

Polymorphism and expression of the Dvl3 gene in the etiology of depressive disorder

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Summary

Aim. The aim of this study was to investigate the role of the Dvl3 gene in the etiology of depression by comparison of Dvl3 mRNA expression, Dvl3 protein levels and polymorphism at locus rs1969253 located in the intron of the Dvl3 gene in patients suffering from depression versus healthy controls, as well as to search for clinical variables related to polymorphism or expression of the analyzed gene.

Material and methods. Study group involved 181 individuals suffering from recurrent depressive disorder or depressive episode. Control group consisted of 102 healthy individuals. Sample of peripheral blood was obtained from each participant to measure Dvl3 mRNA expression, Dvl3 protein levels and polymorphism at locus rs1969253. Patients were examined on study entry with the Hamilton Depression Scale and data on the gender, age and course of disorder were gathered. Obtained data were analyzed statistically.

Results. Significantly decreased Dvl3 mRNA expression and Dvl3 protein levels were found in patients suffering from depression in comparison to healthy individuals. Expression of the Dvl3 gene in depressed patients was not affected by the patients' gender, age, number of episodes, severity of symptoms, duration of the illness or age of onset. The analysis of polymorphism at locus rs1969253 indicated that individuals with genotypes CA and CC had over 3 times higher risk of having depression in comparison to individuals with AA genotype (OR = 3.3; 95% CI = 1.56–6.99). No relationship was observed between the polymorphism and the analyzed clinical variables.

Conclusions. Changes in expression and polymorphism of the Dvl3 gene may play a role in the pathogenetic mechanism of depressive disorder.

Key words: depression, Dvl3 gene

Introduction

Depression is one of the most common and costly health problems [1]. According to the World Health Organization, depressive disorders affects about 350 million people worldwide and is the third cause of disability caused by diseases (measured by DALY – Disability Adjusted Life Years) [2]. Lifetime risk of depression is on average about 8–12% and varies considerably in different countries [3]. The course of the illness is chronic and recurrent [4], associated with shorter life expectancy and high risk of suicide [5].

The etiology of depressive disorders is considered multifactorial and both environmental factors and the complex and interrelated psychological and biological conditions of a given individual are important. The best proven pathogenetic mechanisms of depression are monoaminergic dysfunctions, which is the basis for modern pharmacotherapy, and increased activity of the hypothalamic–pituitary–adrenal axis [6, 7]. In recent years, the evidence for the role of the immune system and inflammatory processes in the etiology of depression has grown rapidly [8, 9]. Patients with depression reveal elevated levels of proinflammatory cytokines, among others, IL-1, IL-6, IFN- γ , TNF- α , acute phase proteins, complement system, and enzymes involved in inflammatory response [10–12]. Other important pathogenetic factors may be neurotrophins that regulate CNS proliferation and survival, such as BDNF [13, 14]. Changes in neurotrophins levels combined with other mechanisms result in structural abnormalities observed in brains of depressed patients: decreased volume of the hippocampus, amygdala, subcortical structures, and reduction in cortical thickness in the frontal, temporal and parietal lobes [15, 16].

The genetic background of depression becomes better understood. Observations of monozygotic twins and families affected by depression indicate the involvement of genetic factors in the etiology of depression as about 36–37% [17, 18]. Furthermore, genome-wide association studies (GWAS) estimate that the genetic component of depression constitutes at least 32% [19]. Although researchers have been trying for years to determine genotypes that predispose to depression, their observations have not provided satisfactory results so far. In the past, the main method of genetic testing used in psychiatry were studies of candidate genes and linkage analysis in families affected by the disorder. The potential association with the risk of depression was attributed first of all to the genes encoding enzymes responsible for the synthesis and degradation of neurotransmitters, transporters and receptors for serotonin, dopamine or other neurotransmitters [20, 21]. These studies were often conducted on small groups of patients and the results were characterized by low reproducibility. New opportunities in psychiatric research have emerged through the use of genome-wide association studies that do not limit the study's gene pool to genes associated with known pathophysiological mechanisms [22]. GWAS method consists in the examination of huge number of single nucleotide polymorphisms (SNP) in the entire genome to identify common genotypes associated with a particular disease or trait. In many psychiatric illnesses such as schizophrenia or bipolar disorder, associative studies have shown a statistically significant relationship at genomic level between polymorphisms of several or more

genes and the occurrence of these illnesses [23, 24]. In terms of depressive disorders, for many years GWAS had not indicate the reliable associations, nor did they confirm the findings of the candidate gene studies [25].

