

Chapter 2

Genetic Influences on Thyroid Function Tests*

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In this review we will discuss the possible effects of polymorphic variation in the genes important for thyroid hormone synthesis, metabolism, and action, on the interindividual variation in thyroid functions tests. The genes involved are summarized in the following outline of thyroid hormone production and action, but their role is discussed in detail in other sections (Chaps. 1 and 4). In addition to these genes, we will also briefly discuss the possible contribution of genetic variation in the thyroid-specific transcription factors which are known to be important for thyroid development and regulation: TTF1, TTF2, and Pax8.

2.1 The Hypothalamus-Pituitary-Thyroid Axis

The production of thyroid hormone occurs in the follicular cells of the thyroid and involves the following steps (Fig. 2.1) [1]:

1. Cellular uptake of iodide by the Na/I symporter (NIS) located in the basolateral membrane.
2. Release of iodide through the apical membrane into the follicular lumen via pendrin and/or other transporters.
3. Generation of H_2O_2 by the enzyme dual oxidase 2 (DUOX2) located in the apical membrane.
4. Iodination of tyrosine residues in thyroglobulin (Tg) with generation of mono- and diiodotyrosine (MIT, DIT); generation of thyroxine (T4) by coupling of two

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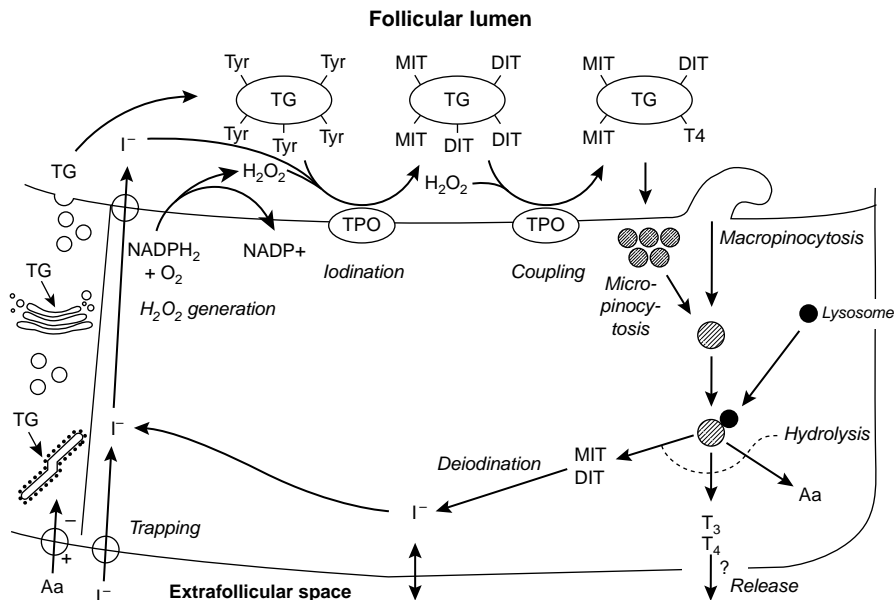


Fig. 2.1 Overview of thyroid hormone synthesis

DITs and of triiodothyronine (T₃) by coupling of MIT and DIT. Both iodination and coupling are catalyzed by thyroid peroxidase (TPO) and require H₂O₂.

- Endocytosis of Tg and hydrolysis by lysosomal enzymes, resulting in the liberation of MIT, DIT, T₄, and T₃.
- Deiodination of excess MIT and DIT residues by iodotyrosine dehalogenase (DEHAL), and reutilization of the iodide liberated for thyroid hormone synthesis.
- Secretion of T₄ and T₃ at the apical membrane by an as yet unknown mechanism.

Under normal conditions the thyroid predominantly secretes the prohormone T₄ and only a small amount of the active hormone T₃. Most T₃ is generated by enzymatic outer-ring deiodination of T₄ in peripheral tissues. Alternative inner-ring deiodination of T₄ results in the generation of the inactive metabolite rT₃. Inner-ring deiodination is also an important route for the degradation of T₃. Three deiodinases (D1-3) are involved in these reactions; D1 and D2 are capable of activating T₄ to T₃, whereas D3 is responsible for inactivation of T₄ to rT₃ and of T₃ to 3,3'-T₂. These deiodinases are expressed in various tissues, including liver, kidney, brain, pituitary, thyroid, and skeletal muscle.

The production of thyroid hormone by the thyroid gland is regulated by the hypothalamus-pituitary-thyroid (HPT) axis (Fig. 2.2) [2]. Thyroid hormone is secreted in response to thyroid-stimulating hormone (TSH), which is synthesized in and released from the pituitary. TSH consists of a (common) α subunit and a TSH-specific β subunit, and exerts its effect via binding to the TSH receptor (TSHR) on the thyroid follicular cells. In turn, TSH production is stimulated by hypothalamic thyrotropin-releasing hormone (TRH). The production of TRH and

