Novel insights into thyroid hormones from the study of common genetic variation

Colin M. Dayan and Vijay Panicker

Abstract | Effects of thyroid hormones in individual tissues are determined by many factors beyond their serum levels, including local deiodination and expression and activity of thyroid hormone transporters. These effects are difficult to examine by traditional techniques, but a novel approach that exploits the existence of common genetic variants has yielded new and surprising insights. Convincing evidence indicates a role of type 1 iodothyronine deiodinase (D1) in determining the serum T_4:T_3 ratio and a role of phosphodiesterase 8B in determining TSH levels. In addition, studies of type 2 iodothyronine deiodinase (D2) variants have shown that thyroid hormones contribute to osteoarthritis and these variants influence Intelligence quotient alterations associated with iodine deficiency. Preliminary evidence suggests associations between TSH-receptor variants and fasting glucose level, D1 variants and insulin-like growth factor I production, and D2 variants and hypertension, psychological well-being and response to T_3 or T_4 treatment. Intriguingly, most of these associations are independent of serum thyroid hormone levels, which highlights the importance of local regulation of thyroid hormones in tissues. Future research might reveal novel roles for thyroid hormones in obesity, cardiovascular disease, osteoporosis and depression and could have implications for interpretation of thyroid function tests and individualization of thyroid hormone replacement therapy.

Dayan, C. M. & Panicker, V. *Nat. Rev. Endocrinol.* **5**, 211–218 (2009); doi:10.1038/nrendo.2009.19

Introduction

Thyroid hormones have a role in a wide range of physiological processes from growth and development to homeostasis in the adult. Variation between individuals in the levels of thyroid hormones in tissues might, therefore, be anticipated to have important effects on many biological systems. Several studies indicated that such variation between individuals does exist, both in the supply of thyroid hormones and in many local factors that determine intracellular thyroid hormone levels separately from serum levels.[1,2] In this Review, we will discuss how important these differences are and how they can be studied.

Local regulation of T_3 and T_4 action

The thyroid gland releases a combination of T_4 and T_3 in a ratio of approximately 17:1.[3] Conversion of T_4 to T_3 is catalyzed by type 1 and type 2 iodothyronine deiodinases (D1 and D2, respectively), which influence the relative balance of these hormones in the circulation (reviewed by Bianco *et al.*[4]). To enter or exit cells, thyroid hormones must be transported across the cell membrane by thyroid hormone transporters,[5] which have different tissue distributions and ligand affinities. The properties of three thyroid hormone transporters (which, unlike many other transporting molecules, have a high affinity and consequently a high specificity for thyroid hormones) are summarized in Table 1. The activity of these transporters in any given tissue is likely to be a key factor in determining the effect of serum T_3 concentration on intracellular T_3 levels. T_4 is also actively transported into cells, but besides the activity of transporters, its contribution to intracellular T_3 levels is also determined by local and temporal variation in the activity of intracellular D2. Notably, the level of circulating free T_4 is typically five times higher than that of T_3, thus the potential contribution of serum T_4 to intracellular T_3 levels is very large. Studies in rats suggested that in tissues with very active uptake and local interconversion of thyroid hormones, such as the cerebral cortex, around 80% of intracellular T_3 is derived from serum T_4, whereas in other tissues (for example, in the kidneys) this fraction is as low as 13%.[6]

Although in rats the ratio of T_4 and T_3 secreted from the thyroid gland and the dependence of serum T_3 levels on the activity of D1 and D2 are different from those in humans, the above data suggest that various tissues are dependent on different amounts of local conversion of T_4 to T_3 in both species. Intracellular T_3 levels are also dependent on the rate of inactivation by conversion to di-iodothyronine (T_2) via the action of type 3 iodothyronine deiodinase (D3), and the rate of efflux from cells by transporters.[7] From the cytoplasm, T_3 enters the cell nucleus and binds to thyroid hormone receptors (THRs), and tissue-specific differences in the distribution and levels of the different isoforms of THRs, as well as of retinoic acid and other coregulator molecules, represent another level of local variation.[8] Figure 1 summarizes the key steps between thyroid hormone release and its effects in individual tissues.

Competing interests
The authors declared no competing interests.

Henry Wellcome Laboratories for Integrative Neurosciences and Endocrinology, University of Bristol, Bristol, UK (**CM Dayan**). Faculty of Medicine, Dentistry and Health Science, University of Western Australia, Australia (**V Panicker**).

Correspondence:
C Dayan,
Henry Wellcome Laboratories for Integrative Neurosciences and Endocrinology, University of Bristol, Dorothy Hodgkin Building, Whitson Street, Bristol BS1 3NY, UK
colin.dayan@bristol.ac.uk

Key points

- Various processes influence the action of thyroid hormones in different tissues, such as local deiodination and transport of thyroid hormones across cell membranes, independently of serum thyroid hormone levels
- Studies of commonly inherited variations have shown that the genes encoding type 1 iodothyronine deiodinase and phosphodiesterase 8B are important determinants of baseline serum thyroid hormone and TSH levels
- Polymorphisms that affect the thyroid hormone pathway influence osteoarthritis and a highly suggestive association has been reported between these polymorphisms and the neurodevelopmental response to iodine deficiency
- Polymorphisms might influence fasting glucose level, insulin-like growth factor I level, hypertension, psychological well-being and response to replacement therapy; many of these effects are independent of serum T_3/T_4 levels
- Studies of common genetic variation in large, diverse cohorts are likely to provide important novel insights into the role of thyroid hormones in health and disease

Table 1 | Characteristics of the specific thyroid hormone transporters

Characteristics	OATP1C1	MCT8	MCT10
Transported molecules	T_4 and rT_3	T_3 and T_4	T_3 and T_4
Tissue location	Brain (capillaries)	Brain, hypothalamus, pituitary gland, liver, heart, placenta	Intestines, liver, kidney, placenta
Possible physiological role	Transport of thyroid hormones across the blood–brain barrier	Transport of T_3 into neurons of the developing brain and transport of thyroid hormones into other tissues	Unknown
Clinical phenotype in its absence	Unknown	Raised T_3, low T_4 and normal TSH levels, hypotonia leading to spasticity, involuntary movements, absence of speech development, severe mental retardation	Unknown

Abbreviations: OATP1C1, organic anion transporter polypeptide 1 C1; MCT, monocarboxylase transporter; rT_3, reverse T_3.

