

# Assessment of type 1 and type 3 deiodinase expression levels in depressive disorders

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A depressive disorder is a disease characterized by a heterogeneous background. The important processes observed and diagnosed in depressed patients indicate that the etiology of depression may include disturbances in thyroid hormone (TH) levels and the occurrence of immune-inflammatory activation. Type 1 (DIO1) and type 3 (DIO3) iodothyronine deiodinases are the enzymes which determine the peripheral and tissue levels of TH, but also interfere with immunological cells and inflammatory processes. We aimed to investigate the levels of DIO1 and DIO3 in the patients suffering from recurrent depressive disorders (rDD). Data collected from 91 rDD patients and 105 healthy controls were analyzed. The diagnoses were made based on the ICD-10 criteria (F33.0–F33.8). The expression levels of DIO1 and DIO3 were estimated using the polymerase chain reaction method and the enzyme-linked immunosorbent assay (ELISA). The expression of DIO1 on mRNA/protein levels in the rDD patients was reduced in comparison to the control subjects, while the expression of DIO3 was higher in the patients suffering from depression. No significant relationship was found between the investigated DIOs and other clinical parameters. Our results indicate and suggest a role of DIO1 and DIO3-related pathways in the pathophysiology of depression. The results represent a promising way to investigate the biological markers of depression.

Key words: type 1 and 3 iodothyronine deiodinases, depressive disorder, thyroid hormone, inflammation

## INTRODUCTION

A depressive disorder is a multifactorial disease the mechanism of which has not been fully examined so far.

Certain observations indicate that disturbances in thyroid hormone (TH) levels, including a decrease or an increase outside normal ranges, are characteristic for the patients suffering from depression (Baumgartner et al. 1988, Eker et al. 2008, Linnoila et al. 1982). Other processes found in unipolar depression in the plasma and cerebrospinal fluid (CSF), and in *postmortem* studies (Raedler 2011), include immune activation as well as increased concentration of pro-inflammatory cytokines and inflammation-related molecules. Moreover, increased levels and activity of monocytes and macrophages are observed in depression (Maes et al. 2009). Selected results also indicate a role of lipopolysaccharides (LPS) and a bacterial or viral infection in the pathophysiology of the said disease (Maes et al. 2008).

Thyroid hormones are widely distributed in the brain and are significant for its proper functioning. Moreover, the limbic system, in which TH receptors play a particularly important role, takes part in the pathogenesis of depression (Bauer et al. 2008). What is more, TH and TH synthesis-related molecules are now confirmed and strongly investigated as immune response and inflammation modulators (de Vito et al. 2012).

The synthesis and levels of TH are regulated by iodothyronine deiodinases type I (DIO1), type II (DIO2) and type III (DIO3), which remove specific iodine moieties from T4 or other iodothyronines. Under normal conditions, most of circulating T3 is produced by DIO1 and its expression is observed mainly in the thyroid and kidneys. DIO1 is also expressed in lymphocytes; it has been confirmed that its expression is influenced by pro-inflammatory cytokines (Köhrle 1999). The enzyme involved in reductive deiodination is DIO3 – it eliminates T4 by way of transformation to reverse T3 (rT3) and diiodothyronine (T2). Under nor-

mal conditions, D3 is expressed in the majority of organs, including the brain – mainly neurons of the hippocampus (Bianco and Kim 2006, Köhrle 1999). On the other hand, aberrant expression of DIO3 is observed in pathological conditions (Richard et al. 1998). For example, high expression of DIO3 is found in activated monocytes and macrophages, which supports the hypothesis that DIO3 plays a role in chemical and bacterial inflammations (Boelen et al. 2008, Boelen et al. 2009). In addition, the protective role of DIO3 against inflammation is suggested (Boelen et al. 2009). To shortly sum up, deiodinases – including DIO1 and DIO3 – interfere with immuno-inflammatory processes and influence the expression and release of inflammatory cytokines (Bartalena et al. 1998).

The exact expression levels and involvement of DIO1 and DIO3 in depression have not been investigated in the patients diagnosed with a depressive disorder/healthy controls yet, and are rather unknown. To the best of our knowledge, only one recently published study has investigated DIO3 gene deficiency on an animal model (Stohn et al. 2016). Considering the fact that the DIO1 and DIO3 enzymes determine TH levels, are involved in other processes observed in depression, and may directly interfere with the immune-inflammatory mechanisms, we aimed to investigate the expression of DIO1 and DIO3 on mRNA and protein levels in the patients suffering from recurrent depressive disorders (rDD).

## MATERIALS AND METHODS

### Subjects

196 individuals, aged 18–64 ( $M=37.41$  years,  $SD=\pm 13.74$ ), were engaged to participate in the study, including patients diagnosed with rDD ( $n=91$ ) and control subjects ( $n=105$ ). The number of depressive episodes and hospitalizations, and the duration of the disease, were recorded for everyone. The diagnosis of rDD was made based on the ICD-10 criteria (F33.0–F33.8). A medical history was obtained in all cases and assessed using the standardized Composite International Diagnostic Interview (CIDI) form (Patten 1997). The Hamilton Depression Rating Scale (HDRS) was applied to estimate the severity of depressive symptoms. The group of control subjects comprised selected healthy community representatives invited to take part in the study based on the absence of diagnostic criteria of the psychiatric CIDI interview. A description of the samples is presented in Table I. We excluded from the study both patients and controls with other psychiatric axis I and II disorders. The exclusion criteria were as follows: (auto)immune-inflammatory diseases and thyroid diseases. All the patients were native inhabitants of central Poland, and were unrelated to one another. All the procedures were reviewed and approved by the Local Bioethics

