Genetic Variations within Metalloproteinases Impact on the Prophylaxis of Depressive Phases in Bipolar Patients

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Key Words

Metalloproteinases · Bipolar disorder · Gene · Single-nucleotide polymorphism

Abstract

Background: The genetic background of the antidepressant response to pharmacological treatment in bipolar disorder (BD) remains elusive. This issue is of primary relevance in that the depressive phases of BD are difficult to treat and they are associated with suicide. **Aim:** We investigated the role of a set of genetic variations (single-nucleotide polymorphisms) harbored by matrix metalloproteinases (MMPs) as predictors of response to treatment in depressed BD patients. **Methods:** 654 BD patients from the publicly available Systematic Treatment Enhancement Program for Bipolar Disorder study were investigated. The outcome was the number of depressive events corrected by the number of times patients were assessed. Clinical and sociodemographic variables were tested as possible stratification factors and included in the analysis if necessary. Genetic predictors were 43 SNPs harbored by 17 MMPs. Imputation, quality check and pruning were conducted according to standards. Results were corrected for multitesting. **Results:** rs486055 (MMP-10) was associated with the outcome. TT homozygotes had 5.08 ± 3.51

events, CT had 3.47 ± 3.18 and CC had 2.57 ± 2.96 depressive events corrected for the times they had been assessed. The time during which BD patients were observed was not significantly different between the rs486055 genotypes. We found evidence that MMP-10 may be a mediator of the number of depressive phases during BD. Due to the limits of the study including the small-to-medium sample size, the naturalistic design and the possible occurrence of false-positive findings, independent analyses are warranted.

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Introduction

Metalloproteinases as Candidates for Bipolar Disorder

Matrix metalloproteinases (MMPs) are key enzymes to the extracellular matrix (ECM) that remodel the pericellular environment. Their activity is pivotal to the communication between cells, and it is central to the orchestration of the activity of the central nervous system [1]. MMPs are activated after the disruption of an interaction between a cysteine residue and the Zn^{2+} ion in their catalytic site. Organomercurial compounds, heavy metals, denaturing agents or oxidants and proteases can disrupt this interaction and activate the MMPs

[2]. The expression of MMPs is enhanced during many physiological conditions including wound healing, ovulation, blastocyst implantation, bone growth and angiogenesis. Inflammatory events enhance the expression of MMPs, and their activity is enhanced in many pathophysiological conditions including tumor invasion, rheumatoid arthritis, periodontal disease and atherosclerosis. The activity of MMPs increases also during blood-brain barrier dysfunction, demyelination, neuroinflammation and neurotoxicity. Consistently, MMPs are involved in a list of neurological diseases including multiple sclerosis, amyotrophic lateral sclerosis, Alzheimer's and Parkinson's diseases [1, 3, 4]. In particular, many ECM proteins, adhesion molecules, chemokines, receptors and growth factors interact during neuronal migration, survival, synaptogenesis and plasticity. Thus, ECMs may be good candidates in the investigation of the diseases of the central nervous system, including bipolar disorder (BD). BD is a complex and frequent (1% prevalence worldwide, which rises up to 6.4% when subthreshold cases are included [5]) neurological disease characterized by pathological mood swings, which results in tremendous social costs [6, 7]. Its biological causes are incompletely understood. As a consequence, the current pharmacological treatments do not target its molecular core and are only partially effective [7, 8]. The identification of the genetic causes of BD could pave the way to the synthesis of new and more efficacious compounds, and some lines of evidence stress the role of MMPs in BD: Rybakowski et al. [9] recently reported that MMP-9 serum levels were increased in a sample of 54 inpatients with BD (19 males, 35 females), aged 42 ± 14 years, compared to 29 subjects (15 males, 14 females), aged 40 ± 11 years, as a control group. Moreover, MMP-9 serum levels significantly correlated with the depressive phases in younger subjects (<45 years). The same group could not show a genetic impact of a variant – the –1562C/T MMP-9 gene polymorphism (rs3918242) – towards lithium prophylaxis in a sample of 109 BD patients [10]. In a previous study, the same group identified an impact of MMP-9 towards cognition in BD patients [11]. Nevertheless, conflicting results from other research groups that also focused on the possible role of MMPs in BD can be found [12], and the small sample size and the incomplete genetic coverage decreased the power of the studies that retrieved positive association findings. Thus, further studies are needed. In the present work, we extended the analysis of the genetic impact of a list of MMPs towards depressive phases of BD patients.

