BJMG 21 (1), 2018 • 5-12
DOI: 10.2478/bjmg-2018-0010
sciendo

ORIGINAL ARTICLE

FAMILY HISTORY AS AN IMPORTANT FACTOR FOR STRATIFYING PARTICIPANTS IN GENETIC STUDIES OF MAJOR DEPRESSION

Zalar B[1,#], Blatnik A[2,#], Maver A[2], Klemenc-Ketiš Z[3], Peterlin B[2,*]

***Corresponding Author:** Professor Borut Peterlin, Clinical Institute of Medical Genetics, Division of Obstetrics and Gynecology, University Medical Center Ljubljana, Šlajmerjeva 3, 1000 Ljubljana, Slovenia. Tel: +386-1-5401-137. E-mail: borut.peterlin@guest.arnes.si

[#]B. Zalar and A. Blatnik contributed equally to this study.

ABSTRACT

Depression is estimated to affect 350 million people worldwide. The World Mental Health Survey conducted in 17 countries found that, on average, about one in 20 people reported having an episode of depression in the previous year. Although depression has been shown to be moderately heritable by studies conducted in the past, the search for its so-called missing heritability has so far been unsuccessful. The difficulty in identifying common genetic variants predisposing to depression could be due to large sample sizes needed to detect small effects on genetic risk and the heterogeneous nature of major depressive disorder (MDD). The aim of our study was to determine whether there was a connection between a family history of depression in MDD patients and the presence of putative risk variants in the well-studied *SLC6A4*, *COMT* and *PCLO* genes. We analyzed 133 patients with MDD (30.0% with a positive family history for MDD and 70.0% sporadic cases) and compared them to 279 healthy controls. When comparing all the depressed patients to controls, no significant differences in genotype and allele distributions were detected. After stratifying patients according to their family history, the *PCLO* rs2522833 C allele was shown to be significantly less common in patients with a positive family history ($p = 0.001$), indicating a possible difference in the genetic structure of MDD between familial and sporadic cases and a less important role of the common genetic risk variants for the development of MDD in familial cases.

Keywords: *COMT* gene; Family history; Major depressive disorder (MDD); *PCLO* gene; *SLC6A4* gene.

INTRODUCTION

Depression is a major cause of disease and disability. It consists of several symptoms, such as depressed mood, loss of interest or pleasure, decreased energy, feelings of guilt or low self-worth, disturbed sleep or appetite, and poor concentration. One-third of all people seeking psychiatric help are depressed; in more economically developed countries, one in five people will be depressed at least once in his/her life, and in poorer ones, this ratio is much worse.

Major depression usually strikes without a a discernible triggering event. This can be confusing and frustrating for both the person affected and his/her surroundings. People expect their illnesses to have clear causes, but many serious diseases, such as cancer, usually have none. Major depression is indeed a serious disease that often causes despair and hopelessness so profound that the person loses all interest in life, becomes incapable of feeling pleasure, and may be unable to get out of bed or eat for days at a time. The total annual cost of depression in Europe is about 253 Euro per inhabitant [1].

Genetic susceptibility to major depressive disorder (MDD) is a field of study that has baffled many researchers; while it has shown great promise, it has actually yielded very few tangible results. Major depressive disorder has long been known to be moderately heritable. Twin studies have repeatedly shown the heritability of major depression to be about 37.0-38.0% [2,3]. Different approaches to

[1] University Psychiatric Hospital Ljubljana, Clinical Institute of Medical Genetics, University Medical Center Ljubljana, Slovenia

[2] Department of Family Medicine, Maribor Medical School, Maribor, Slovenia

[3] Department of Family Medicine, Ljubljana Medical School, Ljubljana, Slovenia

studying the genetics of MDD have so far been consistent only in their inconsistency; linkage studies, candidate gene approaches, studies of gene-environment interaction, and more recent genome-wide association studies (GWAS) have failed to give significant or reproducible results [4,5]. Genome-wide association studies in particular have shattered hopes of finding significant common risk variants. Previously detected associations with genes such as those in the monoamine synthesis pathway have not been replicated in GWAS and no new, clear-cut candidates significantly associated with MDD have been discovered [6,7]. These results are in stark contrast to results for other complex diseases such as diabetes, Crohn's disease, and rheumatoid arthritis, where GWAS have been considerably more successful in identifying potentially interesting loci [8-10]. Researchers are now trying to find novel approaches in their quest for the missing heritability of MDD [11].

