Genetic Determination of the Hypothalamic-Pituitary-Thyroid Axis: Where Do We Stand?

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For a long time it has been known that both hypo- and hyperthyroidism are associated with an increased risk of morbidity and mortality. In recent years, it has also become clear that minor variations in thyroid function, including subclinical dysfunction and variation in thyroid function within the reference range, can have important effects on clinical endpoints, such as bone mineral density, depression, metabolic syndrome, and cardiovascular mortality. Serum thyroid parameters show substantial interindividual variability, whereas the intraindividual variability lies within a narrow range. This suggests that every individual has a unique hypothalamus-pituitary-thyroid axis setpoint that is mainly determined by genetic factors, and this heritability has been estimated to be 40–60%. Various mutations in thyroid hormone pathway genes have been identified in persons with thyroid dysfunction or altered thyroid function tests. Because these causes are rare, many candidate gene and linkage studies have been performed over the years to identify more common variants (polymorphisms) associated with thyroid (dys)function, but only a limited number of consistent associations have been found. However, in the past 5 years, advances in genetic research have led to the identification of a large number of new candidate genes. In this review, we provide an overview of the current knowledge about the polygenic basis of thyroid (dys)function. This includes new candidate genes identified by genome-wide approaches, what insights these genes provide into the genetic basis of thyroid (dys)function, and which new techniques will help to further decipher the genetic basis of thyroid (dys)function in the near future.
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I. Introduction

Adequate thyroid hormone (TH) levels are essential for normal growth and differentiation, for the regulation of energy metabolism, and for the physiological function of virtually all human tissues. This is illustrated by the well-known effects of hypo- and hyperthyroidism. In addition, more recent studies show that also minor variation in serum TH levels, even within the reference range, can have important effects on clinical endpoints, such as bone mineral density (BMD) (1), depression (2), dementia (3), atrial fibrillation (3, 4), metabolic syndrome (5), and cardiovascular mortality (3, 6, 7).

In healthy persons, serum thyroid parameters show substantial interindividual variability, whereas the intraindividual variability lies within a narrow range (Figure 1A) (8). This suggests that every person has a unique hypothalamus-pituitary-thyroid (HPT) axis setpoint that is

Abbreviations: AITD, autoimmune thyroid disease; BMD, bone mineral density; BMI, body mass index; CCR6, chemokine CC motif receptor 6; CH, congenital hypothyroidism; CNV, copy number variation; D1, type 1 deiodinase; D2, type 2 deiodinase; D3, type 3 deiodinase; DIT, diiodotyrosine; DM1, type 1 diabetes mellitus; DM2, type 2 diabetes mellitus; DUOX, dual oxidase; FGF, fibroblast growth factor; FGFR1OP, FGF receptor 1 oncogene partner; FT3, free T_3; FT4, free T_4; GD, Graves' disease; GWAS, genome-wide association studie; HDL, high-density lipoprotein; HPT, hypothalamus-pituitary-thyroid; LAT, L-type amino acid transporter; LD, linkage disequilibrium; LDL, low-density lipoprotein; LT3, liothyronine; LT4, levothyroxine; MCT8, monocarboxylate transporter 8; MIT, monoiodotyrosine; MRI, magnetic resonance imaging; NF1, nuclear factor 1; NIS, sodium/iodide symporter; NTCP, Na^+/taurocholate cotransporting polypeptide; OATP, organic anion transporter; RTH, resistance to TH; SLAM, signaling lymphocytic activation molecule; sORF, short open reading frame; Tg, thyroglobulin; TH, thyroid hormone; TPO, thyroid peroxidase; TPOAb, TPO antibody; TR, TH receptor; T4S, sulfated T_4; TSHR, TSH receptor; UTR, untranslated region; VEGFA, vascular endothelial growth factor A.

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Figure 1.

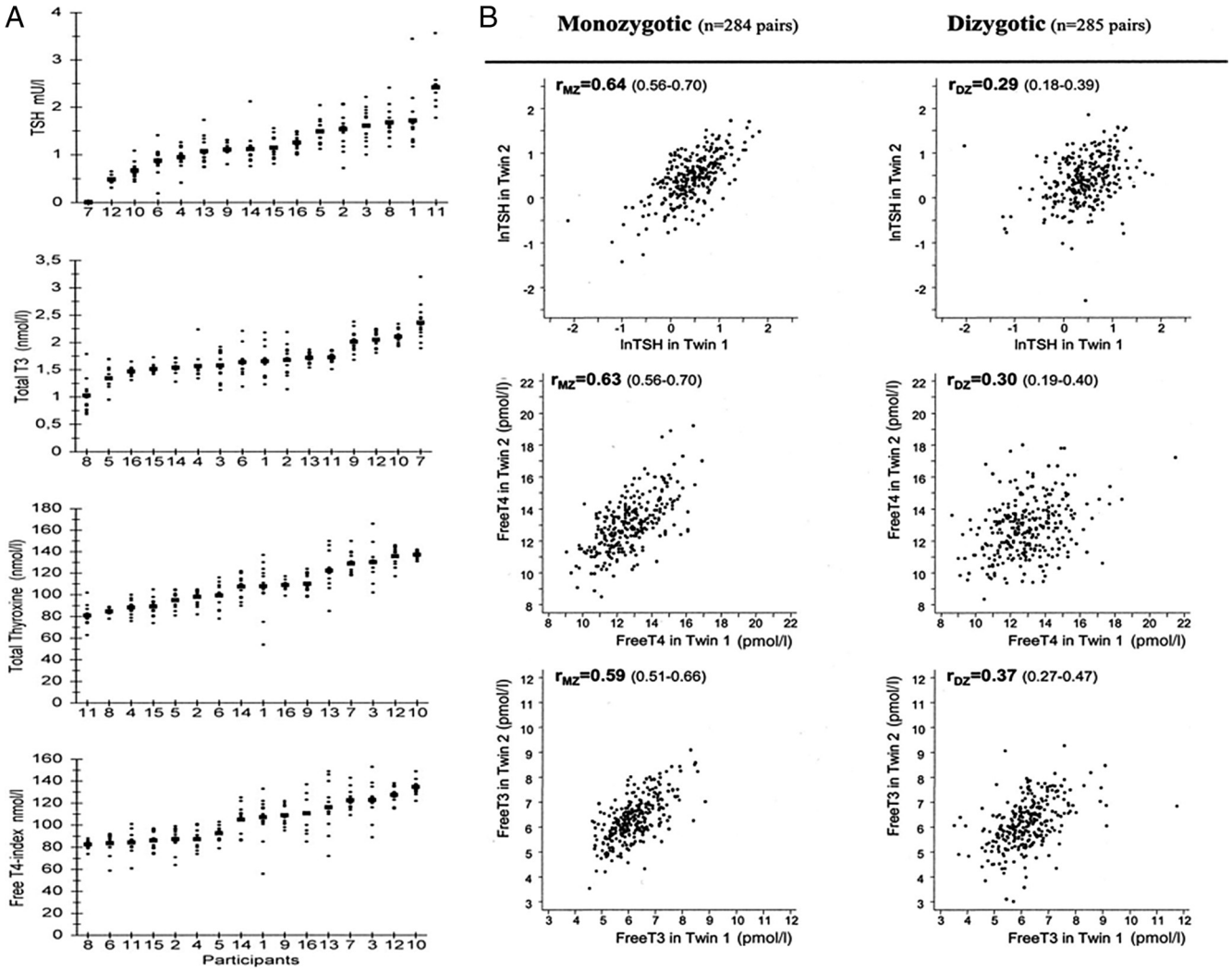

Figure 1. A, Serum TSH, T_3, T_4, and FT4 levels in 16 healthy subjects taken monthly for 12 months. Each dot represents a monthly measurement, and horizontal bars indicate individual parametric means. Laboratory reference ranges are TSH, 0.3–5.0 mU/L; T_3, 1.2–2.7 nmol/L; T_4, 60–140 nmol/L; and FT4 index, 70–140 nmol/L. This study showed substantial interindividual variability in serum thyroid parameters, whereas the intraindividual variability lies within a narrow range. [Adapted from S. Andersen et al: Narrow individual variations in serum T(4) and T(3) in normal subjects: a clue to the understanding of subclinical thyroid disease. *J Clin Endocrinol Metab*. 2002;87:1068–1072 (8), with permission. © The Endocrine Society.] B, Scatterplots and correlations between twins of serum TSH, FT4, and FT3 levels according to zygosity. Correlations were higher in monozygotic twins compared to dizygotic twins, supporting an important role for genetic factors in the HPT axis. Heritability estimates were 64, 65, and 64% for TSH, FT4, and FT3, respectively. [Adapted from P. S. Hansen et al: Major genetic influence on the regulation of the pituitary-thyroid axis: a study of healthy Danish twins. *J Clin Endocrinol Metab*. 2004;89:1181–1187 (11), with permission. © The Endocrine Society.]

mainly determined by genetic factors, in addition to environmental factors such as iodine intake and smoking (9, 10). Indeed, this concept is supported by two classical twin studies from Denmark and the United Kingdom that found an estimated heritability of serum TSH and free T_4 (FT4) levels of 39–65% (Figure 1B) (11, 12). In another study investigating serum thyroid parameters in a Mexican American population, heritability estimates ranged from 26–64% (13). Various studies have shown that persons on T_4 replacement therapy have a decreased well-being, despite having serum TH parameters within the reference range, suggesting that the achieved serum TH parameters may not match the patient's physiological set-point (14, 15).

The genetic architecture of the HPT axis is similar to other complex traits, with contributions from several genes, including rare high-penetrance variants with large effects (mutations) and common low-penetrance variants with small effects (polymorphisms), as illustrated in Figure 2. Various mutations in TH pathway genes have been

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Figure 2.

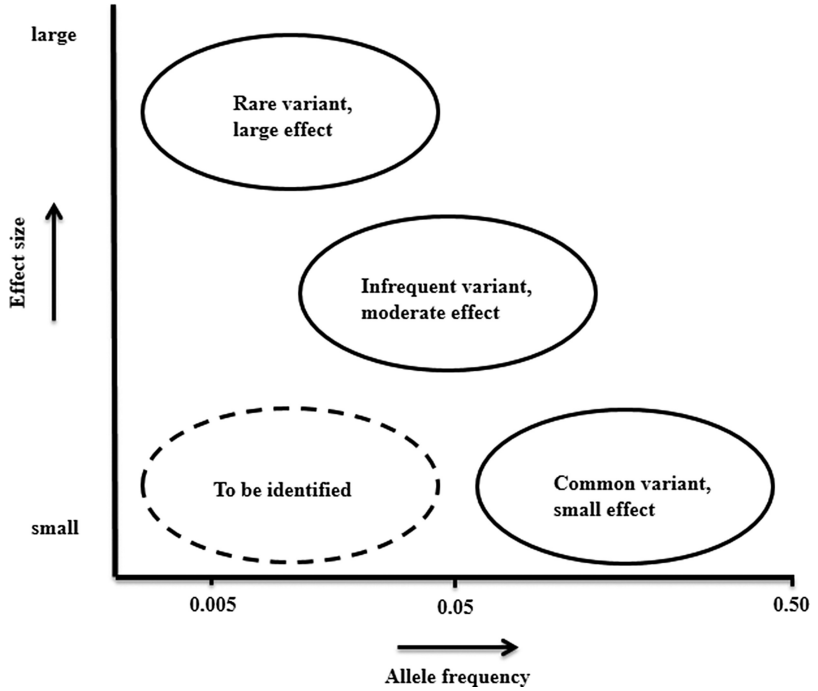

Figure 2. The genetic architecture of the HPT axis, with contributions from various genetic variants with different frequencies and effects. Most of the common variants have small effects, whereas some rare variants have large effects. Common variants with large effects have not been found and probably do not exist. Rare variants with small to moderate effects are likely to be identified by future sequencing efforts. [Adapted from S. H. Ralston and A. G. Uitterlinden: Genetics of osteoporosis. *Endocr Rev*. 2010;31:629–662 (63), with permission. © The Endocrine Society.]

identified as a cause of thyroid dysfunction and altered thyroid function tests (Table 1), as discussed in detail in various reviews (16–35). For example, Ercan-Fang et al (36) showed that patients with resistance to TH (RTH) due to a mutation in the TH receptor (TR) β, the predominant TR in the HPT axis, have an altered setpoint compared to wild-type subjects (Figure 3). Because these monogenic causes are rare, many candidate gene and linkage studies have been performed over the years to identify polymorphisms associated with thyroid (dys)function, but only a limited number of consistent associations have been found. However, in the past 5 years, advances in genetic research have led to the identification of a large number of new candidate genes. Therefore, this review will provide a systematic overview of the polygenic basis of thyroid (dys)function, including the new candidate genes identified by genome-wide association studies (GWAS), and what insights these genes provide into the genetic basis of thyroid (dys)function. Finally, we will discuss new techniques that will help to further unravel the genetic basis of thyroid (dys)function in the near future, which will likely lead to a better understanding of disease identification and treatment. Because various reviews on the genetic basis of autoimmune thyroid disease (AITD) have been published over the years (37–41), we will limit the discussion of AITD candidate gene and linkage studies to one paragraph and will discuss the newly identified loci by GWAS more extensively.

