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Association of the *DIO2* gene single nucleotide polymorphisms with recurrent depressive disorder*

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Genetic factors may play a role in the etiology of depressive disorder. The type 2 iodothyronine deiodinase gene (*DIO2*) encoding the enzyme catalyzing the conversion of T4 to T3 is suggested to play a role in the recurrent depressive disorder (rDD). The current study investigates whether a specific single nucleotide polymorphism (SNP) of the *DIO2* gene, Thr92Ala (T/C); rs 225014 or ORFa-Gly3Asp (C/T); rs 12885300, correlate with the risk for recurrent depression. Genotypes for these two single nucleotide polymorphisms (SNPs) were determined in 179 patients meeting the ICD-10 criteria for rDD group and in 152 healthy individuals (control group) using a polymerase chain reaction (PCR) based method. The specific variant of the *DIO2* gene, namely the CC genotype of the Thr92Ala polymorphism, was more frequently found in healthy subjects than in patients with depression, what suggests that it could potentially serve as a marker of a lower risk for recurrent depressive disorder. The distribution of four haplotypes was also significantly different between the two study groups with the TC (Thr-Gly) haplotype more frequently detected in patients with depression. In conclusion, data generated from this study suggest for the first time that *DIO2* gene may play a role in the etiology of the disease, and thus should be further investigated.

Key words: depressive disorder, iodothyronine deiodinase type II, polymorphism, haplotype

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INTRODUCTION

Depressive disorder is one of the most common psychiatric diseases (Whiteford *et al.,* 2013). The existing evidence suggests a heterogenic etiology with a possible genetic background (Belmaker & Agam, 2008). One of the hypotheses postulates a deregulation of the hypothalamic-pituitary-thyroid (HPT) axis in depression. In adults, thyroid diseases can lead to various clinical manifestations (Bauer, 2008). For example, hypothyroidism causes fatigue, psycho-motor speed, attention and concentration and memory impairment (Samuels, 2008; Bonnin *et al.,* 2010; Almandoz & Gharib, 2012). Hypothyroidism is also associated with bipolar affective disorders, depression, or loss of cognitive functions, especially in the elderly (Bonnin *et al.,* 2010; Bauer *et al.,* 2002; Fountoulakis *et al.,* 2006; Bunevicius & Prange, 2010). The limbic system, where thyroid hormone (TH) receptors play a particularly essential role is implicated in the pathogenesis of depression (Murray *et al.,* 2011; Williams, 2008). Changes in TH levels associated with depression include an increase in thyroxine (T4) concentrations and elevated levels of reversed triiodothyronine (rT3) in the cerebrospinal fluid (CSF) (Kirkegaard & Faber, 1991), as well as elevated levels of circulating T4 (Williams, 2008), and lower levels of circulating T3 (Stipcević *et al.,* 2008).

The peripheral and tissue conversion of T4 into T3 is catalyzed by type I and II iodothyronine deiodinases (D1, D2) (T4 to T3 conversion), while type III (D3) iodothyronine deiodinase inactivates (TH) by converting T3 into T2 and T4 into reverse (rT3) (Köhrle, 1999; Bianco & Kim, 2006).

TH levels may also be affected by pharmacological treatment including antidepressants (Bauer *et al.,* 2008). In the rat, treatment with various antidepressants results primarily in changes of local D2-activities and to a lesser extent of D3-activities (Eravci *et al.,* 2000). Additionally, treatment with antidepressants results in an increase of T3 in the myelin fraction of homogenates of the amygdala, an essential structure implicated in emotion and fear regulation (Pinna *et al.,* 2003). Various hormones of the thyroid axis, including T3 have been used to treat depression as mono-therapy or, more commonly, in combination with standard antidepressants (Cooper-Kazaz *et al.*, 2009; Joffe, 2011). Interestingly, treatment with antidepressants, including a selective serotonin reuptake inhibitor, results in the induction of D2 (Baumgartner *et al.,* 1994).

The D2 protein is mainly expressed in glial cells of various regions of the central nervous system (CNS) and plays an important role in mediating TH action both during CNS development and in the adult brain (Bauer *et al.,* 2008). It is also suggested that D2 protects the thyroid status of the brain under conditions of TH deficiency (Galton *et al.,* 2007).