This intricate system of local regulation allows cells and tissues to receive the appropriate amount of thyroid hormones, at an appropriate time in development, independent of serum thyroid hormone levels, and also provides a mechanism of autoregulation that protects tissues from variation in serum thyroid hormone levels. Note that the expression level of local regulating molecules is not static but can vary widely, depending on local thyroid hormone levels and other factors, such as the stage of development.

Implications of such regulation

From the endocrinologist's point of view, these new insights into local regulation of thyroid hormone action present several challenges. First, serum thyroid hormone levels cannot be assumed to indicate the thyroid hormone status of individual tissues. Second, if genetic differences between individuals determine the activity of elements of the thyroid hormone pathway (for example, transporters and deiodinases), the balance of serum T_3 and T_4 levels might require different interpretation from person to person. Finally, these findings imply that thyroid hormone replacement therapy might have to be individualized. In extreme conditions, such as severe hyperthyroidism or hypothyroidism, individual differences in local regulation become less important than the effects of very high or absent levels of serum thyroid hormones (although variation between individuals might have a role in the wide interindividual variation in the clinical presentation of these conditions). However, thyroid hormones have long-term effects on many processes—some of which endocrinologists were not previously aware (discussed below)—and in these processes, subtle interindividual variation in the thyroid hormone pathway over the lifetime of an individual is proving to be of increasing clinical importance.

Studies of common genetic variation

Common versus rare genetic variation

Recent advances in genetic research have increased our knowledge of the human genome and variation within it. In particular, our knowledge of single nucleotide polymorphisms (SNPs)—commonly inherited single-base changes that occur throughout the genome—has enabled us to obtain an improved map of the human genome and to study the genetic background of important biological parameters that have a polygenic basis, such as body weight or serum lipid levels.

Common genetic variation (mostly attributable to SNPs) usually causes minor alterations in function or activity, rather than mutations that lead to complete or near-complete inactivation of a single molecule in the thyroid hormone pathway. Major mutations (summarized in Table 2) are not only rare (most have not been observed in humans), but their effects are often both quantitatively and qualitatively different from those of common genetic variation, presumably because of the presence of compensatory pathways.

Interpreting studies on genetic variation

Studies of common genetic variation have the potential to answer many of the questions that the existence of local regulation of thyroid hormone presents to endocrinologists. For example, variation in *DIO2* (which encodes D2) or *DIO3* (which encodes D3) might demonstrate the effects of changes in intracellular T_3 levels in tissues without changes in circulating thyroid hormones. As described below, the results of genetic studies have provided new and surprising insights into thyroid hormone physiology, with potentially important clinical implications.

Studies of common genetic variation inevitably involve comparisons of many polymorphisms with many clinical traits, and these multiple comparisons result in an extremely high risk of type I statistical error. Very high levels of significance are, therefore, required to draw robust conclusions from such studies. For example, in genome-wide association studies that assess multiple markers, significance levels of greater than 5×10^{-7} are required.[9] Such a significance level can only be achieved in very large cohorts or by meta-analyses of studies in multiple cohorts.

Common polymorphisms as investigative tools

Up to 65% of baseline thyroid hormone and TSH levels are genetically determined,[10,11] and in any individual limited variation in thyroid hormone levels occurs over time.[12] The identification of common genetic variants that alter thyroid hormone levels provides us with valuable scientific tools in two ways. Firstly, such functional variants provide powerful evidence to identify molecules that have a role in determining serum thyroid hormone levels in humans. Secondly, once a particular polymorphism has been shown to alter the balance of thyroid hormones, it can be used in large cohort studies to explore the effects of changes in thyroid hormone levels on a wide range of biological systems.

Iodothyronine deiodinases

Polymorphisms in the *DIO1* gene (which encodes D1) have been associated with changes in the balance of thyroid hormones in the serum, typically raised T_3, low T_4 and low reverse T_3 levels, but, interestingly, these changes are not associated with any difference in TSH levels. This finding implies that the net effect of these changes in serum thyroid levels are perceived by the hypothalamus and the pituitary gland as 'neutral'.[13–16] These data have been reported at significance levels greater than those required for genome-wide association studies ($P = 3.6 \times 10^{-13}$ for free T_3:free T_4 ratio and $P = 2.1 \times 10^{-9}$ for free T_4).[15] The associations are similar in patients who receive levothyroxine treatment and those who have an intact hypothalamus–pituitary–thyroid axis. By contrast, studies of polymorphisms in *DIO2* and *DIO3* have revealed no effects on thyroid hormone levels except in one study of *DIO2*, the results of which have not yet been replicated (Table 3).[13–15,17–19] In the case of *DIO2,* convincing evidence shows that at least some of the common variants that have been studied are indeed functional in that they have effects on clinical phenotypes (see below). Table 3 summarizes the findings in this area that have been reported so far.