Many reviewers agree that the aforementioned failure of GWAS could be due to the fact they were underpowered [12]. A great number of different loci might play a role in determining a person's predisposition to develop MDD, but if that is indeed the case, the effect of each contributing variant could be exceedingly small. It has been estimated that sample sizes in excess of 50,000 might be needed for variants with genome-wide significance to be discovered [11].

A possible approach that might enable researchers to lower the necessary number of participants in future studies would be to concentrate on the various subtypes of depressive disorders, as there is evidence MDD is a genetically heterogeneous disorder [13]. Depression is more heritable in women and is associated with different genetic variants in both sexes [14,15]. Studying patients with early onset disease, choosing patients with more severe forms of the disease and a higher recurrence rate, as well as focusing on various clinical subtypes, could also help distinguish between different genetic influences. It is also important to acknowledge that there is a considerable overlap between MDD and other psychiatric disorders such as bipolar disorder and generalized anxiety disorder (GAD). Some studies show that the genetic factors underlying both MDD and GAD are essentially the same [16-19].

Another approach to subdividing patients with MDD that has occasionally been used in the past, is to enquire about their family history of depression. A positive family history is the most important risk factor for developing a depressive disorder and is often found in patients with more severe and recurrent disease and an earlier age of onset [20,21]. Linkage studies performed in families with several affected members have shown that many different loci could be associated with the familial form of the disease [22,23]. Estimates of single nucleotide polymorphism (SNP) heritability performed so far seem to indicate that more than 50.0% of MDD heritability is due to common variants [24], but the possibility that rare variants of great effect also account for some MDD heritability cannot be excluded. It is therefore possible that both combinations of common risk variants and specific rare variants are at play in familial cases.

Based on these findings, our study aimed to explore the connection between the family history of depression and the presence of common genetic risk factors for MDD. First, we set out to determine the allele and genotype distributions for the extensively studied SLC6A4, COMT and PCLO polymorphisms in Slovene patients with MDD and to compare them to healthy controls. We then tried to detect any differences in the prevalence of these putative genetic risk variants in MDD patients with a positive family history of depressive disorder compared to sporadic cases with no affected relatives.

MATERIALS AND METHODS

Subjects. Of the 133 patients participating in this study, most were recruited *via* the Outpatient Psychiatry Centre of the University Psychiatric Hospital in Ljubljana, Slovenia. All had been previously diagnosed with either MDD, or in rare cases GAD, by an experienced psychiatrist, in accordance with the DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, 4th edition, 1994; American Psychiatric Association) classification system criteria. A small number of patients was recruited by their general practitioners; they had all been diagnosed as having MDD in the past by a psychiatrist. No patients with a history of schizophrenic, schizoaffective or bipolar disorder or a diagnosis of personality disorder were included in our study. Patients with MDD showing psychotic symptoms were likewise excluded from further analysis.

A total of 279 healthy controls were enrolled in our study while undergoing screening examinations at various occupational medicine departments across Slovenia. Only healthy volunteers with no personal or family history of depression were recruited.

All participants were required to fill in a structured questionnaire enquiring about their general health and habits and about the family history of neuropsychiatric disorders in their first-, second- and third-degree relatives. They also completed the Zung Self-Rating Depression Scale (SDS). No controls that scored 45 or more on the SDS were included in our study.

Blood samples and, in a small number of MDD patients, buccal swabs were obtained from all participants.

All patients and controls were of Caucasian origin. They all signed written informed consent forms. The study was conducted in accordance with the Declaration of Helsinki (1964) and was approved by the Slovenian National Ethics Committee.

