SJIF Impact Factor: 3.458

WORLD JOURNAL OF ADVANCE HEALTHCARE RESEARCH

ISSN: 2457-0400
Volume: 3.
Issue: 1.
Page N. 140-144
Year: 2019

Original Article www.wjahr.com

ASSOCIATION BETWEEN THE RS650727 POLYMORPHISM IN MYO-INOSITOL MONOPHOSPHATASE2 (IMPA2) AND BIPOLAR DISORDER IN A TURKISH POPULATION

[1*]Nisa Uyar Alpaslan, [1]İ. Ömer Barlas and [2]Ayse Devrim Basterzi

[1]*Mersin University Medical Faculty, Department of Medical Biology and Genetics Mersin, TR.
[1]Mersin University Medical Faculty, Department of Medical Biology and Genetics Mersin, TR.
[2]Istanbul, Private Office: Bagdat cad. 270/5 Caddebostan mah. Kadıkoy/ISTANBUL, TR.

Received date: 20 December 2018 **Revised date:** 10 January 2019 **Accepted date:** 31 January 2019

***Corresponding author: Nisa Uyar Alpaslan**
Mersin University Medical Faculty, Department of Medical Biology and Genetics Mersin, TR.
Email ID: nisa.uyar@hotmail.com,

ABSTRACT

Background and objectives: Bipolar disorder (BP) is a heterogeneous disease, with genetic factors being important in its etiology. Although many target genes associated with BP have been studied to date, little is known about the genetic background of the disease. Myo-inositol monophosphatase (IMPAse), encoded by the myo-inositol monophosphatase 2 (IMPA2) gene, plays a role in the phosphatidylinositol 4,5-biphosphate (PIP_2) signaling pathway. IMPase is a key enzyme which catalyzes the dephosphorylation of myo-inositol monophosphate to free myo-inositol. This reduction slows down signal transduction in the PIP_2 cycle, leading to an increase in inositol monophosphate levels. We examined the relationship between IMPA2 gene polymorphisms and BP. *Methods:* The study included 94 individuals with a diagnosis of BP and 99 healthy individuals. Molecular analysis of the IMPA2 gene of each individual was performed by real-time polymerase chain reaction (real-time PCR). *Results:* There was an association between the IMPA2 gene rs650727 polymorphism and patients with BP ($p = 0.038$). By contrast, no association was found between IMPA2 gene polymorphisms rs1787984, rs585247, rs669838, and rs636173 and BP. *Conclusions:* Our results showed that there was a possible association between polymorphism rs650727 in IMPA2 and BP. This represented the first study of IMPA2 gene polymorphisms and BP in a Turkish population.

KEYWORDS: IMPA2, Bipolar Disorder, polymorphism, Real Time PCR, myo-inositol monophosphatase.

INTRODUCTION

Bipolar disorder, is a major psychiatric disorder with a lifetime prevalence of ~1 - 3%, characterized by mania and depression episodes which usually start in adolescence and continue throughout life.[1-5] Family, adoption and twin studies suggest that genetic factors play a key role in BP. [6-8] Genetic studies indicate that the 18th chromosome contains determinants of BP.[9-11] The locus where *IMPA2* is located is the most studied locus for BP.[2,12] At the same time, loci associated with schizophrenia, affective disorder and autism are also associated with human chromosome 18.[13] Linkage and association studies suggest that *IMPA2* could be a genetic susceptibility factor for BP.[14,15]

The *IMPA2* gene encodes myo-inositol monophosphatase (IMPase) which catalyzes the dephosphorylation of various myo-inositol monophosphates to free myo-inositol that acts on the phosphoinositide signaling pathway.[13,14,16] The integrity and sustainability of phosphoinositide signaling pathway, which produces the second messengers 1,4,5-trisphosphate and diacylglycerol (DAG), depends on a continuous supply of free myo-inositol.[17-19] Lithium is the oldest and still the most effective mood stabilizer for the pharmacological treatment of BP, being used in the long-term treatment of associated mania and depression periods.[19-24] This chemical may act by depleting neuronal *myo*-inositol concentrations through the inhibition of IMPase. This represents the "inositol-depletion hypothesis" for the therapeutic action of lithium. [25,26]

