Association between genetic polymorphism and levothyroxine bioavailability in hypothyroid patients

Merve Arici¹, Ezgi Oztas¹, Fatih Yanar², Nihat Aksakal², Beyza Ozcinar² and Gul Ozhan¹

¹) Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Istanbul University, Istanbul, Turkey
²) Department of General Surgery, Faculty of Medicine, Istanbul University, Istanbul, Turkey

Abstract. Thyroid hormones play a vital role in the human body for growth and differentiation, regulation of energy metabolism, and physiological function. Hypothyroidism is a common endocrine disorder, which generally results from diminished normal circulating concentrations of serum thyroxine (fT4) and triiodothyronine (fT3). The primary choice in hypothyroidism treatment is oral administration of levothyroxine (L-T4), a synthetic T4 hormone, as approximately 100–125 μg/day. Generally, dose adjustment is made by trial and error approach. However, there are several factors which might influence bioavailability of L-T4 treatment. Genetic background could be an important factor in hypothyroid patients as well as age, gender, concurrent medications and patient compliance. The concentration of thyroid hormones in tissue is regulated by both deiodinases enzyme and thyroid hormone transporters. In the present study, it was aimed to evaluate the effects of genetic differences in the proteins and enzymes (DIO1, DIO2, TSHR, THR and UGT) which are efficient in thyroid hormone metabolism and bioavailability of L-T4 in Turkish population. According to our findings, rs225014 and rs225015 variants in DIO2, which catalyses the conversion of thyroxine (pro-hormone) to the active thyroid hormone, were associated with TSH levels. It should be given lower dose to the patients with rs225014 TT and rs225015 GG genotypes in order to provide proper treatment with higher effectivity and lower toxicity.

Key words: Levothyroxine, Hypothyroid, Genetic polymorphism
L-T4. DIO1 and DIO2 catalyze activation of thyroid hormone secretion in contrast to DIO3 playing role inactivation of the secretion. Activities of DIO1 and DIO2 play pivotal role in the negative feedback regulation of pituitary TSH secretion [8, 9]. UDP-glucuronosyltransferases (UGTs) are responsible for T4 metabolism in human liver as thyroxine glucuronide. Two common UGT1A subfamily enzymes, UGT1A1 and UGT1A3, provide T4 glucuronidation in humans [10]. It was determined different responses dependently of genetic factors in the L-T4 treatment. The previously studies reported the genetic variations on proteins and enzymes have been played role in thyroid hormones metabolism, serum thyroid hormone concentrations, and bioavailability of L-T4 [8, 11]. Therefore, we aimed to comprehensively evaluate the effect of genetic differences in the proteins and enzymes (DIO1, DIO2, TSHR, THRα and UGT) which are efficient in thyroid hormones metabolism on bioavailability of L-T4.

Material and Methods

Sample collection: This is a cross-sectional study approved by ethical committee of Istanbul University Istanbul Medical Faculty (2015/740), and was carried out in accordance with the Helsinki Declaration of 1975, with all amendments and revisions. A total of 94 unrelated patients with secondary hypothyroidism due to total thyroidectomy were recruited from Endocrine Surgery Clinic of General Surgery Department, Istanbul Medical Faculty, Istanbul University between March 2015 and October 2016. Patients included in this study are all those who completed puberty, aged with 18–75, underwent total thyroidectomy for different causes (multi nodular goitre, suspicion of cancer, pressure effect of large goitre and substernal goitre) and on two types of doses of L-T4 as high and low doses (<1.7 μg/kg/day and ≥1.7 μg/kg/day). In the studied group, L-T4 was administered as a single dose daily. Neoplasm, liver dysfunction, renal failure, and psychiatric condition not related to hypothyroidism symptoms. Also, pregnancy and alcohol abuse patients on L-T4 treatment were accepted as the excluded criteria. TSH, fT3 and fT4 levels were measured by GenWay Biotach Inc. (San Diego, CA, USA) colorimetric ELISA kits according to manufacturer’s instructions by specialists in biochemistry laboratories of Istanbul Medical Faculty.