The meganalysis of GWAS conducted in 2013 for depressive disorders included 18,759 participants, but still the group was too small to detect the statistically significant association between the analyzed SNP and the occurrence of depressive disorders [26]. Results close to the statistical significance level for the whole study group were observed for 2 polymorphisms of autosomal chromosome regions: rs11579964 (chr1: 222 605 563 bp; $P = 1.0 \times 10^{-7}$) and rs7647854 (chr3: 186 359 477 bp; $P = 6.5 \times 10^{-7}$) [26]. Moreover, in the subgroup of women and in the subgroup of patients suffering from recurrent depressive disorders, the region chr3: 185.3Mb rs1969253, located in the intron of the Dvl3 (disheveled 3) gene encoding a protein that is part of the Wnt signaling pathway, was close to achieve a significant result [26]. It is known that as part of the Wnt-dependent signaling pathways, the Dvl3 protein and its isoforms Dvl1 and Dvl2 are involved in embryogenesis, including neurogenesis as well as in the regulation of adult tissue homeostasis [27, 28]. Animal studies also suggest the role of the Dvl3 protein in the modulation of memory processes, social interactions and mood [28–30].

The aim of this work is to gain more knowledge about the Dvl3 gene and its potential importance for the etiology of depressive disorders by evaluation of the rs1969253 polymorphism, Dvl3 mRNA expression and the Dvl3 protein level in people affected by depressive disorders compared to healthy individuals, and to search for the relationship between Dvl3 gene expression, the rs1969253 polymorphism and clinical variables in the course of RDD.

Material

The study covered 283 participants of Polish origin who were unrelated and aged over 18 years. The participants were divided into two groups. The experimental group consisted of 181 patients with depressive disorder (DD) undergoing psychiatric hospitalization diagnosed with depressive episode (F32) or recurrent depressive disorder (F33.0–F33.8) according to ICD-10 (WHO, 1992). Patients suffering from depression who were additionally diagnosed with other mental disorders, addictions (except nicotine and caffeine), traumatic central nervous system injuries, neoplastic diseases, severe neurological diseases, and severe and chronic inflammatory diseases, were excluded from the study. Data on the illness course were obtained using the *Composite International Diagnostic Interview* (CIDI). In addition, information on the age at the moment of diagnosis depression and the number of depressive episodes were obtained, and the severity of depression at inclusion to the study was evaluated using the *Hamilton Depression Rating Scale* (HDRS). These data were gathered by the same physician in the symptomatic phase of the illness at the beginning of hospitalization. With the help of qualified medical staff, 5 ml of venous blood was collected from each patient for genetic testing.

Control group (CG) included 102 mentally healthy participants with negative family history of psychiatric disorders who did not suffer from central nervous system

disorders, neoplastic diseases and severe and chronic inflammatory diseases. The evaluation of mental state was also conducted with the use of CIDI, as it was done in the depressive group.

Participation in the study was voluntary, and the data and results were used only for collective statistical analyzes. Each participant, having familiarized himself/herself with the purpose and procedure of the study, gave his/her written consent for participation. Before the study began, the approval of the Bioethics Committee of the Medical University of Lodz, no. RNN/733/14/KB of October 28, 2014, was obtained.