Two genes encoding human IMPases have so far been isolated, *IMPA1* located on chromosome 8q21.13–21.3,

and *IMPA2*. Analysis of *IMPA2* variants supports linkage of BP to chromosome 18p11.2.[13,14,16] Sjoholt et al. (2004) detected an association between single nucleotide polymorphisms (SNPs) in the *IMPA2* promoter and BP in a family-based association study among Palestinian-Arabs. [14] They reported that *IMPA2* was a candidate gene in *BP* based on convergent evidence from pharmacological, biochemical, linkage, and association analyses.[14] These results provide a basis for further studies on whether *IMPA2* gene plays a role in the etiology of BP and lithium treatment. Lithium may reduce the frequency, duration and severity of recurrent episodes of both mania and depression during maintenance therapy of BP.[25-28] The major aim of this study was to determine the relationship of BP with *IMPA2* gene polymorphisms in the lithium treatment pathway.

MATERIALS AND METHODS

Subjects

Ninety-four individuals with a diagnosis of BP, the experimental group, and 99 healthy individuals without a BP diagnosis, the control group, were included in the study. Diagnosis was based on Diagnostic and Statistical Manual of Mental Irregularities IV (DSM-IV) performed at Mersin University Faculty of Medicine, Department of Psychiatry. The mean age of participants was 40.7 ± 12.6 years; 48.2% were female and 51.8% were male.

The 99 healthy individuals of the control group were selected from an 18 - 65 age range. They were selected to be compatible in terms of gender with the experimental group. Members of the control group had no family history of psychiatric disorders.

Permission from the Ethics Committee of Mersin University was obtained for our study. Signed consent forms were obtained from all participants.

We collected 6 - 7 ml peripheral blood from each individual for DNA isolation. Prior to DNA extraction, the blood was placed in centrifuge tubes containing 1 ml 2% EDTA and stored at +4 ºC.

DNA analyses

DNA isolation was performed on peripheral blood samples from patients and control groups.[29] Molecular analysis of rs1787984, rs585247, rs650727, rs669838 and rs636173 polymorphisms of the *IMPA2* gene of each individual was performed by real-time polymerase chain reaction (real-time PCR; Applied Biosystems, Waltham, MA, USA).

Statistical analyses

Statistical tests were performed in the Biostatistics and Medical Informatics Department, Mersin University Faculty of Medicine, Turkey. Normality test for the continuous variable age was by the Shapiro-Wilks test.

Student's t test was used to test the differences in groups and gender for the age variable. The relationships between categorical variables were tested using Pearson's chi-square and likelihood ratio chi-square tests.

Allele frequencies were calculated, and the population of each was determined by the Hardy-Weinberg equilibrium. Data from genotyping was evaluated statistically with SPSS v.11.5 software (SPSS Inc., Chicago, IL, USA). Genotype frequencies for polymorphisms of the *IMPA2* gene (rs1787984, rs585247, rs650727, rs669838, rs636173) were determined for healthy participants and patients; $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The study included 193 individuals, 93 (48.2%) were female and 100 (51.8%) were male. The mean age of the women and men in the patient and control groups was 39.9 ± 11.7 years and 41.4 ± 13.5 years, respectively. There was no significant statistically difference in the groups in terms of age and gender (Table 1).

Of the 94 patients, 46 (48.9%) were female and 48 (51.8%) were male. Of the 99 healthy participants in the control group, 47 (47.5%) were female and 52 (52.85%) were male. There was no significant statistically difference in the groups in terms of age and gender (Table 2).

