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A preliminary association study between serotonin transporter (5-HTTLPR), receptor polymorphisms (5-HTR1A, 5-HTR2A) and depression symptom-clusters in a north Indian population suffering from Major Depressive Disorder (MDD)

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ABSTRACT

Introduction: Major Depressive Disorder (MDD) is a broad heterogeneous diagnostic construct. Previous studies have shown that it can be resolved into several symptom-clusters which are proposed to be associated with single nucleotide polymorphisms (SNPs) of the serotonergic pathway (5-HTTLPR, 5HTR1A, 5-HTR2A).

Methods and material: In a cross-sectional study conducted at a tertiary level mental health care set-up in north India, 80 out-patients with MDD were evaluated with Montgomery Asberg Depression Rating Scale (MADRS) and then genotyping was done. The different clinical and genetic variables were compared across the factor structures of MADRS. Also, the comparison of the genetic data of cases was done with the pre-existing database of the non-blood related healthy ethnically-matched controls.

Results: There was no significant association between age, gender, other clinical variables, SNPs like 5-HTTLPR SS/SL, rs6295 CC/CG/GG, rs6311GG/GA/AA, rs6313 CC/CT/TT and different factor-structures like 'detachment' consisting of items like concentration difficulty, lassitude, inability to feel; 'psychic anxiety' consisting of suicidal thoughts and inner tension; 'mood-pessimism' consisting of symptoms like apparent sadness, reported sadness, pessimistic thoughts and 'vegetative symptoms' like decreased sleep, poor appetite. Neither there was any association between genotype of the cases compared with the controls.

Conclusions: No significant association was obtained between the four-factor structures of depression in MADRS and serotonin transporter and receptor SNPs in a study with a small sample size. This study evaluates whether depression symptom-clusters have distinct genotypic determinants and necessitates more comprehensive studies for unravelling the genetic determinants of depression.

1. Introduction

Worldwide MDD is one of the commonest causes of disability (Kessler et al., 2003; Lopez and Murray, 1998). As per the latest National Mental Health Survey in India, the life-time prevalence of depressive disorder in India is 5.2% with a high risk of suicide (Gururaj et al., 2016). However, understanding of the aetio-pathogenesis of depression still is inadequate. Its strong heritability and genetic basis are well established from family, twin and adoption studies (Levinson, 2006). But, no definite pattern of familial transmission has so far emerged. So attention turned towards candidate gene research particularly related to the serotonin pathway.

Chromosome position 17q11.1-q12 contains the regulatory serotonin transporter linked polymorphic region (5-HTTLPR) which has an insertion/deletion region of 44 nucleotides. The short protein variant (S) of the polymorphism decreases the transcriptional efficiency of the 5-HTT gene promoter, resulting in reduced turnover of serotonin transporter and uptake of serotonin (Heils et al., 1996). The long protein variant (L) of 5-HTTLPR is more than twice as active as the short variant. Till date, about 150 association studies involving tens of thousands of patients have been conducted, yet the picture is far from being clear (Verhagen et al., 2009). In 2003 Caspi et al., showed that individuals with one or two copies of the S allele of the 5-HTTLPR exhibited more depressive symptoms, diagnosable depression and suicidality in relation to stressful life events than individuals homozygous for the long L allele (Caspi et al., 2003). This opened a new avenue of

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research – however, this was not confirmatory and controversies followed even in a meta-analysis which reported mutually contradictory findings (Risch et al., 2009). Apart from the serotonin transporter, the intronless serotonin-1A receptor gene (HTR1A), mapped on chromosome 5q11.2–13, spanning about 1200 bp, has been extensively studied (Fabbri et al., 2013). Among the different SNPs related to this gene, rs6295C/G, in the promoter region of the gene, has been found to be associated with an altered expression and function of the receptors. When the G allele is present, it prevents the binding of the repressor to DNA, leading to an increase of 5-HT1A auto receptors and to a reduction of serotonergic neurotransmission (Albert, 2012).