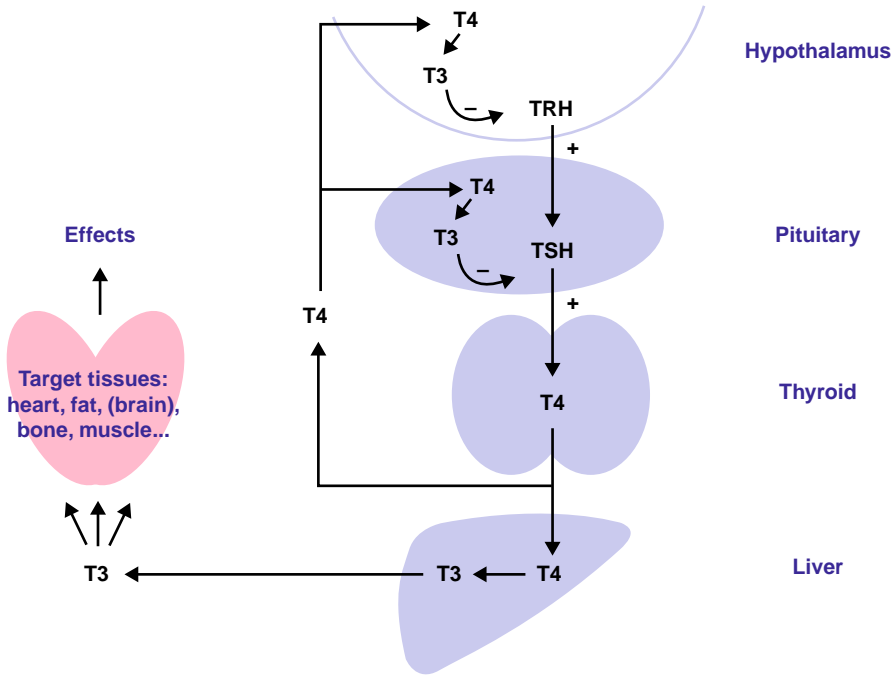


Fig. 2.2 Overview of hypothalamus-pituitary-thyroid-peripheral tissues axis

TSH is down-regulated by thyroid hormones, a process known as negative feedback regulation. Also, other hypothalamic hormones and drugs, such as somatostatin, cortisol, and bromocriptine lower TSH production. Besides the regulation by TSH, thyroid hormone synthesis is also dependent on the availability of iodine.

Thyroid hormone is transported in the circulation tightly bound to different proteins, largely, thyroxine-binding globulin (TBG), transthyretin (TTR), and albumin. However, it is the free fraction of T4 and T3 which is available for metabolism and action in the tissues. The above mentioned deiodinases have their active sites located in the cytoplasm, and most thyroid hormone actions are initiated by binding of T3 to its nuclear receptors (TRs). Cellular uptake of thyroid hormone does not occur by passive diffusion, but is mediated by specific transporters. These include different members of the monocarboxylate transporter (MCT) and organic anion transporting polypeptide (OATP) families. TRs are encoded by two genes: THRA which codes for different TR α isoforms and THRB which codes for different TR β isoforms.

2.2 Influence of Genetic Variation on Thyroid Function Tests

In healthy subjects, serum thyroid parameters show substantial interindividual variability, whereas the intraindividual variability is within a narrow range [3]. Together with environmental factors such as diet and smoking, genetic factors contribute

Table 2.1 Estimates of the genetic contribution to the variation in serum thyroid hormone levels from different studies

	Proportion of variance in serum thyroid hormone levels attributable to genetic effects		
	Hansen et al. [4]	Samollow et al. [5]	Panicker et al. [6]
TSH	64%	37%	65%
FT4	65%	35%	39%
FT3	64%	64%	23%

significantly to this interindividual variability, resulting in a thyroid function set-point that is different for each individual. This is demonstrated by different studies in which heritability accounted for ~30–65% of the overall variation in serum TSH, FT4, and FT3 levels [4–6] (Table 2.1).

Polymorphisms are frequent variations in the nucleotide sequence of the genome that occur in at least 1% of a population, whereas mutations have a lower frequency. These variations seem to play an important role in the interindividual variation in serum thyroid function tests, and contribute to each individual's unique HPT axis setpoint. Since these variations are stable throughout life, they may not only affect serum levels, but also thyroid hormone bioactivity throughout life.

Table 2.2 Genetic defects associated with abnormal thyroid function tests

Phenotype	Gene	Inheritance	Chromosome
Central hypothyroidism	TRHR	AR	8p23
	TSH β	AR	1p13
Resistance to TSH, thyroid hypoplasia, and hypothyroidism	TSHR	AR	14q13
Hyperthyroidism and goiter	TSHR	AD	14q13
Thyroid dysgenesis and hypothyroidism	PAX8	AR	2q12–q14
	TTF1	AD	14q13.3
	TTF2	AD	22q33
Thyroid dyshormonogenesis, hypothyroidism, and goiter	NIS	AR	19p13.2–p12
	Tg	AR	8q24.2–q24.3
	DUOX2	AR	15q15
	TPO	AR	2p25
	DEHAL1	AR	6q24q25
Hypothyroidism, goiter, and deafness	Pendrin	AR	7q31
High total T4/T3, normal FT4/FT3	TBG	X-linked	Xq22
	TTR	AD	18q11
	ALB	AD	4q11
Low total T4/T3, normal FT4/FT3	TBG	X-linked	Xq22
Mental retardation, high serum T3	MCT8	X-linked	Xq13
High TSH/FT4/rT3, low T3	SBP2	AR	9q22

TRHR thyrotropin releasing hormone receptor, *TSH β* thyroid stimulating hormone β subunit, *TSHR* TSH receptor, *PAX8/TTF1/TTF2* thyroid transcription factors, *NIS* sodium iodide symporter, *Tg* thyroglobulin, *DUOX2* dual oxidase 2, *TPO* thyroid peroxidase, *DEHAL1* iodotyrosine dehalogenase 1, *TBG* thyroid binding globulin, *TTR* transthyretin, *ALB* albumin, *MCT8* monocarboxylate transporter 8, *SBP2* selenocysteine *cis* element binding protein 2, *AR* autosomal recessive, *AD* autosomal dominant

Many studies, in which the effects of certain polymorphisms on serum thyroid hormone levels are studied, have been published in the last few years. These studies usually involve comparisons of many polymorphisms with several clinical endpoints, resulting in a high risk of type I statistical errors. For this reason, replication of data in independent cohorts is essential before any conclusions can be drawn. In this chapter we review the different studies that have been published on this subject, and discuss the consequences of polymorphisms and mutations in different thyroid hormone pathway genes on serum thyroid function tests. An overview of the genetic defects associated with abnormal thyroid function tests can be found in Table 2.2.