Phosphodiesterase 8B

Arnaud-Lopez and colleagues[20] performed genome-wide association studies to identify polymorphisms that are associated with altered TSH levels; their findings were confirmed by a meta-analysis that included multiple populations. The investigators found a strong association between rs4704397, an SNP in the gene that encodes phosphodiesterase 8B, and circulating TSH levels ($P = 1.9 \times 10^{-20}$ for a meta-analysis): individuals who carried one or two copies of the rare allele had 0.13 mIU/l or 0.26 mIU/l higher TSH levels, respectively, than those with no copies of it (T_4 and T_3 levels were not assessed in participants in this study).

Phosphodiesterase 8B is a protein that catalyzes the hydrolysis and inactivation of cyclic AMP and is found in the thyroid gland but not in the pituitary gland. Arnaud-Lopez and co-workers[20] suggest that phosphodiesterase 8B could affect TSH levels indirectly (via feedback), by affecting the generation of T_4 and T_3 in the thyroid gland. The SNP rs4704397 and the *DI01* SNPs mentioned above provide robust, novel tools to study the effect of changes in serum thyroid hormone and TSH levels on different body systems.

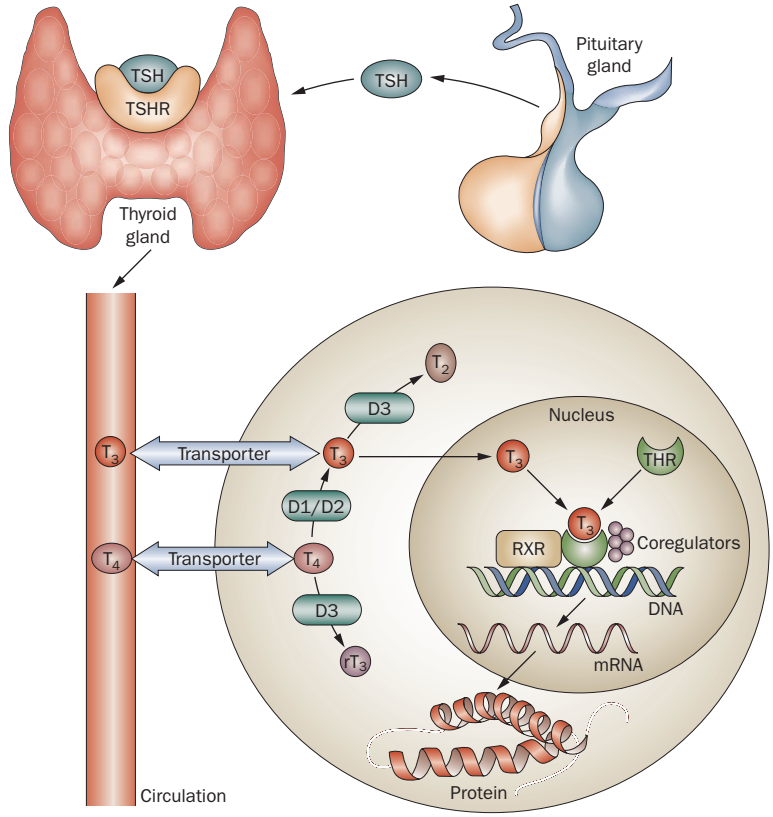

Figure 1 | The thyroid hormone pathway. Abbreviations: D1, deiodinase 1; D2, deiodinase 2; D3, deiodinase 3; rT_3, reverse T_3; RXR, retinoid X receptor; T_2, di-iodothyronine; THR, thyroid hormone receptor; TSHR, TSH receptor.

Thyroid hormone transporters

Several studies have reported associations between variants of thyroid hormone transporters and levels of thyroid hormones and TSH. Polymorphisms in *MCT8* are associated with changes in free T_4 level in men,[21] polymorphisms in *MCT10* with free T_3 and TSH levels,[21] polymorphisms in *OATP1C1* with free T_4, T_3 and reverse T_3 levels,[22] and polymorphisms in *OATP1B1* with free T_4 and reverse T_3 levels.[23] However, these associations have only been studied in small populations and have not yet been replicated.[24,25]

TSH receptor

One SNP in the TSH receptor (Asp727Glu, rs1991517) was associated with serum TSH level but not thyroid hormone levels in three studies that included more than 2,600 individuals in total.[13,26,27] This polymorphism has been proposed to lead to an increased sensitivity of the TSH receptor to TSH, which results in decreased serum TSH level whereas T_4 and T_3 levels do not change.

Table 2 | Effects of single-gene inactivations related to the thyroid hormone pathway in knockout mouse models and humans

Affected protein	T_4 level	T_3 level	TSH level	Phenotype
D1 (in knockout mice)[53]	High	Normal	Normal	Mild,[a] with peripheral tissue euthyroidism
D2 (in knockout mice)[54]	Very high[b]	Normal	Very high	Mild
D3 (in knockout mice)[55]	Very low[c]	Low	Normal or high	Partial perinatal mortality (greater than expected rate of mortality at or preceding birth), growth retardation and impaired fertility. Initial thyrotoxicity followed by central hypothyroidism
MCT8 (in knockout mice)[56]	Very low	Very high	Normal or high	Mild, with no gross neurological defects
TSH receptor (in knockout mice)[57]	Extremely low[d]	Extremely low	Extremely high[e]	Severe, with poor growth and early death postweaning
THRα1/α2 (in knockout mice)[58]	Low	Normal	Low	Severe, with small intestinal pathology, growth arrest, and death at 4 weeks
THRα1 (in knockout mice)[58]	Low	Normal	Low	Mild, with slowed heart rate and lower body temperature
THRβ (in knockout mice)[58]	Very high	High	Extremely high	Mild, with auditory deficit
MCT8 (in humans)[59]	Low	Very high	Normal	Allan–Herndon–Dudley syndrome, characterized by spastic paraplegia and severe cognitive impairment
THRβ (in humans)[60]	Very high	Very high	Normal or high	Resistance to thyroid hormone, characterized by goitre, delayed bone age and tachycardia

[a]Mice with 'mild' phenotypes have normal growth, development and ability to reproduce. [b]Approximately two to three times higher than the normal level; [c]Approximately two to three times lower than the normal level; [d]Approximately four times lower than the normal level; [e]Approximately four times higher than the normal level. Abbreviations: D1, deiodinase 1; D2, deiodinase 2; D3, deiodinase 3; MCT, monocarboxylase transporter; THR, thyroid hormone receptor.