The other important gene is the 5-HT2A (HTR2A), located in position 13q14-q21. Two important common SNPs are rs6311 G/A and rs6313C/T. The serotonin receptor 2A being an important post-synaptic receptor, HT2A receptor gene is one of the most investigated genes in the genetics of depression. Despite a large number of candidate gene studies focusing on genes coding for serotonin receptors, results have been inconsistent. The most replicated findings are the associations between rs6295 G allele or G/G genotype and rs6311 A allele or the A/A genotype and depressive symptoms (Spurlock et al., 1998). Thus we see that the literature regarding the genetic basis of depression is mired in controversy due to different methodological issues (Attia et al., 2009). Recently collaborative efforts have been undertaken to lessen this methodological discrepancy (Little et al., 2009).

One important source of controversy can be the heterogeneity in the very construct of depression. Most of the studies have viewed depression as a discrete construct and neglected its dynamic and versatile nature which extends from mild neurotic depression to very severe depression with melancholia as currently supported by neurobiological studies (Ghaemi et al., 2012; Wakefield, 2012). Examination of the heterogeneity of clinical presentation in depression may be facilitated using symptom-based depression instruments like the 10-item Montgomery and Asberg Depression Rating Scale (MADRS) which is valid as a general severity estimate (Montgomery and Asberg, 1979). Since the items cover a range of signs and symptoms, subscales may exist within the MADRS that might correspond to common groups of depressive symptoms (Suzuki et al., 2005). In a recent study it had been shown that among north Indian patients, baseline principal component analysis (varimax rotation) extracted the following four factor-structures of MADRS (Basu et al., 2017). The first factor denotes 'detachment' comprising of 'concentration difficulty', 'lassitude' and 'inability to feel' items in the MADRS scale. The second factor implied 'psychic anxiety' consisting of 'suicidal thoughts' and 'inner tension', the third factor was for symptoms of 'mood-pessimism' like 'apparent sadness', 'reported sadness', 'pessimistic thoughts' and the fourth one for 'vegetative' symptoms' comprising of 'decreased sleep' and 'decreased appetite'. It has been proposed by Kamata et al. that such different symptom-clusters of depression have distinct genetic and neurobiological basis (Kamata et al., 2011). This underlines the need to understand depression according to its different factor structures and their neurobiologic correlates. Due to a paucity of such studies, this avenue of research needs urgent attention particularly in the Indian context because of the uniqueness of the genetic constitution of the Indian population. It has been shown that frequencies of alleles of neuropsychiatric interest (related to dopamine and serotonin metabolic pathways) in the Indian population differed from others (Mukherjee et al., 2002). Moreover, the recent findings of the Indian Genome Variation Consortium (2008) showed that there is significant genetic variation between different groups of Indian populations that have conglomerated into groups based upon ethnicity and language (Indian Genome Variation Consortium, 2008). Inspite of the overlap between Indian populations and the diversity of the HapMap populations, the genetic uniqueness of India is quite well recognized. So genetic association studies in the different ethnic groups in Indian populations are needed. Now, in the Indian context apart from a few pharmacogenetic studies there are hardly any studies related to the serotonin transporter/receptor in the field of genetics of depression (Manoharan et al., 2016; Basu et al., 2015; Margoob et al., 2008).

So, the aim was to study the different symptoms clusters of depression and their relationship with the genotypes. Hence the objective was to find any possible association between these factor structures and serotonin transporter (5-HTTLPR) and receptor (5-HTR1A, 5-HTR2A) polymorphisms in a north Indian population.

2. Materials and methods

The study population is part of a longitudinal study done on MDD patients originally recruited for a pharmacogenetic study at All India Institute of Medical Sciences, New Delhi. The preliminary findings and methodology have already been published elsewhere (Basu et al., 2017). However, for better understanding of readers the methodology will be described here again briefly.

This cross-sectional study was carried out at the psychiatry outpatient department of AIIMS, New Delhi which is one of the apex tertiary care hospitals in north India. The study duration was between August 2010 and June 2011. Patients of north Indian ethnicity were only selected. They were considered so if they belonged to the states of Delhi, Haryana, Punjab, Uttar Pradesh and Uttarakhand and if the birthplaces of both parents of an individual were in the above states.