Table I. Demographic characteristics of the group with rDD in comparison to controls, and data concerning the course of the disease

Characteristics	rDD ( $n=91$ )			controls ( $n=105$ )			
	n	%	M ( $\pm$ SD)	n	%	M ( $\pm$ SD)	
Sex	Female	53	58.24	–	69	65.71	–
	Male	38	41.76	–	36	34.29	–
Age in years	–	–	47.24 (11.82)	–	–	28.89 (8.69)	
Education level	Primary	8	8.79	–	–	–	
	Vocational	18	19.78	–	–	–	
	Secondary	46	50.55	–	57	54.29	
	College/University	19	20.88	–	48	45.71	
rDD	Disease duration in years	–	–	6.43 (7.72)	–	–	
	Number of depression episodes totalled	–	–	4.44 (5.32)	–	–	
	Number of hospitalization	–	–	2.09 (1.61)	–	–	
	HDRS baseline	–	–	22.79 (6.14)	–	–	
	HDRS final	–	–	6.88 (4.36)	–	–	

rDD – recurrent depressive disorders; HDRS – Hamilton Depression rating Scale; n – number of samples; % – percentage; M – mean;  $\pm$ SD – standard deviation.

Committee. Written informed consent was obtained from all the participants of the study.

### RNA isolation and RT-PCR

Total RNA was extracted from the patients' blood samples using TRIZOL (Invitrogen Life Technologies, Carlsbad, CA, USA), an RNA extraction reagent, according to the standard acid-guanidinium-phenol-chlorophorm method (Chomczyński et al. 1987). The absorbance of isolated RNA was measured using a spectrophotometer (Picodrop, Hinxton, UK) at  $\lambda=260$  nm to determine total RNA concentration.

The quality of total RNA was checked and verified with Agilent RNA 6000 Nano Kit (Agilent Technologies, Santa Clara, CA, USA) in accordance with the manufacturer's recommendations, using 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Complementary DNA (cDNA) was transcribed from total RNA using TaqMan® RNA Reverse Transcription Kit (Applied Biosystems Foster City, CA, USA) based on the manufacturer's recommendations. The samples were incubated (at 16°C for 30 minutes and at 42°C for 30 minutes) in a thermocycler (Biometra, Göttingen, Germany). Reverse transcriptase was inactivated (at 85°C for 5 minutes) and the obtained cDNA was stored at a temperature of -20°C.

Real-Time PCR reaction was conducted using TaqMan® Universal PCR Master Mix, No UNG (Applied Biosystems, Foster City, CA, USA), according to the protocol provided by the manufacturer, using Hs00174944\_m1, Hs00956431\_s1, Hs04194366\_g1 probes delivered by Applied Biosystems, specific for *DIO1*, *DIO3* and *RPL13A*, respectively. The Ct comparative method was applied to calculate expression of mRNA genes. The level of *DIO1* and *DIO3* gene expression was normalized in relation to the *RPL13A* reference gene.

Each target probe was amplified in a separate 96-well plate. All the samples were incubated at 50°C for 2 minutes and at 95°C for 10 minutes, and then cycled at 95°C for 30 seconds, at 60°C for 30 seconds and at 72°C for 1 minute; 40 cycles were performed in total. Fluorescence emission data were captured and mRNA levels were quantified using the critical threshold (Ct) value. Analyses were conducted in ABI Prism 7000 (SDS Software, Applied Biosystems). Controls without RT and with no template cDNA were carried out with each assay. The threshold cycle (Ct) was calculated for each sample. RT-PCR amplification of the *DIO1* and *DIO3* gene was compared to that of *RPL13A*, a house-keeping reference gene, and  $\Delta Ct$  was determined ( $\Delta Ct = Ct_{\text{gene}} - Ct_{\text{RPL13A}}$ ) in each patient and control subject. The results were analyzed according to the  $2^{-\Delta Ct}$  method (Schmittgen and Livak 2008)

### Determination of protein concentration with enzyme-linked immunosorbent assay (ELISA)

Human enzyme-linked immunosorbent assays were used to detect *DIO* and *DIO3* levels in serum. The serum was separated from peripheral blood using clot activating tubes. Next, the samples were allowed to clot for 30 minutes and then centrifuged for 15 minutes at approximately 1000x g. After the centrifugation, the serum was removed and stored aliquot at -80°C. *DIO1* and *DIO3* levels were measured using commercially available Human *DIO1* ELISA Kit (MyBioSource, San Diego, CA, USA) and Human *DIO3* ELISA Kit (MyBioSource, San Diego, CA, USA). The results were calculated according to the instructions and protocols provided by the manufacturers. The absorbance of the samples was measured using Multiskan Ascent Microplate Photometer (Thermo Labsystems, Waltham, MA, USA) at  $\lambda=450$  nm. Analytical curves for the analyzed proteins were worked out to determine protein concentration. Serum *DIO1* and *DIO3* levels were presented as U/L. The detection range kit for deiodinase type 1 produced by MyBioSource totals from 3.12 U/L to 100 U/L. The detection range kit for deiodinase type 3 produced by MyBioSource is from 0.625 U/L to 20 U/L. Both Intra-assay CV(%) and Inter-assay CV(%) is less than 15 [CV(%) = SD/mean x100].

### Statistical analysis

All data analyses were performed using Statistica (version 12.0). A statistical analysis of the collected material included calculation of both descriptive and inferential statistics. The results were presented as percentages (%) or means (M) with standard deviations ( $\pm SD$ ). The chi-square test and Mann-Whitney U-test were used to compare demographic variables (gender and age) between the patients and the controls. The comparison of *DIO1* and *DIO3* expression levels between the subjects with rDD and the controls was performed using non-parametric Mann-Whitney U-test. The Pearson correlation was calculated to evaluate the relationships between the analyzed mRNA/protein levels and the features of depression. Statistical significance was defined as  $P < 0.05$  for all the analyses.

