INVITED REVIEW

Genetic variation in thyroid hormone pathway genes; polymorphisms in the TSH receptor and the iodothyronine deiodinases

Robin P Peeters, Wendy M van der Deure and Theo J Visser

Department of Internal Medicine, Erasmus University Medical Center, Dr Molewaterplein 40, 3015 GD, Rotterdam, The Netherlands

(Correspondence should be addressed to R P Peeters; Email: r.peeters@erasmusmc.nl)

Abstract

Serum thyroid parameters show substantial inter-individual variability, in which genetic variation is a major factor. Findings in patients with subclinical hyper- and hypothyroidism illustrate that even minor alterations in serum thyroid function tests can have important consequences for a variety of thyroid hormone-related clinical endpoints, such as atherosclerosis, bone mineral density, obesity, and heart rate. In the last few years, several studies described polymorphisms in thyroid hormone pathway genes that alter serum thyroid function tests. In this review, we discuss the genetic variation in the TSH receptor and iodothyronine deiodinases. We discuss the possible consequences of these studies for the individual patient and also the new insights in thyroid hormone action that can be obtained from these data.

European Journal of Endocrinology 155 655–662

Introduction

Thyroid hormone is essential for growth and differentiation, for the regulation of energy metabolism, and for the physiological function of virtually all human tissues. The production of thyroid hormone is regulated by the classic hypothalamus–pituitary–thyroid axis, whereas the biological activity of thyroid hormone (i.e. the availability of the active hormone triiodothyronine (T3) for the nuclear thyroid hormone receptors) is mainly regulated at the tissue level by the iodothyronine deiodinases and thyroid hormone transporters.

In healthy subjects, serum thyroid parameters show substantial inter-individual variability, whereas the intra-individual variability is within a narrow range (1). This suggests an important influence of genetic variation, in addition to environmental factors such as food or iodine intake, on the regulation of thyroid hormone bioactivity, resulting in a thyroid function set-point that is different for each individual. This notion is supported by a classical twin study that was recently published (2). In this study, heritability accounted for ~65% of the variation in serum thyroid stimulating hormone (TSH), free thyroxine (FT4), and free T3 (FT3) levels. In a Mexican–American population, total heritability in serum thyroid parameters ranged from 26 to 64% of the total inter-individual variation observed (3).

Findings in patients with subclinical hyper- and hypothyroidism illustrate that even minor alterations

in thyroid hormone levels (and in thyroid hormone bioactivity) can have important consequences for a variety of thyroid hormone-related clinical endpoints, such as atherosclerosis, bone mineral density, obesity, and heart rate (4–6). In the last few years, several studies described polymorphisms in thyroid hormone pathway genes that result in an altered thyroid hormone bioactivity. Some of these polymorphisms are associated with serum TSH and/or thyroid hormone levels in healthy subjects, and/or with thyroid hormone-related clinical endpoints. As DNA variations are stable throughout life, such genetic effects are likely to have an influence during the lifetime of subjects.

In this review, we discuss the genetic variation in thyroid hormone pathway genes, focusing on the polymorphism studies that have emerged in the last few years. For the sake of brevity, we have focused on single nucleotide polymorphisms (SNPs) in the TSH receptor and iodothyronine deiodinases, since only these genes are presently analyzed for possible associations with serum thyroid hormone levels. Besides their relation with serum thyroid hormone levels, we discuss the effects of these polymorphisms on clinical endpoints such as Graves' disease and insulin resistance. Furthermore, we discuss the possible consequences of these studies for the individual patient, and also the new insights in thyroid hormone action that can be obtained from these data.

Polymorphisms in the TSH receptor

Many somatic gain-of-function mutations in the TSH receptor (TSHR) have been described, which result in a phenotype of toxic adenoma or toxic multinodular goiter (7). Germline gain-of-function TSHR mutations that result in congenital hyperthyroidism have also been described. Conversely, germline loss-of-function TSHR mutations are associated with TSH resistance and congenital hypothyroidism (see reference (8) for an illustrative example). However, only three germline TSHR polymorphisms, resulting in amino acid substitutions, have been identified (9–11). Two of these are located in the extracellular domain of the receptor (Asp36His and Pro52Thr) (9, 10), and one is located in the intracellular domain (Asp727Glu) (11) (Fig. 1). In addition, several intronic microsatellite markers and intronic SNPs have been described in TSHR (12–16).