In a sample of convenience, patients of 18 to 65 years of age suffering from depressive symptoms and antidepressant-naive for at least one week were included in this study. Five weeks was the minimum drug-free period for anti-depressants like fluoxetine in view of its long half-life. Patients were evaluated with a structured interview schedule like Mini International Neuropsychiatric Interview (M.I.N.I.) to diagnose MDD according to the Diagnostic and Statistical Manual IV–Text Revision (DSM-IV-TR). The instrument was applied by the first author, psychiatry resident working under the active supervision of the second and third authors who are senior consultants working in the field of psychiatry. The following patients were excluded from this study namely those with severe medical comorbidities, on medications known to influence mood, pregnant and breast-feeding women and the patients with recent clinically significant suicidal attempts. Also past or family history (first or second degree biological relatives) of bipolar disorder (manic episodes), psychotic disorder, severe personality disorder and any substance dependence except tobacco were exclusion criteria. Patients were recruited in the study after they provided a valid informed consent. Ethical clearance from the institute ethics committee was obtained.

All the patients were assessed with a socio-demographic sheet (including birth place of the patient and his/her parents to define ethnicity) and semi-structured clinical proforma. MADRS was used to assess the symptoms of depression and patients with a MADRS of 22 and above only were selected. After the assessment, blood was collected for genetic analysis of the following four SNPs namely rs6295, rs6313, rs6311 and 5-HTTLPR 44 base-pair insertion deletion. These SNPs were selected based upon literature review and because of the fact that they are the commonest SNPs implicated in the pathway to serotonin metabolism. After assessment and investigations the patients were treated as per standard clinical practice.

Initially the cell membranes of the blood cells followed by nuclear membranes were lysed. Thereafter, nuclear proteins were digested followed by saturation with sodium chloride. Finally, DNA was extracted in ethanol (modified salting out procedure) (Miller et al., 1988). After this extraction, the samples were processed for further genotyping. The genotyping platform (SEQUENOME™) was used for genotyping three SNPs namely rs6295, rs6311 and rs6313. At first primer extension reaction was performed. This was followed by Matrix-assisted Laser Desorption/Ionization time-of-flight (MALDI-TOF) mass spectrometry (Sequenom Inc., San Diego, USA). DNA fragment analysis and genotyping of 44bases insertion/deletion was performed with ABI-3130 genetic analyzer. The researchers performing the genotyping were blind

to the clinical data. All the genetic analyses were performed at CSIR-Institute of Genomics and Integrative Biology (CSIR-IGIB), New Delhi.

Statistical Package for Social Sciences (SPSS) version 16.0.0 was used to perform the statistical analysis. At first univariate analysis was performed – continuous values of the factor structures were compared across the different categorical variables related to demography, clinical and genetic constitution using *t*-test and one way ANOVA. The continuous values of the factor structures were also correlated with other continuous variables using Pearsons co-efficient. In the univariate analysis, the p value was considered significant at $p < 0.05$ except for ANOVA where after Bonferroni corrections, p value was considered significant at 0.01. SNPs were also tested for Hardy Weinberg equilibrium using χ^2-test at 0.001 level of significance. In the univariate analysis the independent variables, found to be significant at $p < 0.2$ ($p < 0.05$ in the case of ANOVA) were incorporated in the multivariate linear regression model for further analysis. To rule out population stratification, the genetic data of the study population was compared with a control database of the same ethnicity. The controls were ethnically matched non-blood related healthy individuals (with no past or family history of any mental illness according to self report). The control data was accessed from a database available with IGIB for this purpose only. Controls were not assessed anytime as part of this study.

3. Results

In total 80 depressive patients belonging to north Indian ethnicity and 80 ethnicity-matched controls were evaluated. Socio-demographic and clinical details are described in Table 1. Among the cases, the average age was 36.39 ± 10.30yrs, 48(60%) were male and 32(40%) were female. Majority of the patients were married (83.7%), Hindu by religion (85%), had at least secondary education (87.6%) and were employed (60.1%). The mean age of onset of MDD and duration of the current episode was 33.42 ± 9.98yrs and 17.10 ± 27.67 months respectively. Twenty-eight patients (35%) had melancholic features in the current episode of depression. The number of patients having a past history of depression and a positive family history was 15(18.8%) and 14(17.5%) respectively. The average MADRS score was 27.7 ± 4.62.

