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Prevalence of *Dio2*^{T92A} polymorphism and its association with thyroid autoimmunity

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ABSTRACT. The 3,5,3′-L-triiodothyronine (T_3) partly derives by the deiodination of the prohormone 3,5,3′,5′-L-tetraiodothyronine (T_4) by the type 2 iodothyronine deiodinase (D2). The single-nucleotide polymorphism in the D2 gene at position 92 (*Dio2*^{T92A}), generates an enzyme with a reduced T_4 to T_3 conversion velocity. Because thyroid hormones can modulate the immune response, we hypothesized a pathophysiological role for *Dio2*^{T92A} polymorphism in autoimmunity. The objective of this study was to investigate the *Dio2*^{T92A} polymorphism in relation to thyroid autoimmunity (TA). We compared the prevalence of *Dio2*^{T92A} polymorphism and serum thyroid hormone levels in healthy subjects and subjects with TA. A total of 110 subjects with TA and 106 controls were genotypized for *Dio2*^{T92A} polymorphism. Free T_3 (FT_3), free T_4 (FT_4) and TSH were measured and compared with the *Dio2*^{T92A} polymorphism. *Dio2*$^{92T/A}$, *Dio2*$^{92A/A}$, and *Dio2*$^{92T/T}$ healthy subjects were 40.9%, 46.4%, and 12.7%, respectively. These prevalences were similar to those of some European countries whilst significantly different from that of Brazil. In the two groups of healthy subjects and TA subjects, *Dio2*^{T92A} polymorphism had a similar distribution with non-significant differences. Similarly, no significant differences were observed in the serum concentration of FT_3, FT_4, and TSH between subjects with different *Dio2*^{T92A} polymorphism. The FT_4/FT_3, and TSH/FT_3 ratios were higher in *Dio2*$^{92T/T}$ than in *Dio2*$^{92T/A}$ and *Dio2*$^{92A/A}$ subjects in both TA and healthy groups, but these differences were not significant. In conclusion, the distribution of *Dio2*^{T92A} polymorphism may reflect geographical and ethnic differences, and it is not associated with TA.

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INTRODUCTION

The 3,5,3′-L-triiodothyronine (T_3) is the biologically active thyroid hormone. T_3 is partly produced and released into the serum by the thyroid and partly derives by the deiodination of the prohormone 3,5,3′,5′-L-tetraiodothyronine (T_4) (1). Type 2 iodothyronine deiodinase (D2), catalyzes removal of an outer ring 5′-iodine atom from the circulating T_4 to generate the active metabolite T_3. Thus, D2 in conjunction with thyroid-derived T_3, is an important local modulator of thyroid hormone action. D2-mediated T_3 production by T_4 occurs at the intracellular compartment. Subsequently, T_3 exits the cells and enters the plasma compartment, being responsible for 70% of all extrathyroidal T_3 production in healthy humans (2). Expression of D2 is regulated in a tissue-specific manner, resulting in varying levels of T_3 action in individual tissues, despite relatively constant serum thyroid hormone levels (1). D2 is a protein residing on the endoplasmic reticulum membrane, with a relatively short half-life due to ubiquitination and proteasome degradation (3). D2 expression is regulated on a cell-specific manner by a combination of transcriptional modulation of the *Dio2* gene, post-transcriptional mechanisms regulating *Dio2* mRNA stability, and by post-translational mechanisms such as ubiquitination (1). Several polymorphisms in *Dio2* have been described. The single-nucleotide polymorphism in *Dio2* A92G (D2 Thr92Ala, *Dio2*^{T92A}), generates an enzyme with conserved level of activity. However, the maximal velocity of T_4 to T_3 conversion results decreased by 3- to 10-fold in thyroid and skeletal muscle of carriers of the *Dio2*^{T92A}. This effect was observed in the absence of differences in D2 mRNA level, suggesting that either the Thr92Ala substitution affects protein translation or stability or a functionally relevant polymorphism occurs in linkage disequilibrium in the *Dio2*^{T92A} (4). Recent studies have shown that *Dio2*^{T92A} may have relevant pathophysiological effects. Although discording results have been published, it has been proposed an association of *Dio2*^{T92A} with an approximately 20% lower glucose disposal rate, with obesity and insulin resistance in subjects with Type 2 diabetes mellitus, and with bone mineral density and bone turnover (4-7). It has also been proposed that hypothyroid subjects carrying the *Dio2*^{T92A} polymorphism in T_4 replacement treatment, may experience impaired psychological well-being and would benefit from the T_4/T_3 combination therapy (8). A large body of evidence indicates that thyroid hormones act as modulators of the immune response. In general, thyroid hormones increase the immune response, i.e.: antibody production, cell migration, lymphocyte proliferation, and reactive oxygen species production, whereas it decreases the proinflammatory markers, antioxidant enzymes and their activity (9, 10). Hypothyroidism typically produces the opposite effects on parameters of the immune function; it decreases immune response, antibody production, T cell migration, and lymphocyte proliferation (11, 12). The *Dio2* polymorphism, modulating the level of T_3 in the lymphoid organs or in the thyroid, could