Genotyping. Genomic DNA was extracted from blood samples and buccal swabs using standard protocols. Genotyping of the *COMT* rs4680 and *PCLO* rs2522833 was performed using a real-time polymerase chain reaction (RT-PCR) method on a 7000 Sequenced Detection System (Applied Biosystems, Foster City, CA, USA). KASPar (LGC Ltd., Teddington, Middlesex, UK) SNP genotyping chemistry was utilized according to the manufacturer's protocols. Polymerase chain reaction was carried out in a 10 μL final volume containing 5 μL of a DNA sample, 5 μL of Reaction Mix 2 × and 0.14 μL of Assay Mix. After initial denaturation at 94 °C for 15 min., 20 denaturation cycles (94 °C for 10 seconds, 57 °C for 5 seconds, 72 °C for 20 seconds) were performed, followed by the final extension step of 40 seconds at 72 °C. The allele discrimination analysis was carried out using SDS Software Version 1.2 (Applied Biosystems) [25].

Genotyping of the 5-HTTLPR variant was performed in accordance with procedures previously described in the literature [26]. The *5-HTT* gene regulatory region was amplified by PCR with the following primers: 5'-GGC GTT GCC GCT CTG AAT GC-3' and 5'-GAG GGA CTG AGCT GGA CAA CCA C-3'. Polymerase chain reaction was performed in a 10 μL reaction mixture containing 1 μL of genomic DNA, 0.3 μL of each primer, 0.2 μL of PCR Nucleotide Mix, 2 μL of GoTaq® Flexi Buffer, 0.8 μL of 25 mM MgCl2 solution, 0.1 μL of GoTaq® DNA Polymerase and 5.3 μL of H_2O. Denaturation was performed at 94 °C for 2 min. and was followed by 30 cycles of amplification (98 °C for 10 seconds, 63 °C for 30 seconds and 68 °C for 30 seconds). The PCR products were separated using electrophoresis in a 3.0% agarose gel and visualized by UV after SYBR®Safe staining. A 484 bp band was observed for the short (S) allele, and a 528 bp band for the long (L) allele; heterozygous samples showed both alleles.

Statistical Analyses. Genotype distributions were tested for adherence to the Hardy-Weinberg disequilibrium using the χ^2 distribution test. The χ^2 test was also used to analyze the significance of associations between genotype and allele frequencies and MDD. Patients with MDD were compared to a control group consisting of healthy volunteers. In addition, a group of patients with a positive family history of depressive disorders was compared to patients with no family history of depression, and both groups of patients were separately compared to healthy controls. Analyses were performed using *R* statistical language (*R* 3.1.1 for Windows; https://cran.r-project.org/bin/windows/base/old/3.3.1). A nominal level of significance $p = 0.05$ was regarded as significant and corrected according to Benjamini-Hochberg when multiple tests were performed.

RESULTS

Genotype distributions for tested SNPs in control samples adhered to the Hardy-Weinberg equilibrium. No significant difference in distribution was detected when comparing the MDD cases and healthy controls for any of the variants tested. There were no significant differences using the dominant, recessive or codominant model (data not shown). Genotype and allele distributions for the three tested variants in 133 patients with MDD and 279 healthy controls are shown in Table 1.

A comparison of the genotype and allele frequency distributions was also performed between the group of MDD patients with a positive family history for depression and patients with no such family history. A significant difference in genotype and allele frequencies was detected between the two groups of patients for the *PCLO* rs2522833 polymorphism. The association remained significant even after correction for multiple testing; no other association reached the threshold of statistical significance. Both patients with a positive family history and those with a negative family history were also compared to healthy controls. A significant difference in the allele and genotype frequencies was detected between the familial cases and controls, again for the *PCLO* rs2522833 polymorphism. The results are shown in Table 2.

DISCUSSION

The polymorphic variants included in our analyses have all been thoroughly studied in connection to psychiatric disease. The common Val158Met polymorphism in the *COMT* gene (due to a G>A transition) has been the subject of several association studies performed in different populations and often with conflicting results. Whereas some studies have detected an association between the low activity methionine allele and depression, others have yielded opposite, or, more often, negative results [27,28]. The 5-HTTLPR promoter repeat variant of the *SLC6A4* gene is perhaps the most widely studied of all the common variants that were investigated in MDD using the candidate gene approach. Two variants are usually reported in humans, namely the short (S) and long

Table 1. Genotype and allele distributions of the *PCLO* rs2522833, *COMT* rs4680 and *SLC6A4* 5-HTTLPR polymorphic variants in major depressive disorder/generalized anxiety disorder patients and controls.

