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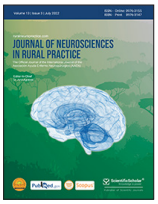

Original Article

Matrixmetalloproteinase-9 gene polymorphism (rs 17576) increases the risk of depressive symptoms in bipolar disorder

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ABSTRACT

Objectives: Plasticity of neural synapses is known to be involved in the complications in bipolar disorder (BD) patients. Matrix metalloproteinases (MMPs) play a role in synaptic plasticity and memory. Even though elevated MMP-9 levels are reported in neuropsychiatric disorders, there is limited data about MMP-9 gene polymorphism in BD. The objectives of the study was to investigate genotype frequency and allele frequency of MMP-9 genetic variant (rs 17576) in BD and its association with disease severity.

Materials and Methods: Eighty BD cases and 80 controls were recruited in the study. MMP-9 genotyping and allele frequency and plasma MMP-9 levels were analyzed in both the groups. Hamilton depression rating scale and Young's Mania Rating Scale (YMRS) were used to evaluate severity of BD.

Results: The genotype and minor allele (G allele) frequency were not significant between BD and controls. MMP-9 levels were significantly increased in BD patients with AG ($P < 0.001$) and GG ($P = 0.022$) genotypes compared to controls. BD patients with GG genotype ($P = 0.038$, OR: 3.26 (1.16–9.09), and G (mutant) allele ($P = 0.013$, OR 2.03(1.18–3.48) confer increased risk of depressive symptoms. MMP-9 was positively correlated with YMRS scale ($r = 0.227$, $P = 0.043$) in BD.

Conclusion: MMP-9 gene polymorphism (rs 17576) is linked with depressive symptoms in BD.

Keywords: Bipolar disorder, Matrix metalloproteinase, Synaptic plasticity

INTRODUCTION

The prevalence of bipolar disorder (BD) is increasing due to its early-onset resulting in considerable morbidity.[1] Disturbed neural development and neural plasticity which involve alteration in the regulation of neurotrophic factors are postulated to be involved in the development of BD.[2,3]

Matrix metalloproteinases (MMPs) are endopeptidases, which cause cleavage of extracellular matrix proteins.[4] MMPs are known to involve in the development of synapses in hippocampus and memory.[3] MMP-9 plays a crucial role in the development of neural plasticity[3] and reported to be associated with the pathophysiology of several neuropsychiatric disorders. Elevated MMP-9 levels are demonstrated in schizophrenia,[5] but the data regarding the same in BD is lacking.

The MMP-9 gene is positioned on chromosome 20 and contains 13 exons and 12 introns.[6] Polymorphisms of matrix metalloproteinase genes in the coding region are known to affect their expression leading to alteration in the activity of MMPs. Among several polymorphisms of MMP-9 gene, rs 17576 variant (A>G) (Arg 279 Gln) has been found to be associated with cardiac disease and diabetes mellitus.[6,7]

MMP-9 gene polymorphism was found to increase the risk of BD in the earlier studies.[8] A recent study has reported elevated serum MMP-9 levels during depression in young patients and indicated that it can be used as a marker for BD staging.[9] To the best of our knowledge, the prevalence of A/G (rs 17576) MMP-9 gene polymorphisms and its association with disease severity has not been previously investigated in BD. The present of the study was designed to analyze the allele frequency and genotype frequency of MMP-9 genetic variant (rs17576) and plasma MMP-9 levels and their relation with disease severity in South Indian population with BD.

MATERIALS AND METHODS

The current study was a case–control genetic study carried out in tertiary care hospital. The Institute Ethics Committee approval was obtained before study (IEC/2017/0352). The study protocol was explained to participants/legally

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acceptable representatives of participants and written informed consent (in English and local language) was obtained from them. Eighty consecutive BD I patients diagnosed as per structured clinical interview for DSM-5 axis 1 disorder (SCID I), aged between 20 and 50 years and 80 apparently healthy controls were included in the study. Subjects with medical and neurological comorbidities, any substances use in the past 12 weeks and previous history of significant head trauma were excluded from the study.