Key-words: *Dio2*^{T92A} polymorphism, thyroid autoimmunity, type 2 iodothyronine deiodinase.

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Table 1 - *Characteristics of the subjects.*

	Healthy subjects (no.=106)	TA patients (no.=110)	*p*
Age (yr)[a]	40.3±9.7	41.7±9.6	0.925
TSH (μIU/ml)[a]	1.98±0.64	1.93±0.69	0.852
FT_4 (ng/ml)[a]	10.68±1.73	9.45±1.58	0.345
FT_3 (ng/ml)[a]	3.34±0.55	3.15±0.76	0.154
TPO-Ab[b]	<100	1254 (100-9300)	0.197

[a]Mean±SD; [b]median (range). Analysis was performed by Student's t-test. TA: thyroid autoimmunity; TPO-Ab: thyroid peroxidase antibodies; FT_4: free T_4; FT_3: free T_3; TPO-Ab: thyroid peroxidase antibodies. Reference values: TSH: 0.27-4.0; FT_4: 7.8-14.2; FT_3: 2.9-5.8; TPO-Ab: <100.

modulate the immune system and thus might affect the tendency to develop thyroid autoimmunity (TA) and its course. An hypothesis never investigated so far. Thus, given the putative role of thyroid hormone as modulators of the immune response, and the emerging pathophysiological importance of the *Dio2*^{T92A} polymorphism, the objective of this study was to investigate the *Dio2*^{T92A} polymorphism in relation to thyroid autoimmunity. We compared the prevalence of *Dio2*^{T92A} polymorphism and serum thyroid hormone levels in healthy and subjects with TA, seeking a possible correlation between this polymorphism and TA.

SUBJECTS AND METHODS

Subjects and biochemical measurements

A total of 216 subjects entered the study following approval from the institutional review board, after giving written consent. Inclusion criteria were female gender, age 25-55 yr, absence of thyroid peroxidase antibodies (TPO-Ab) or thyroglobulin antibodies (TG-Ab) (control group) or TPO-Ab titer ≥100 U/ml (at present and ever documented in the past) (TA group), serum TSH values from 0.27-4.0 μIU/ml. Exclusion criteria were male gender, a body mass index <18.5 or >28, more than 6 month of amenorrhea, pregnancy, a history of pituitary disease, hyperthyroidism or hypothyroidism at present, or use of any medication. Buccal swab and blood samples were drawn for assessment of *Dio2*^{T92A} polymorphism, TSH, free T_4 (FT_4), free T_3 (FT_3), TG-Ab, and TPO-Ab. Serum concentrations of FT_4, FT_3, and TSH were determined by electrochemiluminescent assay using commercially available kits (Roche, Mannheim, Germany). The normal ranges for serum FT_4 and FT_3 were 7.8-14.2 ng/dl and 2.9-5.8 pg/ml, respectively. The normal range for serum TSH was 0.27-4.0 μIU/ml. TG-Ab and TPO-Ab were determined by a radioimmunoassay kit (B.R.A.H.M.S. Diagnostica, Berlin, Germany). TPO-Ab<100 U/ml were considered negative (Table 1).

