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ORIGINAL ARTICLE

Expression of matrix metalloproteinases in patients with bipolar disorder

Fábria Chiarani,[1,2] Gabriel Rodrigo Fries,[1,2] Laura Stertz,[1,2] Keila Maria Ceresér,[1] Angela T. S. Wyse,[2] Flávio Pereira Kapczinski,[1,2] Maurício Kunz[1]

[1]*Molecular Psychiatry Unit and National Science and Technology Institute for Translational Medicine (INCT-TM), Hospital de Clínicas de Porto Alegre (HCPA), Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil.* [2]*Graduate Program in Biological Sciences, Biochemistry, UFRGS, Porto Alegre, RS, Brazil.*

Objective: High cardiovascular mortality rates have been reported in patients with bipolar disorder (BD). Studies indicate that matrix metalloproteinases (MMPs) are implicated in cardiovascular diseases. We evaluated the expression pattern of MMP-2 and MMP-9 in blood from patients with BD during acute mania and after euthymia, in comparison with healthy controls.
Methods: Twenty patients and 20 controls were recruited and matched for sex and age. MMP messenger RNA (mRNA) levels were measured using real-time quantitative polymerase chain reaction (PCR). Body mass index (BMI) was calculated for all subjects.
Results: There were no significant differences in MMP-2 and MMP-9 mRNA expression between patients and controls. mRNA levels were not significantly different during mania and euthymia. However, MMP-2 mRNA levels were negatively associated with BMI in BD patients and positively associated with BMI in controls. There was no difference in the pattern of MMP-9 expression between patients and controls.
Conclusions: Our results suggest a different pattern of association between MMP-2 and BMI in BD patients as compared with controls. Despite some study limitations, we believe that the role of MMPs in BD should be further investigated to elucidate its relationship with cardiovascular risk.

Keywords: Bipolar disorder; matrix metalloproteinases; cardiovascular disease; mania; euthymia

Introduction

Bipolar disorder (BD) is a mood disorder associated with cyclic episodes of mania and depression. BD is estimated to affect 4% of the population,[1] at a significant health care cost to society. It is also associated with high comorbidity rates, which, in turn, are associated with other general medical conditions.[2]

Adults with BD are at increased risk of premature cardiovascular disease, which is prevalent over a decade earlier than in the average population.[3] BD has been associated with high prevalence of cardiovascular disease (about 49%),[4] and patients with BD experience twice the cardiovascular mortality expected from general population estimates.[5] After suicide and accidents, cardiovascular and vascular diseases are the leading causes of death in this patient population.[5]

A prospective cohort study of participants with BD showed that (hypo)manic symptoms independently predicted cardiovascular mortality; the results also showed that mood disorders may mediate vascular disease, and that the mechanisms associated with this mediation of vascular disease are independent of and distinct from traditional risk factors.[6] Moreover, alterations in cardiac variability and predictability in BD may be state-dependent.[7] Although several studies have investigated the mechanisms that underlie high cardiovascular risk in BD patients, these mechanisms are not fully understood.

Matrix metalloproteinases (MMPs) are a group of endopeptidases produced by the structural components of the vascular wall and inflammatory cells, which have the capacity to cleave several components of the extracellular matrix (ECM). MMP-2 and MMP-9 cleave different bioactive molecules, including cytokines, chemokines, cell surface receptors, and growth factors,[8] contributing significantly to the pathogenesis of several cardiovascular diseases, such as atherosclerosis, hypertension, heart failure, and ischemic heart diseases.[9,10]

Pathological processes such as inflammation and oxidative or nitrosative stress play an important role in the expression, secretion, and activation of MMPs.[11] Thus, oxidative and inflammatory pathways may explain some of the clinical features of BD.[12]

In view of the foregoing, this study aimed to evaluate the expression of MMP-2 and MMP-9 messenger RNA (mRNA) in the blood of BD patients during manic episodes and after achievement of euthymia in comparison with healthy subjects.

