Clinical aspects of endogenous hypothyroidism and subclinical hyperthyroidism in patients with differentiated thyroid carcinoma

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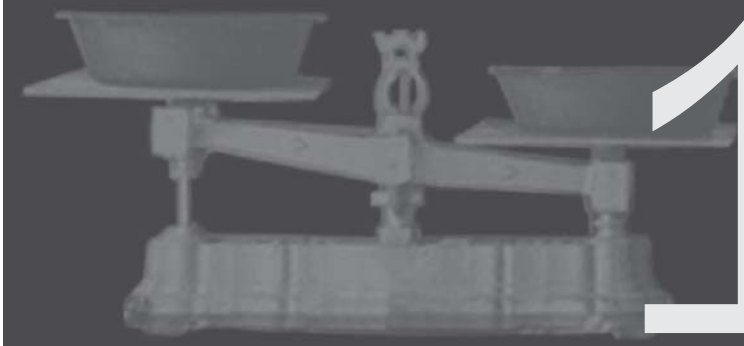

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The Thr92Ala polymorphism in the type 2 deiodinase is not associated with thyroxin dose in athyroid patients or patients with Hashimoto thyroiditis

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Clinical Endocrinology in press

Abstract

Objective: The type 2 deiodinase (D2)-Thr92Ala polymorphism has been associated with decreased D2 activity in some in vitro experiments but not in others. So far no association between the D2-Thr92Ala polymorphism and serum thyroid hormone levels has been observed in humans, but in a recent study in athyroid patients, it was suggested that patients homozygous for the Ala92 allele needed higher thyroxine doses to achieve TSH suppression.

We studied the association between the D2-Thr92Ala polymorphism with thyroid hormone levels and thyroxine dosage, in patients treated for differentiated thyroid carcinoma (DTC) and in a group of patients treated for Hashimoto thyroiditis.

Design: Cross sectional study

Patients: We studied 154 patients with DTC treated with TSH suppressive thyroid hormone replacement therapy for longer than 3 years and 141 patients with Hashimoto thyroiditis treated for at least 6 months with thyroxine.

Measurements: In all patients, serum levels of TSH, free thyroxine, triiodothyronine and reverse T3 were measured and genotypes of the D2-Thr92Ala polymorphism were determined by Taqman assay. Univariate regression analysis was performed to determine the relation between thyroxine dosages and the D2-Thr92Ala polymorphism corrected for age, gender, BMI and serum TSH levels.

Results: Both in DTC patients and Hashimoto patients, no association was observed between serum thyroid hormone levels or thyroxine dosages in presence of the D2-Thr92Ala polymorphism. Categorization of DTC patients according to degree of TSH suppression did not change these results.

Conclusion: The D2-Thr92Ala polymorphism is not associated with thyroid hormone levels or thyroxine dose in patients treated for DTC or Hashimoto thyroiditis.

Background

Most actions of thyroid hormone are mediated by the active form of thyroid hormone, triiodothyronine (T3). Serum and local T3 concentrations are mainly regulated by the iodothyronine deiodinases D1, D2 and D3(1). D2 is essential for the local production of T3 through deiodination of T4. D2 is thus essential for the negative feedback regulation of thyroid hormone on thyrotropin (TSH) production in the pituitary. Several polymorphisms in D2 have been described (2-5). Controversy exists about the functional implications of the D2-Thr92Ala polymorphism, which has been associated with a decreased D2 activity in some in vitro experiments (2) but not in others (5). So far no associations were found between the D2-Thr92Ala polymorphism and serum thyroid hormone levels in studies in healthy subjects(4;6;7).

Torlontano *et al*. reported in thyroidectomized differentiated thyroid carcinoma (DTC) patients that homozygous carriers of the D2-Ala92 allele needed higher dosages of thyroxine(8). This difference was most prominently observed in the group with near-suppressed TSH (TSH values between 0.1 and 0.5 mU/L). Limitations of this study were that actual values of serum TSH levels for wild-type and homozygous groups within the near-suppressed TSH group were not given. It is therefore unclear whether TSH levels in both groups were indeed identical, which would be a key finding to ascribe the slight differences in thyroxine dose indeed to the polymorphism. The fact that serum T4 and T3 levels did not differ between the wild-type group and D2-Thr92Ala homozygotes is also remarkable. Moreover, as TSH is a continuous variable, we believe that the optimal analytic strategy would be by regression analysis, rather than a categorized approach. We therefore performed this study to reconfirm the findings of Torlontano *et al*.