We choose polymorphic regions to examine allelic differences of *IMPA2*. A total of 5 polymorphisms in *IMPA2* gene were evaluated for allelic and genotypic frequencies. We determined allelic discrimination in a multicomponent plot by using real-time PCR. No association was found between *IMPA2* gene polymorphisms rs1787984, rs585247, rs669838, rs636173 and BP. However there was an association between *IMPA2* gene polymorphism rs650727 and BP.

Bipolar disorder is a heterogeneous disorder characterized by periods of mania and depression. The complexity of BP makes it difficult to diagnosis, treat, classify, and understand in terms of pathophysiology.[13,14,16,30] Previous studies have shown that *IMPA2* encodes IMPase, which is involved in the inositol signaling pathway, and this *g*ene may play a role in the therapeutic response of BP to lithium treatment.[9,13,30,31] Lithium acts as an inhibitor without competition, reducing free inositol levels in the phosphoinositide cycle (PIP_2).[17] In this study we investigated the hypothesis that variations in *IMPA2* might increase susceptibility to BP.

Shamir et al. (1998), examining lithium treatment in 77 BP and 29 control lymphoblastoid cell lines, found IMPase activity in BP cell lines to be < 50% of the controls.[32] Sjoholt et al. (2000), identified 9 polymorphisms in 23 patients with BP 7 were located in introns, one in exon 2 (159T>C, quiet transition) and another in exon 5 (443G>A, amino acid substitution, R148Q). They also described the genomic structure of

IMPA2 and characterized some genomic differences between the *IMPA1* and *IMPA2* genes by using genomic sequence analysis of the promoter region. They reported that *IMPA* genes polymorphisms in the 3'UTR region should be investigated for their effects on gene expression.[11] Soares et al. (2000), observed that compared to control individuals, lithium treated BP patients had significantly lower PIP_2 levels.[33] Two previous studies researched the promoter and 5' UTR region of the *IMPA2* gene in BP patients. One study in a Japanese population (Ohnishi et al., 2007) and one in an Arab–Palestinian sample (Sjøholt et al., 2004) found statistically significant relationship between the BP and *IMPA2* promoter –SNPs 207T> C and -461C>T (rs2075824).[2,14] Bloch et al. (2010) found a statistically significant relationship between rs669838 and rs585247 polymorphisms of the *IMPA2* gene and BP, in a study comparing 556 BP patients and 735 controls from a European population.[31] The distribution of the rs585247 polymorphism with T/T genotype indicated differences could be related to the activity of IMPase. In the current stdy, we did not find a relationship to BP for these SNPs. Yoko et al. (2018) examining genetic differences between early-onset BP and late-onset BP for the *IMPA2* gene (rs669838, rs1020294, rs1250171 and rs630110), showed that rs1020294 and rs1250171 were associated with the age of onset in a Caucasian population.[34] Although we studied similar populations, we could not find a significant association for rs669838.

In our study, we investigated 5 polymorphisms of the *IMPA2* gene, rs1787984, rs585247, rs650727, rs669838 and rs636173. We found a significant association between rs650727 polymorphism and BP. These SNPs were localized in the 5' region of the gene near the promoter region. There was no association between *IMPA2* gene polymorphisms rs1787984, rs585247, rs669838, rs636173 and BP. Our results suggested that the rs650727 polymorphism was associated with BP. *IMPA2*-rs650727 genes in patients showed the following genotypes: C/C (45.2%), T/C (47.3%), T/T (7.5%). By comparison, the control group had: C/C genotype (48%) T/C genotypes (33.6%), and T/T genotypes (18.4%; Table 3). The 'C' allele in rs650727 polymorphism might have a functional role in the regulation of the *IMPA2* gene expression. This variation might act on IMPase and affect the phosphoinositide pathway, blocking the formation of free inositol. Reduced free inositol might prevent mania and depression. Differences between reports might be related to population diversity, heterogeneity of the disease, or the number of patients in the study. rs650727 might be responsible for transcriptional up-regulation of mRNA. An expanded study with a larger patient population is necessary to examine the effects of polymorphisms in the 3' UTR region of IMPA genes, and their effect on gene expression.