Dio2^{T92A} *polymorphism genotyping*

Cotton buccal swabs were stored dry at room temperature up to 3 months before usage. To obtain genomic DNA, buccal swabs were immersed in 400 μl Lysis buffer and resuspended in 10 μl diethylpyrocarbonate (DEPC) water. DNA concentration was quantitated by A 260 absorbance with a BioPhotometer (Eppendorf, Hamburg, Germany). PCR amplification and pyrosequencing analysis PCR were performed with 50-100 ng genomic DNA, forward primer and reverse 5-biotinylated primer (Table 2) at 10M concentration, and 2.5 U Taq Polymerase Recombinant (Fermentas, Thermo Scientific, Canada). All primers were obtained from Primm (Milan, Italy). All PCR were performed separately in a TC-4000 Thermal Cycler (Bibby Scientific, Milan, Italy), including an initial denaturation of 5 min at 94 C and subsequent denaturation for 20 sec at 94 C, annealing for 20 sec at 56 C, and extension for 30 sec at 72 C. The PCR products were electrophoresed in a 2.5% agarose gel containing ethidium bromide to confirm successful amplification of the PCR products. Preparation of the single-stranded DNA template for pyrosequencing was performed using the PSQ Vacuum Prep Tool (Diatech, Ancona, Italy) according to the manufacturer's instructions. Twenty microliters of biotinylated PCR product were immobilized on streptavidin-coated Sepharose high-performance beads (Diatech), processed to obtain a single-stranded DNA using the PSQ 96 Sample Preparation Kit (Diatech), according to the manufacturer's instructions, and incubated under shaking at room temperature for 10 min in binding buffer. Subsequently, samples were hybridized to 13.5 μM sequencing primers (Table 2) in annealing buffer at 80 C for 2 min in a PSQ96 plate, followed by cooling to room temperature. The sequencing-by-synthesis reaction of the complementary strand was automatically performed on a PSQ 96MA instrument (Biotage, Uppsala, Sweden) at room temperature using PyroGold reagents (Diatech). As nucleotides were dispensed, a light signal was generated proportional to the amount of each incorporated nucleotide. These light signals were detected by a charge-coupled device camera and converted to peaks in a sequencing pyrogram that was automatically generated in real time for each sample.

Statistical analysis

Values are presented as percentage or mean±SD. Comparisons between groups were analyzed by Student's t-test, analysis of variance or chi-square tests. All calculations were performed using SPSS 12.0 for windows (SPSS, Inc., Chicago, IL, USA). The level of significance was set at <0.05.

RESULTS

A total of 216 subjects, 110 with TA documented by the presence of serum autoantibodies and 106 controls were genotypized for *Dio2*^{T92A} polymorphism by pyrosequencing. Sufficient DNA was extracted from all the swabs and sequencing was always successful. *Dio2*$^{92T/A}$, *Dio2*$^{92A/A}$ and *Dio2*$^{92T/T}$ subjects were 41.2%, 44.9%, and 13.9%, respectively. In the two groups of healthy subjects and TA subjects, *Dio2*^{T92A} polymorphism had a similar distribution and non significant differences were ob-

Table 2 - *PCR primers and sequencing primers for Dio2.*

	PCR primers	Sequencing primers
Forward:	5'-ATTCCAGTGTGGTGCATGTC -3'	5'-TGGTGCATGTCTCCA-3'
Reverse:	5'-biotin-GCTCGTGAAAGGAGGTCAAG -3'	

Table 3 - *Prevalence of Dio2 polymorphism in healthy subjects and thyroid autoimmunity (TA) patients. Percentages.*

	Healthy subjects	TA patients	*p*
Dio2$^{92T/T}$	40.9	38.7	0.829
Dio2$^{92T/A}$	46.4	47.2	1.000
Dio2$^{92A/A}$	12.7	14.2	0.710

Analysis was performed by chi-square of independence test.

served (Table 3). Similarly, no significant differences were observed in the serum concentration of TSH and FT_4 between subjects with different *Dio2*^{T92A} polymorphism. Serum FT_3 in *Dio2*$^{92T/T}$ subjects was slightly lower than in *Dio2*$^{92T/A}$ and *Dio2*$^{92A/A}$ subjects in both groups. However, the differences were not significant. Similarly, the FT_4/FT_3 and TSH/FT_3 ratios were higher in *Dio2*$^{92T/T}$ subjects than in *Dio2*$^{92T/A}$ and *Dio2*$^{92A/A}$ subjects in both groups, but these differences were not significant.

DISCUSSION

Recent studies proposed an association of *Dio2*^{T92A} polymorphism with metabolic traits, Type 2 diabetes, bone mineral density, and Alzheimer disease. Thus, given the emerging physio-pathological importance of D2, the objective of this study was to investigate the *Dio2*^{T92A} polymorphism in relation to TA, and circulating thyroid hormones in a large cohort of women from the same geographical area.

The first objective of this study was to investigate whether *Dio2* polymorphism at position 92 is associated with TA. The distribution of *Dio2*^{T92A} polymorphism in our Italian cohort of healthy subjects is comparable to the one reported previously in subjects from Denmark and The Netherlands, while the percentage of *Dio2*$^{92A/A}$ subjects (12.7%) was significantly lower than in subjects from Brazil (36.0%) (4, 6, 7). All subjects of our cohort were resident in Campania, thus geographical and ethnic factors could account for the different prevalence observed. No significant association of the *Dio2*^{T92A} polymorphism with TA was observed. Our analysis considered subjects with TA as documented by the presence of TPO-Ab, and normal level of serum TSH and iodothyronines. Thus, while polymorphism of *Dio2*^{T92A} was not associated with TA, its association with Hashimoto's thyroiditis or consequential thyroidal status should be investigated.

