

Sex-dependent differences in matrix metalloproteinase-9 and inflammatory markers in postmortem brain of depressed subjects: Effect of pharmacological treatment and suicide

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Abstract

Major depressive disorder (MDD) is a complex neuropsychiatric disorder affected by genetic and environmental factors and associated with inflammation and impaired neuroplasticity. This study investigated the role of matrix metalloproteinase-9 (MMP-9), inflammatory markers (STAT3 and NF- κ B), and the neuroplasticity marker BDNF in the prefrontal cortex of subjects with MDD and their matched controls. The results showed increased MMP-9 expression and activity in MDD, particularly in those without detectable antidepressant treatment and in subjects who died by suicide. The association with suicide was especially pronounced in women, suggesting sex-dependent differences. Regarding inflammation, untreated subjects showed higher STAT3 and NF- κ B levels, while antidepressant-treated subjects showed reduced STAT3 levels, particularly men, suggesting a possible anti-inflammatory effect of antidepressants. In addition, treated men showed higher BDNF levels, which may reflect enhanced neuroplasticity. Overall, this study suggests that MMP-9 may be a potential marker of depression severity and suicidal behavior, especially in women, while neuroinflammation may play a more prominent role in men. These findings further highlight the importance of considering sex differences when investigating the role of MMP-9 in the pathophysiology of MDD.

INTRODUCTION

Major depressive disorder (MDD) is a neuropsychiatric disease that currently affects more than 332 million people worldwide, with women affected 50% more than men¹. The severity of anxiety and depressive symptoms is associated with suicide attempts². In 2021, more than 740 thousand people committed suicide³. The etiopathogenesis of MDD is not fully known, but several factors are involved in the complexity of the disease, including genetic and environmental factors^{4,5}.

Metalloproteinases (MMPs) are endopeptidases responsible for cleaving components from the extracellular matrix, including cell adhesion molecules, cell surface receptors, growth factors, cytokines, and other proteases, thereby regulating intracellular signaling networks⁶. MMP-9 is a gelatinase that plays a pivotal role in regulating synaptic plasticity, allowing the growth and maturation of dendritic spines^{7,8}. In fact, this matrix metalloproteinase can activate the brain-derived neurotrophic factor (BDNF)⁹ and modulate inflammatory processes by cleaving the inactive precursor forms of pro-inflammatory cytokines, such as the tumor necrosis factor- α (TNF α) and interleukin-1 β (IL-1 β)¹⁰. Previous preclinical studies suggested the implication of MMP-9 in the etiopathogenesis of depression. Different studies report increased MMP-9 mRNA and protein expression levels in blood samples of patients with depression^{11–13}, which were reverted following electroconvulsive therapy¹⁴. In *postmortem* hippocampal samples of MDD patients, Bijata et al.¹⁵ described increased MMP-9 activity, while Alaiyed et al.¹⁶ reported no differences in MMP-9 protein levels in the prefrontal cortex of untreated MDD patients, but an increase in antidepressant-treated MDD patients. An increase in the expression or activity of MMP-9 has also been reported in the brain of different animal models of depression, such as the chronic restraint stress model¹⁷, the chronic unpredictable stress model in mice¹⁵, the chronic

corticosterone mouse model¹⁸, and a neuroinflammation model induced by chronic lipopolysaccharide administration¹⁹. Moreover, we recently reported a sex-dependent role of MMP-9 in anxious and depressive-related behavior in MMP-9 knockout and overexpressing transgenic mice²⁰.

The *neuroinflammatory* hypothesis of depression advocates the role of inflammation in this disease. Several studies demonstrate the interrelation between inflammation and neuropsychiatric diseases, including depression^{21,22}. Patients with MDD present high levels of proinflammatory markers^{23,24}, which correspond to a reduction in markers associated with synaptic plasticity, such as BDNF^{25–28}. On the other hand, some anti-inflammatory agents displayed an antidepressant effect alone or as adjuvant treatment in MDD patients^{29–31}. In this regard, the mTOR pathway mediates the inhibition of nuclear factor- κ B (NF- κ B), thereby decreasing the level of pro-inflammatory cytokines, and the activation of the signal transducer and activator of transcription 3 (STAT3) pathway, which increases the level of anti-inflammatory cytokines³². It has been reported that the IL-6-induced activation of the STAT3 pathway reduces SERT expression in the hippocampus, which contributes to depressive-like behavior³³. In addition, the activation of the STAT3 pathway correlates with an increase in MMP-9 expression³⁴. Moreover, Xu et al.³⁵ have identified the HMGB1/STAT3/NF- κ B p65 axis as a potential mediator of microglial activation and autophagy in *postmortem* samples of the dorsolateral prefrontal cortex of men diagnosed with MDD. In fact, preclinical models of depression, such as the chronic corticosterone³⁶ and the lipopolysaccharide³⁷ mouse models, show an increase in NF- κ B. Moreover, high NF- κ B expression has been described in the serum of MDD patients³⁸. Interestingly, NF- κ B is not only involved in inflammatory processes but also regulates neurogenesis and synaptic plasticity, as it regulates the transcription of several genes³⁹.