## RESULTS

No significant differences were found between the rDD patients and the controls with respect to gender ( $\chi^2=1.16$ ,  $P=0.28$ ). The groups were gender-matched but varied significantly with respect to age distribution

Table II. Expression on the protein level and on the level of mRNA for DIO1 and DIO3 in the examined group

Variable	rDD n=91; M (±SD)	Controls n=105; M (±SD)	Mann - Whitney U-test (P)
DIO1 protein (U/L)	20.03 (5.14)	22.72 (4.46)	= 0,000212
DIO1 mRNA ( $(2^{-\Delta ct})$ )	0.07 (0.02)	0.08 (0.015)	= 0,000159
DIO3 protein (U/L)	16.70 (3.90)	13.36 (6.35)	= 0,000001
DIO3 mRNA ( $(2^{-\Delta ct})$ )	0.06 (0.014)	0.046 (0.02)	= 0,000001

DIO1 – deiodinase type 1; DIO3 – deiodinase type 3; n – number of subjects; rDD – recurrent depressive disorders; M – mean; SD – standard deviation; P – level of statistical significance.

( $Z=8.65$ ,  $P<0.001$ ). There were significant differences in DIO1 and DIO3 expression between the patients and the controls on both the mRNA and protein level (Mann-Whitney U-test  $P<0.001$ ). The obtained results indicated that DIO1 expression levels were significantly lower in the patients diagnosed with rDD in comparison

to the controls, while DIO3 expression was significantly higher in the individuals affected by depression. Detailed results can be found in Table II.

The significant differences on both the mRNA and protein level were also observed between the group of males with rDD and healthy males, and between fe-

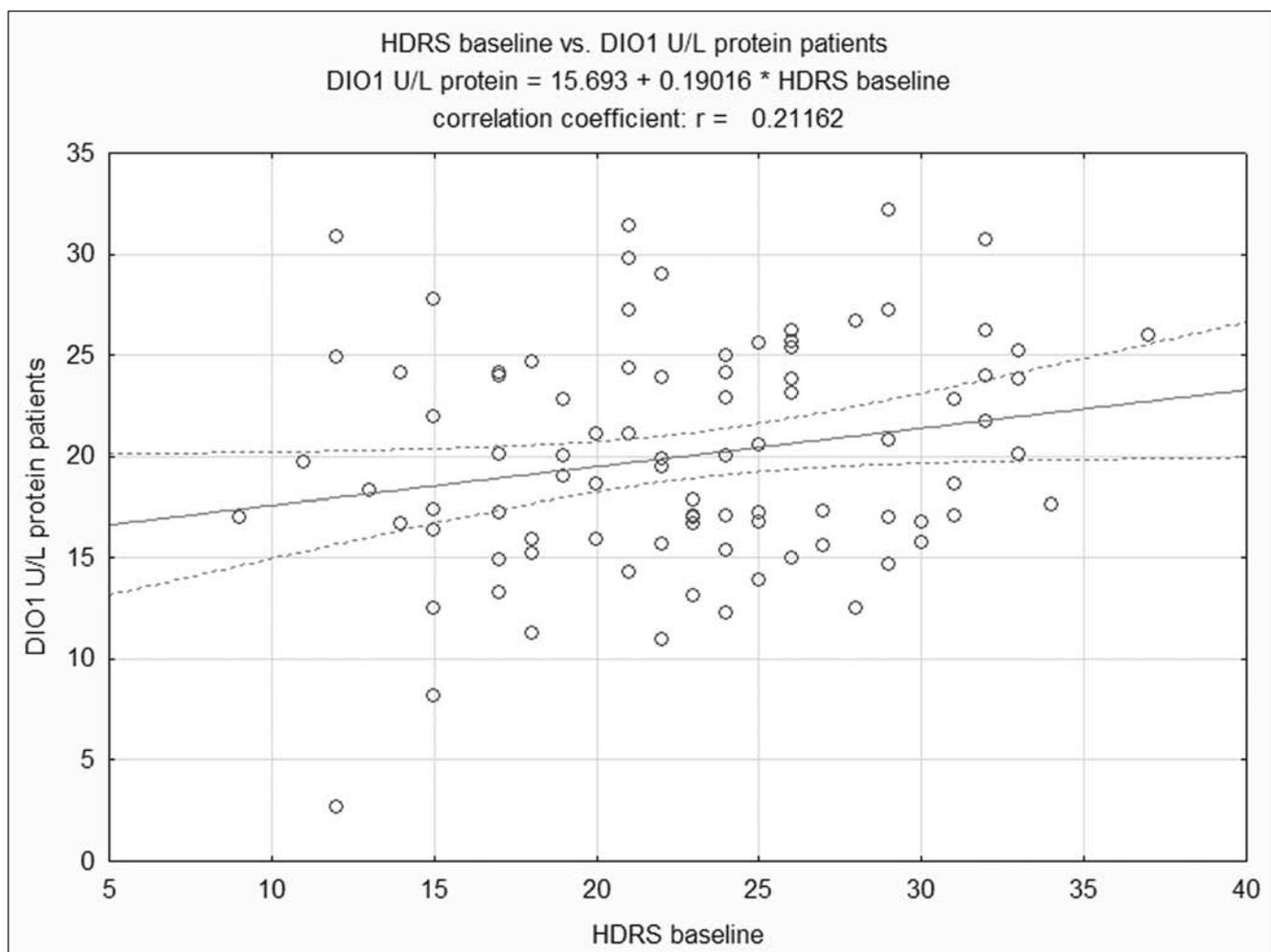


Fig. 1. Correlation between Hamilton Depression Rating Scale (HDRS) baseline and deiodinase type 1 (DIO1) protein levels in patients with recurrent depressive disorder.

