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Study of Deiodinase Type 2 Polymorphisms in Graves' Disease and Ophthalmopathy in a Swedish Population

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Abstract
Background: Deiodinase type 2 (DIO2) is an enzyme that catalyzes the production of the active form of thyroid hormone triiodothyronine (T3) from thyroxine (T4) and is important for maintaining intracellular T3 levels. Single nucleotide polymorphisms (SNPs) in DIO2 were associated with several diseases. The association of SNPs in DIO2 with Graves' disease (GD) was suggested in 2 Russian studies. ***Objectives:*** The aim of the study was to examine whether SNPs in DIO2 are associated with GD or Graves' ophthalmopathy (GO). ***Methods:*** Seven SNPs in the DIO2 gene – rs225014 (Thr92Ala), rs12885300, rs2267872, rs225011, rs224995, rs225015, and rs2267873 – were studied to assess their association with GD and GO. In total, 712 patients with GD with (n = 311) or without (n = 399) ophthalmopathy and 1,183 sex-matched controls from Malmö, Sweden were analyzed. In GD patients with available data, the SNPs were examined for association with the levels of free T3, free T4, thyroid-stimulating hormone receptor antibodies (TRAb), and thyroid-peroxidase antibodies (TPOAb). ***Results:*** Rs225011 was nominally associated with GD (OR 1.18, CI 1.01–1.37, p = 0.036). None of the SNPs were associated with GO. In GD patients, none of the SNPs were associated with the free-T4 (fT4), TRAb, or TPOAb levels. A weak, nonsignificant association was observed between free-T3 (fT3) levels and rs225014 and rs12885300, separately. ***Conclusions:*** Rs225011 in DIO2 was weakly associated with GD. The mechanism behind this association requires further study. None of the investigated common SNPs in DIO2 was significantly associated with GO, fT3, fT4, TRAb, or TPOAb in GD patients.

Introduction

Deiodinase type 2 (DIO2) is an enzyme that catalyzes the production of the active form of thyroid hormone triiodothyronine (T3) from thyroxine (T4) and is important for maintaining intracellular T3 levels [1]. DIO2 is also responsible for the production of a fraction of serum T3

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in euthyroid and hypothyroid individuals [2]. DIO2 is expressed in the pituitary, brain, thyroid, myocardium, skeletal muscle [3, 4], and adipose tissue [5]. We demonstrated the expression of DIO2 in intraorbital adipose tissue in euthyroid individuals and in intraorbital adipose tissue in patients with active and chronic Graves' ophthalmopathy (GO) [6]. In this study, DIO2 was downregulated in chronic but not active GO compared with controls. We speculated that a consequence of the decreased level of DIO2 in intraorbital tissues may be the limited local bioavailability of T3, thus promoting GO. In the present study, we hypothesize that single nucleotide polymorphisms (SNPs) in DIO2 could play a role in individual susceptibility to develop local hypothyroidism and GO.

The SNP Thr92Ala (rs225014) in DIO2 was linked to and associated with Graves' disease (GD) in 2 Russian studies [7, 8]. The Ala92 allele of the Thr92Ala SNP caused a delayed secretion of T3 following a TRH-mediated acute rise in TSH, which is consistent with a reduced intrathyroidal conversion of T4 into T3 [9]. In contrast, the –258G/x variant of rs12885300 was associated with a reduced rate of acute TSH-stimulated free T4 secretion with normal T3 release from the thyroid [10]. These findings suggest that common polymorphisms in DIO2 can subtly affect the circulating levels of thyroid hormone and might modulate the thyroid hormone homeostasis, which is further supported by the association of the Thr92Ala SNP with psychological well-being and response to T3 or T4 treatment [11].

SNPs in DIO2 have been associated with obesity, insulin resistance, and type 2 diabetes [12–16]; however, some studies could not confirm such associations [17, 18]. Thr92Ala SNP has also been associated with decreased bone mass, increased bone turnover [19], and increased risk for osteoarthritis [20] and hypertension [21]. Most of these associations were independent of serum thyroid hormone levels, and this underscores the importance of peripheral regulation of thyroid hormone production.

The aim of the present study was to examine whether SNPs in DIO2 are associated with GD and/or GO in a Swedish population.