Methods

Sample under Investigation

The sample under investigation was retrieved from the publicly available Systematic Treatment Enhancement Program for Bipolar Disorder (STEP-BD) [13]. During the study, BD patients of every subtype with ages ≥ 15 years were entered into a study registry. All patients received a systematic assessment battery at entry and were treated by a psychiatrist using a series of model practice procedures consistent with expert recommendations. At every follow-up visit, the treating psychiatrist completed a standardized assessment and assigned an operationalized clinical status based on DSM-IV criteria. Patients had independent evaluations at regular intervals throughout the study and remained under the care of the same treating psychiatrist while making transitions between randomized care studies and the standard care treatment pathways. In the present study, we only considered records from the naturalistic first phase of the study. Table 1 reports the characteristics of the sample under analysis. BD I and BD II patients were all included in the analysis to increase the power of the study. No differentiation between BD I and BD II could be made due to the lack of data (number of manic and hypomanic phases in the past). This may be considered as a limitation of the study. Nevertheless, major depressive episodes are common traits between BD I and BD II patients, which offers a rationale to include all the patients in the same genetic association study when investigating depressive phases.

Definition of the Phenotype

The ratio between the times patients were found to be definitively depressed and the times patients were observed during the phase of the study under analysis was the outcome of choice. The correlations between the phenotype of choice and a set of other phenotypes calculated at standard time points (number of depressive phases from 30, 90, 120 and so forth days from the beginning of the study) – a more classical approach to this kind of studies – were calculated. We had confirmation that the phenotype under analysis significantly correlated with almost all the phenotypes at different time points, with the advantage of having 0% of missing values. Results are reported in table 2. The only time point at which the correlation was not significant was after 30 days from the beginning of the study. Nevertheless, the number of missing information for this time point (94%) may be held accountable for the lack of association. All patients were treated in a naturalistic environment according to the international guidelines for the treatment of BD. Thus, the authors interpreted the phenotype under analysis as a measure of the efficacy of the pharmacological treatments administered to patients. This phenotype could also be related to depressive phases per se, rather than to the impact of the pharmacological treatments. The genetic effect could then be related to the course of disease rather than to the impact of the pharmacological treatments.

Study of Stratification Factors

The sociodemographic and clinical variables were investigated as possible stratification factors. A story of treatment for alcohol-related problems was found to be significantly associated with the outcome as was included in the analysis. Treatment (combination of treatments) correlated with the number of depressive episodes during the period of observation and was included in the analysis.

Table 1. Sample description

Variable	Result	Association with outcome
Age	41.48±12.6 years	t = -0.6241, d.f. = 652, p value = 0.5328
Gender	female = 353 (54%) male = 300 (45.9%) transgender = 1 (0.2%)	f = 0.08, d.f. = 2, p value = 0.923
Ethnicity	Asian or Pacific Islander = 0 (0%) Black or African-American = 0 (0%) Native American, Eskimo or Aleut = 0 (0%) no primary race = 0 (0%) other, specify = 0 (0%) White or Caucasian = 654 (100%)	not calculable
Diagnosis	BD = 654 (100%)	not calculable
Times depressed/ times seen	2.86±3.06	
Past treatment for alcohol-related problems	not available = 43 (6.6%) no = 244 (37.3%) not on original form = 266 (40.7%) unknown = 26 (4%) yes = 75 (11.5%)	f = 2.54, d.f. = 4, p value = 0.038

d.f. = Degrees of freedom.

Table 3 reports the details of the treatments delivered to subjects under analysis during phase 1 of the study. The higher the number of depressive events during the period of observation, the higher the rate of administration of a combination of treatments with antipsychotics, mood stabilizers and antidepressants. The administration of an antidepressant alone did not correlate with the number of depressive events, consistent with the literature and guidelines in the field.

Power of the Study

We had sufficient power (0.80) to detect a small-to-medium effect size (0.22) between two allelic frequencies, each represented by at least 300 subjects (R-cran pwr package used for the analysis).

Selection of Genes

Genes that belong to the MMP family that were used for the analysis are reported in table 4. Imputation was run for the genes that belong to the pathway under analysis in order to decrease the computational effort. The CEU HapMap 1,000 genomes served for the analysis. From the original (not pruned) set of 203 single-nucleotide polymorphisms from the pathway under analysis we obtained 43 that passed the imputation quality control (info > 0.9) and the pruning ($r^2 > 0.2$). Pruning was undertaken after imputation.