For this reason, we studied the association between the D2-Thr92Ala polymorphism and thyroid hormone levels and thyroxine dosage in 154 patients treated for DTC and 141 patients substituted with thyroxine for Hashimoto thyroiditis, using a linear regression model. In addition, we performed a categorized analysis to allow maximal comparability with the Torlontano study.

Patients and Methods

Patients

Patients treated for DTC were recruited from the outpatient clinic of the Department of Endocrinology of the Leiden University Medical Center. All patients had been treated by near-total thyroidectomy followed by radioiodine ablation. 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. The Local Ethics Committee of the Leiden University Medical Centre approved the study, and written informed consent was obtained from all subjects.

We also included 141 patients treated for at least 6 months with L-thyroxine for Hashimoto thyroiditis. Serum TSH levels were between 0.11 and 4.0 mU/L. These patients were described earlier by Appelhof *et al* (9).

Study design

After an overnight fast, patients had a physical examination, including, height (meters [m]) and weight (kilograms [kg]). Blood was collected for determination of TSH, free thyroxine (FT4), T3 and reverse T3 (rT3). Serum samples were handled immediately and stored at –80 oC in Sarstedt tubes. DNA was collected for genotyping of the D2-Thr92Ala polymorphism. To be able to compare our study with the study of Torlontano *et al*. (8), patients were categorized in groups with a suppressed TSH (< 0.1 mU/L), near-suppressed TSH (0.1-0.5 mU/L) or non-suppressed TSH (>0.5 mU/L).

Serum biochemistry

In the patients treated for DTC, serum FT4 and TSH were measured using a chemoluminescence immunoassay with a Modular Analytics E-170 system (intraassay CV of 1.6-2.2 % and 1.3-5.0 % respectively (Roche, Almere, The Netherlands). Serum T3 was measured with a fluorescence polarization immunoassay, CV 2.5-9.0 %, on an ImX system (Abbott, Abbott Park, IL, USA). Reverse T3 was measured using a RIA as described previously (10).

In the patients treated for Hashimoto thyroiditis, serum TSH and FT4 were measured by time-resolved fluoroimmunoassay and serum T4 and T3 by in-house RIA methods (6).

Genotyping

DNA was isolated from peripheral leucocytes by the salting out procedure (11). Genotypes of the D2-Thr92Ala polymorphism (rs 225014) 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 TaqMan® 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 Hardy-Weinberg Equilibrium was analysed using a X2-test. Dominant (Thr/Thr vs. Ala/X) and recessive (Thr/X vs. Ala/Ala) effects of the polymorphism were analyzed. The association between D2-Thr92Ala genotypes and thyroxine dosages or thyroid hormone levels were analyzed using multivariate regression analyses. This was corrected for age, gender, BMI and the natural logarithm of TSH levels. In addition, differences between the different D2 genotype groups were analyzed using unpaired t-test or ANCOVA. All calculations were performed using SPSS 12.0 for windows (SPSS, Inc., Chicago, IL). Differences were considered statistically significant at $P<0.05$.

Results

Patient characteristics

We studied 154 DTC patients. Mean duration of TSH suppressive therapy was 9.2 years (range 0.5-42.6 years). Median duration of cure was 8.9 years (range 1.0-41.8 years). The mean dose of thyroxine was 183 ± 51 µg/day. Mean thyroxine dose was 2.2 ± 1.0 µg/kg body weight. We also studied 141 patients with Hashimoto thyroiditis on thyroxine replacement therapy. Genotyping of the D2-Thr92Ala polymorphism failed in two subjects. The remaining 139 patients were treated with thyroxine for a mean duration of 7.3 ± 5.8 years. Mean thyroxine dose was 125 ± 46 µg/day.