Table 1
Socio-demographic and clinical profile of cases and controls.

Cases(n = 80)	Gender	Male	48(60%)
		Female	32(40%)
	Mean age		36.39 ± 10.30yrs
	Marital status	Unmarried	13(16.3%)
		Married	67(83.7%)
	Religion	Hindu	68(85%)
		Muslim & others	12(15%)
	Occupation	Unemployed	4(5.0%)
		Home-maker	28(35%)
		Students	6(7.5%)
		Unskilled	22(27.5%)
		Skilled/semi-skilled	13(16.3%)
		Professional	7(8.8%)
	Education	Primary	10(12.5%)
		Secondary	11(13.8%)
		Higher-secondary	33(41.3%)
		Graduate/ above	26(32.5%)
	mean age of onset		33.42 ± 9.98yrs
	Presence of past depressive episode		15(18.8%)
	Positive family history		14(17.5%)
	Melancholic features in current episode		28(35%)
	Mean MADRS score		27.7 ± 4.624
Controls (n = 80)	**Mean age**		23.54 ± 3.48years
	Gender	Male	40(50%)
		Female	40(50%)

Allele frequencies of the polymorphisms in the sample are as follows: rs6295 C(0.53)/G(0.47), rs6311 G(0.6)/A(0.4), rs6313 C(0.59)/T(0.41) and S(0.72)/L(0.28). Genotypic frequencies of the polymorphisms are provided in Table 4. According to the univariate analysis in Table 2, statistically significant association was found between 'vegetative functions' and gender which, however, did not persist in the multivariate analysis (Table 3). No statistically significant association was found between any of the factor structures and the genotypes. However, there was a trend towards a significant association of the L allele with 'detachment' as the concerned p-value was 0.07 with an odds ratio of 1.2 (95% CI 2.5-0.9) after adjusting for other variables in multivariate analysis.

4. Discussion

In this study in a north Indian population the factor structures of MADRS and their genotypic correlates have been studied. This study found no statistically significant association between the SNPs and cases with respect to the controls, neither there was any association with the symptom clusters. Though there was a trend towards significance for the association of the L allele of 5-HTTLPR with the 'detachment' factor, yet no conclusions can be drawn.

The alleles were in Hardy Weinberg equilibrium. The allelic frequencies were similar to a previous study with a smaller sample of the same cohort where the S allele frequency was 0.7 and there was no homozygous L allele (Basu et al., 2015). For 5HTTLPR, the S allele frequency is variable across populations – from 0.35-0.40 in White European origin populations to 0.454 among a Brazilian sample and 0.64-0.66 among American Indians (Hu et al., 2006). In Indian population also the variation is seen from 0.6 in a predominantly south Indian sample to 0.66 in an eastern Indian sample and 0.73 in a north-eastern sample (Tibrewal et al., 2010). Apart from the current cohort from AIIMS centre, there is only another north Indian study from Kashmir which yielded a S allele frequency of 0.51 (Margoob et al., 2008). The present study population had a S allele frequency of 0.72 and there was no LL genotype. Whether this is the true S allele frequency or a distorted value due to the selection of the lesser number of subjects in a non-randomized non-stratified manner can only be decided by large population-based studies.

For rs6295C/G the allelic frequency was 0.53 for the C allele. Though no Indian study with this allele frequency was found, it is similar to Caucasian studies with a frequency of 0.5 but less than Japanese Asian studies with the frequency of 0.79 (Yu et al., 2006). For rs6311 and rs6313, the allelic frequencies are similar to the Caucasian, Japanese Asians and a lone Indian study done from eastern India. However, a single small study is not enough to draw any definite conclusion (Guhathakurta et al., 2006).

The results of this study are in contradiction to a similar study by Kamata et al. who on a sample of 132 Japanese patients with MDD showed that among the three factors of MADRS namely dysphoria, retardation and vegetative symptom, 5HT2A (A allele) was related with higher scores in the vegetative functions domain($p = 0.001$). The 5HTTLPR (S allele) was associated with higher scores in the 'dysphoria' domain ($p = 0.012$) (Kamata et al., 2011). Also it needs to be remembered that the symptoms of depression are influenced by social and cultural influences (Kleinman, 2004). Hence, rather than correlating with the symptoms clusters as has been done in this study, biological endophenotypes are a better choice as by definition endophenotypes are more stable, heritable factors. Other causes of the lack of correlation between the different symptom-clusters and genotypes may be the limited sample size in this study. Moreover, the controls were not evaluated with any structured interview schedule and were mentally healthy as per their self-report only. The controls were only ethnically matched and no

Table 2
Univariate analysis: Relationship between independent variables (socio-demographic, clinical and genetic) and the factors.

