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Original Article

Association between 15 Insertion/Deletion Polymorphisms and the Risk of Type 1 Bipolar Disorder

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Abstract

Background and objectives: Type 1 bipolar disorder (BP) is a mental illness characterized by extreme shifts in mood, oscillating between manic and depressive episodes. It ranks as the sixth most prevalent psychiatric disorder globally, often emerging in the teenage years. This study aimed to identify associations between BP and 15 insertion/deletion (Indel) polymorphisms in the human genome, examining genes including *TPA*, *UCP2*, *HLA-G*, *FADS2*, *ADRA2B*, *VEGF*, *PDCD6IP*, *SLC6A4*, *TLR2*, *APOB*, *TP53*, *LRPAP1*, *DHFR*, *MDM2*, and *DBH*.

Methods: This case-control study involved 226 patients with BP and 235 healthy controls. Allele frequencies for each polymorphism in cases and controls were estimated using pooled samples. Polymerase chain reaction was performed for each Indel polymorphism using pooled samples as templates to estimate allele frequencies.

Results: The data presented herein demonstrate a significant association between a 14bp Ins/Del polymorphism in the *HLA-G* gene and the risk of BP. The deletion allele of this polymorphism increased the risk of BP (odds ratio = 1.434, 95% confidence interval = 1.106–1.859, $p = 0.007$). Other 14 Indel polymorphisms were not associated with the risk of BP.

Conclusions: The *HLA-G* 14bp Indel polymorphism exhibits a significant correlation with the risk of BP in this study. This finding contributes to understanding the etiology of BP.

Introduction

Bipolar disorder (BP) is a complex mental illness that involves rapid mood swings between manic and depressive states, often interspersed with periods of normalcy. Within the bipolar spectrum of mental disorders, bipolar type 1 and type 2 are commonly diagnosed subtypes. Affecting more than 1% of the global population, BP remains a significant mental health problem.[1] The exact etiology of BP remains unclear. However, research has shown that the disorder is complex and multifactorial, with multiple genetic elements. According to family, twin, and adoptee studies, the heritability of the disorder is in the range of 70–90%, indicating a significant hereditary role in its pathogenesis.[2,3]

Genetic polymorphisms can be categorized into several classes, including insertion/deletion (Indel). Indels are defined as short insertions and deletions (ranging from one to 10,000 bp) in the genome.[4,5] In the human genome, Indels are the second most common group of genetic variation after single nucleotide polymorphisms. The 1000 Genomes Project results reveal the identification of over 3.4 million Indels in the human genome.[6] Due to the identification of Indels in exons and promoters of known genes, where gene function is expected to be affected,[6] these have become important sources for genetic association studies. The association between Indel genetic variations and the risk of various diseases has been extensively studied, leading to a wealth of available meta-analyses.[7–10] However, only a limited number of published articles have explored the relationship between Indel polymorphisms and the risk of bipolar disorder.[11–16]

Several genetic polymorphisms in genes such as *APOB*,[17,18] *FADS2*,[19,20] *LRPAP1*,[21] *VEGF*,[22] *DBH*,[23] *SLC6A4*,[24–26] *HLA-G*,[15,16] and *TLR2* have been associated with increased risks of BP in genome-wide scans or case-control studies.[27] Literature has demonstrated that the expression of *UCP2* and *TPA* genes is decreased in BP patients.[28,29] While *TP53* gene expression is increased. In addition, proteomic analysis has indicated differences in PDCD6IP levels between BP patients and controls.[30,31] The serum level of vascular endothelial growth factor (VEGF) was found to be heightened in individuals with BP.[32] Multiple sources indi-

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cate that although both BP and schizophrenia share common signs and symptoms, they also share certain genetic and non-genetic risk factors.[33,34] However, it is worth noting that the polymorphic loci associated with the risk of these two disorders are not entirely identical.[35] Of course, not all polymorphic loci are the same; several studies demonstrate the relationship between the same polymorphic variant and the risk of developing both BP and schizophrenia, such as the rs2228145 polymorphism in *IL6R* and the rs1801133 in *MTHFR*.[36,37] Polymorphisms in the *MDM2*,[38] *MTHFR*,[37] and *ADRA2B* genes have also been associated with an increased risk of schizophrenia.[39] Taken together, it is reasonable to suggest that the Indel polymorphisms identified in the aforementioned genes may be associated with an increased risk of developing BP.