Methods

Methods of genetic testing

Evaluation of the Dvl3 gene mRNA expression

Total RNA isolation from the patients' peripheral blood mononuclear cells was performed with the use of RNA extraction reagent – TRIZOL (Invitrogen Life Technologies), according to the standard of single-step method of RNA isolation (acid-guanidinium-phenol-chlorophorm method – AGPC) [31]. Absorbance of isolated RNA was measured using a spectrophotometer (Picodrop) at $\lambda = 260$ nm in order to determine total RNA concentration. The quality of total RNA was checked with Agilent RNA 6000 Nano Kit (Agilent Technologies) in accordance with the manufacturer's recommendations, using 2100 Bioanalyzer (Agilent Technologies). Reverse transcription (RT) reaction was carried out using TaqMan[®]RNA Reverse Transcription Kit (Applied Biosystems) in accordance with the manufacturer's recommendations, using specific fluorogenic probes (Applied Biosystems): Hs00610261_m1, Hs04194366_g1 probes, specific respectively for *Dvl3* and *RPL13A* genes. Real-time PCR reaction was conducted using TaqMan[®] Universal PCR Master Mix, No UNG (Applied Biosystems) according to the protocol provided by the manufacturer. To calculate relative expression of mRNA genes, the Ct comparative method was used [32, 33]. In this method, the level of expression of a given gene (RQ value) in the tested sample was normalized in relation to the selected endogenous control. The level of *Dvl3* gene expression in each probes was normalized in relation to the *RPL13A* reference gene selected out of previously analyzed genes (GAPD – Glyceraldehyde 3-phosphate dehydrogenase, cyclophilin, 18S rRNA, *RPL13a* – ribosomal protein L13a). Fluorescence emission data were captured and mRNA levels were quantified using the critical threshold (Ct) value. Analyses were performed using ABI Prism 7900 HT Fast Real Time PCR System (SDS Software). No template control (NTC), in which cDNA was replaced with deionized water, was performed for each sample. Relative gene expression levels were obtained using the standard $2^{-\Delta\Delta Ct}$ calculations and expressed as a change rate in the control sample [32, 33].

Determination of Dvl3 protein concentration in peripheral blood

Dvl3 protein concentration in the serum of patients and controls was determined by ELISA test, using the Dvl3 ELISA kit (MyBiosource, San Diego, CA, USA) according to the protocols provided by the manufacturer. β -actin was used as endogenous control of protein concentrations in the samples and was labeled with Human Actin Beta Kit (ACTb) ELISA Kit (BMASSAY) as recommended by the manufacturer. 100 μ l of cytosol fraction ($\rho_{\text{protein}} = 0.5$ mg/ml) was added to the wells coated with antibodies specific to the analyzed proteins and incubated. The contents were removed and the wells were washed with three changes in 10 mM PBS buffer and incubated with 100 μ l of biotinylated antibodies specific for the analyzed proteins. Subsequently the content was removed and the wells were washed with three changes in 10 mM PBS buffer and incubated with 100 μ l ABC Working Solution. The contents were removed and the wells were washed with five changes in 10 mM PBS buffer and incubated with 90 μ l of TMB substrate. After adding 100 μ l of TMB Stop Solution, the sample absorbance was measured using a Multiskan Ascent microplate reader (Thermo Lab-systems) at $\lambda = 450$ nm.

Genotyping SNP rs1969253 of the DVL3 gene

Genomic DNA was extracted from peripheral blood leukocytes by the standard procedure according to manufacturer's protocol (A & A Biotechnology, Gdansk, Poland). The quantity and quality of the isolated DNA was analyzed via NanoDrop (Thermo Fishr Scientific Inc., DE, USA). The genotypes of polymorphisms were identified by research assistants blinded to the clinical status of participants, using the TaqMan discrimination assay. Primers specific for TaqMan SNP (Dvl3) assay code C_316872_10 for rs1969253 were used. The PCR reaction was performed using the HT Real Time PCR system 7900 (Applied Biosystems) following standard procedure. Allelic discrimination was performed on the post-PCR product. Analyses of amplification products were performed using SDS software, version 1.2. The efficiency of amplification was calculated from the slope of the standard curves generated on the basis of individual measurement by each probe when testing diluted heterozygote samples. A sample without a template was used as a negative control.

Evaluation of the severity of depression

The 21-item *Hamilton Depression Rating Scale* (HDRS) was used to assess the severity of depressive disorder in the experimental group, and was performed on each patient at admission. The severity of symptoms was assessed on a four-point scale (from 0 to 4 points in 13 items, in the rest from 0 to 2 points). Scoring from the last 4 questions was not included in the total score. The result was classified according to a standard score [34].