Material and Methods

Study Subjects

The study consisted of 712 patients (127 males and 585 females) with GD with (n = 311) or without (n = 399) ophthalmopathy and 1,183 controls (211 males and 972 females) from Malmö in southern Sweden (Table 1). A total of 521 patients (73%) were selected from the registry GD2002 in which clinical data on patients with GD have been collected since 2002 by a single endocrinologist. In total, 119 patients were recruited from the studies MFM (Malmö Preventive Project) [22] and MKC (Malmö Diet and Cancer Study) [23] on the basis of an ICD-10 diagnosis of E05.0 (GD) and H06.2 (GO). These studies were performed in the 1990s as a primary preventive project (MFM) and a study investigating the association of diet and risk of cancer (MKC). Seventy-two patients were recruited from the TT-96 study as described previously [24]. GD diagnosis was made by an endocrinologist based on clinical symptoms and signs and biochemical hyperthyroidism in combination with the presence of thyroid-stimulating hormone (TSH)-receptor antibodies (TRAb) and/or diffuse uptake by technetium scintigraphy. GO diagnosis was made by an endocrinologist and/or ophthalmologist based on the presence of clinical signs, as previously described [24]. Eyelid retraction alone was not classified as GO. The controls were recruited from the MFM and MKC databases. The case and control groups were matched for sex. Regarding ethnicity, the origin of the individuals was assigned as Swedish, born in a European country other than Sweden, or born outside of Europe.

Table 1. Characteristics of the study population

	Cases	%	Controls	%
Number	712		1,183	
Age at inclusion, years[a]	49+14		57+6	
Gender				
Male	127	17.8	211	17.8
Female	585	82.2	972	82.2
Ethnicity				
Swedish	531	74.6	833	70.4
European	117	16.4	177	15.0
Other	61	8.6	83	7.0
Missing	3	0.4	90	7.6
Smoking				
Yes	288	40.4	343	29.0
No	395	55.5	801	67.7
Missing	29	4.1	39	3.3
Ophthalmopathy				
Yes	311	43.7	0	0.0
No	399	56.0	1,183	100.0
Missing	2	0.3	0	0

[a] Data for age are presented as the mean ± SD.

Free-T4 (fT4; 12–22 pmol/L), free-T3 (fT3; 3.6–6.3 pmol/L), thyroid peroxidase antibodies (TPOAb; <34 kIE/L), and TRAb (<1.2 IE/L) levels at diagnosis of GD were measured with ElectroChemi-Luminiscence Immunoassay on Cobas (Roche) at the Department of Clinical Chemistry, Skåne University Hospital, Sweden.

All patients provided written informed consent, and the local research Ethics Committee approved the study. For characteristics of the subjects, see Table 1.

SNP Selection and Genotyping

SNPs were selected using data from the Hap Map consortium [25]. Applying the tag SNP approach reduces the amount of SNPs that must be genotyped to cover the genetic variation in a region.

Therefore, the region surrounding each of the selected genes, including an extra 10 kb upstream and downstream, was analyzed by Tagger in the Haplowiew program [26], providing tag SNPs. In total, 7 SNPs in the DIO2 gene, including rs225014, rs12885300, rs2267872, rs225011, rs224995, rs225015, and rs2267873, were selected for the analysis. The minor allele frequency for all SNPs was >0.05. DNA was extracted from whole blood using the MaxiPrep Kit (QIAGEN, Sweden), and SNPs were genotyped using the Sequenom platform (MALDI-TOF) and TaqMan SNP Genotyping using Quantstudio 7 Flex system. The genotyping was performed in our laboratory. SNPs included in the analysis exhibited a >90% success rate.

Power Calculations

As the allele frequencies of the SNPs in our population were unknown prior to genotyping in this study, we based our power calculations on the rs225014 SNP where the allele frequencies were known from other studies [13, 15] and the Hap Map consortium using the Genetic Power Calculator [27]. Given a control/case ratio of 1.7, minor allele frequency 0.40 and OR 1.3, the number of cases required for 80% power and 0.05 significance is 710.

Statistical Analysis

Logistic regression using age, smoking and ethnicity as covariates was used to estimate SNP associations with GD and GO. In GD patients with available data, we examined possible associations of the DIO2 SNPs with the serum levels of fT3, fT4, TRAb, and anti-TPO upon diagnosis of GD. Linear regression adjusted for age was used to estimate associations with fT3 given that age was highly associated with fT3 levels in our material. Unadjusted associations were used for fT4, TRAb, and anti-TPO. The *p* values are based on additive models for the genetic variants. Correction for multiple testing was performed using permutations (maxT permutation method in PLINK). All statistical calculations were performed using PLINK version 1.07 (http://pngu.mgh.harvard.edu/~purcell/plink/index.shtml) [28].

Results

Association with GD

Using logistic regression with age, smoking, and ethnicity as covariates, we found that rs225011 was associated with GD (OR 1.18, CI 1.01–1.37, $p = 0.036$). The remaining SNPs (i.e., rs225014, rs12885300, rs2267872, rs224995, rs225015, and rs2267873) were not associated with GD (Table 2). After correction for multiple testing, the association was no longer significant.

Associations with GO, fT4, fT3, TRAb, and TPOAb

None of the SNPs were associated with GO and serum levels of fT4, TRAb, or TPOAb. A weak, non-significant association for rs225014 (Thr92Ala) and rs12885300 with serum levels of fT3 was noted in GD patients with available data on fT3 at diagnosis ($n = 377$; Table 3).