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Abbreviations: CIDI, Composite International Diagnostic Interview; CI, confidence interval; CNS, central nervous system; CSF, cerebrospinal fluid; DIO2, deiodinase type 2 gene; D1, type I iodothyronine deiodinase; D2, type II iodothyronine deiodinase; D3, type III iodothyronine deiodinase; HDRS, Hamilton Depressive Rating Scale CG, control group; HPT, hypothalamic-pituitary-thyroid; OR_{dis}, the disease odds ratio; LD, linkage disequilibrium; PCR, polymerase chain reaction; rDD, recurrent depressive disorder, rT3, reversed triiiodothyronine; S.D., standard deviation; SNP, single nucleotide polymorphism; SNPs, sinle nucleotide polymorphisms; TH, thyroid hormone; T4, thyroxine; TSH, thyroid stimulating hormone

There are results showing that single nucleotide polymorphisms (SNPs) in *DIO2* gene may be associated with TH levels (Peeters *et al.,* 2005) and that there is an association between the single nucleotide polymorphism (SNP) within the *DIO2* gene, the D2 protein level and its enzymatic activity. For example, allele T of the Thr92Ala (T/C) variant was found to be related with higher D2 activity/TH levels, while C allele of the ORFa-Gly3Asp (C/T) polymorphism resulted in lower D2 activity/TH levels (Bauer *et al.,* 2008; Peeters *et al.,* 2005; Canani *et al.,* 2005). The *DIO2* gene polymorphism is linked to a bipolar disorder, mental retardation and well-being (Guo *et al.,* 2004; He *et al.,* 2009; Panicker *et al.,* 2009).

Considering the possible changes in TH levels in depression, and the role of the D2 enzyme in maintaining the active TH levels, the current study examined a potential genetic contribution of the *DIO2* gene to the etiology of recurrent depressive disorders (rDD).

The aim of the study was to investigate whether two common SNPs in the gene, Thr92Ala and ORFa-Gly3Asp, linked to expression/stability of the D2 protein (Peeters *et al.,* 2005; Canani *et al.,* 2005) are associated with rDD.

MATERIALS AND METHODS

Subjects. The study enrolled 179 patients diagnosed and treated for rDD (110 females — 61.45% and 69 males — 38.55%). The diagnosis was established according to ICD-10 (1992) criteria (F33.0–F33.8). A medical history was obtained and assessed using the standardized Composite International Diagnostic Interview (CIDI) form (Patten 1999). The Hamilton Depression Rating Scale (HDRS) was used to assess the level of depressive symptoms. Next, the number of depressive episodes, the duration of disease and the age of patient at disease onset were recorded for each individual. The control group (CG) consisted of 152 healthy subjects (87 females — 57.24% and 65 males — 42.76%) with a negative family history for psychiatric disorders. Healthy controls constituted of healthy community volunteers, enrolled in the study on the basis of a CIDI psychiatric interview (Patten, 1999).

Individuals (both patients and CG) with other psychiatric diagnoses within the axis I and II disorders were excluded from the current study. Severe or chronic diseases with confirmed inflammatory or autoimmune etiology served as an additional exclusion criteria. All study subjects (patients and CG) were unrelated individuals from central Poland. To avoid a population stratification effect, genotypes were determined only in individuals of Polish origin, i.e., all four grandparents identified themselves to be of Polish origin. The study protocol had earlier been approved by the Local Bioethics Committee.

Genotyping of SNPs. Peripheral blood was collected and genomic DNA was extracted using a commercial isolation kit according to the manufactures' protocol (A&A Biotechnology, Gdańsk, Poland).

The rs225014 SNP is a polymorphism at nucleotide 674 of the D2 sequence predicting a threonine (Thr) to Alanine (Ala) substitution at codon 92 (Thr92Ala). The rs12885300, C/T polymorphism is in the most up-

Table 1. Descriptive statistics for demographic and clinical data in rDD patients and the CG for different genotypes of Thr92Ala and ORFa–Gly3Asp polymorphisms
p stands for *p*-value of appropriate statistical test: χ2 or Kruskal-Wallis Anova

Genotype	Sex	Age of onset in years	Duration of rDD in years	Number of hospitaliztion	Number of episodes	HDRS before treatment	HDRS after treatment
			Thr92Ala–rDD				
TT-83	(50F, 33M)	46±10	6±7	2±2	4±5	23±7	7±4
TC-95	(59F, 36M)	50±10	6±7	2±2	5±1	24±6	7±4
CC-1	(1F, 0M)	22	5	1	2	27	5
	p=0.71	*p*=0.005	*p*=0.95	*p*=0.80	*p*=0.48	*p*=0.43	*p*=0.57
			Thr92Ala–CG				
TT-65	(38F, 27M)	32±9					
TC-76	(43F, 33M)	31±9					
CC-11	(6F, 5M)	34±11					
	p=0.96	*p*=0.64					
			ORFa–Gly3A*sp*–rDD				
CC-51	(25F, 26M)	46±13	6±5	2±2	6±7	24±6	6±4
TC-89	(61F, 28M)	49±9	6±8	2±2	5±5	24±7	7±4
TT-39	(4F, 15M)	50±11	6±8	2±2	3±3	23±6	8±5
	p=0.07	*p*=0.40	*p*=0.64	*p*=0.91	*p*=0.13	*p*=0.84	*p*=0.07
			ORFa–Gly3A*sp*–CG				
CC-53	(30F, 23M)	30±8					
TC-65	(39F, 26M)	33±10					
TT-34	(8F, 16M)	33±9					
	p=0.79	*p*=0.17					