We searched MEDLINE articles, personal files, references of relevant articles and textbooks published from 1961 to 2014. Only English-language articles were studied, and the following search terms were used: thyroid, thyroid function, HPT axis, hypothyroidism, hyperthyroidism, subclinical, thyroid autoimmunity, genetic, mutation, polymorphism, candidate gene, genome wide association, and linkage. A critical assessment of the literature was performed by M.M. and R.P.P.

II. Key Players in the HPT-axis and Local Thyroid Hormone Regulation

Mutations or polymorphisms have been identified in almost every step of TH synthesis, serum protein binding, metabolism, transport and action, leading to subtle or more pronounced changes in thyroid function tests. We will therefore first briefly discuss these key players in TH synthesis, transport, uptake and action, which are also shown in a simplified overview of the HPT axis in Figure 4.

Hypothalamic TRH stimulates TSH secretion from the anterior pituitary. TSH binds to the TSH receptor (TSHR), a G protein-coupled receptor essential for the proliferation, differentiation, and function of the thyroid gland. The synthesis of TRH and the two genes encoding the α- and β-subunits of TSH is inhibited at the transcriptional level by TH, which also inhibits post-translational modification and release of TSH (42).

The synthesis of TH involves multiple steps. Ingested iodine, which is present in the circulation as iodide, is actively transported across the basolateral membrane of the thyroid follicular cells by the sodium/iodide symporter (NIS). The energy released by the inward translocation of Na^+ down to its electrochemical gradient, as generated by

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Table 1. Monogenic Causes of Thyroid Dysfunction and Altered Thyroid Function Tests

	Subtype	Gene	Affected Process	Inheritance	Serum Thyroid Parameters	Clinical and Other Biochemical Characteristics
Hypothyroidism	Central hypothyroidism	*TRHR*	TRH signaling	AR	Normal TSH, low T_4	Growth retardation and low IQ.
		β-TSH	TSH signaling	AR	Variable TSH, low T_4 and T_3	Mild to severe mental retardation.
		POU1F1	Thyrotrophic pituitary cell development	AR or AD	Low TSH and T_4, normal T_3	Mild to severe mental retardation, growth retardation, low GH and PRL.
		Prop-1	Thyrotrophic pituitary cell development	AR	Low TSH and T_4	No or delayed puberty, growth retardation, low GH, PRL, LH, and FSH.
		LHX3	Thyrotrophic pituitary cell development	AR	Low TSH and T_4	Cervical spine rigidity, growth retardation, low GH, PRL, LH, and FSH.
		IGSF1	Unknown	X-linked	Low TSH and T_4 in men (subset of women)	Growth retardation, delayed puberty, macroorchidism, increased BMI and low PRL (men).
	TSH resistance	*TSHR* (inactivating mutations)	TSHR	AR	Overt hypothyroidism	Growth retardation and developmental delay.
	Thyroid dysgenesis	*PAX8*	Thyroid development	AD	High TSH, low T_4	Varying from thyroid ectopy/hypoplasia with severe CH to eutopic thyroid with mild CH. Kidney dysgenesis.
		FOXE1 (TTF1)	Thyroid development	AR	High TSH, low T_4	Developmental delay, cleft palate, choanal atresia, bifid epiglottis and spiky hair (Bamforth-Lazarus syndrome).
		NKX2.1 (TTF2)	Thyroid development	AD	From high TSH to CH	Global developmental delay, hypotonia, ataxia, microcephaly, choreoathetosis, neonatal respiratory distress syndrome and pulmonary infections (brain-thyroid-lung syndrome).
	Thyroid dyshormonogenesis	*NIS*	Iodide trapping	AR	From euthyroid to hypothyroid (dependent on iodine intake). Low or absent RAI uptake. Low iodine saliva/serum ratio	Goiter and phenotypic spectrum from euthyroid to hypothyroid.
		SLC26A4	Intrathyroidal iodide transport. Mild organification defect.	AR	From euthyroid to hypothyroid (dependent on iodine intake)	Deafness and goiter. Phenotypic spectrum from euthyroid to hypothyroid (Pendred syndrome).
		Tg	Tg synthesis	AR	High TSH, low Tg and T_4, variable T_3	Goiter and varying from no to severe psychomotor delay.
		TPO	Impaired organification	AR	Transient/permanent mild to severe CH, high RAI uptake	Goiter and phenotypic spectrum from mild to severe hypothyroidism.
		DUOX2	Impaired organification	AR	Permanent mild to severe CH, high RAI uptake	Goiter and phenotypic spectrum from mild to severe hypothyroidism.
		DUOXA2	Impaired organification	AR	Permanent mild to severe CH, high RAI uptake	Goiter and phenotypic spectrum from mild to severe hypothyroidism.
		DEHAL	MIT and DIT deiodination	AR	CH (varying time of onset in childhood)	Goiter, growth and psychomotor retardation. Higher urinary MIT and DIT concentrations.
		GLIS3	Unknown	AR	CH (varying anatomy on ultrasound: ranging from athyreosis to normal)	Varying from neonatal diabetes and facial anomalies to hepatic fibrosis, polycystic kidney disease, glaucoma, osteopenia, bilateral sensorineural deafness, and pancreatic insufficiency.
Hyperthyroidism		*TSHR* (activating mutations)	TSHR	AD and sporadic	Overt hyperthyroidism (varying age of onset)	Varying from no complications to craniosynostosis, advanced bone age, neurodevelopmental delay, jaundice, and cerebral ventriculomegaly.
Disturbed TSH action		*TSHR* (inactivating and activating mutations)	TSHR	AR or AD	Inactivating mutation: high TSH, normal T_4. Activating mutation: low TSH, normal T_4	None
Disturbed TH protein binding		*TBG* (deficiency and excess)	Protein binding (serum)	X-linked	Deficiency: normal TSH, FT4, and FT3; low TT4 and TT3. Excess: normal TSH, FT4, and FT3, high TT4 and TT3	None
		TTR (increased and decreased TH binding)	Protein binding (serum)	AD	Normal TSH and FT4, high to low TT4 (depending on type of mutation)	Amyloidosis with polyneuropathy and/or cardiomyopathy.
	FDH	*ALB*	Protein binding (serum)	AD	Normal TSH, high TT4 and TT3	None
		CRYM	Protein binding (cytosol)	AD	Normal	Deafness

(*Continued*)

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Table 1. Continued

	Subtype	Gene	Affected Process	Inheritance	Serum Thyroid Parameters	Clinical and Other Biochemical Characteristics
Disturbed TH metabolism		*SBP2*	Deiodinase synthesis	AR	Normal-high TSH, high T_4 and rT_3, low-normal T_3	Growth retardation, (mild) mental and motor retardation, muscle weakness, hypoglycemia, impaired hearing, and infertility.
Disturbed TH transport		*MCT8*	TH transport	X-linked	Normal-high TSH, low T_4 and rT_3, high T_3	Severe psychomotor retardation with cognitive impairment, hypotonia in childhood followed by spastic quadriplegia.
Disturbed TH action	RTHα	*THRA*	TH receptor	AD	Normal TSH, low T_4 and rT_3, high T_3	Growth retardation; delayed bone, motor, and mental development; and constipation.
	RTHβ	*THRB*	TH receptor	AD	Normal-high TSH, high T_4 and T_3	Diffuse goiter and sinus tachycardia.

AR, autosomal recessive; AD, autosomal dominant; PRL, prolactin; FDH, familial dysalbuminemic hyperthyroxinemia; RAI, radioactive iodine.

the Na^+-K^+-ATPase, is coupled to the inward iodide translocation against its electrochemical gradient (19). The efflux of iodide into the follicular lumen is facilitated by the SLC26A4 transporter (Pendrin). Recent studies have also shown a contributing role for anoctamin-1, a Ca^{2+}-activated iodide channel (43, 44). Although its exact role still needs to be established, these data even suggest a larger contribution to iodide efflux than by Pendrin. Thyroglobulin (Tg) is a glycoprotein whose tyrosine residues are a substrate for iodination and iodothyronine formation. Thyroid peroxidase (TPO) catalyzes the oxidation of iodide by H_2O_2 and the iodination of the tyrosine residues. Dual oxidase 2 (DUOX2) and its maturation factor (DUOXA2) are responsible for generating H_2O_2 (45). Subsequently, T_4 is formed by the coupling of two diiodotyrosines (DITs), and T_3 is formed by coupling of one monoiodotyrosine (MIT) and one DIT. T_4 and T_3 are released into the circulation by transporters, including monocarboxylate transporter 8 (MCT8). Iodide is recycled by deiodination of iodotyrosines by iodotyrosine deiodinase/dehalogenase (18). In the circulation, only 0.03% of T_4 and 0.3% of T_3 are present in unbound form, whereas the rest is bound to the TH binding proteins T_4-binding globulin, transthyretin, and albumin.

Figure 3.

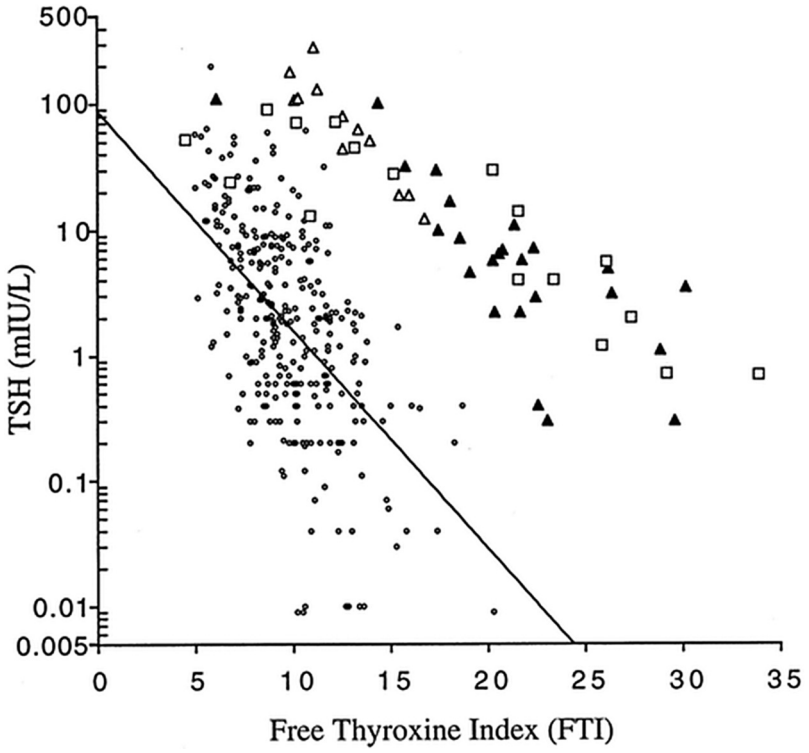

Figure 3. Relationship between FT4 index (FTI) and TSH in wild-type subjects (open dots), subjects from two families with a TRβ E460K mutation (open triangles and squares), and subjects from a family with an A317T mutation (filled triangles). This study showed that the HPT-axis setpoint in wild-type individuals is different from subjects with a TRβ mutation. [Adapted from S. Ercan-Fang S et al: Quantitative assessment of pituitary resistance to thyroid hormone from plots of the logarithm of thyrotropin versus serum free thyroxine index. *J Clin Endocrinol Metab*. 2000;85: 2299–2303 (36), with permission. © The Endocrine Society.]

The cellular uptake of T_4 and T_3 is mediated by a number of plasma membrane transporters. OATP1C1 is an organic anion transporter (OATP) family member, which is mainly expressed in brain capillaries, choroid plexus, and astrocytes, whereas MCT8 and MCT10 are expressed in various tissues (33, 47). Peripheral TH metabolism is mediated importantly by the three deiodinases that catalyze the iodothyronine deiodination (48–50). Type 1 deiodinase (D1) is present in the liver, kidney, and thyroid and plays a key role in the production of serum T_3 from T_4 and in the breakdown of the metabolite rT_3. Type 2 deiodinase (D2) is present in the brain, anterior pituitary, brown adipose tis-

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Figure 4.