Genotyping: DNA was isolated from venous blood samples by High Pure PCR Template Preparation Kit (Roche, Germany). The single nucleotide polymorphisms (SNPs) analysis was performed on real-time PCR platform (LightCycler 480, Roche, Germany) using LightCycler FastStart DNA Master HybProbe and Roche LightSNP assay probes (Roche, Germany). The studied SNPs were rs11206244, rs2235544 in DIO1; rs225014, rs225015, rs12885300 in DIO2; rs939348 in TSHR; rs4903957, rs1991517, rs2239610, rs2268458 in TSHR; rs1983023, rs3806596 in UGT1A3 and rs8175347 in UGT1A1*28.

Statistical analysis: All statistical analyses were performed using Statistical Package for Social Sciences (SPSS) software (Version 20, Chicago, USA). The Hardy-Weinberg Equilibrium (HWE) analysis was performed to compare the observed and expected genotype frequencies of subject by using the chi-square ($\chi^2$) test. It was confirmed that the studied population was randomized by the results obtained from HWE analysis. Sample size was calculated with 95% confidence, and a margin of error 5% for an assuming population proportion of 0.5, and unlimited population size. Continuous variables were expressed as mean ± standard deviation (SD) whereas discrete variables were expressed as frequencies. The differences were accomplished by comparison with one way ANOVA. Post Hoc Tukey test and Independent t Test was applied to evaluate for the association between the clinical and biochemical characteristics and the studied genes. A significant difference is considered at $p < 0.05$.

Results

It was evaluated the effects of 13 SNPs of DIO1, DIO2, TSHR, TSHR and UGT1A on thyroid hormones metabolism and bioavailability of L-T4. The mean age of patients was 51.35 (±1.53) years, the mean body mass index (BMI, kg/m$^2$) was 29.16 (±0.65) and of the all participants 82.61% were female and 48.89% have familial background of thyroid disorders. Of the all participants, 22.83% were dyslipidemic, 23.92% were diabetic, and 20.41% were hypertensive. The patients were also evaluated by dividing into two groups based on current replacement L-T4 dose: low dose group (<1.7 μg/kg/day) and high dose group (≥1.7 μg/kg/day). Age distribution between two groups did not show any difference. As it is expected, the hypothyroid patients who received high dose of LT-4 had a slightly lower BMI. Also, it was found that high dose of LT-4 treatment was associated with lower levels of TSH, and higher levels of fT3 and
fT4 ($p < 0.05$) (Table 1). The typical reference ranges for thyroid function parameters are between 0.4–4 mIU/L for TSH, 3.5–7.8 pmol/L for fT3, and 9–25 pmol/L for fT4 [12]. Similarly, the mean values of the parameters were $1.54 \pm 0.44 \mu g/kg/day$ for L-T4 daily dose, $4.69 \pm 1.02$ pmol/L for fT3, and $19.75 \pm 4.22$ pmol/L for fT4 in the studied group.

Genotype distributions of studied SNPs are shown in Table 2. All genotypes were found to be consistent with the HWE ($p > 0.05$). Heterozygous type $[AT/-]$ for UGT1A1*28 (rs8175347) was not observed. Interestingly, we observed that DIO2 rs225015 $G$ and TSHR rs1991517 $C$ alleles were wild types with the 66.1% and 88.9% frequencies, respectively. However, the ancestral alleles were indicated as $A$ and $G$ according to NCBI SNP database [https://www.ncbi.nlm.nih.gov/projects/SNP].