2.3 Genetic Variation in Thyroid-Regulating Genes: TRH, TRHR, TSH, TSHR

Central hypothyroidism is a rare disorder with an estimated frequency of 0.005% in the general population [7]. It is characterized by insufficient TSH secretion resulting in low levels of thyroid hormones, caused by either pituitary or hypothalamic defects. Theoretically, it could result from mutations in the TRH, TRHR, and TSH β genes. Several clinical reports have suggested isolated TRH deficiency as a cause of central hypothyroidism [8, 9]. However, to date, no patients with mutations in the TRH gene have been described. Collu et al. described the first patient with a mutation in the TRHR causing central hypothyroidism. A 9-year-old boy was found to have compound heterozygous mutations in the 5'-part of the gene [10]. Recently, a family has been described with complete resistance to TRH due to a homozygous nonsense mutation in the TRH receptor [11].

Central hypothyroidism due to a mutation in the TSH β gene was first described in 1990 [12]. In these patients, TSH is undetectable or very low, and the administration of TRH does not result in a rise in serum TSH. Among the currently known mutations, most are located in the coding region of the gene [12, 13]. However, a mutation that led to TSH deficiency caused by exon skipping has also been described [14]. All affected patients were homozygous. No data regarding the influence of polymorphisms in TRH, TRHR, and TSH β genes on thyroid function tests are available.

Many mutations in TSHR have been described which can be divided into germline or acquired mutations. Acquired gain-of-function mutations result in a phenotype of toxic adenoma or toxic multinodular goiter [15]. Germline gain-of-function TSHR mutations were first identified in two French families in 1992 [16]. It causes autonomous thyroid growth and function, resulting in a phenotype of hyperthyroidism and goiter. On the other hand, germline loss-of-function TSHR mutations are associated with TSH resistance and congenital hypothyroidism [17]. In a subset of these patients, the mutations in the TSHR are partially inactivating [18]. In partial resistance TSH is elevated, but the peripheral hormone levels are normal: a condition known as euthyroid hyperthyrotropinemia [18]. In these patients, the size of the thyroid is normal or enlarged.

Polymorphisms in TSHR have been extensively studied in the context of the development of autoimmune thyroid disease. Although early studies investigating TSHR polymorphisms in Graves' disease proved inconclusive, more recent studies have provided convincing evidence for association of the TSHR region with Graves' disease. Strongest associations were obtained for two SNPs (single nucleotide polymorphisms) (rs179247 and rs12101255), both located in intron 1 of the TSHR. [19–21]. Data on the influence of TSH polymorphisms on thyroid function tests, on the other hand, are sparse: so far only one polymorphism has been shown to influence serum thyroid hormone levels. In several Caucasian populations, the TSHR-Asp727Glu polymorphism is associated with lower levels of plasma TSH, but not with FT4 [22–24]. This could point toward a higher sensitivity of the variant versus the wild-type TSHR, since less TSH is needed to produce normal FT4 levels. Although there is one *in vitro* study showing that the TSHR-Glu⁷²⁷ variant results in an increased cAMP response of the receptor to TSH [25], others have not been able to replicate this [26, 27]. A different explanation would be that the Asp727Glu polymorphism is linked to another polymorphism elsewhere in the gene. The TSHR-Asp727Glu polymorphism is found to be within a linkage disequilibrium block starting at intron 8 and extending about 10 kb beyond the 3'-UTR of the TSHR gene [28].

2.4 Genetic Variation in Thyroid Transcription Factors: PAX8, TTF1, TTF2

The paired-box gene PAX8 is important for the development of the thyroid gland and for the regulation thyroid-specific gene expression, including NIS, Tg, and TPO [29]. The PAX8 gene is located on human chromosome 2q12–q14. Homozygous inactivation of the *Pax8* gene in mice results in a complete lack of the development of thyroid follicles [30]. Most animals die within the first 3 weeks of life unless they are treated with thyroid hormone. The first patient with thyroid dysgenesis resulting from a heterozygous nonsense mutation in PAX8 was reported in 1998 by the group of Di Lauro [31]. Since then several other patients with congenital hypothyroidism have been identified with heterozygous mutations in the paired-box domain of the PAX8 protein.

No associations have been reported between polymorphisms in PAX8 and thyroid function tests. A nonsynonymous Phe329Leu SNP has been identified, but it lies outside the paired-box domain and probably has little effect on PAX8 function [32]. In addition to the thyroid, PAX8 is also expressed in the central nervous system and the kidneys.

TTF1 is a homeobox-containing protein belonging to the NKX2 family of transcription factors and is also referred to as NKX2.1. TTF1 is expressed predominantly in the thyroid, lung, and brain, in particular the basal ganglia. Homozygous *Ttf1* knockout mice are born without thyroid gland and also lack lung parenchyma [33]. In patients, different mutations in TTF1 result in varying dysfunction of the

organs where TTF1 is expressed, including congenital hypothyroidism, respiratory distress, and choreoathetosis [34–36]. In the deCODE population study in Iceland, polymorphisms in the region of the TTF1 gene on chromosome 14q13.3 have recently been associated with an increased risk of thyroid cancer as well as with lower serum TSH levels [37].