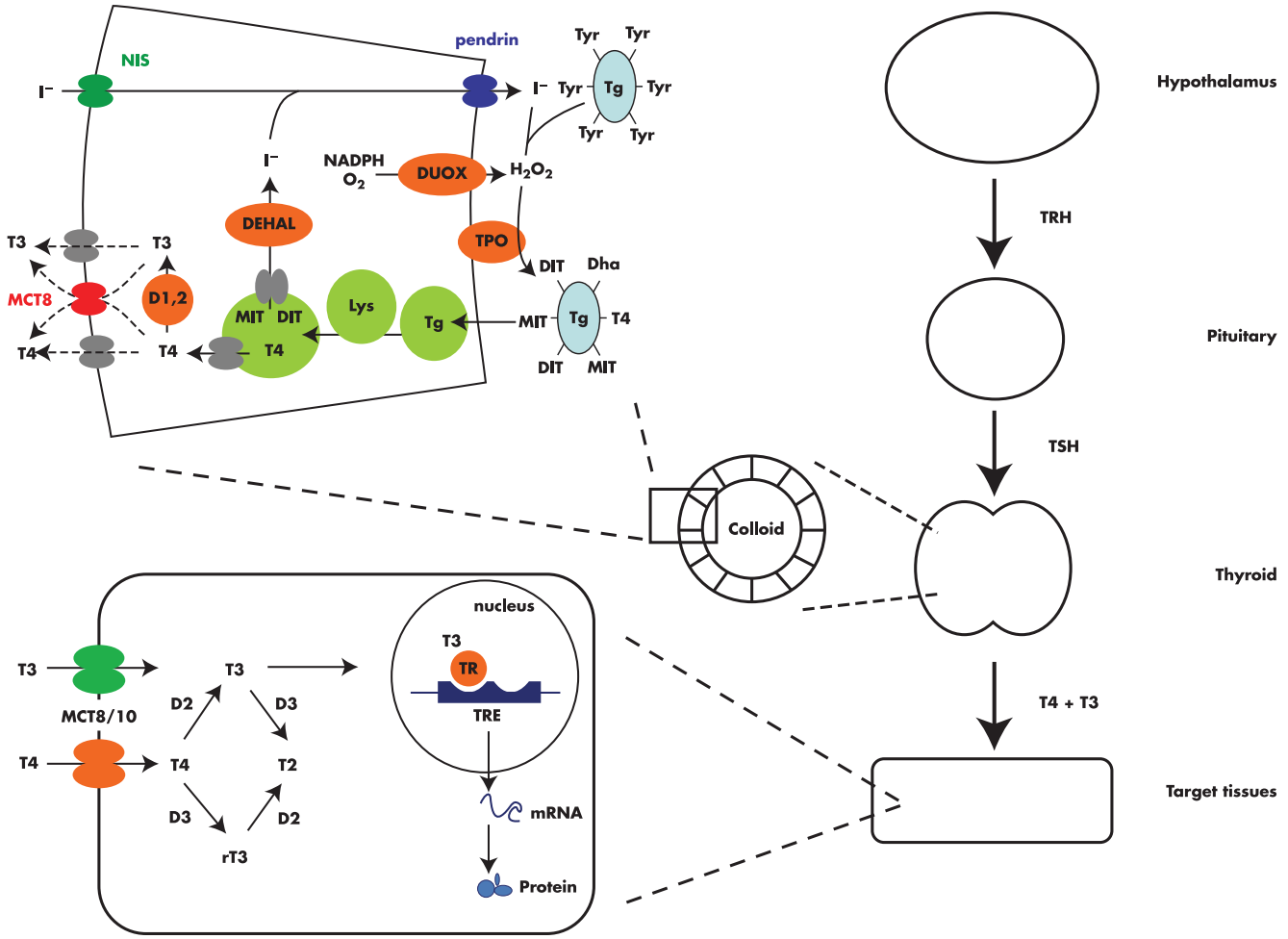

Figure 4. Simplified overview of the HPT axis, TH synthesis, and TH action in target tissues. Dha, dehydroalanine; Lys, lysosome; TRE, TH response element.

sue, thyroid, and skeletal muscle. D2 only has outer ring deiodinase activity and catalyzes the conversion of T_4 to T_3 and of rT_3 to 3,3′-diiodothyronine. In tissues such as the brain, D2 is important for local production of T_3, whereas D2 in skeletal muscle may also contribute to plasma T_3 production. Type 3 deiodinase (D3) is mainly present in various fetal tissues, the placenta, and pregnant uterus, as well as in the adult brain and skin. D3 has only inner ring deiodinase activity, and it catalyzes the inactivation of T_4 and T_3 by deiodination to rT_3 and 3,3′-diiodothyronine, respectively. It is the major T_3- and T_4-inactivating enzyme and contributes to TH homeostasis by protecting tissues from excess TH (49).

T_3 is considered to be the major bioactive TH, which exerts its effects by binding to the intracellular receptors TRα and TRβ. Both receptors have a wide expression pattern, with a predominance of TRα1 in the brain, heart, and bone; of TRβ1 in the liver, kidney, and thyroid; and of TRβ2 in the retina, cochlea, and pituitary (20).

The importance of these TH pathway genes is illustrated by the fact that mutations in many of these genes can lead to thyroid dysfunction. Table 1 provides an overview of these monogenic causes of hypothyroidism, hyperthyroidism, and other alterations in thyroid function tests. Of note, mutations in genes involved in TH metabolism and action have also been identified in nonthyroidal tumors, such as TRα and TRβ mutations in hepatic and renal cell carcinomas and TRβ mutations in TSH-secreting pituitary tumors (52–55). Furthermore, hypothyroidism due to D3-overexpressing tumors ("consumptive hypothyroidism") has been described in large hepatic hemangiomas, and more recently also in gastrointestinal stromal tumors (56–58). The further discussion of these tumors, as well as the monogenic causes of thyroid dysfunction shown in Table 1, is beyond the scope of this review, and we therefore refer to a number of excellent reviews (16–35, 59, 60). It is interesting to note that for part of the genes in Table 1, not only mutations but also polymorphisms

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have been associated with thyroid (dys)function, as discussed in Sections III and IV of this review.

III. Genetic Variants Associated With Thyroid (Dys)function (Pre-GWAS)

Because the monogenic causes of thyroid dysfunction and altered thyroid function tests are rare, a large number of studies have been performed in the last two decades to identify more common variants with a minor allele frequency >1% (polymorphisms) associated with thyroid (dys)function. These studies included both linkage and candidate gene studies, which will be discussed below.

A. Linkage studies

Linkage studies use polymorphic markers spread across the entire genome to detect cosegregation with the phenotype of interest (Table 2). This technique has been very successful in identifying rare causative variants with large effects for monogenic diseases. To date, only one linkage study on serum TH parameters has been published (61). This study by Panicker et al was performed in 613 dizygotic female twin pairs, and linkage peaks were detected on chr 2q36, 4q32, and 9q34 for TSH; on chr 14q13 and 18q21 for FT4; and on chr 7q36, 8q22, and 18q21 for free T_3 (FT3). No further mutational screening was done to identify the causative variants within the detected linkage region. Furthermore, Liu et al (62) performed a linkage scan in a Chinese family with nonautoimmune hyperthyroidism and detected a linkage peak on chr 14q24.2–31.3. Further mutational screening in this region led to the identification of a new mutation in the *TSHR* gene.

B. Candidate gene analyses for HPT-axis setpoint

Candidate gene association studies (Table 2) have been widely used to study the genetics of complex diseases in the last 10–15 years. Regarding the HPT-axis setpoint, they involve the analysis of polymorphic variants in candidate genes (ie, genes with a role in the regulation of TH production and/or activity) in relation to serum thyroid function tests, thyroid disease, and/or TH-related endpoints. These studies are relatively easy to perform and can be powered to detect small effects of specific alleles, but replication in independent cohorts is mandatory to avoid false-positive results. Causes of false-positive findings may be small sample size, lack of standardized phenotyping and genotyping, and population stratification when insufficient care has been paid to matching cases and controls (63). However, this can usually be avoided by careful study design and statistical correction for confounding factors (64). Although most studies nowadays take a genome-wide approach by genotyping large numbers of polymorphisms across the genome instead of focusing on a single candidate gene, candidate gene analyses can still be very useful. This is especially the case for variants in which the effects on gene function have been demonstrated in vitro.

The first candidate gene study analyzing the effect of genetic variation in relation to the HPT axis studied several TH pathway genes, ie, all three deiodinases, *TSHR*, *MCT8*, and *THRB* (65). Since then, multiple studies have been published analyzing the association between polymorphisms in candidate genes and the HPT-axis setpoint (29, 66–94). Studies vary in quality, and not all findings have been replicated in independent cohorts. Below we aim to give an overview of the consistent findings in the literature, as well as the controversies for the different candidate genes that have been analyzed. For genetic variants in genes that were initially identified by GWAS, and that were subsequently confirmed or studied in specific populations by candidate gene analysis, the reader is referred to Section IV.

Table 2. Techniques Used to Identify Genomic Loci and Gene Variants in Medical Genetics

Method	Description
Linkage analysis	Use of polymorphic markers spread across the entire genome to detect cosegregation with the phenotype of interest.
Candidate gene analysis	Analysis of polymorphic gene variants in genes with a role in the (patho)physiology of the respective phenotype. The carriage of these gene variants is tested against this phenotype.
GWAS	Genotyping of 100 000 to 500 000 single nucleotide polymorphisms across the entire genome. Nongenotyped variants are predicted based on their LD with surrounding genotyped variants, leading to the genotypes of a total of 2.5 to 9.5 million gene variants. The carriage of each allele is tested against the phenotype of interest.
Exome chip analysis	Genotyping of >250 000 putative functioning exonic variants, which are selected from various whole exome and whole genome sequencing efforts. The identified variants are tested against the phenotype of interest.
Whole exome sequencing analysis	Sequencing the protein-coding part of the genome (ie, exome) to obtain a complete catalog of all variants in these regions. The identified variants are tested against the phenotype of interest.
Whole genome sequencing analysis	Sequencing the entire genome to obtain a complete catalog of all variants in the genome. The identified variants are tested against the phenotype of interest.

LD is the non-random association of alleles at two loci, with a high LD meaning that the presence of an allele at one locus is strongly predictive for the presence of an allele at the other locus.

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1. TSH receptor

As shown in Table 1, activating as well as inactivating mutations have been identified within the *TSHR* gene (95). One of the best-studied polymorphisms within the *TSHR* gene is a C-to-G transition at position 2281 resulting in a p.727D>E substitution in the cytoplasmic tail of the TSHR. Several candidate gene analyses have suggested that this genetic variant is associated with serum TSH levels (65, 72, 94). A study analyzing the effect of this particular polymorphism in a healthy population of twins demonstrated that, although the effect on TSH was clearly significant, the proportion of genetic variation that could be accounted for by this TSHR-p.727D>E variant was very small (0.91% of the overall variation in TSH) (72). No statistically significant evidence was found for interaction between the genotype and environmental factors such as iodine intake and cigarette smoking. This polymorphism is associated with lower TSH levels but normal FT4 levels in all three studies (65, 72, 94), a finding we were able to replicate in another independent Dutch population of more than 1000 subjects (W. M. van der Deure, R. P. Peeters, and T. J. Visser, unpublished data). A study in preeclamptic women also showed lower levels of TSH, but no data on FT4 were provided in this study (84). These results suggest that the setpoint of the HPT axis is affected by this particular polymorphism, due to an altered sensitivity of the receptor. An increased activity of the TSHR in carriers of the TSHR-p.727E variant would require less TSH to produce normal FT4 levels. There is indeed one in vitro study showing that the TSHR-p.727E variant results in an increased cAMP response of the receptor to TSH (96). However, others have not been able to replicate this finding (97, 98). This suggests that the TSHR-p.727D>E variant might also be linked to another functional polymorphism elsewhere in the *TSHR* gene.

It should be noted, however, that the association of the p.727D>E variant with TSH levels was not confirmed in a large-scale association analysis of 68 TH pathway genes or in several GWAS (see Section IV) (76, 99–102).

2. TH transporters

The first TH transporter identified at the molecular level was rat OATP subtype 3 (103). In subsequent years, it has been demonstrated that TH is transported by various types of transporters, including the Na^+/taurocholate cotransporting polypeptide (NTCP; SLC10A1) (104, 105), the heterodimeric L-type amino acid transporters LAT1 and LAT2 (106), various members of the OATP family (29, 107), MCT8 (SLC10A2), and MCT10 (SLC16A10) (108, 109). Most of these transporters accept a variety of ligands, with MCT8 and, to a lesser extent, MCT10 and OATP1C1 as exceptions. Very little is known about the possible effects of genetic variation in NTCP, LAT1, and LAT2 (93). For this reason we will only discuss MCT8, MCT10, and several members of the OATP family.

a. MCT8 and MCT10. Only a few studies exist on the relationship between polymorphisms in *MCT8*, located on the X-chromosome, and serum TH levels (75, 76, 87, 93). The largest and most recent study by Roef et al (87) found that two polymorphisms in *MCT8* were related to circulating TH levels in men but not in women. The rs5937843 polymorphism in intron 5 of the *MCT8* gene was inversely associated with FT4 levels (87). This is in line with a previous, smaller study in which carriers of another polymorphism in intron 5 of the *MCT8* gene (rs5937843) had lower FT4 levels than wild-type male subjects. This finding could not be replicated in the homozygous female carriers in the same population (93). Roef et al (87) also found that a nonsynonymous polymorphism (rs6647476 [p.107S>P]) was significantly associated with lower serum FT3 levels in males, which is in contrast to previous smaller studies (75, 93). No in vitro effects of this polymorphism could be demonstrated so far (75, 93).