There was no significant correlation among genotypes and L-T4 dose ($p > 0.05$), the biochemical parameters including thyroid function test (TSH, fT3 and fT4), and body mass index. A statistical significance was found between DIO2 rs225014 and TSH levels. Homozygous wild type ($TT$, $Thr/Thr$) for DIO2 rs225014 was associated with higher levels of TSH ($p < 0.05$). Besides, DIO2 rs225015 $GG$ genotype was found to be associated with higher levels of TSH compared with the $AA$ genotype ($p < 0.05$). Homozygous mutant types of TSHR rs4903957 ($AA$), rs1991517 ($CC$), rs2239610 ($CC$) and rs2268458 ($CC$) were related with higher levels of TSH. Although obtained significant differences in the values, the association did not reach statistical significance. Probably the reason is their excessive SDs. Additionally, homozygous variant types of THRα rs939348 ($TT$) and UGT1A3 rs1983023 ($CC$) were related with lower levels of TSH (Table 3).

### Table 1  Characteristics of hypothyroid patients based on the dose of LT-4 replacement

| Variable          | Low dose <1.7 μg/kg/day ($n = 66$) | High dose ≥1.7 μg/kg/day ($n = 28$) | $p$ value |
|-------------------|-----------------------------------|------------------------------------|-----------|
| Age               | 52.00 ± 4.06                      | 45.08 ± 2.03                       | 0.101     |
| BMI (kg/m$^2$)    | 29.82 ± 0.81                      | 26.99 ± 1.41                       | 0.079     |
| TSH (mIU/L)       | 1.93 ± 0.37                       | 0.51 ± 0.29                        | 0.029     |
| fT3 (pmol/L)      | 4.71 ± 0.16                       | 5.39 ± 0.27                        | 0.048     |
| fT4 (pmol/L)      | 19.73 ± 0.66                      | 22.09 ± 0.75                       | 0.045     |

*Bold values mean $p < 0.05$*

Discussion

The present study is a comprehensive cross-sectional study, and first study to evaluation the genetic profiles of Turkish people in the genes related to thyroid metabolism (Table 3). There are limited publications about the relationship between the gene variants and drug-response in hypothyroidism treatment [1, 2, 8, 10, 13–20].

Panicker et al. [14] suggested that DIO1 rs2235544 has a correlation with circulating fT3/fT4 levels both in the population with thyroid hormone replacement and in the general population. Rs2235544 $C$ allele was associated with increased fT3 and decreased fT4 levels, however not associated with serum TSH levels. They also reported no association between DIO1 rs11206244, which increase enzyme activity, and the levels of mentioned serum parameters. In the previous studies, it was suggested that the patient with carrying $C$ allele of rs2235544 should be treated higher doses of L-T4 because $C$ allele increases the function of DIO1. However, Santoro et al. [10] stated no association between dose and these genes. Similar to Santoro et al. [10], in the present study, we observed that the values of TSH, fT3 and fT4 were in normal range in patients with variant and wild alleles of DIO1 rs11206244 and rs2233544. There was no association between DIO1 (rs2233544) genotype and the TSH, fT3 and fT4 levels.

Heemestra et al. [19] observed no differences between DIO2 rs225014 and thyroid hormones levels and L-T4 doses adjusted for age, gender, BMI and serum TSH levels. Similar results were observed by Panicker et al. [14] and Al-Azzam et al. [8]. However, Torlontano et al. [15] stated that the patient with homozygous variant type ($CC$, $Ala/Ala$) for DIO2 rs225014 needed the higher dose of L-T4 to provide favourable TSH levels. They reported that an approximate 20% higher dose was needed in patient with homozygous variant type for target TSH levels to be reached. And, they suggested a reduced pituitary feedback due to abnormal DIO2 hypothalamic/pituitary activity. As it is known, DIO2 activity plays an important role in the negative feedback regulation of
pituitary TSH secretion [15]. Similarly, in the present study, a statistical significance was found between DIO2 rs225014 and TSH levels; homozygous wild type (TT, Thr/Thr) was associated with higher levels of TSH (p < 0.05). Also, DIO2 rs225015 GG genotype was found to be associated with higher levels of TSH compared with the AA genotype (p < 0.05). For DIO2 rs225014 and rs225015, their effected genotypes were observed in different frequencies. The common homozygous type for DIO2 rs225014 (TT) and the mutant homozygous types for rs225015 (GG) were associated with higher levels of TSH compared with their other types. Therefore, the patients with for DIO2 rs225014 (TT) and rs225015 (GG) variants have higher TSH levels, and should take higher L-T4 dose.