Table III. Expression on the protein level and on the level of mRNA for DIO1 and DIO3 in females and males with rDD and controls

Variable	rDD females n=53	control females n=69	Mann – Whitney U-test	rDD males n=38	control males n=36	Mann – Whitney U-test
	M (±SD)	M (±SD)	p	M (±SD)	M (±SD)	P
DIO1 protein (U/L)	20.09 (5.56)	22.72 (4.54)	0.0028	19.74 (5.32)	22,71 (4,37)	0.0107
DIO1 mRNA (2 <sup>-Δct</sup> )	0.069 (0.02)	0.079 (0.016)	0.00098	0.07 (0.017)	0,079 (0,014)	0.014
DIO3 protein (U/L)	16.52 (3.38)	13.91 (6.47)	0.0026	17.57 (4.5)	12,31 (6,05)	0.000009
DIO3 mRNA (2 <sup>-Δct</sup> )	0.057 (0.014)	0.048 (0.023)	0.0022	0.06 (0.016)	0,042 (0,02)	0,000007

DIO1 – deiodinase type 1; DIO3 – deiodinase type 3; n – number of subjects; rDD – recurrent depressive disorders; M – mean; SD – standard deviation; P – level of statistical significance.

males with rDD and healthy female subjects on both the mRNA and protein levels. Detailed results are presented in Table III.

No differences were found between rDD males and rDD females as well as between healthy control males

and females (Mann-Whitney U-test  $P > 0.05$ ). Pearson's statistics show no correlation between DIO1 and DIO3 expression on mRNA/protein levels, and clinical variables, in rDD patients, except for baseline HDRS for DIO1 protein ( $P < 0.05$ ; Fig. 1).

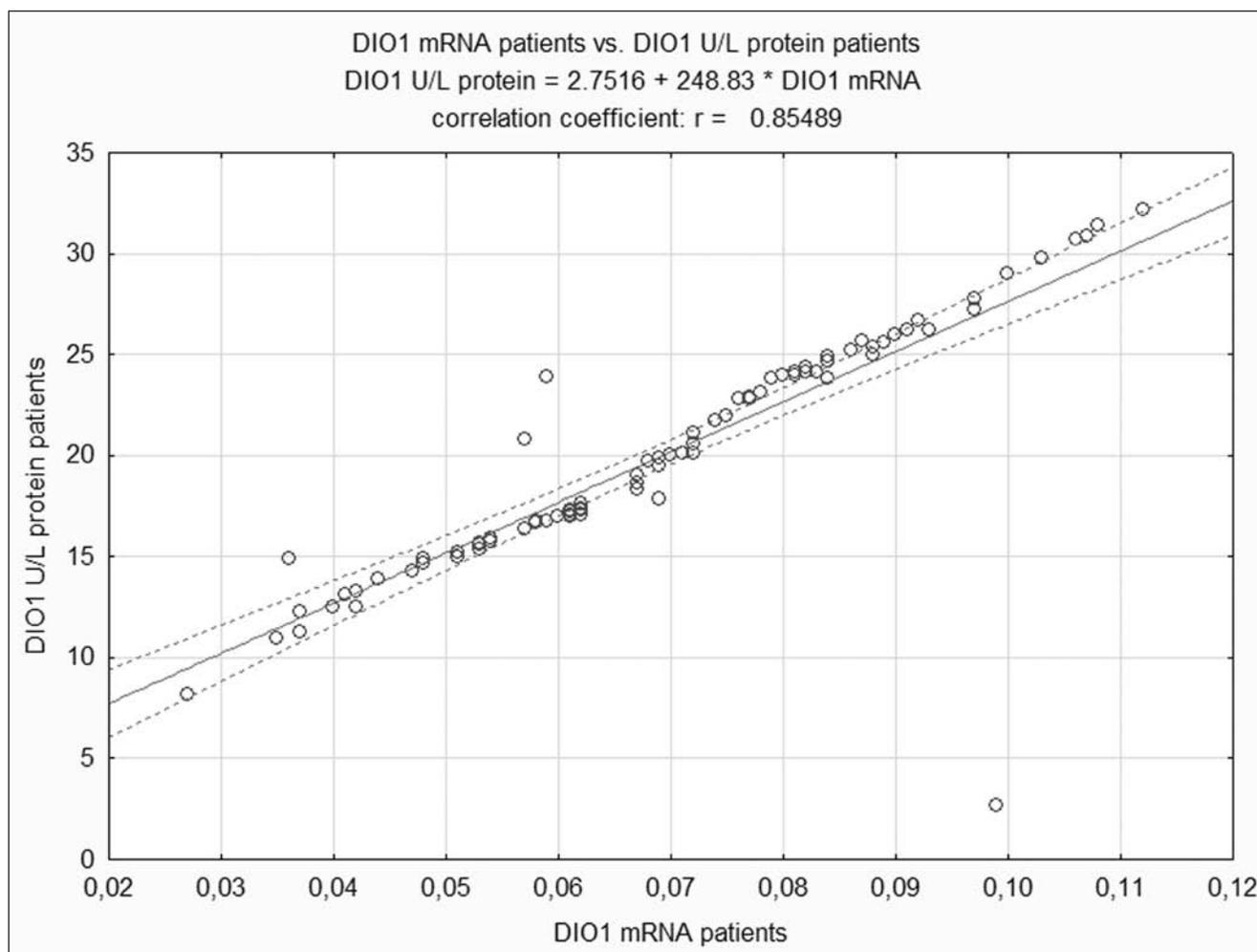


Fig. 2. Correlation between deiodinase type 1 (DIO1) mRNA and DIO1 protein in patients with recurrent depressive disorder.

