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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

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Background: The common Thr92Ala D2 polymorphism has been associated with changes in pituitary–thyroid axis homeostasis, but published results are conflicting. To investigate the effects of the Thr92Ala polymorphism on intrathyroidal thyroxine (T4) to triiodothyronine (T3) conversion, we designed prospective pharmacogenomic intervention aimed to detect differences in T3 levels after thyrotropin (TSH)-releasing hormone (TRH)–mediated TSH stimulation of the thyroid gland.
Methods: Eighty-three healthy volunteers were screened and genotyped for the Thr92Ala polymorphism. Fifteen volunteers of each genotype (Thr/Thr, Thr/Ala, and Ala/Ala) underwent a 500 mcg intravenous TRH stimulation test with serial measurements of serum total T3 (TT3), free T4, and TSH over 180 minutes.
Results: No differences in baseline thyroid hormone levels were seen among the study groups. Compared to the Thr/Thr group, the Ala/Ala group showed a significantly lower TRH-stimulated increase in serum TT3 at 60 minutes (12.07 ± 2.67 vs. 21.07 ± 2.86 ng/dL, $p = 0.029$). Thr/Ala subjects showed an intermediate response. Compared to Thr/Thr subjects, the Ala/Ala group showed a blunted rate of rise in serum TT3 as measured by mean time to 50% maximum delta serum TT3 (88.42 ± 6.84 vs. 69.56 ± 6.06 minutes, $p = 0.028$). Subjects attained similar maximal (180 minutes) TRH-stimulated TT3 levels. TRH-stimulated TSH and free T4 levels were not significantly different among the three genotype groups.
Conclusions: The commonly occurring Thr92Ala D2 variant is associated with a decreased rate of acute TSH-stimulated T3 release from the thyroid consistent with a decrease in intrathyroidal deiodination. These data provide a proof of concept that the Thr92Ala polymorphism is associated with subtle changes in thyroid hormone homeostasis.

Introduction

The circulating pool of thyroid hormones is the result of a dynamic equilibrium between thyroidal secretion of thyroxine (T4) and triiodothyronine (T3), their tissue uptake and degradation, the peripheral conversion of T4 into T3 or reverse-T3, and the conversion of T3 into T2 (1,2). The conversion of the pro-hormone T4 into T3 or rT3 represents an important step in the maintenance of circulating and tissue levels of T3, thus acting as a pre-receptor modulator of thyroid hormone action. Both type-1 (D1) and type-2 (D2) deiodinase enzymes are present in the thyroid gland and, by regulating intrathyroidal conversion of T4 into T3, play an important role in the secretion of thyroid hormone (3), especially in pathologic conditions in which the thyrotropin (TSH) receptor or its downstream pathway is overstimulated, resulting in T3 toxicosis (3–6).

The common Thr92Ala D2 polymorphism (7) has been associated with traits of impaired thyroid hormone action at various end-organ targets (7–10), the hypothalamic-pituitary level (11), and in circulating levels of thyroid hormones (12). Nonetheless, results are conflicting (13–16) and the data refer

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to steady-state serum thyroid hormone levels. In this prospective pharmacogenomic study, we characterize the effects of the Thr92Ala D2 polymorphism on acute thyroidal T3 release by analyzing the dynamic response to TSH-releasing hormone (TRH)–stimulated acute secretion of TSH.

Materials and Methods

Participants and study design

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 (Clinical Trials.gov identifier number NCT00812149).

Healthy volunteers aged 18–65 were eligible for the study. Exclusion criteria included thyroid disease (by history, TSH <0.4 or >4.0 mcIU/mL, positive antithyroid peroxidase antibodies or positive thyroid-stimulating immunoglobulin), hypertension (by history or blood pressure $>140/90$ mmHg), pregnancy, history of liver disease, kidney disease, diabetes, coronary artery disease, asthma, seizures, chronic headache, depression, use of prescription medications, or use of supplements potentially affecting thyroid function.

Eligible subjects 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 for analysis of Thr92Ala D2 polymorphism status. Subjects then underwent a TRH test.

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 23°C–25°C. Women of reproductive age had a repeat pregnancy test at admission. A saline lock IV catheter was inserted 15 minutes before the first blood draw. At time 0, 500 μg TRH was given intravenously over 1 minute followed by a 10 cc normal saline flush. Blood samples were taken at -15, 0, 5, 10, 15, 20, 30, 60, 120, and 180 minutes for measurement of TSH, free T4 (FT4), and total T3 (TT3). Prolactin levels were measured at -15, 0, 60, and 180 minutes as an independent marker of TRH action. Blood pressure was monitored before administration of TRH, and after the 30, 60, 120, and 180 blood draws. Side effects (nausea, urinary urgency, and overall malaise) were assessed at the end of the study using a 10-cm visual-analog scale.

