CLINICAL STUDY

The –258A/G (SNP rs12885300) polymorphism of the human type 2 deiodinase gene is associated with a shift in the pattern of secretion of thyroid hormones following a TRH-induced acute rise in TSH

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Abstract

Objective: Type 2 deiodinase gene (*DIO2*) polymorphisms have been associated with changes in pituitary–thyroid axis homeostasis. The –258A/G (SNP rs12885300) polymorphism has been associated with increased enzymatic activity, but data are conflicting. To characterize the effects of –258A/G polymorphism on intrathyroidal thyroxine (T_4) to triiodothyronine (T_3) conversion and thyroid hormone (TH) secretion pattern, we studied the effects of acute, TRH-mediated, TSH stimulation of the thyroid gland.

Design: Retrospective analysis.

Methods: The TH secretion in response to 500 μ g i.v. TRH injection was studied in 45 healthy volunteers.

Results: Twenty-six subjects (16 females and ten males, 32.8 ± 10.4 years) were homozygous for the ancestral (–258A/A) allele and 19 (11 females and eight males, 31.1 ± 10.9 years) were carriers of the (–258G/x) variant. While no differences in the peak TSH and T_3 levels were observed, carriers of the –258G/x allele showed a blunted rise in free T_4 (FT₄; $P < 0.01$). The –258G/x92Thr/Thr haplotype, compared with the other groups, had lower TSH values at 60 min ($P < 0.03$). No differences were observed between genotypes in baseline TH levels.

Conclusions: The –258G/x *DIO2* polymorphism variant is associated with a decreased rate of acute TSH-stimulated FT₄ secretion with a normal T_3 release from the thyroid gland consistent with a shift in the reaction equilibrium toward the product. These data indicate that the –258G *DIO2* polymorphism causes changes in the pattern of hormone secretion. These findings are a proof of concept that common polymorphisms in *DIO2* can subtly affect the circulating levels of TH and might modulate the TH homeostasis.

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Introduction

The serum levels of thyroid hormones (THs) are tightly regulated throughout the adult life by multiple mechanisms, and the intraindividual variability of TH levels is minimal (1). The serum levels of triiodothyronine (T_3), which is the biologically active form of TH, are the net results of secretion from the thyroid gland, the peripheral conversion of the pro-hormone thyroxine (T_4) into T_3 by the two 5'-deiodinases, D1 and D2, and its catabolism and excretion (2, 3). Apart from their activity in the peripheral tissues, D1 and D2 are active in the thyroid gland and play an important role in

modulating the release of T_3 from the gland both in physiological and in pathological states (4, 5, 6, 7). Intrathyroidal D2 activity is positively stimulated by the thyrotropin (thyroid-stimulating hormone (TSH)) receptor pathway by increasing the intracellular cAMP levels (8, 9), ultimately leading to an activation of the T_4 to T_3 intrathyroidal conversion. This phenomenon is contributing to the preferential T_3 secretion during states of sustained activation of the TSH receptor pathway (7). Thus, genetic variants in the deiodinases can affect the pattern of TH secretion. Recently, we demonstrated that the common polymorphism of the type 2 deiodinase gene (*DIO2*;

Thr92Ala DIO2, SNP rs225014) causes a differential response in the TH secretion pattern following the acute rise in serum TSH levels in response to an injection of thyrotropin-releasing hormone (TRH) (10). In this secondary analysis of the original dataset, we characterize the role of the $-258A/G$ (*D2-ORFa-Gly3Asp*, SNP rs12885300) (11) polymorphism in the pattern of TH release and explore the interaction between the two polymorphisms.

Materials and methods

Participants and study design

This study is a secondary analysis of a previously described dataset (10). Briefly, the study was approved by the NIDDK-NIAMS Institutional Review Board and conducted at the National Institutes of Health Clinical Center in Bethesda, MD. The research protocol was designed as a prospective cohort study (ClinicalTrials.gov identifier number NCT00812149).