On the other hand, stress induces a reduction in the expression of neurotrophic factors in the limbic system, such as BDNF, decreased synaptic density, and high cortical and hippocampal neuronal death, which would be responsible for depression⁴⁰. Clinical studies describe a decrease in the number of glial cells, loss of synapses, and neuronal density in the hippocampus and prefrontal cortex of depressed patients^{41,42}. Low BDNF levels in blood are correlated with depressive symptomatology and are reversed after treatment with antidepressant drugs⁴³. In parallel, BDNF levels are also decreased in several *postmortem* brain areas of MDD patients^{40,44–50}, and antidepressant treatments reverse this effect^{40,45}. Preclinical studies also report atrophy of neurons, glial loss, and reduced neurogenesis, especially in the hippocampus and prefrontal cortex of animal models of depression exposed to chronic stress^{51–53}.

Therefore, the objective of this study was to evaluate the activity and expression of matrix metalloproteinase 9 (MMP-9), and some key inflammatory and neuroplasticity markers in the prefrontal cortex of patients diagnosed with MDD, considering the potential effect of sex, drug treatment, and the commitment of suicide.

MATERIALS AND METHODS

1. HUMAN BRAIN SAMPLES

1. *POSTMORTEM* HUMAN BRAIN SAMPLES

Human samples from the prefrontal cortex (Brodmann's area 9) were collected at the autopsy performed by the Basque Institute of Legal Medicine, Bilbao, Spain. This was in accordance with protocols approved by the Human Studies Biomedical Research Ethics Committee of Cantabria. The brain samples were stored at -80°C until use. Cases ($n = 26$) had a prior diagnosis of MDD determined by their own psychiatrists and were classified as treated (AD treated) or non-treated (AD free) with antidepressant drugs, according to the *postmortem* toxicology report. MDD subjects were also classified by their cause of death into those who committed suicide and those who suffered a natural death (non-suicide). Control subjects ($n = 25-26$) were free of psychiatric or neurological disorders in anamnesis and suffered a natural death. Both groups were matched for sex, age, and *postmortem* interval. The demographic and clinical characteristics of each subject can be found in Table S1.

2. GEL ZYMOGRAPHY

2.1. Zymography protein extraction

Protein extraction was performed as previously described by Szklarczyk et al.⁵⁴. Briefly, the tissue was homogenized 1:20 (w/v) in sample buffer (10 mM CaCl_2 , 0.25% Triton X-100) and then centrifuged at 6,000xg for 30 min, at 4°C . The pellet was resuspended in 150 μl of pellet buffer (50 mM Tris-HCl, pH 7.4, 0.1 M CaCl_2), heated for 15 min at 60°C , and then centrifuged at 10,000xg for 30 min, at 4°C . Four μl of pellet buffer, containing 10% Triton X-114, was added to the supernatant. The protein was quantified using the DC™ Protein Assay, according to the manufacturer's protocol, and the samples were prepared using 4x Laemmli buffer. As a positive control, 2% FBS (Fetal Bovine Serum) was prepared in extraction buffer (2% Triton X-114, 10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 20 $\mu\text{l}/\text{ml}$ aprotinin, 10 $\mu\text{l}/\text{ml}$ PMSF) and 4x Laemmli buffer.

2.2. Gel zymography

One hundred μg of protein were loaded per duplicate on an 8.5% SDS-PAGE gel containing 0.1% gelatin. The gel was run at 100 V for 15 min, then 160 V for 50 min, in running buffer (20 mM Tris, 0.2 M glycine, 1% SDS) at 4°C . After the electrophoresis, the gel was washed twice in washing buffer (2.5% Triton X-100) at room temperature, for 15 min. The gel was incubated for 30 min at room temperature in the incubation buffer (50 mM Tris-HCl, pH 7.4, 200 mM NaCl, 6.7 mM CaCl_2 , 1 μM ZnCl_2 , 0.2% Brij35®). Later, the gel was incubated with fresh incubation buffer at 37°C for 7 days. Following this incubation, the gel was washed 3x5 min with distilled water, at room temperature. The gel was stained with staining solution (0.5% Coomassie Blue R-250, 5% methanol, and 10% acetic acid) for 1 h at room temperature and then destained (10% methanol, 5% acetic acid) at room temperature until bands of proteolytic activity were clearly visible. Finally, the gel was scanned using an Odyssey® CLx Imaging System (LI-

COR Biosciences, Lincoln, USA). The bands were quantified using the Image Studio™ Software (LI-COR Biosciences, Lincoln, USA).

3. WESTERN BLOT

3.1. Protein extraction

The protein extraction was performed according to the protocol previously described by Li et al.⁵⁵. Briefly, the prefrontal cortices were homogenized 1:15 (w/v) in homogenization buffer (0.32 M sucrose, 20 mM HEPES pH 7.4, 1 mM EDTA, 1:100 protease inhibitor cocktail, 5 mM NaF, and 1 mM Na₃VO₄), and centrifuged at 800xg for 10 min, at 4°C. The pellet (nuclear fraction) was resuspended in 150 µl of protein lysis buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1% Triton X-100, 0.1% SDS, 2 mM EDTA, 1 mM Na₃VO₄, 5 mM NaF, and 1:100 protease inhibitor cocktail) and sonicated for 1 min. The supernatant was centrifuged at 15,300xg for 10 min, at 4°C. Subsequently, the pellet (synaptoneurosomal fraction) was resuspended in 150 µl of protein lysis buffer and sonicated for 1 min. The protein concentration was quantified using the DC™ Protein Assay, according to the manufacturer's protocol. The samples were prepared with 2x Laemmli buffer containing β-mercaptoethanol, boiled at 100°C for 5 min, cooled in ice for 3 min, and centrifuged at 1,000xg for 5 min, at 4°C. The supernatant was stored at -20°C until used.