Blood sampling

All blood samples were drawn under sterile technique through the intravenous catheter using the Vacutainer® system (Becton Dickson and Co.).

Laboratory testing

Screening tests (Chem 20, lipid panel, complete blood count [CBC], TSH, FT4, T3, antithyroid antibody panel, and urine human chorionic gonadotropin [HCG] in women) were performed in the NIH Department of Laboratory Medicine; thyroid-stimulating immunoglobulin testing was performed by Mayo Medical Labs (Rochester, MN). The TRH test blood samples were analyzed by the NIH Department of Laboratory Medicine.

DNA isolation

Genomic DNA was isolated from peripheral mononuclear cells from the screening blood samples using the QIAmp® system (Qiagen, Inc.) in our laboratory. After isolation, DNA concentration was measured at 260 nm using a NanoDrop™ spectrophotometer (Thermo Fisher Scientific, Inc.). Purity was determined by measuring the 260/280-nm ratio.

Restriction fragment length polymorphism analysis

Thr92Ala D2 polymorphism status was characterized using established methods (7) by polymerase chain reaction restriction fragment–length polymorphism analysis. The

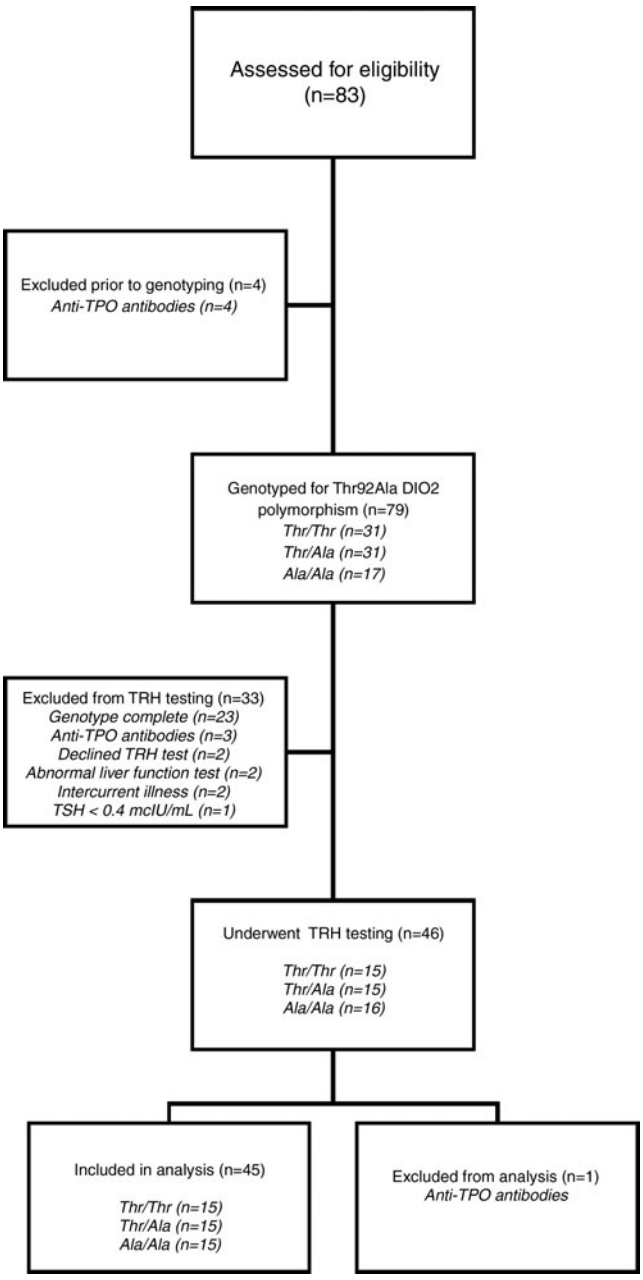

FIG. 1. CONSORT chart describing subject recruitment and testing.

polymerase chain reaction product resulting from a 5′ CTCAGGGCTGGCAAAGTCAAG 3′ sense primer and a 5′ CCACACTCTATTAGAGCCAATTG 3′ antisense primer was digested with Bsg-1 (New England BioLabs®, Inc.), and the digestion products were separated on a 1.5% agarose gel by electrophoresis.