Healthy volunteers aged 18–65, with normal baseline thyroid function and negative antithyroid peroxidase antibodies or thyroid-stimulating immunoglobulin, were invited to participate in the study. The study volunteers' accrual was designed to achieve three groups of 15 subjects with the following *DIO2* codon 92 genotype: *Thr/Thr*, *Thr/Ala*, *Ala/Ala*.

Study volunteers underwent an outpatient screening visit, at which a medical history was taken and physical examination performed. A blood sample was taken for basic metabolic and thyroid function, and a DNA sample was collected for *DIO2* polymorphisms status. Eligible subjects were then invited to undergo a TRH stimulation test.

Blood sampling and laboratory testing

Blood samples were drawn under sterile technique through the i.v. catheter using the Vacutainer system (Becton Dickinson & Co. Franklin Lakes, NJ, USA). Screening tests, TSH, free T_4 (FT_4), T_3 , antithyroid antibody panel, prolactin, and urine human chorionic gonadotropin in women were performed daily in the NIH Department of Laboratory Medicine. TSH, FT_4 , and T_3 were analyzed daily by the Department of Laboratory Medicine by immunoassay on a Siemens Immulite 2500 analyzer platform (Siemens, Tarrytown, NY, USA). Intra- and interassay coefficients of variability for TSH were 4.2 and 3.5%, T_3 11.2 and 5.4%, and FT_4 16.43 and 2.58% respectively. Thyroid-stimulating immunoglobulin testing was performed by Mayo Medical Labs (Rochester, MN, USA). Cell pellets were stored in -80°C and processed in batch for genomic DNA isolation.

DNA isolation and *DIO2* restriction fragment polymorphism analysis

Genomic DNA was isolated from peripheral mononuclear cells from the screening blood samples using the QIAamp system (Qiagen). After isolation, DNA concentration was measured using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Purity was determined by measuring the 260/280 nm ratio. The $-258A/G$ *DIO2* polymorphism status was characterized using established methods (12) by PCR restriction fragment length polymorphism (RFLP) analysis. Ten nanograms of genomic DNA were amplified with the following primers: forward 5'-AAAGCTGGCGTACTCGTC-3' and reverse 5'-AAA-GAGCATAGAGACAATGAAAG-3'. After purification, PCR products were digested with CviKI-1 (New England BioLabs, Ipswich, MA, USA), restriction enzyme for 4 h at 37°C . The digestion products were separated on 4.5% agarose gel by electrophoresis and visualized by ethidium bromide staining. The *Thr92Ala DIO2* polymorphism status was characterized using established methods (13) by PCR RFLP analysis. Ten nanograms of genomic DNA were amplified with the following primers: forward 5'-CTCAGGGCTGGCAAAGTCAAG-3' and reverse 5'-CCACACTCTATTAGAGCCAATTG-3'. After purification, PCR products were digested with Bsg-1 (New England BioLabs, Ipswich, MA, USA), and the digestion products were separated on a 1.5% agarose gel by electrophoresis.

TRH test procedure

The TRH test was performed at the NIH Clinical Center day hospital. After an overnight fast, subjects underwent testing while resting supine on a comfortable bed in a room maintained at a temperature of $23\text{--}25^\circ\text{C}$. Women of reproductive age had a repeat pregnancy test at admission. A saline lock i.v. catheter was inserted 15 min before the first blood draw. At time 0, 500 μg TRH was given i.v. over 1 min followed by a 10 cc normal saline flush. Blood samples were taken at -15 , 0, 5, 10, 15, 20, 30, 60, 120, and 180 min for measurement of TSH, FT_4 , and total T_3 (TT_3). Prolactin levels were measured at -15 , 0, 60, and 180 min as an independent marker of TRH action. Blood pressure was monitored before administration of TRH and 30, 60, 120, and 180 min after the blood draws.

