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ORIGINAL ARTICLE

Association of single nucleotide polymorphism in TPH2 Gene with age of MDD (Major Depressive disorder) cases and controls.

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ABSTRACT… Objective: To determine association of TPH2 (rs7305115) gene polymorphism with age of MDD cases and controls in Pakistani population. **Study Design:** Case-control study. **Setting:** Multidisciplinary Lab in Islamic International Medical College Trust in Collaboration with Psychiatry Department, Railway General Hospital, and Rawalpindi. **Period:** September 2019 to September 2020. **Material & Methods:** After taking written informed consent blood samples were drawn from all the participants followed by extraction of DNA using Chelex™ method. Allelic frequencies of TPH2 (rs7305115) gene polymorphism in both cases and controls were determined using Multiplex PCR. **Results:** Number of participants included in the study were 240, out of which 120 were diagnosed cases of MDD whereas 120 were age and gender matched controls. Both the cases and controls were divided in to three groups on the basis of their ages. In group 1 both subjects aged 15 to 35 years were included and a total of 128 subjects fall under this age group out of which 64 (53.33%) were cases and 64 (53.33%) were controls. In group 2 subjects aged 36 to 55 years were included and a total of 86 individuals were found to be present in this age group out of which 45 (37.5%) were cases and 41(34.1%) were controls and in group 3 subjects aged 56 to 75 years were included and a total of 26 individuals were present in this group out of which 11(9.16%) were cases and 15(12.5%) were controls. Significant association was found between age group 1(15-35 years) and GG genotype with risk of development of MDD (OR: 0.3778, 95% CI: 0.1512-0.9349, P= 0.0338). **Conclusion:** We have found in the present study that age group 1(15-35 years) with GG genotype of TPH2 gene polymorphism have a significantly increased risk of developing MDD as compared to other age groups in our study.

Key words: Major Depressive Disorder, Polymerase Chain Reaction, Tryptophan Hydroxylase Gene.

INTRODUCTION

Major Depressive disorder (MDD) is characterized by at least a single episode that is lasting for at least two weeks that involves changes in one's interests and pleasure, mood and as well as changes in cognition and vegetative symptoms.[1] MDD is a complex disorder with considerable number of interconnected etiologic pathways. Previous studies have suggested a large number of risk factors to be associated with onset of MDD.[2] Epidemiological studies have unfolded that depression is one of the most predominant psychiatric condition all over the world and is measured to be the most important public health problem.[3] Prevalence rates are still rising and at a global level, approximately over 300 million people are expected to have depression which is corresponding to almost 4.4 percent of population all over the world.[4] Many studies have revealed the high prevalence of depressive disorder in Pakistan and it is estimated to be 22% to as high as 60% according to a study done in 2016.[5]

The etiology and pathophysiology of depression is still not fully understood. However, studies suggest that this might be due to disturbances in the Tryptophan Catabolite (TRYCATs) pathway.[6] Increased levels of the harmful tryptophan metabolites which are xanthurenic, quinolinic acid, kynurenine were present in the plasma of patients with depression.[5] These metabolites can cause destruction of neurons and as well

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as the postsynaptic structures through the apoptosis of hippocampal cells and necrosis of selective granular cells.[5] Moreover, studies done recently have revealed that patients of depression show an increased plasma level of enzymes involved in tryptophan catabolism as compared to healthy individuals.[7] These enzymes include 2,3-dioxygenase indoleamine (IDO) and 2.3-dioxygenase tryptophan (TDO) and convert tryptophan to harmful metabolites thus causing an increased levels of these enzymes in the plasma.[8] Therefore resulting in decreased levels of serotonin, that is associated with depressed mood.[8] Serotonin is synthesized by an enzyme called tryptophan Hydroxylase (TPH), which catalyzes the initial and rate regulatory step in the synthesis of serotonin. There are two different genes that encode TPH enzyme in humans labelled as TPH1 and TPH2. Studies have shown that altered expression levels of these TPH genes may be related to various diseases including depression.[7]