	MDD/GAD Patients (*n* = 133)	**Controls (*n* = 279)**	***p* Value**
PCLO rs2522833 Genotype frequencies: AA AC CC	 *n* (%) 59 (0.44) 58 (0.43) 16 (0.12)	 *n* (%) 122 (0.44) 112 (0.40) 44 (0.16)	0.567
Alleles: A C	 176 (0.66) 90 (0.34)	 356 (0.64) 176 (0.36)	0.549
COMT rs4680 Genotype frequencies: GG GA AA	 *n* (%) 26 (0.21) 73 (0.56) 31 (0.23)	 *n* (%) 67 (0.24) 154 (0.56) 55 (0.20)	0.51
Alleles: G A	 125 (0.48) 135 (0.52)	 288 (0.52) 264 (0.48)	0.276
SLC6A4 50HTTLPR Genotype frequencies: LL LS SS	 *n* (%) 44 (0.34) 70 (0.52) 19 (0.14)	 *n* (%) 100 (0.36) 131 (0.48) 45 (0.16)	0.614
Alleles: L S	 158 (0.59) 108 (0.41)	 331 (0.60) 221 (0.40)	0.877

MDD: major depressive disorder; GAD: generalized anxiety disorder.

(L) allele, although the repeat region is complex and can be subdivided into several allelic variants. The S allele has been repeatedly linked to depression or to the personality traits predisposing to depression, most famously in the setting of gene-environment interaction [29,30]. Even though some meta-analyses appear to confirm its role in the etiology of MDD, there is poor replication between similarly designed studies [31]. The *PCLO* gene was first identified as a candidate MDD gene in a GWAS study in which no findings of genome-wide significance were detected, but a suggestive result was obtained for the rs2522833 *PCLO* polymorphism [32]. Again, the attempts to replicate these results have not been completely successful. However, some studies have linked the C allele to an increased vulnerability to depression, both directly and through its influence on those personality traits which increase the risk of MDD [33,34].

Our study conducted in Slovene MDD patients found no evidence of a link between the presence of putative high risk alleles in *COMT*, *PCLO* or *SLC6A4* and the likelihood of either having depression or having a family history of depressive disorder. On the contrary, our results for the *PCLO* rs2522833 polymorphism show that homozygosity for the presumed high risk C allele is less common in patients with a positive family history for a depressive disorder compared to those with no family history of depression or compared to the controls. The small number of participants in this study prohibits any definite conclusions to be drawn from these results as there is a possibility that the association we have detected has arisen due to chance. Nevertheless, these findings would appear to suggest that a polymorphism previously associated with both MDD and endophenotypes seen as predisposing factors for depression is not a contributing factor for MDD in families with several affected members. It therefore seems possible that the genetic factors involved in the etiology of depression in families with several affected family members differ from those involved in sporadic cases of depression. Rare variants of great affect would appear to be the most likely candidates, especially in families with a greater number of affected members, a recognizably Mendelian pattern of inheritance, and early disease onset. Such patients would therefore seem more appropriate candidates for next generation sequencing (NGS)-based techniques of investigation rather than SNP genotyping.

The genetic architecture of the most common diseases, including depression, is likely to be complex, with rare cases of clear monogenic inheritance and a some-

Table 2. Genotype and allele distribution of the *PCLO* rs2522833, *COMT* rs4680 and *SLC6A4* 5-HTTLPR polymorphic variants in major depressive disorder/generalized anxiety disorder patients with a positive or negative family history for depressive disorders.