Clinical workup

Symptom severity of BD was assessed by following questionnaires.

For Mania – Youngs Mania Rating score (YMRS) was used.

For depression – Hamilton Depression rating scale (HDRS) was used.

The diagnosis and assessment of disease severity were made by a consultant in Psychiatry department.

Collection of samples

A 5 ml of peripheral venous blood was collected from each study participants and blood sample was transferred to a polypropylene centrifuge tube (Tarson-15 ml) containing 100 µl of 10% Na_2EDTA (Disodium Ethylenediamine-tetra-acetic acid). After centrifugation, the supernatant plasma was stored at −40°C and used for the estimation of MMP-9 by ELISA. The cellular component of the blood sample was stored in −40°C deep freezer and used for DNA extraction and genotyping.

DNA extraction and quantification

DNA was extracted using reagent kits from QIAamp DNA Mini Kit lot number160044812 (Qiagen, Germany). The quality and quantity of the extracted DNA were checked using Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, DE 19810, USA).

Genotyping of MMP-9 (rs17576) by real-time PCR

Screening of the MMP-9 (rs17576) polymorphism was done using TaqMan 5′nuclease assay. The allele specific primers (CTCCTCGCCCCAGGACTCTACACCC[A/G] GGACGGCAATGCTGATGGGAAACCC) and fluorogenic oligonucleotide probes were procured from Helini Biomolecules, India. The genotyping was carried out using standard protocol and analyzed using CFX96 Touch™real time PCR detection system (Bio-Rad Laboratories, California, USA). The genotypes were discriminated based on the emitted fluorescence from the corresponding fluorescent dyes (FAM and HEX). Technical or observational error was ruled out by replicating 30% of the randomly selected samples.

Estimation of MMP-9

MMP-9 was estimated by ELISA using reagent kit from Elabscience, USA.

Statistical analysis

Genotyping and allele frequencies in cases and controls were analyzed by direct counting and tested for Hardy–Weinberg equilibrium by Chi-square test. Chi-square test was used to calculate Odds ratios and 95% confidence intervals. One-way ANOVA was used to assess MMP-9 levels among various genotypes of BD.

The results of continuous variables such as MMP-9, age, and duration of disease were expressed as mean ± Standard deviation or median (range). The normality of the data was tested by Kolmogorov–Smirnov test. The Mann–Whitney U-test was done to assess the differences in plasma MMP-9 levels between control and cases. Spearman correlation test was used to assess the association of MMP-9 with disease severity, age, and duration of disease. $P < 0.05$ was considered significant. SPSS, Version 16.0 (Armonk, New York, USA), and GraphPad Prism version 5.00 (San Diego, California, USA) were used for analysis of data.

RESULTS

[Table 1] shows general characteristics, clinical characteristics, and MMP-9 levels in controls and cases. In the present study, age and body mass index were reduced in BD compared to controls. About 95% of controls and 86% of cases were of poor socioeconomic status and 43 cases and 66 controls were non-graduates. MMP-9 levels were elevated ($P < 0.001$) in BD cases in comparison with controls.

Genotyping and allele frequency data of rs 17576 polymorphism between controls and BD cases are shown in [Table 2]. The genotype frequencies in MMP-9 SNP (rs 17576) were found to be AA (20%), AG (42%), and GG (38%) in patients with BD and in controls, it was found to be AA (31%), AG (40%), and GG (29%), respectively. We did not observe any significant difference in genotype frequencies of reference genotype (AA) with mutant genotype (GG) ($P = 0.71$) and heterozygote genotype (AG) ($P = 0.28$). The minor allele frequency (G) was found to be 49% in controls and 59% in cases and it was not significant ($P = 0.09$).

MMP-9 levels were assessed between various genotypes among patients with BD and healthy controls. MMP-9 was significantly increased in BD patients with AG ($P < 0.001$) and GG genotype ($P = 0.022$) compared to controls. Within

Table 1: Age, body mass index, and general characteristics in controls and bipolar disorder cases.