3.2. Western blot

Seventy µg of protein per sample was loaded per duplicate on 8.5% or 15% SDS-PAGE gels. The electrophoresis was run at 100 V for 15 min, followed by 160 V for 50 min, and then transferred to nitrocellulose membranes (GE Healthcare Europe GmbH, Munich, Germany). The membranes were blocked with 5% (w/v) powder skimmed milk in TBS-T (50 mM Tris-HCl pH 7.6, 150 mM NaCl, 0.05% Tween-20) for 1 h. For the anti-NF-κB antibody, the blocking solution used was 5% BSA in TBS-T 0.1 (with 0.1% Tween-20). The following primary antibodies were incubated with the corresponding blocking solution at 4°C overnight: BDNF (1:250, ab108319, abcam, Cambridge, MA, USA), MMP-9 (1:6.000, #102-16681, RayBiotech, Georgia, USA), NF-κB (1:1.000, #6959, Cell Signaling, Massachusetts, USA), STAT3 (1:1.000, #9139, Cell Signaling, Massachusetts, USA), PCNA (1:1 000, PA5-27214, Invitrogen, California, USA), and β-tubulin III (1:20.000, T2200/T8660, Sigma-Aldrich, Missouri, USA). The membranes were washed with TBS-T and then incubated with the corresponding secondary antibody conjugated to a NIR fluorophore (1:15,000, LI-COR Biosciences, Lincoln, NE, USA) for 1 h at room temperature. After washing, the specific signal was visualized using an Odyssey® CLx Imaging System (LI-COR Biosciences, Lincoln, USA) and quantified using the Image Studio™ Software (LI-COR Biosciences, Lincoln, USA). The densitometric values were normalized to the housekeeping protein β-tubulin III or PCNA. The expression of these housekeeping proteins is stable across experimental groups.

4. DATA ANALYSIS

Values are expressed as mean $\pm$ standard error of the mean (S.E.M.). The mean of the sample replicates was normalized *versus* the control group of the total cohort (100%). Data were analyzed using a paired Student's *t*-test to compare with the corresponding control group, while an unpaired Student's *t*-test was used to compare the effect of the antidepressant treatment and the cause of death. The statistical analyses and the identification of outliers were performed using GraphPad Prism 10 (GraphPad Software, Inc., California, USA). Statistical significance was considered at $p < 0.05$.

RESULTS

1. MMP-9 expression and activity in the synaptoneurosomal fraction of the prefrontal cortex of depressed subjects

MMP-9 expression was assessed in Brodmann's area 9 of a cohort of MDD subjects, who were subdivided according to whether they had measurable plasma levels of antidepressant drugs following the toxicology report, or by cause of death, in both men and women.

The analysis of MMP-9 expression showed increased levels of MMP-9 in all MDD individuals ($p < 0.05$) and in individuals in whom no blood antidepressant levels were detected at the moment of death ($p < 0.05$), compared to their respective control groups, while no differences were observed in the AD-treated group. Likewise, MDD individuals who committed suicide displayed higher MMP-9 levels than the control group ($p < 0.05$). No differences were observed in individuals who suffered a natural death (non-suicide) (Fig. 1A).

When only men were analyzed, higher MMP-9 levels remained present across the total MDD cohort compared to the control group ($p < 0.05$). Additionally, there was a tendency for the AD-free group to display higher MMP-9 levels than their respective control group ($p = 0.074$). Also, the suicide group showed a strong tendency to present elevated levels of MMP-9 ($p = 0.057$). No alterations in MMP-9 levels were observed in AD-treated men subjects (Fig. 1B).

MDD women showed a great tendency toward increased MMP-9 levels ($p = 0.058$). Among women, a tendency toward higher MMP-9 levels was observed in AD-free individuals ($p = 0.079$) and women who committed suicide ($p = 0.057$), compared with their respective control group. No differences in MMP-9 levels were observed in AD-treated or non-suicide women groups (Fig. 1C).

MMP-9 functionality in the prefrontal cortex was also analyzed in the same cohort. Increased activity of MMP-9 was found in the complete cohort of MDD subjects ($p < 0.05$). No differences in MMP-9 activity levels were observed between AD-free individuals and their matched controls, while increased activity of MMP-9 was observed in AD-treated subjects ($p < 0.05$). Besides, higher MMP-9 activity was found in the PFC of those MDD subjects who committed suicide ($p < 0.05$), with no differences in non-suicide subjects (Fig. 2A).

In men, the MMP-9 activity in the PFC did not show significant differences in any of the experimental groups studied (Fig. 2B). On the other hand, increased activity of MMP-9 was observed in women diagnosed with MDD ($p < 0.05$) and in AD-treated women ($p < 0.05$), with no differences in non-treated women. Moreover, a strong tendency toward increased MMP-9 activity was observed in those MDD women who committed suicide ($p = 0.056$), with no significant differences in women who died by natural causes (Fig. 2C).

The activity of the matrix metalloproteinase-2 (MMP-2) was also assessed. No statistical differences were observed in any of the experimental groups studied (Supplementary Figure S1).

2. Inflammatory markers in the nuclear fraction of the prefrontal cortex of depressed subjects

No statistical differences were found in STAT3 levels of the whole cohort of MDD subjects compared to the control group. Once differentiated by the drug treatment presence, higher levels of STAT3 were observed in the AD-free group ($p < 0.05$, Fig. 3A), and a trend toward lower STAT3 levels was observed in AD-treated individuals, compared to their matched controls ($p = 0.070$). No significant differences were observed in STAT3 levels related to their cause of death (Fig. 3A).

