Differentiated thyroid carcinoma : treatment and clinical consequences of therapy

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The type 2 deiodinase ORFa-Gly3Asp polymorphism influences the setpoint of the hypothalamus-pituitary-thyroid axis in patients treated for differentiated thyroid carcinoma

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Abstract

Context

Iodothyronine deiodinases D1, D2 and D3 play an important role in synthesis and degradation of T3. The relationship between serum TSH and T3 levels is determined by an individual setpoint of the hypothalamus-pituitary-thyroid axis.

Objective

Several polymorphisms have been described in D1 and D2 of which some are associated with serum TSH and iodothyronine levels. In this study we investigate whether polymorphisms of D1 and D2 influence the setpoint of the hypothalamus-pituitary-thyroid-axis.

Design

We collected 1905 serum FT4 and TSH measurements during 11.5±8.8 years of follow-up in patients treated for differentiated thyroid carcinoma (DTC). We determined these polymorphisms: D1-rs11206244, D1-rs12095080, D2-rs225014 and D2-rs12885300. Effects of these polymorphisms on the setpoints of the hypothalamus-pituitary-thyroid-axis were analysed with a linear mixed model.

Patients

151 consecutive patients treated and cured for DTC were included

Results

DTC patients homozygous for the D2-rs12885300 T allele have an altered setpoint of the hypothalamus-pituitary-thyroid axis. The slope of the regression line (corrected for age, BMI and gender) for wild-type patients was -0.32±0.028 (ln[TSH mU/l]/[FT4 pmol/l]), the intercept 4.95. For heterozygous patients the slope was -0.30±0.028 (ln[TSH mU/l]/[FT4 pmol/l]), the intercept 4.23. The slope of homozygous patients was -0.35±0.026 (ln[TSH mU/l]/[FT4 pmol/l]) and the intercept 6.07 (P =0.036 compared to wild-type and heterozygous patients).

Conclusion

Our data suggest that the negative feedback of FT4 on TSH is weaker in patients homozygous for the D2-ORFa-Gly3Asp than in wild-type and heterozygous subjects.

Background

Thyroid hormone has an important role in a wide range of physiological processes: from growth and development in children to energy homeostasis in the adult (1). Most actions of thyroid hormone are mediated by the active form of thyroid hormone, triiodothyronine (T3). Serum T3 levels are relatively constant in healthy subjects. Serum and local T3 concentrations are mainly regulated by the iodothyronine deiodinases D1, D2 and D3 (2). D1 is mainly involved in serum T3 production. In addition, it plays a role in the breakdown of rT3 (3,4). D2 catalyzes local T3 production through deiodination of T4 production in various tissues and is necessary for the negative feedback regulation of thyroid hormone on thyrotropin (TSH) production in the pituitary (5). Approximately 80% of T3 is produced by extrathyroidal pathways (2). D3 inactivates T3 and T4 and thus regulates the clearance of T3 and T4. It is thought to contribute to thyroid hormone metabolism by protecting tissues from excess thyroid hormone. The deiodinases adjust the thyroid hormone levels of individual tissues in response to various conditions (6,7).

Several polymorphisms have been described in D1 and D2 of which some are associated with circulating levels of T4, T3 and TSH (8,9,10,11). Two polymorphisms in the D1 gene, D1-rs11206244 (previously D1-C785T) and D1-rs12095080 (previously D1-A1814G), have been associated with changes in the balance of thyroid hormones in the serum, with raised T3, low T4 and low rT3 levels, but these changes have not been associated with differences in TSH. This implies that the net effect is perceived by the hypothalamus and pituitary as "neutral" (1,12,13,14). However, the D1-rs11206244 is in linkage disequilibrium with another single-nucleotide polymorphism (SNP) rs2235544 which did show a distinct association with circulating FT3/FT4 ratio (11).

Controversy exists about the functional implications of the D2-rs225014 (previously D2-Thr92Ala) polymorphism, which has been associated with a decreased D2 activity in some in-vitro experiments (9), but not in others (13). So far only one study found an association between the D2-rs225014 polymorphism and serum thyroid hormone levels (15), however a previous study performed by our department could not confirm these data (16). A recent study by Butler *et al*. performed a prospective intervention study which aimed at demonstrating in-vivo effects of the Thr92Ala D2 variant. They found subtle differences in the serum changes of total T3 in the Ala/Ala subjects 60 minutes after TRH injection. However, there were no significant differences in the response of FT4 and TSH after TRH injection (17).