Variable		Factor 1 'Detachment' (Mean)	P	Factor 2 'Psychic-anxiety' (mean)	P	Factor 3 'Mood-pesimism' (mean)	P	Factor 4 'Vegetative functions' (mean)	P
Gender	male	8.51	0.44	3.23	0.06	10.72	0.13	4.43	**0.05**
	female	8.06		4.12		11.21		5.64	
melancholia	present	8.93	0.12	3.71	0.72	11.07	0.50	5.50	0.17
	absent	8.00		3.54		10.85		4.62	
Past history	present	7.73	0.32	4.27	0.17	11.60	0.4	4.80	0.85
	absent	8.46		3.45		10.77		4.95	
Family history	present	7.71	0.33	3.57	0.96	11.29	0.30	4.86	0.92
	absent	8.45		3.61		10.85		4.94	
5-HTTLPR	SL	9.26	0.07	−3.58	0.96	10.63	0.31	5.37	0.42
	SS	8.03		3.61		−11.02		4.79	
rs6295*	CC	8.53	0.77	3.58	0.45	10.63	0.34	4.63	0.86
	CG	8.14		3.82		11.14		5.05	
	GG	8.59		3.06		10.71		4.94	
rs6311*	GA	8.38	0.87	4.11	0.13	11.03	0.59	5.51	0.03
	GG	8.41		3.10		10.97		4.97	
	AA	8.00		3.29		10.57		3.29	
rs6313*	CC	8.32	0.59	2.97	0.45	10.90	0.62	4.71	0.55
	CT	8.61		4.18		11.03		5.70	
	TT	7.75		3.63		10.75		3.75	
Pearson's Correlation coefficient		R		r		R		r	
Age		0.09	0.40	0.13	0.24	0.13	0.25	0.02	0.88
Age of onset		0.14	0.23	0.96	0.4	0.09	0.44	0.11	0.34

Bold letters represent statistical significance ($p < 0.05$).
* For these variables because of Bonferroni corrections, the p was considered significant at < 0.015.

Table 3
Multivariate analysis: Relationship between independent variables (socio-demographic, clinical and genetic) and factors.

Variable	Factor 1 'Detachment' (p-value)	Factor 2 'Psychic-anxiety' (p-value)	Factor 3 'Mood-pessimism' (p-value)	Factor 4 'Vegetative (p-value)
gender	–	0.09	0.13	0.09
melancholia	0.13	–	–	0.16
past history	–	0.24	–	–
5-HTTLPR	0.07	–	–	–
rs6313CT/TT (reference rs6313CC)	–	–	–	0.44/0.11

Table 4
Genotypic frequency of studied polymorphisms in cases and controls.

SNP(rsID)	Patients	Controls	χ^2	p-value
rs-6295 (C/G)	CC(0.26)/CG(0.41)/ GG(0.23)	CC(0.28)/CG(0.54)/ GG(0.18)	0.34	0.84
rs6311(G/A)	GG(0.36)/GA(0.45)/ AA(0.19)	GG(0.38)/GA(0.47)/ AA(0.15)	0.40	0.82
rs6313(C/T)	CC(0.39)/CT(0.4)/ TT(0.21)	CC(0.34)/CG(0.51)/ GG(0.15)	2.25	0.32
rs44INSDEL(S/L)	SS(0.75)/SL(0.25)	SS(0.69)/SL(0.31)	0.91	0.33

matching was done for other socio-demographic variables. However, there was no statistically significant difference between the cases and the controls with respect to gender ($p = 0.2$) which may act as a confounder.