The TSHR-Glu727 allele was associated with lower levels of plasma TSH in a population of healthy blood donors, but had no effect on FT4 (17). Recently, we have confirmed this observation in unrelated study populations (18, 19). This could point toward a higher sensitivity of the variant versus the wild-type TSHR, since less TSH is needed to produce normal FT4 levels. Although there is one *in vitro* study showing that the TSHR-Glu727 variant results in an increased cAMP response of the receptor to TSH (11), others have not been able to replicate this (20, 21). A different explanation would be that the Asp727Glu polymorphism is linked to another polymorphism

elsewhere in the gene. The TSHR-Asp727Glu polymorphism is found to be within a linkage disequilibrium block starting at intron 8 and extending about 10 kb beyond the 3'UTR of the *TSHR* gene (22).

Conflicting data are also available regarding the response of the TSHR-Pro52Thr variant to TSH stimulation (23–25). This might reflect the subtle effects of these polymorphisms. The TSHR-Pro52Thr and -Asp36His polymorphisms were not associated with changes in serum TSH or iodothyronine levels in healthy blood donors. However, this could be attributable to the low allele frequency of these SNPs (6 and 0.6% respectively) resulting in a lack of power.

No data on polymorphisms resulting in a relative loss of function of the TSHR are yet available. Subjects with a heterozygous loss of function mutation appear to have a dominant transmission of partial TSH resistance, which is due to intracellular entrapment and reduced maturation of the wild-type TSHR by the inactive mutants (26). Similarly, polymorphisms resulting in a relative loss of function could have an impact via this mechanism, and possibly account for some of the so-called 'euthyroid outliers' with elevated TSH determinations.

Besides effects on serum thyroid hormone levels, polymorphisms in the TSHR may also have effects on the development of autoimmune thyroid disease. The *TSHR* gene is located on chromosome 14q31, an area in which a Graves' disease susceptibility locus (GD-1) has been mapped (27). The GD-1 locus is

Figure 1 Exonic polymorphisms in the thyroid stimulating hormone receptor (TSHR) and the deiodinases that are described in this study (D1–3). The coding sequence is represented by , the 5' and 3'UTR by , whereas represents an alternatively spliced exon. A UGA codon, coding for selenocysteyl, is depicted by . Finally, selenocysteine insertion sequence elements are indicated by .

specifically linked to Graves' disease, but not Hashimoto's thyroiditis or autoimmune thyroid disease in general. Several case-control studies have been carried out analyzing the possible association between one or more of the previously mentioned TSHR polymorphisms and autoimmune thyroid disease. An overview of the 14 studies up to 2002 that analyzed the possible association of TSHR polymorphisms with Graves' disease has been presented by Ban *et al.* (28).

All studies analyzing the TSHR-Pro52Thr or Asp36His variant showed no association, apart from one in which an association of the TSHR-Pro52Thr variant with Graves' disease was described in US Caucasian females ($n=100$ females with autoimmune thyroid disease versus 69 controls) (29). These same authors later described two subjects who were homozygous for the Thr52 allele and had normal thyroid function tests, on the basis of which they suggested that the variant receptor is able to respond normally to TSH (23). Obviously, more subtle effects of this polymorphism cannot be excluded by the last study. In a multiethnic (Chinese, Malays, and Indians) cohort of patients with Graves' disease, TSHR-Asp36His was absent, and TSHR-Pro52Thr and -Asp727Glu were not associated with Graves' disease (30). Unfortunately, no data are presently available if the two variants in the extracellular domain of the receptor show altered binding of thyroid-stimulating antibodies. Nor are any data available regarding altered binding or altered cAMP response of the variant TSH receptor to a different TSHR ligand termed thyrostimulin that has recently been identified (31).

Three case-control studies in Caucasians showed no association between the TSHR-Asp727Glu polymorphism and Graves' disease (11, 28, 32). However, meta-analysis of these three studies (28), as well as a study in Russian patients ($n=78$ vs 93 controls), showed a weak association of the variant receptor with Graves' disease (33, 34). These Graves' patients showed a significantly higher frequency of the TSHR-Glu727 allele than did healthy subjects (33, 34). A recent transmission disequilibrium test (TDT) study in Russian families showed that the D2-Thr92Ala (see below) and TSHR-Asp727Glu polymorphisms are in weak linkage disequilibrium (35), and that the D2-Ala92/TSHR-Glu727 haplotype allele was preferentially transmitted from parents to affected siblings with Graves' disease (35). However, TDT is not the best design to analyze linkage disequilibrium.

A recent study in Japanese patients with autoimmune thyroid disease showed that several SNPs in intron 7 of the *TSHR* gene are significantly associated with Graves' disease (14). Another polymorphism in intron 4 of *TSHR* was associated with Graves' disease in a multiethnic population of patients from Singapore (30). Recently, a study in which common haplotype tagging SNPs in *TSHR* were analyzed showed a significant

association of intronic SNPs in one linkage disequilibrium block with Graves' disease (22).

