Rom J Morphol Embryol 2021, 62(2):491–496 ISSN (print) 1220–0522, ISSN (online) 2066–8279 doi: 10.47162/RJME.62.2.15

ORIGINAL PAPER

RJME Romanian Journal of Morphology & Embryology http://www.rjme.ro/

Association between genetic variants and depression in a Romanian cohort

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Abstract

Major depressive disorder (MDD) is beyond doubt a common, disabling, and costly condition. MDD associates hypothalamic–pituitary–adrenal (HPA) axis alterations. We sought to investigate two candidate variants which could have a role in the genetic susceptibility for stress or corticoid-induced MDD: glucocorticoid receptor (GR) – nuclear receptor subfamily 3 group C member 1 (*NR3C1*) *rs41423247* and brain-derived neurotrophic factor *rs6265 BDNF:c.442G>A Val66Met*. We enrolled 82 Romanian subjects, 1:2 male to female ratio, 53.54±8.98 years old, diagnosed with an episode of major depression at the Clinical Neuropsychiatry Hospital in Craiova, Romania, and 286 healthy controls, 34.28±16.34 years old. All subjects were genotyped using specific ThermoFisher Scientific assays on a ViiA™ 7 real-time polymerase chain reaction (PCR) system. The impact of certain genetic variants may be ethnic-specific. In our Romanian cohort, *rs41423247 NR3C1:c.1184+646C>G* has a minor allele frequency of 29.2%, and *rs6265 BDNF:c.442G>A* of 22.2%. Neither reached significance in our study, under any of the association models – dominant, recessive, or allelic. Interpretation of our negative findings requires caution: literature provides arguably more evidence for the association between the analyzed polymorphisms; our study has sample size challenges, from which refined phenotyping limitations derive.

Keywords: depression, polymorphisms, *rs6265 BDNF:c.442G>A Val66Met*, *rs41423247 NR3C1:c.1184+646C>G*.

Introduction

The *Global Burden of Disease* study estimated around 258 million people worldwide being affected by depression in 2017 [1], and it is especially of notice under the recent circumstances of the current pandemic [2, 3]. Depression is considered the leading cause of disability when measuring using years lived with disability (YLDs) [4]. A chronic condition, depression lowers the quality of life [5], having an even more distinct and impactful individual cost, as depression is a significant risk factor for suicidal ideation and suicidal behavior [6].

The etiology of depression is complex and incompletely understood. Most studies identify a gradient of genetic contribution in interaction with a boon of environmental [7] and psychological factors for mental illnesses. Genetic factors can explain around 35% of the heritability [8, 9], while other authors propose higher percentages [10]. Epidemiological studies support environmental factors and the gene–environment interactions as accountable of a major part of the unexplained heritability [11, 12].

Major depressive disorder (MDD) pathology involves many biological systems: neural (structure, function, neurotransmitters), gastrointestinal, immune, endocrine, to name several [13]. Numerous studies have suggested an association between the dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis in adults [14–17]. The hyperactivity of the HPA axis is a consistent finding in MDD [18]. To strengthen our case, out of a wide range of predictors, strong prospective evidence was found only for cortisol, as potential biomarker for MDD [13].

Altered or impaired signaling through the glucocorticoid receptor (GR) leads to a disruption of negative feedback and thus dysregulation of normal HPA axis activity [19, 20].

The *rs41423247* polymorphism of the nuclear receptor subfamily 3 group C member 1 (*NR3C1*) gene, *NR3C1: c.1184+646C>G*, which encodes the GR has been linked to depression [21], is associated with anomalies in the HPA axis [22].

Brain-derived neurotrophic factor (BDNF) is generally found in the central nervous system (CNS), more specifically in the amygdala, hippocampus, neocortex, and cerebellum, all of which are regions that are relevant for mood regulation. BDNF has been found to play a significant role in cognition and the processing of emotions factor and has been linked to brain structure abnormalities observed in depressed patients [23–26]. The *rs6265 BDNF:c.442G>A Val66Met* polymorphism, has been linked not only to depression [26] but has also been studied as a candidate

for association with dysregulation of the HPA axis and cortisol [23].

Aim

Our study aimed to evaluate *rs41423247 NR3C1:c.1184+646C>G* and *rs6265 BDNF:c.442G>A* polymorphisms by real-time polymerase chain reaction (PCR) in a cohort of Romanian subjects with the aim to test their association with susceptibility to MDD.

Participants, Materials and Methods

Our study enrolled patients admitted to the hospital with a diagnosis of major depressive episode established using the Hamilton Rating Scale for Depression (HAM-D) following approval granted by the Medical Ethics Committee of the University of Medicine and Pharmacy of Craiova, Romania (No. 22/26-02-2016).