Recently, we investigated the association between 15 common Indel polymorphisms and the risk of schizophrenia.[17] However, there has been no similar study on BP. To further elucidate the genetic basis of BP and to identify new candidate risk factors among common genetic polymorphisms, we investigated the association of these 15 different Indel polymorphisms in the genes mentioned in the previous paragraph with the risk of BP. Utilizing pooled samples to estimate allele frequency is a straightforward, economical, and quick approach. This study analyzed the allele frequencies of these 15 Indel polymorphisms among BP patients and control subjects employing this technique.

Materials and methods

Participants

The current research study is a case-control study conducted in Shiraz, Southern Iran. A comprehensive overview of the study participants is available in earlier publications.[11,22] BP type 1 diagnoses were established by a psychiatrist according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) criteria. The patients' status was confirmed as symptomatic cases. Patients belonging to ethnicities other than Persian, such as Arabs, Afghans, Azaris, and Turkmen, were excluded from the study.

Two hundred and twenty-six participants diagnosed with type 1 BP (167 males, 59 females; mean age ± SD = 29.0 ± 10.6 years) and 235 healthy individuals (179 males, 56 females; mean age ± SD = 29.2 ± 10.3 years) were previously reported to be considered in the study.[11,40] No significant difference in gender ($\chi^2 = 0.31$, df = 1, $p = 0.572$) and age distribution (t = 0.21, df = 459, $p = 0.837$) was apparent between cases and controls, signifying appropriate group matching.

The sample size was determined using Epi Info software (version 7.2.2.6). To establish a significant difference in the allelic frequency of Indel polymorphisms between patients and controls, a minimum sample size of 201 patients was required. This calculation was based on an assumed frequency of 35% for Del alleles, equal numbers of cases and controls, an expected strength of association of 1.50, a statistical power of 0.80, and a significance level of $\alpha = 0.05$. The study ultimately included 226 patients and 235 controls.

Considering that the gene pool of the Iranian population is highly heterogeneous,[41,42] in order to rule out this point in our study findings, we selected both BP patients and control subjects from a single gene pool. All study participants were Caucasian/Muslim residents of Shiraz (Fars province of Iran). The Shiraz University Ethics Committee approved the study, and informed consent was obtained from each participant.

Genes studied

The frequencies of Ins and Del alleles of the following genes were compared between BP cases and controls: *DBH* 19bp, *TPA* 311bp, *HLA-G* 14bp, *UCP2* 45bp, *MDM2* 40bp, *DHFR* 19bp, *LRPAP1* 37bp, *TP53* 16bp, *APOB* 9bp, *TLR2* 22bp, *SLC6A4* (*5-HTT*) 44bp, *PDCD6IP* 15bp, *VEGF* 18bp, *ADRA2B* 9bp, and *FADS2* 22bp.

Estimation of allelic frequencies

Genomic DNA was extracted from blood samples using the boiling method.[43] Here we employed a pooled method for estimating allelic frequency. This method relies on a strong relationship between the intensity of the insertion/deletion allele bands on gel electrophoresis and the relative amount of genomic DNA. Therefore, the accuracy of the correlation between DNA amount and band intensity was investigated using serial dilutions of DNA samples. The linear regression slope was calculated using a no-intercept model and was determined to be 0.998, demonstrating the high efficacy of the method in estimating the relative amount of DNA by analyzing band intensity. Band intensity was measured using ImageJ software version 1.53e. To estimate allelic frequencies, the intensity of Ins and Del bands was analyzed as described previously.[44]

Two sets of pooled genomic DNA samples were prepared by combining equal amounts of extracted DNA: one from the control group and the other from the patient group. For estimating allelic frequencies, pooled DNA samples were used for polymerase chain reaction (PCR) from BP patients and controls along with DNA from a heterozygous individual. The heterozygous genotype for each Indel polymorphism served as a calibration sample.[44]

The PCR-specific forward and reverse primers are available in Table 1. After PCR, two insertion and deletion (Ins and Del) allele bands were observed on gel electrophoresis. The intensities of the bands were measured in both pooled patient and control samples, as well as a heterozygote sample. The allele frequencies in the pooled samples were estimated utilizing the previously described formula.[44] Three replicates of PCR, gel electrophoresis, and band intensity measurements were performed.