TTF2 is a forkhead gene which is now also termed FOXE1. Like PAX8 and TTF1, TTF2 is an essential transcription factor for the development of the thyroid gland, but it is not involved in the regulation of thyroid function. Mice with homozygous inactivation of the *Foxe1* gene exhibit an ectopic or absent thyroid gland and a cleft palate [38]. Heterozygous missense mutations have been identified in patients with thyroid dysgenesis, cleft palate, choanal atresia and spiky hair [39, 40]. After the first report, this is also referred to as the Bamforth–Lazarus syndrome.

In the deCODE study, polymorphisms in the FOXE1 locus on human chromosome 22q33 have been associated with an increased risk for thyroid cancer as well as with lower serum TSH and T4 levels and higher serum T3 levels [37]. Contradictory reports have appeared on the possible association of the poly-Ala stretch (14 or 16 residues) in TTF2 with thyroid dysgenesis [41, 42]. Furthermore, an SNP in the 5'-UTR of FOXE1 has been associated with cleft palate [43].

2.5 Genetic Variation in Thyroid Hormone Synthesis Genes: NIS, Pendrin, Tg, TPO, DUOX2, DEHAL

The cloning and characterization of NIS was reported in 1996 by the group of Carrasco [44, 45]. It mediates the electrogenic thyroidal uptake of I^- together with Na^+ in a stoichiometry of 1:2. NIS is also involved in iodide transport in other tissues such as the breast and intestine [46, 47]. The NIS gene is located on chromosome 19p13.2–p12 and consists of 15 exons. Different homozygous and compound heterozygous NIS mutations have been reported in patients with congenital hypothyroidism because of a thyroid hormone synthesis defect [48]. To our knowledge, no studies have been reported regarding the possible effects of polymorphisms in the NIS gene.

The precise role of pendrin in the transport of iodide in the thyroid follicle is still subject to debate [48, 49]. Pendrin earned its name because mutations in this gene have been identified in patients with Pendred syndrome, which is a recessive disorder characterized by sensorineural deafness and hypothyroidism resulting from a thyroid hormone synthesis defect (dysmorphogenesis) [48, 49]. However, pendrin mutations may also result in a selective hearing defect without thyroid dysfunction [50]. The hearing impairment is the result of a malformation of the cochlea, where pendrin plays an important role in the secretion of bicarbonate into the endolymph [51]. As far as we know, there have been no reports of an association of polymorphisms in the pendrin gene with thyroid function.

Human Tg is a large 660 kDa protein consisting of two identical subunits consisting of 2,748 amino acids. The gene covers ~300 kb on chromosome 8q24.2–q24.3

and contains 37 exons; the mature mRNA is ~8.7 kb in size [52, 53]. Tg provides the substrate for the synthesis of thyroid hormone and is the most abundant protein in the follicular lumen. It is not surprising, therefore, that Tg is a major antigen against which antibodies are produced in patients with autoimmune thyroid disorders.

Many mutations in Tg have been identified in patients with congenital hypothyroidism due to dysmorphogenesis. The interested reader is referred to the OMIM section of the NCBI website (<http://www.ncbi.nlm.nih.gov/omim>). Also, the *cog/cog* mouse has severe hypothyroidism because of a homozygous missense mutation in Tg [54]. Polymorphisms in the Tg gene have been associated with a risk for autoimmune thyroid disease [53, 55, 56], but to our knowledge no evidence has been reported for the association of Tg polymorphisms with thyroid function tests.

Human DUOX2 is a large and complex protein containing 1,548 amino acids, the sequence of which indicates the presence of 7 transmembrane domains, an NADPH-binding domain, an FAD-binding domain, a heme-binding domain, two calcium-binding EF hands, and a peroxidase domain. It catalyzes the oxidation of NADPH from the cytoplasm and delivers its product (H_2O_2) to the luminal surface of the apical membrane where it is utilized as a substrate for TPO [57]. Proper expression of DUOX2 requires the presence of the maturation factor DUOX2A, a protein consisting of 320 amino acids and five transmembrane domains [58]. They are encoded by genes located in a cluster on human chromosome 15q15 which also contains the homologous DUOX1 and DUOX1A genes [58].

A variety of mutations have been identified in DUOX2 [59, 60] and recently also in DUOX2A [60, 61] in patients with thyroid dysmorphogenesis. However, associations of polymorphisms in these genes with thyroid function tests have so far not been reported.

TPO is a glycoprotein consisting of 933 amino acids and containing a single transmembrane domain. A short C-terminal domain is located in the cytoplasm, but most of the protein is exposed on the luminal surface of the apical membrane which also contains a heme-binding domain, the active center of the enzyme [62]. TPO is encoded by a gene which covers about 150 kb on chromosome 2p25, distributed over 17 exons. In addition to full-length TPO-1, different splice variants have been characterized, including the TPO-2 variant which is generated by skipping of exon 10, resulting in the loss of 57 amino acids in the middle of the protein [62]. TPO-2 has no enzyme activity and its function is unknown.

Many TPO mutations have been identified in patients with thyroid dysmorphogenesis, see for instance [63, 64]. To our knowledge association have not been reported so far of polymorphisms in TPO with thyroid function tests.

DEHAL1 is a 289-amino acid protein containing an N-terminal membrane anchor and a conserved nitroreductase domain with an FMN-binding site [65–67]. Functional DEHAL1 probably exists as a homodimer. The DEHAL1 gene is located on chromosome 6q24–q25 and consists of 5 exons. DEHAL1, also termed IYD, catalyzes the reductive deiodination of MIT and DIT by NADH. Since DEHAL1 lacks an NADH-binding sequence, iodotyrosine deiodinase activity requires the involvement of a reductase, which has not yet been identified.