In men, no statistical differences were observed in the cohort of men with MDD compared to the control group. However, a trend to increase STAT3 levels was observed in AD-free men ($p = 0.074$) and a tendency to decrease STAT3 levels in those AD-treated ($p = 0.079$). No differences in STAT3 levels were observed regarding the cause of death of men (Fig. 3B). Women did not show significant differences in STAT3 levels in any of the groups studied (Fig. 3C).

The NF- κ B levels were not altered in MDD subjects compared to their matched controls, regardless of the presence of antidepressant levels in blood or their cause of death (Fig. 4A).

In men, no statistical differences were observed in the whole cohort of MDD subjects or subjects treated with antidepressants, whilst increased levels of NF- κ B were observed in MDD men free of antidepressants ($p < 0.01$). No statistical differences were found in NF- κ B levels when men were classified by their cause of death (Fig. 4B). Women did not present differences in the NF- κ B levels in any experimental group studied (Fig. 4C).

3. BDNF levels in the synaptoneurosomal fraction of the prefrontal cortex of depressed subjects

The BDNF levels were not altered in MDD subjects compared to their matched controls, regardless of their blood antidepressant levels or the cause of death (Fig. 5A).

In men, no statistical differences were observed in the whole cohort of MDD subjects or subjects free of antidepressant drugs. Instead, higher BDNF levels were observed in MDD men with antidepressant treatment ($p < 0.05$). No differences were found in suicidal individuals (Fig. 5B). Women did not show differences in BDNF levels in any of the experimental groups studied (Fig. 5C).

4. Heatmap

To visualize the enrichment of protein levels and activity in each group, and compare the subgroups with each other, the $\log_2$ of the ratio of each individual to their matched control was plotted in a heatmap (Fig. 6).

The heatmap revealed lower MMP-9 expression levels in the women MDD non-suicide group compared with the women suicide group ($p = 0.07$). In the group of individuals who committed suicide, MDD women presented a greater increase in MMP-9 levels than MDD men ($p < 0.05$).

The heatmap also revealed a down-regulation of STAT3 ($p < 0.01$) in all the individuals treated with AD drugs compared to MDD subjects free of AD drugs. MDD men with AD treatment displayed lower STAT3 levels ($p < 0.01$) than AD-free men. Two-way ANOVA revealed a significant effect of the treatment [$F_{(1,19)} = 12.1, p < 0.01$].

Similarly, a trend toward low NF- κ B levels ($p = 0.08$) was observed in AD-treated individuals compared to the AD-free group, as well as a down-regulation of NF- κ B ($p < 0.05$) in AD-treated men compared to AD-free men. A two-way ANOVA revealed a trend toward the interaction sex $\times$ treatment [$F_{(1,19)} = 3.53, p = 0.08$].

DISCUSSION

Herein, we focused on the presence of MMP-9 alterations and markers related to inflammation and neuroplasticity in *postmortem* brain samples from individuals diagnosed with major depressive disorder (MDD), considering the influence of sex, treatment, and the commitment of suicide.

To the best of our knowledge, this is the first time that both the MMP-9 expression and activity levels are evaluated in the same cohort of MDD subjects and their matched controls. The analysis of MMP-9 expression in the prefrontal cortex revealed increased MMP-9 protein levels in MDD subjects, as previously described in blood samples^{11–13}. This increase was observed only in the MDD subject group free of antidepressant medications, while MMP-9 levels were not modified in the antidepressant-treated group, which aligns with the decreased MMP-9 expression levels observed in individuals who did not relapse after electroconvulsive therapy¹⁴. In contrast, Alaiyed et al.¹⁶ report increased MMP-9 levels in prefrontal cortex samples. These discrepancies may be due to (1) the different experimental techniques used to analyze the protein levels, (2) the different doses/types of antidepressants used, and (3) the

brain region analyzed. While our study focused on Brodmann area 9 (BA9), Alaiyed¹⁶ examined Brodmann area 10 (BA10).

On the other hand, when analyzing the cause of death, MDD subjects who committed suicide also displayed higher MMP-9 expression levels, which are in agreement with previous reports in which there is a positive correlation between blood MMP-9 levels and the severity of depression⁵⁷, or in the cerebrospinal fluid⁵⁸ and blood of patients^{13,59} who attempted to commit suicide. Also, the lack of increased MMP-9 levels in the non-suicide group compared to controls agrees with the low MMP-9 levels observed in individuals who did not attempt suicide or whose suicide attempt was not recent¹³. For the cause of death, suicide women presented higher MMP-9 expression levels than men, providing the first evidence of a sex-dependent regulation of MMP-9 associated with suicidal behavior in humans.

Parallel to the higher MMP-9 expression in the prefrontal cortex of individuals diagnosed with MDD, an increased MMP-9 activity was observed, which aligns with previous studies in other brain regions in MDD subjects, such as the CA1 subfield of the hippocampus¹⁵. Notably, the increase in MMP-9 activity in our cohort was evident in subjects treated with antidepressants, but not in those who were free of antidepressant drugs in their toxicological screenings. Similarly, Alaiyed et al.¹⁶ reported an elevated pro-MMP-9/TIMP-1 ratio in the prefrontal cortex of antidepressant-treated MDD individuals.