Statistical Methods

Covariate linear regression was the statistical model for the analysis. PLINK was used for the analysis [14]. The p value for a significant result was set at $0.05/43 = 0.001$ (Bonferroni correction).

Results

rs486055 in the *MMP10* gene was found associated with the number of depressive events corrected for the number of total visits during the period of observation ($p = 0.0004$; $t = 3.68$). In particular, TT homozygotes ($n = 10$) had 5.08 ± 3.51 events, CT ($n = 131$) had 3.47 ± 3.18 and CC ($n = 359$) had 2.57 ± 2.96 depressive events corrected for times they had been assessed during the period of observation (Hardy-Weinberg equilibrium, $p = 0.58$). The time during which BD patients were observed during the period included in the analysis was not significantly different between the rs486055 genotypes (TT = 15 ± 10.28 ; CT = 20.49 ± 13.47 ; CC = 17.98 ± 13.57 ; $t = 1.18$; $p = 0.23$).

Discussion

We found evidence that the rs486055 C/T nonsynonymous (R53K) exonic variant in MMP-10 impacts on the number of depressive events during BD. The implication of this extracellular enzyme in response to treatment during bipolar depression is new in the literature. In the brain, MMPs comprise a family of over 20 endo-

Table 2. Analysis of the correlation between the number of depressive events per unit visit and the number of depressive events per defined time points

Days from baseline	Mean depressive events per time	Missing, %	Mean depressive events per visit unit	Pearson correlation coefficient	t	p	conf1	conf2
30	1.24±0.51	94.22	2.26±2.92	0.1	0.87	0.39	-0.13	0.32
60	1.41±0.77	88.85	2.26±2.92	0.22	3.12	0.002	0.08	0.35
90	1.48±0.98	83.72	2.26±2.92	0.3	5.4	0.0000001	0.19	0.4
120	1.61±1.14	78.61	2.26±2.92	0.4	8.63	<0.0000001	0.32	0.48
150	1.68±1.17	74.40	2.26±2.92	0.48	11.48	<0.0000001	0.4	0.54
180	1.77±1.24	70.53	2.26±2.92	0.55	14.49	<0.0000001	0.48	0.61
210	1.87±1.35	66.47	2.26±2.92	0.6	17.28	<0.0000001	0.54	0.65
240	1.95±1.42	62.90	2.26±2.92	0.62	18.97	<0.0000001	0.57	0.67
270	2.03±1.46	59.52	2.26±2.92	0.65	20.76	<0.0000001	0.6	0.69
300	2.13±1.53	55.98	2.26±2.92	0.68	23.26	<0.0000001	0.64	0.72
330	2.18±1.58	52.82	2.26±2.92	0.68	23.85	<0.0000001	0.64	0.72
360	2.22±1.63	49.97	2.26±2.92	0.69	24.25	<0.0000001	0.64	0.72
390	2.27±1.66	47.03	2.26±2.92	0.69	25.08	<0.0000001	0.65	0.73
420	2.31±1.71	44.37	2.26±2.92	0.71	26.88	<0.0000001	0.67	0.75
450	2.34±1.73	41.86	2.26±2.92	0.73	28.35	<0.0000001	0.69	0.76
480	2.37±1.76	39.42	2.26±2.92	0.73	29.11	<0.0000001	0.7	0.76
510	2.44±1.81	37.19	2.26±2.92	0.74	30.12	<0.0000001	0.71	0.77
540	2.49±1.85	34.88	2.26±2.92	0.75	30.68	<0.0000001	0.71	0.78
570	2.54±1.89	32.49	2.26±2.92	0.76	32.59	<0.0000001	0.73	0.79
600	2.58±1.94	30.47	2.26±2.92	0.78	34.37	<0.0000001	0.75	0.8
630	2.63±1.99	28.43	2.26±2.92	0.79	35.64	<0.0000001	0.76	0.81
660	2.68±2.04	26.59	2.26±2.92	0.79	35.95	<0.0000001	0.76	0.81
690	2.71±2.05	24.91	2.26±2.92	0.8	37.3	<0.0000001	0.77	0.82
720	2.74±2.07	23.29	2.26±2.92	0.81	39.17	<0.0000001	0.79	0.83
750	2.77±2.1	21.52	2.26±2.92	0.82	41.07	<0.0000001	0.8	0.84
780	2.81±2.12	20.03	2.26±2.92	0.83	42.78	<0.0000001	0.81	0.85
810	2.84±2.14	18.71	2.26±2.92	0.85	44.97	<0.0000001	0.82	0.86
840	2.86±2.16	17.34	2.26±2.92	0.85	45.75	<0.0000001	0.83	0.87
870	2.89±2.2	16.12	2.26±2.92	0.86	47.32	<0.0000001	0.84	0.87
900	2.93±2.24	14.96	2.26±2.92	0.86	49.28	<0.0000001	0.85	0.88
930	2.95±2.25	13.75	2.26±2.92	0.87	50.21	<0.0000001	0.85	0.88
960	2.97±2.27	12.68	2.26±2.92	0.87	51.75	<0.0000001	0.86	0.89
990	3±2.31	11.73	2.26±2.92	0.88	54.58	<0.0000001	0.87	0.9