TPH2 gene is situated on chromosome 12q15 and it contains 11 exons which are covering approximately 93.5 kb. According to a study conducted on polymorphism situated in exons 7 of TPH2, rs7305115, is found to be associated with increased risk of MDD and response to its treatment.[9] Only a limited number of studies have been conducted worldwide to establish the genetic etiology of Major Depressive disorder and Moreover in Pakistan no study so far has been done to find association of TPH2 gene polymorphism with development of MDD in the Pakistani population but depression is however considered to be a hereditary disorder as several studies suggesting done showed a heritability of MDD to be 40%.[10] Since MDD is a major concern nowadays as it can promote a persistent damaging impact on the quality of life.[11] So the present study was done to establish association of TPH2 (rs7305115) gene polymorphism with MDD in Pakistani population. Purpose of the study was to find individuals who are prone to develop MDD, followed by their periodic evaluation for early diagnosis and early treatment, in order to decrease morbidity as well as mortality and thus decreasing the economic burden of the disease.

MATERIAL & METHODS

The study was done in the Biochemistry Department in Islamic International Medical College Trust in cooperation with Psychiatry Department, Railway General Hospital, Rawalpindi after being approved from Ethical Review Committee (ERC) (Ripah/IRC/19/0382), from October 2019 to September 2020.

Subjects recruited in the study were diagnosed cases (n=120) of Major Depressive disorder and healthy controls (n= 120) with age and gender matched. Those subjects who refused to give informed consent, with history of mental disorders in family other than recurrent depressive disorders, having systemic disorders that can lead to Depression, with history of injuries to central nervous system or controls with history of any Psychiatric illness were all excluded from the study. Individuals recruited for study aged between 15 to 75 years and from almost all ethnic background found in Pakistan.

Samples of blood were taken from study subjects for TPH2 (rs7305115) gene polymorphism genotype analysis. Extraction of DNA was done from blood samples by using Chelex™ method. After extraction DNA was stored at -70 degrees Celsius in labelled Eppendorf tubes till further analysis. Primers were designed using an article. Sequences of primers used are given in Table-I.[12]

Primer	Gene Locus	Primer Sequences, 5´ to 3´
TPH2(F)	TPH2	5 ″ TTAGAAAGGTCTGGCTTCACGGTGAG 3″
TPH2 (R)	TPH2	5 ″ AGGAGTCTGATCCTTCAGTGAGCCC 3″(Outer primers)
TPH2 (F)	TPH2	5 ″ GGCTCAGATCCCCTCTACACCACA 3″ (A-allele)
TPH2 (R)	TPH2	5″ GGCTTTAATGTAGGTACTCACGGTGCC 3″ (G- allele) (inner primers)

Table-I. Primer sequence of TPH2 genes

The PCR reaction was carried out using Multiplex PCR procedure in a PCR tube consisting of outer and inner primers which were specific to TPH2 Gene (rs7305115). The volume present in each PCR reaction was 24.5 µL including 8.5 µl PCR water, 12.5 µl thermo scientific™ master mix (consisting of thermus aquaticus (Taq) polymerase, dNTP's and MgCl2 as per manufacturer's specifications), 0.5 µl of each of the primer was added to reaction mixture from all the four primers. Finally 3µl of DNA was added to this reaction mixture from sample to be genotyped.

The process of PCR amplification initiated with an initial step of denaturation at 94°C for 7 minutes, which was followed by 40 amplification cycles that included denaturation at 94°C for 50 seconds, annealing at 62°C for 45 seconds, and extension at 72°C for 52 seconds. The final step of extension was done at 72°C for 12 minutes, and cycle was terminated to hold at 4°C. Amplified genes present in reaction mixture were then subjected to electrophoresis containing 2% agarose gel which is premixed with 0.5 µg/ml concentration of ethidium bromide in 1x TBE buffer solution. Electrophoresis was continued for about 65 minutes with current settings at 700 mA and voltage setting at 100 V. Amplified bands were visualized using UV320 trans-illumination under UV camera in Gene Box™ by Gene Sys™. Gene Ruler™ 100 bp (base pair) DNA reference ladder was used as a reference in order to determine size of amplified bands in base pairs. Statistical analysis was performed using IBM™ SPSS version 21. Frequencies and percentages were measured using descriptive statistics. Significance between variables of our study groups and genotype of gene of interest that is TPH2 were determined using Chi square test. Possible association between TPH2 gene polymorphism and MDD was computed using OR and 95% confidence interval and p value equal to and less than 0.05 was taken to designate the statistically significant results.