Correspondence: Fábria Chiarani, Hospital de Clínicas de Porto Alegre, Laboratório de Psiquiatria Molecular, Rua Ramiro Barcelos, 2350, CEP 90035-903, Porto Alegre, RS, Brazil.
E-mail: fabriachiarani@yahoo.com.br
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Methods

Patients with BD were recruited from Hospital de Clínicas de Porto Alegre (HCPA), Porto Alegre, Brazil. All participants underwent a comprehensive clinical interview by a psychiatrist. The diagnosis of BD was established on the basis of the available clinical information and confirmed with a Structured Clinical Interview for DSM-IV – Axis I (SCID-I) and a standard protocol to assess patient psychopathology and clinical features. Baseline assessment, including blood sampling, was performed at admission to the psychiatric unit. Patients had to fulfill DSM-IV criteria for a manic episode for inclusion in the study. All patients received naturalistic treatment at the discretion of the attending psychiatrist and had no significant comorbid medical conditions. Mood symptoms were assessed using the Young Mania Rating Scale (YMRS)[13] and the Hamilton Depression Rating Scale (HAM-D), 21-item version.[14] A second assessment was carried out after remission of manic symptoms. Patients were considered euthymic if they presented scores < 7 on both the YMRS and HAM-D scales. BMI was defined as weight divided by height squared (kg/m^2).

Healthy controls had no personal history of neurological or psychiatric illness and no first-degree relatives with psychiatric disorders. Patients and controls were matched for age and sex and were free of clinically apparent cardiovascular diseases. The assessment of cardiovascular status was based on the patient's clinical history (self-reported data and data obtained from medical records) and no intake of medications used for the treatment of cardiovascular diseases.

Twenty patients and 20 healthy controls (10 males and 10 females in each group) participated in the study. Mean age was 38.35 ± 3.33 years in the BD group and 37.07 ± 3.64 years in the control group. All patients were taking at least one mood stabilizer (lithium, valproic acid) and one antipsychotic. Fifty percent of the patients were being treated with first-generation antipsychotics (haloperidol and chlorpromazine), and the others with atypical antipsychotics (risperidone, olanzapine, and clozapine).

The study was approved by the local Ethics Committee, and informed consent was obtained from all participants prior to their inclusion in the study.

RNA extraction and cDNA synthesis

Peripheral blood was collected from patients and healthy volunteers in PAXGene Blood RNA Tubes (PreAnalytiX). Total RNA was isolated from whole blood using the PAXGene Blood RNA Kit (Qiagen) according to manufacturer instructions. RNA concentrations were assessed using NanoDrop (Uniscience) equipment. RNA was converted to cDNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems) in a total reaction volume of 10 μL containing 2 μL of 10x RT buffer, 0.8 μL of 25x dNMT Mix (100 mM), 2 μL of 10x RT Random Primers, and 1 μL of MultiScribe Reverse Transcriptase (50 units/mL) (all according to manufacturer instructions). Reactions were carried out for 10 min at 25°C, 2 h at 37°C, and 5 s at 85°C. Subsequently, cDNA was kept at -20°C until it was used for polymerase chain reaction (PCR) amplification. MMP-2 and MMP-9 gene expression were measured by real-time quantitative reverse transcription-PCR (RT-PCR) using specific TaqMan FAM/MGB assays (Applied Biosystems, ID assay Hs01548727_m1 for MMP-2 and Hs00234579_m1 for MMP-9).

Expression values were normalized by β2M endogenous control expression using a TaqMan VIC/MGB endogenous control inventoried assay (Applied Biosystems, 4326319E). Reactions were performed in an Applied Biosystems 7500 Real-Time PCR System, which detects the PCR product directly without downstream processing. Reactions were carried out in a total volume of 12 μL with 6 μL of 2x TaqMan Gene Expression Master Mix (containing ROX, Amplitaq Gold DNA polimerase, AmpErase UNG, dATP, dCTP, dGTP, dUTP, and MgCl2), 0.6 μL of 20x TaqMan Gene Expression Assay, 0.6 μL of 20x TaqMan Endogenous Control, 3.8 μL of water, and 1 μL of cDNA solution. The cycling program consisted of 2 min at 50°C and 10 min at 95°C followed by 40 cycles of 15 s at 95°C and 1 min at 60°C. All reactions were performed in triplicate. Relative expression levels were determined by the ddCt method as described by Livak & Schmittgen.[15]

Statistical analysis

Statistical analyses were performed using SPSS version 18.0. Results were considered significant when $p < 0.05$. Normality tests were performed using the Shapiro-Wilks test. All comparisons between and within BD patients were performed using generalized estimating equations (GEE). Bonferroni's test was used for post-hoc analysis of the data. The gamma distribution was used due to the asymmetric profile of variables. BMI was included as a covariate in a regression model, and results are presented with and without the BMI factor. Data are expressed as mean and standard deviation (SD) or as median and interquartile range (IQR).