Other reasons for this discrepancy with the previous study can be linkage disequilibrium and lack of haplotype analysis. For e.g. an SNP (rs25531) located just upstream of 5-HTTLPR can affect the expression of the serotonin transporter (Hu et al., 2007). Other similar polymorphisms are low expression L allele (L_G) and 17bp variable nucleotide tandem repeats (VNTR) within intron 2(STin2) (Hranilovic et al., 2004). From literature it is known that methodological issues like selection criteria of patients, associated co-morbidities, ethnic influence on the genetic constitution, lack of haplotype analysis in most studies, influence of linkage disequilibrium, low sample size leading to underpowered studies, influence of confounding factors/ stressors and finally the small effect size of individual genes are important causes behind such contradictory findings in genetic association studies (Attia et al., 2009).

Inspite of these limitations, the methodology of this study has several strengths. Though a small sample size decreases the power of the study and limits generalizability, studies with large sample sizes are also at a disadvantage because of the inherent heterogeneity due to population stratification. Confounders were avoided by selecting study subjects free of possible psychiatric and medical co-morbidities. Patients in extremes of age group were avoided and those with MADRS values below 22 to rule out sub-syndromal depression and dysthymia. As far as genotyping is considered standard procedures were followed and the study design is in accordance with Strengthening the Reporting of the Genetic Association studies (STREGA) guidelines excepting some short-comings (Little et al., 2009).

Overall, this study had important implications. First of all it indicated that different factor structures of depression might not be related to the serotonergic SNPs unlike the study by Kamata et al. Such a finding is not new in literature and several times the initial euphoria over such genetic associations die prematurely due to non-replication in future studies. Moreover, the symptoms clusters of depression are having several neurobiologic determinants like different neurotransmitters, genes and neuroendocrine responses along with complicated gene-gene and gene-environment interactions as illustrated by the example of psychomotor retardation (Buyukdura et al., 2011). Due to the importance of gene-environment interactions, epigenetic studies may give us some headway. So to unravel the genetic underpinnings of depression large-scale studies are needed in future to explore the relationship between the symptom-clusters of depression and a large number of genotypes in preferably genome-wide association studies after adjusting for several confounders.

Conflict of interest

Author declare that neither nor any one of the co-authors have any conflict of interest.

Financial disclosure

Author declare that neither nor any one of the co-authors have any financial disclosure to make.

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References

Albert, P.R., 2012. Transcriptional regulation of the 5-HT1A receptor: implications for mental illness. Philos. Trans. R. Soc. B Biol. Sci. 367, 2402. https://doi.org/10.1098/rstb.2011.0376.

Attia, J., Ioannidis, J.P.A., Thakkinstian, A., McEvoy, M., Scott, R.J., Minelli, C., Thompson, J., Infante-Rivard, C., Guyatt, G., 2009. How to use an article about genetic association: B: are the results of the study valid? JAMA 301, 191–197. https://doi.org/10.1001/jama.2008.946.

Basu, A., Chadda, R.K., Sood, M., Kaur, H., Kukreti, R., 2015. Association of serotonin transporter (SLC6A4) & receptor (5HTR1A, 5HTR2A) polymorphisms with response to treatment with escitalopram in patients with major depressive disorder: a preliminary study. Indian J. Med. Res. 142 (1), 40–45.

Basu, A., Chadda, R., Sood, M., Rizwan, S.A., 2017. Pre-treatment factor structures of the Montgomery and Åsberg Depression Rating scale as predictors of response to escitalopram in Indian patients with non-psychotic major depressive disorder. Asian J. Psychiatry 28, 154–159. https://doi.org/10.1016/j.ajp.2017.04.029.

Buyukdura, J.S., McClintock, S.M., Croarkin, P.E., 2011. Psychomotor retardation in depression: biological underpinnings, measurement, and treatment. Prog. Neuropsychopharmacol. Biol. Psychiatry 35, 395–409. https://doi.org/10.1016/j.pnpbp.2010.10.019.

Caspi, A., Sugden, K., Moffitt, T.E., Taylor, A., Craig, I.W., Harrington, H., McClay, J., Mill, J., Martin, J., Braithwaite, A., Poulton, R., 2003. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science 301, 386–389. https://doi.org/10.1126/science.1083968.