RESULTS

In our study a total of 240 participants were included. There were 120 diagnosed cases of MDD and 120 normal healthy age and gender matched controls according to criteria previously described. Both the cases and controls were divided in to three groups on the basis of their ages. In group 1 both subjects aged 15 to 35 years were included and a total of 128 subjects fall under this age group out of which 64 (53.33%) were cases and 64 (53.33%) were controls. In group 2 subjects aged 36 to 55 years were included and a total of 86 individuals were found to be present in this age group out of which 45 (37.5%) were cases and 41(34.1%) were controls and in group 3 subjects aged 56 to 75 years were included and a total of 26 individuals were present in this group out of which 11(9.16%) were cases and 15(12.5%). In cases out of 120 subjects there were 101(84%) females and 19 males (6%). In controls, we had 106 females (88%) and 14 males (12%).

In terms of genotype frequencies genotype AA was present in 62 (51.7%) cases and 81 (67.5%) controls, AG Genotype was present in 25 (20.8%) cases and 18 (15%) controls whereas GG genotype was present in 33 (27.5%) cases and 21(17.5%) controls. In terms of Allele frequency, A Allele was present in 74 (63%) cases and 90 (75%) controls whereas G Allele was present in 45 (37%) cases and 30 (25%) controls.

Total N=240	Cases =120 n= (%)	Controls=120 n= (%)
Genotype		
AA	62(51.7%)	81(67.5%)
AG	25(20.8%)	18(15%)
GG	33(27.5%)	21(17.5%)
Allele		
A	149(63%)	180(75%)
G	91(37%)	60(25%)

Table-II. Genotype and allele characteristics of cases and controls.

DISCUSSION

Depression is a condition with a very inconstant progression and non-responsiveness to treatment.[13] Even though a number of possible genes have been nominated so far but variation in their timings and the type of adversative environmental condition have hindered identical studies of single nominee genes.[14]

Total N=240	Cases N=120 n= (%)	Controls N=120 n= (%)	OR(95%CI)	P-Value
Age(years)				
15-35	64(53.33%)	64(53.33%)		
AA	34(53.1%)	45(70.3%)	Ref	
AG	12(18.8%)	10(15.6%)	0.6296(0.2435-1.6282)	0.337
GG	18(28.1%)	9(14.1%)	0.3778(0.1512-0.9439)	0.0338*
36-55	45(37.5%)	41(34.16%)		
AA	20(44.4%)	26(63.4%)	Ref	
AG	12(26.7%)	7(17.1%)	0.4487(0.1494-1.3473)	0.1492
GG	13(28.9%)	8(19.5%)	0.4734(0.1646-1.3611)	0.1615
56-75	11(9.16%)	15(12.5%)		
AA	8(72.7%)	10(66.7%)	Ref	
AG	1(9.1%)	1(6.7%)	0.8(0.043-14.88)	---
GG	2(18.2%)	4(26.7%)	1.6(0.23-11.08)	---

Table-III. Association of genotype of TPH2 (rs7305115) gene polymorphism with age of MDD cases and controls. OR- Odds Ratio, CI- confidence interval, P< 0.05- statistically significant