Missing: percentage of subjects that lack the investigated value at a certain time point; conf1 = upper confidence limit; conf2 = lower confidence limit.

Table 3. Combination of drug classes during the period of observation and correlation with the outcome

Drug	Total	Dep.	Nondep.	Association with depressive events
0/0/ad	22.42±115	23.01±115.74	21.98±114.49	F = 2.019; p = 0.156
0/ap/0	29.7±137.62	25.99±127.57	32.46±144.61	F = 2.48; p = 0.116
0/ap/ad	16.03±96.29	17.33±103.11	15.07±90.91	F = 18.28; p = 2.07e-05
st/0/0	379.93±404.28	367.79±405.49	388.95±403.25	F = 5.688; p = 0.0172
st/0/ad	218.06±344.87	225.72±350.47	212.38±340.64	F = 39.82; p = 4.07e-10
st/ap/0	180.55±311.57	179.7±315.06	181.18±309.04	F = 26.72; p = 2.8e-07
st/ap/ad	97.83±240.76	105.28±248.1	92.3±235.07	F = 28.2; p = 1.33e-07

0 = No drug; ad = antidepressants; ap = antipsychotics; st = mood stabilizers; dep. = administered during depression; nondep. = administered outside depressive phases. Association: statistics of the association between the number of depressive events per visit unit and the kind of treatment administered to subjects. Any combinations that included antidepressants but not antidepressants alone were significantly (italicized in the table) associated with the number of depressive events per visit unit.

Table 4. Genes and single-nucleotide polymorphisms (SNPs) under analysis

Gene name	Gene name extended	Position	Length, bp	Start, bp	End, bp	Known SNPs, n	Available in the original data set, n	Available SNPs after quality control, imputation and pruning, n
<i>MMP10</i>	matrix metalloproteinase 10 (stromelysin 2)	11q22.2	10,127	102,641,233	102,651,359	300	16	13
<i>MMP11</i>	matrix metalloproteinase 11 (stromelysin 3)	22q11.23	16,091	24,110,413	24,126,503	281	6	4
<i>MMP12</i>	matrix metalloproteinase 12 (macrophage elastase)	11q22.2	12,301	102,733,464	102,745,764	401	7	0
<i>MMP13</i>	matrix metalloproteinase 13 (collagenase 3)	11q22.2	12,743	102,813,721	102,826,463	299	9	8
<i>MMP14</i>	matrix metalloproteinase 14 (membrane-inserted)	14q11.2	12,471	23,305,766	23,318,236	296	3	3
<i>MMP15</i>	matrix metalloproteinase 15 (membrane-inserted)	16q21	21,524	58,059,282	58,080,805	416	4	0
<i>MMP16</i>	matrix metalloproteinase 16 (membrane-inserted)	8q21.3	296,018	89,044,237	89,340,254	4,702	36	0
<i>MMP17</i>	matrix metalloproteinase 17 (membrane-inserted)	12q24.33	23,391	132,312,938	132,336,328	541	14	0
<i>MMP1</i>	matrix metalloproteinase 1 (interstitial collagenase)	11q22.2	8,326	102,660,641	102,668,966	269	12	8
<i>MMP20</i>	matrix metalloproteinase 20	11q22.2	48,498	102,447,566	102,496,063	1,158	19	0
<i>MMP21</i>	matrix metalloproteinase 21	10q26.13	9,369	127,455,022	127,464,390	233	3	0
<i>MMP24</i>	matrix metalloproteinase 24 (membrane-inserted)	20q11.22	50,348	33,814,457	33,864,804	871	10	0
<i>MMP2</i>	matrix metalloproteinase 2 (gelatinase A)	16q12.2	116,992	55,423,612	55,540,603	675	19	0
<i>MMP3</i>	matrix metalloproteinase 3 (stromelysin 1)	11q22.2	8,007	102,706,528	102,714,534	225	12	7
<i>MMP7</i>	matrix metalloproteinase 7 (matrilysin)	11q22.2	10,246	102,391,239	102,401,484	291	16	0
<i>MMP8</i>	matrix metalloproteinase 8 (neutrophil collagenase)	11q22.2	15,256	102,582,526	102,597,781	446	14	0
<i>MMP9</i>	matrix metalloproteinase 9 (gelatinase B)	20q13.12	7,654	44,637,547	44,645,200	426	3	0
Total, n						11,830	203	43
bp = Base pairs.								