Data pooling

Eligible publications were identified by searching PubMed, Scopus, and DOAJ databases using the following keywords: "*HLA-G* 14 bp Indel" and "bipolar disorder". The last search was updated on July 15, 2023. The search was not limited by language. For each study, the following information was extracted: author's surname, year of publication, country in which the study was conducted, number of cases and controls, and number of cases and controls with respect to the genotypes of the *HLA-G* 14 bp Indel polymorphism. The data were extracted by the investigators, and there was no discrepancy between the two extractions. Heterogeneity among studies was measured by Cochran's Q and I2 statistics. The random-effects model was used when heterogeneity between studies was present, and the fixed-effects model was used when heterogeneity was not present.

Statistical analysis

For each polymorphic site, we calculated the number of Ins and Del alleles by multiplying the relative frequency of each allele by twice the number of participants. We used the odds ratio (OR) and its corresponding 95% confidence interval (CI) to estimate the strength of the association between each polymorphic site and the risk of bipolar disorder type 1. It is important to note that for all comparisons, the insertion (Ins) allele was used as the reference

Table 1. The specific forward and reverse primers and PCR product

PCR product length (bp)	Primer sequences	OMIM	Loci
93/84	F: 5′ ACCGGCCCTGGCGCCCGCCAGCA 3′ R: 5′ CAGCTGGCGATGGACCCGCCGA 3′	107730	*APOB*
112/103	F: 5′ CAAGCTGAGGCCGGAGACACT 3′ R: 5′ AGGGTGTTTGTGGGGCATCT 3′	104260	*ADRA2B*
227/212	F: 5′ CCTTAAGGTCTGTGTCAACC 3′ R: 5′ TATTCCTCCACTCGAACAAC 3′	308074	*PDCD6IP*
222/185	F: 5′ AGTGTGCGTGGAGCCTATG 3′ R: 5′ GGTGTTTCTGGACACAAAGGA 3′	104225	*LRPAP1*
286/264	F: 5′ CACGGAGGCAGCGAGAAA 3′ R: 5′ CTGGGCCGTGCAAAGAAG 3′	603028	*TLR2*
119/100	F: 5′ CGCAAGTCTGGCCCCATC 3′ R: 5′ TCAGGTATCTGCCGGGCC 3′	126060	*DHFR*
230/212	F: 5′ GCTGAGGATGGGGCTGACTAGGTA 3′ R: 5′ GTTTCTGACCTGGCTATTTCCAGG 3′	199240	*VEGF*
224/210	F: 5′ GTGATGGGCTGTTTAAAGTGTCACC 3′ R: 5′ GGAAGGAATGCAGTTCAGCATGA 3′	142871	*HLA-G*
424/113	F: 5′ GTAAGAGTTCCGTAACAGGACAGCT 3′ R: 5′ CCCCACCCTAGGAGAACTTCTCTTT 3′	173370	*TPA*
166/147	F: 5′ TGCAAAAATCAGGCACATGC 3′ R: 5′ TCCAATAATTTGGCCTCAATC 3′	609312	*DBH*
502/457	F: 5′ CAGTGAGGGAAGTGGGAGG 3′ R: 5′ GGGGCAGGACGAAGATTC 3′	601693	*UCP2*
629/607	F: 5′ TTTCTCAAAGGCCGTGGTGT 3′ R: 5′ AGTGCTAACCACTCCTGGAA 3′	606149	*FADS2*
262/222	F: 5′ TTTCCTTTCTGGTAGGCTGG 3′ R: 5′ CACCTACTTTCCCACAGAGA 3′	164785	*MDM2*
195/179	F: 5′ TCAAATCATCCATTGCTTGG 3′ R: 5′ TGGGACTGACTTTCTGCTCTT 3′	191170	*TP53*
310/266	F: 5′ GACATAATCTGTCTTCTGGCCTCTCAAG 3′ R: 5′ CAATGTCTGGCGCTTCCCCTACATAT 3′	182138	*SLC6A4*

OMIM, Online Mendelian Inheritance in Man; PCR, polymerase chain reaction.

allele (OR = 1). Data analysis was conducted utilizing SPSS software version 19, with a significance level set at a *p*-value below 0.05.

Ethical statement

The procedures followed in this case-control study were approved by the Ethics Committee of Shiraz University in correspondence with the ethical guidelines of the Declaration of Helsinki (as revised in 2013). Informed consent was obtained from all participants before enrolled in this study.

