situation mirrors the trend in the DS literature, where the causes of thyroid dysfunction are still being hypothesised.

4.7.7 CONSEQUENCES OF SUBCLINICAL THYROID DYSFUNCTION

None of the patients were tested for the reported SCH-related side-effects. Thus, there is no available data to document whether the patients with SCH have lipid dysfunction. Additionally, since the prevalence of cardiac dysfunction is higher in children with DS due to their chromosome abnormality, it would be difficult to document how much of the dysfunction could be attributed to the SCH. The same is true for the neurobehavioural difficulties experienced by children with SCH, which may be viewed as part of the DS phenotype.

4.8 EFFECT OF DEMOGRAPHIC VARIABLES ON THE TSH VALUES

The analysis in 3.7 showed that older children were less likely to have TSH values greater than 10mIU/L. This is not in keeping with what is known about thyroid function in DS,

where the trend is the exact opposite. In fact, TSH seems to increase with age in individuals with DS (Pueschel and Pezullo, 1985; Pueschel *et al*., 1991; Karlsson *et al.*, 1999). However, this finding should be interpreted with caution, as there were very few results for children in the older age categories. Once more, this re-emphasises the need for testing in older children. The analysis also showed no influence of other demographic variables, including gender, on the TSH levels. As very few patients belonged to other race groups or DS-subtype groups, larger samples will be needed for a more accurate analysis.

4.9 PATIENT FOLLOW-UP

Every effort is made by the doctors and genetic counsellors in the GC clinics to ensure that annual thyroid function testing is performed on all patients with DS. Few data are available as to whether these tests are being performed. In this study, a total of twelve (3.7%) of the 327 patients who had a TFT done, had regular annual testing. The majority of these tests were still being performed within the tertiary hospital setting, showing that the TFT surveillance has not been devolved to the secondary and primary care levels.
A large proportion of the patients who were included in the present study were lost to follow-up (80%; 155/194), despite the fact that the GC clinic file documents at least two telephone numbers and a street and postal address for every patient. This statistic is disturbing. A more effective means of maintaining contact with the patients needs to be found. It is hoped that the patients have received the GC letters, and are accessing medical care and surveillance wherever they are.

Furthermore, a more practical and streamlined process needs to be implemented in order to enable better patient compliance with the thyroid surveillance protocol. This may include establishing a specialised DS Clinic, which would run once a month and offer endocrine, genetic, cardiac, physiotherapy, occupational and speech therapy on the same day. Currently, patients need to make several trips a month to the tertiary hospital to access these services, which is inconvenient and costly. With a dedicated DS clinic, patients would only need to travel to the tertiary hospital once a year for a thyroid and cardiac check-up, if the latter was also necessary. The therapists could make sure that the parents are able to continue effective therapy at home, while regular therapies could continue at their local hospitals/clinics. More frequent visits to the tertiary centre would only be necessary for more regular surveillance in certain patients. This approach may enhance not only the compliance with the medical surveillance, but will provide a platform for parents of children with DS to meet one another and to discuss issues surrounding DS.

4.10 REFERRAL INFORMATION

There were many patients with abnormal TFT results, who were never referred to the Endocrine Clinics. Overall, more than a third of patients (36.6%) with a TSH level greater than 10mIU/L were not referred to the endocrinologist immediately as far as could be ascertained. The figure is even higher (53,9%) for those patients who had a TSH level between the upper limit of the reference ranges and 10mIU/L. Although the problem of referral seems less pronounced at the CMJAH and RMH, the fact remains that there are still many patients who have remained without adequate endocrine assessment and treatment at all the tertiary hospitals. This can partly be explained by the lack of a standardised guideline, outlining the need for active thyroid surveillance of children with

DS. Testing, as the first step in the surveillance protocol, cannot benefit the patient if it is not followed by appropriate referral practices.

Delaying immediate referral of the two hypothyroid neonates mentioned in 3.8.1, meant that the opportunity to treat their congenital hypothyroidism was missed. Both patients are currently on treatment, which was instituted later in infancy, but their cases highlight the need for a lower threshold for referral and treatment in neonates with DS. Another seven neonates were found to be hypothyroid at CHBH; no treatment information about them was available during the course of this study.

Whether the patients were tested by the geneticists or other specialists did not seem to make a difference to the referral practices. Although the preferences of the Endocrine Clinics have been documented here, this information is not widely available to the GC Clinic staff or other healthcare professionals caring for children with DS. It is hoped that wider publication of these preferences will prompt doctors to have a lower threshold for referral to the specialist endocrinologist.

4.11 TREATMENT PRACTICES

The controversy surrounding SCH seemed to extend to the present study cohort as well. While the paediatric endocrinologists believe that the SCH is a true reflection of the patients' thyroid status, they differ as to the appropriate management thereof.

These two different approaches from the tertiary Endocrine Clinics at CMJAH and RMH (as previously detailed in the Introduction) could each be justified. Presently, one could

not judge which approach has a better outcome for the children with DS. One way to solve the controversy would be to design a study to compare the long-term developmental outcomes of the two groups of children.