Statistical analysis and sample size

An *a priori* analysis for the primary outcome was conducted using data from the original FDA Investigational New Drug application for the use of TRH at the National Institutes of Health Clinical Center (Protocol 03-DK-0098, Principal Investigator Monica C. Skarulis). From this analysis a sample size of 15 subjects per group was calculated to confer a >80% power to detect a significant difference (20%, i.e., one standard deviation) in the primary endpoint, that is, the change in serum concentration of TT3 (delta TT3) after TRH stimulation, by underlying Thr92 AlaD2 polymorphism (Thr/Thr vs. Ala/Ala), at an α error of 0.05 using a two-tailed analysis.

Statistical analysis was performed using Prism 5® (GraphPad) using the two-tailed paired *t*-test and one-way analysis of variance. Data were assessed for normality using the D'Agostino and Pearson omnibus normality test. Nonparametric data were analyzed using the Mann–Whitney test or Kruskal–Wallis test. Area under the curve was calculated using the trapezoidal rule. Results are expressed as mean ± one standard error; an α error of 0.05 was considered the threshold for statistical significance. Time to 50% maximum delta serum TT3 was calculated by solving second-order polynomial nonlinear regression curve fits for individual delta serum TT3 levels between 30 and 180 minutes after TRH injection for each subject.

Results

Patient recruitment and characteristics

Forty-six eligible individuals from 83 study participants completed TRH testing during the January 2009–November 2009 (Fig. 1). One patient was removed from analysis due to the presence of serum thyroid peroxidase antibodies.

Baseline parameters

As illustrated in Table 1, no significant difference was found in any of the studied baseline parameters among the three genotype groups. Specifically, no significant differences were noted in baseline serum TSH, FT4, or TT3 levels by genotype group.

Response to TRH testing

Serum TT3. A robust rise in serum TT3 level was noted in all participants undergoing TRH testing starting at 30 minutes after TRH injection (Fig. 2). The three groups achieved similar maximal (180 minutes) TRH-stimulated levels of serum TT3. However, subjects homozygous for the Thr92Ala D2 polymorphism (Ala/Ala) showed a blunted rate of rise in serum TT3 levels after TRH stimulation compared to Thr/Thr subjects, as measured by mean time to 50% maximum delta serum TT3 (88.42 ± 6.84 vs. 69.56 ± 6.06 minutes, $p = 0.028$) (Fig. 3). As compared to the homozygous groups, Thr/Ala subjects showed a significantly different intermediate response (79.07 ± 5.36 minutes, $p = 0.048$, analysis of variance). Mean TRH-stimulated delta serum TT3 at 60 minutes was significantly lower in Ala/Ala (12.07 ± 2.67 ng/dL) versus Thr/Thr (21.07 ± 2.86 ng/dL) ($p = 0.029$); Thr/Ala subjects showed a nonsignificantly different intermediate response (17.00 ± 4.18 ng/dL, $p = 0.31$). A similar trend was observed in the area under the curve for delta serum TT3 between 30 and 180 minutes after TRH stimulation [Thr/Thr 5970 ± 664 ng/(dLmin) vs. Ala/Ala 4538 ± 314 ng/(dLmin), $p = 0.062$].

Serum FT4. There was no significant difference in maximal (180 minutes) TRH-stimulated levels of serum FT4 among the three groups; however, Ala 92 homozygous subjects showed a nonsignificant trend toward higher FT4 levels (mean delta serum FT4 at 180 minutes: Ala/Ala 0.29 ± 0.03 ng/dL vs. Thr/Thr 0.24 ± 0.02 ng/dL, $p = 0.154$).

Serum TSH. There was no significant difference in maximal (30 minutes) TRH-stimulated levels of serum TSH among the three groups; however, Ala 92 homozygous subjects showed a nonsignificant trend toward higher TSH levels

Table 1. Baseline Patient Characteristics by Threonine92Alanine Type 2 Deiodinase Polymorphism Genotype