Table 3 | Association of deiodinase SNPs and serum thyroid hormone parameters

SNP	Free T_4 level	T_3 or free T_3 level	T_3:T_4 ratio	rT_3 level	TSH level
In the DIO1 gene					
rs11206244[13–16]	Increased	Decreased	Decreased	Increased	No effect seen
rs12095080[13,14]	No effect seen	Increased	Results of association not published	rT_3:T_4 ratio decreased	No effect seen
rs2235544[15]	Decreased	Increased	Increased	Decreased	No effect seen
In the DIO2 gene					
rs225014[13–15,17–19]	No effect seen	No effect seen	No effect seen	No effect seen	No effect seen
rs12885300[17]	Decreased[a]	No effect seen	Increased[a]	Decreased[a]	No effect seen
In the DIO3 gene					
rs945006[13]	No effect seen	No effect seen	No effect seen	No effect seen	No effect seen

[a]Early, not replicated findings. Abbreviations: rT_3, reverse T_3; SNP, single nucleotide polymorphism.

Thyroid hormone receptor

Only one polymorphism affecting THR has been studied, in *THRB*, and no relationship was observed between this polymorphism and serum thyroid hormone levels.[13] This finding contrasts with the observation that a major mutation of this gene leads to resistance to thyroid hormone (Table 2).

Common polymorphisms and clinical phenotypes

Common polymorphisms that affect the thyroid hormone pathway could influence biological systems in two ways: either by changing serum thyroid hormone levels or by changing the intracellular availability of T_3. The genes described in the previous section (*DIO1* and the gene encoding phosphodiesterase 8B) provide tools to study the former effect, whereas other genes, notably *DIO2,* provide tools to study the effects of changes in intracellular thyroid hormone levels, independently of changes in their serum levels. The effects of common genetic variation on biological systems need to be studied in larger cohorts than those needed to study their effects on thyroid hormone levels, as the more downstream an effect is from the effector molecules (for example, thyroid hormones), the less accurately it can be measured. As the majority of the latest findings in this area were obtained from relatively small cohorts, they require replication;

nonetheless, these studies have provided intriguing insights (Box 1).

Bone and joints

The first example in which a robust effect of thyroid hormone-related polymorphisms has been confirmed is osteoarthritis. Thyroid hormones are vital for normal bone development and maintenance.[28] Meulenbelt and colleagues[29] performed a genome-wide linkage scan and found an association between the *DIO2* polymorphism rs225014 and generalized osteoarthritis. Further linkage scans in three other cohorts that included more than 4,500 individuals in total revealed associations between a haplotype containing the rare allele of rs225014 and the common allele of rs12885300 (also in *DIO2*) and osteoarthritis ($P = 2.02 \times 10^{-5}$). The authors proposed that impaired function of this gene could lead to relative hypothyroidism in tissues and the development of osteoarthritis, as D2-catalyzed conversion of T_4 to T_3 in the growth plate is required for chondrocyte differentiation and bone matrix synthesis. The rs225014 SNP is a coding-sequence polymorphism, located at a site of *DIO2* that is important for D2 turnover because it affects ubiquitin-mediated degradation of D2 within cells.[30]

Polymorphisms in the TSH-receptor gene affect serum TSH levels[13,26] and TSH levels are related to bone formation.[31] Van der Deure and co-workers[27] assessed 1,089 individuals and found that those who carried the allele associated with the Glu727 variant of the TSH receptor had 2.3% higher femoral neck BMD ($P = 0.03$) and 12.6% lower TSH level ($P = 0.04$) that those who were homozygotes for the wild-type allele (Asp727). The association with BMD persisted when TSH was added as a covariate. These findings suggest that TSH might contribute to the regulation of bone formation through the action of TSH receptors.

Brain development and neurocognitive function

The developing brain is particularly sensitive to local T_3 levels and D2 is likely to be responsible for maintaining brain T_3 levels in iodine deficiency. This hypothesis is supported by the work of Guo and colleagues,[32] who used a case–control design (which is different from that of other association studies mentioned here) to compare the prevalence of *DIO2* SNPs in children who had mental retardation with that in children with normal intelligence quotients in a iodine-deficient region of China. The investigators found significant differences between the two groups in the frequency of the haplotype combination of rs225010 and rs225012 ($P = 0.0005$). These results suggest that individual differences in the tolerance of low iodine levels during development might be explained by genetic variation in *DIO2*.

A study that used MRI to assess morphological features of early Alzheimer dementia did not find any association between *DIO1* or *DIO2* polymorphisms and the volume of the most affected areas in this condition, the hippocampus and the amygdala.[14]

Box 1 | Effects of SNPs on clinical phenotypes

***DIO2* polymorphisms**
- Osteoarthritis[a]
- Mental retardation in iodine-deficiency
- Psychological well-being in patients on levothyroxine treatment
- Response to combination therapy in patients on levothyroxine treatment

***DIO1* polymorphisms**
- IGF-I level

TSH-receptor gene polymorphisms
- Bone density
- Insulin resistance and metabolic parameters

***OATP1C1* polymorphisms**
- Psychological well-being in patients on levothyroxine treatment

[a]Confirmed at high level of significance ($P = 2 \times 10^{-5}$). Abbreviation: SNP, single nucleotide polymorphism.