Table 2. Association of 7 DIO2 SNPs with Graves' disease

SNP	Allele	OR	95% CI	*p* value
rs225014	C	1.13	0.96–1.32	0.15
rs12885300	T	0.93	0.79–1.1	0.39
rs2267872	A	1.11	0.86–1.44	0.42
rs225011	C	1.18	1.01–1.37	0.04
rs224995	C	1.09	0.88–1.35	0.41
rs225015	A	1.10	0.93–1.29	0.27
rs2267873	G	0.98	0.76–1.25	0.85

Analysis was performed using logistic regression with age, smoking, and ethnicity as covariates.

Table 3. Association between DIO2 SNPs and serum fT3 (linear regression)

SNP	Allele	Beta	95% CI	*p* value
rs225014	C	1.40	–0.13 to 2.94	0.07
rs12885300	T	–1.58	–3.31 to 0.15	0.07
rs2267872	A	–0.29	–2.40 to 1.81	0.78
rs225011	C	1.16	–0.32 to 2.65	0.12
rs224995	C	–0.74	–2.72 to 1.25	0.46
rs225015	A	1.21	–0.31 to 2.74	0.12
rs2267873	G	–0.88	–2.82 to 1.06	0.38

Beta, regression coefficient.

Discussion

In this large Swedish case-control study of GD, we examined possible associations of common variants in the DIO2 gene and GD and GO, respectively. Our study is the first to examine associations of DIO2 SNPs and GD in a Scandinavian population and the first to study associations with GO. The only positive finding was an association between the rs225011 and GD, which, however, disappeared after correction for multiple testing. None of the SNPs were associated with GO. In GD patients, we did not observe any association between any of the SNPs and TRAb, TPOAb, or fT4 levels. An association was noted between 2 DIO2 SNPs, Thr92Ala (rs225014), and rs12885300, and circulating fT3 levels; however, the association was not statistically significant.

Our study reports similar conclusions as the findings of 2 earlier studies examining associations between SNPs in DIO2 and GD. In the Russian family study, SNPs located within the GD-1 susceptibility locus on chromosome 14q23-q32 were examined for predisposition to GD using the transmission disequilibrium test in 126 simplex

Russian families affected with GD. Among the SNPs tested, a significant preferential transmission of the Ala allele of the Thr92Ala SNP from parents to affected children was identified, suggesting the association of the Thr92Ala SNP with GD [7]. In the more recent association study, the AA genotype of the Ala92Thr polymorphism of DIO2 gene was found to be protective regarding the development of GD, severity of disease, and remission rate in GD patients [8]. In our study, rs225011 but not Thr92Ala was weakly associated with GD. However, these 2 SNPs are in high linkage disequilibrium ($D' \geq 0.9$) and therefore are most likely inherited together [16]. The mechanism behind this association remains unknown as lack of association was noted between rs225011 and TRAb, TPOAb, fT4, and fT3 levels in GD patients. Rs225011 was modestly associated with early-onset type 2 diabetes and hepatic glucose output in Pima Indians, the subjects with the risk allele exhibiting lower rates. The authors hypothesize that lower physiologically active plasma or intracellular T3 levels in the carriers of the risk allele could explain the reduced hepatic gluconeogenesis and association with diabetes [16]. GD is a multifactorial disease caused by interplay among endogenous, genetic, and environmental factors. Based on the weak OR of the associations in all studies, including ours, the SNPs in DIO2 probably contribute to the development and modulate the course of GD in the presence of TRAb rather than cause the disease. TRAb increases intrathyroidal DIO2 expression and activity, and increased intrathyroidal DIO2 activity may contribute significantly to the relative increase in thyroidal T3 production in GD patients [4]. Two Japanese studies aiming at characterizing the T3-predominant type of GD observed overexpression and increased thyroidal DIO2 activity as well as a high serum fT3/fT4 ratio in patients with T3-predominant GD [29, 30]. Thus, DIO2 gene polymorphisms could further modulate these responses. In our study, Thr92Ala and rs12885300 exhibited weak, non-significant associations with circulating fT3 levels. It is still possible that a stronger association with T3 levels exists in the peripheral tissues, including the thyroid or orbital tissues.

The development of hypothyroidism in GD patients is a known risk factor for the development or worsening of GO. Therefore, we hypothesized that polymorphisms in the DIO2 gene could affect intraorbital levels of T3, thus influencing the risk of the development of GO. In the GD cohort, we did not observe any associations between the SNPs studied and the presence of GO. This mechanism does not seem to modulate the risk of GO in our population.

In conclusion, rs225011 was weakly associated with GD in our study. The mechanism behind this association is currently not known. None of the investigated common SNPs in DIO2 were significantly associated with GO, fT4, TRAb, or TPOAb in GD patients.

Disclosure Statement

The authors declare that there is no conflict of interest regarding the publication of this article.

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