rDD, recurrent depressive disorder; CG, control group; *p*, level of statistical significance; F, female; M, male; ±standard deviation, HDRS, Hamilton Depression Rating Scale

Table 2. Comparison of genotypes and allele frequencies of Thr92Ala and ORFa-Gly3Asp polymorphisms in rDD patients and the control group.
Odds ratios (OR) with 95% confidence intervals (95%CI) were calculated by the method of logistic regression with age and sex adjustment.

Genotypes/allelles	rDD (n=179)	CG (n=152)	rDD vs. CG OR (95% CI)	P
Thr92Ala T/C (χ^2=10.5; df=2; p=0.005; 1-β=83.5%)				
TT	83 (46.4%)	65 (42.8%)	1.43 (0.80; 2.54)	0.22
TC	95 (53.1%)	76 (28.5%)	0.92 (0.52; 1.63)	0.78
CC	1 (0.6%)	11 (7.2%)	0.09 (0.01; 0.82)	0.03
Allelle (χ^2=2.1; df=1; p=0.15; 1-β=30.0%)				
T	261 (72.9%)	206 (67.8%)	1.47 (0.94; 2.31)	0.09
C	97 (27.1%)	98 (32.2%)	0.68 (0.43; 1.06)	
ORFa-Gly3Asp C/T (χ^2=1.9; df=2; p=0.38; 1-β=22.0%)				
CC	51 (28.5%)	53 (34.9%)	0.72 (0.38; 1.36)	0.31
TC	89 (49.7%)	65 (42.8%)	1.11 (0.62; 1.99)	0.72
TT	39 (21.8%)	34 (22.4%)	0.79 (0.40; 1.56)	0.49
Allelle (χ^2=0.6; df=1; p=0.46; 1-β=12.0%)				
T	167 (46.7%)	133 (43.8%)	0.80 (0.53; 1.21)	0.29
C	191 (53.3%)	171 (56.2%)	1.25 (0.83; 1.88)	

rDD, recurrent depressive disorder; CG, control group; *p*, level of statistical significance; F, female; M, male; ± standard deviation; HDRS, Hamilton Depression Rating Scale; n, number of samples; χ^2, Chi-square statistics; df, degrees of freedom; OR, odds ratio; 95% CI, 95% confidence interval; 1-β, post-hoc power of Chi-square test; %, percentages

stream short open reading frame (ORFa-Gly3Asp) of the 5'untranslated region of *DIO2*. The region containing the Thr92Ala and the ORFa-Gly3Asppolymorphism was amplified by PCR-based method as described by He and coworkers (2009) and Dora and coworkers (2010) with some modifications.

Statistical analysis. The results are reported as percentages (%) or means with standard deviations (±S.D.). In order to determine the association between SNPs within the *DIO2* gene and recurrent depression disorder rDD, χ^2 test was used. A *Post-hoc* power analysis was performed with the use of non-central χ^2 distribution. The analysis of association was based on 95% confidence interval (CI) for the disease odds ratio (OR_{dis}), calculated with the use of logistic regression model including sex and age as covariates. Deviations from the Hardy-Weinbergs equilibrium were determined by comparison of observed genotype prevalence rates with the expected ones. The Hardy–Weinberg equilibrium for genotype frequencies in rDD group was calculated using χ^2 tests. In all the analyses, $p \leq 0.05$ was accepted as the level of statistical significance.

RESULTS

No significant differences were found between rDD patients and CG with respect to gender ($p > 0.05$). Groups were gender matched (p=0.44), but varied significantly with respect to the age distribution ($p < 0.0001$). The mean age was higher for rDD patients than for CG: 48.5y ± 10.8y *vs* 31.7y ± 9.1 (Table 1).