Fabbri, C., Marsano, A., Serretti, A., 2013. Genetics of serotonin receptors and depression: state of the art. Curr. Drug Targets 14, 531–548.

Ghaemi, S.N., Vöhringer, P.A., Vergne, D.E., 2012. The varieties of depressive experience: diagnosing mood disorders. Psychiatr. Clin. North Am. 35, 73–86. https://doi.org/10.1016/j.psc.2011.11.008.

Guhathakurta, S., Ghosh, Sagarmoy, Sinha, S., Chatterjee, A., Ahmed, S., Chowdhury, S.R., Gangopadhyay, P.K., Ghosh, Saurabh, Singh, M., Usha, R., 2006. Serotonin transporter promoter variants: analysis in Indian autistic and control population. Brain Res. 1092, 28–35. https://doi.org/10.1016/j.brainres.2006.03.078.

Gururaj, G., Varghese, M., Benegal, V., Rao, G.N., Pathak, K., Singh, L.K., Misra, R., 2016. National Mental Health Survey of India, 2015-16: Summary. Natl. Inst. Ment. Health Neurosci., Bengaluru.

Heils, A., Teufel, A., Petri, S., Stöber, G., Riederer, P., Bengel, D., Lesch, K.P., 1996. Allelic variation of human serotonin transporter gene expression. J. Neurochem. 66, 2621–2624.

Hranilovic, D., Stefulj, J., Schwab, S., Borrmann-Hassenbach, M., Albus, M., Jernej, B., Wildenauer, D., 2004. Serotonin transporter promoter and intron 2 polymorphisms: relationship between allelic variants and gene expression. Biol. Psychiatry 55, 1090–1094. https://doi.org/10.1016/j.biopsych.2004.01.029.

Hu, X.-Z., Lipsky, R.H., Zhu, G., Akhtar, L.A., Taubman, J., Greenberg, B.D., Xu, K., Arnold, P.D., Richter, M.A., Kennedy, J.L., Murphy, D.L., Goldman, D., 2006. Serotonin transporter promoter gain-of-function genotypes are linked to obsessive-compulsive disorder. Am. J. Hum. Genet. 78, 815–826. https://doi.org/10.1086/503850.

Hu, X.-Z., Rush, A.J., Charney, D., Wilson, A.F., Sorant, A.J.M., Papanicolaou, G.J., Fava, M., Trivedi, M.H., Wisniewski, S.R., Laje, G., Paddock, S., McMahon, F.J., Manji, H., Lipsky, R.H., 2007. Association between a functional serotonin transporter promoter polymorphism and citalopram treatment in adult outpatients with major depression. Arch. Gen. Psychiatry 64, 783–792. https://doi.org/10.1001/archpsyc.64.7.783.

Indian Genome Variation Consortium, 2008. Genetic landscape of the people of India: a canvas for disease gene exploration. J. Genet. 87, 3–20.

Kamata, M., Suzuki, A., Yoshida, K., Takahashi, H., Higuchi, H., Otani, K., 2011. Genetic polymorphisms in the serotonergic system and symptom clusters of major depressive disorder. J. Affect. Disord. 135, 374–376. https://doi.org/10.1016/j.jad.2011.08.027.

Kessler, R.C., Berglund, P., Demler, O., Jin, R., Koretz, D., Merikangas, K.R., Rush, A.J., Walters, E.E., Wang, P.S., National Comorbidity Survey Replication, 2003. The epidemiology of major depressive disorder: results from the National Comorbidity Survey Replication (NCS-R). JAMA 289, 3095–3105. https://doi.org/10.1001/jama.289.23.3095.

Kleinman, A., 2004. Culture and depression. 2004. N. Engl. J. Med. 351 (10), 951–953. https://doi.org/10.1056/NEJMp048078.

Levinson, D.F., 2006. The genetics of depression: a review. Biol. Psychiatry 60, 84–92. https://doi.org/10.1016/j.biopsych.2005.08.024.