Statistical methods

The statistical calculations were performed using the STATISTICA PL, version 12. Qualitative features in the studied population were described by selected structure indicators; the frequency of occurrence of features was given in percent. For quantitative features, the arithmetic mean (M) was calculated. The standard deviation (SD) and the range of values (giving the minimum and maximum values) were assumed as the measure of the dispersion. The Shapiro-Wilk test was used to assess the normality of the variables' distribution – for none of the variables the distributions were normal. For non-parametric variables, appropriate non-parametric tests were used for statistical comparisons between the studied groups: Pearson's χ^2 test (for qualitative variables), Mann-Whitney *U* test and Kruskal-Wallis test (for quantitative variables). The Spearman's rank correlation coefficient was used to assess the relationships between the selected quantitative or ordinal variables.

Hardy-Weinberg equilibrium (HWE) was determined using a SNPstats [35]. Three genotypes were considered for the rs1969253 polymorphism: AA, AC and CC. Differences on genotype distribution between study and control groups were assessed by the odds ratio (OR), 95% confidence interval (CI) and χ^2 test, using a linear regression model adjusted for sex and age. 5 models of inheritance were tested. In order to choose the best model of inheritance Akaike's information criterion (AIC) was used. For statistical comparison of different genotypes in relation to quantitative variables the nonparametric Kruskal–Wallis test was used. The statistically significant differences were considered at $p < 0.05$.

Results

Characteristics of the studied group

The mean age of all examined individuals ($N = 283$) was: $M = 40.94$ years, standard deviation (SD) = 13.67, minimum age – 18 years, maximum – 67 years. The groups differed significantly in terms of age, there were no statistically significant differences between the examined groups in terms of gender. The characteristics of the group with depressive disorders and the control group in terms of age and gender are shown in Table 1. Statistical analysis showed that there is a statistically significant difference in the level of Dvl3 gene expression at mRNA and protein levels between patients suffering from recurrent depressive disorder and healthy controls (Table 1). In patients with DD, mean level of the mRNA expression and Dvl3 protein concentration was lower than in healthy controls.

Table 1. Comparison of the examined groups in terms of age, gender, Dvl3 gene mRNA expression and Dvl3 protein level

Variable	DD (n = 181)	CG (n = 102)	DD vs. CG
Age (M±SD)	47.59±11.29	29.16±8.68	Z = 10.56; p < 0.01*
Male gender (n, %)	72 (39.78%)	35 (34.31%)	$\chi^2 = 0.83$; p = 0.36

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Dvl3 mRNA expression (M±SD)	0.11±0.03	0.15±0.06	Z = - 4.26; p < 0.01*
Concentration of Dvl3 protein (M±SD)	13.03±3.04	16.58±5.59	Z = - 4.68; p < 0.01*

DD – group of patients with depressive disorders; CG – control group; n – number of patients; M – mean; SD – standard deviation; Z – Z value of the Mann-Whitney U test; χ^2 – value of Pearson’s χ^2 test; p – level of statistical significance; * – statistically significant p

The study and the control group were significantly different in terms of age, while age did not correlate with the level of mRNA expression or Dvl3 protein concentration in the study group. Significant relationship between age and Dvl3 gene expression was observed among healthy subjects and, interestingly, it was a positive correlation (older people showed higher level of expression of the Dvl3 gene than younger people). Taking into consideration the fact that mean age in the control group was significantly lower than in the experimental group, the age-matched control group would probably reveal even greater differences in Dvl3 mRNA expression and Dvl3 protein level than in our study. Gender was not related to the expression of the Dvl3 gene in any of the analyzed groups (Table 2).