peptidases synthesized and secreted by both neuronal and glial cells. These enzymes serve as effectors of many brain cellular functions, such as migration, cytotoxicity, tissue remodeling, cell behavior and cell-cell communication via degradation of ECM components. In particular, they play a critical role in the degradation and/or proteolytic processing of many ECM factors, including growth factors, cytokines, chemokines, adhesion molecules and other proteins of the cell surface, proteases, intercellular junction proteins and structural molecules. Moreover, MMPs have been implicated in numerous neurological diseases, including multiple sclerosis, amyotrophic lateral sclerosis and other neuromuscular diseases, neurodegenerative diseases, such as Alzheimer's disease and Huntington's disease, as well as epilepsy, addiction, fragile-X syndrome and autism [15–23]. The association of a polymorphism in an *MMP* gene and the BD is not surprising, as it has been previously demonstrated that the functional –1562C/T polymorphism of the *MMP9* gene segregates in BD [24]. Moreover, a polymorphism in *MMP9* was associated with schizophrenia, suggesting that the *MMP9* gene may be a common susceptibility gene to major psychoses [25], further supporting the overlap between the genetic loci and alleles that

predispose to these disorders [26, 27]. While the role of many MMPs, including MMP-2, MMP-3, MMP-7 and especially MMP-9, in synaptic plasticity, remodeling and spine morphology is well established [28–36], and their alteration is known to be associated with neuropathological and psychiatric conditions [22, 32, 33, 37, 38], MMP-10 has never been correlated before either with brain plasticity or with neuropathologies. On the other hand, MMP-10 is known to be upregulated in brain tumors [39, 40], ischemic neurons after stroke [41, 42] and cerebrospinal fluid of cerebral adrenoleukodystrophy [43], sustaining the role of this MMP in neurodegeneration. In agreement with that, a role of MMP-10 has been demonstrated in Huntington's disease, as MMP-10 is involved in the pathological cleavage of huntingtin, leading to the production of toxic huntingtin fragments in striatal neurons [19]. However, considering that there are now many lines of evidence showing that synaptic plasticity is dysfunctional in BD [44, 45] and that MMPs, including MMP-10, are involved in the tightly regulated proteolysis of many ECM substrates involved in synaptic plasticity, it is tempting to speculate that the correlation between the MMP-10 exonic variants and the number of depressive events could be related to the possible role of

MMP-10 in brain plasticity, through proteolysis of the ECM proteins involved in neurotransmission between neurons or between neurons and glial cells. In particular, the contribution of MMP-10 to brain inflammation, which is mainly related to the activity of the glial cells [46–48], and the relevance of neuroinflammation in BD [49–51], leads to a possible hypothesis of an MMP-10-related imbalance in neuron-glia interactions in synaptic plasticity alteration in BD somehow linked to neuroinflammation.

The present study is limited by the small-to-medium sample size that may have decreased the power of the study to the point that small but true genetic effects went undetected. To limit this caveat we designed a specific output for the analysis in order to enroll as many patients as possible. The demonstration that this outcome was significantly correlated with a number of other possible outcomes calculated with more classical methods, confirmed the validity of our analysis. Nevertheless, more independent studies are needed to confirm the role of rs486055 in shaping the risk of a nonresponse to common pharmacological treatments during the depressive phases of BD. Another possible limit of the study depends on the naturalistic design of the STEP-BD study. We could only control statistically for this caveat. Moreover, a higher external validity versus a lower internal validity is a classical advantage of a naturalistic design. For a more thorough analysis of this relevant methodological issue, please refer to Tunis et al. [52]. Our findings should be considered as suggestive, waiting for a confirmation by a more strict and classic pharmacogenetic approach. Finally, this kind of investigations is biased towards false-positive findings

[53]. We tried to face this limit by applying a strict threshold. Nevertheless, this approach, despite being statistically sound, may be ineffective concerning the true biological correlations.

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Disclosure Statement

The authors declare no conflicts of interest.

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