Liver function and IGF-I level

Adults with hypothyroidism are reported to have low insulin-like growth factor I (IGF-I) levels, whereas hyperthyroid individuals have normal or high IGF-I levels. The level of IGF-I normalizes on attainment of euthyroidism.[33] Peeters *et al.*[34] found an association between a haplotype of two *DIO1* SNPs and increased IGF-I levels in 156 blood donors ($P = 0.02$) and 350 elderly men ($P = 0.01$), which suggests a possible local effect of D1 activity in the liver. In elderly men, this haplotype was also associated with increased lean body mass ($P = 0.03$) and improved muscle strength ($P = 0.047$). These findings suggest that the increase in IGF-I level was clinically relevant.

Insulin resistance and diabetes mellitus

Previous studies have reported conflicting results on the association between insulin resistance and D2 polymorphisms, particularly rs225014. Three small studies showed associations between measures of insulin resistance and glucose utilization and rs225014 in both healthy individuals and people with diabetes mellitus.[35–37] Furthermore, one of these studies demonstrated decreased activity of D2 in biopsy samples that were obtained from the thyroid gland and skeletal muscle in patients with diabetes mellitus who were homozygous for the rare allele (Ala92).[36] Three large studies[18,38,39] (total $n > 10{,}000$), however, have demonstrated no association between rs225014 and any metabolic markers. Taken together, currently available data do not provide sufficient evidence of associations between this polymorphism and type 2 diabetes mellitus, BMI or insulin sensitivity.

In one study, which requires replication, a significant gene–gene interaction was observed between the rs225014 (Thr92Ala) polymorphism of *DIO2* and the Pro12Ala polymorphism of the *PPARγ2* gene—a gene that is thought

to have a role in determining insulin-resistant phenotypes. This interaction was associated with elevated systolic ($P = 0.01$) and elevated diastolic ($P = 0.02$) blood pressure and an increased risk of metabolic syndrome ($P = 0.02$) in 590 nondiabetic individuals. Among these patients, carriers of the 92Ala variant of rs225014 and the Ala12 variant of *PPARγ2* displayed the most severe symptoms.[40]

Peeters *et al.*[37] have demonstrated associations between the 727Glu variant of the TSH receptor and levels of fasting glucose ($P = 0.01$), fasting insulin ($P = 0.001$), HbA_{1c} ($P = 0.002$), leptin ($P = 0.008$) and homeostasis model assessment (HOMA) scores ($P = 0.001$). This study implies that in those who carry the 727Glu variant, the increased activity of the TSH receptor (which is known to be expressed in human adipose tissue) might cause increased adipogenesis, which subsequently increases leptin level and insulin resistance. However, as this study was performed in a population of elderly men, replication in large, diverse samples is required.

Hypertension

DIO2 is expressed in vascular smooth muscle cells and T_3 acts as a vasodilator, hence relative tissue hypothyroidism could lead to hypertension. Gumieniak *et al.*[19] found an increased frequency of the rare allele of the *DIO2* polymorphism, rs225014, in 372 hypertensive individuals (odds ratio 2.11, $P = 0.01$). Another study, however, did not find any association between rs225014 and blood pressure,[36] and a third one found an association only for rs225014 combined with the Pro12Ala polymorphism of *PPARγ2*,[40] hence the relation between this polymorphism and hypertension is yet to be clarified.

Thyroid hormone replacement therapy

In the brain, thyroid hormone levels are closely regulated by the action of thyroid hormone transporters and local deiodination.[41,42] Thyroid hormones must be first transported across the blood–brain barrier (by OATP1C1 and possibly by other transporters as well). T_4 is then converted to T_3 within astrocytes by D2, and T_3 is transported into neurons by MCT8.[7] Animal models have shown that this process can protect brain cells from changes in serum thyroid hormone levels very effectively.[43]

Individuals who receive thyroid hormone replacement therapy are unable to respond to low serum T_3 levels by increased production of T_3 in the thyroid gland. Insufficient compensation of low T_3 level in some individuals might have detrimental effects especially in those tissues where close regulation of thyroid hormone levels is particularly important, such as the brain.[6] These effects might explain the fact that patients who receive thyroid hormone replacement therapy have reported decreased well-being.[44,45] To investigate this phenomenon further, our research group has studied the effects of 16 tag SNPs (representative SNPs in a region of the genome with high linkage disequilibrium, which were chosen from the 'HapMap' haplotype database of the human genome) in the three deiodinase genes in 552 individuals on levothyroxine treatment. We found that SNPs in *DIO2*, particularly rs225014, were associated with impaired psychological well-being ($P = 0.02$).[46] We found no association between SNPs in *DIO1* or *DIO3* and well-being, which is consistent with the fact that D2 is the only activating deiodinase that is present in the brain.[6] The detrimental effect of *DIO2* polymorphisms on well-being might result from a reduced ability to upregulate D2 function in the brain in response to low local T_3 levels, which would be particularly apparent in individuals who receive T_4 replacement alone, as these patients have a decreased T_3:T_4 serum ratio.[47,48] In a small study that included 141 patients on T_4 replacement, Appelhof *et al.*[49] observed a similar trend towards an association between rs225014 and impaired well-being, although it had low statistical significance ($P = 0.11$ to 0.13, with limited power due to sample size). Van der Deure *et al.*[50] investigated the effect of polymorphisms in *OATP1C1,* which encodes a brain-specific thyroid hormone transporter, on well-being in the same 141 patients and showed an association between rs10770704 and increased fatigue and depression. Interestingly, the investigators found no association between this polymorphism and results in neurocognitive tests in these individuals, which suggests that thyroid hormones affect well-being and cognitive function via distinct mechanisms.