In healthy blood donors the D2-ORFa-Asp variant (rs12885300) was associated with lower levels of serum T4, fT4 and rT3, but not with plasma T3 and TSH levels (8). This suggests that the D2-rs1288530 polymorphism leads to higher activity of D2 at the pituitary level. However, this association was not seen in elderly men (8).

Intraindividual variation in serum T4, T3 and TSH is narrow; however there is a considerable interindividual variability (18). A large body of evidence suggests that every individual has a unique thyroid function setpoint, compatible with a genetic influence on the regulation of the pituitary-thyroid axis (18,19,20). We hypothesized that polymorphisms in D1 and D2 could influence the setpoint of the hypothalamus-pituitary-thyroid axis. We tested this hypothesis in 151 patients treated for differentiated thyroid carcinoma (DTC). These individuals have no endogenous thyroid hormone production and thus no interference of the intrinsic T3 production of the thyroid. During follow-up of DTC, patients are treated with thyroxine substitution therapy in a dose intended to suppress TSH level, which results in a constant and precisely measurable supply of T4. During this period they regularly come for routine measurements of TSH and FT4, moreover they are regularly withdrawn from thyroxine to perform TSH stimulated radioactive iodine-131 whole body imaging, which leads to a wide individual range of combined measurements of TSH and FT4. These patients are therefore an ideal group to assess the relationship between polymorphisms in deiodinases and the setpoint of the hypothalamus-pituitary-thyroid axis.

Patients and Methods

Patients

One hundred and fifty-one consecutive patients treated for DTC were recruited from the outpatient clinic of the Department of Endocrinology of the Leiden University Medical Center, a tertiary referral center for thyroid carcinoma. All patients had been treated by near-total thyroidectomy followed by radioiodine ablation between 1992 and 2007. After initial treatment, thyroxine therapy was started in a dose intended to suppress TSH levels below 0.4 mU/l for 15 years. All patients were cured as defined by the absence of I-131 accumulation at diagnostic scintigraphy, serum thyroglobulin (Tg) concentrations below 2 µg/l after TSH stimulation in the absence of Tg antibodies, a normal neck ultrasound and no other indication for disease. Patients with tumor relapse were only included if they had been subsequently cured. Patients that used corticosteroids or psychopharmaca that could alter serum TSH or FT4 levels (amiodarone, dopamine, phenytoin, lithium) were excluded from the study. The Local Ethics Committee of the Leiden University Medical Centre approved the study, and written informed consent was obtained from all subjects.

Study design

We collected all FT4 and TSH measurements since the initial diagnosis of the patients with the exception of recombinant human (Thyrogen) stimulated TSH levels. Blood

was collected for regular outpatient clinic appointments from 1992 till 2007. In 2007, blood was collected for DNA isolation for genotyping of the deiodinase type 1 and 2 polymorphisms, and for measurements of T3 and rT3 in addition to TSH and FT4.

Serum biochemistry

Serum free T4 concentrations were measured throughout the study period on an IMx system (Abbott, Abbott Park, IL) (intra-assay variability of 2.5–7.6% and interassay variability of 5.6–12.4% at different levels). Serum TSH concentrations were determined throughout the study period with Elecsys E-170 on a Modular Analytics E-170 system (Roche Diagnostic Systems, Basel, Switzerland; reference range 0.4–4.5 mU/liter, detection limit 0.005 mU/liter, intraassay variability 0.9–10.7%, and interassay variability 0.9–12.1%). Serum T3 was measured with a fluorescence polarization immunoassay, CV 2·5–9·0%, on an ImX system (Abbott, Abbott Park, IL). Reverse T3 was measured using a RIA as described previously (20).

Genotyping

DNA was isolated from peripheral leucocytes by the salting out procedure (21). Genotypes of the D1 and D2 polymorphisms were determined using 5 ng genomic DNA in a 5′ fluoregenic Taqman assay and reactions were performed in 384-wells format on ABI9700 2x384well PCR machines with endpoint reading on the ABI 7900HT TaqManÒ machine (Applied Biosystems, Nieuwerkerk aan den IJssel, The Netherlands). Primer and probe sequences were optimized using the single nucleotide polymorphism assay-by-design service of Applied Biosystems.