These data together suggest that genetic variation in *TSHR* is, albeit weakly, associated with the development of Graves' disease, but it is yet unclear whether the associated polymorphisms are functional or whether they are linked to functional variants elsewhere in the gene or in the nearby genome that are still to be discovered. Meta-analysis, detailed linkage disequilibrium analysis, and haplotype tagging approaches (as well as the HapMap project, www.hapmap.org) should be able to resolve this issue (36).

TSHR is not only expressed in the thyroid, but also in adipose tissue (37), brain (38), orbital tissue (37, 39), lymphocytes (40), and bone (41). Evidence is accumulating for direct effects of TSH via the TSHR on these tissues. TSH is able to induce proliferation and inhibit differentiation in cultured rat pre-adipocytes (42), and *TSHR* knockout mice have a severe phenotype of osteoporosis, independent of their thyroid hormone levels (41). Genetic variation in *TSHR* may thus not only be important for the development of autoimmune thyroid disease (more specifically Graves' disease), but may also be associated with more common clinical endpoints such as osteoporosis (19), either via its influence on thyroid hormone levels, or via direct effects of TSH on bone.

Polymorphisms in the iodothyronine deiodinases

No patients with inactivating mutations in any of the iodothyronine deiodinases have yet been described. Whether this means that these mutations are not compatible with life, that they have little or no consequences, or that they result in unexpected phenotypes is unclear. Based on the phenotypes of mice with targeted deletions of D1, D2, or D3, the most severe effects would be expected of mutations in D3 (43–45). In the last few years, several polymorphisms in deiodinases have been described (17, 46, 47) (Fig. 1). Based on the physiological role of the three different deiodinases (48) (Table 1), one can speculate about the possible consequences of polymorphisms in these enzymes. D1 is present in liver, kidney, and thyroid, and plays a key role in the production of the active hormone T3 from T4 and in the clearance of the metabolite reverse T3 (rT3) (48, 49). D2 is present in brain, pituitary, brown adipose tissue, thyroid, skeletal muscle, aortic smooth muscle cells, and osteoblasts; D2 mRNA has also been detected in the human heart (48). In tissues such as the brain, D2 is important for local production of T3, whereas D2 in skeletal muscle may also contribute to plasma T3 production. D3 is present in brain, skin, placenta, pregnant uterus, and various fetal tissues, and is induced in critical illness (48, 50). D3 is the major T3 and T4 inactivating enzyme and

age of 69 years (RP Peeters, WM van der Deure, TJ Visser, unpublished observations).

Haplotype analysis showed that the D1-C785T and D1-A1814G polymorphisms appear on different haplotype alleles (17, 51). The haplotype allele containing D1-785T is not only associated with changes in serum iodothyronine levels, but also with increased levels of free insulin-like growth factor-I (IGF-I) in two unrelated populations (51). This was substantiated by the association of this haplotype allele with several IGF-I-related endpoints, such as increased muscle strength and muscle mass (51). As IGF-I has a stimulatory effect on D1 expression (55), these higher levels of free IGF-I might be seen as an adaptation to normalize D1 activity in carriers of the D1a-T haplotype allele. Conversely, thyroid hormone stimulates the expression of IGF-binding protein-I (IGFBP-I) in human hepatoma cells (56). A lower activation of thyroid hormone by liver D1 could result in lower levels of IGFBP-1, and thus a higher level of free IGF-I (51), as the majority of IGFBP-1 is produced in the liver.

D2

D2 is important in the production of local T3, but D2 in skeletal muscle also contributes to serum T3 production (48, 57). The above-mentioned association of D2-ORFa-Gly3Asp with the serum T3/T4 ratio also points toward an important role of D2 in serum T3 production (47, 51).

The first polymorphism described in any of the deiodinases was D2-Thr92Ala (46) (Fig. 1). Although this polymorphism does not seem to be associated with serum iodothyronine levels, it has been associated with insulin resistance in three different populations (46, 58, 59). The mechanism behind this association is yet unclear, but it might involve expression of D2 in skeletal muscle and/or in (brown) fat in humans (48). T3 stimulates the transcription of the muscle/fat-specific insulin-sensitive glucose transporter GLUT4 (60). In addition, thyroid hormone augments catecholamine-stimulated lipolysis (61), and a particular inactivating thyroid hormone receptor α mutation results in insulin resistance in mice (62). A decreased D2 activity in insulin-sensitive tissues such as adipose tissue and skeletal muscle, resulting in a decreased availability of local T3, may thus explain the association of D2-Thr92Ala with relative insulin resistance (46, 58, 59). Furthermore, it was recently shown that administration of bile acids to mice can increase energy expenditure, and thereby prevent obesity and insulin resistance via the induction of D2 (63). Alternatively, hypothalamic D2, which regulates the T3 content of brain stem neurons projecting to white adipose tissue, may be involved (64). Although D2 activity does not differ between different cells that are transfected with the D2-92Thr or the D2-92Ala variant (17, 58), a lower activity of D2 has been reported in muscle and thyroid

homogenates of carriers of the D2-Ala92 allele (58). This suggests that the consequences of the D2-Thr92Ala polymorphism are caused by linkage with another polymorphism. Haplotype analysis has shown that the D2-Thr92Ala polymorphism and the previously mentioned D2-ORFa-Gly3Asp polymorphism appear on different haplotype alleles (47). So far, there is no evidence of any relationship between the D2-ORFa-Gly3Asp polymorphism and insulin resistance.