	Thr/Thr (n = 15)	*Thr/Ala* (n = 15)	*Ala/Ala* (n = 15)	*ANOVA*, p *value*
Female (*n*)	8	9	10	
Age (years)	32.5 ± 10.1	33.5 ± 9.5	30.3 ± 11.7	0.41
Body mass index (kg/m^2)	25.9 ± 3.9	26.2 ± 5.4	24.3 ± 3.8	0.47
Resting HR (bpm)	65 ± 13	68 ± 10	62 ± 12	0.33
Fasting serum glucose (mg/dL)	81.6 ± 6.5	82.7 ± 9.9	77.3 ± 9.3	0.21
Fasting serum insulin (mcU/mL)	4.4 ± 3.4	4.7 ± 3.4	3.6 ± 1.9	0.71
HOMA	0.89 ± 0.70	0.99 ± 0.83	0.68 ± 0.38	0.43
Fasting serum total cholesterol (mg/dL)	149.3 ± 31.0	159.3 ± 29.4	157.5 ± 46.4	0.73
Fasting serum triglyceride (mg/dL)	74.4 ± 47.2	77.6 ± 63.8	66.1 ± 24.5	0.98
Fasting serum high density lipoprotein (mg/dL)	50.5 ± 15.6	51.9 ± 9.9	50.3 ± 12.0	0.77
Fasting serum low density lipoprotein (mg/dL)	83.8 ± 21.1	92.9 ± 21.4	94.2 ± 38.5	0.55
Serum thyrotropin (mcIU/mL)	1.24 ± 0.63	1.60 ± 0.89	1.69 ± 0.94	0.27
Serum FT4 (ng/dL)	1.25 ± 0.14	1.32 ± 0.20	1.31 ± 0.13	0.46
Serum TT3 (ng/dL)	105.2 ± 22.2	108.9 ± 24.2	102.7 ± 20.8	0.87

Data are shown as mean ± standard deviation.

ANOVA, analysis of variance; FT4, free thyroxine; HR, heart rate; HOMA, homeostatic model assessment of insulin resistance; TT3, total triiodothyronine.

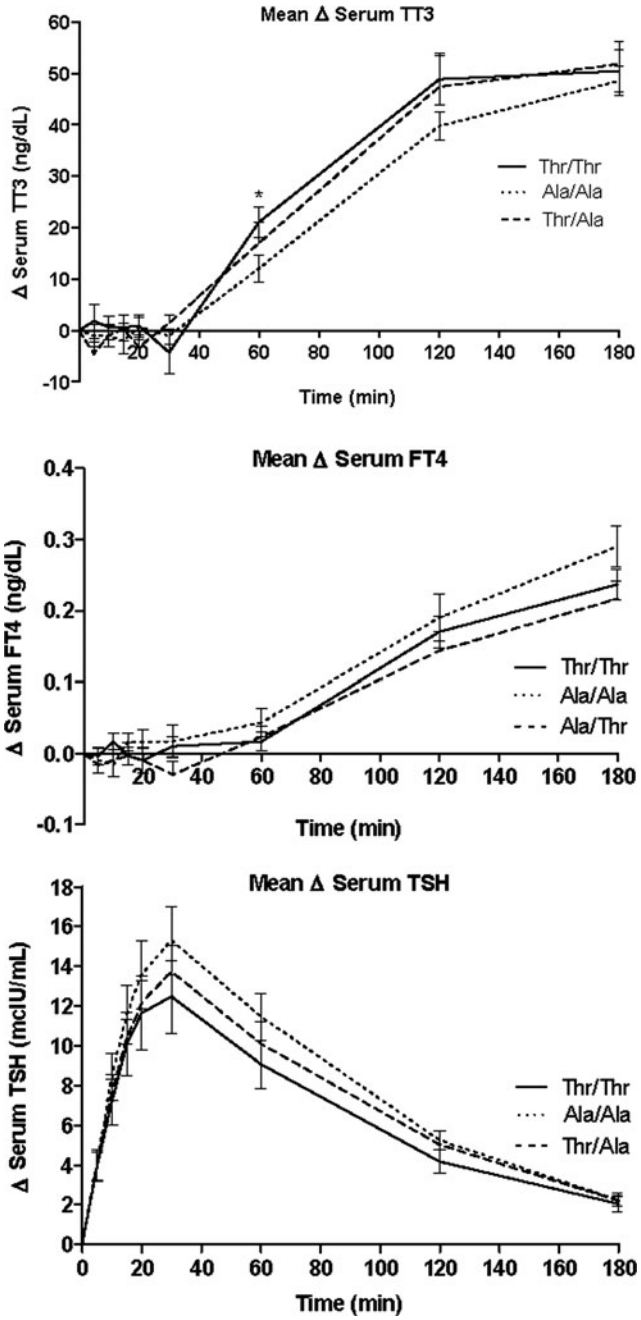

FIG. 2. Mean change in serum thyroid hormone and thyrotropin levels after the intravenous injection of 500 μg of thyrotropin-releasing hormone (TRH), by Thr92Ala deiodinase type 2 genotype. Serum levels were measured at −15, 0, 5, 10, 15, 20, 30, 60, 120, and 180 minutes of TRH injection. Error bars represent the standard error of the mean at each time point. *$p = 0.029$ Thr/Thr versus Ala/Ala.