Statistical analysis

This is a secondary analysis of the original dataset (10), which was by design enriched in carriers of the *Ala92 DIO2* allele. The $-258A/G$ *DIO2* (rs12885300) polymorphism is common in the general population (allele frequency 0.34) and it is not in linkage disequilibrium with the *Thr92Ala* variant (11). Thus, we reckoned *a priori* that $\sim 50\%$ of the study population

would carry the *DIO2* –258G/G ancestral allele and the rest of the population carry the minor allele gene polymorphism either as homozygous or heterozygous (A/A or G/x). TRH stimulation test data are presented as delta from baseline (average –15 and 0 min). Data were log transformed where indicated. A two-tailed Student's *t*-test was performed as primary analysis for changes in TH levels following the TRH injection. Nonparametric data were analyzed using Mann–Whitney *U* test. An α error of 0.05 was considered the threshold for statistical significance.

Results

Patient recruitment and characteristics

Forty-six eligible individuals from 83 study participants completed TRH testing. The dataset of one volunteer who underwent the TRH stimulation test was omitted from the analysis because of the presence of antithyroid peroxidase antibodies. Thus, the data of 45 volunteers who were given an i.v. injection of 500 µg TRH with serial measurements of serum TT₃, FT₄, and TSH over 180 min were analyzed. The study population was composed of 26 subjects with the –258A/A genotype, age 32.8 ± 10.4 years (16 females and ten males) and 19 subjects with the –258G/x genotype, age 31.1 ± 10.9 years (11 females and eight males). The –258/codon 92 haplotypes were as follows: ten subjects with 92Trh/Thr –258A/A, age 31.8 ± 7.5 years (six females and four males), 16 subjects with 92Ala/x –258A/A, age 33.5 ± 11.5 years (ten females and six males), five subjects with 92Thr/Thr –258G/x, age 34.0 ± 14.9 years (two females and three males), and 14 subjects with 92Ala/x –258G/x, age 30.1 ± 9.5 years (nine females and five males; Table 1).

Baseline parameters

As illustrated in Table 1, no significant difference was found in any of the studied baseline parameters among the genotype groups. Specifically, no significant differences between genotypes were noted in baseline serum TSH, FT₄, or TT₃ levels or in fasting glucose, fasting insulin, or HOMA levels.

Response to TRH testing

There was no significant difference in maximal (30 min) TRH-stimulated levels of serum TSH between the two groups (–258A/A 14.28 ± 6.93 vs –258G/x 13.23 ± 5.39 mIU/ml, $P=0.845$; Fig. 1A). Similarly, no significant difference between groups were observed in serum TSH level at 60 min (–258A/A 10.43 ± 4.99 vs –258G/x 9.91 ± 3.94 mIU/ml, $P=0.863$), 120 min (–258A/A 4.80 ± 2.46 vs –258G/x 4.81 ± 2.07 mIU/ml, $P=0.845$), and 180 min (–258A/A 2.10 ± 1.57 vs –258G/x 2.22 ± 1.22 mIU/ml, $P=0.364$). When the analysis was performed among –258/codon 92 haplotypes, the –258G/x 92Thr/Thr showed, compared with the other haplotypes, lower TSH values at 30 min (8.90 ± 3.39 vs 14.45 ± 6.31 mIU/ml, $P<0.05$), 60 min (6.44 ± 2.29 vs 10.68 ± 4.54 mIU/ml, $P=0.03$), and nonsignificant trend at 120 min (3.24 ± 1.27 vs 5.00 ± 2.31 mIU/ml, $P=0.07$; Fig. 1B).