The effect of polymorphisms on the response to levothyroxine monotherapy or combination T_4 and T_3 therapy is still a controversial area, as clinical trials have not shown any benefit from combination therapy as compared with monotherapy.[51] In 552 patients (270 on T_4 and T_3, 282 on T_4 only) we found a significantly better response to T_4 and T_3 therapy than to T_4 therapy in those with the rare allele of rs225014 (which was associated with the lowest baseline scores for well-being on T_4 therapy) as measured by the general health questionnaire (GHQ-12; $P = 0.03$), thyroid symptom questionnaire ($P = 0.03$) and satisfaction scores ($P = 0.02$). Combination therapy was associated with a mean improvement in GHQ-12 scores of 2.3 points after 3 months of treatment.[46] Consistent with these findings, another study has demonstrated that patients with thyroid cancer who carried rs225014 required a higher dose of levothyroxine to suppress TSH than patients with the same TSH levels who did not carry this polymorphism. This finding might reflect decreased D2 activity in the pituitary gland of patients with rs225014.[52]

In two relatively small studies, no association was found between either version of thyroid hormone replacement therapy and D2 or *OATP1C1* polymorphisms.[49,50] All these studies retrospectively genotyped the participants, which means that these studies were not set up to investigate genotypic differences and their results have to be confirmed in prospective studies that are randomized by the genotype of participants.

Conclusions

Genetic association studies have increased our understanding of thyroid hormone regulation and action. The D1 deiodinase seems to have a key role in determining

the balance of T_3 and T_4 in the serum and phosphodiesterase 8B has been demonstrated to determine TSH levels. A robust association has been observed between *DIO2* polymorphisms and generalized osteoarthritis, and one study suggests an association of these polymorphisms with the ability of the brain to compensate for iodine deficiency. Furthermore, possible associations have been reported between TSH-receptor polymorphisms and osteoporosis and fasting glucose, *DIO1* polymorphisms and IGF-1 production, and *DIO2* polymorphisms and hypertension, psychological well-being and response to thyroid hormone treatment. Intriguingly, in the majority of these body systems, the effects of genetic variation were seen independently of serum thyroid hormone levels, highlighting the importance of local regulation of thyroid hormone in tissues. For the endocrinologist, genetic association studies provide new tools to examine the long-term effects of small changes in thyroid function on different tissues as well as to deepen our knowledge of thyroid hormone replacement therapy. Our current views of the importance of thyroid hormone and how to replace it seem likely to be challenged by surprising new findings in the near future.

Review criteria

PubMed was searched for articles published between 1970 and August 2008, using the search terms "thyroid", "genetic", "polymorphism" and "SNP".