Little, J., Higgins, J.P.T., Ioannidis, J.P.A., Moher, D., Gagnon, F., von Elm, E., Khoury, M.J., Cohen, B., Davey-Smith, G., Grimshaw, J., Scheet, P., Gwinn, M., Williamson, R.E., Zou, G.Y., Hutchings, K., Johnson, C.Y., Tait, V., Wiens, M., Golding, J., van Duijn, C., McLaughlin, J., Paterson, A., Wells, G., Fortier, I., Freedman, M., Zecevic, M., King, R., Infante-Rivard, C., Stewart, A., Birkett, N., 2009. STrengthening the REporting of genetic association studies (STREGA)–an extension of the STROBE statement. Genet. Epidemiol. 33, 581–598. https://doi.org/10.1002/gepi.20410.

Lopez, A.D., Murray, C.C., 1998. The global burden of disease, 1990-2020. Nat. Med. 4, 1241–1243. https://doi.org/10.1038/3218.

Manoharan, A., Shewade, D.G., Rajkumar, R.P., Adithan, S., 2016. Serotonin transporter gene (SLC6A4) polymorphisms are associated with response to fluoxetine in south Indian major depressive disorder patients. Eur. J. Clin. Pharmacol. 72, 1215–1220. https://doi.org/10.1007/s00228-016-2099-9.

Margoob, M.A., Mushtaq, D., Murtza, I., Mushtaq, H., Ali, A., 2008. Serotonin transporter gene polymorphism and treatment response to serotonin reuptake inhibitor (escitalopram) in depression: an open pilot study. Indian J. Psychiatry 50, 47–50. https://doi.org/10.4103/0019-5545.39759.

Miller, S.A., Dykes, D.D., Polesky, H.F., 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 16, 1215.

Montgomery, S.A., Asberg, M., 1979. A new depression scale designed to be sensitive to change. Br. J. Psychiatry J. Ment. Sci. 134, 382–389.

Mukherjee, O., Saleem, Q., Purushottam, M., Anand, A., Brahmachari, S.K., Jain, S., 2002. Common psychiatric diseases and human genetic variation. Public Health Genomics 5, 171–177.

Risch, N., Herrell, R., Lehner, T., Liang, K.-Y., Eaves, L., Hoh, J., Griem, A., Kovacs, M., Ott, J., Merikangas, K.R., 2009. Interaction between the serotonin transporter gene (5-HTTLPR), stressful life events, and risk of depression: a meta-analysis. JAMA 301, 2462–2471. https://doi.org/10.1001/jama.2009.878.

Spurlock, G., Heils, A., Holmans, P., Williams, J., D'Souza, U.M., Cardno, A., Murphy, K.C., Jones, L., Buckland, P.R., McGuffin, P., Lesch, K.P., Owen, M.J., 1998. A family based association study of T102C polymorphism in 5HT2A and schizophrenia plus identification of new polymorphisms in the promoter. Mol. Psychiatry 3, 42–49.

Suzuki, A., Aoshima, T., Fukasawa, T., Yoshida, K., Higuchi, H., Shimizu, T., Otani, K., 2005. A three-factor model of the MADRS in major depressive disorder. Depress. Anxiety 21, 95–97. https://doi.org/10.1002/da.20058.

Tibrewal, P., Kiran, K.H., Shubha, G.N., Subhashree, D., Purushottam, M., Thennarasu, K., Reddy, Y.C., Jain, S., 2010. Association of serotonin transporter gene polymorphisms with obsessive-compulsive disorder (OCD) in a south Indian population. Indian J. Med. Res. 132 (6), 690.

Verhagen, M., van der Meij, A., Janzing, J.G.E., Arias-Vásquez, A., Buitelaar, J.K., Franke, B., 2009. Effect of the 5-HTTLPR polymorphism in the serotonin transporter gene on major depressive disorder and related comorbid disorders. Psychiatr. Genet. 19, 39–44. https://doi.org/10.1097/YPG.0b013e3283208061.

Wakefield, J.C., 2012. DSM-5: proposed changes to depressive disorders. Curr. Med. Res. Opin. 28, 335–343. https://doi.org/10.1185/03007995.2011.653436.

Yu, Y.W., Tsai, S.J., Liou, Y.J., Hong, C.J., Chen, T.J., 2006. Association study of two serotonin 1A receptor gene polymorphisms and fluoxetine treatment response in Chinese major depressive disorders. Eur. Neuropsychopharmacol. 16 (7), 498–503.