A rise in serum TT₃ level was noted in all participants undergoing TRH testing starting at 30 min after TRH injection (Fig. 2). No differences between groups were observed in TT₃ at 60 min (–258A/A 19.35 ± 12.89 vs –258G/x 14.44 ± 12.44 ng/dl, $P=0.206$), 120 min (–258A/A 46.58 ± 20.91 vs –258G/x 44.91 ± 15.29 ng/dl, $P=0.769$), and at 180 min (–258A/A 49.51 ± 13.61 vs –258G/x 52.54 ± 15.54 ng/dl,

Table 1 Study participants' characteristics. Clinical data of the study participants were reported according to either the –258 status (columns 1 and 2) or the –258/codon 92 haplotype (columns 3–6). No significant differences were observed among the groups.

Parameter	–258		–258/codon 92 haplotype			
	–258A/A	–258G/x	92Thr/Thr –258A/A	92Ala/x –258A/A	92Thr/Thr –258G/x	92Ala/x –258G/x
<i>n</i>	26	19	10	16	5	14
Sex (M/F)	16/10	11/8	6/4	10/6	2/3	9/5
Age (years)	32.8 ± 10.0	31.1 ± 10.9	31.8 ± 7.5	33.5 ± 11.5	34.0 ± 14.9	33.1 ± 9.5
BMI (kg/m ²)	25.9 ± 4.7	24.9 ± 4.0	25.4 ± 3.7	26.2 ± 5.3	26.7 ± 4.5	24.2 ± 3.7
Baseline TSH (µIU/ml)	1.4 ± 0.9	1.5 ± 0.6	1.2 ± 0.6	1.6 ± 1.1	1.2 ± 0.5	1.6 ± 0.6
Baseline T ₃ (ng/dl)	107.4 ± 19.8	101.8 ± 24.0	111.8 ± 24.6	103.8 ± 17.6	90.6 ± 6.9	105.2 ± 24.5
Baseline FT ₄ (ng/dl)	1.3 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	1.3 ± 0.2	1.3 ± 0.2	1.3 ± 0.1
Baseline T ₃ /FT ₄ (ng/dl)	84.2 ± 18.7	80.0 ± 21.1	91.5 ± 21.3	79.9 ± 14.3	71.7 ± 6.8	83.3 ± 23.6
Cholesterol (mg/dl)	151.0 ± 40.0	161.3 ± 29.7	144.3 ± 36.3	154.8 ± 42.6	158.2 ± 18.2	162.4 ± 33.4
LDL (mg/dl)	86.1 ± 29.4	96.1 ± 25.5	78.6 ± 21.1	90.7 ± 33.3	94.2 ± 18.7	96.8 ± 28.1
HDL (mg/dl)	52.1 ± 12.8	49.2 ± 12.2	51.8 ± 17.0	52.2 ± 9.9	47.8 ± 13.8	49.1 ± 12.4
Triglycerides (mg/dl)	64.3 ± 38.1	83.7 ± 55.9	72.6 ± 56.9	59.7 ± 23.1	77.8 ± 27.1	85.8 ± 63.9
Glucose (mg/dl)	79.3 ± 9.9	82.3 ± 7.0	80.3 ± 7.2	78.6 ± 11.4	84.2 ± 4.7	81.6 ± 7.7
Insulin (µIU/ml)	4.1 ± 2.9	4.0 ± 2.9	3.0 ± 1.4	4.8 ± 3.4	5.8 ± 5.0	3.3 ± 1.5
HOMA	0.8 ± 0.7	0.8 ± 0.6	0.6 ± 0.3	1.0 ± 0.8	1.2 ± 1.0	0.7 ± 0.3

Figure 1 Changes in serum TSH levels from baseline (Δ) following the i.v. injection of 500 μ g TRH. Serum levels were measured at $-15, 0, 5, 10, 15, 20, 30, 60, 120$, and 180 min after TRH injection. Error bars represent the S.E.M. at each time point. (A) Data reported according to the $-258A/G$ *DIO2* genotype. No significant differences were observed between genotypes. (B) Data reported according to the $-258/\text{codon } 92$ haplotype. Compared with the other haplotypes, the $-258G/x$ 92Thr/Thr showed significantly lower TSH values 30 and 60 min after TRH injection.