The control group included 286 healthy individuals, 34.28±16.34 years old, 186 females and 100 males. We enrolled a total of 82 subjects with MDD, 53.54±8.98 years old, 30 men and 52 women. The study group consisted of patients of the Clinical Neuropsychiatry Hospital in Craiova. Participation was strictly voluntary, included a medical evaluation by a psychiatrist, a questionnaire, and a biological sample of 2 mL ethylenediaminetetraacetic acid (EDTA) blood. The questionnaire included self-reported information that regarded history of previous depressive episodes, prior treatment administration, the presence of anxiety, suicidal thoughts, and suicidal gestures.

Inclusion criteria consisted of a diagnosis of MDD quantified using the HAM-D rating system, age of over 18. Exclusion criteria were age, history of violence, psychiatric comorbidities.

Molecular testing was performed at the Laboratory of Human Genomics, University of Medicine and Pharmacy of Craiova. Deoxyribonucleic acid (DNA) extraction was performed automatically using Maxwell® 16 Blood DNA Purification kits running on a Maxwell 16 Research Instrument machine, obtaining a DNA concentration of 40±10 µg/µL. Genotyping experiments by real-time PCR were performed on a ViiA™ 7 real-time PCR system with 384 well blocks.

Both the *rs41423247 NR3C1:c.1184+646C>G* polymorphism (a G/C transversion), as well as the *rs6265 BDNF:c.442G>A* polymorphism (a C/T transition) were evaluated through real-time PCR using specific Thermo Fisher Scientific assays (*rs41423247* – assay ID: C__865 07873_10; *rs6265* – assay ID: C__11592758_10). The total reaction mix had a volume of 5 µl comprising of 2.5 µL of Applied Biosystems TaqMan™ Genotyping Master Mix, 0.25 µL of Assay Working Stock and 2.25 µL of DNA sample normalized at 10 ng/µL.

Data analysis was performed using the Applied Biosystems QuantStudio™ v1.6.1 proprietary software system. We compared our genotyping results with data already available from 1000 Genomes, *Exome Aggregation Consortium* (ExAC) exomes and genomes available at https://www.ncbi.nlm.nih.gov/.

Further statistical analysis was performed using basic statistics for genetic association using the tools available online at https://www.genecalculators.net/associatorrr-cc.html.

Hardy–Weinberg equilibrium (HWE) was tested for both cases and controls before calculating the association tests: odds ratio (OR) with 95% confidence interval (CI), χ^2 (*chi*-squared) and *p*-values under each classical association models – dominant, recessive, and allelic.

Results

Several clinical characteristics of the MDD group are presented in Table 1. We are showing the MDD group differences by gender. We further stratified the MDD group based on self-reported patient characteristics of relevance to MDD evaluation: family history of depression up to third degree relatives, the occurrence of multiple depressive episodes before the time of enrollment, a positive personal history of suicidal thoughts, a positive personal history of suicidal gestures, feelings of anxiety associated with MDD and a history of adverse life events.

Table 1 – *Patient characteristics*

	Percentage % (*n*)	**Percentage % (*n*)**	
Gender	63.42% (*n*=52) females	36.58% (*n*=30) males	
	Percentage % (*n*) of the females	**Percentage % (*n*) of the males**	**Percentage % (*n*) of total patients**
Family history of depression	26.92% (*n*=14)	40.00% (*n*=12)	31.70% (*n*=26)
Multiple depressive episodes at the time of enrollment	84.61% (*n*=44)	83.33% (*n*=25)	84.15% (*n*=69)
Suicidal thoughts	71.15% (*n*=37)	76.66% (*n*=23)	73.17% (*n*=60)
Suicidal gestures	15.38% (*n*=8)	30.00% (*n*=9)	20.73% (*n*=17)
Associated anxiety	48.07% (*n*=25)	60.00% (*n*=18)	52.43% (*n*=43)
Adverse life events*	21.15% (*n*=11)	20.00% (*n*=6)	20.73% (*n*=17)

n: No. of cases. *Includes any adverse events during childhood and adult life.

A higher percentage of the enrolled males (40.00%) had a family history of depression compared to the opposite gender (26.92%). There is a gender difference in the case of suicidal gestures too, where the male rate (30.00%) was nearly double that of the females (15.38%). Finally, the last notable difference was in the presence of associated feelings of anxiety, where the female group (48.07%) had a lower rate than the males did (60.00%). Gender does not seem to discriminate when it comes to history of multiple depressive events (84.61% *vs* 83.33%), the presence of suicidal thoughts (71.15% *vs* 76.66%) and the presence of adverse life effects (21.15% *vs* 20.00%), which had comparable frequencies in both male and female participants.

Genotyping results for the study groups are summarized in Table 2 for each of the evaluated single nucleotide polymorphism (SNPs).