The few studies of genetic variation in the *MCT10* gene failed to show any significant association with serum thyroid parameters (76, 87, 93).

b. OATPs. The OATPs are a large family of transporters responsible for Na^+-independent transmembrane transport of amphipathic organic compounds, including bile salts, bromosulfophthalein, steroid conjugates, and numerous drugs (29). In humans, 11 OATPs have been identified, all containing 12-transmembrane domains. Although most OATP proteins are expressed in multiple tissues, some members show a more tissue-specific distribution (110). OATP1B1 and OATP1B3 are exclusively expressed in liver (111, 112), whereas OATP1C1 is present in the brain, cochlea, retina, Leydig cells, and placenta (113).

Several members of the large OATP family facilitate uptake of TH, including members of the OATP1 subfamily: OATP1A2 (114), OATP1B1 (115, 116), OATP1B3 (116), and OATP1C1 (113). This family has best been studied in relation to genetic variation and thyroid function.

Although OATP1A2-p.172E>D (rs11568563) showed decreased transport activity in vitro, this variant was not associated with serum thyroid parameters in two Caucasian populations (29).

Polymorphisms in the *OATP1B1* and *OATP1B3* genes have been extensively studied as they impact on the interindividual variability of drug disposition and drug response (117). To date, only one study has focused on associations between a polymorphism in the *OATP1B1*

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gene, OATP1B1-p.174V>A (rs4149056), and serum TH levels (90). OATP1B1 preferentially transports sulfated hormones, ie, T4S, T3S, rT3S, and estrone sulfate, and OATP1B1-p.174A shows a 40% lower transport of these substrates than OATP1B1-p.174V (90). This is in line with a decreased activity of this variant in transporting other substrates as well (118). These in vitro data are supported by population-based data, showing that this polymorphism is associated with higher serum T4S and estrone sulfate levels (90). No studies have yet been published on associations between genetic variation in the *OATP1B3* gene and serum TH levels.

OATP1C1, which is capable of T_4, T4S, and rT_3 transport, is expressed at the blood–brain barrier, suggesting a critical role for T_4 uptake into the brain. Polymorphisms in the *OATP1C1* gene are not consistently associated with serum TH levels (87, 91).

None of the variants in these transporters showed a significant association in the previously mentioned large-scale association analysis of 68 TH pathway genes or the GWAS performed so far (76, 99–102).

3. Deiodinases

a. DIO1. Candidate gene analyses of *DIO1* have predominantly focused on three polymorphisms, two located in the 3′-untranslated region (UTR) (rs11206244 and rs12095080), and one located in intron 3 (rs2235544). Initial studies focused on the two polymorphisms in the 3′UTR. In general, the *DIO1*-rs11206244-T allele is associated with higher serum FT4, T_4 and rT_3 levels in combination with lower serum FT3 and T_3 concentrations (65, 68, 76, 78, 80, 83, 85, 86, 92). As a consequence, this variant allele is associated with lower T_3/rT_3 and T_3/T_4 ratios. These data suggest a negative effect of the *DIO1*-rs11206244-T variant on D1 activity because liver D1 plays a major role in the production of serum T_3 from T_4 and in the breakdown of rT_3. The *DIO1*-rs12095080 polymorphism has been investigated in fewer studies, showing opposite results from the *DIO1*-rs11206244-T allele. The *DIO1*-rs12095080-G allele was associated with a higher T_3/rT_3 ratio in two independent cohorts, suggesting that this variant is associated with an increased D1 activity (65, 68). Based on stronger effects of these variants on serum T_3 levels in elderly subjects, it has been proposed that the relative contribution of D2 to serum T_3 production decreases with an increase in age (81), but this hypothesis remains to be confirmed. No effects on mRNA levels, mRNA decay rate, or enzyme activity have been demonstrated for any of these two variants in the 3′UTR of *DIO1* (92).

The intronic polymorphism rs2235544 is in high linkage disequilibrium (LD) with rs11206244 ($r^2 = 0.76$) (119). LD is the nonrandom association of alleles at two loci, with a high LD meaning that the presence of an allele at one locus is strongly predictive for the presence of an allele at the other locus. It is therefore not surprising that a candidate gene analysis using a set of nine tagging polymorphisms to capture most common variations across the *DIO1* gene demonstrated that rs2235544 is associated with decreased levels of FT4 and rT_3 and an increased FT3/FT4 ratio (78). Given this high LD, the exact causative variant driving these associations still needs to be identified, which could also be a rare variant in high LD with rs2235544 or rs11206244, whereas these associations could also be due to gene-gene interactions (epistasis). In 2011, a large-scale candidate gene analysis of 68 genes was performed with a similar tagging approach, including the genes in Table 1 and Figure 4 (76). One of the significant findings included an association of rs2235544 with FT4 in the same direction as in the previously mentioned reports. This association was recently confirmed in a meta-analysis of GWAS for TSH and FT4 (see Section IV) (100). None of the *DIO1* polymorphisms is associated with serum TSH levels, which is likely due to the fact that circulating T_4 and T_3 are affected in opposite directions.

b. DIO2. Candidate gene analyses of *DIO2* have predominantly focused on two polymorphisms, located in exon 1 (rs12885300 [p.3G>D]) and exon 2 (rs225014 [p.92T>A]). DIO2-p.92T>A, the best-studied polymorphism in DIO2 in vitro as well as in vivo, is not associated with any change in circulating TH and/or TSH levels. This has been demonstrated in multiple populations with and without thyroid disease (65, 66, 68, 70, 71, 77–79, 86). The DIO2-p.92T>A variant is located in a part of the protein that is important for stability (120), but in vitro studies have produced inconsistent results about its functionality. DIO2-p.92A was associated with decreased D2 activity in skeletal muscle and thyroid samples of homozygous patients with type 2 diabetes mellitus (121). In contrast, cells transiently expressing the p.92T>A form of D2 display similar kinetic properties with either T_4 or rT_3 as substrate as compared to wild-type D2 (65, 121). This discrepancy might be explained by linkage to a functional variant elsewhere in the genome.

In contrast, the *DIO2*-rs12885300 polymorphism was associated with an increased T_3/T_4 ratio in one study, suggesting an increase in deiodinase activity (79). However, this finding has not been replicated in other cohorts (68). This could be due to differences in population characteristics such as age (79, 81), or it could be due to a chance finding.

DIO2-p.3G>D is located in a short open reading frame (sORF) within the 5′UTR. This sORF is considered to be

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primarily responsible for the inhibitory effect of the 5′UTR on DIO2 translation because mutation of the start codon of the sORF completely abolished this inhibitory effect (122). In vitro analysis of the *DIO2*-p.3G>D polymorphism showed that the minor p.3D variant was associated with an increased gene transcription and increased D2 activity (123), suggesting that the observed associations with the T_3/T_4 ratio may very well be true effects.

Although none of the D2 variants were associated with serum TSH levels, there is evidence that the HPT-axis setpoint is affected. Hoftijzer et al (74) studied the relations between serum TSH and FT4 levels in patients treated for differentiated thyroid carcinoma and showed that the negative feedback of FT4 on TSH was weaker in homozygous carriers of the DIO2-p.3D variant. Furthermore, homozygous subjects showed a delayed rise in serum T_3 for DIO2-p.92A and a blunted rise in FT4 for DIO2-p.3D, respectively, indicating subtle alterations in intrathyroidal conversion of T_4 to T_3 (67, 82). Finally, there is one study in patients with differentiated thyroid cancer after thyroidectomy reporting that carriers of the DIO2-p.92A variant need a higher dose of levothyroxine (LT4) to suppress TSH (89). However, the fact that serum FT4 and FT3 levels were not different between the genotype groups in this study, and the fact that the findings could not be replicated in a similar cohort of patients with differentiated thyroid carcinoma, does not support an altered pituitary setpoint in these patients (73).

c. DIO3. The *DIO3* gene is an imprinted gene (124), hampering candidate gene analysis studies. The few candidate studies that have studied the *DIO3* gene in relation to the HPT-axis setpoint did not find any association (65, 76, 78).

4. TH receptors

Although many studies have been published on associations between clinical endpoints and polymorphisms in other nuclear receptors, such as the estrogen and glucocorticoid receptors, relatively little is known about functional polymorphisms in TRs. As shown in Table 1, patients with mutations in TRα and TRβ have clear alterations in serum thyroid function tests (20, 30, 125). Therefore, polymorphisms in these receptors may also be associated with alterations in the HPT-axis setpoint.

By sequencing all *THRA* and *THRB* exons and their flanking regions in more than 50 subjects, eight polymorphisms were identified in *THRA* and seven in *THRB* (65, 69, 88). These variants in *THRA* were not associated with serum thyroid parameters. One polymorphism in *THRB* was associated with higher levels of TSH in one population, but this could not be replicated in a second, older population (88). Genetic variation in these receptors did not show significant associations with TSH or FT4 levels in the large-scale association analysis of 68 TH pathway genes or the GWAS performed so far (76, 100–102).

C. Candidate gene analyses for clinical thyroid-related endpoints

Because TH is a pleiotropic hormone, with effects on almost all tissues and organ systems, it can be expected that polymorphic variants affecting local TH action may well have consequences for a variety of clinical phenotypes (81, 126). Although the effects of common variants are usually very small, they exert their effects throughout life. In recent years, multiple studies have investigated the association between genetic variation in TH pathway genes (especially *DIO1* and *DIO2*) and a large variety of clinical endpoints, a summary of which is provided in Table 3. Interestingly, most of the effects of genetic variation were seen independently of serum TH levels, highlighting the importance of local regulation of TH in tissues.

1. Neurocognitive function

a. Neurocognitive function in euthyroid patients. The brain is particularly sensitive to relatively small changes in TH, as is illustrated by the increased risk of cognitive complaints and depression in patients with clinical and subclinical thyroid disease (2, 127). As shown in Table 3, genetic variation in D1 has been associated with an increased risk of lifetime major depression in white females and a better response to potentiation of sertraline by T_3 addition in depressed patients (83, 119). Whereas DIO2-p.92T>A was not associated with response to paroxetine treatment in patients with a major depression, this variant was associated with an increased risk of bipolar disorder in a Chinese population, but these data need replication in a separate cohort (128, 129).

Thyroid disorders have also been suggested to be a risk factor for dementia (130, 131). However, studies using magnetic resonance imaging (MRI) did not find any association between *DIO1*, *DIO2*, or *THRA* polymorphisms and markers of early Alzheimer's' dementia (132, 133).

b. Neurocognitive function in patients receiving TH replacement therapy. A small but significant proportion of thyroidectomized patients on LT4 replacement have low serum T_3 despite normal TSH and high-normal FT4 levels (134). These patients may be more vulnerable to genetic variants affecting local T_3 production. Perhaps in these patients a reduced D2 activity cannot fully compensate for the absence of the thyroidal T_3 production (126). This might explain why a subgroup of patients who receive TH

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Table 3. TH Pathway Genes and Clinical Outcomes

Clinical Endpoint	Gene	First Author, Year (Ref)	n[a]	Summary of Findings
Neurocognitive function (euthyroids)	*DIO1*	Cooper-Kazaz, 2009 (119)	35/29	rs11206244 but not rs12095080 associated with enhanced potentiation of sertraline by T_3.
		Philibert, 2011 (83)	1555	rs11206244 but not rs2294512 and rs2235544 associated with increased risk of major depression in white middle-aged women.
		de Jong, 2007 (68)	473	rs11206244 and rs12095080 not associated with MRI markers of dementia (hippocampus/amygdala volume) in Dutch elderly subjects.
	DIO2	Brouwer, 2006 (129)	98	rs225014 (p.92T>A) not associated with response to paroxetine in patients with major depression.
		Cooper-Kazaz, 2009 (119)	35/29	rs225014 (p.92T>A) and rs12885300 (p.3G>D) not associated with enhanced potentiation of sertraline by LT3.
		He, 2009 (128)	278/284	rs225014 (p.92T>A) and rs12885300 (p.3G>D) associated with increased risk of bipolar disorder in Chinese middle-aged subjects.
		de Jong, 2007 (68)	473	rs225014 (p.92T>A) and rs12885300 (p.3G>D) not associated with MRI markers of dementia in Dutch elderly subjects.
	THRA	Medici, 2012 (133)	454	rs868150, rs7502966, rs1568400, rs939348, rs2230701 (c.351C>T) and rs3744805 not associated with MRI markers of small vessel disease or neurodegeneration in Dutch elderly.
Neurocognitive function (TH replacement therapy)	*DIO1*	Panicker, 2009 (135)	552	rs11206237, rs11206244, rs2235544, rs2268181, rs2294511, rs2294512, rs4926616, rs731828 and rs7527713 not associated with psychological well-being on LT4 and response to combination LT3/LT4 in trial comparing LT4 with LT3/LT4 combination therapy.
	DIO2	Panicker, 2009 (135)	552	rs225014 (p.92T>A) but not rs225011 and rs225015 associated with impaired psychological well-being on LT4 and better response to combination LT3/LT4 therapy.
		Appelhof, 2005 (66)	141	rs225014 (p.92T>A) and rs12885300 (p.3G>D) not associated with well-being, cognitive function and response to LT3/LT4 combination therapy in trial comparing LT4 with LT3/LT4 combination therapy
	DIO3	Panicker, 2009 (135)	552	rs17716499, rs7150269, rs8011440, and rs945006 not associated with psychological well-being on LT4 and response to combination LT3/LT4 therapy.
	OATP1C1	van der Deure, 2008 (91)	141	rs10770704 and rs10444412, but not rs36010656 (p.143P>T), associated with fatigue and depression but not with neurocognitive functioning or preference for combination LT3/LT4 therapy in trial comparing LT4 with LT3/LT4 combination therapy.
Mental retardation	*DIO2*	Guo, 2004 (137)	96/331	rs225010 and rs225012, but not rs225014 (p.92T>A), associated with mental retardation in Chinese children from an iodine-deficient area.
		Zhang, 2012 (138)	1461	rs225015, rs2267872, and rs1388378, but not rs225014 (p.92T>A) and rs225012, associated with mental retardation in Chinese children from an iodine-deficient area.
	TSHR	Guo, 2005 (139)	94/326	rs2284716, rs917986, rs2075173, and rs2075179 (c.561T>C) not associated with mental retardation in Chinese children from an iodine-deficient area.
Osteoporosis and osteoarthritis	*DIO2*	Heemstra, 2009 (73)	154	rs225014 (p.92T>A) associated with lower BMD and higher bone turnover in thyroidectomized thyroid carcinoma patients on LT4 replacement.
		Meulenbelt, 2008 (155)	2209/1199	rs225014 (p.92T>A) associated with generalized osteoarthritis in Caucasian and Asian subjects.
	DIO3	Meulenbelt, 2011 (157)	3252/2132	rs945006, but not rs1190715 and rs8011440, associated with knee and hip osteoarthritis in European subjects.
	THRA	Medici, 2012 (154)	19 195	rs868150, rs7502966, rs1568400, rs939348, rs2230701 (c.351C>T), and rs3744805 not associated with BMD, osteoporotic fractures, or bone geometry in Caucasian subjects.
	TSHR	van der Deure, 2008 (94)	4934	rs1991517 (p.727D>E) associated with increased femoral neck BMD in Dutch elderly subjects.
		Liu, 2012 (148)	150	rs1991517 (p.727D>E), but not rs61747482 (p.36D>H), associated with osteoporosis in Chinese subjects.