Table 2. Analysis of the relationship between age and gender versus Dvl3 gene mRNA expression and Dvl3 protein level in the studied groups

Variables	DD		CG	
	RS	p	RS	p
Age & Dvl3 gene mRNA expression	-0.06	0.43	0.25	p = 0.01*
Age & Dvl3 protein level	-0.06	0.42	0.26	p < 0.01*
	Z	p	Z	p
Gender & Dvl3 gene mRNA expression	0.62	0.53	0.9	p = 0.37
Gender & Dvl3 protein level	0.74	0.46	0.82	p = 0.41

DD – group of patients with depressive disorders; CG – control group; Z – Z value of the Mann-Whitney U test; RS – R value of Spearman’s rank correlation coefficient; p – level of statistical significance; * – statistically significant p

Table 3 presents the clinical characteristics of the DD group in terms of the number of hospitalizations, the age of onset, the duration of the illness, the number of depressive episodes, and the HDRS result on the day of enrollment. There were no statistically significant correlations between any of the analyzed clinical variables and the mRNA expression of the Dvl3 gene, Dvl3 protein level and the rs1969253 polymorphism, these results are also shown in Table 3.

Table 3. Clinical characteristics of a group of patients suffering from depressive disorders and analysis of relationships between clinical variables and Dvl3 mRNA expression, Dvl3 protein level and the rs1969253 polymorphism

Variable	Characteristics of DD group (n = 181)				Dvl3 mRNA expression	Dvl3 protein level	rs1969253 polymorphism
	M	Min.	Max.	SD	RS (p)	RS (p)	H (2, N = 181) (p)
Number of hospitalizations	2.12	1	12	1.98	-0.08 (p = 0.26)	-0.091 (p = 0.22)	2.09 (p = 0.35)
Age of onset	41.19	14	66	11.83	-0.07 (p = 0.32)	-0.073 (p = 0.33)	5.16 (p = 0.08)
Duration of the illness	6.39	1	40	7.32	-0.03 (p = 0.67)	-0.031 (p = 0.68)	3.81 (p = 0.15)
Number of depressive episodes	4.72	1	20	5.50	-0.07 (p = 0.35)	-0.070 (p = 0.35)	0.16 (p = 0.92)
HDRS on admission	22.58	5	37	6.39	-0.07 (p = 0.36)	-0.068 (p = 0.37)	1.45 (p = 0.49)

DD – group of patients with depressive disorders; n – number of patients; M – mean; Min. – minimum; Max. – maximum; SD – standard deviation; RS – R value of Spearman's rank correlation coefficient; H – H value of Kruskal – Wallis test; p – level of statistical significance; HDRS – Hamilton Depression Rating Scale; * – statistically significant p

Dvl3 gene expression was also compared between patients who experienced the first episode of depression ($N = 67$) and those with recurrent depressive disorder ($N = 113$), but no significant differences were observed for Dvl3 gene mRNA expression ($Z = 0.56$; $p = 0.58$) or for the expression of the Dvl3 protein ($Z = 0.42$; $p = 0.67$). These patients did not differ also as to the rs1969253 polymorphism ($Z = 0.65$; $p = 0.72$).

Polymorphism exact test for Hardy-Weinberg equilibrium showed that genotype distribution in control group fulfils HWE expectations ($p = 0.21$). The frequency for the C allele at locus rs1969253 within the Dvl3 gene is significantly higher in DD cases than in controls (55% vs. 39%; $p < 0.001$) (Table 4). The linear regression model, adjusted for sex and age, showed statistically significant differences in genotype distribution between depressed patients and healthy controls. Several genotypic models were tested with the lowest AIC value for dominant model, in which individuals with CA and CC genotype were at over three times higher risk of developing depression (OR = 3.30; 95% CI = 1.56–6.99) when compared with the AA genotype (Table 4).