1. Saravanan, P. & Dayan, C. M. Understanding thyroid hormone action and the effects of thyroid hormone replacement—just the beginning not the end. *Hot Thyroidology* [http://www.hotthyroidology.com/editorial_135.html] (accessed 5 February 2009).
2. Schweizer, U., Weitzel, J. M. & Schomburg, L. Think globally: act locally. New insights into the local regulation of thyroid hormone availability challenge long accepted dogmas. *Mol. Cell. Endocrinol.* **289**, 1–9 (2008).
3. Pilo, A. *et al.* Thyroidal and peripheral prodsuction of 3, 5, 3'-triiodothyronine in humans by multicompartmental analysis. *Am. J. Physiol.* **258**, E715–E726 (1990).
4. Bianco, A. C. & Kim, B. W. Deiodinases: implications of the local control of thyroid hormone action. *J. Clin. Invest.* **116**, 2571–2579 (2006).
5. Hennemann, G. *et al.* Plasma membrane transport of thyroid hormones and its role in thyroid hormone metabolism and bioavailability. *Endocr. Rev.* **22**, 451–476 (2001).
6. Bianco, A. C., Salvatore, D., Gereben, B., Berry, M. J. & Larsen, P. R. Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. *Endocr. Rev.* **23**, 38–89 (2002).
7. Visser, W. E., Friesema, E. C., Jansen, J. & Visser, T. J. Thyroid hormone transport in and out of cells. *Trends Endocrinol. Metab.* **19**, 50–56 (2008).
8. Yen, P. M. Physiological and molecular basis of thyroid hormone action. *Physiol. Rev.* **81**, 1097–1142 (2001).
9. WTCCC. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* **447**, 661–678 (2007).
10. Hansen, P. S., Brix, T. H., Sorensen, T. I., Kyvik, K. O. & Hegedus, L. Major genetic influence on the regulation of the pituitary-thyroid axis: a study of healthy Danish twins. *J. Clin. Endocrinol. Metab.* **89**, 1181–7118 (2004).
11. Panicker, V. *et al*. Heritability of serum TSH, free T4 and free T3 concentrations: a study of a large UK twin cohort. *Clin. Endocrinol. (Oxf.)* **68**, 652–659 (2008).
12. Andersen, S., Pedersen, K. M., Bruun, N. H. & Laurberg, P. 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.* **87**, 1068–1072 (2002).
13. Peeters, R. P. *et al*. Polymorphisms in thyroid hormone pathway genes are associated with plasma TSH and iodothyronine levels in healthy subjects. *J. Clin. Endocrinol. Metab.* **88**, 2880–2888 (2003).
14. de Jong, F. J. *et al*. The association of polymorphisms in the type 1 and 2 deiodinase genes with circulating thyroid hormone parameters and atrophy of the medial temporal lobe. *J. Clin. Endocrinol. Metab.* **92**, 636–640 (2007).
15. Panicker, V. *et al*. A common variation in deiodinase 1 gene *DIO1* is associated with the relative levels of free thyroxine and triiodothyronine. *J. Clin. Endocrinol. Metab.* **93**, 3075–3081 (2008).
16. van der Deure, W. M. *et al*. The effect of genetic variation in the type 1 deiodinase gene on the inter-individual variation in serum thyroid hormone levels. An investigation in healthy Danish twins. *Clin. Endocrinol. (Oxf.)*, [doi:10.1111/j.1365–2265200803419.x] (2008).
17. Peeters, R. P. *et al*. A new polymorphism in the type II deiodinase gene is associated with circulating thyroid hormone parameters. *Am. J. Physiol. Endocrinol. Metab.* **289**, E75–E81 (2005).
18. Mentuccia, D. *et al*. The Thr92Ala deiodinase type 2 (*DIO2*) variant is not associated with type 2 diabetes or indices of insulin resistance in the old order of Amish. *Thyroid* **15**, 1223–1227 (2005).
19. Gumieniak, O. *et al*. Ala92 type 2 deiodinase allele increases risk for the development of hypertension. *Hypertension* **49**, 461–466 (2007).
20. Arnaud-Lopez, L. *et al*. *Phosphodiesterase 8B* gene variants are associated with serum TSH levels and thyroid function. *Am. J. Hum. Genet.* **82**, 1270–1280 (2008).
21. van der Deure, W. M., Peeters, R. P. & Visser, T. J. Genetic variation in thyroid hormone transporters. *Best Pract. Res. Clin. Endocrinol. Metab.* **21**, 339–350 (2007).
22. van der Deure, W. M. *et al*. Thyroid hormone transport and metabolism by organic anion transporter 1C1 and consequences of genetic variation. *Endocrinology* **149**, 5307–5314 (2008).
23. van der Deure, W. M. *et al*. Organic anion transporter 1B1: an important factor in hepatic thyroid hormone and estrogen transport and metabolism. *Endocrinology* **149**, 4695–4701 (2008).
24. Dominguez-Gerpe, L. *et al*. Study of a possible association between serum levels of T4, T3 or TSH and the SNP S33P of the monocarboxylate transporter 8 (MCT8) *Thyroid* **16** (**Suppl. 835**), P78 (2006).
25. Lago-Leston, R. *et al*. Prevalence and functional analysis of the S107P polymorphism (rs6647476) of the monocarboxylate transporter 8 (*SLC16A2*) gene in the male population of Northwest Spain (Galicia). *Clin. Endocrinol. (Oxf.)*, [doi:10.1111/j.1365–2265200803377.x] (2008).
26. Hansen, P. S. *et al*. The impact of a TSH receptor gene polymorphism on thyroid-related phenotypes in a healthy Danish twin population. *Clin. Endocrinol. (Oxf.)* **66**, 827–832 (2007).
27. van der Deure, W. M. *et al*. Effects of serum TSH and FT4 levels and the TSHR-Asp727Glu polymorphism on bone: the Rotterdam Study. *Clin. Endocrinol. (Oxf.)* **68**, 175–181 (2008).
28. Murphy, E. & Williams, G. R. The thyroid and the skeleton. *Clin. Endocrinol. (Oxf.)* **61**, 285–298 (2004).
29. Meulenbelt, I. *et al*. Identification of *DIO2* as a new susceptibility locus for symptomatic osteoarthritis. *Hum. Mol. Genet.* **17**, 1867–1875 (2008).
30. Dentice, M. *et al*. The Hedgehog-inducible ubiquitin ligase subunit WSB-1 modulates thyroid hormone activation and PTHrP secretion in the developing growth plate. *Nat. Cell Biol.* **7**, 698–705 (2005).
31. Abe, E. *et al*. TSH is a negative regulator of skeletal remodeling. *Cell* **115**, 151–162 (2003).
32. Guo, T. W. *et al*. Positive association of the *DIO2* (deiodinase type 2) gene with mental retardation in the iodine-deficient areas of China. *J. Med. Genet.* **41**, 585–590 (2004).
33. Miell, J. P. *et al*. Effects of hypothyroidism and hyperthyroidism on insulin-like growth factors (IGFs) and growth hormone- and IGF-binding proteins. *J. Clin. Endocrinol. Metab.* **76**, 950–955 (1993).
34. Peeters, R. P. *et al*. A polymorphism in type I deiodinase is associated with circulating free insulin-like growth factor I levels and body composition in humans. *J. Clin. Endocrinol. Metab.* **90**, 256–263 (2005).
35. Mentuccia, D. *et al*. 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* **51**, 880–883 (2002).