(mean delta serum TSH at 30 minutes Ala/Ala 15.31 ± 1.72 mcIU/mL vs. Thr/Thr 12.48 ± 1.83 mcIU/mL, $p = 0.269$).

Serum prolactin. Subjects in all groups showed a robust elevation in serum prolactin levels after TRH injection with a maximal level at 60 minutes (Fig. 4). There was no significant difference in mean 60-minute prolactin levels among the

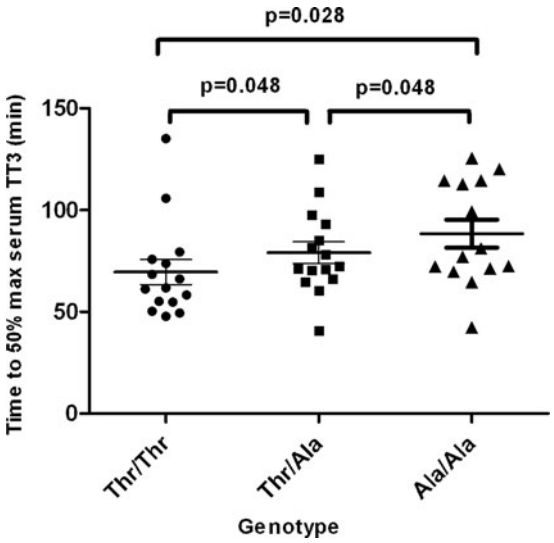

FIG. 3. Time to 50% of maximum rise in serum total triiodothyronine (TT3) levels after the injection of 500 μg of TRH by Thr92Ala deiodinase type 2 genotype. These values were derived from a nonlinear (second order polynomial) regression analysis of the primary data. Horizontal bars represent the group mean ± standard error.

three groups (Thr/Thr 33.4 ± 5.7mcg/L vs. Thr/Ala 31.8 ± 3.7 mcg/L vs. Ala/Ala 39.1 ± 5.4 mcg/L, $p = 0.58$).

Discussion

Clinical and experimental data have demonstrated that intrathyroidal conversion of T4 into T3 makes an important contribution to the overall maintenance of the pool of circulating thyroid hormones (3,17). This is particularly evident in states of overactivation of the TSH pathway such as in Graves' disease, toxic thyroid adenoma, or McCune-Albright syndrome (3–6). These conditions result in an increased serum T3:T4 ratio (18), indicating sustained activation of deiodinase-mediated T4 to T3 conversion within the thyroid gland;

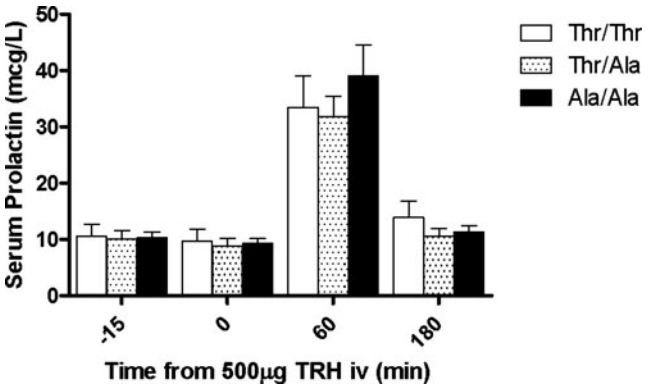

FIG. 4. Mean serum prolactin concentration after the injection of 500 μg of TRH by Thr92Ala deiodinase type 2 genotype. No significant differences were observed at any time point among genotypes. Error bars represent the standard error of the mean at each time point.

the role of D2 is particularly important since its transcription is directly stimulated by cAMP (19), which is the main signal transduction pathway of the G-protein-coupled TSH receptor (20).

The common Thr92Ala D2 polymorphism has been associated with indices of reduced thyroid hormone action at various end-organ targets (7–10,21), and some *ex vivo* data strongly suggest that the Ala allele encodes a defective enzyme (8). The results are, nonetheless, conflicting (12) and the clinical data are mostly derived from secondary analyses of cross-sectional studies aimed to analyze other endpoints.