Based on the expression pattern of D2, and since D2 is crucial in the regulation of local T3 concentrations, one can speculate about other possible consequences of these and other D2 polymorphisms. Guo *et al.* studied the relation of the *DIO2* gene with mental retardation in iodine-deficient areas of China in a case-control study ($n=96$ vs 331 controls) (65). They found a positive association of two intronic D2 polymorphisms (but not of D2-Thr92Ala) with mental retardation in these areas (65), and concluded that genetic variation in D2 may determine the risk of developing mental retardation in an iodine-deficient area, probably by affecting the local amount of T3 available in the brain (65). Appelhof *et al.* addressed the questions whether genetic variation in the *DIO2* gene is a determinant of well-being and neurocognitive functioning in hypothyroid patients on levothyroxine substitution, and whether D2 polymorphisms were associated with a preference for T4/T3 combination therapy over substitution with T4 alone (66). No differences in well-being, neurocognitive functioning or appreciation of T4/T3 combination therapy were detected in these thyroid hormone-replaced hypothyroid patients (66).

D3

Until now, only one polymorphism has been identified in D3 (D3-T1546G), located in the 3'-UTR (17). This polymorphism does not result in altered thyroid hormone levels in healthy individuals. D3 plays an important role in thyroid hormone homeostasis in critical illness and during fetal development, providing protection against thyroid hormone excess (50, 67). Possible effects of this polymorphism on development and under pathophysiological conditions therefore remain to be investigated in future studies. A major obstacle in these studies is that the *D3* gene is an imprinted gene, with preferential expression from the paternal allele, as has been studied in a mouse model (43). Therefore, the effects of polymorphisms in the *DIO3* gene on thyroid hormone homeostasis depend on the parental origin of the variant allele.

Concluding remarks and future perspectives

Here, we have discussed several polymorphisms in TSHR and the iodothyronine deiodinases that affect

serum thyroid hormone levels and/or have effects on thyroid hormone-related physiological endpoints. These polymorphism studies are important for several reasons. First, new insight can be obtained about the physiological function of thyroid hormone pathway genes. The hypothesis regarding a relative decrease in the contribution of D2 to serum T3 production (Fig. 2), based on the different associations of D1 and D2 polymorphisms in younger and elder populations, is an example of this (17, 51), as is the role of D2 activity in the development of insulin resistance (46, 47, 58). Second, genetic variation is important in inter-individual variation in thyroid hormone bioactivity (1–3). It seems that each individual has a different, genetically determined thyroid function set-point, and that small variations around this set-point, even within the normal range, can have important consequences on, for example, body weight (6). A better selection of subjects, by excluding subjects with autonomous thyroid nodules (68, 69), and standardized (regarding time of day) and perhaps multiple TSH measurements to better define an individual's set-point (1), would increase the power of such association studies. This raises the possibility of estimating an individual's set-point based on his/her genetic make-up of thyroid hormone pathway genes. The decision of whether a patient with subclinical changes in thyroid parameters should be treated might then be made on that individual patient's normal values. In addition, the decision to treat patients with subclinical thyroid disease is based on the risk of these patients developing complications. If the genetic profile makes a patient more vulnerable, then this might be an indication to initiate treatment in an earlier phase.

In addition to peripheral metabolism of thyroid hormone by the deiodinases, transmembrane transport of iodothyronines and expression of thyroid hormone receptors are other key processes in the regulation of thyroid hormone bioactivity. Surprisingly, no studies have yet been published investigating the association of polymorphisms in these transporters and receptors with clinical endpoints. This area of research remains to be explored, and it is likely that exciting new insights will be obtained in the upcoming years.

Acknowledgements

This work was supported by ZonMw Grant: 920-03-146 (RPP).

References

- 1 Andersen S, Pedersen KM, Bruun NH & Laurberg P. Narrow individual variations in serum T(4) and T(3) in normal subjects: a clue to the understanding of subclinical thyroid disease. *Journal of Clinical Endocrinology and Metabolism* 2002 **87** 1068–1072.