Table 4. Differences in genotype distribution of genetic polymorphism rs1969253 between patients suffering from depression and control group (n = 283, adjusted for sex and age)

Model of inheritance	Genotype rs1969253	DD (n, %)	CG (n, %)	OR (95% CI)	p	AIC
Codominant	AA	38 (20.99%)	41 (40.2%)	1.00	p < 0.01*	230
	CA	86 (47.51%)	42 (41.18%)	2.90 (1.29–6.56)**		
	CC	57 (31.49%)	19 (18.63%)	3.98 (1.62–9.81)**		
Dominant	A/A	38 (20.99)	41 (40.2)	1.00	p < 0.01*	228.6
	C/A-C/C	143 (79.01)	61 (59.8)	3.30 (1.56–6.99)**		
	Allele	DD (n, allele frequency)	CG (n, allele frequency)			
	A	162 (0.45)	224 (0.61)	1.00	p < 0.01*	
	C	200 (0.55)	80 (0.39)	3.46 (2.49–4.80)**		

DD – group of patients with depressive disorders; CG – control group; n – number of patients; OR – odds ratio; CI – confidence interval; AIC – Akaike's information criterion; p – level of statistical significance; * – statistically significant p; ** – value above 1 is a significant risk factor

Discussion

In literature, there are very few reports of Dvl3 gene polymorphism and expression in the context of depressive disorders. Our study demonstrated a significant difference in Dvl3 mRNA expression and Dvl3 protein levels as well as differences in the rs1969253 polymorphism between healthy and depressed individuals. Jansen et al. [36] in a study of 1,848 individuals measured the expression of several hundred peripheral blood genes in patients during a depressive episode compared to those in remission and healthy individuals. This study showed that during depressive episodes the expression of the Dvl3 gene was altered (interestingly, they observed an increase in Dvl3 expression during depressive episode), the expression of genes involved in the pathways of NK (natural killer) activation was decreased and the expression of interleukin 6 (IL-6) was increased. In a postmortem study, in individuals suffering from depression reduced transcription of the Dvl3 protein was observed in the nucleus accumbens, which is part of the reward system and plays a significant role in the enjoyment and cognitive processing of aversion, motivation and pleasure [29]. Similar observations were made on the animal model of depressive disorder – in the nucleus accumbens of stress-prone mice, transcription of Dvl1, Dvl2 and Dvl3 isoforms was reduced compared to stress-resistant mice, and pharmacological blockade of Dvl proteins activity promoted depressive behavior in mice [29]. Mutations of the Dvl1 and Dvl3 genes can lead to transient embryonic brain expansion during deep cortex formation and changes in social interactions as well as stereotypic behavior in adult animals, which can be prevented by pharmacological activation of the canonical Wnt pathway during early corticogenesis [30].

Dvl proteins play an important role in the proliferation, differentiation and migration of cells during embryogenesis [37, 38]. In the neural system, they also participate in neurite formation, synaptogenesis, neuronal polarization, as well as in the anterior-posterior axis formation and dorsal-ventral polarity of the neural tube [39, 40]. The Wnt signaling pathway is also one of the major regulators of adult hippocampal neurogenesis, suggesting that isoforms of the Dvl protein are involved in learning and memory processes [41]. Activation of Wnt pathway improves episodic memory, while its blocking prevents long-term memory consolidation without impact on short-term memory, which is most likely a result of the dysfunction of dopaminergic pathways [42, 43]. At the cellular level, turning off the function of Wnt pathway leads to impairment of long-term synaptic potentiation process that is necessary for permanent memorization, including movement reactions and memories in formation of which the reward system is involved [44]. Downregulation of Wnt signaling in humans may be associated with cognitive impairment associated with aging as well as with the pathophysiology of Alzheimer's disease [45]. Experimental animal model of neurodegenerative changes proved relationship between the lower levels of nuclear Dvl3 and neuronal loss in the hippocampus, and the activation of the Wnt pathway impeded memory loss and improved synaptic functions [42].

However, overexpression of the Dvl3 gene was observed in leukocytes of socially isolated people, which is probably due to the participation of the Dvl3 gene in the process of cell proliferation during the activation of inflammatory mechanisms [46]. Elevated levels of Dvl3 have also been reported in the prefrontal cortex and striatum of rats after treatment with antipsychotic drugs, and – as suggested by the authors – may be associated with the observed neurotrophic effects of these drugs [47, 48].