Both the MDD study group and the control group are in HWE.

rs41423247 NR3C1:c.1184+646C>G has a minor allele frequency (MAF) of 29.19% G in the Romanian healthy group and slightly higher at 33.53% in the MDD group. Although the incidence of *GG* genotype for *rs41423247*

NR3C1:c.1184+646C>G is slightly higher in the MDD group, the OR for the dominant model is OR=1.13 (p=0.72), OR=1.86 for the recessive model (p=0.16) and OR=1.22 for the allelic model (p=0.33).

Table 2 – *Genotyping results and inheritance models calculations*

rs41423247 NR3C1:c.1184+646C>G			***rs6265 BDNF:c.442G>A***		
Genotype	***MDD (%, n)***	***Control (%, n)***	***Genotype***	***MDD (%, n)***	***Control (%, n)***
CC	46.34%, *n*=38	49.30%, *n*=141	*GG*	63.41%, *n*=52	60.69%, *n*=173
CG	40.24%, *n*=33	43.01%, *n*=123	*GA*	34.15%, *n*=28	34.42%, *n*=99
GG	13.41%, *n*=11	7.69%, *n*=22	*AA*	2.44%, *n*=2	4.90%, *n*=14
	HWE, p=0.38	HWE, p=0.50		HWE, p=0.43	HWE, p=0.97
Dominant model OR=1.13, 95%CI: (0.69, 1.84); χ^2=0.12, p=0.72			Dominant model OR=0.88, 95%CI: (0.53, 1.47); χ^2=0.12, p=0.73		
Recessive model OR=1.86, 95%CI: (0.86, 4.01); χ^2=1.90, p=0.16			Recessive model OR=0.49, 95%CI: (0.11, 2.18); χ^2=0.43, p=0.51		
Allelic model OR=1.22, 95%CI: (0.84, 1.77); χ^2=0.94, p=0.33			Allelic model OR=0.85, 95%CI: (0.55, 1.31); χ^2=0.40, p=0.53		

BDNF: Brain-derived neurotrophic factor; CI: Confidence interval; HWE: Hardy–Weinberg equilibrium; MDD: Major depressive disorder; *n*: No. of cases; NR3C1: Nuclear receptor subfamily 3 group C member 1; OR: Odds ratio.

MAF for *rs6265 BDNF:c.442G>A* is 22.20% in the healthy group and 19.51% in the MDD group. The AA genotype of *rs6265 BDNF:c.442G>A* is lower in the MDD group, but OR values are OR=0.88 (p=0.73) for the dominant model, OR=0.49 (p=0.51) for the recessive model and OR=0.85 (p=0.53) for the allelic model.

Statistical significance has therefore not been reached for any of the run models: dominant ('AA + AB' *vs* 'BB'), recessive ('AA' *vs* 'AB + BB') or allelic ('A' *vs* 'B') for either of the evaluated SNPs.

Discussions

Allele frequency aggregator (ALFA) for *rs41423247 NR3C1:c.1184+646C>G* from the database of Genotypes and Phenotypes (dbGaP) show a MAF varying greatly across European (EUR) 36.95%, East Asian (EAS) 16.00% and African (AFR) 22.37% populations, with similar trends reported by 1000 Genomes, ExAC exomes and genomes (https://www.ncbi.nlm.nih.gov/snp/rs41423247#frequency_tab, accessed on January 31, 2021). Our study reports 29.19% for allele G for the control group, slightly lower than the EUR cited frequency. Frequency data for this polymorphism have not been reported to the mentioned genomic databases for the Romanian population to date, to the best of our knowledge.

ALFA frequencies for *rs6265 BDNF:c.442G>A* are EUR 19.39%, EAS 44.49% and AFR 4.34%, with similar trends reported by 1000 Genomes, ExAC exomes and genomes (https://www.ncbi.nlm.nih.gov/snp/rs6265#frequency_tab, accessed on January 31, 2021). In this case, MAFs for the control group was 22.2%, comparable to the EUR reported frequency. *rs6265 BDNF:c.442G>A* has been previously reported in healthy individuals of Romanian ethnicity having a MAF ranging from 22.18% [27], 19.26% [28], and 16.53% [29].

Literature provides convincing evidence to link stress response through HPA axis to depression and other conditions sharing the same mechanism, *e.g.*, post-traumatic stress disorder (PTSD) [30].

GR, encoded by the *NR3C1* gene, is crucial for stress response as it plays a role in the negative feedback mechanism in the HPA axis. Stress elicits an adaptive change to the HPA axis and stimulates glucocorticoid secretion, whose effects are mediated by GR. Activated GR sends negative feedback to the hypothalamus and pituitary gland. GR function inhibition leads to a decrease of the negative feedback resulting in high stress hormone levels that are persistent over time in the blood; GR function activation may also lead to symptoms of depression [21].