Although the number of patients used in the current study was too small to represent the Turkish population, there was a statistically significant relationship between the rs650727 polymorphism and BP.

To better reflect the Turkish population, it would be necessary to increase the number of patients and controls. In addition, investigation of other gene polymorphisms in the PIP_2 pathway might contribute to further understanding the etiology of BP.

There were some limitations in the current study. The mania and depression status of individuals in the patient group was not defined. In addition, we did not investigated clinical subphenotypes, such as age of onset, family history and frequency of mood episodes.

Table 1: The mean age of the male and female patients (n = number of subjects and individual).

	Gender	***n***	***Average age***	***P***
Patient and control group	Women	93	39.9 ± 11.7	0.435
	Men	100	41.4 ± 13.5	

Table 2: The mean age of the patient and control group (n = number of subjects / individual).

Gender	***Patient***		***Control***		χ^2	***P***
	n	The mean of age / (%)	n	The mean of age / (%)		
Women	46	43,52 / (48,9)	47	40,25 / (47,5)	0,041	0,0839
Men	48	43,72 / (51,1)	52	39,17 / (52,85)		

Table 3: Genotype and allele frequencies of variations in the *IMPA2* gene.

SNP	Sample	*n*	Genotype frequency			**p*	Allele frequency	***p*	HWE χ^2
SNP 1			**AA**	**AG**	**GG**		**A G**		
rs1787984	Bipolar	94	56	34	4		0.78 0.22	0.681	0.169
	Controls	99	50	42	7	0.392	0.72 0.28	0.649	0.207
SNP 2			**TT**	**TC**	**CC**		**T C**		
rs585247	Bipolar	92	56	31	5		0.78 0.22	0.795	0.068
	Controls	99	66	29	4	0.690	0.81 0.19	0.720	0.129
SNP 3			**TT**	**TC**	**CC**		**T C**		
rs650727	Bipolar	93	7	44	42	**0.038**	0.31 0.69	0.324	0.975
	Controls	98	18	33	47		0.35 0.65	**0.010**	6.722
SNP 4			**TT**	**TG**	**GG**		**T G**		
rs669838	Bipolar	91	16	44	31	0.950	0.42 0.58	0.955	0.003
	Controls	99	19	46	34		0.42 0.58	0.657	0.236
SNP 5			**AA**	**AG**	**GG**		**A G**		
rs636173	Bipolar	93	17	47	29	0.903	0.44 0.56	0.788	0.072
	Controls	99	20	47	32		0.44 0.56	0.718	0.131

Bold indicates p<0.05.
**p* values for comparison of genotype frequencies between bipolar individuals and controls.
***p* values for comparison of allele frequencies between bipolar individuals and controls.

CONCLUSION

We demonstrated an association between the rs650727 polymorphism of the *IMPA2* gene and BP. While the study requires further replication in a larger sample, it provides additional information on the chromosome 18p genomic region. Further functional experiments are necessary to investigate the role of the *IMPA2* gene and its involvement in regulation of the inositol pathway.

Funding
The study was supperted by the Mersin University Coordination Unit for Scientific Research Projects. Grant number is BAP-SBE TTB (NU) 2011-3 YL.

Conflict Of Interest
No conflicts of interest have been declared.