THRa rs939348 was associated with L-T4 replacement doses [1]. It was suggested that T allele carrying patients should be taken more doses of L-T4 because of the increased enzyme function. Similarly, we observed that the TSH level was lower in the patient with THRa rs939348 TT genotype in same dose L-T4 treatment.

The previous studies stated there was no association between TSHR and L-T4 dose [20, 21]. In the present study, it was found that homozygous mutant type of

| SNP            | Genotype | n (%)  |
|----------------|----------|--------|
| DIO1 rs11206244 | CC       | 47 (52.2)  |
|                | CT       | 35 (38.9)  |
|                | TT       | 8 (8.9)    |
| DIO1 rs2235544 | CC       | 33 (36.7)  |
|                | CA       | 44 (48.9)  |
|                | AA       | 13 (14.4)  |
| DIO2 rs225014  | TT       | 38 (45.2)  |
|                | TC       | 34 (40.5)  |
|                | CC       | 12 (14.3)  |
| DIO2 rs225015  | AA       | 13 (14.4)  |
|                | AG       | 35 (38.9)  |
|                | GG       | 42 (46.7)  |
| DIO2 rs12885300| CC       | 39 (43.8)  |
|                | CT       | 43 (48.3)  |
|                | TT       | 7 (7.9)    |
| THRa rs939348  | CC       | 49 (54.4)  |
|                | CT       | 35 (38.9)  |
|                | TT       | 6 (6.7)    |
| TSHR rs4903957 | GG       | 50 (55.6)  |
|                | GA       | 36 (40)    |
|                | AA       | 4 (4.4)    |
| TSHR rs1991517 | GG       | 3 (3.3)    |
|                | GC       | 14 (15.6)  |
|                | CC       | 73 (81.1)  |
| TSHR rs2239610 | GG       | 56 (62.2)  |
|                | GC       | 32 (35.6)  |
|                | CC       | 2 (2.2)    |
| TSHR rs2268458 | TT       | 56 (62.2)  |
|                | TC       | 32 (35.6)  |
|                | CC       | 2 (2.2)    |
| UGT1A3 rs1983023| TT       | 41 (45.6)  |
|                | TC       | 37 (41.1)  |
|                | CC       | 12 (13.3)  |
| UGT1A3 rs3806596| AA      | 28 (31.1)  |
|                | AG       | 41 (45.6)  |
|                | GG       | 21 (23.3)  |
| UGT1A1*28 rs8175347| [AT] | 24 (26.7) |
|                | [AT/–]  | 0 (0)      |
|                | [–]      | 66 (73.3)  |
| Genotype | BMI (kg/m²) | L-T4 daily dose (μg/day) | L-T4 daily dose (μg/kg) | TSH (mIU/L) | fT3 (pmol/L) | fT4 (pmol/L) |
|----------|-------------|-------------------------|------------------------|-------------|-------------|-------------|
| rs11206244 |
| CC       | 28.48 ± 0.99 | 111.1 ± 4.6 | 1.45 ± 0.06 | 1.91 ± 0.38 | 4.70 ± 0.21 | 19.85 ± 0.65 |
| CT       | 30.08 ± 1.09 | 133.4 ± 10.4 | 1.67 ± 0.13 | 1.60 ± 0.39 | 4.72 ± 0.15 | 19.53 ± 0.72 |
| TT       | 28.42 ± 1.14 | 116.4 ± 11.9 | 1.56 ± 0.16 | 1.82 ± 0.56 | 4.36 ± 0.28 | 20.19 ± 1.08 |
| rs2235544 |
| CC       | 27.26 ± 1.32 | 111.3 ± 5.5 | 1.50 ± 0.74 | 1.91 ± 0.62 | 4.97 ± 0.28 | 20.72 ± 1.15 |
| CA       | 30.12 ± 1.04 | 135.3 ± 8.9 | 1.67 ± 0.11 | 1.18 ± 0.39 | 4.84 ± 0.22 | 20.22 ± 0.60 |