Results of polymorphism frequencies analysis revealed that for the Thr92Ala polymorphism, the Hardy-Weinberg equilibrium in the rDD and the control group was rDD χ^2=21.1, p<0.001; CG χ^2=0.0002, p>0.05, respectively. While for the ORFa–Gly3Asp polymorphism, the Hardy–Weinberg equilibrium in the rDD and the CG group was as: rDD χ^2=3.17, p>0.05; CG χ^2=2.61, p>0.05, respectively.

No significant difference in the distribution of demographic and clinical characteristics for different genotypes was observed, except for the age difference for the Thr92Ala polymorphism between rDD patients and the control group (Kruskal–Wallis test; p=0.048). The observed differences in age and gender distribution between groups and certain genotypes caused necessity of age and sex adjustment using a logistic regression.

A distribution comparison between rDD patients and the control group with respect to genotypes/alleles of

Table 3. The *DIO2* gene haplotype analysis for rDD patients and CG

Thr92Ala and ORFa-Gly3Asp	rDD	CG	OR (95%CI)	p
TT	98 (27.4%)	80 (26.3%)	1.18 (0.75; 1.85)	0.47
TC	163 (45.5%)	126 (41.5%)	1.59 (1.05; 2.38)	0.03
CT	69 (19.3%)	53 (17.4%)	1.15 (0.68; 1.46)	0.60
CC	28 (7.8%)	45 (14.8%)	0.51 (0.28; 1.05)	0.07
χ^2=8.3; df=3; p=0.04				

rDD, recurrent depressive disorder; CG, control group; *p*, level of statistical significance; χ^2, Chi-square statistics; df, degrees of freedom; %, percentages; 95% CI, 95% confidence interval; OR, odds ratio

Thr92Ala and ORFa-Gly3Asp polymorphisms did not reveal a significant difference except for the CC genotype of Thr92Ala polymorphism. The CC genotype of the Thr92Ala polymorphism was significantly less frequent in rDD patients than in the controls. A summary of results for genotypes and allele frequencies within the examined SNPs is presented in Table 2.

In a haplotype analysis conducted separately for the tested SNPs, Thr92Ala and ORFa-Gly3Asp, a linkage disequilibrium (LD) in genotype frequencies was observed between rDD patients and controls (rDD patients $\chi^2=32.2$; $p<0.0001$), (control group, $\chi^2=6.1$; $p=0.014$). Therefore, further analysis was done for the combined SNPs ($\chi^2=33.1$, $p<0.0001$). The combined SNPs haplotype analysis revealed that significant differences in haplotype frequencies between rDD patients and controls ($p=0.04$).

The results of the *DIO2* gene haplotype analysis indicate a significant difference in the distribution of haplotypes for the combined SNPs between rDD patients and controls (Table 3).

DISCUSSION

This study is the first to test the hypothesis whether *DIO2* polymorphisms are linked to depressive disorders.

Our results suggest that common variation in the *DIO2* gene may be linked to a lower risk for rDD. The CC genotype is present in a small proportion of the population, approximately 4% of our study population. In a group of patients we found only 1 person with CC genotype. Interestingly, a relatively low prevalence of the CC genotype (16%) was reported by Panicker *et al.* (2009) for a larger group from Bristol, United Kingdom. Similarly, Babenko and coworkers (2012) observed an 8% presence of Ala/Ala (CC) homozygous in the Caucasian population. In the cohort consisting of 946 subjects Zevenbergen *et al.,* (2014) found CC genotype also to be less frequent (16.6%).

The Thr92Ala genotype frequency differences were noted between the Caucasian and two other Asian and Indian populations (He *et al.,* 2009; Nair *et al.,* 2012). Ethnical and geographical differences are also suggested by Guerra *et al.* (2013) who investigated the Thr92Ala polymorphism in the Brazilian population. By contrast to the above effect, neither the ORFa-Gly3Asp polymorphism nor its genotype frequencies were significantly different between two study groups. However, the frequency distribution of ORFa-Gly3Asp polymorphism genotypes in our study remains in agreement with previous observations for the Caucasian population (Peeters *et al.,* 2005; de Jong *et al.,* 2007; Hoftijzer *et al.,* 2011).

The obtained results show that the TC (Thr-Gly) haplotype is statistically more frequently detected in rDD patients. The ORFa-Gly3Asp is located in 5' untranslated region and is a functional variant related to the enzyme activity. Our findings of TC haplotype increasing rDD risk may possibly support both the fact that depressive disorder is a low T3 syndrome (Baumgartner *et al.,* 1998; Premachandra *et al.,* 2006) and the suggestion on the supplementation of antidepressant therapy, especially in some cases.