Recently, homozygous missense mutations in *DEHAL1* have been identified in patients with hereditary hypothyroidism [68, 69]. Remarkably, patients are not always identified at neonatal screening, and hypothyroidism develops later in life, probably depending on the iodine intake. Since this may occur in the first year(s) and is not immediately recognized, it may result in mental retardation. So far, associations of polymorphisms in *DEHAL1* with thyroid function tests have not been reported.

2.6 Genetic Variation in Thyroid Hormone Receptor Genes: $TR\alpha$, $TR\beta$

Thyroid hormone action is initiated by binding of T3 to its receptor (TR), which is located in the nucleus. TRs are associated with T3 response elements of target genes, and binding of T3 to the TR leads to stimulation or suppression of gene transcription. TRs are encoded by the *THRA* gene located on chromosome 17q11.2 and the *THRB* gene located on chromosome 3p24.3. *THRA* encodes five proteins, of which only $TR\alpha 1$ has intact DNA and T3 binding domains. *THRB* encodes three proteins that can bind DNA and T3. $TR\alpha$ is the predominant TR in brain, heart, and bone, whereas $TR\beta$ is the predominant TR in the liver, kidney, thyroid, and pituitary [70, 71].

Mutations in $TR\beta$ can lead to thyroid hormone resistance syndrome. Over 100 different heterozygous $TR\beta$ mutations have been identified, almost all located in the ligand-binding domain. The estimated frequency is 1/50,000 [72, 73]. As $TR\beta$ is the predominant TR in the negative feedback regulation of the HPT axis, increased serum thyroid hormones and nonsuppressed TSH are the hallmarks of the diagnosis. Amongst others, clinical features include goiter, short stature, decreased weight, tachycardia, cardiomyopathy, hearing loss, attention deficit hyperactivity disorder, decreased IQ, and dyslexia. These are due to a relative hypothyroid state in $TR\beta$ -expressing tissues and a relative hyperthyroid state in $TR\alpha$ -expressing tissues [74].

A limited number of studies have been published about the association of genetic variation in $TR\beta$ and serum thyroid parameters [23, 75]. Sørensen et al. found the *THRB*-intron9-G>A polymorphism to be associated with higher serum TSH in a Danish twin population. Although replication in a Caucasian population showed a similar trend, it did not reach statistical significance [75].

No patients with mutations in $TR\alpha$ have been identified yet. Various mouse models with knock-in mutations in $TR\alpha$ have been generated [76–79]. In all models, TSH was (moderately) elevated. Depending on the mutation, T3 and T4 levels ranged from slightly decreased to slightly increased. In general, these mice showed a higher mortality, delayed growth, reduced fertility, delayed bone development and signs of impaired cardiac function and neuropsychiatric abnormalities (e.g., ataxia and anxiety-related behavior).

Sørensen et al. studied the possible relationship of two polymorphisms in *THRA* (i.e., *THRA*-A2390G, rs12939700) with serum thyroid parameters in a large

population of Danish twins and found no significant associations [75]. Furthermore, no studies have been published on the association of genetic variation in TR α and serum thyroid parameters.

2.7 Genetic Variation in Serum TH Transport Proteins: TBG, TTR, and Albumin

Thyroid hormones circulate in serum bound to the carrier proteins TBG, TTR (formerly known as thyroxine-binding prealbumin or TBPA) and albumin. These proteins provide a large and stable pool of circulating thyroid hormone, distributing the water-insoluble hormone to all tissues. In humans TBG, TTR, and albumin carry about 75%, 15%, and 10%, respectively, of T4 and T3 [80–82].

The TBG gene is located on the long arm of the X chromosome (Xq22.2) and is composed of five exons [83]. Mutations in the TBG gene can lead to three different phenotypes according to serum TBG concentrations in affected hemizygous males: complete TBG deficiency, partial TBG deficiency, and TBG excess [84]. TBG deficiency is associated with very low levels of total T4 and T3. Since unbound hormone levels are normal, patients are euthyroid and TSH levels are normal. TBG excess gives rise to increased levels of total T4 and T3, again with normal levels of FT4 and FT3. To date, approximately 30 variants have been associated with TBG defects [83]. These are either nonsense or missense mutations, randomly distributed throughout the TBG gene. Gene duplications or triplications have been shown to be the cause of TBG excess [85].

The TTR gene is located on chromosome 18q11.2–q12.1. Circulating TTR is a tetramer of identical 127-amino acid subunits. More than 80 different mutations in this gene have been reported. Most mutations are related to amyloid deposition, affecting predominantly peripheral nerves and/or the heart. These mutations can lead to diseases such as amyloidotic polyneuropathy, amyloidotic vitreous opacities, cardiomyopathy, oculoleptomeningeal amyloidosis, meningocerebrovascular amyloidosis, and carpal tunnel syndrome [86]. Interestingly, only a small portion of the gene mutations is nonamyloidogenic. Some mutations increase the affinity of T4, leading to a state called euthyroid hyperthyroxinemia: increased total T4 and T3 levels, with normal levels of FT4, FT3, and TSH. This has been shown for the Ala109Thr, Ala109Val and Thr119Met mutations [87, 88]. A decreased concentration or affinity of TTR is not associated with variations in serum concentrations of thyroid hormones [89].

Familial dysalbuminemic hyperthyroxinemia (FDH) was first identified in 1979 by Hennemann et al. [90]. Like other conditions associated with euthyroid hyperthyroxinemia, it is characterized by increased levels of total T4, but normal levels of FT4 and TSH. It is caused by mutations in the albumin gene. The most common mutation in this respect is Arg218His: it produces an albumin molecule with 10- to 15-fold higher affinity for T4 than wild-type albumin, and a fivefold increase in affinity for T3 [91]. Two other mutations have been identified, i.e., Arg218Pro and Leu66Pro [91]. The latter induces a selective increase in affinity for T3.