The *Dio2*$^{92T/T}$ genotype is associated with a lower D2 enzyme velocity (4). Because extra-thyroidal T_4 deiodination by D2 significantly contributes to global T_3 production in healthy subjects, we asked whether *Dio2*^{T92A} polymorphism was associated with a different serum level of iodothyronine and TSH/T_3 ratio. To analyze a more homogeneous population, we excluded elderly or young subjects, or subjects with a body mass index >28. This restriction was operated because muscle mass largely expresses D2 and it is age-related, while obesity can influence or can be associated with the level of circulating iodothyronines (4, 5). In both healthy subjects and patients with thyroid autoimmunity, *Dio2*^{T92A} polymorphism was not associated with a variation in TSH or FT_4 serum concentration. Serum FT_3 level was slightly lower in *Dio2*$^{92T/T}$ subjects than in the subjects with the two other polymorphisms, a difference that affected the TSH/FT_3 and FT_4/FT_3 ratios. However these differences were not significant. The lack of a different TSH/FT_3 ratio as a consequence of *Dio2*^{T92A} polymorphism suggests the possibility that an increased T_3 of thyroidal origin counteracts the reduced peripheral T_4 to T_3 conversion in *Dio2*$^{92A/A}$ subjects. Whilst this may reflect a physiological situation in healthy individuals, a less efficient peripheral T_4 to T_3 conversion could have metabolic and clinical relevance in hypothyroid subjects treated with substitutive T_4 therapy.

A fair proportion (about 13-15%) of hypothyroid patients treated with oral administration of T_4 alone, deplores the persistence of symptoms such as feeling tired and lethargic, putting on weight, clumsiness, fatigue, reduced exercise tolerance, muscle weakness (13, 14). It has been postulated that in these patients, deiodination of T_4 is less efficient than in others and, although the serum T_3 is within the normal range, the final concentration of T_3 is low in some tissues. Different studies compared the effects of T_4 alone with T_4 plus T_3 treatment in hypothyroid patients, showing controversial results. Bunevicius et al. reported that the T_4/T_3 combined replacement therapy of hypothyroidism resulted in improved scores in mood scales and neurocognitive tests in patients under combined treatment while no changes were found in cardiovascular function, body weight and composition, and endocrine functions (15, 16). The remarkable finding of advantageous effects of T_4/T_3 combined therapy on well-being and neurocognitive functions was further studied in other clinical trials, showing inconstant differences between

Table 4 - *Correlation of serum TSH and iodothyronine levels with Dio2 polymorphism in healthy subjects and thyroid autoimmunity (TA) patients.*

	Healthy subjects			TA patients		
	Dio2$^{92T/T}$	*Dio2*$^{92T/A}$	*Dio2*$^{92A/A}$	*Dio2*$^{92T/T}$	*Dio2*$^{92T/A}$	*Dio2*$^{92A/A}$
Age, mean yr	46.3	49.1	44.5	44.3	45.4	48.3
TSH (μIU/ml)[a]	1.99±0.64	1.97±0.75	1.96±0.76	1.95±0.69	1.98±0.83	1.92±0.70
FT_4 (ng/ml)[a]	11.48±2.05	10.65±1.58	9.89±1.09	9.68±1.42	9.86±1.46	9.29±1.74
FT_3 (ng/ml)[a]	3.48±0.54	3.35±0.41	2.90±1.00	3.26±0.75	3.19±0.67	2.65±1.11
FT_4/FT_3[a]	3.4±1.01	3.23±0.76	4.12±2.34	3.2±1.15	3.20±0.66	4.40±2.06
TSH/FT_3[a]	0.59±0.20	0.61±0.23	0.80±0.57	0.66±0.33	0.66±0.39	0.98±0.67

[a]mean±SD. All parameters of healthy subjects *vs* TA patients were compared, for all, $p>0.05$.

T_4 monotherapy and combined therapy (17-19). Recently, Panicker et al. found that hypothyroid patients sharing *Dio2*$^{92A/A}$ genotype had a poorer psychological well-being under T_4 monotherapy but they shared a greater psychological improvement under combined T_4/T_3 therapy (8). In conclusion, the distribution of *Dio2*^{T92A} polymorphism may reflect geographical and ethnic differences, and it is not associated with thyroid autoimmunity. Its association with Hashimoto's thyroiditis, its clinical course and substitutive treatment should be investigated.