| AA       | 29.49 ± 1.24 | 112.1 ± 10.2 | 1.43 ± 0.13 | 1.65 ± 0.47 | 4.64 ± 0.13 | 20.32 ± 1.36 |
| rs225014 |
| TT       | 28.69 ± 1.09 | 117.8 ± 5.9 | 1.49 ± 0.75 | 2.33 ± 0.51* | 4.63 ± 0.24 | 20.07 ± 0.78 |
| TC       | 29.39 ± 1.36 | 117.3 ± 8.3 | 1.75 ± 0.12 | 0.31 ± 0.09 | 5.06 ± 0.18 | 21.66 ± 0.91 |
| CC       | 30.09 ± 1.13 | 111.7 ± 16.9 | 1.45 ± 0.22 | 0.17 ± 0.16 | 4.71 ± 0.22 | 18.67 ± 0.93 |
| rs225015 |
| AA       | 31.59 ± 1.27 | 144.5 ± 19.2 | 1.66 ± 0.22 | 0.16 ± 0.05 | 4.74 ± 0.16 | 20.07 ± 1.47 |
| AG       | 29.34 ± 1.28 | 136.7 ± 9.8 | 1.67 ± 0.12 | 0.95 ± 0.31 | 5.04 ± 0.15 | 21.37 ± 0.96 |
| GG       | 28.32 ± 1.01 | 112.7 ± 5.3 | 1.48 ± 0.07 | 2.44 ± 0.51* | 4.78 ± 0.24 | 20.01 ± 0.72 |
| rs12885300 |
| CC       | 29.32 ± 1.07 | 119.2 ± 8.6 | 1.53 ± 0.11 | 1.22 ± 0.52 | 4.81 ± 0.26 | 20.75 ± 0.79 |
| CT       | 28.87 ± 1.06 | 121.8 ± 7.1 | 1.55 ± 0.09 | 1.74 ± 0.43 | 4.84 ± 0.19 | 19.63 ± 0.81 |
| TT       | 29.16 ± 2.02 | 132.6 ± 8.8 | 1.65 ± 0.11 | 1.27 ± 0.54 | 5.09 ± 0.66 | 22.58 ± 0.99 |
| rs939348 |
| CC       | 28.93 ± 0.98 | 126.1 ± 7.1 | 1.60 ± 0.09 | 1.63 ± 0.42 | 4.71 ± 0.15 | 19.93 ± 0.69 |
| CT       | 29.55 ± 1.13 | 118.5 ± 6.4 | 1.49 ± 0.08 | 1.54 ± 0.45 | 4.91 ± 0.29 | 20.74 ± 0.96 |
| TT       | 27.27 ± 3.25 | 116.7 ± 16.0 | 1.61 ± 0.22 | 0.23 ± 0.16* | 5.88 ± 0.61 | 23.05 ± 1.26 |
| rs4910957 |
| GG       | 29.09 ± 1.02 | 125.1 ± 6.3 | 1.58 ± 0.08 | 1.73 ± 0.43 | 5.07 ± 0.23 | 20.31 ± 0.87 |
| GA       | 29.52 ± 1.01 | 125.2 ± 8.1 | 1.54 ± 0.10 | 1.07 ± 0.35 | 4.66 ± 0.19 | 20.54 ± 0.60 |
| AA       | 33.91 ± 2.48 | 136.1 ± 5.4 | 1.51 ± 0.06 | 2.53 ± 0.23 | 4.93 ± 0.49 | 20.38 ± 2.90 |
| rs1991517 |
| GG       | 27.34 ± 0.01 | 152.9 ± 2.9 | 2.13 ± 0.04 | 0.08 ± 0.01 | 4.91 ± 0.02 | 20.61 ± 0.05 |
| GC       | 31.13 ± 1.83 | 124.3 ± 8.9 | 1.54 ± 0.11 | 0.53 ± 0.29 | 5.41 ± 0.31 | 22.92 ± 1.28 |
| CC       | 28.66 ± 0.78 | 125.4 ± 5.7 | 1.55 ± 0.07 | 1.78 ± 0.35* | 4.71 ± 0.16 | 19.82 ± 0.57 |
| rs2239610 |
| GG       | 29.94 ± 0.91 | 119.9 ± 5.6 | 1.49 ± 0.07 | 1.55 ± 0.38 | 4.78 ± 0.17 | 20.29 ± 0.81 |
| GC       | 28.46 ± 1.17 | 130.2 ± 9.3 | 1.68 ± 0.12 | 1.21 ± 0.41 | 5.02 ± 0.27 | 20.63 ± 0.61 |
| CC       | 35.82 ± 2.76 | 141.9 ± 0.9 | 1.51 ± 0.01 | 3.77 ± 0.21* | 4.62 ± 0.66 | 20.07 ± 5.01 |
| rs2268458 |
| TT       | 28.74 ± 0.93 | 115.4 ± 5.3 | 1.53 ± 0.07 | 1.48 ± 0.39 | 4.86 ± 0.18 | 20.40 ± 0.82 |
| TC       | 28.76 ± 1.12 | 129.9 ± 8.8 | 1.62 ± 0.11 | 1.31 ± 0.41 | 4.88 ± 0.26 | 20.46 ± 0.61 |
| CC       | 35.82 ± 2.76 | 145.4 ± 3.9 | 1.51 ± 0.04 | 3.77 ± 0.22* | 4.61 ± 0.65 | 20.07 ± 5.01 |
| rs1983023 |
| TT       | 28.37 ± 1.07 | 118.1 ± 8.6 | 1.51 ± 0.11 | 1.65 ± 0.45 | 4.52 ± 0.24 | 20.72 ± 0.83 |
| TC       | 29.73 ± 1.18 | 130.1 ± 6.7 | 1.55 ± 0.08 | 1.77 ± 0.49 | 5.11 ± 0.19 | 19.92 ± 0.85 |
| CC       | 28.74 ± 1.46 | 139.6 ± 10.4 | 1.74 ± 0.13 | 0.31 ± 0.09* | 4.84 ± 0.26 | 21.22 ± 1.18 |