The effects of the Thr92Ala polymorphism on circulating levels of thyroid hormones are somewhat controversial: Peeters *et al*. found significantly lower plasma TSH levels and TSH/T4 ratios in heterozygous subjects, but no association with circulating iodothyronine levels (13) and confirmed this finding in a follow-up study (12) and also in elderly subjects (12,13,22). Recently, however, Torlontano *et al*. demonstrated that athyreotic patients homozygous for the Ala variant require higher doses of T4 replacement therapy to achieve near-suppression of serum TSH (11). These findings are in keeping with reduced D2 activity in pituitary thyrotrophs. On the other hand, although suggestive, these findings were not confirmed in another study based on a regression analysis model (16).

To characterize the role of the Ala92 variant in the homeostasis of circulating levels of thyroid hormones, we designed a prospective pharmacogenomic intervention to study the dynamic response of T3 secretion to TSH stimulation as a function of intrathyroidal D2-mediated T4 to T3 conversion. To achieve this objective, we exploited the characteristics of the TRH-stimulation test to produce a reliable acute raise of TSH within the physiologic range (23), ultimately leading to a preferential secretion of T3 due to at least in part to the intrathyroidal deiodination of T4 to T3 (3–6).

Our data indicate that Ala92 homozygous subjects show a delayed serum T3 rise in response to TRH-mediated TSH secretion, whereas the Cmax was similar among the study groups. In the heterozygous group the T3 data were suggestive of an intermediate response. Not surprisingly, the results of this latter group were marginally significant since the study was not powered to detect this outcome. Taken together, the data are in keeping with the original hypothesis that the Ala92 variant encodes an enzyme with a decreased activity ultimately causing a blunted rise in serum T3 due to a reduced rate of intrathyroidal T4 to T3 conversion after acute TSH stimulation. On the other hand, we cannot exclude the possibility that the Ala 92 variant causes a slower turnover of the enzyme, rather than a reduction of the activity. The nonsignificant trend toward an elevated rate of T4 secretion observed in Ala92 homozygotes further strengthens this interpretation, suggesting a relative accumulation of substrate in the presence of a reduced amount of product of the enzymatic reaction. Further, the trend toward an increase in delta TSH in the Ala/Ala group is in keeping with the hypothesis that this polymorphism results in a state of relative pituitary insensitivity to the suppressive effects of T4 on TSH secretion (11).

We did not observe any significant differences in body mass index, insulin sensitivity, lipid profile, and baseline thyroid hormones levels, suggesting that in young healthy individuals the Ala92 variant has minimal or no effects on these parameters.

The main limitation of the study is represented by the small number of study subjects, which may prevent the demonstration of subtle differences in baseline parameters such as indices of insulin sensitivity or cholesterol, thyroid hormone levels, or the effects of the heterozygous state. Further, the acute effects of TSH on the thyroid gland are not limited to the activation of D2, and the rise in T3 is also secondary to mobilization of T3 directly from the thyroglobulin and D1-mediated T4 is conversion into T3. Despite these confounder factors this protocol allowed us to demonstrate a significant difference in the primary endpoint, that is, the role of the Ala92 D2 variant in the thyroid gland secretion of T3 in response to and acute TSH stimulation. The major strength of our study is represented by the prospective, hypothesis-driven, pharmacogenomic study design. Moreover, the use of the TRH injection as a means of producing a predictable rise of TSH within physiologic levels (23) allowed us to characterize the dynamic response to acute thyroidal stimulation in terms of T3 secretion. Finally, the use of strict inclusion and exclusion criteria in the selection of our cohort of healthy individuals has provided further validity to our findings.

In conclusion, to the best of our knowledge, this is the first prospective interventional study aimed at demonstrating *in vivo* the effects of the Thr92Ala D2 variant on circulating thyroid hormone homeostasis. The small but significant changes observed further strengthen the hypothesis that this polymorphism has the potential to affect thyroid hormone homeostasis at the level of organ targets of T3 action in which D2 is important in providing the T3 locally. By extension, this polymorphism may play a modulatory role in thyroid hormone action and its influence may be clinically relevant only in pathological states, such as primary hypothyroidism, where compensatory mechanisms are deficient. Further, our data demonstrate the necessity of an accurate study design and dynamic testing of sensitive indices of thyroid hormone action to unravel the effects of such polymorphisms at a target tissue level *in vivo*.

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Disclosure Statement

The authors have nothing to disclose.

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