Although polymorphisms have been identified in the TBG, TTR, and albumin genes, none has been linked to alterations in serum thyroid hormone levels.

2.8 Genetic Variation in TH Transporters: MCT8, MCT10, OATPs

Both TRs and deiodinases are located intracellularly. Therefore, transport of thyroid hormone across the cell membrane is required for hormone action and metabolism. Based on the lipophilic structure of thyroid hormones, it was assumed that they enter cells through passive diffusion. However, it has become increasingly clear that there are specific thyroid hormone transporters, and that the activity of these transporters in part determines the intracellular thyroid hormone concentration [92].

2.8.1 MCT8 and MCT10

Monocarboxylate transporter 8 (MCT8) has been characterized as an active and specific thyroid hormone transporter [93]. The MCT8 gene is located on the X chromosome (Xq13.2) and contains six exons. Mutations in the MCT8 gene cause a syndrome of severe psychomotor retardation and high serum T3 levels in affected male patients, known as the Allan–Herndon–Dudley syndrome [94, 95]. The neurological deficits are probably explained by an impeded uptake of T3 in MCT8-expressing central neurons and, hence, an impaired brain development. This has been reviewed in detail elsewhere [94, 95].

Since mutations in the MCT8 gene have such profound effects, the question arises whether small changes in the MCT8 gene may affect transport activity as well. Only two studies exist on the relationship between MCT8 polymorphic variants and serum thyroid hormone levels [96–98] (Table 2.3). Dominguez-Gerpe and colleagues studied the Ser107Pro polymorphism (rs6647476), which is the only established nonsynonymous polymorphism in MCT8 [98]. In their study, 276 healthy Spanish men were genotyped for this polymorphism. They found no association with serum thyroid hormone levels or with mRNA levels coding for MCT8 or thyroid hormone-responsive genes in white blood cells or in T3-stimulated

Table 2.3 Effect of common variation in MCT8 and MCT10 on thyroid function tests

Gene	Polymorphism	Location	Change	Effect on serum thyroid hormone levels	<i>In vitro</i> effect
MCT8	–	Exon	Ser33Pro	No effect observed	Not determined
	rs6647476	Exon	Ser107Pro	No effect observed	No effect observed
	rs5937843	Intron	G > T	Not consistent	Not determined
MCT10	rs14399	3'-UTR	C > A	No effect observed	Not determined

fibroblasts. We also genotyped this polymorphism in a population of 156 healthy men and women and found no association between this variant and serum thyroid parameters. Hemizygous carriers of a different polymorphism, i.e., rs5937843, located in intron 5 of the MCT8 gene, had lower FT4 levels compared to wild-type male subjects. However, we failed to replicate these findings in the homozygous female carriers in the same population.

MCT10 has been characterized by Kim et al. in 2002 [99] as a T-type amino acid transporter, facilitating the cellular uptake and efflux of aromatic amino acids. Although this was not immediately clear, we have later shown that MCT10 is an active iodothyronine transporter [100]. The MCT10 gene is located on chromosome 6q21–q22 and has the same gene structure as MCT8. The MCT10 protein also has ~50% amino acid identity with the MCT8 protein.

To date, only one study has been published regarding the possible association of genetic variation in the MCT10 gene with serum thyroid parameters. We showed that a common polymorphism (rs14399) in the 3'-UTR region of the MCT10 gene is not associated with serum thyroid hormone levels [97]. The only established nonsynonymous polymorphism identified in human MCT10 is Lys508Gln (rs17072442), with a minor allele frequency of ~2%. Considering the type of amino acid change, it would be interesting to investigate the association of this polymorphism with serum thyroid parameters and other thyroid-related endpoints.

So far, patients with mutations in MCT10 have not been identified. Considering the wide tissue distribution of MCT10 expression and its swift T3 transport, it is quite likely that MCT10 mutations are associated with significant alterations in tissue and/or serum thyroid hormone concentration. As is the case with MCT8, mutations in MCT10 may well result in a significant impairment of tissue T3 uptake and, thus, in manifestations of thyroid hormone resistance. To predict the phenotype of patients with MCT10 mutations, it would be highly interesting to study MCT10 knockout mice, although it should be realized that MCT8 knockout mice do not show any neurological abnormality in sharp contrast with the clinical condition of patients with MCT8 mutations [101–103].

2.8.2 *OATP1A2, 1B1, 1B3, and 1C1*

The organic anion transporting polypeptides (OATPs) are a large family of transporters responsible for Na⁺-independent transmembrane transport of amphipathic organic compounds, including bile salts, bromosulfophthalein, steroid hormones and numerous drugs [104]. Among the many ligands transported by OATPs, several members of this large family also facilitate uptake of thyroid hormone. These include members of the OATP1 subfamily: 1A2 [105, 106], 1B1 [105, 107], 1B3 [105], and 1C1 [108]; a member of the OATP2 subfamily: 2B1 [105]; and members of the OATP4 subfamily: 4A1 [106] and 4C1 [109]. The focus in this review will be on OATP1A2, 1B1, 1B3, and 1C1, since data regarding the effect of genetic

variants on serum thyroid hormone levels is available for only these four genes. These transporters show high sequence homology and are encoded by a gene cluster located on chromosome 12p12.