New advances in research and data suggest that when tryptophan catabolite pathway is damaged then it may lead to the development of depression.[14] These abnormalities may be related to decreased functioning of pathway enzyme such as TPH. This enzyme has two isoforms TPH1 and TPH2 both are encoded by two different genes i.e TPH1 and TPH2 genes respectively.[15] TPH2 gene is possibly important nominee gene for causing MDD, as it has been shown to have expression in large areas of brain including brain stem, the main locus of the serotonin-producing neurons. Several studies have effectively established the association between TPH2 gene polymorphism with MDD in African Americans as well as Southwestern American Indians.[16] The exonal variant rs7305115 was highly associated with MDD and disturbed sleep.[17]

In our current study we genotyped SNP (rs7305115) of TPH2 gene. In present study there was a difference in the gender distribution among MDD cases as there were increased percentage of female MDD cases as compared to men. This is supported by a study, according to which women have an greater risk of developing MDD than men after adolescence.[18] Another study shows that women have a higher lifetime risk of most of the mood disorders, specifically MDD and dysthymic disorder in comparison to men.[19] In our present study we have found that the frequency of A allele was greater in controls in comparison to cases and may predict protective factor against MDD whereas G allele frequency was greater in cases and is a risk factor towards the development of MDD. In contrast to our study an association was observed between A allele of TPH2 gene and Psychiatric disorders including MDD linked to suicide attempts in the Mexican population.[20] We have found significant association between GG genotype and increased risk of MDD development in subjects aging 15 to 35 years. In agreement with our study, a study done by Kessler RC et al showed significant association of age with MDD.[21]

In our current study we have genotyped only one SNP variant of TPH2 gene further studies should be conducted to see the association of other SNPs of TPH2 gene with MDD in our population as no study so far has done according to my knowledge in Pakistan to establish the association between various variants of TPH2 gene and MDD.

CONCLUSION

We have found in the present study that age group 1(15-35 years) with GG genotype of TPH2 gene polymorphism have a significantly increased risk of developing MDD as compared to other age groups in our study.

REFERENCES

1. Otte C, Gold SM, Penninx BW, Pariante CM, Etkin A, Fava M, et al. **Nature disease primer: Major Depressive Disorder.** Nat Publ Gr [Internet]. 2016; 2(Mdd):1–21. Available from: http://www.nature.com/articles/nrdp201665%0Ahttp://dx.doi.org/10.1038/nrdp.2016.65

2. Rudaz DA, Vandeleur CL, Gebreab SZ, Gholam-Rezaee M, Strippoli MPF, Lasserre AM, et al. **Partially distinct combinations of psychological, metabolic and inflammatory risk factors are prospectively associated with the onset of the subtypes of major depressive disorder in midlife.** J Affect Disord [Internet]. 2017; 222:195–203. Available from: http://dx.doi.org/10.1016/j.jad.2017.07.016

3. S.R. S, A. A, R.K. G. **Epidemiology of depression in adults.** Indian J Psychiatry [Internet]. 2014; 56(SUPPL. 1):S48. Available from: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed15&NEWS=N&AN=71618852

4. Ferrari AJ, Somerville AJ, Baxter AJ, Norman R, Patten SB, Vos T, et al. **Global variation in the prevalence and incidence of major depressive disorder: A systematic review of the epidemiological literature.** Vol. 43, Psychological Medicine. 2013. p. 471–81.

5. Iqbal Z, Murtaza G, Bashir S. **Depression and anxiety: A Snapshot of the Situation in Pakistan.** Int J Neurosci Behav Sci. 2016;4(2):32–6.

6. Wigner P, Czarny P, Synowiec E, Bijak M, Białek K, Talarowska M, et al. **Association between single nucleotide polymorphisms of TPH1 and TPH2 genes, and depressive disorders.** J Cell Mol Med. 2018; 22(3):1778–91.

7. Maes M, Leonard B, Myint A. **Activation induces indoleamine 2, 3-dioxygenase, which leads to lower plasma tryptophan and an increased synthesis of detrimental tryptophan catabolites (TRYCATs. Prog Neuro- … [Internet].** 2011; 35(3):702–21. Available from: http://www.sciencedirect.com/science/article/pii/S0278584610005117

8. Bromet E, Andrade LH, Hwang I, Sampson NA, Alonso J, de Girolamo G, et al. **Cross-national epidemiology of DSM-IV major depressive episode.** (Research article)(Survey). BMC Med. 2011; 9:90.