Significant relationships were observed between the expression of DIO1 on the mRNA and protein levels, and between the DIO3 gene and protein expression in the patients as well as the controls ( $P < 0.05$ ; Figs 2, 3, 4, 5).

## DISCUSSION

To our knowledge, this is the first study ever to examine the expression of DIO1 and DIO3 on mRNA/protein levels in the patients diagnosed with depression.

DIO1 expression in the mononuclear cells of human peripheral blood was investigated by Nishikawa et al. (1998) in the patients suffering from the Graves' disease. During our investigation, we observed lower levels of DIO1 in depressed patients when comparing them with healthy subjects. The results obtained may be used in a discussion regarding the role and involvement of the enzyme (molecule) mentioned above in the development and course of depression. Lower levels of DIO1 can partially explain

a drop in T3 in depressed patients (Stipcević et al. 2008), but also may explain the occurrence of depressive symptoms in hypothyroid patients (Bathla et al. 2016). The data obtained support a study conducted by Hickie et al. (1996) who suggested that hypothyroidism could play a role in the development of some treatment-resistant depressive disorders. The authors found that hypothyroidism might have an impact on the course of the disorder since the presence of lower levels of T3 correlated with treatment-resistant depression. In addition, low DIO1 expression may partially be responsible for the presence of depressive symptoms in the patients with the non-thyroidal illness syndrome. The syndrome is characterized by low levels of T3 without any changes in the thyroid-stimulating hormone (TSH), and is often observed in critically ill patients (de Vries et al. 2015) in whom depressive syndrome symptoms are described frequently (Davydow et al. 2009).

Our assumption that lower levels of DIO1 may determine low levels of T3 is based on the fact that Nishikawa et al. (1998) observed increased mRNA for DIO1 in the patients

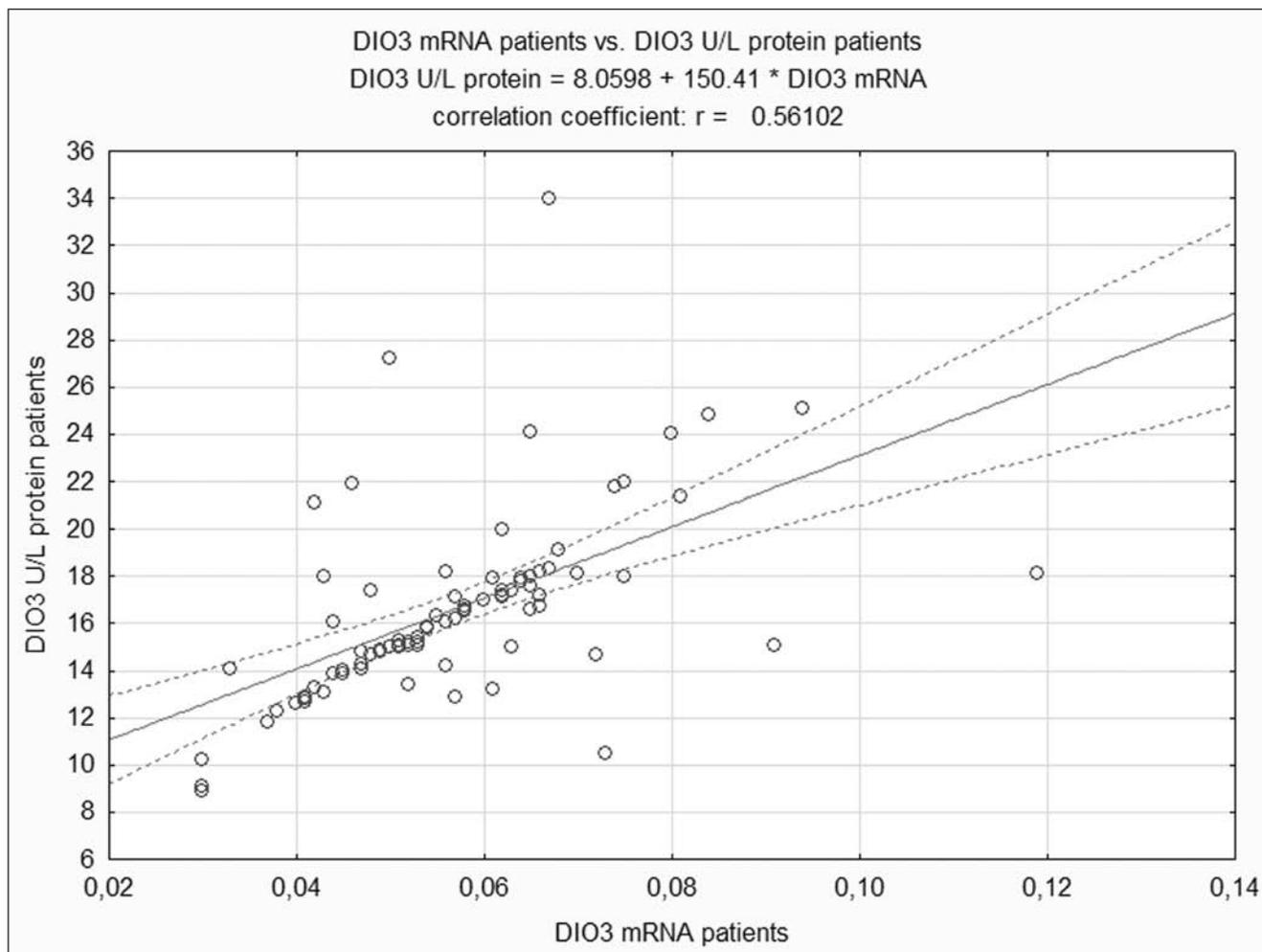


Fig. 3. Correlation between deiodinase type 3 (DIO3) mRNA and DIO3 protein levels in patients with recurrent depressive disorder.

suffering from the Graves' disease, which may explain the occurrence of hyperthyroiditis during the said disease.