(*Continued*)

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Table 3. Continued

Clinical Endpoint	Gene	First Author, Year (Ref)	n[a]	Summary of Findings
Diabetes and insulin sensitivity	*DIO2*	Mentuccia, 2002 (158)	135	rs225014 (p.92T>A) associated with insulin resistance in subjects undergoing euglycemic-hyperinsulinemic clamps.
		Mentuccia, 2005 (77)	747	rs225014 (p.92T>A) not associated with DM2 or impaired glucose tolerance in middle-aged Amish.
		Gumieniak, 2007 (71)	372	rs225014 (p.92T>A) not associated with HOMA index in middle-aged subjects.
		Grarup, 2007 (160)	7342	rs225014 (p.92T>A) not associated with DM2, glucose intolerance, or fasting plasma glucose levels in middle-aged Danish subjects.
		Maia, 2007 (161)	1633	rs225014 (p.92T>A) not associated with DM2, fasting plasma glucose or insulin levels, HbA1c levels, or insulin resistance in elderly subjects.
		Peeters, 2005 (80)	349	rs225014 (p.92T>A) not associated with DM2, fasting plasma glucose or insulin levels, HbA1c levels, or HOMA index in Dutch elderly men.
		Nair, 2012 (162)	150/150	rs225014 (p.92T>A), rs225011, rs225015, and rs6574549 not associated with early-onset DM2, hepatic glucose output, fasting insulin, and insulin action in Pima Indians.
		Dora, 2010 (164)	2811/8661	rs225014 (p.92T>A) associated with increased risk of DM2 in middle-aged subjects.
		Canani, 2005 (121)	183	rs225014 (p.92T>A) associated with fasting insulin levels in Brazilian patients with DM2.
		Estivalet, 2011 (165)	246	rs225014 (p.92T>A) associated with fasting insulin levels and HOMA in middle-aged European patients with DM2.
	TSHR	Peeters, 2007 (163)	349	rs1991517 (p.727D>E) associated with higher glucose, insulin, HbA1c, and HOMA in elderly Dutch men.
Blood pressure	*DIO2*	Gumieniak, 2007 (71)	372	rs225014 (p.92T>A) associated with hypertension in euthyroid middle-aged subjects.
		Dora, 2010 (164)	1057	rs225014 (p.92T>A) not associated with diastolic or systolic blood pressure in Brazilian patients with DM2.
		Canani, 2007 (166)	315	rs225014 (p.92T>A) not associated with hypertension or mean blood pressure levels in Brazilian patients with DM2.
		Maia, 2008 (167)	1557	rs225014 (p.92T>A) not associated with hypertension or mean blood pressure levels in general population.
		van der Deure, 2009 (168)	2294	rs225014 (p.92T>A) and rs12885300 (p.3G>D) not associated with hypertension or mean blood pressure levels in general population.
	THRH	Garcia, 2001 (169)	183/185	−221G>C promoter variant (no rs number available) associated with increased risk of essential hypertension.
	TSHR	van der Deure, 2009 (168)	2294	rs1991517 (p.727D>E) not associated with hypertension or mean blood pressure levels in Dutch elderly.
Dyslipidemia	*DIO2*	Mentuccia, 2005 (77)	747	rs225014 (p.92T>A) not associated with triglycerides, total, LDL, or HDL cholesterol levels in middle-aged Amish.
		Canani, 2005 (121)	183	rs225014 (p.92T>A) not associated with triglycerides, total or HDL cholesterol levels in patients with DM2.
		Grarup, 2007 (160)	5843	rs225014 (p.92T>A) not associated with triglycerides, total, LDL, or HDL cholesterol levels in Danish middle-aged subjects.
		Estivalet, 2011 (165)	714	rs225014 (p.92T>A) not associated with triglycerides, total or HDL cholesterol levels in middle-aged European patients with DM2.
BMI	*DIO1*	Peeters, 2005 (80)	350	rs11206244 and rs12095080 not associated with BMI in Dutch elderly men.
	DIO2	Mentuccia, 2005 (77)	747	rs225014 (p.92T>A) not associated with BMI in middle-aged Amish.
		Canani, 2005 (121)	183	rs225014 (p.92T>A) not associated with BMI in middle-aged Brazilian patients with DM2.
		Torlontano, 2008 (89)	191	rs225014 (p.92T>A) not associated with BMI in middle-aged Italian thyroid cancer patients on LT4 replacement.
		Heemstra, 2009 (73)	295	rs225014 (p.92T>A) associated with BMI in middle-aged Dutch Hashimoto's thyroiditis patients on LT4 replacement (n = 141), but no association with BMI in thyroid cancer patients on LT4 replacement (n = 154).
		Grarup, 2007 (160)	7342	rs225014 (p.92T>A) not associated with BMI in middle-aged Danish subjects.
		Maia, 2007 (161)	1633	rs225014 (p.92T>A) not associated with BMI in elderly subjects.

(*Continued*)

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Table 3. Continued

Clinical Endpoint	Gene	First Author, Year (Ref)	n[a]	Summary of Findings
Sepsis susceptibility	*DIO2*	Ma, 2011 (51)	336/375	rs225014 (p.92T>A), but not rs12885300 (p.3G>D), rs225017, rs225011, rs2267872, rs2267873, rs8009555 or rs6574551, associated with lower risk of severe sepsis and related acute lung injury in European Americans (217 cases, 188 controls). No associations in African Americans were found (119 cases, 187 controls).

[a] Number of patients or number of patients/controls.

replacement have decreased well-being (15). As shown in Table 3, two studies analyzed the effects of genetic variation in deiodinases in hypothyroid patients (66, 135). Both studies were secondary analyses of prospective trials comparing liothyronine (LT3)/LT4 combination therapy to LT4 alone in primary hypothyroidism. The largest study in 552 patients showed that genetic variation in DIO2 (rs225014) was associated with impaired psychological well-being at baseline (135). It should be noted that although 16 polymorphisms were tested in this study, no multiple testing correction was applied because the study was powered to detect only large gene-treatment interactions. Therefore, these results need replication in an independent cohort. Interestingly, this polymorphism was also associated with response to combination LT3/LT4 treatment. These effects were not found in the smaller second study (141 patients), but the DIO2-p.92A variant did show a similar but nonsignificant trend with impaired well-being (66).

The third study analyzed OATP1C1, a T_4 transporter expressed at the blood–brain barrier (see Section III.B), in the same 141 patients (136). Polymorphisms in this transporter were associated with fatigue and depression, but not with neurocognitive functioning or preference for LT3/LT4 combination therapy. Because all of these studies retrospectively genotyped the participants, results have to be confirmed in randomized prospective studies.

c. Mental retardation. TH and its essential trace element iodine are crucial for normal brain development. Two studies investigated whether the risk of mental retardation was associated with genetic variation in *DIO2*, both of which were conducted in an iodine-deficient area (137, 138). As shown in Table 3, several *DIO2* polymorphisms were associated with an increased risk of mental retardation, but these associations were not present in both study populations, and no associations with *DIO2*-rs225014 were found. Genetic variation in the *TSHR* was also studied in one of these populations, but no associations with mental retardation were detected (139).

2. Osteoporosis and other bone-related phenotypes

TH is crucial for bone development and maintenance. Hyperthyroidism results in bone loss, osteoporosis, and an increased risk of fractures, whereas hypothyroidism has been reported to result in increased cortical thickness (140). Subclinical hyper- and hypothyroidism have both been related to fracture risk as well, although conflicting results have been reported (127, 141).

Several studies have investigated variation in TH pathway genes and osteoporosis. Genetic variation in D2 has been associated with more bone turnover and decreased BMD in thyroidectomized patients with thyroid cancer on replacement therapy (142), suggesting a role in bone homeostasis. This is in line with data from *Dio2* knockout mice, which have increased fracture susceptibility due to an essential role for D2 in osteoblasts in reaching optimal bone strength and mineralization (143).

The *TSHR* is also expressed in bone. Although various mouse studies have been performed to investigate the independent effects of TSH on bone, controversy remains concerning its exact role in bone metabolism (144–147). A large population-based study showed that TSH, in contrast to FT4, was positively correlated with BMD (94). Polymorphisms in the TSHR have been related to bone formation as well. The p.727D variant, which is thought to result in a lower TSHR activity, has been associated with a lower femoral neck BMD in the general population and is more frequent among males with osteoporosis (94, 148).

THRA is the predominant TR in bone, and RTHα patients have a delayed bone development (32, 149–153). The associations between *THRA* polymorphisms, BMD, bone geometry, and fracture risk have therefore been studied in a large population-based sample, but no associations were found (154).

Finally, genetic variation in the deiodinases has also been associated with osteoarthritis. A genome-wide linkage scan identified an association between the DIO2-p.92T>A variant and generalized osteoarthritis (155). The same authors later demonstrated increased D2 protein in cartilage of patients with osteoarthritis, as well as

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allelic imbalance of the *DIO2* mRNA. In heterozygous carriers, mRNA from the variant allele was more abundant than from the wild-type allele (156). Genetic variation in *DIO3* has also been implicated in knee and hip osteoarthritis (157), but these findings still require independent replication.

3. Metabolic syndrome

The first study that associated a TH pathway gene with a clinical endpoint concerned the DIO2-p.92T>A variant in relation to insulin resistance (158). Since then, various studies have analyzed polymorphisms in different TH pathway genes in relation to parameters of the metabolic syndrome, including insulin resistance, high blood pressure, dyslipidemia, and body composition.

a. Diabetes and insulin resistance. The initial study by Mentuccia et al (158) analyzed a population of 135 nondiabetic women undergoing euglycemic-hyperinsulinemic clamps to determine insulin sensitivity. In these women, a consistent strong relationship between the *DIO2*-rs225014 variant and lower glucose disposal rate was observed, pointing toward an increased risk for insulin resistance. Subsequent studies showed conflicting results in larger populations with different characteristics. In multiple nondiabetic cohorts of varying size but with a total of more than 10 000 subjects, no association of this polymorphism with diabetes or insulin resistance could be demonstrated (71, 77, 159–163). However, in one case-control study of 1057 type 2 diabetic and 516 nondiabetic subjects, the *DIO2*-rs225014 variant was associated with a significantly increased risk of type 2 diabetes mellitus (DM2) (164). A subsequent meta-analysis of the available case-control studies in 2010 resulted in a significantly increased risk as well, with a pooled odds ratio of 1.18 (95% confidence interval, 1.03–1.36; $P = .02$) (164).

Three studies analyzed insulin sensitivity in patients with DM2 using homeostasis model of assessment-estimated insulin resistance and fasting insulin. All three studies showed an increased insulin resistance in homozygous carriers of the DIO2-p.92A variant allele (121, 164, 165). Altogether, these data suggest that genetic variation in *DIO2* is indeed associated with a mild increase in insulin resistance. So far, however, large GWAS of diabetes have not identified the *DIO2* gene as a susceptibility locus. One study found an association between the TSHR-p.727D>E variant (TSHR is known to be expressed in adipose tissue) and insulin resistance in nondiabetic elderly men, but this finding needs replication in an independent cohort (163).

b. Blood pressure. Studies analyzing the association of the DIO2-p.92T>A variant in relation to blood pressure show conflicting results as well. A study in a relatively small population showed a positive association between this variant and blood pressure, but this finding was not replicated in four larger studies of nondiabetic and diabetic patients (164, 166–168).