When analyzing the cause of death, we also observed increased MMP-9 activity in suicide victims, but not in those who died of natural causes. These results align with the positive correlation between the severity of depression and the MMP-9 levels in the hippocampus¹⁵ and blood^{57,60–63}. Moreover, this association is also linked with the lack of antidepressant efficacy, as subjects who relapsed after electroconvulsive therapy presented high MMP-9 levels¹⁴. Moreover, it should be noted that in our study, eleven of the fourteen individuals receiving AD treatment committed suicide, which may explain the increased MMP-9 activity in the AD-treated group.

In our study, the MMP-9 activity differences in MDD subjects seem to follow a sex-dependent effect, suggesting a different regulation that affects MMP-9 catalytic activity. We have previously described this gender bias associated with MMP-9 activity in preclinical studies²⁰, supporting the sex-dependent differences reported for other molecular markers in MDD subjects^{64–67}. In addition, sex-related differences in MMPs have been suggested for other neurological and cardiovascular diseases⁶⁸. It would be of interest to investigate the mechanisms underlying those sex-dependent alterations. In this sense, there is a series of metalloproteinase inhibitors, such as α 2-macroglobulin⁶⁹ or tissue inhibitor of matrix metalloproteinases 1 (TIMP-1)⁷⁰, whose dysregulation has been proposed to be associated with depression^{16,71,72}. TIMP-1 expression shows sex-dependent differences associated with peripheral aneurysms⁷³. In fact, it has been reported that estrogen can regulate TIMP-1 expression in a sex-specific manner⁷⁴ and, specifically, that estradiol can induce MMP-9 activity without modulating its expression in the hippocampus of female mice⁷⁵.

The specific association between MMP-9 and depression is supported by the lack of differences observed in MMP-2 activity in the prefrontal cortex in our MDD cohort, regardless of the presence or absence of antidepressant drugs in the toxicological screening and the cause of death. This finding is consistent with previous results reported in the hippocampus of MDD individuals who committed suicide¹⁵, while studies in peripheral samples report contradictory data, including increased⁷⁶, decreased⁵⁶, or no changes⁷⁷ in MMP-2 levels.

Overall, our findings support a specific role for cortical MMP-9—but not MMP-2—in the severity of MDD, particularly in women.

Neuroinflammation is also associated with the neurobiology of depression, the severity of the symptomatology, and the response to treatment. Moreover, MMP-9 has an important role in inflammatory processes⁷⁸. Here we found increased STAT3 and NF-κB nuclear levels in AD-free individuals and decreased STAT3 levels mainly in samples of men diagnosed with MDD, supporting the importance of neuroinflammation in the neurobiology of depression⁷⁹, and suggesting an anti-inflammatory effect exerted by the antidepressant drugs⁸⁰. Franzen et al.⁸¹ highlighted the putative role of the STAT3 signaling pathway in MDD, stating the lack of studies on this matter. The activation of the HMGB1/STAT3/p65 axis in the dorsolateral PFC of men with MDD acts as a mediator of the neurobiology of depression³⁵. In addition, increased peripheral NF-κB levels have been described in MDD patients⁸². However, other authors report a reduction in NF-κB levels in the prefrontal cortex of MDD individuals, both AD-treated and AD-free. These authors suggest that this finding may be attributable to the higher body mass index observed in the control subjects compared to the MDD group⁸³. Regarding the effect of antidepressant drugs, a recent study elucidates that some antidepressant drugs, such as venlafaxine and vortioxetine, induce an anti-inflammatory effect and specifically downregulate STAT3 and NF-κB⁸⁴. Classical antidepressant treatments also promote a reduction of proinflammatory markers such as TNF-α and IL-6, and an increase in anti-inflammatory cytokine expression (e.g., IL-10)⁸⁵. This reduction in brain inflammatory markers observed in the AD-treated male group does not seem to correlate with an adequate response to treatment, as most of the male MDD subjects committed suicide.

The modulation of inflammatory markers observed in men, but not in women, supports the sex-dependent importance of inflammatory markers in the pathophysiology of MDD, as previously described⁶⁵. In this sense, it is known that men and women show distinct neuroinflammatory signatures in depression, derived through the regulation of different molecular pathways^{86,87}.

Regarding BDNF, we observed increased protein levels in the PFC of AD-treated men, but not women, consistent with increased cortical⁸⁸ and hippocampal⁸⁹ BDNF levels in antidepressant-treated individuals but not in untreated subjects. In addition, several studies describe the upregulation of peripheral BDNF expression following chronic antidepressant treatment^{90–92}. However, other findings

suggest no significant changes in BDNF levels in the prefrontal cortex⁴⁴ or hippocampus⁴⁶ following chronic treatment with antidepressants.

Even though several studies reported low levels of peripheral BDNF in MDD subjects^{57,93} and an association with suicidal behavior⁹⁴, our data do not display differences in those categories. Similarly, Tripp et al.⁴⁸ did not observe differences in BDNF levels in the subgenual anterior cingulate cortex of men and women diagnosed with depression, but reported decreased BDNF signaling, which was more pronounced in men than in women. Therefore, we cannot exclude a dysregulation of BDNF function in our MDD cohort, suggesting a differential role of BDNF in drug treatment between men and women. These different changes between men and women with depression have been described in other synapse-related genes⁶⁵.

Our study supports the role of MMP-9 activity as a potential marker of suicidal behavior, especially in women with MDD, and the role of neuroinflammation in men with MDD. However, this study presents some limitations, such as the relatively small sample size and the heterogeneity of the antidepressant drug treatments, which could differentially affect protein expression. Additional studies will be needed to disentangle the discrepancies observed between MMP-9 expression and activity, which would help us to deepen into the role of this metalloproteinase in depression.