Statistical Analysis

Values are presented as mean ± standard deviation (SD), median (range) or as numbers or proportions of patients. Deviation from the Hardy-Weinberg Equilibrium was analysed using a X^2-test. For the comparison of setpoints of the hypothalamus-pituitary-thyroid axis, we used a regression analysis with a general mixed model with a random intercept and random slope to assess the correlation between the natural logarithm of TSH (lnTSH) and FT4 for the different alleles of the polymorphisms. Herewith, we tested if the regression lines (with the equation $\ln TSH = \beta \cdot FT4 + \alpha$, where β is the slope and α is the intercept) were similar in slopes given a random intercept. We had different numbers of combined TSH/FT4 measurements for each individual. With our model the regression lines of patients were compared independently of the number of blood samples (combined TSH/FT4 measurements) available. However,

regression lines are expected to be more accurate if an individual patient had more TSH/FT4 measurements. In our model, we corrected for sex, BMI and age at time of the blood sampling, since these parameters can influence the lnTSH/FT4 relationship.

All calculations were performed using SPSS 17.0 for windows (SPSS, Inc., Chicago, IL). Differences were considered statistically significant at $P<0.05$.

Results

Patient characteristics

Patient characteristics are summarized in **Table 1**. We studied 151 DTC patients, 28 males and 123 females. At the timepoint of the blood sampling for the determination of the different alleles of the deiodinase type 1 and 2 polymorphisms, mean age was 49.1 ± 12.9, mean dose of thyroxine 183 ± 51 µg/day and mean duration of follow-up 11.5 ± 8.8 years.

We identified a total of 3121 blood samples routinely obtained during follow up between 1992 and 2007. In 1905 of these samples both TSH and FT4 were measured and these values were used for this study. Mean number of measurements per patient was 12.53 ± 5.49 (range 4-32).

During this period, patients were regularly withdrawn from thyroxine for routine TSH stimulated radioiodine-131 whole body scanning. Therefore, the TSH (and FT4) values have a very wide range (see **Table 2**).

Genotyping failed in 3 patients for the D1-rs11206244 polymorphism, in 4 patients for the D1-rs12095080 polymorphism, in 3 patients for the D2-rs225014 polymorphism and in 4 patients for the D2-rs12885300 polymorphism.

Polymorphisms in deiodinases and values of TSH and FT4

Detailed information on genotype frequencies of the two deiodinases is given in Table 2.

For the D1-rs11206244 (D2-C785T) polymorphism 66 patients were wild type (C/C), 58 patients were heterozygous (C/T) and 24 patients were homozygous variant carriers (T/T). For the D1-rs12095080 (D1-A1814G) polymorphism, 125 patients were wild type (A/A), 19 patients were heterozygous (A/G) and only 3 were homozygous (G/G). The D2-rs225014 polymorphism (D2-Thr92Ala) was more equally distributed: 56 patients were wild type (Thr/Thr), 64 heterozygous (Thr/Ala) and 28 homozygous (Ala/Ala). For the D2-rs12885300 (D2-ORFa-Gly3Asp) polymorphism, 70 patients were wild type (Gly/Gly), 64 patients were heterozygous (Gly/Asp) and 13 patients were homozygous (Asp/Asp). The genotype distributions did not deviate from the Hardy-Weinberg equilibrium.

Table 1: Patient characteristics

	Patients (n=151)
Age (yr, mean ± SD)	49.10 ± 12.93
Sex (M/F)	28/123
Tumor stage	
Unknown	6
T0 N1 M0	1
T0 N1 M1	1
IA T1 N0 M0	8
IB T2 N0 M0	72
IIA T1 N1 M0	7
IIB T2-3 N0-1 M0	28
IIIA T1-3 N1-2 M0	6
IIIB T1-3 N3 M0, T4, any N M0	18
IV Any T, any N, M1	4
Histology Tumor	
Papillary (n)	104
Papillary-follicular variant (n)	21
Follicular (n)	25
Follicular-Hurtle cell (n)	1
Duration of follow-up (yr) (mean ± SD)	11.45 ± 8.86
Total number of blood samples	3121
Number of blood samples with both TSH and FT4	1905
Number of measurements per patient	12.53 ± 5.49 (range 4-32)
L-thyroxin dose (µg/day) (mean±SD)	182.87 ± 50.84