4.12 SUMMARY OF THE MAIN PROBLEMS IDENTIFIED IN THIS STUDY

The number of patients not referred after an abnormal TFT result is alarming. Often, the TFT was repeated, but no further action was taken as regards referral and treatment. The problem is of greatest concern, when the thyroid dysfunction is detected in the sensitive neonatal period.

Many patients with abnormal results, and some of those, who had never had a TFT, were lost to follow-up: of these 194 patients, only 22 (11.3%) came for a repeat TFT.

Despite all efforts to ensure that annual TFTs are being performed on children with DS following discharge from the GC clinics, very few have regular thyroid function testing. These are generally not performed outside of the tertiary hospital setting.

Due to the statistical difference observed between the laboratory at CHBH and the other two hospitals, questions have arisen as to the manner in which TFT results are interpreted at the different hospitals. This discrepancy needs to be addressed, in order for difficulties with interpretation of results to be resolved.

4.13 CONCLUSION

This study highlighted several important issues regarding the diagnosis, referral and management of children with DS as regards thyroid function.

Given the resource constraints placed on the healthcare system by the burden of other diseases like HIV and TB, an overall thyroid testing rate of 84% in this cohort is remarkable. However, these tests are still only being requested by specialists and sub-specialists. Importantly, although most of the children in the study were tested, there seemed to be a less structured approach to referral of those children found to have abnormal TFT results. This has left a large proportion of children who needed referral and treatment, without the necessary management and care. It is hoped that the broader knowledge of the Endocrine Clinic preferences will allow for more timeous referrals in future.

More research is needed to establish whether it is feasible in our setting to change the TFT testing modality to that of a finger/heel prick. This may ensure better parental and patient compliance with thyroid surveillance, especially outside the tertiary hospital setting.

The establishment of a DS Specialist Clinic would also allow for the multidisciplinary care of children with DS and facilitate suitable health surveillance in this population of children, at least for the short-term. An ideal long-term solution would include devolving the thyroid surveillance of children with DS to the secondary or primary healthcare settings. This would necessitate national guidelines for the health supervision of children with DS to be adopted by all hospitals. However, difficulties with staff numbers, available qualified

phlebotomists, biochemical analyses and reference ranges needs to be borne in mind, along with the need for education of the healthcare providers at such institutions.

Difficulties with interpretation of TFT results in the Gauteng population of children with DS were discussed. Special mention was made of the fact that the TFT paediatric reference ranges were derived on a particular machine using European children; thus interpretation of TFTs analysed on different machines and on South African children in general, and South African children with DS in particular, may prove challenging. Additionally, there is an urgent need for the discrepancy between the laboratories at the various tertiary hospitals in Johannesburg to be resolved.

Increased communication and inter-disciplinary discussion among health professionals caring for children with DS will improve the overall care provided and ensure that all parties are aware of the necessary surveillance and other medical and social issues faced by children with DS.

Finally, there is a need for a uniform, standard protocol, outlining the health supervision needs of children with DS, which includes thyroid function testing, referral and treatment guidelines. Thyroid dysfunction can be easily and cost-effectively treated. There is thus no justification for leaving thyroid dysfunction untreated in this vulnerable population of children, especially as it affects their cognitive abilities and developmental potential.

5. CONCLUSION

5.1 CHALLENGES

Left untreated, hypothyroidism causes irreversible mental and physical handicaps. It is therefore desirable to detect hypothyroidism as early as possible in any individual, but even more so in children who already have a predisposition to learning disabilities and growth impairment.

Optimising the care for children with DS should be prioritised. It is vital that a standardised, national surveillance protocol be established to specifically address the health needs of children with DS in South Africa, while taking into account the country's limited resources. The guideline would need to include, among others, protocols for thyroid surveillance, which would standardise the monitoring of thyroid function across the country. Ideally, this would include TFT testing for all newborns with DS, regular thyroid surveillance of all children with DS, active and appropriate referral for monitoring and treatment, less invasive testing techniques, and the facilities to have these tests as close to their homes as possible, preferably in the primary or secondary hospital systems.

However, the immediate implementation of such a guideline is hampered by several issues. The first of these issues is the discrepancy between the laboratories noted in this study. Urgent studies are needed to delineate the exact causes of the differences. Should the laboratories not be able to reconcile the differences and standardise the TFT analyses, alternatives like the heel prick tests should be urgently optimised. The supervision guidelines would not be feasible in the absence of a standardised test, which delivers easily and reliably interpretable results. Whether establishing DS-specific TFT reference ranges

would prove beneficial in the South African health care setting, also requires further consideration.

Secondly, the absence of a NBS policy in South Africa means that children with DS who have CHT are not being diagnosed during the neonatal period, which is the ideal time to institute treatment. Instituting a national NBS to test for thyroid dysfunction at birth, will not only benefit newborns with DS, but the general population of neonates as well.

Furthermore, little is currently known about the referral and treatment and surveillance practices at peripheral hospitals. It was also noted that although children with DS are given a letter upon discharge from the GC clinics requesting annual TFTs from their local hospital, there is no record that these tests are being performed. Enhanced communication is needed amongst the professionals who care for children with DS, so that the goal of standardizing their care can be achieved.