In sum, this study highlights the importance of considering sex differences in research. Our results underscore significant sex differences in the neurobiology of MDD, the response to antidepressant treatment, and suicidal behavior—an area that remains poorly understood and warrants further investigation.

Abbreviations

AD
antidepressant drugs
BDNF
brain-derived neurotrophic factor
FBS
fetal bovine serum
MDD
major depressive disorder
MMP-9
matrix metalloproteinase 9
NF-κB
Nuclear factor-κB
PCNA
Proliferating cell nuclear antigen
STAT3

signal transducer and activator of transcription 3

Declarations

CONFLICT OF INTERESTS

The authors declare no competing financial interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

CONSENT FOR PUBLICATION

“Not applicable”.

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AUTHORS’ CONTRIBUTIONS

J.S.: performing experiments, analyzing and interpreting data, writing the original draft; T.G.: performing experiments and data analysis; E.F-Z.: performing experiments, methodology and data analysis; A.D.: conceptualization and writing review; A.P.: funding acquisition, writing review; L.F.C.: resources and writing review; J.J.M.: resources and writing review; E.C.: conceptualization, design of the study, interpreting data and writing the original draft; and F.P-C.: conceptualization, design of the study, funding acquisition, project administration, writing original draft.

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AVAILABILITY OF DATA AND MATERIALS

The data that support the findings of this study are available on request from the corresponding author.

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Figures

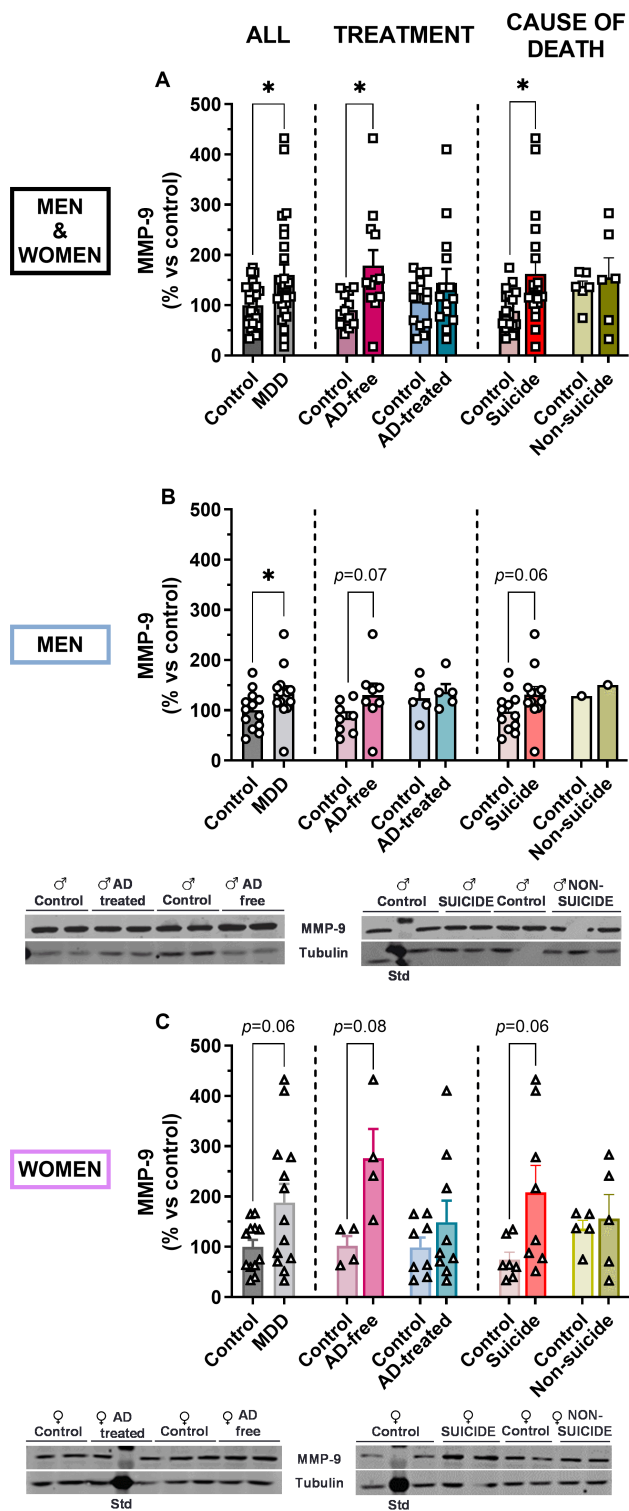


Figure 1

MMP-9 expression in the synaptoneurosomal fraction of the prefrontal cortex of *postmortem* depressed patients (MDD) (left), considering the treatment (AD-treated and AD-free) (center) and the cause of death (suicide and non-suicide) (right). (A) Both sexes, (B) men, and (C) women. Representative Western blot bands are shown. Results are expressed as a percentage vs the control MDD-matched group and as

mean $\pm$ S.E.M. Paired Student's *t*-test. **p*<0.05 vs control group. n = 26 samples per control or MDD group. AD: antidepressant drugs, MDD: major depressive disorder.