$P=0.490$; Fig. 2A). No differences were observed among haplotypes (Fig. 2B).

A rise in serum FT_4 level was noted in all participants undergoing TRH testing starting at 60 min after TRH injection. Compared with $-258A/A$ group, the $-258G/x$ group showed a significantly blunted TRH-stimulated increase in serum FT_4 at 120 min ($-258A/A$ 0.20 ± 0.10 vs $-258G/x$ 0.12 ± 0.09 ng/dl, $P<0.01$) and at 180 min ($-258A/A$ 0.28 ± 0.09 vs $-258G/x$ 0.20 ± 0.09 ng/dl, $P<0.01$; Fig. 3A). When the analysis was performed among haplotypes, no significant differences were observed (Fig. 3B).

Discussion

The interaction between TSH and its receptor on the thyrocyte plays a central role in regulating the development, growth, and function of the thyroid gland. In particular, the interaction with the downstream G-protein signal pathways directly affects the secretion of TH from the gland (14). Sustained

activation of this pathway by congenital or acquired conditions is associated with a shift in the ratio of serum T_3/T_4 (15) secondary to activation of intrathyroidal conversion of T_4 into T_3 by both deiodinases type 1 and type 2 (7). While deiodinase type 1 gene transcription is primarily driven by positive feedback of increased intracellular levels of T_3 (16), thyrocyte transcription of the *DIO2* gene is driven by the TSH-mediated cAMP pathway (8, 9). Hence, the latter has a primary role in regulating intrathyroidal TH conversion and the pattern of TH secretion after TSH stimulation.

Genetic variants of *DIO2* have been associated with changes in indices of TH action at various end organs (12, 13, 17, 18, 19) and also with changes in circulating levels of TH (11, 20), but the results are controversial (21, 22). Clinical and *in vitro* studies suggest that the two most common polymorphisms have opposite effects on enzymatic activity. In fact, while the *Thr92Ala* variant is associated with a decreased activity (23) and indices of reduced TH at the end organ level (13), the $-258A/G$ has been associated with increased enzymatic activity (11, 24). With respect to the effects of these polymorphisms on circulating levels of TH, the effects of *Thr92Ala* are minimal (20), while the $-258A/G$ is associated with a change in the T_3/FT_4 ratio (11), confirming *in vitro* observations that the minor G allele confers an increase in transcription and ultimately activity (24). It is worth noting that the changes in TH levels observed at a steady state are small, and not all studies confirmed the original observations (22). Conversely, a secondary analysis of a large $\text{L-T}_3/\text{L-T}_4$ combination therapy trial suggests that the common *Ala92* variant is associated with worse baseline indices of quality of life, and an improved response to $\text{L-T}_3/\text{L-T}_4$ combination therapy (19). These observations suggest that subtle changes in *DIO2* activity may play an important role in the individual response to replacement therapy.

The injection of TRH allows the dynamic assessment of the hypothalamus–pituitary–thyroid axis and is a sensitive tool to demonstrate small differences in its pathophysiology; hence, we exploited the characteristics of this test to analyze the differences secondary to polymorphisms in the *DIO2* gene in acute TSH secretion and in the pattern of TH secretion after TRH injection. Indeed, we previously demonstrated that the *Ala92* allele causes a blunted secretion of T_3 following a TRH-mediated acute rise in TSH, consistent with a reduced intrathyroidal conversion of T_4 into T_3 (10).