- 2 Hansen PS, Brix TH, Sorensen TI, Kyvik KO & Hegedus L. Major genetic influence on the regulation of the pituitary–thyroid axis: a study of healthy Danish twins. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 1181–1187.
- 3 Samollow PB, Perez G, Kammerer CM, Finegold D, Zwartjes PW, Havill LM, Comuzzie AG, Mahaney MC, Goring HH, Blangero J, Foley TP & Barmada MM. Genetic and environmental influences on thyroid hormone variation in Mexican Americans. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 3276–3284.
- 4 Toft AD. Clinical practice. Subclinical hyperthyroidism. *New England Journal of Medicine* 2001 **345** 512–516.
- 5 Cooper DS. Clinical practice. Subclinical hypothyroidism. *New England Journal of Medicine* 2001 **345** 260–265.
- 6 Knudsen N, Laurberg P, Rasmussen LB, Bulow I, Perrild H, Ovesen L & Jorgensen T. Small differences in thyroid function may be important for body mass index and the occurrence of obesity in the population. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 4019–4024.
- 7 Krohn K & Paschke R. Somatic mutations in thyroid nodular disease. *Molecular Genetics and Metabolism* 2002 **75** 202–208.
- 8 Sunthornthepvarakul T, Gottschalk ME, Hayashi Y & Refetoff S. Brief report: resistance to thyrotropin caused by mutations in the thyrotropin-receptor gene. *New England Journal of Medicine* 1995 **332** 155–160.
- 9 Sunthornthepvarakul T, Hayashi Y & Refetoff S. Polymorphism of a variant human thyrotropin receptor (hTSHR) gene. *Thyroid* 1994 **4** 147–149.
- 10 Gustavsson B, Eklof C, Westermark K, Westermark B & Heldin NE. Functional analysis of a variant of the thyrotropin receptor gene in a family with Graves' disease. *Molecular and Cellular Endocrinology* 1995 **111** 167–173.
- 11 Gabriel EM, Bergert ER, Grant CS, Van Heerden JA, Thompson GB & Morris JC. Germline polymorphism of codon 727 of human thyroid-stimulating hormone receptor is associated with toxic multinodular goiter. *Journal of Clinical Endocrinology and Metabolism* 1999 **84** 3328–3335.
- 12 Villanueva R, Inzerillo AM, Tomer Y, Barbesino G, Meltzer M, Concepcion ES, Greenberg DA, Maclaren N, Sun ZS, Zhang DM, Tucci S & Davies TF. Limited genetic susceptibility to severe Graves' ophthalmopathy: no role for CTLA-4 but evidence for an environmental etiology. *Thyroid* 2000 **10** 791–798.
- 13 Sale MM, Akamizu T, Howard TD, Yokota T, Nakao K, Mori T, Iwasaki H, Rich SS, Jennings-Gee JE, Yamada M & Bowden DW. Association of autoimmune thyroid disease with a microsatellite marker for the thyrotropin receptor gene and CTLA-4 in a Japanese population. *Proceedings of the Association of American Physicians* 1997 **109** 453–461.
- 14 Hiratani H, Bowden DW, Ikegami S, Shirasawa S, Shimizu A, Iwatani Y & Akamizu T. Multiple SNPs in intron 7 of thyrotropin receptor are associated with Graves' disease. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 2898–2903.
- 15 Akamizu T, Sale MM, Rich SS, Hiratani H, Noh JY, Kanamoto N, Saijo M, Miyamoto Y, Saito Y, Nakao K & Bowden DW. Association of autoimmune thyroid disease with microsatellite markers for the thyrotropin receptor gene and CTLA-4 in Japanese patients. *Thyroid* 2000 **10** 851–858.
- 16 De Roux N, Misrahi M, Chatelain N, Gross B & Milgrom E. Microsatellites and PCR primers for genetic studies and genomic sequencing of the human TSH receptor gene. *Molecular and Cellular Endocrinology* 1996 **117** 253–256.
- 17 Peeters RP, Van Toor H, Klootwijk W, De Rijke YB, Kuiper GG, Uitterlinden AG & Visser TJ. Polymorphisms in thyroid hormone pathway genes are associated with plasma TSH and iodothyronine levels in healthy subjects. *Journal of Clinical Endocrinology and Metabolism* 2003 **88** 2880–2888.
- 18 Hansen PS, Van Der Deure WM, Peeters RP, Iachine I, Fenger M, Sorensen TIA, Kyvik KO, Visser TJ & Hegedus L. The impact of a TSH receptor gene polymorphism (Asp727Glu) on thyroid function and size in a healthy Danish twin population. 31st Annual Meeting of the European Thyroid Association, Naples, Italy, 2006 (Abstract P 168).