One study has reported on the association of genetic variation in the *TRHR* gene and hypertension (169). However, in a population-based study of normotensive subjects, we did not find any associations of these *TRHR* polymorphisms with blood pressure (W. M. van der Deure, unpublished results).

c. Dyslipidemia. Hypothyroidism results in a marked increase in total and low-density lipoprotein (LDL) cholesterol levels, and several (but not all) cross-sectional studies have suggested an association of subclinical hypothyroidism with total and LDL cholesterol levels as well (127). As a consequence, several studies analyzing TH pathway genes (more specifically *DIO2*) in relation to insulin resistance also studied its relation with total, high-density lipoprotein (HDL), and LDL cholesterol as well as triglycerides, but none of these studies found significant associations (77, 121, 159, 160, 165). However, considering the fact that not D2 but D1 is the predominant deiodinase in liver, and the fact that the DIO2-p.92T>A variant does not affect circulating TH levels, a substantial effect of this polymorphism on cholesterol metabolism would not be expected either.

To the best of our knowledge, no candidate gene studies have been published on the association between genes involved in TH uptake or metabolism in the liver, such as *NTCP*, *MCT8*, *OATP1B1*, and *DIO1*, and dyslipidemia. Interestingly, *MCT8* knockout mice have increased hepatic T_3 levels and decreased cholesterol levels (170), whereas lipid profiles have not yet been extensively studied in human MCT8-deficient patients (Allan-Herndon-Dudley syndrome). Furthermore, OATP1B1 is also involved in the liver uptake of statins. A GWAS in patients using statins revealed that a polymorphism in this transporter (rs4363657) is associated with statin-induced myopathy (171). This variant is in almost complete LD ($r^2 = 0.97$) with rs4149056 (p.174V>A), which is associated with increased serum T4S levels (90).

d. Body composition. The only study reporting on the association between genetic variation in *DIO1* and body mass index (BMI) was negative (80). However, *DIO1* polymorphisms putatively associated with decreased D1 activity were also associated with higher serum free IGF-1 levels in two independent populations (80). The pathophysiological significance of this association with IGF-1 was supported by increased muscle strength and muscle mass in

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elderly carriers of this variant allele. However, these data have not yet been replicated, nor have other studies been published to date on IGF-1-related endpoints such as body length. Almost all studies of the *DIO2*-p.92T>A polymorphism in relation to body composition showed a lack of association with BMI (73, 77, 89, 121, 159–161), except for one small study that showed that treated Hashimoto's thyroiditis patients homozygous for the p.92A allele have an increased BMI (73).

Because the phenotype of RTHα patients includes growth retardation (Table 1), one may expect that polymorphisms in *THRA* could affect height. In the previously mentioned study on *THRA* polymorphisms and bone parameters (154), we therefore tested the association with height but did not find any relations, suggesting that these common *THRA* variants are not functional (M. Medici, T. J. Visser, and R. P. Peeters, unpublished results).

D. Linkage and candidate gene studies in autoimmune thyroid disease

Since the 1970s, a number of loci have been consistently associated with the risk of AITD, including *HLA* classes I and II, *CTLA4*, *PTPN22*, *IL2RA*, *TSHR*, and *FCRL3*. The discussion of these studies is beyond the scope of this review, and we therefore refer to a number of comprehensive reviews published over the years (38–41, 172–175). For the results of a large AITD candidate gene analysis using the ImmunoChip, please see the original publication by Cooper et al (176) and the recent review by Simmonds (39). The new loci more recently discovered by GWAS are covered in Section IV.

IV. Genetic Variants Associated With Thyroid (Dys)function (GWAS)

As discussed above, linkage and candidate gene studies have identified only a limited number of genes consistently associated with thyroid function or dysfunction. More recently, GWAS have had much more success in identifying genetic variants associated with thyroid-related traits. These studies have been made possible by advances in genotyping techniques, in which 100 000 to 500 000 variants are genotyped across the whole genome and tested against the phenotype of interest (Table 2). A stringent *P* value threshold of $P < 5 \times 10^{-8}$ is used to prevent false-positive results due to multiple testing. As expected for "common" variants with an allele frequency >1%, effect sizes are small, and therefore large populations and often meta-analyses of populations are needed to reach sufficient statistical power. In this way, in the last 7 years, GWAS have identified many genetic variants associated with thyroid-related traits, the results of which are discussed below.

A. GWAS on hypothyroidism

Only two GWAS on hypothyroidism have been published. Using electronic medical records for case identification, Denny et al (177) published the first GWAS on hypothyroidism in 2011. A drawback of the second hypothyroidism GWAS by Eriksson et al (178) was that the identification of hypothyroidism cases was based on web-based questionnaires. The five loci found to be significant in these GWAS are discussed below.

1. Loci with an established role in autoimmunity

Four of the significant hypothyroidism loci had an established role in autoimmunity and include the *HLA* class I region, *PTPN22*, *SH2B3*, and *VAV3* loci. The *HLA* class I region emerged as a candidate region in the hypothyroidism GWAS by Eriksson et al (178). The detected rs2517532 polymorphism is located between the *HLA-E* and *HLA-C* genes. Variation in *HLA-C* has previously been associated with Graves' disease (GD) (179). These HLA class I region molecules are important for antigen presentation, including viral antigens, which have been suggested to play an important role in triggering AITD (39, 180). However, the exact effects of these variants on antigen presentation remain unclear at present.

PTPN22 is a lymphoid-specific intracellular phosphatase involved in the T-cell receptor signaling pathway. As discussed, early candidate gene studies had already associated genetic variation in this gene with AITD (39, 40, 173). Eriksson et al (178) also detected a significant association with rs6679677, which is located near *PTPN22*. This polymorphism is in high LD with the missense variant rs2476601 (p.620R>W), which has been previously associated with Hashimoto's thyroiditis (181). The results of a study by Menard et al (182) suggested that this mutation results in impaired removal of autoreactive B cells, as well as the up-regulation of genes such as *CD40*, *TRAF1*, and *IRF5*, which encode proteins that promote B-cell activation and have been identified as susceptibility genes also associated with other autoimmune disorders. Furthermore, variations in PTPN22, in particular p.620R>W, have been associated with various autoimmune disorders, such as type 1 diabetes mellitus (DM1), rheumatoid arthritis, and systemic lupus erythematosus (183–186).

SH2B3 encodes the adaptor protein LNK, a key negative regulator of T-cell cytokine signaling, which plays a critical role in hematopoiesis (187). Eriksson et al (178) were the first to find an association of genetic variation in *SH2B3* with hypothyroidism. The identified variant rs3184504 causes a p.262W>R substitution and had al-

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ready been associated with other autoimmune diseases, such as celiac disease, DM1, vitiligo, and rheumatoid arthritis (183, 188–190). It remains to be determined how this mutation affects the protein structure and its function.

Finally, the *VAV3* locus emerged as a candidate locus in the hypothyroidism GWAS by Eriksson et al (178). VAV3 is a guanine nucleotide exchange factor for Rho and Rac family GTPases. VAV3 is expressed in the thyroid and has been shown to be down-regulated in some subtypes of thyroid tumors (191), but there is no clear role for VAV3 in human thyroid physiology or autoimmunity. However, Fujikawa et al (192) have shown in mice that the VAV family proteins, including VAV3, play an important role in lymphocyte development and activation. Mouse *VAV3* has furthermore been suggested as a candidate gene for DM1 (193). Future human studies should investigate the potential role of VAV3 in thyroid autoimmunity.

2. FOXE1

FOXE1 is a transcription factor essential in thyroid development, mutations in which can lead to congenital hypothyroidism, as shown in Table 1 (194). *FOXE1* was also identified as a candidate gene for hypothyroidism in the GWAS by Denny et al (177). Associations were detected with four polymorphisms that were in strong LD, located 58–71 kb upstream from the *FOXE1* gene. The strongest association was with rs7850258, which was replicated in an independent set. One of the four polymorphisms (rs925489) was also the strongest hit in the hypothyroidism GWAS by Eriksson et al (178). Several studies have detected associations between *FOXE1* polymorphisms and the risk of follicular and papillary thyroid cancer (195–197). In this context, it is interesting to note that higher TSH levels have been associated with an increased risk of thyroid cancer and advanced-stage disease (198). As a second part of the hypothyroidism GWAS of Denny et al (177), a so-called phenome-wide association analysis was performed of the identified top *FOXE1* variant (rs7850258). In this way, associations were found with thyroiditis, nodular and multinodular goiters, and thyrotoxicosis. However, the associations in this phenome-wide approach need replication in independent cohorts, and the underlying biological mechanisms need to be clarified in future studies.

B. GWAS on hyperthyroidism

Two GWAS have been published for GD. Chu et al (199) performed the first GD GWAS in the Chinese Han population, and an extension of this study was published in 2013 by Zhao et al (200). These studies confirmed previously identified candidate genes, including *HLA* class II region genes, *TSHR*, *CTLA4*, and *FCRL3*, but also identified new candidates, which are discussed below.

1. Loci with an established role in autoimmunity

Similar to the hypothyroidism-associated loci, most of the loci found to be significant in GWAS for hyperthyroidism have a known role in autoimmunity or immunity in general, including *Tg*, *GPR174-ITM2A*, the *C1QTNF6-RAC2* locus, *SLAMF6*, and the 6q27 and 14q32.2 loci.

Various studies have investigated the relation between genetic variation in the *Tg* locus and AITD (40, 41, 201) and have led to conflicting results. Zhao et al (200) was the first to report an association between the *Tg* gene and GD in a GWAS setting. The authors additionally showed that the top polymorphism, rs2294025, influenced Tg splicing, skipping exon 46. Tg is located on chr 8q24.22 and is a key auto-antigen in the pathogenesis of GD with 40–70% of the GD patients having Tg antibodies (202). The role of Tg in AITD is underlined by the fact that Jacobson et al (203) generated an AITD mouse model by immunizing mice with human Tg. Furthermore, Nielsen et al (204) have shown that Tg antibodies promote the formation of complement-activating complexes, binding of immune complexes to B-cells, and the proliferation of B- and T-cell subsets.

The *GPR174-ITM2A* was the most significant new hit in the GD GWAS by Zhao et al (200). It is located on chr Xq21.1, which is of interest because GD is more prevalent among women. The top polymorphism, rs5912838, lies between the *GPR174* and *ITM2A* genes. *ITM2A* (integral membrane protein 2A) has been shown to escape X-chromosome inactivation and is induced during thymocyte selection and T-cell activation (205–207). At that time, little was known about GPR174, a G protein-coupled receptor. However, an X-chromosome-specific follow-up study on the GWAS by Chu et al (208) showed that a nonsynonymous variant located in *GPR174* (rs3827440 [p.162S>P]) was significantly associated with GD and affected *GPR174* mRNA levels in peripheral blood cells. GPR174 was furthermore shown to be widely expressed in immune-related tissues such as spleen, lymph nodes, thymus, bone marrow, and leukocytes, with a moderate expression in the thyroid. Therefore, future studies should investigate the distinct roles of GPR174 and ITM2A in the pathogenesis of GD.

Also, genetic variation in the *C1QTNF6-RAC2* locus has been associated with GD in the GWAS by Zhao et al (200). This locus has previously been associated with various autoimmune diseases, such as Crohn's disease, DM1, and multiple sclerosis (209, 210). RAC2 has been shown to play an important role in both T- and B-cell development and signaling (211–214), and *RAC2* mutations have

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been detected in the human neutrophil immunodeficiency syndrome (215). However, the role of *C1QTNF6* in (thyroid) autoimmunity remains to be established.

It has already been known for several years that the signaling lymphocytic activation molecule (SLAM) pathway members, including SLAMF6, have an important role in T-cell stimulation as well as in the pathogenesis of lupus in mice (216, 217). Recently, it has been shown by Menard et al (218) that this pathway also influences B-cell tolerance in humans, which is important for the development of autoimmunity. *SLAMF6* is located on chr 1q23.2, and this locus was also among the newly identified loci in the GWAS by Zhao et al (200) in which rs1265883 in intron 1 of *SLAMF6* was associated with an increased risk of GD.

The most significant new hit in the GD GWAS by Chu et al (199) was the 6q27 locus. This locus contains the *RNASET2*, *FGFR1OP* (fibroblast growth factor receptor 1 oncogene partner), and *CCR6* (chemokine CC motif receptor 6) genes. The top polymorphism, rs9355610, was associated with RNASET2 and FGFR1OP expression levels in peripheral blood mononuclear cells. Little is known about *RNASET2* and *FGFR1OP* in (auto)immunity, whereas CCR6 is a chemokine receptor preferentially expressed in immature dendritic cells and memory T cells (219). CCR6-deficient mice are resistant to autoimmune encephalitis, but there are no data available on their thyroid phenotype (220). Previous GWAS have also associated this locus with other autoimmune diseases, including rheumatoid arthritis, Crohn's disease, and vitiligo (190, 221, 222). Therefore, further studies are needed to determine the exact molecular mechanisms behind the observed associations with these various autoimmune diseases.