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REFERENCES

1. Bianco AC, Kim BW. Deiodinases: implications of the local control of thyroid hormone action. J Clin Invest 2006, 116: 2571-9.
2. Bianco AC, Salvatore D, Gereben B, Berry MJ, Larsen PR. Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. Endocr Rev 2002, 23: 38-89.
3. Baqui M, Botero D, Gereben B, et al. Human type 3 iodothyronine selenodeiodinase is located in the plasma membrane and undergoes rapid internalization to endosomes. J Biol Chem 2003, 278: 1206-11.
4. Canani LH, Capp C, Dora JM, 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 2005, 90: 3472-8.
5. Mentuccia D, Proietti-Pannunzi L, Tanner K, 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 2002, 51: 880-3.
6. Heemstra KA, Hoftijzer H, van der Deure WM, et al. The type 2 deiodinase Thr92Ala polymorphism is associated with increased bone turnover and decreased femoral neck bone mineral density. J Bone Miner Res 2010, 25: 1385-91.
7. Grarup N, Andersen MK, Andreasen CH, et al. Studies of the common DIO2 Thr92Ala polymorphism and metabolic phenotypes in 7342 Danish white subjects. J Clin Endocrinol Metab 2007, 92: 363-6.
8. Panicker V, Saravanan P, Vaidya B, 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 2009, 94: 1623-9.
9. De Vito P, Incerpi S, Pedersen JZ, Luly P, Davis FB, Davis PJ. Thyroid hormones as modulators of immune activities at the cellular level. Thyroid 2012, 21: 879-90.
10. Barreiro Arcos ML, Sterle HA, Paulazo MA, et al. Cooperative nongenomic and genomic actions on thyroid hormone mediated-modulation of T cell proliferation involve up-regulation of thyroid hormone receptor and inducible nitric oxide synthase expression. J Cell Physiol 2011, 226: 3208-18.
11. Klecha AJ, Genaro AM, Lysionek AE, Caro RA, Coluccia AG, Cremaschi GA. Experimental evidence pointing to the bidirectional interaction between the immune system and the thyroid axis. Int J Immunopharmacol 2000, 22: 491-500.
12. Marino F, Guasti L, Cosentino M, et al. Thyroid hormone regulation of cell migration and oxidative metabolism in polymorphonuclear leukocytes: clinical evidence in thyroidectomized subjects on thyroxine replacement therapy. Life Sci 2006, 78: 1071-7.
13. Saravanan P, Chau WF, Roberts N, Vedhara K, Greenwood R, Dayan CM. Psychological well-being in patients on 'adequate' doses of l-thyroxine: results of a large, controlled community-based questionnaire study. Clin Endocrinol (Oxf) 2002, 57: 577-85.
14. Walsh JP. Dissatisfaction with thyroxine therapy - could the patients be right? Curr Opin Pharmacol 2002, 2: 717-22.
15. Bunevicius R, Prange AJ. Mental improvement after replacement therapy with thyroxine plus triiodothyronine: relationship to cause of hypothyroidism. Int J Neuropsychopharmacol 2000, 3: 167-74.
16. Bunevicius R, Kazanavicius G, Zalinkevicius R, Prange AJ Jr. Effects of thyroxine as compared with thyroxine plus triiodothyronine in patients with hypothyroidism. N Engl J Med 1999, 340: 424-9.
17. Siegmund W, Spieker K, Weike AI, et al. Replacement therapy with levothyroxine plus triiodothyronine (bioavailable molar ratio 14 : 1) is not superior to thyroxine alone to improve well-being and cognitive performance in hypothyroidism. Clin Endocrinol (Oxf) 2004, 60: 750-7.
18. Slawik M, Klawitter B, Meiser E, et al. Thyroid hormone replacement for central hypothyroidism: a randomized controlled trial comparing two doses of thyroxine (T4) with a combination of T4 and triiodothyronine. J Clin Endocrinol Metab 2007, 92: 4115-22.
19. Escobar-Morreale HF, Botella-Carretero JI, Gomez-Bueno M, Galan JM, Barrios V, Sancho J. Thyroid hormone replacement therapy in primary hypothyroidism: a randomized trial comparing L-thyroxine plus liothyronine with L-thyroxine alone. Ann Intern Med 2005, 142: 412-24.