* indicates significance of difference between genotypes.
TSHR rs4903957 (AA), rs1991517 (CC), rs2239610 (CC) and rs2268458 (CC) were related with higher levels of TSH. However, the association did not reach statistical significance probably due to their excessive SDs.

The previous studies have showed a direct or indirect correlation between L-T4 dose and UGT1A. It was also indicated the importance of glucuronidation in T4 homeostasis and UGT1A1 and UGT1A3. UGT1A1 had a higher affinity than UGT1A3 for T4 in T4 glucuronidation while UGT1A3 had higher capacity for T4 glucuronidation. It was stated a significant correlation between UGT1A1*28 and T4 glucuronidation [13]. Graber et al. [13] pointed out hypothyroid patients with homozygous UGT1A1*28 variant should receive a lower L-T4 dose. Santoro et al. [10] and Vargens et al. [16] reported that polymorphic individuals of UGT1A should be received lower doses of L-T4 to reach appropriate levels of TSH due to lower expressions of related SNPs of UGT1A. In our findings, homozygous variant type (CC) of UGT1A3 rs1983023 were related with lower levels of TSH; however, the association did not reach statistical significance.

In conclusion; there was a significant correlation between rs225014 and rs225015 in DIO2 and TSH levels. However, DIO1, DIO3, THRα, TSHR and UGT1A3 genotypes were not associated L-T4 treatment. It should be lower dose in the individuals with DIO2 rs225014 (TT) and rs225015 (GG) in order to provide the more effectively treatment with lower toxicity. The observed significant correlations should be sensitive and precious biomarkers of thyroid function and treatment among patients. Therefore, personalised medicine should be tendered optimal treatment according to patients’ genetic background.

Acknowledgements

This work was supported by Istanbul University (BAP-53871).

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