The results of this study indicate that while the TSH and T_3 levels were similar to those observed in the ancestral $-258A/A$ alleles, carriers of the $-258G$ minor allele displayed a significantly blunted rise in FT_4 . These findings suggest an increase in enzymatic activity, resulting in a change of T_3/FT_4 ratio consistent with a shift of the reaction equilibrium toward the product. This is in keeping with the initial observations of Peeters *et al.* (11), as well as with our *in vitro* data indicating

Figure 2 Changes in serum T₃ levels from baseline (Δ) following the i.v. injection of 500 μg TRH. Serum levels were measured at –15, 0, 5, 10, 15, 20, 30, 60, 120, and 180 min after TRH injection. Error bars represent the s.e.m. at each time point. (A) Data reported according to the –258A/G DIO2 genotype. No significant differences were observed between genotypes. (B) Data reported according to the –258/codon 92 haplotype. No significant differences were observed among haplotypes.

that the –258G allele causes an increase in transcription of the DIO2 gene by decreasing the binding of a repressor factor in the 5'-UTR of the gene (24). One can speculate that the –258A/G polymorphism also generates a shifted equilibrium in the thyrotroph, thus preventing an overstimulation of the thyroid gland, which would otherwise result in increased T₃ levels. Although no differences were found between alleles in the dynamic response of TSH to TRH injection, when the analysis was performed according to the combination of the –258/codon 92 polymorphisms, the –258G/x Thr/Thr92 was associated with a significant decrease in the peak of TSH, consistent with a state of

local 'hyperthyroidism' in the thyrotroph, in keeping with an overall increase in deiodinase activity due to the additive effects of the activating –258G polymorphism and the Thr92 'wild-type' allele.

The strengths of this pharmacogenomic intervention reside in the study of well-characterized healthy volunteers devoid of thyroid disease and the use of dynamic testing in a controlled environment able to amplify relatively small differences between the genotypes. On the other hand, the major limitation of the study is related to the secondary analysis of a previously collected dataset, hence the inability to perform the analysis in homozygous –258G/G subjects. Conversely, it is worth noting that the outcomes tested fall within the physiological pathway explored, the results confirm the study hypothesis, and are supported by *in vitro* and *in vivo* observations (24, 25).

Figure 3 Changes in serum free T₄ levels from baseline (Δ) following the i.v. injection of 500 μg TRH. Serum levels were measured at –15, 0, 5, 10, 15, 20, 30, 60, 120, and 180 min of TRH injection. Error bars represent the s.e.m. at each time point. (A) Data reported according to the –258A/G DIO2 genotype. Compared with –258A/A ancestral allele, carriers of the –258G/x minor allele showed a significantly blunted TRH-stimulated increase in serum free T₄ at 120 and at 180 min after TRH injection. (B) Data reported according to the –258/codon 92 haplotype. No significant differences were observed among haplotypes.

In conclusion, the data indicate that the $-258A/G$ and $Thr92Ala$ *DIO2* polymorphisms affect the dynamic response of the thyroid after TRH-induced TSH stimulation, and in particular, the $-258G$ allele causes a shift in the substrate/product ratio, consistent with an increased activation of the enzyme. Furthermore, the analysis of the haplotype suggests that the combination of the activating $-258G$ allele, with the wild-type $Thr92$ allele, confers a blunted TSH response to TRH stimulation, in keeping with a 'priming' of the thyrotroph (26) with T_3 derived by an increased state of conversion of T_4 into T_3 .

These findings are a proof of concept that common polymorphisms in the *DIO2* can subtly affect the circulating levels of TH and might modulate TH homeostasis, particularly in the case of replacement therapy when the entire pool of T_3 derives from the deiodinase activity (20). Further studies are needed to demonstrate the relevance of these observations in relation to clinically meaningful endpoints.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References