- 19 Van Der Deure WM, Uitterlinden AG, Pols HAP, Peeters RP & Visser TJ. The TSH receptor Asp727Glu polymorphism is associated with higher bone mineral density and bone mineral content. 13th International Thyroid Congress, Buenos Aires, Argentina, 2005 (Abstract O 57).
- 20 Nogueira CR, Kopp P, Arseven OK, Santos CL, Jameson JL & Medeiros-Neto G. Thyrotropin receptor mutations in hyperfunctioning thyroid adenomas from Brazil. *Thyroid* 1999 **9** 1063–1068.
- 21 Sykietis GP, Neumann S, Georgopoulos NA, Sgourou A, Papachatzopoulou A, Markou KB, Kyriazopoulou V, Paschke R, Vagenakis AG & Papavassiliou AG. Functional significance of the thyrotropin receptor germline polymorphism D727E. *Biochemical and Biophysical Research Communications* 2003 **301** 1051–1056.
- 22 Dechairo BM, Zabaneh D, Collins J, Brand O, Dawson GJ, Green AP, Mackay I, Franklyn JA, Connell JM, Wass JA, Wiersinga WM, Hegedus L, Brix T, Robinson BG, Hunt PJ, Weetman AP, Carey AH & Gough SC. Association of the TSHR gene with Graves' disease: the first disease specific locus. *European Journal of Human Genetics* 2005 **13** 1223–1230.
- 23 Cuddihy RM, Bryant WP & Bahn RS. Normal function *in vivo* of a homozygotic polymorphism in the human thyrotropin receptor. *Thyroid* 1995 **5** 255–257.
- 24 Loos U, Hagner S, Bohr UR, Bogatkewitsch GS, Jakobs KH & Van Koppen CJ. Enhanced cAMP accumulation by the human thyrotropin receptor variant with the Pro52Thr substitution in the extracellular domain. *European Journal of Biochemistry* 1995 **232** 62–65.
- 25 Tonacchera M & Pinchera A. Thyrotropin receptor polymorphisms and thyroid diseases. *Journal of Clinical Endocrinology and Metabolism* 2000 **85** 2637–2639.
- 26 Calebiro D, De Filippis T, Lucchi S, Covino C, Panigone S, Beck-Peccoz P, Dunlap D & Persani L. Intracellular entrapment of wild-type TSH receptor by oligomerization with mutants linked to dominant TSH resistance. *Human Molecular Genetics* 2005 **14** 2991–3002.
- 27 Tomer Y, Barbesino G, Keddache M, Greenberg DA & Davies TF. Mapping of a major susceptibility locus for Graves' disease (GD-1) to chromosome 14q31. *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 1645–1648.
- 28 Ban Y, Greenberg DA, Concepcion ES & Tomer Y. A germline single nucleotide polymorphism at the intracellular domain of the human thyrotropin receptor does not have a major effect on the development of Graves' disease. *Thyroid* 2002 **12** 1079–1083.
- 29 Cuddihy RM, Dutton CM & Bahn RS. A polymorphism in the extracellular domain of the thyrotropin receptor is highly associated with autoimmune thyroid disease in females. *Thyroid* 1995 **5** 89–95.
- 30 Ho SC, Goh SS & Khoo DH. Association of Graves' disease with intragenic polymorphism of the thyrotropin receptor gene in a cohort of Singapore patients of multi-ethnic origins. *Thyroid* 2003 **13** 523–528.
- 31 Nakabayashi K, Matsumi H, Bhalla A, Bae J, Mosselman S, Hsu SY & Hsueh AJ. Thyrostimulin, a heterodimer of two new human glycoprotein hormone subunits, activates the thyroid-stimulating hormone receptor. *Journal of Clinical Investigation* 2002 **109** 1445–1452.
- 32 Muhlberg T, Herrmann K, Joba W, Kirchberger M, Heberling HJ & Heufelder AE. Lack of association of nonautoimmune hyperfunctioning thyroid disorders and a germline polymorphism of codon 727 of the human thyrotropin receptor in a European Caucasian population. *Journal of Clinical Endocrinology and Metabolism* 2000 **85** 2640–2643.
- 33 Chistiakov DA, Savost'anov KV, Turakulov RI, Petunina N, Balabolkin MI & Nosikov VV. Further studies of genetic susceptibility to Graves' disease in a Russian population. *Medical Science Monitor* 2002 **8** CR180–CR184.
- 34 Chistiakov DA. Thyroid-stimulating hormone receptor and its role in Graves' disease. *Molecular Genetics and Metabolism* 2003 **80** 377–388.