Results

Table 2 summarizes the results of the study, demonstrating a significant association between BP and the *HLA-G* polymorphism. Specifically, the deletion allele is associated with an increased risk of BP (OR = 1.43, 95% CI = 1.10–1.85, $p = 0.007$). The remaining 14 Indel polymorphisms show no significant association with BP risk.

A search of PubMed, Scopus, and DOAJ databases revealed two studies investigating the association between the *HLA-G* 14 bp Indel and BP risk, conducted in France and India.[15,16] Although the researchers reported that they observed a significant difference in genotype prevalence between BP patients and controls, they failed to find a significant difference in allelic frequencies. We extracted the raw data of allelic frequencies from these reports and performed a small meta-analysis. Our analysis showed that there was no significant heterogeneity among these three studies (Q statistic = 0.53, df = 2, $p = 0.838$, $I^2 = 0.00$). The fixed-effect model was used to estimate the pooled OR and its 95% CI, which showed a strong association between the Del allele and BP risk (OR = 1.34, 95% CI: 1.15–1.57, $p < 0.001$; Fig. 1). A sensitivity test was performed by removing each study from the analysis, and the pooled ORs remained unchanged.

Discussion

Our findings indicate a significant association between the 14bp *HLA-G* polymorphism and BP risk. BP is a multifactorial disorder arising from a combination of genetic and environmental

Table 2. Association between 15 Indel polymorphisms and the risk of bipolar disorder

Polymorphisms	Alleles	Cases	Controls	OR	95% CI	*p*-value
MDM2 Indel 40bp	Ins	(75.56) 342	(72.70) 342	1.0	–	–
	Del	(24.43) 110	(27.30) 128	0.859	0.639–1.155	0.315
VEGF Indel 18bp	Ins	(40.37) 182	(42.00) 197	1.0	–	–
	Del	(59.62) 270	(58.00) 273	1.071	0.823–1.392	0.611
TP53 Indel 16bp	Ins	(30.94) 140	(34.42) 162	1.0	–	–
	Del	(69.05) 312	(65.57) 308	1.172	0.890–1.544	0.259
PDCD6IP Indel 15bp	Ins	(26.40) 119	(29.23) 137	1.0	–	–
	Del	(73.59) 333	(70.76) 333	1.151	0.862–1.537	0.339
HLA-G Indel 14bp	Ins	(46.15) 209	(55.15) 259	1.0	–	–
	Del	(53.84) 243	(44.87) 211	1.427	1.101–1.850	0.007
TLR2 Indel 22bp	Ins	(77.16) 349	(74.17) 349	1.0	–	–
	Del	(22.83) 103	(25.82) 121	0.851	0.630–1.151	0.296
UCP2 Indel 45bp	Ins	(25.50) 115	(25.83) 121	1.0	–	–
	Del	(74.51) 337	(74.16) 349	1.016	0.756–1.366	0.916
DHFR Indel 19bp	Ins	(60.00) 271	(61.02) 287	1.0	–	–
	Del	(40.00) 181	(39.07) 183	1.047	0.804–1.364	0.731
FADS2 Indel 22bp	Ins	(53.90) 244	(54.03) 254	1.0	–	–
	Del	(46.10) 208	(45.96) 216	1.002	0.774–1.299	0.985
DBH Indel 19bp	Ins	(52.27) 236	(51.70) 243	1.0	–	–
	Del	(47.72) 216	(48.30) 227	0.980	0.757–1.296	0.877
ADRA2B Indel 9bp	Ins	(56.07) 253	(51.26) 241	1.0	–	–
	Del	(43.93) 199	(48.73) 229	0.828	0.639–1.073	0.153
TPA Indel 311bp	Ins	(52.20) 236	(46.32) 218	1.0	–	–
	Del	(47.80) 216	(53.67) 252	0.792	0.611–1.026	0.077
SLC6A4 Indel 44bp	Ins	(61.34) 277	(61.82) 291	1.0	–	–
	Del	(38.65) 175	(38.17) 179	1.027	0.788–1.339	0.844
LRPAP1 Indel 37bp	Ins	(14.67) 66	(14.55) 68	1.0	–	–
	Del	(85.32) 386	(85.44) 402	0.989	0.686–1.427	0.954
APOB Indel 9bp	Ins	(71.35) 323	(73.40) 345	1.0	–	–
	Del	(28.64) 129	(26.59) 125	1.102	0.826–1.472	0.509

CI, confidence interval; OR, odds ratio.