Finally, the 14q32.2 locus emerged as a GD candidate gene in the GWAS by Zhao et al (200). The top variant was located in an intergenic region, where the authors identified two new noncoding RNAs that they designated C14orf64 and "GD candidate gene at 14q32.2" (*GDCG14q32.2*). These were shown to be highly expressed in immune-related tissues, including thymus and CD4+ and CD8+ T cells. Finally, it is noteworthy that the 14q32.2 locus has previously been identified as a DM1 susceptibility locus (183).

2. Other loci

The *ABO* and 4p14 loci were also detected as susceptibility loci for hyperthyroidism (199, 200). The *ABO* gene encodes a glycosyltransferase that catalyzes the transfer of carbohydrates to the H antigen, forming the antigenic structure of the ABO blood groups. In recent years, the *ABO* gene has been associated with a remarkably wide range of diseases, such as myocardial infarction (223), ischemic stroke (224), venous thromboembolism (225), and esophageal and pancreatic cancer (226, 227). However, there is no clear explanation for these pleiotropic effects of genetic variation in ABO (228). Neither is there a documented role for ABO in thyroid physiology or autoimmunity. The mechanisms by which genetic variation in *ABO* alters the risk of GD therefore remain to be explored.

The 4p14 locus was identified as a new GD candidate locus in the GWAS by Chu et al (199). The top polymorphism, rs683215, is located between the *CHRNA9* and *RHOH* genes but is not in LD with variants in these genes. However, the authors identified a new gene 5 kb downstream of rs683215, which they designated "GD candidate gene at 4p14" (*GDCG4p14*), which was shown to be highly expressed in CD4+ and CD8+ T-cell subsets. Finally, rs683215 was shown to be correlated with *GDCG4p14* and *CHRNA9* expression levels in peripheral blood mononuclear cells. However, the exact roles of these genes in the pathogenesis of GD remains to be clarified.

C. GWAS on TPO antibodies

Whereas the previously discussed GWAS included cases with GD or hypothyroidism, we recently took a different approach to identify new AITD susceptibility loci, namely by performing a GWAS on TPO antibodies (TPOAbs), including 27 200 subjects from 16 populations (229). Because TPOAb positivity is associated not only with an increased risk of hypothyroidism (Hashimoto's thyroiditis), but also with an increased risk of hyperthyroidism (GD), loci found to be significant in this GWAS were additionally tested in relation to hypo- and hyperthyroidism. The most significantly associated polymorphism was located on chr 2p25 (rs11675434), 9 kb upstream of the *TPO* gene. As discussed, TPO plays an important role in TH synthesis, and mutations can lead to congenital hypothyroidism (CH) (Table 1). More recently, the only significant finding in a Korean TPOAb GWAS in two populations (n = 4238) was a variant 75 bp upstream of the TPO translation start site (rs2071403), which was associated with lower TPOAb levels (230). Interestingly, the authors also showed that this variant was associated with lower *TPO* mRNA levels in the thyroid. Whereas these *TPO* variants were strongly associated with TPOAbs, none of the studies detected associations with subclinical or overt hypo- or hyperthyroidism, suggesting less clinical relevance of these antibodies.

Some of the hits were associated not only with TPOAbs, but also with a higher risk of clinical thyroid disease, such as *BACH2*, which was associated with an increased risk of GD, as well as a borderline significantly increased risk of hypothyroidism. *BACH2* is specifically expressed in early stages of B-cell differentiation, represses different Ig genes,

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Figure 5.

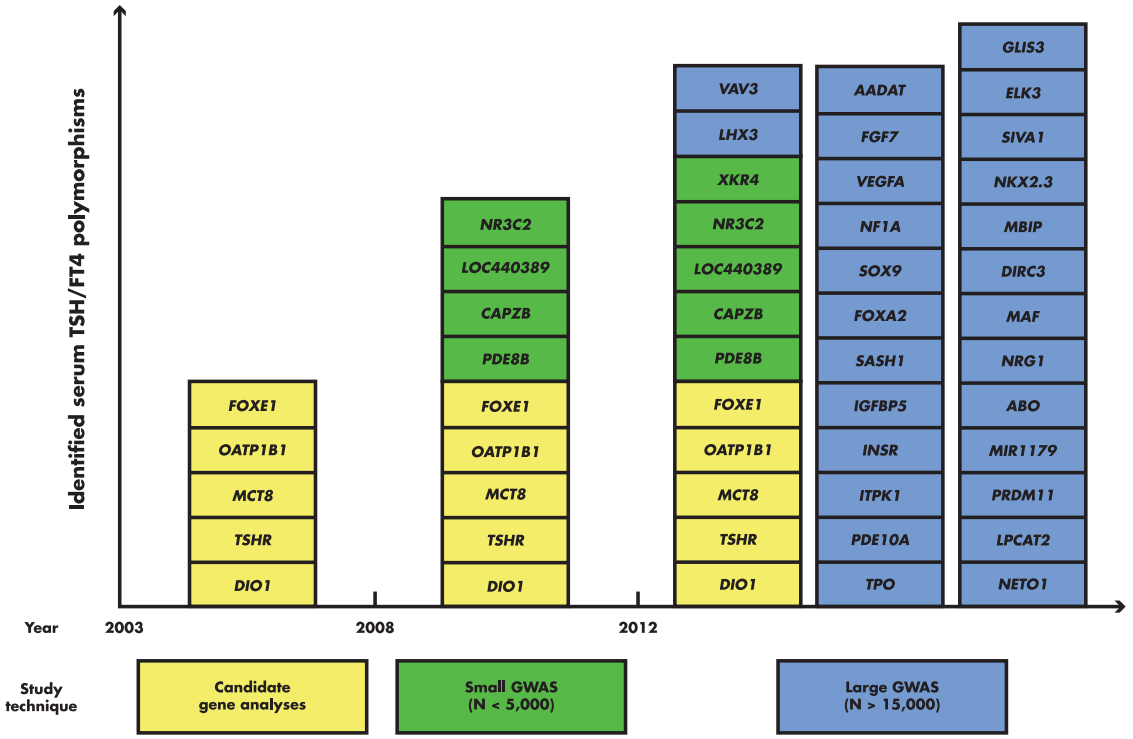

Figure 5. Identified serum TSH and/or FT4 associated polymorphisms over time, using different study techniques. Loci found to be significant in candidate gene analyses were included when associations were replicated in at least one independent population (n > 500) or in case of in vitro evidence for functionality.

and binds to the corepressor SMRT (silencing mediator of retinoid and thyroid receptor), suggesting a more direct effect on TH secretion and action as well (231). It has been associated with TPOAbs and AITD in previous candidate gene studies (176, 232) and implicated in the susceptibility to several other autoimmune diseases, including DM1, Crohn's disease, and multiple sclerosis (183, 233, 234).

Associations with TPOAbs, GD, and hypothyroidism were also found for a variant in the *MAGI3* gene. MAGI3 is a protein that modulates the activity of the AKT/PKB pathway (235), which plays a role in regulating apoptosis in the thyroid, a key step in the development of AITD (236, 237). However, rs1230666 is in LD with rs2476601, causing a p.620R>W substitution in *PTPN22*. As discussed, PTPN22 plays an important role in the T-cell receptor signaling pathway, genetic variants in which have been associated with various autoimmune diseases, including GD (183, 185, 186). Future studies should therefore clarify whether the observed associations could also be driven by linkage with disease-associated variants in *PTPN22*.

Finally, the *ATXN2* and *KALRN* loci were also identified as susceptibility loci for TPOAbs. *ATXN2* encodes the Ataxin-2 protein, which has no known role in the thyroid and has been implicated in the pathogenesis of spinocerebellar ataxia and Parkinson's disease (238, 239). However, the top associated polymorphism, rs653178, is in high LD with rs3184504, causing a p.262W>R substitution of SH2B3. As previously mentioned, SH2B3 is a key negative regulator of cytokine signaling with a critical role in hematopoiesis. *SH2B3* variants are associated with TPOAbs in DM1 patients, as well as with susceptibility to several other autoimmune diseases (183, 190, 232). Although the *KALRN* (*Kalirin*) gene has been suggested to play a role in megakaryopoiesis and platelet formation (240), there are no other data further clarifying the biological mechanism behind the association between *KALRN* and TPOAbs. This should therefore be investigated in future studies.

D. GWAS on HPT-axis setpoint

In the last 6 years, seven GWAS on serum TSH and/or FT4 levels have been published (99–102, 230, 241, 242), the largest being the GWAS by Porcu et al (100) and Gudmundsson et al (99). These GWAS have led to an enormous increase in the number of identified susceptibility loci for serum TSH and FT4 levels, as illustrated in Figure 5. Porcu et al (100) performed a GWAS on normal-range TSH and FT4 levels in 26 400 and 17 500 individuals, respectively, from 18 populations, resulting in the identi-

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fication of 26 genome-wide significant hits. A genetic risk score was calculated based on these new hits, indicating that carrying multiple risk alleles was associated with a higher risk of an increased TSH level. Gudmundsson et al (99) performed a GWAS on TSH in 27 700 Icelanders and identified 22 significant loci, three of which were also associated with thyroid cancer. Part of the identified loci in these GWAS included genes that were already known to affect thyroid parameters, such as *DIO1*, *FOXE1*, *GLIS3*, *LHX3*, *TPO*, and *VAV3*, but also included a large number of new candidate genes, which are discussed below.

1. Hits in the TSH signaling cascade

Part of the recently discovered candidate genes for serum thyroid parameters include genes with a role in the TSH signaling cascade. After binding of TSH to the TSHR, the cAMP signaling cascade is activated, and the family of phosphodiesterases is responsible for cAMP degradation, thereby inactivating this pathway. Genetic variation in intron 1 of the *PDE8B* gene is the most consistently reported significant hit in the various GWAS on serum TSH levels (99–101, 241). PDE8B is highly expressed in the thyroid and has the highest affinity for cAMP of any known phosphodiesterase (243). Its association with serum FT4 levels remains controversial (76, 99, 100, 244–246), but it is speculated that the identified variants increase PDE8B activity, resulting in lower cAMP levels in response to TSH. Consequently, a higher TSH level is required to maintain normal levels of TH. More recent studies have shown that genetic variation in *PDE8B* is associated with subclinical hypothyroidism in pregnancy and recurrent miscarriage (247, 248). In addition, Jorde et al (244) found in a large population study in Norway that genetic variation in *PDE8B* was associated not only with higher serum TSH levels, but also with reduced height and an increased risk of myocardial infarction.

PDE10A is another phosphodiesterase that is also highly expressed in the thyroid and has been associated with TSH levels in two GWAS (99, 100). It has been shown to degrade both cAMP and cGMP (249). Furthermore, genetic variation in *PDE10A* was associated with increased serum TSH levels in a large Alpine population (250).

Genetic variation in *CAPZB* has been related to serum TSH levels in various GWAS (12, 99, 100, 241). *CAPZB* (capping protein β) is highly expressed in the thyroid and encodes the two β-subunit isoforms of the barbed-end actin-binding protein. The TSH-induced extension of microvilli and filopodia protruding from the thyrocyte surface in the follicular lumen is a key step in TH production, in which CAPZB is thought to play an important role. In this way, Tg is endocytosed, endocytotic vesicles fuse with lysosomes, and proteolysis of Tg leads to the release of the iodothyronines. Teumer et al (251) postulated that the *CAPZB* variants result in an altered capping capacity, thereby affecting TH synthesis and leading to altered TSH levels. Depending on the directions of the effects, one could also expect compensatory hypo- or hyperplasia of the thyroid. In this respect, it is noteworthy that *CAPZB* was indeed one of the genome-wide significant hits in the thyroid volume and goiter GWAS by Teumer et al (251).

Another candidate gene in the TSH signaling cascade was identified in the GWAS by Porcu et al (100). An intergenic variant on chr 14q31 (rs11624776) was associated with serum TSH levels. This locus contained *ITPK1*, encoding the enzyme inositol 1,3,4-trisphosphate 5/6-kinase, which catalyzes the rate-limiting step in the formation of phosphorylated forms of inositol. It has been known for several years that TSHR couples not only to G_s, leading to cAMP activation, but also to G_q, activating the inositol phosphatase pathway (252). These data therefore suggest that ITPK1 plays a role in the TSH signaling cascade.

2. Hits in genes encoding transcription factors expressed in the thyroid

Two of the newly identified loci in GWAS of serum thyroid parameters included transcription factors expressed in the thyroid. In the GWAS by Porcu et al (100), a polymorphism on chr 17q23 located 5 kb downstream of *SOX9* (rs9915657) was associated with serum TSH levels. Besides being a transcription factor involved in chondrocyte differentiation and male sex determination, SOX9 is highly expressed in the thyroid. In 2002, Zhou et al (253) showed that SOX9 interacts with TRAP230, a component of the T_3 receptor-associated protein complex, suggesting an interaction between the TH signaling and SOX9 pathways.