9. Pusceddu MM, Nolan YM, Green HF, Robertson RC, Science N, Food M, et al. **us cr ip t Ac ce Ac ce ed cr t.** 2015; 1–37.

10. Manuscript A. **Nihms384745.Pdf.** 2012; 379(9820):1045–55.

11. Vancampfort D, Firth J, Schuch FB, Rosenbaum S, Mugisha J, Hallgren M, et al. **Sedentary behavior and physical activity levels in people with schizophrenia, bipolar disorder and major depressive disorder: a global systematic review and meta-analysis.** World Psychiatry. 2017; 16(3):308–15.

12. Nazree NE, Loke AC, Zainal NZ, Mohamed Z. **Lack of association between TPH2 gene polymorphisms with major depressive disorder in multiethnic Malaysian population.** Asia-Pacific Psychiatry. 2015 Mar 1; 7(1):72–7.

13. Vermetten E, Bremner J. **Role of serotonergic and noradrenergic systems in the pathophysiology of depression and anxiety disorders.** Depress Anxiety. 2002;16(1):14–38.

14. Renard CE, Dailly E, Nic Dhonnchadha BÁ, Hascoet M, Bourin M. **Is dopamine a limiting factor of the antidepressant-like effect in the mouse forced swimming test?** Prog Neuro-Psychopharmacology Biol Psychiatry. 2004; 28(8):1255–9.

15. Nielsen DA, Jenkins GL, Stefanisko KM, Jefferson KK, Goldman D. **Sequence, splice site and population frequency distribution analyses of the polymorphic human tryptophan hydroxylase intron 7.** Mol Brain Res. 1997; 45(1):145–8.

16. Zhou K, Bellenguez C, Sutherland C, Hardie G, Palmer C, Donnelly P, et al. Zhou et al. **reply. Vol. 44, Nature Genetics.** 2012. p. 361–2.

17. Zupanc T, Pregelj P, Paska AV. **Tryptophan hydroxylase 2 (TPH 2) single nucleotide polymorphisms, suicide, and alcohol-related suicide.** Psychiatr Danub. 2013; 25(SUPPL.2).

18. Kendler K, Bigdeli T, Cai N, Webb T, Peterson R, Bacanu S, et al. **Sparse whole genome sequencing identifies two loci for major depressive disorder in han Chinese women.** Eur Neuropsychopharmacol [Internet]. 2017; 27(Supplement 2):S119–20. Available from: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed18&NEWS=N&AN=621381536

19. Seedat S, Scott KM, Angermeyer MC, Berglund P, Bromet EJ, Brugha TS, et al. **Cross-national associations between gender and mental disorders in the World Health Organization World Mental Health Surveys.** Arch Gen Psychiatry. 2009; 66(7):785–95.

20. López-narváez ML, Tovilla-zárate CA, González-castro TB, Juárez-rojop I, Pool-garcía S, Genis A, et al. **ScienceDirect Association analysis of TPH-1 and TPH-2 genes with suicidal behavior in patients with attempted suicide in Mexican population.** Compr Psychiatry [Internet]. 2015; 61:72–7. Available from: http://dx.doi.org/10.1016/j.comppsych.2015.05.002

21. Kessler RC, Bromet EJ. **The epidemiology of depression across cultures.** Vol. 34, Annual Review of Public Health. 2013. p. 119–38.

AUTHORSHIP AND CONTRIBUTION DECLARATION

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1	Sidra Ashfaq	Study design, Data collection, Data analysis, Statistical analysis, write up and literatur ecitation and is responsible for integrity of research.	
2	Amena Rahim	Did final review and approval manuscript.	
3	Nurain Baiq	Helped in write up and data analysis.	
4	Maria Sarfraz	Helped in write up and data analysis.	
5	Aqsa Tazarrat	Helped in write up and data analysis.	
6	Aneela Shabbir	Helped in write up and data analysis.