The *DIO2* gene polymorphisms were linked to psychiatric disorders. For example, the *DIO2* gene is linked with bipolar disorder in the Asian population (He *et al.,* 2009). Activity of the D2 in the brain may be a determinant of well-being and neurocognitive function (Panicker *et al.,* 2009). It is known that the CC genotype of the Thr92Ala polymorphism is relevant to TH metabolism, as it is linked to a greater therapeutic improvement in hypothyroid patients on T4/T3 combination hormone replacement as compared with T4 mono-therapy. It is also associated with poorer psychological well-being, as measured by General Health Questionnaire (GHQ) score (Panicker *et al.,* 2009).

The possible effect of the *DIO2* gene polymorphism on TH metabolism could be related to the effect of a small amino acid substitution in the D2 protein, thus influencing its level and/or activity. Studies on subclinical hypothyroidism show that subtle changes in TH bioavailability may have clear effects on well-being and neurocognitive function (Cooper, 2001; Toft, 2001). Previous studies have shown that Thr92Ala polymorphism is related to minimal effects on circulating levels of TH (Canani *et al.,* 2005; Torlontano *et al.,* 2008), whereas the ORFa-Gly3Asp polymorphism was associated with lower serum T4 and free T4 but unaltered TSH and T3 levels (Peeters *et al.,* 2005; Canani *et al.,* 2005; Peltsverger *et al.,* 2012). It was also postulated that the Thr92Ala substitution may result in the instability loop in D2 protein. Such instability may affect ubiquitination, prompting D2 protein degradation and impairing the ability of D2 enzyme to increase its activity in the presence of low T4 levels, reducing the ability to maintain homeostasis and increasing dependence on serum T3 as a source of T3 in the brain (Dentice *et al.,* 2005). Thus, the contribution of *DIO2* polymorphisms to the etiology of depressive disorders could be related to the D2 increased activity and subsequently to TH levels in the brain.

The findings that the CC genotype may act as a protective factor appears to be contradictory to previous observations that found low TH levels in patients with depression (Rao *et al.,* 1996). The results can also enlarge discussion on the hyperthyroidism involvement in the depressive disorder. Hyperthyroidism is accompanied by some psychiatric symptoms including depressive symptoms (Tylor, 1975). The possible protective role of CC genotype may be related to the lower levels of TH as C allele is associated with decreased thyroid stimulating hormone-stimulated (TSH) release of T3 and the lower levels of TSH (Butler *et al.,* 2010; Zevenbergen *et al.,* 2014). On the other hand, it is possible that the Thr92Ala variant may have no specific function or may be in linkage equilibrium with other variants. Given the involvement of the inflammatory process in the etiology of depression (Anisman, 2011; Maes *et al.,* 2011) and taking into consideration more recent data that suggests the role of iodothyronine deiodinase in the inflammation process (Boelen *et al.,* 2011), another potential explanation for the obtained results could be proposed. A significant increase in both *DIO2* expression and D2 levels was observed after lipopolysaccharide-induction (LPS) in increased lung injury, while the Ala (C) allele was considered a protective factor in sepsis and severe lung injury (Ma *et al.,* 2011). Further, a markedly higher amount of D2 protein positive cells are characteristically found in osteoarthritis (Bos *et al.,* 2012). Thus, an increase in D2 activity would be characteristic for the inflammation process. Similar to the above mentioned diseases, depression is characterized by an increased number of leukocytes and pro-inflammatory cytokine levels in the peripheral blood (Anisman, 2011; Maes *et al.,* 2011). In addition, LPS which is associated with an increased levels of both DIO2 and D2, was shown to be able to induce depression (Maes & Leunis, 2008). Thus, our results showing a significantly higher frequency of the CC genotype of Thr92Ala in healthy subjects with no inflammatory com-

ponent involved, which potentially could be linked to lower D2 activity, would be in agreement with the above hypothesis.

There are several limitations associated with our study. First, a modest study cohort used to determine genetic variants associated with rDD. It is acknowledged that a relatively larger study cohort would be required in order to confirm trends observed in the current study. Second, the current study focuses on two functionally known polymorphisms and other *DIO2* polymorphisms should be investigated in order to fully characterize the potential involvement of the *DIO2* gene in the etiology of depression disorder. Further studies involving larger cohort, characterization of TH levels, as well as markers of the inflammation process should provide a better understanding of the role of *DIO2* polymorphisms in depression.

CONCLUSIONS

DIO2 polymorphisms may constitute potential risk factors in depression.

Depression is not simply a thyroid-related illness but should be rather considered as a complex process with an inflammatory component involved.

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