OATP1A2 has been shown to transport T3 and T4 with K_m values of 7 μ M and 8 μ M, respectively [106]. In addition, it was demonstrated that OATP1A2 facilitates not only transport of T4, T3, and rT3, but also of their sulfates T4S, T3S, and rT3S in transfected cells [110]. We analyzed the OATP1A2-Ile13Thr and -Glu172Asp polymorphisms for association with serum thyroid hormone levels. For the Ile13Thr polymorphism, no consistent associations with serum thyroid hormone levels were found. In addition, no differences in thyroid hormone transport were observed between this variant and wild-type OATP1A2 *in vitro*. However, cells transfected with the Glu172Asp variant showed decreased transport compared to cells transfected with wild-type OATP1A2. This variant was, however, not associated with serum thyroid parameters in two populations of Caucasians. It could, therefore, be concluded that this polymorphism might affect tissue thyroid hormone concentrations independent of serum levels. Alternatively, OATP1A2 might not play an important role in thyroid hormone transport in a physiological situation.

OATP1B1 and OATP1B3 are exclusively expressed in the liver and share approximately 80% amino acid identity with each other [107, 111]. Recent studies have shown that OATP1B1 markedly stimulates uptake of the iodothyronine sulfates T4S, T3S, and rT3S but has little activity toward nonsulfated T4, T3, and rT3 [112]. Like OATP1B1, OATP1B3 preferentially transports the sulfated iodothyronines as well as rT3 [110]. Polymorphisms in the OATP1B1 and OATP1B3 genes have been extensively studied as they impact on the interindividual variability of drug disposition and drug response [113]. To date, only one study has focused on associations between a polymorphism in the OATP1B1 gene, Val174Ala, and serum thyroid hormone levels. This polymorphism has been studied extensively: Niemi and colleagues have shown that the Val174Ala polymorphism leads to decreased function of OATP1B1 and thereby increases the systemic bioavailability of lipid-lowering drugs [114].

As we saw that OATP1B1 preferentially transports sulfated hormones, i.e., T4S, T3S, rT3S, and E1S [112], we expected that the Val174Ala polymorphism would be associated with serum levels of iodothyronine sulfates and E1S. Indeed, this polymorphism was associated with higher serum T4S levels in 155 blood donors, while in a larger cohort of elderly Caucasians this same polymorphism was associated with 40% higher serum E1S levels. *In vitro*, OATP1B1-Ala¹⁷⁴ showed a 40% lower induction of transport and metabolism of these substrates than OATP1B1-Val¹⁷⁴ [112]. Decreased hepatic uptake of T4S and E1S by OATP1B1-Ala¹⁷⁴ compared with OATP-Val¹⁷⁴ *in vivo* thus gives rise to higher T4S and E1S levels.

To date, no associations have been found between genetic variation in the OATP1B3 gene and serum thyroid hormone levels [110]: the OATP1B3-Ser112Ala and Met233Ile polymorphisms showed no association with serum thyroid parameters in a population of Caucasian blood donors. OATP1C1 shows a high preference for

T4 and rT3 [108]. In addition, T4S uptake is also facilitated by OATP1C1, although less effectively than T4 [115]. Together with the almost exclusive expression at the blood–brain barrier, this suggests that OATP1C1 is critical for T4 uptake into the brain. This important role is substantiated by Sugiyama and colleagues who showed that expression levels of *Oatp1c1* in isolated rat brain capillaries are regulated by thyroid hormone concentrations [116]. *Oatp1c1* is up-regulated in hypothyroid rats and down-regulated in hyperthyroid rats [116]. Considering the presumed function of T4 transport across the blood–brain barrier, mutations in OATP1C1 are expected to have a significant impact on brain development and function. Loss of OATP1C1 function may well lead to neuronal deficits similar to that seen in subjects with untreated congenital hypothyroidism or in patients with MCT8 mutations [116]. It seems worthwhile to study this in *Oatp1c1* knockout mice.

OATP1C1 is capable of T4, T4S, and rT3 transport, but polymorphisms in the OATP1C1 gene are not consistently associated with serum thyroid hormone levels [115]. Although, the OATP1C1-Pro143Thr and C3035T polymorphisms were associated with serum thyroid parameters in 156 blood donors, we could not replicate these findings in a much larger cohort of Danish twins. Nor did we observe any differences in uptake and metabolism of T4 and rT3 between these variants and wild-type OATP1C1. In addition, no associations were found between the OATP1C1-intron3C>T polymorphism and serum thyroid hormone levels [115]. However, both intron3C>T and C3035T polymorphisms, but not Pro143Thr, were associated with symptoms of fatigue and depression in a population of adequately treated hypothyroid patients [117]. This is of interest as recently a number of papers have reported on effects of polymorphisms in thyroid hormone pathway genes, independent of an effect on serum thyroid hormone levels [118, 119]. Many associations between polymorphisms in thyroid hormone pathway genes and different clinical endpoints are independent of serum thyroid hormone levels, highlighting the importance of local regulation of thyroid hormones in tissues [120].

Table 2.4 presents a summary of the studies discussed above regarding the possible effects of polymorphisms in the different OATP1 transporters on serum thyroid hormone levels *in vivo* and on the rate of iodothyronine transport *in vitro*.