Results

The BMI of BD patients and controls was not statistically different, with a median BMI of 27.2 (19.5-58.9) for patients and 24.2 (17.1-31.6) for controls.

Median time between acute episodes and remission of symptoms in BD patients was 29.50 (20.50-58.00) days. The median HAM-D and YMRS scores in manic state were 3.50 (1.75-5.00) and 27.50 (19.00-30.70), respectively, vs. 1.00 (0.00-2.50) and 1.00 (0.00-4.00) in euthymia.

Table 1 shows the mRNA levels from BD patients and controls. MMP-2 and MMP-9 mRNA levels were not significantly different between BD patients and controls ($p = 0.933$ and $p = 0.268$, respectively). However, when adjusted for BMI, the expression pattern of MMP-2 was different between BD patients and controls ($p = 0.004$). Our results showed a negative association between BMI and MMP-2 mRNA levels (Figure 1). There was no

Table 1 Matrix metalloproteinase messenger RNA levels for patients with bipolar disorder and controls

	Bipolar disorder				
	Mania	Euthymia	Controls	p-value	Adjusted p-value*
MMP-2	1.183±0.410	1.000±0.367	1.026±0.218	0.933	0.004†
MMP-9	0.879±0.232	0.817±0.267	1.399±0.302	0.268	0.526

MMP = matrix metalloproteinase.
Results expressed as mean ± standard deviation.
* Adjusted by body mass index.
† Difference significant for $p < 0.05$.

difference in the expression pattern of MMP-9 between patients and controls when adjusted for BMI (p = 0.483) (Figure 2). MMP mRNA levels did not differ between the manic and euthymic states in BD patients.

Discussion

In this study, the expression pattern of MMP-2 was different between BD patients and controls when adjusted for BMI. The different MMP-2 mRNA levels found here may explain the connection between BD and somatic illnesses such as cardiovascular disease.

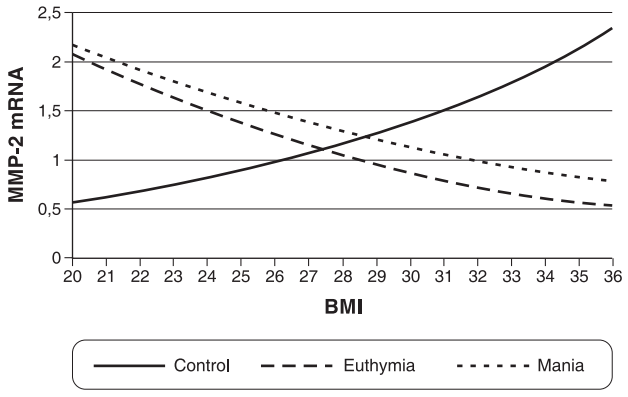

Figure 1 Effect of body mass index (BMI) on matrix metalloproteinase 2 expression in patients with bipolar disorder (during mania and euthymia) and controls

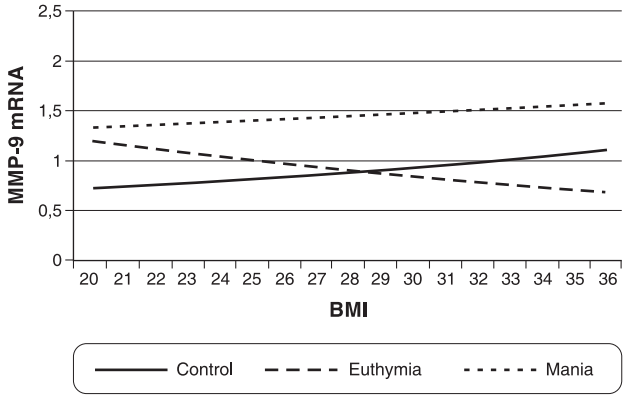

Figure 2 Effect of body mass index (BMI) on matrix metalloproteinase 9 expression in patients with bipolar disorder (during mania and euthymia) and controls

Tissue remodeling and inflammation are central to the process of cardiovascular diseases both in acute events, such as myocardial infarction and sudden death, and in chronic conditions, such as vascular atherosclerosis and heart failure.[16] Several MMPs have been implicated in tissue remodeling, and the equilibrium between MMPs and their endogenous inhibitors is critical for the maintenance of the integrity of the cardiovascular system.[17]

Inflammatory abnormalities have been associated with BD, and increased levels of inflammatory cytokines, particularly tumor necrosis factor-alpha (TNF-α) may play a critical role in BD.[18-20] Shifts in mood states are a key feature of BD. It has been suggested that immune system activation may vary across affective states.[21] Furthermore, oxidative stress plays a critical role in the pathophysiology of BD.[22] The formation of reactive oxygen species as well as the induction of signaling molecules, such as cytokines, cause the activation of MMPs,[23] which may represent a possible mechanism linking BD to the biological activation of MMP.