The *rs41423247 NR3C1:c.1184+646C>G* homozygous state increases the risk for depression. The strongest evidence to date is coming from a meta-analysis based on 1630 MDD and 3362 healthy control (HC) analysis from the nine studies it includes [21]. Smaller, more recent studies seem to concur [31] or even suggest the polymorphisms modulated depression severity [32]. Despite not reaching significance, we do note the tendency for *rs41423247* effects in our own dataset.

It could very well be that the effects of *rs41423247* genotype are seem more clearly in certain circumstances. This polymorphism may be linked to development of emotional and behavioral problems in children with maternal psychological symptoms during pregnancy [33]. Our study was underpowered to subset and look carefully at adverse life events (Table 1), but such endeavors are warranted.

BDNF has been linked to several neuropsychiatric disorders, such as MDD, PTSD, schizophrenia, eating disorders, panic disorders. This association however seems not uniform amongst all populations as different populations express different frequencies for the *Val66Met* polymorphism [34]. Petryshen *et al.* [35] have shown that the frequency of the *Met* allele can be extremely variable between populations, with an interval range between 0% and 72%. Stratification by ethnicity may not in all circumstances be able to yield significant associations [36]. Low frequencies expressed in certain populations might derive from studies simply not having a large enough sample size. It can also be that this specific polymorphism interacts differently with other environmental or genetic factors in each specific population [34].

Though our study fails to identify the association, there is substantial supportive evidence that *Met* allele of *BDNF Val66Met* polymorphism plays a role in moderating the relationship between stress and depression [37]. A few recent studies have supported a positive link between *rs6265 BDNF:c.442G>A*, childhood events and depression [37]; evidence is stronger for stressful life events [38].

BDNF Met allele may confer increased risk for depression

as individual age [26]. *rs6265 BDNF:c.442G>A* impact could be significantly different in men than they are in women [36]. There may be gender specific regions of the genome associated with MDD [39, 40]. Another explanation might involve the differences in environmental factors that men and women experience throughout their lives [36]. Note differences in our study group (Table 1) between men and women: men seem to have a higher incidence of family history of depression, more suicidal gestures, and more often associated anxiety.

Research approaches in MDD should attempt to overcome several limitations conditioned by the biology of the illness and practical implementation constraints.

Population-oriented studies may be needed to fully understand the role these polymorphisms play in influencing susceptibility to disease. We acknowledge sample size as a major limitation to many genetic association studies in MDD, including to our contribution. Reaching statistical significance can be also hampered by low effects of individual SNPs. Whereas an increase in sample size may increase power, implementation is challenging. Alternatively, functional studies (including for instance protein level measurements) are a different approach studies could take to provide answers.

Most studies address depression under a unified umbrella. Nonetheless, different endophenotypes with distinct pathophysiological mechanisms seems to be a more accurate description of the phenomenon [41]. This could, in fact, mean that relevant associations remain hidden in over-simplified study designs. For instance, to identify association for SNP, such as the ones of interest for the current study, a gender specific evaluation of the SNPs could be warranted, with much stricter inclusion criteria to filter in stress-related depression only.

Reducing the depression model to a discrete approach may mean missing complex interactions. Single-marker analysis may not be able to provide much needed insight into the etiopathology of depression. To integrate and position correctly the role for genetics in MDD, we have mentioned above the poly-gene–environmental–psychological causation paradigm. Candidate polymorphisms will not reveal the whole picture. For instance, *NR3C1* and *BDNF* are sites of interest for epigenetic changes at the interplay of environment and genetic susceptibility in MDD [42, 43]. DNA methylation is associated with depressive symptoms, suggesting that early life traumatic experiences are biologically embedded, even more so in a gender-specific manner [44, 45].

Conclusions

In our limited study on a Romanian population, the evaluated *rs41423247 NR3C1:c.1184+646C>G* and *rs6265 BDNF:c.442G>A* did not significantly associate to susceptibility to depression. However, we encourage future studies designs to follow the endeavor to bring these interactions to light with a larger sample, careful phenotyping (gender, ethnicity, a measure for subtyping stress-induced depression, etc.) and combining with other layers of data such as transcriptomics, epigenetics, etc.

Conflict of interests

No conflict of interests to declare.

Acknowledgments

This research was kindly supported by the Laboratory of Human Genomics, University of Medicine and Pharmacy of Craiova, Romania. The authors would like to thank the involved Departments for the openness in collaboration.

Author's contribution

AC, IU, MP, MI and IS worked on conceptualization. ALR, IS and MI performed validation, formal analysis, and investigation. AC and IU were responsible for resource. AC, ALR, MP, MED, IMV and DGG were involved in writing – original draft preparation, as well as review and editing. All authors read and approved the final manuscript.

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Received: April 3, 2021

Accepted: November 12, 2021