Table 2.4 Effect of common variation in OATP1A2, OATP1B1, OATP1B3, and OATP1C1 on thyroid function tests

Gene	Polymorphism	Location	Change	Serum thyroid hormone levels	<i>In vitro</i> transport
OATP1A2	rs57921276	Exon	Ile13Thr	No effect	No effect
	rs57550534	Exon	Glu172Asp	No effect	↓
OATP1B1	rs4149056		Val174Ala	T4S↑, E1S↓ T3/rT3↓	↓
OATP1B3	rs4149117	Exon	Ser112Ala	No effect	ND
	rs7311358	Exon	Met233Ile	No effect	ND
OATP1C1	rs10770704	Intron	C/T	No effect	ND
	rs36010656	Exon	Pro143Thr	rT3↑ (not consistent)	No effect
	rs10444412	3'-UTR	C3035T	FT4↑, rT3↑ (not consistent)	No effect

2.9 Genetic Variation in Deiodinases: D1, D2, D3

All the three deiodinases have a different physiological role [121]. D1 is present in liver, kidney, and thyroid, and plays a key-role in the production of the active hormone T3 from T4 and in the clearance of the metabolite rT3. D2 is present in brain, pituitary, brown adipose tissue, thyroid, skeletal muscle, aortic smooth muscle cells, and osteoblasts; D2 mRNA has also been detected in the human heart. In tissues such as the brain, D2 is important for local production of T3, whereas D2 in skeletal muscle may also contribute to plasma T3 production. D3 is present in brain, skin, placenta, pregnant uterus, and various fetal tissues, and is induced in critical illness. D3 is the major T3 and T4 inactivating enzyme and contributes to thyroid hormone homeostasis by protecting tissues from excess thyroid hormone.

The DIO1 gene has four exons and is located on human chromosome 1p33–p32, the DIO2 gene has two exons and is located on chromosome 14q24.3, and the DIO3 gene consists of a single exon and is located on chromosome 14q32. No patients with inactivating mutations in any of the iodothyronine deiodinases have yet been described. Whether this means that these mutations are not compatible with life, that they have little or no consequences, or that they result in unexpected phenotypes is still unclear. Based on the phenotypes of mice with targeted deletions of *Dio1*, *Dio2*, or *Dio3*, the most severe effects would be expected of mutations in DIO3 [122–125]. All three deiodinases are selenoproteins, and contain a selenocysteine residue in the catalytic center, which is crucial for enzymatic activity. Interestingly, mutations that result in an incomplete loss of function of SECISBP2, which is essential for the incorporation of selenocysteine, lead to a thyroid phenotype [126]. TSH, FT4, and rT3 are high in these patients, whereas T3 levels are low. Similar thyroid function tests are observed in *Dio1xDio2* knockout mice, although these mice are still able to maintain normal levels of serum T3 [127].

In the last few years, several studies on polymorphisms in deiodinases and their association with thyroid function tests have been published (see [128] and [120] for reviews). Polymorphisms in DIO1 (rs11206244, rs12095080, rs2235544) have consistently been associated with altered thyroid hormone levels (especially T3 and rT3 levels) in different populations, without an effect on serum TSH [23, 120, 128–130]. The associations are similar in hypothyroid patients who receive levothyroxine treatment and those who are euthyroid without medication. Interestingly, a randomized placebo controlled study investigating the effect of T3 addition in the treatment of depression, showed an enhanced response to T3 in depressed patients which was associated with the rs11206244 and rs2235544 polymorphisms [131]. In other words, depressed patients who have a genetically determined lower T4 to T3 conversion may be more likely to benefit from T3 supplementation. However, these findings need replication in an independent study cohort.

On the other hand, polymorphisms in DIO2 and DIO3 show no associations with serum thyroid hormone levels [23, 120, 128, 129], except for one study of DIO2 in younger subjects (rs12885300), the results of which have not been replicated [132]. A different polymorphism in DIO2 (rs225014) has been associated with different

clinical endpoints independent of serum thyroid hormone levels, such as osteoarthritis, mental retardation in iodine deficient areas, and insulin resistance [120, 128]. This suggests that an altered D2 activity may lead to certain clinical phenotypes, without affecting serum thyroid hormone levels.

2.10 Genome-Wide Association (GWA) Studies

Besides the classical candidate gene approach, an increasing number of studies use a hypothesis-free approach by performing a genome-wide association (GWA) analysis. In such a GWA study the genome of each individual in the population is typed for more than 500,000 polymorphisms to search for variants that are associated with the phenotype of interest. GWA analysis will be very useful to identify novel loci involved in the regulation of thyroid hormone levels. Although such a study is complicated, as it requires large sample sizes, replication, and reliable geno- and phenotyping, it will unravel previously unknown pathways involved in thyroid hormone metabolism. Panicker et al. identified several loci associated with serum FT4 and TSH by a genome-wide linkage scan with 737 microsatellite markers [133], but as expected from an underpowered linkage scan in related subjects, they did not identify the actual genes explaining the variation in serum thyroid hormone levels. Arnaud-Lopez and colleagues recently demonstrated that polymorphisms in the Phosphodiesterase 8B gene are associated with serum TSH levels and thyroid function [134]. Probably, GWA studies will provide more candidate genes involved in thyroid function.

2.11 Concluding Remarks

Genetic variation has an important contribution to the overall variation in thyroid function tests, with estimates varying from 30 to 65%. Although many studies have been published in which the effects of polymorphisms on serum thyroid hormone levels have been demonstrated, only a minor fraction of the overall genetic variation is yet explained. This is clearly illustrated by two studies, in which the contribution of two polymorphisms (rs11206244 in DIO1 and rs1991517 in TSHR) to the overall genetic variation was calculated [24, 135]. Although both polymorphisms show a very significant association with serum thyroid function tests in different independent populations, the proportion of genetic influence explained by these particular polymorphisms is very small (~1%). Genome wide association strategies (and in the near future probably whole genome sequencing), made possible due to the rapid technical progress and the advancement of new techniques, will undoubtedly unravel previously unknown pathways involved in thyroid hormone metabolism, and provide new insights about their physiological function. Whether these techniques will enable us to better estimate an individual's HPT setpoint, remains to be elucidated.

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