REFERENCES

1. Diagnostic and Statistical Manual of Mental Disorders-Text Revision. 4th Ed. 2000.
2. Ohnishi T, Kazuo Y, Ohba H, Iwayama Y, Toyota T, Hattori E, Inada T, Kunugi T, Tatsumi M, Ozaki N, Iwata N, Sakamoto K, Iijima Y, Iwata Y, Tsuchiya K J, Sugihara G, Nanko S, Osumi N, Detera Wadleigh S D, Kato T, Yoshikawa T. A Promoter haplotype of the inositol monophosphatase 2 gene (IMPA2) at 18p11.2 confers a possible risk for bipolar disorder by enhancing transcription. *Neuropschopharmacolgy*, 2007; 32: 1727-1737.
3. Janardhan Reddy Y C. Prodromal symptoms of recurrences of mood episodes in bipolar disorder. *Indian Journel of Medical Research*, 2012; 135(2): 154–156.
4. Merikangas KR, Akiskal HS Angst J, Greenberg PE, Hirschfeld RM, Petukhova M, Kesler R. Lifetime and 12-month prevalence of bipolar spectrum disorder in the national comorbidity survey replication. *Archieves of General Psychiatry*, 2007; 64(5): 543–552.
5. Barnett JH, Smoller JW. The Genetics of bipolar disorder. *Neuroscience*, 2009; 164: 331–343.
6. Agama G, Shamir A, Shaltiela G, Greenbergb ML. Myo-inositol-1-phosphate (MIP) synthase: a possible new target for antibipolar drugs. *Bipolar Disorders*, 2002; 4(1): 15–20.
7. Shastry BS. Bipolar disorder: an update. *Neurochemistry International*, 2005; 46: 273–279.
8. Merikangas K, Yu K. Genetic epidemiology of bipolar bisorder. *Clinical Neuroscience Research*, 2002; 2: 127-141.
9. Shamir A, Sjùholtc G, Ebstein RP, Agama G, Steenc VM. Characterization of two genes, IMPA1 and IMPA2 encoding mouse myo-inositol monophosphatases. *Gene*, 2001; 271(2): 285-291.
10. Chen X, Mcmahon EG, Gulves EA. Stimulatory effect of lithium on glucose transport in rat adipocytes is not mediated by elevation of IP1. *American Journal of Physiology Endocrinology and Metababolism*, 1998; 275: 272-277.
11. Sjøholt G, Gulbrandsen AK, Løvlie R, Berle JØ, Molven A, Sten VM. A Human myo-inositol monophosphatase gene (IMPA2) localized in a putative susceptibility region for bipolar disorder on chromosome 18p11.2: genomic structure and polymorphism screening in manic-depressive patients. *Molecular Psychiatry*, *2000*; 5: 172–180.
12. Ohnishi T, Ohba H, Seo KC, Im J, Sato Y, Iwayama Y, Furuichi T, Chung SK, Yoshikawa T. Spatial expression patterns and biochemical properties distinguish a second *myo*-ınositol monophosphatase IMPA2 from IMPA1. *The Journal of Biological Chemistry*, 2007; 282(1): 637-646.
13. Sjøholt G, Molven A, Løvlie R, Wilcox A, Sikela JM, Sten VM. Genomic structure and chromosomal localization of a human myo-ınositol