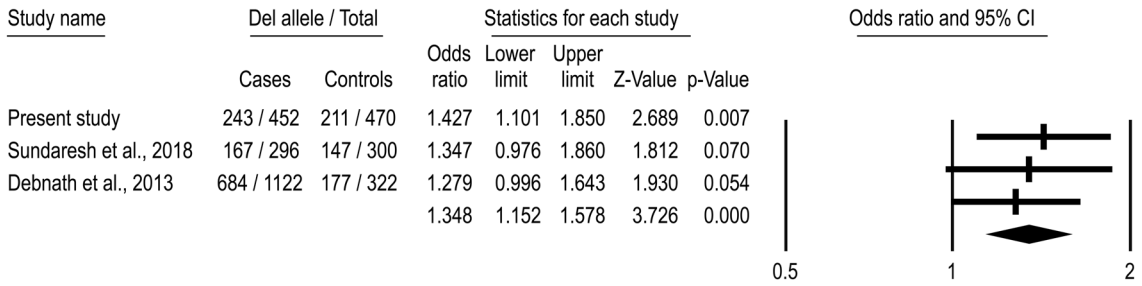

Fig. 1. Forest plot of case-control studies used for meta-analysis to examine the association between the Del versus Ins allele of the 14 bp Indel in the *HLA-G* gene and the risk of bipolar disorder.

factors.[2,3] A non-classical HLA class I molecule critical to the immune system is encoded by the *HLA-G* gene, playing an important role in viral infections.[45] Furthermore, evidence suggests that infectious diseases and impaired immune regulation could be implicated in BD pathogenesis.[46–50] Therefore, it is reasonable to suggest that modifications in gene function may heighten BP risk. Studies indicate that this genetic polymorphism impacts the stability of HLA-G mRNA species and consequently the level of circulating sHLA-G. The Del allele is linked to lower levels of both HLA-G mRNA and circulating sHLA-G isoforms, as suggested by researchers.[51–53] Moreover, some studies have suggested that reduced HLA-G expression may impair the effectiveness of immune tolerance mechanisms.

Recently, we found a positive association between the Del allele of the *HLA-G* 14bp Indel and schizophrenia risk.[17] Our current research provides further evidence suggesting *HLA-G* as a common polymorphic locus for both BP and schizophrenia, alongside previously identified loci.[33,34]

The *SLC6A4* polymorphism, previously referred to as the 44-bp insertion/deletion 5-*HTTLPR*, has been extensively studied in multifactorial traits, including BP.[24–26] Consistent with our current finding, a number of studies have failed to find a significant association between this polymorphism and BP risk.[24–26] The other Indel polymorphic loci examined in the current study, not significantly associated with BP risk, have not been previously studied.

Finally, it should be noted that this study has an important limitation that needs to be addressed. As described in the Methods section, pooled samples were used to estimate allele frequencies in both cases and controls. Although the use of this pooled sample method is associated with cost and time savings, researchers can easily study a larger number of genetic polymorphisms. In this study, we investigate the association between a substantial number of indel polymorphisms and the risk of BP. However, without information on the genotypes and risk factors of the subjects, it is impossible to study potential interactive effects between associated genes and risk factors.

Conclusions

In the current study, the Del allele of the *HLA-G* 14bp Indel polymorphism was positively associated with the risk of BP. The *HLA-G* gene encodes an important protein involved in the immune response, playing a significant role in viral infections. The Del allele is associated with lower levels of both HLA-G mRNA and circulating sHLA-G isoforms, potentially impairing the effectiveness of immune tolerance mechanisms. Further genetic association studies using case-control designs are needed to establish Indel polymorphisms as possible risk factors for bipolar disorder.

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Conflict of interest

The authors declare no conflict of interests.

Author contributions

Genotyping and data collection, data analysis, and paper writing (FZ), data interpretation (FZ, MS), study conception and design (MS). All authors have read and approved the final version of the manuscript.

Data sharing statement

No additional data or information is available for this paper.

Ethical statement

The procedures followed in this case-control study were approved by the Ethics Committee of Shiraz University in correspondence with the ethical guidelines of the Declaration of Helsinki (as revised in 2013). Informed consent was obtained from all participants before enrolled in this study.

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