Correlation between TSH and FT4 for the different alleles of the D1 and the D2 polymorphisms

We used a linear mixed model analysis to compare the slopes of the regression lines between lnTSH and FT4 for the different alleles of the D1 and D2 polymorphisms. Interestingly, the regression lines of the D2-rs12885300 (D2-ORFa-Gly3Asp) polymorphism for wild type and heterozygous patients were significantly different from the regression line of homozygous subjects (Table 2, Figure 1). The slopes were comparable; however the intercept for homozygous patient was significantly higher. The slope of the regression line for wild type patients was -0.322 ± 0.028 (ln[TSH mU/l]/[FT4 pmol/l]) with an intercept of 4.95. For heterozygous patients the slope was also -0.299 ± 0.028 (ln[TSH mU/l]/[FT4 pmol/l]) with an intercept of 4.23. The slope of the homozygous patients was -0.347 ± 0.026 (ln[TSH mU/l]/[FT4 pmol/l]), whereas the intercept was 6.07 ($p = 0.036$ vs. wild-type and heterozygous subjects).

Table 2: Distribution of sample size, thyroid hormone parameters

Deiodinase type 1 C785T polymorphism			
Setpoint analysis 1992-2007	**Wild type**	**Heterozygous**	**Homozygous**
N (3 missing polymorphisms)	66	58	24
Number of blood samples	885	739	245
FT4 (pmol/l) (mean ± SD)	21.17 ± 5.63	21.27 ± 6.26	22.08 ± 5.219
TSH (mU/l) (median(range))	0.13 (0.004-289)	0.14 (0.005-365)	0.07 (0.005-256)
LnTSH (median(range))	-2.04 (-5.52-5.67)	-1.96 (-5.3-5.9)	-2.66 (-5.3-5.55)
β (slope) (ln[TSH mU/l]/[FT4 pmol/l]) (mean ± SD)	-0.269 ± 0.023	-0.275 ± 0.024	-0.296 ± 0.021
Intercept (mean ± SD)	5.82 ± 0.58	5.53 ± 0.58	5.08 ± 0.82
Cross-sectional data 2007			
TSH (mU/l)	0.63 ± 1.36	0.28 ± 0.64	0.41 ± 1.01
T4 (nmol/l)	138.33 ± 33.67	141.78 ± 45.07	133.04 ± 41.51
T3 (nmol/l)	1.47 ± 0.31	1.50 ± 0.36	1.37 ± 0.33
T3/T4 ratio	0.011 ± 0.002	0.011 ± 0.002	0.011 ± 0.004
rT3 (nmol/l)	0.57 ± 0.24	0.51 ± 0.19	0.54 ± 0.20
Deiodinase type 1 A1814G polymorphism			
Setpoint analysis 1992-2007	**Wild type**	**Heterozygous**	**Homozygous**
N (4 missing polymorphisms)	125	19	3
Number of blood samples	1570	260	33
FT4 (pmol/l) (mean ± SD)	21.61 ± 5.83	21.27 ± 5.73	22.96 ± 6.57
TSH (mU/l) (median(range))	0.12(0.04-364)	0.17 (0.05-324)	0.10 (0.05-93)
LnTSH (median(range))	-2.12 (-5.52-5.90)	-1.77 (-5.30-5.78)	-2.32 (-5.30-4.53)
β (slope) (ln[TSH mU/l]/[FT4 pmol/l]) (mean ± SD)	-0.338 ± 0.061	-0.323 ± 0.023	-0.317 ± 0.008
Intercept (mean ± SD)	4.96 ± 1.48	5.70 ± 0.55	5.52 ± 0.67
Cross-sectional data 2007			
TSH (mU/l)	0.44 ± 1.01	0.63 ± 1.23	0.09 ± 0.13
T4 (nmol/l)	137 ± 37.64	151.70 ± 50.05	115.37 ± 19.09
T3 (nmol/l)	1.46 ± 0.33	1.46 ± 0.40	1.55 ± 0.43
T3/T4 ratio	0.011 ± 0.003	0.010 ± 0.002	0.013 ± 0.003
rT3 (nmol/l)	0.56 ± 0.23	0.52 ± 0.14	0.41 ± 0.05
Deiodinase type 2 Thr92Ala polymorphism			
Setpoint analysis 1992-2007	**Wild type**	**Heterozygous**	**Homozygous**
N (3 missing polymorphisms)	56	64	28
Number of blood samples	781	777	311
FT4 (pmol/l) (mean ± SD)	21.73 ± 5.93	21.63 ± 5.79	21.10 ± 5.73
TSH (mU/l) (median(range))	0.12 (0.05-364)	0.10 (0.04-365)	0.14 (0.05-324)
LnTSH (median(range))	-1.85 (-5.30-5.67)	-2.30 (-5.50-5.90)	-1.96 (-5.30-5.78)
β (slope) (ln[TSH mU/l]/[FT4 pmol/l]) (mean ± SD)	-0.313 ± 0.022	-0.319 ± 0.022	-0.315 ± 0.018
Intercept (mean ± SD)	5.64 ± 0.54	5.47 ±0.53	5.29 ± 0.77