	Positive Family History (*n* = 40)	***p* Value: Positive Family History *vs.* Controls**	**Negative Family History (*n* = 93)**	***p* Value: Negative Family History *vs.* Controls**	***p* Value: Positive *vs.* Negative Family History**
PCLO rs2522833 Genotype frequencies: AA AC CC	*n* (%) 26 (0.65) 14 (0.35) 0 (0.00)	**0.007**	*n* (%) 33 (0.36) 44 (0.47) 16 (0.17)	0.354	**0.001**
Alleles: A C	66 (0.82) 14 (0.18)	**0.001**	110 (0.59) 76 (0.41)	0.232	**0.000**
COMT rs4680 Genotype frequencies: GG GA AA	*n* (%) 10 (0.25) 18 (0.45) 12 (0.30)	0.297	*n* (%) 16 (0.18) 55 (0.61) 19 (0.21)	0.439	0.232
Alleles: G A	38 (0.48) 42 (0.52)	0.434	87 (0.48) 93 (0.52)	0.371	0.901
SLC6A4 50HTTLPR Genotype frequencies: LL LS SS	*n* (%) 18 (0.45) 18 (0.45) 4 (0.10)	0.436	*n* (%) 26 (0.28) 52 (0.56) 15 (0.16)	0.302	0.148
Alleles: L S	54 (0.67) 26 (0.33)	0.197	104 (0.56) 82 (0.44)	0.332	0.078

MDD: major depressive disorder; GAD: generalized anxiety disorder.

what greater number of familial cases associated with oligogenic inheritance. In most patients with common diseases, however, a great number of common risk variants is thought to contribute to the likelihood of developing the disease. Our findings raise an interesting question for those searching for such common variants associated with depression: if the failure of past studies is due to the heterogeneous nature of genetic causes involved in different patients, could excluding patients with similarly affected relatives increase the chance of positive findings in sporadic cases? Although no statistically significant differences were discovered when comparing our patients with no family history of depression to the controls, there was a trend towards a greater S allele frequency for the *SLC6A4* 5-HTTLPR variant and a trend towards a greater C allele frequency and CC homozygosity for the *PCLO* rs2522833 polymorphism in these patients; both variants have also been linked to MDD in the past. This trend was undetectable when all depressed patients were compared to the controls, suggesting that the admixture of familial cases changed the genotype and allele distributions. Our results show that focusing on sporadic cases of depression might provide a more homogenous sample for future association studies. The number of participants included in GWAS would no longer have to be prohibitively large and such studies might stand a better chance of detecting variants with a small contribution to the overall disease risk.

Some of the previous studies which subdivided the participants according to their family history only enquired about first-degree relatives [28]. As there is evidence that the presence of MDD in second-degree relatives can influence the risk of developing depression, we would suggest that one should also enquire about the disease status in second- and perhaps even third-degree relatives [21].

One of the limitations of our study is the small number of participants. The study was therefore underpowered to detect significant associations. Furthermore, as all of the data concerning the family history of our participants was collected by patient recall, some of it was bound to be unreliable and incomplete.

Major depressive disorder is an important health problem worldwide and studying its genetic etiology might one day lead to better diagnostic and treatment options for those affected [35,36]. However, no genetic variants identified

so far seem to offer hope of a clinically useful screening test for the near future [37]. As researchers try out different approaches to genetic studies of depression, stratifying patients into subgroups when performing a GWAS becomes increasingly important [38,0]. We would argue that enquiring about the patients' extended family history of this disease could prove a simple and effective way of differentiating patients with different genetic etiologies.

CONCLUSIONS

In our study, no difference in the genotype and allele distributions for the above-mentioned three common polymorphisms was detected when comparing patients with MDD to healthy controls. However, our results seem to indicate that there is a difference in the genetic make-up of MDD patients with a positive family history of depressive disorder compared to sporadic cases. Further studies involving a much greater number of participants and a greater number of tested genetic variants would be necessary to either confirm or refute these findings.

ACKNOWLEDGMENTS

The authors would like to thank all the volunteers who participated in this study and the staff at the various institutions involved who helped us recruit the participants and collect the samples.