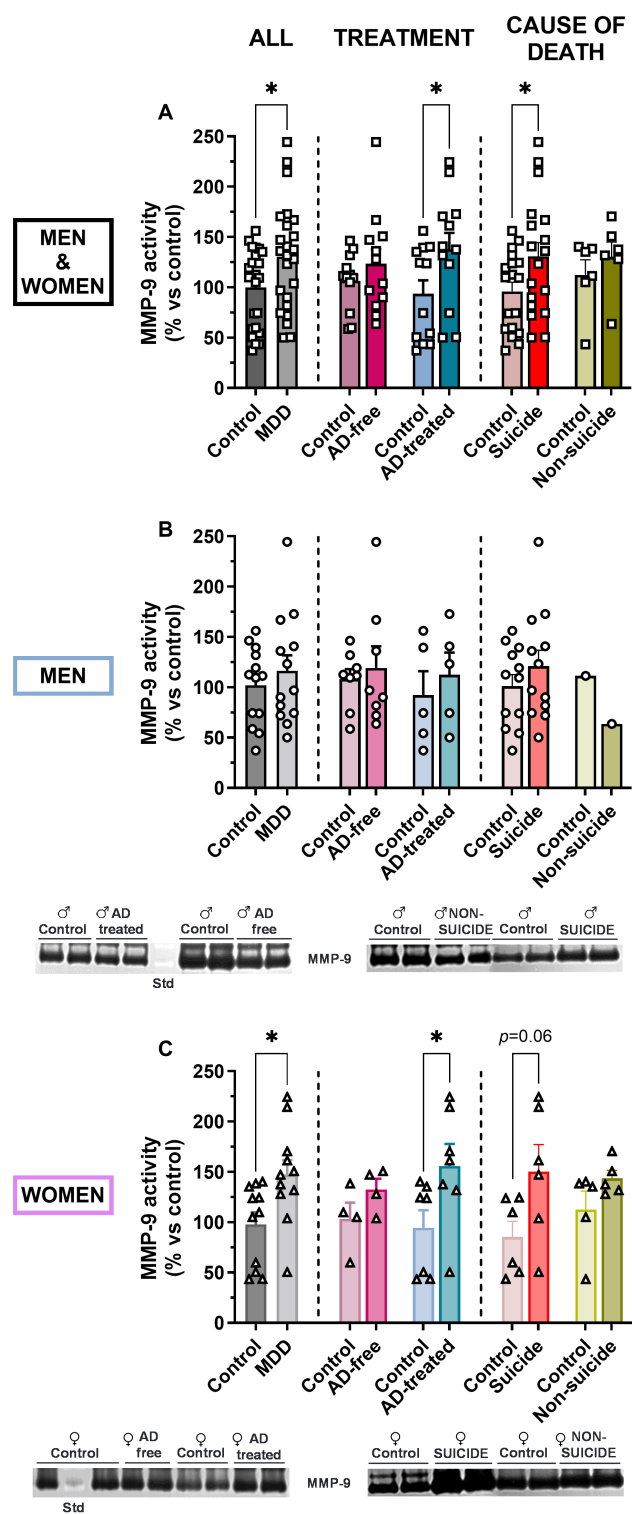


Figure 2

MMP-9 activity assessed by gel zymography in the synaptoneurosomal fraction of the prefrontal cortex of *postmortem* depressed patients (MDD) (left), considering the treatment (AD-treated and AD-free)

(center) and the cause of death (suicide and non-suicide) (right). (A) Both sexes, (B) men, and (C) women. Representative bands from the gel zymography are shown. Results are expressed as a percentage vs the control MDD-matched group and as mean $\pm$ S.E.M. Paired Student's *t*-test. **p*<0.05 vs control group. n = 24 samples per control or MDD group. AD: antidepressant drugs, MDD: major depressive disorder.