- 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. (doi:10.1210/jc.87.3.1068)
- Gereben B, Zavacki AM, Ribich S, Kim BW, Huang SA, Simonides WS, Zeold A & Bianco AC. Cellular and molecular basis of deiodinase-regulated thyroid hormone signaling. *Endocrine Reviews* 2008 **29** 898–938. (doi:10.1210/er.2008-0019)
- Maia AL, Kim BW, Huang SA, Harney JW & Larsen PR. Type 2 iodothyronine deiodinase is the major source of plasma T_3 in euthyroid humans. *Journal of Clinical Investigation* 2005 **115** 2524–2533. (doi:10.1172/JCI25083)
- Salvatore D, Tu H, Harney JW & Larsen PR. Type 2 iodothyronine deiodinase is highly expressed in human thyroid. *Journal of Clinical Investigation* 1996 **98** 962–968. (doi:10.1172/JCI118880)
- Murakami M, Araki O, Hosoi Y, Kamiya Y, Morimura T, Ogiwara T, Mizuma H & Mori M. Expression and regulation of type II iodothyronine deiodinase in human thyroid gland. *Endocrinology* 2001 **142** 2961–2967. (doi:10.1210/en.142.7.2961)
- Brtko J, Bobalova J, Podoba J, Schmutzler C & Kohrle J. Thyroid hormone receptors and type I iodothyronine 5'-deiodinase activity of human thyroid toxic adenomas and benign cold nodules. *Experimental and Clinical Endocrinology & Diabetes* 2002 **110** 166–170. (doi:10.1055/s-2002-32147)
- Celi FS, Coppotelli G, Chidakel A, Kelly M, Brillante BA, Shawker T, Cherman N, Feuillan PP & Collins MT. The role of type 1 and type 2 5'-deiodinase in the pathophysiology of the 3,5,3'-triiodothyronine toxicosis of McCune-Albright syndrome. *Journal of Clinical Endocrinology and Metabolism* 2008 **93** 2383–2389. (doi:10.1210/jc.2007-2237)
- Canettieri G, Celi FS, Baccheschi G, Salvatori L, Andreoli M & Centanni M. Isolation of human type 2 deiodinase gene promoter and characterization of a functional cyclic adenosine monophosphate response element. *Endocrinology* 2000 **141** 1804–1813. (doi:10.1210/en.141.5.1804)
- Bartha T, Kim SW, Salvatore D, Gereben B, Tu HM, Harney JW, Rudas P & Larsen PR. Characterization of the 5'-flanking and 5'-untranslated regions of the cyclic adenosine 3',5'-monophosphate-responsive human type 2 iodothyronine deiodinase gene. *Endocrinology* 2000 **141** 229–237. (doi:10.1210/en.141.1.229)
- Butler PW, Smith SM, Linderman JD, Brychta RJ, Alberobello AT, Dubaz OM, Luzon JA, Skarulis MC, Cochran CS, Wesley RA, Pucino F & Celi FS. The $Thr92Ala$ 5' type 2 deiodinase gene polymorphism is associated with a delayed triiodothyronine secretion in response to the thyrotropin-releasing hormone-stimulation test: a pharmacogenomic study. *Thyroid* 2010 **20** 1407–1412. (doi:10.1089/thy.2010.0244)
- 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. (doi:10.1152/ajpendo.00571.2004)
- He B, Li J, Wang G, Ju W, Lu Y, Shi Y, He L & Zhong N. Association of genetic polymorphisms in the type II deiodinase gene with bipolar disorder in a subset of Chinese population. *Progress in Neuro-Psychopharmacology & Biological Psychiatry* 2009 **33** 986–990. (doi:10.1016/j.pnpbp.2009.05.003)
- 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. (doi:10.2337/diabetes.51.3.880)
- Corvilain B, Laurent E, Lecomte M, Vansande J & Dumont JE. Role of the cyclic adenosine 3',5'-monophosphate and the phosphatidylinositol- Ca^{2+} cascades in mediating the effects of thyrotropin and iodide on hormone synthesis and secretion in human thyroid slices. *Journal of Clinical Endocrinology and Metabolism* 1994 **79** 152–159. (doi:10.1210/jc.79.1.152)
- Laurberg P. Mechanisms governing the relative proportions of thyroxine and 3,5,3'-triiodothyronine in thyroid secretion. *Metabolism* 1984 **33** 379–392. (doi:10.1016/0026-0495(84)90203-8)
- Jakobs TC, Schmutzler C, Meissner J & Kohrle J. The promoter of the human type I 5'-deiodinase gene – mapping of the transcription start site and identification of a DR+4 thyroid-hormone-responsive element. *European Journal of Biochemistry* 1997 **247** 288–297. (doi:10.1111/j.1432-1033.1997.00288.x)
- Dora JM, Machado WE, Rheinheimer J, Crispim D & Maia AL. Association of the type 2 deiodinase $Thr92Ala$ polymorphism with type 2 diabetes: case-control study and meta-analysis. *European Journal of Endocrinology* 2010 **163** 427–434. (doi:10.1530/EJE-10-0419)
- Meulenbelt I, Min JL, Bos S, Riyazi N, Houwing-Duistermaat JJ, van der Wijk HJ, Kroon HM, Nakajima M, Ikegawa S, Uitterlinden AG, van Meurs JB, van der Deure WM, Visser TJ, Seymour AB, Lakenberg N, van der Breggen R, Kremer D, van Duijn CM, Kloppenburg M, Loughlin J & Slagboom PE.