Our study, apart from differences in expression between people suffering from depression and healthy controls, also showed a statistically significant correlation between the occurrence of depression and the rs1969253 polymorphism of the Dvl3 gene in the study group. The GWAS meta-analysis from 2013 did not show that the rs1969253 polymorphism was significant at the genomic level in the etiology of depression, but it was one of the SNPs coming close to the statistical significance threshold in the subgroup of women and people suffering from RDD [26]. Subsequent GWAS studies did not confirm the significance of the rs1969253 polymorphism, however, only five of the GWAS studies carried out so far gave statistically significant results. The first one was performed on a homogeneous group of Chinese women with severe recurrent depressive disorders and indicated two SNPs: one near the *SIRT1* gene and the other within the intron of the *LHPP* gene [49]. The second study included 75,607 depressed participants of European origin and a control group consisting of 231,747 subjects and indicated 15 significant SNPs [50]. Another study, using a different methodology based on the HRHM method (Haplotype-block-based regional heritability mapping), showed the importance of the *TOX2* gene [51]. By combining the results of the two previous meta-analyses, which allowed to involve over 70,000 participants, it was possible to detect the relationship between the occurrence of depression and locus rs9825823, located in the intron of the *FHIT* gene [52]. In contrast, the analysis taking into account the age of onset confirmed

a statistical significance for the polymorphism of another region of chromosome 3 – rs7647854 suggested in the mega analysis from 2013, which was associated with 50% of depression cases with a late onset (above 27 years of age) [53]. According to the authors of this study, the genetic basis of depressive disorders with onset at a very young age is similar to bipolar disorder and schizophrenia [53]. In our study, we did not observe the relationship between the age of onset and the polymorphism or expression of the Dvl3 gene.

A recent study, published in April 2018, included a group of patients with depression including 135,458 cases and a control group including 344,901 individuals, and identified 44 loci associated with depression, among which the 4 most important loci were located within the following genes: *OLFM4*, *NERG1*, *RBFOX1*, *LRFN5*. This study also indicated the relationship between the risk of depression and a low level of education, higher BMI as well as a shared genetic background with schizophrenia [54].

It is worth to answer the question why GWAS, which allowed to detect the genetic basis of many other diseases that are inherited polygenically, including schizophrenia or bipolar disorder, find it difficult to find genes predisposing to depressive disorder? There are several reasons. First, GWAS typically include frequent polymorphisms, i.e., those that occur in at least 5% of the population, and their effect in shaping the phenotype is usually small. In the case of depressive disorders, the number of loci with a small effect may be extraordinary large and exceed the number of SNPs significant in other psychiatric disorders such as schizophrenia or bipolar disorder [55]. In addition, depression is an illness with a high prevalence [56] and heterogeneous etiology (seasonal, postpartum, atypical, with anxiety, with psychotic symptoms, in patients after traumatic experiences, accompanying somatic diseases, etc.), thus probably there are also differences in the contribution of genetic factors as well as different genetic variants predisposing to different endophenotypes of depression [57, 58]. In addition, some patients are incorrectly classified as suffering from depression because depressive episodes may be a prelude to the development of other psychiatric disorders, while the control group may encompass people who will develop unipolar disorder in the future, which is important for the results of GWAS [59]. The above-mentioned factors cause that a very large group of participants is necessary to obtain statistically significant results in GWAS, and the first GWAS in depression were conducted on too small populations and gave results that were not repeatable [22]. Finally, we cannot forget about the influence of environmental factors in the etiology of depression and their mutual interactions with genetic factors [60].

Our study has several important limitations, first of all statistically significant difference in age between the group of people with depressive disorder and the control group. Moreover the study was conducted on a small study group, which could affect the lack of relationship between clinical variables and polymorphism or Dvl3 gene expression. However, despite these limitations, we observed a significant difference in the expression of the Dvl3 gene between depressed and healthy people. The obtained results are consistent in terms of mRNA expression and protein level. We also demonstrated the importance of the Dvl3 gene polymorphism for the risk of depression in the study group.

Conclusions

Disclosure of frequent SNPs associated with depression reveal areas for further research to explain the role of genes and their expression in the pathogenesis of this common illness, which may lead to new methods of prevention, diagnosis and treatment of depression. Earlier observations and the results presented in this study suggest that the Dvl3 gene is one of many genes whose polymorphism and alterations in expression play a significant role in the etiology of depression.

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