Furthermore, genetic variation in *NF1A* has also been associated with serum TSH levels (100). *NF1A* encodes a member of the NF1 (nuclear factor 1) family of transcription factors, which play a role in various developmental processes (254). Several lines of evidence support an important role of these transcription factors in the control of TH synthesis. Fernández et al showed that NF1 binds simultaneously with FOXE1 to the *NIS* upstream enhancer region, activating the *NIS* promoter (255). Nakazato et al (256) have found that NF1 proteins, including NF1A, control constitutive repression of *TTF1* expression. Finally, NF1 proteins have been shown to interact with TTF-2 to control the expression of TPO (257).

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3. Hits encoding growth factors expressed in the thyroid

Vascular endothelial growth factor A (VEGFA) is a growth factor with a well-established role in angiogenesis, and it has been associated with benign and malignant tumors of the thyroid for a long time (258). However, in the GWAS by Gudmundsson et al (99) and Porcu et al (100), genetic variation in *VEGFA* was also found to be associated with serum TSH levels. Angiogenesis is particularly essential for the thyroid because the microvasculature supplies iodine and TSH, whereas iodine deficiency stimulates VEGFA secretion from thyrocytes via reactive oxygen species/hypoxia-inducible factor-1-dependent pathways (259). In addition, it has been shown that in the developing mouse thyroid, epithelial VEGFA production is necessary for endothelial cell recruitment and expansion, controlling epithelial reorganization in follicles and C-cell differentiation (260).

Fibroblast growth factor 7 (*FGF7*) has also been identified as a candidate gene for serum TSH levels. FGFs play an important role in the development of the thyroid, as well as in the progression of thyroid cancer (261, 262). Furthermore, *FGF7* was one of the significant hits in the goiter GWAS by Teumer et al (251). Future studies should clarify the exact molecular mechanism behind the observed associations between genetic variation in *FGF7*, serum thyroid parameters, and goiter.

It has been known for a long time that the GH/IGF-1 and TH signaling pathways interact (263, 264). Thyroid follicular cells have been shown to be the main source of IGF-1 in the thyroid (265). However, IGF-1 not only stimulates thyroid growth and TH production in an autocrine manner, but also influences production of TH-binding proteins and peripheral deiodination (263, 264). In turn, TH influences GH/IGF-1 production and signaling at various levels. It is therefore interesting that a number of hits in the serum TSH and FT4 GWAS were located in or near members of the GH/IGF-1 signaling pathway. Hits included the *INSR*, encoding the insulin receptor, and *IGFBP5*, a member of the IGF-1 binding protein family (99, 100). Enhanced thyroidal IGFBP5 production is correlated with inhibition of thyroid function, and has been shown to be significantly down-regulated in GD patients with ophthalmopathy compared to GD patients without ophthalmopathy (266, 267). Further hits associated with serum TSH levels included SASH1 and FOXA2 (99, 100), which are downstream targets of the GH/IGF-1 signaling pathway, whereas Lantz et al (268) have shown that FOXA2 also regulates insulin secretion (268–270).

Finally, there are a number of loci found to be significant in GWAS on serum thyroid parameters that do not have a documented role in thyroid signaling pathways, including *AADAT*, *NETO1/FBXO15*, *LPCAT2/CAPNS2*, *PRDM11*, *MIR1179*, *NRG1*, *MAF*, *DIRC3*, *NR3C2*, *MBIP*, *NKX2.3*, *SIVA1*, *XKR4*, and *ELK3* (99, 100, 242). We refer to the respective GWAS for further details on these genes (99, 100, 242). Future studies should obviously clarify the biological mechanisms behind the observed associations, possibly elucidating new pathways in thyroid (patho)physiology.

V. Discussion and Future Perspectives

Over the past few years, the introduction of GWAS has led to the identification of a large number of new candidate genes for thyroid (dys)function. As a proof of concept, it is reassuring to note that the hits also included well-known genes that had already been identified in candidate and linkage analyses. Because effect sizes are small, the individual variants have no direct clinical relevance in predicting thyroid disease, but the observed associations could identify new pathways in the pathogenesis of thyroid dysfunction. Many of the identified new variants are noncoding, located in intergenic regions or in loci that have no known role in TH signaling or autoimmunity. Unfortunately, for most of these variants, no attempts have been made to further understand the exact biological mechanism behind the observed associations, which is a crucial step in unraveling the pathogenesis of thyroid diseases.

Identification of new candidate genes and associated pathways can be of clinical importance for a number of reasons. First of all, new pathways may provide a focus for the design of new drugs for the treatment of thyroid diseases. Genetic variation may furthermore have a role in the choice and prediction of drug dosing and response. This has been nicely shown for a number of drugs in the cardiovascular field, including the cytochrome P450 (CYP)2C9 and vitamin K epoxide reductase (VKORC1) for warfarin treatment (271). Despite the few reports on the effects of D1 and D2 on treatment response, much remains to be learned about the role of genetic variation in TH pathway genes in the treatment of thyroid disorders (66, 135, 136).

The identification of new candidate gene variants can also be of potential use in the field of thyroid diagnostics. Porcu et al (100) have shown that subgroups with a substantially increased risk of hypothyroidism can be identified by combining multiple risk alleles. In our GWAS on TPOAbs, we have shown that with the use of only five polymorphisms, a large subgroup with an increased risk of TPOAb-positivity as well as increased TSH levels can be identified (229). Despite this, these currently available genetic markers for thyroid disorders lack sensitivity and

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specificity to be clinically useful. Further studies are therefore required to explain the remainder of the variation.

Studies have shown that patients on LT4 replacement therapy have a decreased well-being, despite having serum TH parameters within the reference range (14, 15). This suggests that these "normal" serum TH parameters do not match the patient's physiological setpoint. Therefore, the ultimate application of genetics in the treatment of thyroid diseases would be the use of genetic markers to reliably estimate an individual's setpoint, toward which then a personalized treatment can be directed. We are still very far from personalized treatment, although many risk loci have been identified in GWAS over the last few years. This is illustrated by the fact that, when combining all identified risk loci in the GWAS by Porcu et al (100), only 5.6 and 2.3% of the total variation in serum TSH and FT4 levels, respectively, could be explained.

Various approaches can be taken to clarify the unexplained part of the genetic heritability of thyroid (dys-)function. One obvious way is performing GWAS including a larger number of samples, thereby increasing power. The benefits of increasing sample size in GWAS have been comprehensively reviewed by Lindquist et al (272), who estimated that only one-fifth of all GWAS detectable polymorphisms underlying chronic diseases have been detected by GWAS so far. They furthermore conclude that increasing sample size has a much larger impact than increasing coverage on the potential of future GWAS to detect additional polymorphism-disease associations and heritability. This also seems to hold true for the thyroid field, where the benefits of increasing GWAS sample size have been illustrated by the fact that the more recent GWAS including more than 15 000 samples have been much more successful in identifying risk loci than the first GWAS including 2000 to 4000 samples (Figure 5) (99–102, 241).

It has been known for long that there are substantial differences in the prevalence of thyroid diseases between men and women, and the GWAS by Porcu et al (100) on serum TSH and FT4 levels detected a number of loci with sex-specific effects. It would therefore be interesting to also include the X-chromosome in these analyses, which has not been studied in most of the published thyroid GWAS. Furthermore, fine mapping involves screening all known risk variants from any available data sources, including HapMap, sequencing data etc, around the GWAS-identified variant. In this way, one can determine whether the identified effect is actually driven by another (more rare) marker that is in LD with the identified variant. As previously mentioned, fine mapping is important not only to further unravel the molecular mechanism underlying the observed associations but also to determine the true effects of a locus on thyroid function.

In recent years, it has become increasingly clear that various autoimmune diseases have a shared genetic basis (273). This is illustrated by the fact that a large part of the discussed loci found to be significant in GWAS in Section IV had also been associated with other autoimmune diseases. Newly identified susceptibility genes for other autoimmune diseases should therefore also be considered as potential candidate genes for thyroid (dys)function.

However, besides candidate gene analyses, GWAS, and fine mapping, a number of novel methods and approaches have emerged that will further improve our understanding of the genetic basis of thyroid (dys)function in the coming years. Copy number variations (CNVs) are genetic variations of a larger part of the genome, including insertions, duplications, and deletions. For a long time, it has been known that CNVs play an important role in the genetic basis of intellectual deficiencies, congenital anomalies, and autism spectrum disorders. However, very little is known about the role of CNVs in human thyroid (dys-)function. Huber et al (274) studied the effects of CNVs in *PTPN22* and *CD40* on GD, but these were too rare to be informative. Therefore, the potential role of CNVs in thyroid (dys)function still needs to be clarified by large-scale studies.

The fact that various loci associated with thyroid (dys-)function are located within the same pathways suggests that epistasis could also occur. However, gene-environment interactions should also be taken into consideration in explaining the remaining part of the susceptibility and variability of thyroid (dys)function. Despite the various challenges involved in these kinds of studies (275), including the requirement of even larger sample sizes, studies on gene-environment interactions would be especially interesting for thyroid disease, considering the multiple environmental factors that play a role in its pathogenesis, such as iodine status, smoking, and viral and bacterial infections (9, 39, 180, 276, 277).

Furthermore, GWAS only assesses 0.1% of the nucleotides of the genome, and therefore much can be expected from exome and whole-genome sequencing (Table 2), providing a complete catalog of all variants within the studied genomic region, rather than relying on markers or LD. Sequencing only the part of the genome that is protein coding (ie, the exome) is more cost-effective and targets the part that is most likely to directly affect protein structure/function, simplifying its biological interpretation. This technique has already proved itself in the thyroid field, elucidating mutations involved in the pathogenesis of familial goiter and thyroid cancer (278–280). Furthermore, it has been successfully used in the detection of mutations

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in *THRA* as a novel cause of RTH, mutations in *IGSF1* in patients with central hypothyroidism, and in the screening of patients with psychomotor retardation for *DIO2* variants (149, 150, 152, 281, 282). Besides higher laboratory and computational costs, more challenges have to be faced when sequencing the whole genome, mainly because of the fact that this technique identifies thousands of new variants in each individual. Sequencing nonaffected related family members is an effective way to filter out all shared mutations that are not relevant in causing the proband phenotype (283). Despite these challenges, much is expected from this approach, given its success in identifying the genetic causes of many other human disorders in the past few years (284). Furthermore, part of the results of these sequencing efforts are already available in public databases, such as variants detected in the NHLBI GO Exome Sequencing Project, thereby stimulating access and further use of these data by the general scientific community (285).

The techniques discussed above will likely lead to the identification of variants over the entire spectrum depicted in Figure 2, ranging from rare variants with large effects causing monogenic thyroid diseases to common variants with small effects causing polygenic thyroid diseases and variations in thyroid function tests (Figure 2).

Finally, besides investigating genetic variants, new technologies have emerged that investigate gene expression and its regulation. Studies of the epigenome characterize the control of gene expression, including DNA methylation, micro-RNAs, and histone modification, whereas transcriptomics analyzes the actual RNA levels. These techniques have also entered the thyroid field. For example, Ambrosio et al (286) have shown that LSD-1 and FoxO3 play an important role in the epigenetic control of *DIO2* and *DIO3* in myogenesis. As discussed, after *DIO2* had been suggested as a susceptibility locus for osteoarthritis, Bos et al (156) showed an increased amount of D2 protein in osteoarthritic vs normal cartilage. Although it has been known for a long time that *DIO3* is an imprinted gene, this imprinting has recently been shown to be tissue-dependent, the epigenetic control of which needs to be clarified in future studies (46). The addition of these dynamic expression data to the available data on genetic variation of the static DNA backbone is a crucial next step in unraveling the molecular mechanisms underlying thyroid function and dysfunction.

Addendum: Recently, Taylor et al. (287) meta-analyzed whole-genome sequence-based data and deeply imputed datasets from 7 cohorts (n = 16 335) for TSH and FT4 levels. For TSH, a new independent variant in PDE8B (rs2928167) was found, as well as a novel variant in synapsin 2 (SYN2) (rs310763). SYN2 is a member of a family of neuron-specific phosphoproteins involved in the regulation of neurotransmitter release and is expressed in the pituitary and hypothalamus. For FT4, a novel variant near B4GALT6 (rs113107469) was found. B4GALT6 (β-1,4-galactosyltransferase 6) plays a role in the ceramide metabolic pathway, which inhibits cAMP production in TSH-stimulated cells. However, rs113107469 also tags the p.139T>M substitution in transthyretin (TTR; rs28933981) and may therefore be a marker for this functional change in TTR. Finally, genome-wide complex trait analyses estimated that all common variants (minor allele frequency $\geq$1%) explained 24% and 20% of the variance in TSH and FT4, respectively. This study therefore showed that whole-genome sequence-based analysis is an effective technique to further unravel the genetic basis of thyroid function, while studies with larger sample sizes are needed to identify more rare variants with potentially large effects.

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