Table 2: Continued

Cross-sectional data 2007			
TSH (mU/l)	0.44 ± 0.99	0.37 ± 0.87	0.66 ± 1.60
T4 (nmol/l)	144.80 ± 34.96	135.30 ± 44.38	133.26 ± 34.29
T3 (nmol/l)	1.49 ± 0.28	1.46 ± 0.38	1.40 ± 0.33
T3/T4 ratio	0.011 ± 0.001	0.011 ± 0.003	0.011 ± 0.002
rT3 (nmol/l)	0.58 ± 0.23	0.52 ± 0.21	0.55 ± 0.19
Deiodinase type 2 ORFa-Gly3Asp polymorphism			
Setpoint analysis 1992-2007	**Wild type**	**Heterozygous**	**Homozygous**
N (3 missing polymorphisms)	70	64	13
Number of blood samples	865	818	199
FT4 (pmol/l) (mean ± SD)	21.90 ± 5.95	21.44 ± 5.68	21.02 ± 5.71
TSH (mU/l) (median(range))	0.12 (0.05-364)	0.11(0.05-218)	0.24 (0.05-131)
LnTSH (median(range))	-2.12 (-5.30-5.90)	-2.21 (-5.52-5.39)	-1.42 (-5.30-4.88)
β (slope) (ln[TSH mU/l]/[FT4 pmol/l]) (mean ± SD)	-0.322 ± 0.028	-0.299 ± 0.028	-0.347 ± 0.026
Intercept (mean ± SD)	4.95 ± 0.66	4.23 ± 0.66	6.07 ± 0.86
Cross-sectional data 2007			
TSH (mU/l)	0.51 ± 1.15	0.39 ± 1.05	0.52 ± 0.90
T4 (nmol/l)	138.03 ± 42.98	136.68 ± 37.29	152.67 ± 20.13
T3 (nmol/l)	1.45 ± 0.33	1.45 ± 0.36	1.58 ± 0.23
T3/T4 ratio	0.011 ± 0.003	0.011 ± 0.002	0.010 ± 0.001
rT3 (nmol/l)	0.56 ± 0.21	0.50 ± 0.19	0.71 ± 0.33

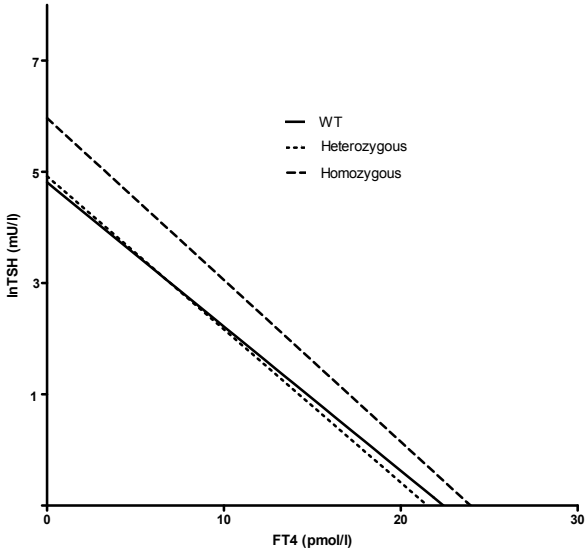

Figure 1
Correlation between the natural logarithm of serum levels of TSH and serum levels of FT4 for the D2-ORFa-Gly3Asp polymorphism in 151 patients with differentiated thyroid carcinoma.