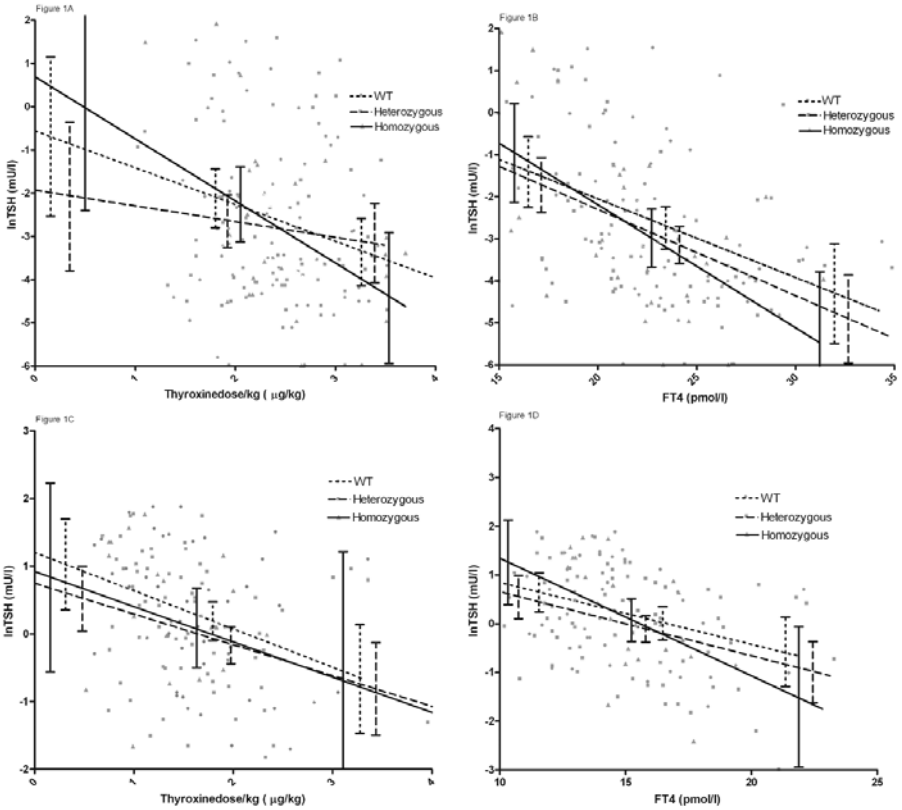
Thyroid hormone parameters and D2-Thr92Ala

Allele frequencies of the D2-Thr92Ala polymorphism in the DTC patients and Hashimoto thyroiditis patients were 39.6% and 40.3%, respectively. The genotype distributions did not deviate from Hardy-Weinberg equilibrium. Thyroid hormone levels and thyroxine dose for patients with DTC and Hashimoto thyroiditis are presented in Table 1. No differences were observed in thyroid hormone levels and thyroxine dose, corrected for BMI and TSH levels between wild-type, heterozygous and homozygous carriers of the D2-Thr92Ala polymorphism. Analyses were comparable when thyroxine dose was corrected for BMI. No differences were observed in the correlation between lnTSH and thyroxine dose/kg or FT4 level for the different carriers of the D2-thr92ala polymorphism (figure 1 A-D).

No differences were observed either in thyroid hormone levels or thyroxine dose when patients were categorized according to the degree of TSH suppression in the cohort of DTC patients.

Linear regression analysis showed no association between the D2-Thr92Ala polymorphism and thyroid hormone levels or thyroxine dose corrected for TSH levels and BMI (DTC: p=0.960, r=0.564, Hashimoto thyroiditis: p=0.274, r=0.302)

Figure 1.



a. Correlation between the natural logarithm of TSH and thyroxine dosage/kg for the different alleles of D2-Thr92Ala polymorphism in 154 patients with differentiated thyroid carcinoma. Lines: regression lines; bars: 95% confidence intervals of regression lines.

b. Correlation between the natural logarithm of TSH and FT4 for the different alleles of D2-Thr92Ala polymorphism in 154 patients with differentiated thyroid carcinoma. Lines: regression lines; bars: 95% confidence intervals of regression lines.

c.c. Correlation between the natural logarithm of TSH and thyroxine dosage/kg for the different alleles of D2-Thr92Ala polymorphism in 139 patients treated for Hashimoto thyroiditis.

d.d. Correlation between the natural logarithm of TSH and FT4 for the different alleles of D2-Thr92Ala poly morphism in 139 patients treated for Hashimoto thyroiditis.

Table 1. Deiodinase type 2 genotypes and thyroid hormone parameters.