36. Canani, L. H. *et 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. *J. Clin. Endocrinol. Metab.* **90**, 3472–3478 (2005).
37. Peeters, R. P. *et al*. The Asp727Glu polymorphism in the TSH receptor is associated with insulin resistance in healthy elderly men. *Clin. Endocrinol. (Oxf.)* **66**, 808–815 (2007).
38. Maia, A. L. *et al*. The type 2 deiodinase (*DIO2*) A/G polymorphism is not associated with glycemic traits: the Framingham Heart Study. *Thyroid* **17**, 199–202 (2007).
39. Grarup, N. *et al*. Studies of the common *DIO2* Thr92Ala polymorphism and metabolic phenotypes in 7342 Danish white subjects. *J. Clin. Endocrinol. Metab.* **92**, 363–366 (2007).
40. Fiorito, M. *et al*. Interaction of *DIO2* T92A and *PPARγ2* P12A polymorphisms in the modulation of metabolic syndrome. *Obesity (Silver Spring)* **15**, 2889–2895 (2007).
41. Leonard, J. L., Kaplan, M. M., Visser, T. J., Silva, J. E. & Larsen, P. R. Cerebral cortex responds rapidly to thyroid hormones. *Science* **214**, 571–573 (1981).
42. Silva, J. E. & Larsen, P. R. Comparison of iodothyronine 5'-deiodinase and other thyroid-hormone-dependent enzyme activities in the cerebral cortex of hypothyroid neonatal rat. Evidence for adaptation to hypothyroidism. *J. Clin. Invest.* **70**, 1110–1123 (1982).
43. Escobar-Morreale, H. F., Obregon, M. J., Escobar del Rey, F. & Morreale de Escobar, G. Replacement therapy for hypothyroidism with thyroxine alone does not ensure euthyroidism in all tissues, as studied in thyroidectomized rats. *J. Clin. Invest.* **96**, 2828–2838 (1995).
44. Saravanan, P. *et al*. Psychological well-being in patients on 'adequate' doses of l-thyroxine: results of a large, controlled community-based questionnaire study. *Clin. Endocrinol. (Oxf.)* **57**, 577–585 (2002).
45. Engum, A., Bjoro, T., Mykletun, A. & Dahl, A. A. An association between depression, anxiety and thyroid function—a clinical fact or an artefact? *Acta Psychiatr. Scand.* **106**, 27–34 (2002).
46 Panicker, V. *et al.* Common variation in the *DIO2* gene predicts baseline psychological well-being and response to combination thyroxine plus triiodothyronine therapy in hypothyroid patients. *J. Clin. Endocrinol. Metab.*, doi:10.1210/jc.2008-1301 (2009).
47. Woeber, K. A. Levothyroxine therapy and serum free thyroxine and free triiodothyronine concentrations. *J. Endocrinol. Invest.* **25**, 106–109 (2002).
48. Jonklaas, J., Davidson, B., Bhagat, S. & Soldin, S. J. Triiodothyronine levels in athyreotic individuals during levothyroxine therapy. *JAMA* **299**, 769–777 (2008).
49. Appelhof, B. C. *et al*. Polymorphisms in type 2 deiodinase are not associated with well-being, neurocognitive functioning, and preference for combined thyroxine/3, 5, 3'-triiodothyronine therapy. *J. Clin. Endocrinol. Metab.* **90**, 6296–6299 (2005).
50. van der Deure, W. M. *et al*. Polymorphisms in the brain-specific thyroid hormone transporter OATP1C1 are associated with fatigue and depression in hypothyroid patients. *Clin. Endocrinol. (Oxf.)* **69**, 804–111 (2008).
51. Grozinsky-Glasberg, S., Fraser, A., Nahshoni, E., Weizman, A. & Leibovici, L. Thyroxine-triiodothyronine combination therapy versus thyroxine monotherapy for clinical hypothyroidism: meta-analysis of randomized controlled trials. *J. Clin. Endocrinol. Metab.* **91**, 2592–2599 (2006).
52. Torlontano, M. *et al*. Type 2 deiodinase polymorphism (threonine 92 alanine) predicts L-thyroxine dose to achieve target thyrotropin levels in thyroidectomized patients. *J. Clin. Endocrinol. Metab.* **93**, 910–913 (2008).
53. Schneider, M. J. *et al*. Targeted disruption of the type 1 selenodeiodinase gene (*Dio1*) results in marked changes in thyroid hormone economy in mice. *Endocrinology* **147**, 580–589 (2006).
54. Schneider, M. J. *et al*. Targeted disruption of the type 2 selenodeiodinase gene (*Dio2*) results in a phenotype of pituitary resistance to T_4. *Mol. Endocrinol.* **15**, 2137–2148 (2001).
55. Hernandez, A., Martinez, M. E., Fiering, S., Galton, V. A. & St Germain, D. Type 3 deiodinase is critical for the maturation and function of the thyroid axis. *J. Clin. Invest.* **116**, 476–484 (2006).
56. Trajkovic, M. *et al*. Abnormal thyroid hormone metabolism in mice lacking the monocarboxylate transporter 8. *J. Clin. Invest.* **117**, 627–635 (2007).
57. Marians, R. C. *et al*. Defining thyrotropin-dependent and -independent steps of thyroid hormone synthesis by using thyrotropin receptor-null mice. *Proc. Natl Acad. Sci. USA* **99**, 15776–15781 (2002).
58. Hsu, J. H. & Brent, G. A. Thyroid hormone receptor gene knockouts. *Trends Endocrinol. Metab.* **9**, 103–112 (1998).
59. Schwartz, C. E. *et al*. Allan-Herndon-Dudley syndrome and the monocarboxylate transporter 8 (*MCT8*) gene. *Am. J. Hum. Genet.* **77**, 41–53 (2005).
60. Refetoff, S., Weiss, R. E. & Usala, S. J. The syndromes of resistance to thyroid hormone. *Endocr. Rev.* **14**, 348–399 (1993).

Acknowledgments

The authors are grateful for very helpful discussions with the following people: J Evans, G Lewis, A Hattersley and T Frayling from the Peninsula Medical School, Exeter, UK; P Saravanan at the University of Warwick, UK; R Greenwood at United Healthcare Bristol, UK; O Bjerkeset, T Bjoro and B Asvold, HUNT research center, Norway; and T Visser and his research team at Erasmus University Medical Centre, The Netherlands. V Panicker is supported by an A&A Saw Medical Research Fellowship through the Faculty of Medicine, Dentistry and Health Sciences at the University of Western Australia.