Recent studies have shown that patients with BD have a significant preponderance of the T allele vs. C allele of the 1562C/T polymorphism of the MMP-9 gene, suggesting that this polymorphism may mediate the association between BD and cardiovascular disease.[24,25] However, to our knowledge, this is the first study to report the expression of the *MMP2* and *MMP9* genes in whole blood of patients with BD, which is a group at increased risk of cardiovascular disease. Although mean BMI in our sample was not different between BD patients and controls, when we consider MMP-2 mRNA levels individually in relation to BMI, the expression pattern appears to be influenced differently by BMI. The results show that MMP-2 gene transcription changes as a function of BMI in BD patients, indicating that the profile of expression in patients is the opposite of that in controls. The influence of BMI suggests that MMP-2 expression in total blood is dependent on obesity status, corroborating the hypothesis that adipose tissue, in addition to storing energy, is an important endocrine organ that produces and secretes a variety of inflammatory cytokines, hormones, and other metabolic factors involved in the pathogenesis of atherosclerosis.[26]

Downregulation of MMP-2 activity has been demonstrated in premenopausal obese women, and is associated with attenuated ECM degradation and abnormalities of left ventricular function.[27] Although MMP-2 and MMP-9 are similar enzymes, our results corroborate a study that showed distinct promoter

elements and different spatial and temporal expression patterns of these proteases during ischemia/reperfusion injury.[11] It is possible that the type of upstream stimuli operative in BD will ultimately determine the transcriptional activity of selected MMP types.

Nonpharmacologic and pharmacologic factors alike contribute to higher BMI in mental illnesses. In BD patients, atypical depression, eating habits, behavior, physical inactivity, and lower basal metabolic rate during depression can influence the BMI.[28]

Some potential limitations of the present study include the small number of subjects, the lack of information about lifestyle factors (e.g., eating habits and physical activity), the heterogeneous of time to remission of symptoms, and the interference of drugs used by BD patients in the expression pattern of MMPs. Studies have shown that some antidepressants and mood stabilizers may regulate the expression and activity of MMP-2 and MMP-9 and of tissue inhibitors of the metalloproteinases (TIMPs) 1-4.[29,30]

An interesting future research direction would be study of the gene expression of major MMP-9 and MMP-2 inhibitors (TIMPs) and evaluation of MMP-9/TIMP-1 and MMP-2/TIMP-2 ratios. In a study by Rybakowski et al.,[31] increased levels of serum MMP-9 in bipolar illness were observed only in depressive episodes, and not in manic ones. Further studies should also be carried out to assess the circulating levels of MMP-2 in BD patients.

Belo et al.[32] found lower pro-MMP-2/TIMP-2 ratios in obese children and adolescents when compared to non-obese controls; conversely, the pro-MMP-9/TIMP-1 ratios were comparable in these groups. Patients with essential hypertension exhibit decreased MMP-2 and MMP-9 plasma levels and activity; this may reflect abnormal ECM metabolism. The increase in ECM deposition components on the arterial wall facilitates vascular fibrosis.[33] Derosa et al.[34] demonstrated that both MMP-2 and MMP-9 plasma levels are significantly higher in obese patients than in controls. The decrease in MMP-2 mRNA in response to a BMI increase may reflect feedback inhibition of MMP-2 transcription by the increase in circulating active MMP-2.[35]

Our study suggests a different pattern of expression of MMP-2 associated with BMI in patients with BD in comparison to healthy controls. The study contributes to understanding the association between inflammation and clinical comorbidity in BD. Nevertheless, further trials are necessary to clarify the relationship between MMPs and cardiovascular risk in BD. In addition, improved therapeutic methods are needed to address the effects of obesity on individuals with this mental illness. The involvement of adipose tissue and immune system abnormalities appears to be a potential biological target that will likely contribute to a better understanding of the mechanisms involved in BD pathophysiology.

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Disclosure

The authors report no conflicts of interest.

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