The level of expression is affected by distinct factors and, obviously, lower expression of DIO1 may be caused by the action of other molecules. For example, glucocorticoids (GR) and cytokines, such as interleukin-1 (IL-1) and interleukin-6 (IL-6), may reduce the levels of DIO1 (Davies et al. 1996, Jakobs et al. 2002, Yu and Koenig 2000, Xu et al. 2014). This fact is worth mentioning, especially since the levels of GR, IL-1 and IL-6 are increased in depression, and both GR and inflammation could participate in cumulative damage (Horowitz et al. 2013). Oxidative stress, which is also characteristically observed in depression (Czarny et al. 2015), is an important factor that may affect and reduce DIO1 levels. Chen et al. (2016) suggest that oxidative stress may reduce expression of DIO1 on the mRNA level and downregulates the conversion of T4 to T3 through the function of DIO1. Similarly to our findings, lower expression of DIO1 was also observed in the patients who died of cardiovascular collapse and renal failure (Peeters et al.

2003, Peeters et al. 2005). An inflammation is a pathology which often appears in depression and is considered a mechanism linking the diseases (Halaris 2013, Oyekçin et al. 2012). DIO1 catalyzes deiodination of T4 to produce T3 and T2 (Köhrle 1999). The involvement of lower levels of DIO1 in depression may be in line with the fact that T2 takes part in the mediation of antidepressant effects (Markova et al. 2013), while lower levels of DIO1 determine lower concentrations of T2. Stimulation of the mitochondrial function, including respiratory chain (Pinna et al. 2003) and mitochondrial biogenesis (de Lange et al. 2011), is a suggested mechanism by which T2 – alternatively from other TH – positively influences an antidepressant therapy, which is of great importance, especially due to the fact that mitochondrial alterations are implicated in depression (Marazziti et al. 2011) and antidepressants stimulate mitochondrial respiration processes (Ignácio ZM et al. 2015). Moreover, damage to mitochondria and mitochondrial DNA as well as a reduced activity of respiratory chain enzymes and adenosine triphosphate