- 35 Chistiakov DA, Savost'anov KV & Turakulov RI. Screening of SNPs at 18 positional candidate genes, located within the GD-1 locus on chromosome 14q23–q32, for susceptibility to Graves' disease: a TDT study. *Molecular Genetics and Metabolism* 2004 **83** 264–270.
- 36 Ioannidis JP. Genetic associations: false or true? *Trends in Molecular Medicine* 2003 **9** 135–138.
- 37 Bell A, Gagnon A, Grunder L, Parikh SJ, Smith TJ & Sorisky A. Functional TSH receptor in human abdominal preadipocytes and orbital fibroblasts. *American Journal of Physiology. Cell Physiology* 2000 **279** C335–C340.
- 38 Crisanti P, Omri B, Hughes E, Meduri G, Hery C, Clauser E, Jacquemin C & Saunier B. The expression of thyrotropin receptor in the brain. *Endocrinology* 2001 **142** 812–822.
- 39 Bahn RS, Dutton CM, Natt N, Joba W, Spitzweg C & Heufelder AE. Thyrotropin receptor expression in Graves' orbital adipose/connective tissues: potential autoantigen in Graves' ophthalmopathy. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 998–1002.
- 40 Paschke R & Geenen V. Messenger RNA expression for a TSH receptor variant in the thymus of a two-year-old child. *Journal of Molecular Medicine* 1995 **73** 577–580.
- 41 Abe E, Marians RC, Yu W, Wu XB, Ando T, Li Y, Iqbal J, Eldeiry L, Rajendren G, Blair HC, Davies TF & Zaidi M. TSH is a negative regulator of skeletal remodeling. *Cell* 2003 **115** 151–162.
- 42 Haraguchi K, Shimura H, Kawaguchi A, Ikeda M, Endo T & Onaya T. Effects of thyrotropin on the proliferation and differentiation of cultured rat preadipocytes. *Thyroid* 1999 **9** 613–619.
- 43 Hernandez A, Fiering S, Martinez E, Galton VA & Germain D. St. The gene locus encoding iodothyronine deiodinase type 3 (Dio3) is imprinted in the fetus and expresses antisense transcripts. *Endocrinology* 2002 **143** 4483–4486.
- 44 Schneider MJ, Fiering SN, Pallud SE, Parlow AF, Germain DL, St & Galton VA. Targeted disruption of the type 2 selenodeiodinase gene (DIO2) results in a phenotype of pituitary resistance to T4. *Molecular Endocrinology* 2001 **15** 2137–2148.
- 45 Berry MJ, Grieco D, Taylor BA, Maia AL, Kieffer JD, Beamer W, Glover E, Poland A & Larsen PR. Physiological and genetic analyses of inbred mouse strains with a type I iodothyronine 5' deiodinase deficiency. *Journal of Clinical Investigation* 1993 **92** 1517–1528.
- 46 Mentuccia D, Proietti-Pannunzi L, Tanner K, Bacci V, Pollin TI, Poehlman ET, Shuldiner AR & Celi FS. Association between a novel variant of the human type 2 deiodinase gene Thr92Ala and insulin resistance: evidence of interaction with the Trp64Arg variant of the beta-3-adrenergic receptor. *Diabetes* 2002 **51** 880–883.
- 47 Peeters RP, Van Den Beld AW, Attalki H, Toor H, De Rijke YB, Kuiper GG, Lamberts SW, Janssen JA, Uitterlinden AG & Visser TJ. A new polymorphism in the type II deiodinase gene is associated with circulating thyroid hormone parameters. *American Journal of Physiology. Endocrinology and Metabolism* 2005 **289** E75–E81.
- 48 Bianco AC, Salvatore D, Gereben B, Berry MJ & Larsen PR. Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. *Endocrine Reviews* 2002 **23** 38–89.
- 49 Leonard JL & Koehle J. *Intracellular Pathways of Iodothyronine Metabolism* Philadelphia, PA, USA: Lippincott Williams & Wilkins, 2000.
- 50 Peeters RP, Wouters PJ, Kaptein E, Van Toor H, Visser TJ & Van Den Berghe G. Reduced activation and increased inactivation of thyroid hormone in tissues of critically ill patients. *Journal of Clinical Endocrinology and Metabolism* 2003 **88** 3202–3211.
- 51 Peeters RP, Van Den Beld AW, Van Toor H, Uitterlinden AG, Janssen JAMJL, Lamberts SWF & Visser TJ. A polymorphism in type I deiodinase (D1) is associated with circulating free IGF-I levels and body composition in humans. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 256–263.
- 52 Steinsapir J, Bianco AC, Buettner C, Harney J & Larsen PR. Substrate-induced down-regulation of human type 2 deiodinase