Declaration of Interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

REFERENCES

1. Sobocki P, Jonsson B, Angst J, Rehnberg C. Cost of depression in Europe. J Ment Health Policy Econ. 2006; 9(2): 87-98.
2. Sullivan PF, Neale MC, Kendler KS. Genetic epidemiology of major depression: Review and meta-analysis. Am J Psychiatry. 2000; 157(10): 1552-1562.
3. Kendler KS, Gatz M, Gardner CO, Pedersen NL. A Swedich national twin study of lifetime major depresssion. Am J Psychiatry. 2006; 163(1): 109-114.
4. Cohen-Woods S, Craig IW, McGuffin P. The current state of play on the molecular genetics of depression. Psychol Med. 2013; 43(4): 673-687.
5. Bosker FJ, Hartman CA, Nolte IM, Prins BP, Terpstra P, Posthuma D, *et al.* Poor replication of candidate genes for major depressive disorder using genome-wide association data. Mol Psychiatry. 2011; 16(5): 516-532.
6. Ripke S, Wray NR, Lewis CM, Hamilton SP, Weissman MM, Breen G, *et al.*; Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium. A mega-analysis of genome-wide association studies for major depressive disorder. Mol Psychiatry. 2013; 18(4): 497-511.
7. Shi J, Potash JB, Knowles JA, Weissman MM, Coryell W, Scheftner WA, *et al.* Genome-wide association study of recurrent early-onset major depressive disorder. Mol Psychiatry. 2011; 16(4): 193-201.
8. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature. 2007; 447(7145): 661-678.
9. Grover MP, Ballouz S, Mohanasundaram KA, George RA, Sherman CD, Crowley TM, *et al.* Identification of novel therapeutics for complex diseases from genome-wide association data. BMC Med Genomics. 2014; 7(Suppl 1): S8.
10. Shyn SI, Hamilton SP. The genetics of major depression: moving beyond the monoamine hypothesis. Psychiatr Clin North Am. 2010; 33(1): 125-140.
11. Flint J, Kendler KS. The genetics of major depression. Neuron. 2014; 81(5): 1214.
12. Wray NR, Pergadia ML, Blackwood DH, Penninx BW, Gordon SD, Nyholt DR, *et al.* Genome-wide association study of major depressive disorder: New results, meta-analysis, and lessons learned. Mol Pschiatry. 2012: 17(1): 36-48.
13. Kendler KS, Aggen SH, Neale MC. Evidence for multiple genetic factors underlying DSM-IV criteria for major depression. JAMA Psychiatry. 2013; 70(6): 599-607.
14. Weissman MM, Bland R, Joyce PR, Newman S, Wells JE, Wittchen HU. Sex differences in rates of depression: Cross-national perspective. J Affect Disord. 1993; 39(2-3): 77-84.
15. Verhagen M, van der Meij A, van Deurzen PA, Janzing JG, Arias-Vásquez A, Buitelaar JK, *et al.* Meta-analysis of the BDNF Val66Met polymorphism in major depressive disorder: Effects of gender and ethnicity. Mol Psychiatry. 2010; 15(3): 260-271.
16. Middeldorp CM, Cath DC, Van Dyck R, Boomsma DI. The co-morbidity of anxiety and depression in the perspective of genetic epidemiology. A review of

twin and family studies. Psychol Med. 2005; 35(5): 611-624.