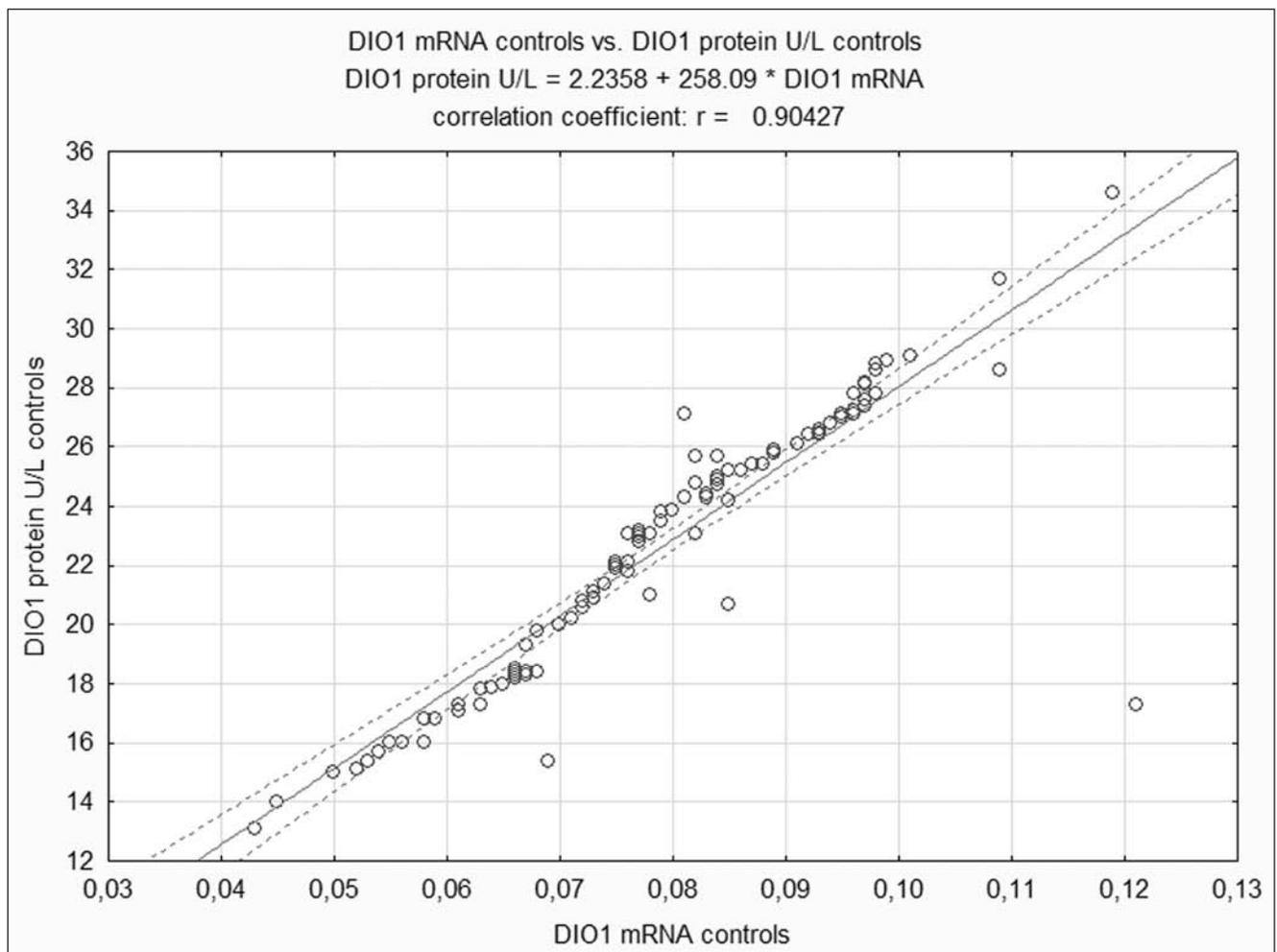


Fig. 4. Correlation between deiodinase type 1 (DIO1) mRNA and DIO1 protein levels in controls.

production are a new target of antidepressant treatment (Maes et al. 2012).

On the other side, the levels of DIO1 in the patients who died of brain damage were higher than in the control group (Peeters et al. 2005), which needs to be discussed shortly. The main deiodinase in the brain is type 2 deiodinase (DIO2) (Köhrle, 1999). In this case one may suggest that the higher levels of DIO1 in certain areas of the brain may be considered a compensatory mechanism for a drop in DIO2.

The next objective of our study was to investigate the levels of DIO3. We observed that its expression (on the mRNA and protein levels) was increased in the patients.

Since immune activation plays a role in depression pathomechanism, and deiodinases, including DIO3, are known to affect immune-inflammatory processes, our results are in line with other findings. Studies on animal models revealed that an inflammation strongly induces DIO3 in inflammatory cells (Boelen et al. 2005). Increased expression of DIO3 by granulocytes and macrophages was found in spinal cord inflammatory lesions in experimen-

tal autoimmune encephalomyelitis in rats (Boelen et al. 2009). Similarly, DIO3 was highly expressed in infiltrating neutrophilic granulocytes in response to a bacterial infection (Boelen et al. 2008), while the absence of DIO3 resulted in the elimination of damaged bacteria (Boelen et al. 2009). An observation that LPS induces depressive-like behavior is particularly interesting (Maes et al. 2008). On the contrary, according to Boelen et al. (2006), a chronic local inflammation results in decreased DIO3 expression in hypothalamic paraventricular nucleus. The importance of DIO3 and its expression were investigated on an animal model (Stohn et al., 2016). The results revealed that *DIO3*<sup>-/-</sup> mice had increased thyroid hormone levels in the brain, which was associated with reduced anxiety and depression-like behaviors. The data in this case confirmed that the status of the thyroid hormone can be considered a determinant of behavior. Taking these results into account, another mechanism of respective deiodinase should be discussed and investigated considering peripheral and/or brain presence.