Discussion

This study demonstrates that thyroidectomised DTC patients on thyroxine substitution who are homozygous for the D2-rs12885300 (D2-ORFa-Gly3Asp) polymorphism have an altered setpoint of the hypothalamus-pituitary-thyroid axis. This study comprises a unique series of 1905 combined TSH and FT4 measurements. The mixed model analysis of the TSH/FT4 ratios is a precise approach to determine differences in individual setpoints. Our data suggest that the negative feedback of T4 on TSH is weaker in patients homozygous for the D2-rs12885300 (D2-ORFa-Gly3Asp) than in wild-type and heterozygous subjects. This is demonstrated by a higher lnTSH in combination with equal FT4 levels for homozygous patients.

Patients treated for DTC are ideal to investigate thyroid hormone metabolism, because they have been treated with total thyroidectomy and radioiodine ablation therapy. Because of this treatment they have no intrinsic T3 production. Therefore T3 levels are dependent on production at the tissue level through deiodination of exogenous T4 by D1 and D2. The negative feedback regulation of pituitary TSH secretion by T3, which in our patients is completely produced outside the thyroid, is mainly dependent on pituitary D2.

Although we have found a clear difference in the setpoint of the hypothalamus-pituitary-thyroid axis for the different D2-rs12885300 (D2-ORFa-Gly3Asp) polymorphisms, there are some unknown factors that could have also influenced TSH/FT4 ratios. We will discuss these factors briefly. First, unfortunately, because samples were collected as routine clinical follow-up, only TSH and FT4 levels were available, hence T3 and rT3 are only measured at one time point in 2007. Therefore we are not able to speculate about the serum values of T3 and rT3, and with that not the complete metabolic cycle of thyroid hormones during the entire period of the sample collection.

Second, we did not correct for a possible seasonal influence on setpoints. Two studies showed higher serum T3 and T4 values during the winter and lower values of TSH and total T3 during spring in healthy volunteers (23,24). However, given the huge number of random samples we assume that the seasons in which blood samples were collected will probably be equally distributed. Moreover, a previous study suggests a very limited effect of seasonal variation in thyroid function tests (20). In addition to this, we think that healthy volunteers in these studies and our thyroidectomized patients are not easily compared, since healthy persons have intrinsic thyroid hormone production possibly dependent on the seasonal feedback variation, whereas our patient population is treated with a fixed dose of thyroxine substitution. We therefore think that the potential contribution of seasonal influence is limited.

Our observations are however in contrast to the findings of Coppotelli *et al.* (25) who found an increased D2 activity of the D2-rs12885300 polymorphism in an in-vitro study and with the results of the study by Peeters *et al.* (8), who found that healthy blood donors with a D2-rs12885300 mutation needed less T4 to produce local T3 for the negative feedback action on the pituitary. These results were not confirmed in a group of healthy elderly men (8). However their observations in healthy blood donors with intrinsic thyroid function cannot be easily compared to DTC patients on TSH-suppressive thyroxine therapy.

Our patients are treated with a TSH suppressive dose of thyroxine (26,27). Another factor could be that long term subclinical hyperthyroidism may result in down-regulation of D1 and D2 and/or upregulation of D3 (2). However, we did not find a significant contribution of follow-up time and age at presentation to the observed effects of the D2-rs12885300 polymorphism on the setpoint of the hypothalamus-pituitary-thyroid axis.

In conclusion, we have found an altered setpoint of the hypothalamus-pituitary-thyroid axis for patients homozygous for the D2-rs12885300 polymorphism. However, it is unknown what the clinical significance of this altered setpoint will be. In the future, it would be interesting to investigate the proof of functionality of this D2 polymorphism and differences in biological variability in cell lines containing the different alleles of the D2-rs12885300 polymorphism.

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