17. Lee SH, Ripke S, Neale BM, Faraone SV, Purcell SM, Perlis RH, *et al.*; Cross-Disorder Group of the Psychiatric Genomics Consortium. International Inflammatory Bowel Disease Genetics Consortium (IIBDGC) Genetic relationship between five psychiatric disorders estimated from genome-wide SNPs. Nat Genet 2013; 45(9): 984-994.
18. McGuffin P, Rijsdijk F, Andrew M, Sham P, Katz R, Cardno A. The heritability of bipolar affective disorder and the genetic relationship to unipolar depression. Arch Gen Psychiatry. 2003; 60(5): 497-502.
19. Demirkan A, Penninx BW, Hek K, Wray NR, Amin N, Aulchenko YS, *et al.* Genetic risk profiles for depression and anxiety in adult and elderly cohorts. Mol Psychiatry. 2011; 16(7): 773-783.
20. Weissman MM, Wickramaratne P, Nomura Y, Warner V, Pilowsky D, Verdeli H. Offspring of depressed parents: 20 years later. Am J Psychiatry. 2006; 163(6): 1001-1008.
21. Weissman MM, Wickramaratne P, Nomura Y, Warner V, Verdeli H, Pilowsky DJ, *et al.* Families at high and low risk for depression: A 3-generation study. Arch Gen Psychiatry. 2005; 62(1): 29-36.
22. Levinson DF, Evgrafov OV, Knowles JA, Potash JB, Weissman MM, Scheftner WA, *et al.* Genetics of recurrent early-onset major depression (GenRED): Significant linkage on chromosome 15q25-q26 after fine mapping with single nucleotide polymorphism markers. Am J Psychiatry. 2007; 164(2): 259-264.
23. Zubenko GS, Maher B, Hughes HB 3rd, Zubenko WN, Stiffler JS, Kaplan BB, *et al.* Genome-wide linkage survey for genetic loci that influence the development of depressive disorders in families with recurrent, early-onset, major depression. Am J Med Genet B Neuropsychiatr Genet. 2003; 123B(1): 1-18.
24. Lubke GH, Hottenga JJ, Walters R, Laurin C, de Geus EJ, Willemsen G, *et al.* Estimating the genetic variance of major depressive disorder due to all single nucleotide polymorphisms. Biol Psychiatry. 2012; 72(8): 707-709.
25. Hodžić A, Ristanović M, Zorn B, Tulić C, Maver A, Novaković I, *et al.* Genetic variation in circadian rhythm genes CLOCK and ARNTL as risk factor for male infertility. PLoS One. 2013; 8(3): e59220.
26. Tomoda A, Nishitani S, Matsuura N, Fujisawa TX, Kawatani J, Toyohisa D, *et al.* No interaction between serotonin transporter gene (5-HTTLPR) polymorphism and adversity on depression among Japanese children and adolescents. BMC Psychiatry. 2013; 13: 134.
27. Antypa N, Drago A, Serretti A. The role of COMT gene variants in depression: Bridging neuropsychological, behavioral and clinical phenotypes. Neurosci Biobehav Rev. 2013; 37(8): 1597-1610.
28. Massat I, Souery D, Del-Favero J, Nothen M, Blackwood D, Muir W, *et al.* Association between COMT (Val158Met) functional polymorphism and early onset in patients with major depressive disorder in a European multicenter genetic association study. Mol Psychiatry. 2005; 10(6): 598-605.
29. Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, *et al.* Influence of life stress on depression: Moderation by a polymorphism in the 5-HTT gene. Science. 2003; 301(5631): 386-389.
30. Fergusson DM, Horwood LJ, Miller AL, Kennedy MA. Life stress, 5-HTTLPR and mental disorder: Findings from a 30-year longitudinal study. Br J Psychiatry. 2011: 198(2): 129-135.
31. López-León S, Janssens AC, González-Zuloeta Ladd AM, Del-Favero J, Claes SJ, Oostra BA, *et al.* Meta-analyses of genetic studies on major depressive disorder. Mol Psychiatry. 2008; 13(8): 772-785.
32. Sullivan PF, de Geus EJ, Willemsen G, James MR, Smit JH, Zandbelt T, *et al.* Genome-wide association for major depressive disorder: A possible role for the presyn-aptic protein piccolo. Mol Psychiatry. 2009; 14(4): 359-375.
33. Hek K, Mulder CL, Luijendijk HJ, van Duijn CM, Hofman A, Uitterlinden AG, Tiemeier H. The PCLO gene and depressive disorders: replication in a population-based study. Hum Mol Genet. 2010; 19(4): 731-734.
34. Minelli A, Scassellati C, Cloninger CR, Tessari E, Bortolomasi M, Bonvicini C, *et al.* PCLO gene: Its role in vulnerability to major depressive disorder. J Affect Disord. 2012; 139(3): 250-255.
35. Ustün TB, Ayuso-Mateos JL, Chatterji S, Mathers C, Murray CJ. Global burden of depressive disorders in the year 2000. Br J Psychiatry. 2004; 184: 386-392.
36. Collins PY, Patel V, Joestl SS, March D, Insel TR, Daar AS, *et al.*; Scientific Advisory Board and the Executive Committee of the Grand Challenges on Global Mental Health. Grand challenges in global mental health. Nature. 2011; 475(7354): 27-30.

37. Lacerda-Pinheiro SF, Pinheiro Junior RF, Pereira de Lima MA, Lima da Silva CG, Santos MD, Teixeira Júnior AG, *et al.* Are there depression and anxiety genetic markers and mutations? A systematic review. J Affect Disord. 2014; 168: 387-398

38. Levinson DF. The genetics of depression: A review. Biol Psychiatry. 2006; 60(2): 84-92.