While most children with DS are being actively tested at least once for thyroid dysfunction, the next steps in the management chain are conspicuously lacking. Children are not being referred timeously and are therefore not receiving treatment. Increased education of health care professionals and parents is needed to ensure that not only regular surveillance, but appropriate referral and treatment, takes place to guarantee the best care for children with DS.

Without the above-mentioned prerequisites, a national DS surveillance guideline would be of little practical value. It is hoped that the results of this study will increase awareness of the current challenges, and form the basis for discussion around the management and care

of children with DS in South Africa, finally leading to more structured protocols regarding health screening and treatment of complications associated with DS.

5.2 FUTURE RESEARCH

This study highlighted several challenges faced by doctors caring for children with DS. These challenges should be viewed as opportunities for future research. The first urgent study which needs to be conducted should focus on resolving the discrepancies between the hospital laboratories' TFT assays, so that TFT result interpretation can be standardised. The results from such a study would provide the necessary impetus to investigate whether DS-specific TFT reference ranges should be proposed.

Further studies in the field of thyroid function in DS could focus on

- investigating the cause of the thyroid dysfunction in this population of children with DS,
- exploring the viability of alternative testing techniques,
- investigating whether SCH has other adverse effects on children with DS,
- examining whether thyroxine treatment in the absence of overt hypothyroidism affords children with DS a developmental or growth advantage,
- reproducing this study in other areas/hospitals/provinces to identify if similar challenges are faced in other parts of South Africa

Results from these studies would increase our knowledge of thyroid dysfunction in the South African population of children with DS, and contribute positively towards improving their care.

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Appendix A: *Summary of the country-specific guidelines for the thyroid surveillance of children with DS*

COUNTRY	0-1 MONTH	1-12 MONTHS	1-5 YEARS	>5 YEARS
UNITED STATES OF AMERICA (American Academy of Pediatrics – Bull, 2011)	Routine NBS[1]	TSH at 6 months and 1 year	TSH annually	TSH annually
UNITED KINGDOM (Down Syndrome Medical Interest Group – 2005)	Routine NBS[1]	a. Full TFT with thyroid antibody tests at 1 year and 2 yearly thereafter or b. TSH finger prick annually [2]	a. Full TFT with thyroid antibody 2 yearly OR b. TSH finger prick annually [2]	a. Full TFT with thyroid antibody tests 2 yearly OR b. TSH finger prick annually [2]
CANADA (Down Syndrome Research Foundation- 2006)	Routine NBS[1]	TSH and FT4 at 6 months and 1 year	TSH and FT4 annually	TSH and FT4 annually
IRELAND (Down Syndrome Association- 2006)	Routine NBS[1]	Nil	a. Full TFT OR b. TSH finger prick (if available) annually	a. Full TFT OR b. TSH finger prick (if available) annually
EUROPE (European Down Syndrome Association- 2002)	Routine NBS[1]	TFT[3] at 6 months and 1 year	TFT[3] annually	TFT[3] annually

[1] NBS (Newborn screening programme): may differ from country to country, and within the USA from state to state; generally includes TSH and FT4

[2] Finger prick must be followed by venous TSH, FT4 and thyroid antibodies if initial TSH is raised

[3] Different European countries have different practices: some may do a full TFT, other may only test the TSH levels

Appendix B: *NHLS Paediatric TFT reference ranges*

AGE	TSH mlU/L	FT4 pmol/L	FT3 pmol/L
1- 3 days	0.13-9.23	10.8-26.8	2.3-8.1
4 - 30 days	0.16-8.48	10.9-25.5	2.4-7.9
31 - 60 days	0.19-7.78	11.0-24.3	2.5-7.8
2 months-1 year	0.3-5.88	11.4-20.9	2.7-7.3
1 - 5 years	0.42-4.79	11.4-19.0	3.1-6.9
6 -10 years	0.48-4.67	11.0-18.8	3.3-6.8
11 -14 years	0.53-4.58	10.8-18.7	3.5-6.7
15 - 18 years	0.56-4.53	10.7-18.7	3.6-6.7

Appendix C: *Data Collection Sheet*

CODE	HOSP	GENDER	RACE	DATE OF BIRTH	AGE	DATE OF TEST	TEST REQ	CHROM. RES	TSH	FT4	REF Y/N	ON Rx AT ENDO Y/N

CODE: anonymous patient code

HOSP: hospital

TEST REQ: test requester

CHROM RES: chromosome result

TSH: thyroid stimulating hormone

FT4: free thyroxine

Ref Y/N: referred to Endocrine Clinic, yes or no

On Rx at EndoY/N: on treatment at Endocrine Clinic, yes or no

Appendix D: *Ethics Approval Certificate*

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Dr Shahida Moosa

CLEARANCE CERTIFICATE	**M090710**
PROJECT	An Audit of Thyroid Function Test in a Cohort of South African Children with Down Syndrome
INVESTIGATORS	Dr Shahida Moosa.
DEPARTMENT	Division Human Genetics/School of Pathology
DATE CONSIDERED	09.07.31
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 09.07.31 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Dr N Gregersen

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

.....