	Genotype	Patients (n)	Age (yr)	Gender (m/f)	Weight (kg)	BMI (kg/m²)	TSH (mU/l)	FT4 (pmol/l)	T3 (nmol/l)	rT3 (nmol/l)	T4 dose (µg/day)	T4 dose (µg/kg)	Dose / kg x lnTSH
DTC	WT Thr/Thr	60	47.2±12.4	13/47	76.1±15.3	25.6±4.7	0.05 (0.003-4.6)	22.72±3.89	1.49±0.28	0.60±0.23	186.3±58.2	2.09±1.04	-6.74±5.28
	HeZ Ala/Thr	66	51.5±13.5	11/55	75.7±12.2	26.2±3.5	0.03 (0.003-4.9)	22.42±4.48	1.46±0.38	0.51±0.21	178.2±41.5	2.22±0.87	-6.81±4.96
	HoZ Ala/Ala	28	48.3±10.2	5/23	74.7±14.8	25.8±5.9	0.05 (0.003-6.8)	21.66±4.27	1.40±0.33	0.56±0.19	185.9±58.4	2.19±1.07	-7.82±5.67
	P-value	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS		
HT	WT Thr/Thr	47	46.6±8.6	5/42	78.5±17.8	27.8±5.4	2.02±1.76	14.46±2.87	1.73±0.36	ND	124.2±41.4	1.64±0.62	0.24±1.81
	HeZ Ala/Thr	72	47.3±10.9	11/61	80.4±18.3	28.2±5.6	1.59±1.41	15.16±3.05	1.73±0.30	ND	127.6±50.6	1.64±0.71	-0.22±2.01
	HoZ Ala/Ala	20	52.1±8.2	4/16	91.0±31.3	32.2±9.1	1.96±1.68	14.42±2.48	1.71±0.36	ND	116.3±38.1	1.36±0.51	0.17±1.45
	P-value	NS	NS	0.046	0.026	NS	NS	NS	NS	NS	NS	NS	NS

Table 1. Deiodinase type 2 genotypes and thyroid hormone parameters

DTC= Differentiated thyroid carcinoma, HT= Hashimoto thyroiditis, WT= wild-type, HeZ= Heterozygous, HoZ= Homozygous

Data are expressed as mean ± SD or number of patients, except for TSH which is median (range)

Analyses for TSH, FT4, T3 and T4 dose in HT patients are corrected for age, gender and BMI.

Discussion

We studied the association between the D2-Thr92Ala polymorphism and thyroid hormone levels and thyroxine dose in 2 separate groups of patients, treated for DTC or Hashimoto thyroiditis. Frequencies of the alleles of D2-Thr92Ala are in agreement with previous studies varying between 30-38.8 % in patient with normal thyroid function or not taking thyroid replacement or thyreostatic medication (3;4;6;7). The D2-Thr92Ala polymorphism was not associated with thyroid hormone parameters or thyroxine dosages in the 2 separate group of patients included in our analyses. This is in accordance with previous studies (4-7). Torlontano *et al*. found that homozygous DTC carriers of the D2-Ala92 allele need higher thyroxine dosages (8). This association was observed in the near-suppressed TSH group, but not in the suppressed group. The study of Torlontano *et al*. has however several limitations. TSH levels in the near-suppressed group of the different alleles were not given, which would have been useful to investigate whether the differences in thyroxine dose are not caused by alterations in TSH levels. In our study, no differences were observed in TSH levels or thyroxine dose for the different alleles with and without categorization according to the degree of TSH suppression in the DTC patients or Hashimoto patients. In addition, we believe that the analysis strategy should be primarily based on regression analysis rather than TSH categories, because for alterations in TSH levels should be corrected.

Remarkably, they did not find any differences in thyroid hormone levels suggesting that patients with D2-Ala92 alleles need a higher thyroxine dose to reach the same serum FT4 level. By inference, the Ala allele would not affect T4 feedback but rather T4 resorption. Torlontano *et al*. explain the discrepancies of their findings with previous studies by two arguments. First, they state that in previous studies thyroid hormone levels were within the wide reference range, which makes is difficult to detect subtle differences in thyroid hormone levels for different carriers of the D2-Thr92Ala polymorphism. However, they found this difference only in the near-suppressed group, which is an ill-defined group with a wide plasma TSH range including patients with normal TSH levels. Second, Torlontano *et al*. argue that the difference between their finding and earlier studies may be explained by the absence of a thyroid gland in their patients. However, in our analysis with athyroid DTC and Hashimoto patients, we could not confirm this. A posthoc power analysis for thyroxine dose and thyroxine/kg showed a sufficient power of 100 %. Therefore, it seems unlikely that underpowering of our study plays a major role in the negative findings.

In summary, we found no association between the D2-Thr92Ala polymorphism and thyroid hormone levels and thyroxine dose in 2 separate groups of patients treated for DTC or Hashimoto thyroiditis.

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