- (hD2) is mediated through proteasomal degradation and requires interaction with the enzyme's active center. *Endocrinology* 2000 **141** 1127–1135.
- 53 Donda A & Lemarchand-Beraud T. Aging alters the activity of 5'-deiodinase in the adenohypophysis, thyroid gland, and liver of the male rat. *Endocrinology* 1989 **124** 1305–1309.
 - 54 Gereben B, Kollar A, Harney JW & Larsen PR. The mRNA structure has potent regulatory effects on type 2 iodothyronine deiodinase expression. *Molecular Endocrinology* 2002 **16** 1667–1679.
 - 55 Hussain MA, Schmitz O, Jorgensen JO, Christiansen JS, Weeke J, Schmid C & Froesch ER. Insulin-like growth factor I alters peripheral thyroid hormone metabolism in humans: comparison with growth hormone. *European Journal of Endocrinology* 1996 **134** 563–567.
 - 56 Angervo M, Leinonen P, Koistinen R, Julkunen M & Seppala M. Tri-iodothyronine and cycloheximide enhance insulin-like growth factor-binding protein-1 gene expression in human hepatoma cells. *Journal of Molecular Endocrinology* 1993 **10** 7–13.
 - 57 Maia AL, Kim BW, Huang SA, Harney JW & Larsen PR. Type 2 iodothyronine deiodinase is the major source of plasma T3 in euthyroid humans. *Journal of Clinical Investigation* 2005 **115** 2524–2533.
 - 58 Canani LH, Capp C, Dora JM, Meyer EL, Wagner MS, Harney JW, Larsen PR, Gross JL, Bianco AC & Maia AL. The type 2 deiodinase A/G (Thr92Ala) polymorphism is associated with decreased enzyme velocity and increased insulin resistance in patients with type 2 diabetes mellitus. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 3472–3478.
 - 59 Peeters RP, Van Den Beld AW, Attalki H, Toor H, Kuiper GG, Lamberts SW, Janssen JA, Uitterlinden AG & Visser TJ. Polymorphisms in the type 2 deiodinase (D2) are associated with serum thyroid parameters and insulin resistance. 76th annual meeting of the American Thyroid Association, Vancouver, Canada, 2004 (P162).
 - 60 Casla A, Rovira A, Wells JA & Dohm GL. Increased glucose transporter (GLUT4) protein expression in hyperthyroidism. *Biochemical and Biophysical Research Communications* 1990 **171** 182–188.
 - 61 Viguerie N, Millet L, Avizou S, Vidal H, Larrouy D & Langin D. Regulation of human adipocyte gene expression by thyroid hormone. *Journal of Clinical Endocrinology and Metabolism* 2002 **87** 630–634.
 - 62 Liu YY, Schultz JJ & Brent GA. A thyroid hormone receptor alpha gene mutation (P398H) is associated with visceral adiposity and impaired catecholamine-stimulated lipolysis in mice. *Journal of Biological Chemistry* 2003 **278** 38913–38920.
 - 63 Watanabe M, Houten SM, Matak C, Christoffolete MA, Kim BW, Sato H, Messaddeq N, Harney JW, Ezaki O, Kodama T, Schoonjans K, Bianco AC & Auwerx J. Bile acids induce energy expenditure by promoting intracellular thyroid hormone activation. *Nature* 2006 **439** 484–489.
 - 64 Romijn JA & Fliers E. Sympathetic and parasympathetic innervation of adipose tissue: metabolic implications. *Current Opinion in Clinical Nutrition and Metabolic Care* 2005 **8** 440–444.
 - 65 Guo TW, Zhang FC, Yang MS, Gao XC, Bian L, Duan SW, Zheng ZJ, Gao JJ, Wang H, Li RL, Feng GY, Clair D, St & He L. Positive association of the DIO2 (deiodinase type 2) gene with mental retardation in the iodine-deficient areas of China. *Journal of Medical Genetics* 2004 **41** 585–590.
 - 66 Appelhof BC, Peeters RP, Wiersinga WM, Visser TJ, Wekking EM, Huyser J, Schene AH, Tijssen JGP, Hoogendijk WJG & Fliers E. Polymorphisms in type 2 deiodinase are not associated with well-being, neurocognitive functioning and preference for combined T4/T3 therapy. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 6296–6299.
 - 67 Kester MH, Martinez De Mena R, Obregon MJ, Marinkovic D, Howatson A, Visser TJ, Hume R & Morreale De Escobar G. Iodothyronine levels in the human developing brain: major regulatory roles of iodothyronine deiodinases in different areas. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 3117–3128.
 - 68 Berghout A, Wiersinga WM, Smits NJ & Touber JL. Interrelationships between age, thyroid volume, thyroid nodularity, and thyroid function in patients with sporadic nontoxic goiter. *American Journal of Medicine* 1990 **89** 602–608.
 - 69 Rieu M, Bekka S, Sambor B, Berrod JL & Fombeur JP. Prevalence of subclinical hyperthyroidism and relationship between thyroid hormonal status and thyroid ultrasonographic parameters in patients with non-toxic nodular goitre. *Clinical Endocrinology (Oxf)* 1993 **39** 67–71.

Received 9 June 2006

Accepted 29 August 2006