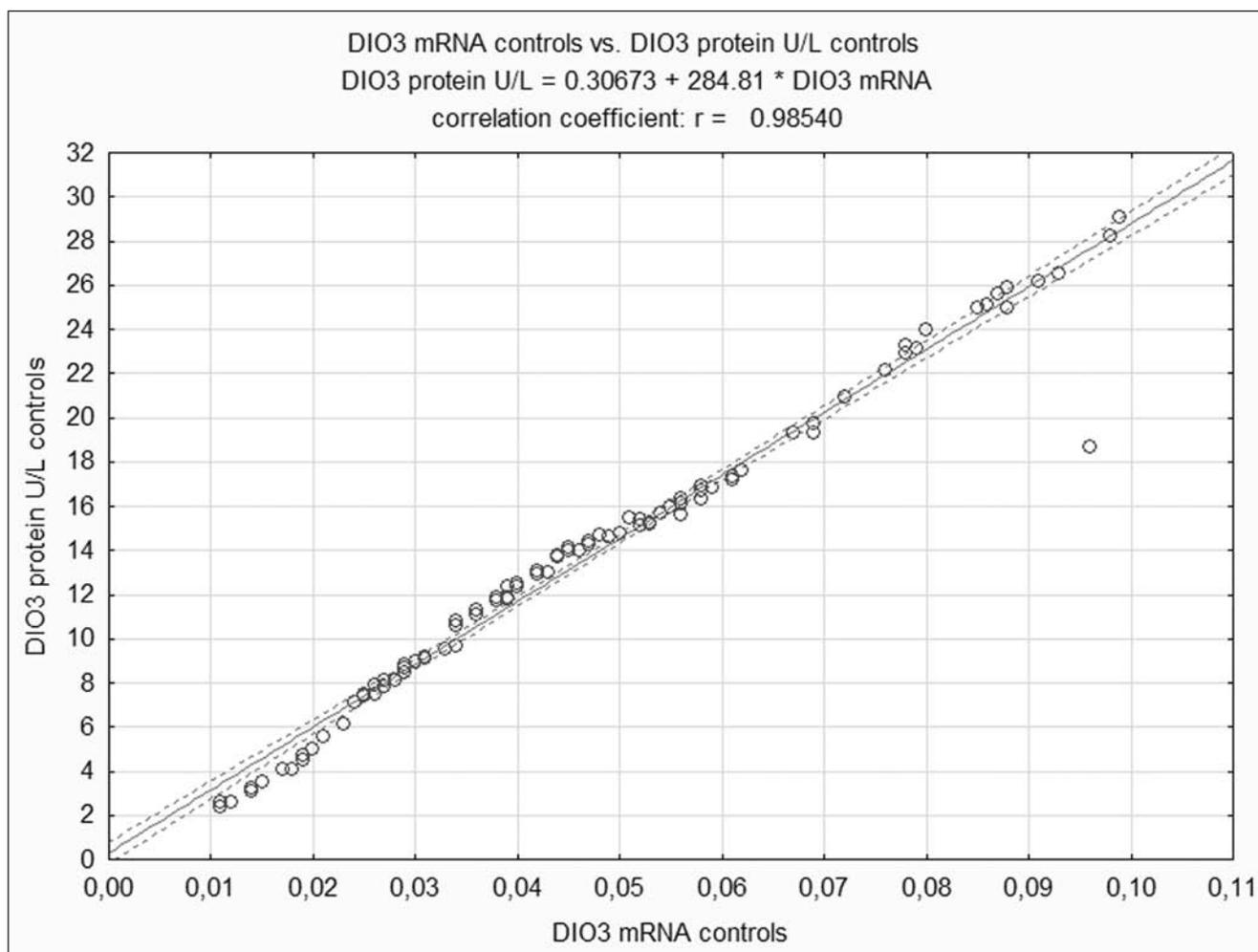


Fig. 5. Correlation between deiodinase type 3 (DIO3) mRNA and DIO3 protein levels in controls.

The data concerning the involvement of DIO1 and DIO3 in depressive disorder are sparse. Recently we have investigated genetic polymorphism within *DIO1* and *DIO3* genes in patients with rDD. No significant relations were found between genetic variants and rDD. There were no important association between genotypes distribution and clinical/demographical variables (Gałecka et al., 2016). In particular, it should shortly be discussed that we observed changes in the levels of both DIO1 and DIO3 in this study. Polymorphic site within the gene is a stable feature. According to our results we can draw a cautious conclusion that genetic variant within DIO1 and DIO3 genes is not a risk factor for the rDD. Nevertheless, changes in expression levels of both DIOs observed in this study may participate in the disease development and/or management.

The presented study may have certain limitations related to the fact that mRNA/protein expression levels can be affected by many factors and mechanisms (i.e. transcriptional and post-transcriptional), depending on the type of illness and the presence of other processes, and may be also time- and cell-specific. In addition, the activation of different signal transduction pathways may modulate the levels of expression. For example, selenium deficiency may reduce DIO1 expression and activity by decreasing mRNA (Yang et al. 2006), while sustained activation of the mitogen-activated protein kinase pathway or leptin administration may modulate the levels of DIO3 expression (Romitti et al. 2016, Kwakkel et al. 2006, Boelen et al. 2012).

It might be valuable to present information about the status of thyroid hormones. Nevertheless, we are aware of the fact that no such data are demonstrated, which limits the study. The information regarding the levels of T3 and T4 could obviously expand and improve the knowledge about a link between respective deiodinase type 1 and 3 and the status of thyroid hormones. We did not exclude the influence of DIO1 and DIO3 as important modulators of TH levels and their impact on the function of the brain, particularly since there is evidence for the interaction between TH and the neurotransmitter involved in the development and course of depression (Bauer et al., 2008). From the point of view of research and a scientific discussion, no clear information is provided whether an increase or a decrease in T3 and/or T4 levels is associated with the risk of depression. Both low and high levels of T3 and T4 can be related to depressive disorders (Premachandra et al. 2006, Bauer 2008), yet changes in the thyroid hormone are not very common (Fava et al. 1995).

The main aim of the study was to investigate the levels of DIO1 and DIO3 in the patients who were diagnosed with rDD. We would like to explain that we decided to investigate DIO1 and DIO3 because these molecules are

related to inflammatory processes and are known to be immune players. Certain reports indicate a correlation between DIO1 and DIO3 and inflammation and bacterial infections (Pappa et al. 2011, Wajner and Maia 2012, van der Spek et al. 2016). The possible mechanism explaining the role of DIO 3 is the fact that the enzyme is a source of iodide, which together with hydrogen peroxide is utilized by myeloperoxidase (MPO). As a result of such a reaction, hypiodite is created, i.e. a toxic compound that can kill bacteria (Klebanoff 1967, Boelen et al. 2011). It is also worth emphasizing that DIO3 has been recently found in human neutrophils in the intracellular granules participating in the process of bacteria elimination (van der Spek et al. 2016). There is much less information regarding DIO1; nevertheless, the expression of this molecule is also present in human neutrophils (van der Spek et al., 2016). We decided to focus on DIO1 and DIO3 because depressive disorders are characterized by an inflammation, immune cells activation, a higher level of proinflammatory cytokines (Anisman 2009), and increased expression of MPO (Gałecki et al. 2012); moreover, lipopolysaccharides are capable of inducing depressive-like behavior (Maes et al. 2008).

The data recorded by us may be of importance in the inflammatory theory of cytokine-induced depression, as many inflammation markers, including proinflammatory cytokines, affect gene expression for the enzymes mentioned above. In our opinion, the results may serve to confirm that different multidirectional molecules can participate in the immuno-inflammatory mechanism of depression. The results may be considered an introduction to a discussion about the participation of the investigated deiodinases in the etiology of depression.

## CONCLUSION

Our study is the first to present that peripheral DIO1 and DIO3 expression on mRNA/protein levels may be associated with depressive disorders, while deiodinase-related mechanisms may participate in the development and course of the disease. Further investigations of DIO1 and DIO3 levels on a more diverse population of patients are required to explore the complex role of the molecules and examine the changes of expression during depression-related pathologic mechanisms.

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