- Identification of DIO2 as a new susceptibility locus for symptomatic osteoarthritis. *Human Molecular Genetics* 2008 **17** 1867–1875. (doi:10.1093/hmg/ddn082)
- 19 Panicker V, Saravanan P, Vaidya B, Evans J, Hattersley AT, Frayling TM & Dayan CM. Common variation in the DIO2 gene predicts baseline psychological well-being and response to combination thyroxine plus triiodothyronine therapy in hypothyroid patients. *Journal of Clinical Endocrinology and Metabolism* 2009 **94** 1623–1629. (doi:10.1210/jc.2008-1301)
- 20 Torlontano M, Durante C, Torrente I, Crocetti U, Augello G, Ronga G, Montesano T, Travascio L, Verrienti A, Bruno R, Santini S, D'Arcangelo P, Dallapiccola B, Filetti S & Trischitta V. Type 2 deiodinase polymorphism (threonine 92 alanine) predicts L-thyroxine dose to achieve target thyrotropin levels in thyroidectomized patients. *Journal of Clinical Endocrinology and Metabolism* 2008 **93** 910–913. (doi:10.1210/jc.2007-1067)
- 21 Grarup N, Andersen MK, Andreasen CH, Albrechtsen A, Borch-Johnsen K, Jorgensen T, Auwerx J, Schmitz O, Hansen T & Pedersen O. Studies of the common DIO2 Thr92Ala polymorphism and metabolic phenotypes in 7342 Danish white subjects. *Journal of Clinical Endocrinology and Metabolism* 2007 **92** 363–366. (doi:10.1210/jc.2006-1958)
- 22 de Jong FJ, Peeters RP, den Heijer T, van der Deure WM, Hofman A, Uitterlinden AG, Visser TJ & Breteler MM. The association of polymorphisms in the type 1 and 2 deiodinase genes with circulating thyroid hormone parameters and atrophy of the medial temporal lobe. *Journal of Clinical Endocrinology and Metabolism* 2007 **92** 636–640. (doi:10.1210/jc.2006-1331)
- 23 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. (doi:10.1210/jc.2004-1977)
- 24 Coppotelli G, Summers A, Chidakel A, Ross JM & Celi FS. Functional characterization of the 258 A/G (D2-ORFa-Gly3Asp) human type-2 deiodinase polymorphism: a naturally occurring variant increases the enzymatic activity by removing a putative repressor site in the 5' UTR of the gene. *Thyroid* 2006 **16** 625–632. (doi:10.1089/thy.2006.16.625)
- 25 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. (doi:10.1210/jc.2002-021592)
- 26 Faglia G. The clinical impact of the thyrotropin-releasing hormone test. *Thyroid* 1998 **8** 903–908. (doi:10.1089/thy.1998.8.903)

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