monophosphatase gene (*IMPA*). *Genomics*, 1997; 45: 113-122.
14. Sjøholt G, Ebstein RP, Lie RT, JØ Berle, J Mallet, JF Deleuze, DF Levinson, C Laurent, M Mujahed, I Bannoura, I Murad, A Molven1, VM Sten. Examination of IMPA1 and IMPA2 genes in manic-depressive patients: association between IMPA2 promoter polymorphisms and bipolar disorder. *Molecular Psychiatry*, 2004; 9: 621–629.
15. Kamnasaran D.Genetic analysis of psychiatric disorders associated with human chromosome 18. *Clin Invest Med.* 2003; 26(6): 285-302.
16. Bone R, Springer JP, John R. Atack JR. Structure of inositol monophosphatase, the putative target of lithium therapy. *Biochemistr*, 1992; 89: 10031-10035.
17. Jope RS, Song L, Li PP, Young LT, Kish SJ, Pacheco MA, Warsh JJ. The phosphoinositide signal transduction system is impaired in bipolar affective disorder brain. *J Neurochem*, 1996; 66(6): 2402- 2409.
18. John R., Howard B., Scott J. Structure of mechanism of ınositol monophosphatase. *FEBS Letters*, 1995; 361: 1-7.
19. Marmol F. Lithium: bipolar disorder and neurodegenerative diseases possible cellular mechanisms of the therapeutic effects of lithium. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, 2008; 32: 1761–1771.
20. Fountoulakis KN, Grunze H, Panagiotidis P, Kaprinis G. Treatment of bipolar depression: an update. *Journal of Affective Disorders*, 2008; 109: 21–34.
21. Li PP. Transcriptional mechanisms of lithium action: therapeutic ımplications. *Clinical Neuroscience Research*, 2004; 4: 271–280.
22. King J, Keim M, Teo R, Weening KE, Kapur M, McQuillan K, Ryves J, Rogers B, Dalton E, Williams RSB, Harwood AJ. Genetic control of lithium sensitivity and regulation of ınositol biosynthetic genes. *Plos One*, 2010; 5(6): 11151.
23. Karege F, Buresi C, Golaz J, Schwald M, Malafosse A. Decreased expression of Gs mRNA and Protein levels in lithium treated bipolar affective disorder. *Human Psychopharmacolgy*, 2000; 15: 191-197.
24. Nemanov L, Ebstein RP, Belmaker RH, Osher Y, Agam G. Effect of bipolar disorder on lymphocyte ınositol monophosphatase mRNA levels. *International Journal of Neuropsychopharmacology*, 1999; 2: 25-29.
25. Agama G, Shatiela G, Kozlovskya N, Shimonb H, Belmakera RH. Lithium ınhibitable enzymes in postmortem brain of bipolar patients. *Journal of Psychiatric Research*, 2003; 37: 433–442.
26. Atack JR. Inositol monophosphatase ınhibitors lithium mimetics? *Medicinal Research Reviews*, 1997; 17(2); 215–224.
27. Parthasarathy R, Parthasarathy L, Vandal R. Brain ınositol monophosphatase ıdentified as a galactose 1-phosphatase. *Brain Research*, 1997; 778: 99–106.
28. Chen X, Mcmahon EG, Gulves EA. Stimulatory effect of lithium on glucose transport in rat adipocytes is not mediated by elevation of IP1. *American Journal of Physiology Endocrinology and Metababolism*, 1998; 275: 272-277.
29. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research*, 1988; 16(3): 1215.
30. Dimitrova A, Milanova V, Krastev S, Nikolov I, Toncheva D, Owen MJ, Kirov G. Association study of myo-inositol monophosphatase 2 (IMPA2) polymorphisms with bipolar affective disorder and response to lithium treatment. *Pharmacogenomics Journal*, 2005; 5(1): 35- 41.
31. Bloch PJ, Weller AE, Doyle GA, Ferraro TN, Berrettini WH, Hodge R, Lohoff FW. Association analysis between polymorphisms in the myo-inositol monophosphatase 2 (IMPA2) gene and bipolar disorder. *Progress in Neuropsychopharmacology & Biological Psychiatry*, 2010; 34(8): 1515-1519.
32. Shamir A, Ebstein RP, Nemanov L, Zohar A, Belmaker RH, Agama G. Inositol monophosphatase in immortalized lymphoblastoid cell lines ındicates susceptibility to bipolar disorder and response to lithium therapy. *Scientific Correspondence*, 1998; 3(6): 481- 482.
33. Soares JC, Chen G, Dippold CS, Wells KF, Frank E, Kupfer DJ, Manji HK, Mallinger AG. Concurrent measures of protein kinase c and phosphoinositides in lithium- treated bipolar patients and healthy ındividuals: a preliminary study. *Psychiatry Research*, 2000; 95(2): 109-118.
34. Tomioka Y, Jiménez E, Salagre E, Arias B, Mitjans M, Ruiz V, Sáiz P, García-Portilla MP, Fuente L, Gomes-da-Costa SP, Bobes J, Vieta E, Benabarre A, Grande I. Association between genetic variation in the myo-inositol monophosphatase 2 (*IMPA2*) gene and age at onset of bipolar disorder. *Journal of Affective Disorders*, 2018; 232: 229-236.