Parameters	Control (*n*=80)	Bipolar disorder (*n*=80)	*P*-value
Age (years)	37.6±7.6	34.2±9.8	0.015*
Gender (Male/Female)	34/46	31/49	
Body mass index (kg/m^2)	26.46±2.8	24.58±3.3	0.029*
Socioeconomic status (APL/BPL)	4/76	11/69	
Education (graduate/non-graduate)	14/66	37/43	
Duration of disease (years)	----	5 (1–31)	
Age at onset (years)	----	25 (18–44)	
YMRS	----	2.35±2.99	
HDRS	----	2.9±3.1	
Suicidal ideation/behavior	----	10	
Treatment	----		
Lithium monotherapy		47	
Lithium combination therapy		33	
Matrix metalloproteinase-9 (µg/L)	571.47 (54.87–2067.7)	963.75 (179.20–7816.2)	<0.001*

HDRS: Hamilton depression rating scale

Table 2: Genotyping and allele frequency data of rs17576 polymorphism between controls and bipolar disorder cases.

rs17576	Controls (*n*=80) (%)	Bipolar disorder (*n*=80) (%)	*P*-value	OR (95% CI) (%)
Genotype				
AA	25 (31)	16 (20)		
AG	32 (40)	34 (42)	0.2893	0.6062 (0.2729–1.330)
GG	23 (29)	30 (38)	0.7126	1.228 (0.5934–2.539)
Allele				
A (wild)	82 (51)	66 (41)	0.0926	0.6679 (0.4293–1.039)
G (Mutant)	78 (49)	94 (59)		
Dominant model				
GG+AG	55 (69)	64 (80)	0.1474	1.818 (0.8817–3.750)
AA	25 (31)	16 (20)		
Recessive model				
AA+AG	57 (71)	50 (62)	0.3135	0.6725 (0.3466–1.305)
GG	23 (29)	30 (38)		
Co-dominant model				
AG	32 (40)	34 (42)	0.8724	0.9020 (4.804–1.693)
AA+GG	48 (60)	46 (58)		
Homozygote model				
AA	25 (31)	16 (20)	0.1381	0.4907 (0.2139–1.125)
GG	23 (29)	30 (38)		

CI: Confidence interval

the control (P = 0.589) and BD group (P = 0.772), we did not findf any significant difference among various genotypes.

Genotype and allele frequency of MMP-9 gene in controls and BD patients with depressive symptoms are shown in [Table 3]. The genotype frequencies in MMP-9 SNP (rs 17576) were found to be AA (16%), AG (36%), and GG (48%) in BD patients with depressive symptoms and in controls, it was found to be AA (31%), AG (40%), and GG (29%), respectively. The significant increase in the frequency of GG genotype confers increased risk of depressive symptoms in BD patients (P = 0.038, OR: 3.26 [1.16–9.09]). Furthermore, we found that BD patients with G (mutant) allele are at higher risk of developing depressive symptoms (P = 0.013, OR 2.03 [1.18–3.48]).

When MMP-9 levels were analyzed in BD patients with depressive and maniac symptoms, we found that MMP-9 levels were significantly increased in BD patients with depressive symptoms (P = 0.041) and maniac symptoms (P <0.001) when compared with controls. Within the BD group, patients with maniac symptoms had significantly higher MMP-9 levels compared to those with depressive symptoms (P = 0.008).

Table 3: Genotype and allele frequency of MMP-9 gene in controls and bipolar disorder patients with depressive symptoms.

Genotype	Controls (*n*=80) (%)	Bipolar disorder with depressive symptoms (*n*=44) (%)	Odds ratio (Confidence interval)	*P*-value
AA	25 (31)	7 (16)		
AG	32 (40)	16 (36)	1.78 (0.63–5.0)	0.39
GG	23 (29)	21 (48)	3.26 (1.16–9.09)	0.038*
A	82 (51)	30 (24)		
G	78 (49)	58 (47)	2.03 (1.18–3.48)	0.013*

Genotype or allele frequency was not significantly altered with maniac symptoms of BD when compared with controls (Data not shown).

When we did correlation analysis of MMP-9 with disease severity, we found that MMP-9 was significantly associated with YMRS (r = 0.227, $P = 0.043$) in patients with BD. MMP-9 was not significantly correlated with HDRS, BMI, age, and duration of disease. Furthermore, we found positive association between age and duration of disease in BD (r = 0.652, $P = 0.001$).

DISCUSSION

MMPs are involved in organizing the activity of nervous system.[10] Enhanced activity of MMPs has been reported during demyelination, neuroinflammation, and neurotoxicity and neurological conditions such as Parkinson disease and Alzheimer's disease.[10,11]

Several case–control molecular-genetic studies have reported conflicting findings regarding MMP-9 gene polymorphism and the schizophrenia risk,[12,13,14] but there is paucity of data about the same in BD. A recent study has analyzed the MMP-9 gene polymorphism in BD and did not find any significant association with treatment response.[8]

The present study evaluated the single nucleotide polymorphisms of MMP-9 gene, rs 17576 variant (A>G) (Arg 279 Gln), and its association with severity of BD. Hardy–Weinberg equilibrium showed that the genetic frequencies of all the genotypes were in concordance with it. There was no significant difference in genotype frequencies of reference genotype (AA) with mutant genotype (GG) ($P = 0.71$) and heterozygote genotype (AG) ($P = 0.28$). The minor allele frequency (G) was found to be 49% in controls and 59% in cases and it was not significant ($P = 0.09$).

Any mutation in the gene can influence the expression of the gene leading to alteration in its plasma levels. MMP-9 levels were found to be increased in BD in comparison with controls ($P < 0.001$) in our study and these findings were similar to an earlier study.[9] When MMP-9 levels were compared among various genotypes between controls and BD, we found that MMP-9 was enhanced in BD patients with AG ($P < 0.001$) and GG genotype ($P = 0.022$) compared to controls suggesting mutation in these genotypes may upregulate the expression of MMP-9, leading to its enhanced plasma levels. Within the control ($P = 0.589$) and BD group ($P = 0.772$), we did not find any significant difference among various genotypes.

To assess the association of genetic variants of MMP-9 (rs 17576) with disease severity, we analyzed genotype frequencies in BD patients with maniac and depressive episodes. The significant increase in the frequency of GG genotype confers increased risk of depressive symptoms in BD patients ($P = 0.038$, OR: 3.26 [1.16–9.09]). Furthermore, we found that BD patients with G (mutant) allele are at higher risk of developing depressive symptoms ($P = 0.013$, OR 2.03 [1.18–3.48]). We did not observe any association of genotype or allele frequency with maniac symptoms of BD.

When MMP-9 levels were compared between subgroups of BD and controls, we found that MMP-9 levels were significantly increased in BD patients with depressive symptoms ($P = 0.041$) and maniac symptoms ($P < 0.001$) when compared with controls. Within the BD group, patients with manic symptoms had significantly higher MMP-9 levels compared to those with depressive symptoms ($P = 0.008$). These findings were in contrast to an earlier study by Rybakowski *et al*. who reported elevated MMP-9 levels only in depressive episodes, but not in maniac episodes.[9] Spearman correlation test (r = 0.227, $P = 0.043$) and linear regression analysis revealed an association between MMP-9 and YMRS score indicating MMP-9 may increase disease severity in BD.

The main limitation of the study was newly diagnosed that BD cases were not included in the study as the patients were on treatment on lithium, valproate, risperidone, etc. Hence, the effect of these drugs on MMP-9 levels was not established. We studied only one genetic variant of MMP-9 due to financial constraints. Since the sample size was less, we did not apply any corrections for type I and type II errors.

CONCLUSION

The data from the present study show that MMP-9 is elevated in BD patients with maniac and depressive symptoms. Association of MMP-9 with YMRS score indicates MMP-9 increases severity of BD. Even though there was no significant difference in genotype and allelic frequency between BD and controls, we observed that BD patients with GG genotype

and G (mutant) allele of MMP-9 gene (rs 17576) are at higher risk of developing depressive symptoms. Furthermore, BD patients with AG and GG genotype exhibited increased MMP-9 levels compared to controls.

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Declaration of patient consent

Patients consent not required as there is no patient in this study.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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