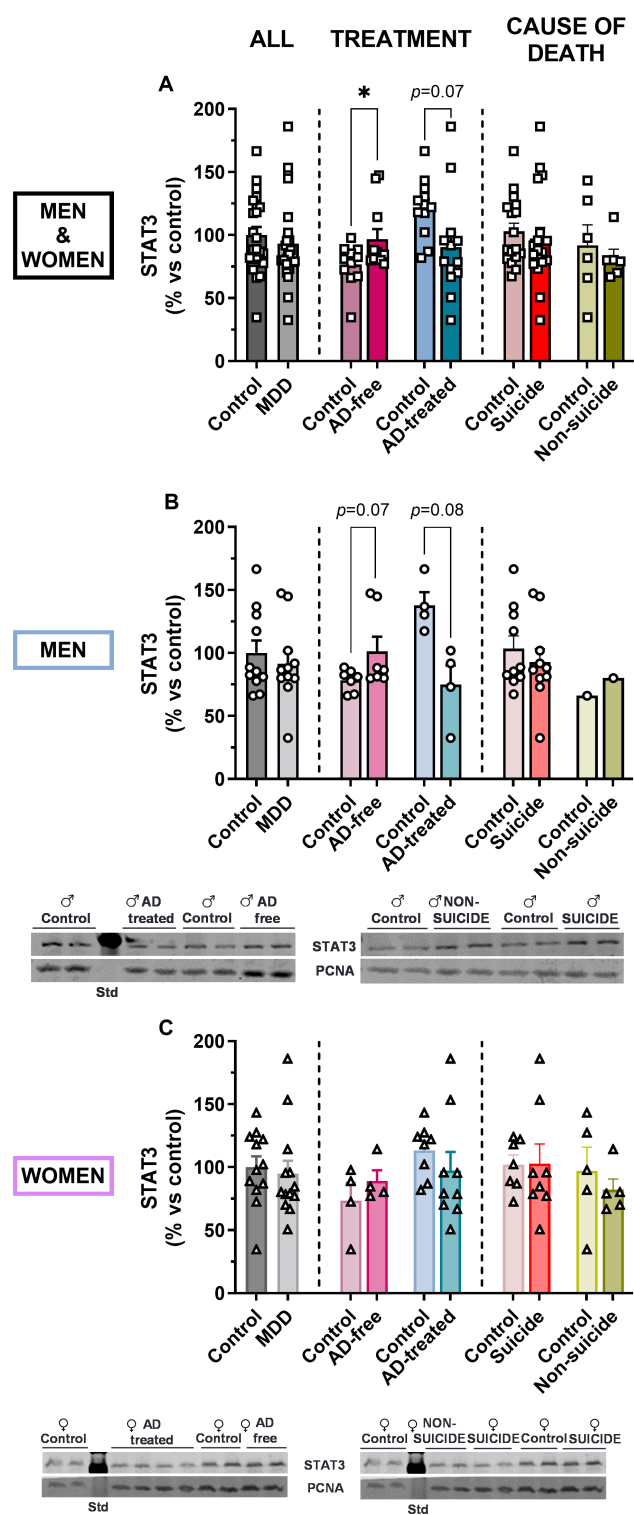


Figure 3

STAT3 expression in the nuclear fraction of the prefrontal cortex *postmortem* of depressed patients (MDD) (left), considering the treatment (AD-treated and AD-free) (center) and the cause of death (suicide and non-suicide) (right). (A) Both sexes, (B) men, and (C) women. Representative Western blot bands are shown. Results are expressed as a percentage vs the control MDD-matched group and as mean $\pm$ S.E.M. Paired Student's *t*-test. **p*<0.05 vs control group. n = 24 samples per control or MDD group. AD: antidepressant drugs, MDD: major depressive disorder.

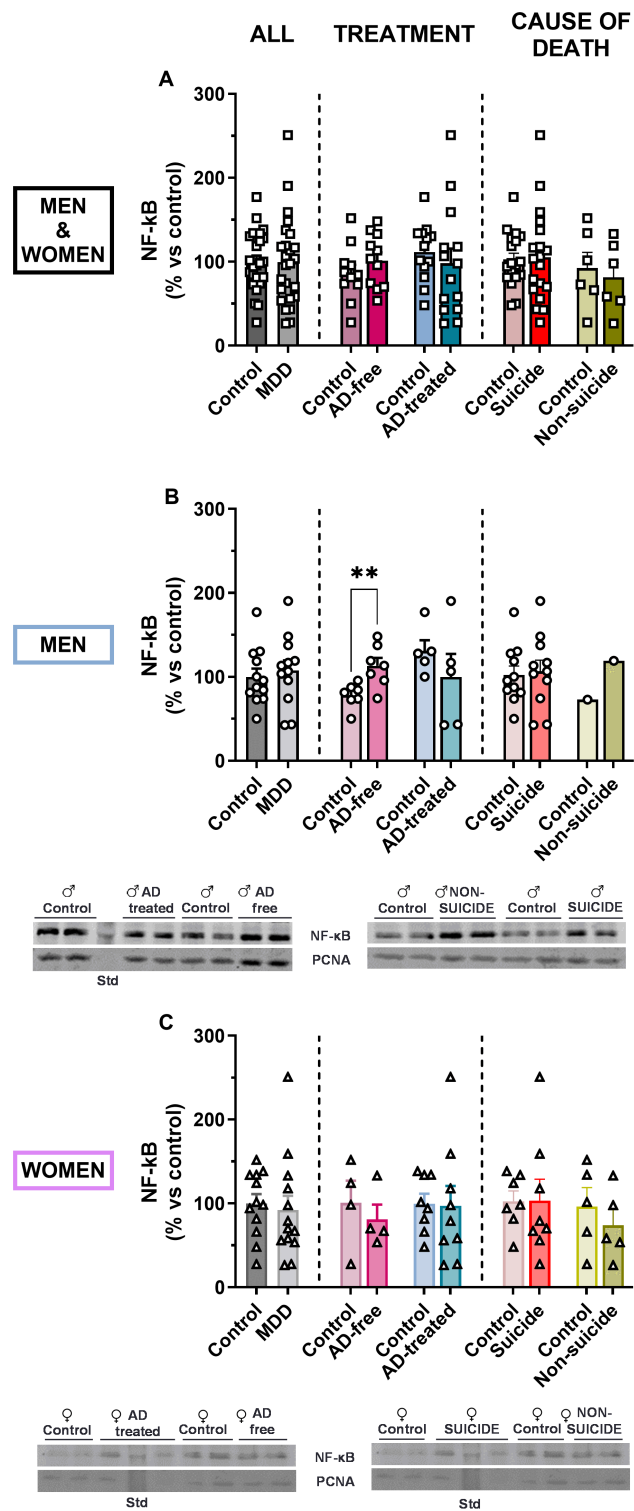


Figure 4

NF- κ B expression in the nuclear fraction of the prefrontal cortex of *postmortem* depressed patients (MDD) (left), considering the treatment (AD-treated and AD-free) (center) and the cause of death (suicide and non-suicide) (right). (A) Both sexes, (B) men, and (C) women. Representative Western blot bands are shown. Results are expressed as a percentage vs the control MDD-matched group and as mean $\pm$ S.E.M. Paired Student's *t*-test. ** $p < 0.01$ vs their control group. *n* = 25 samples per control or MDD group. AD: antidepressant drugs, MDD: major depressive disorder.



mean ± S.E.M. Paired Student's *t*-test. **p*<0.05 vs control group. n = 23 samples per control or MDD group. AD: antidepressant drugs, MDD: major depressive disorder.

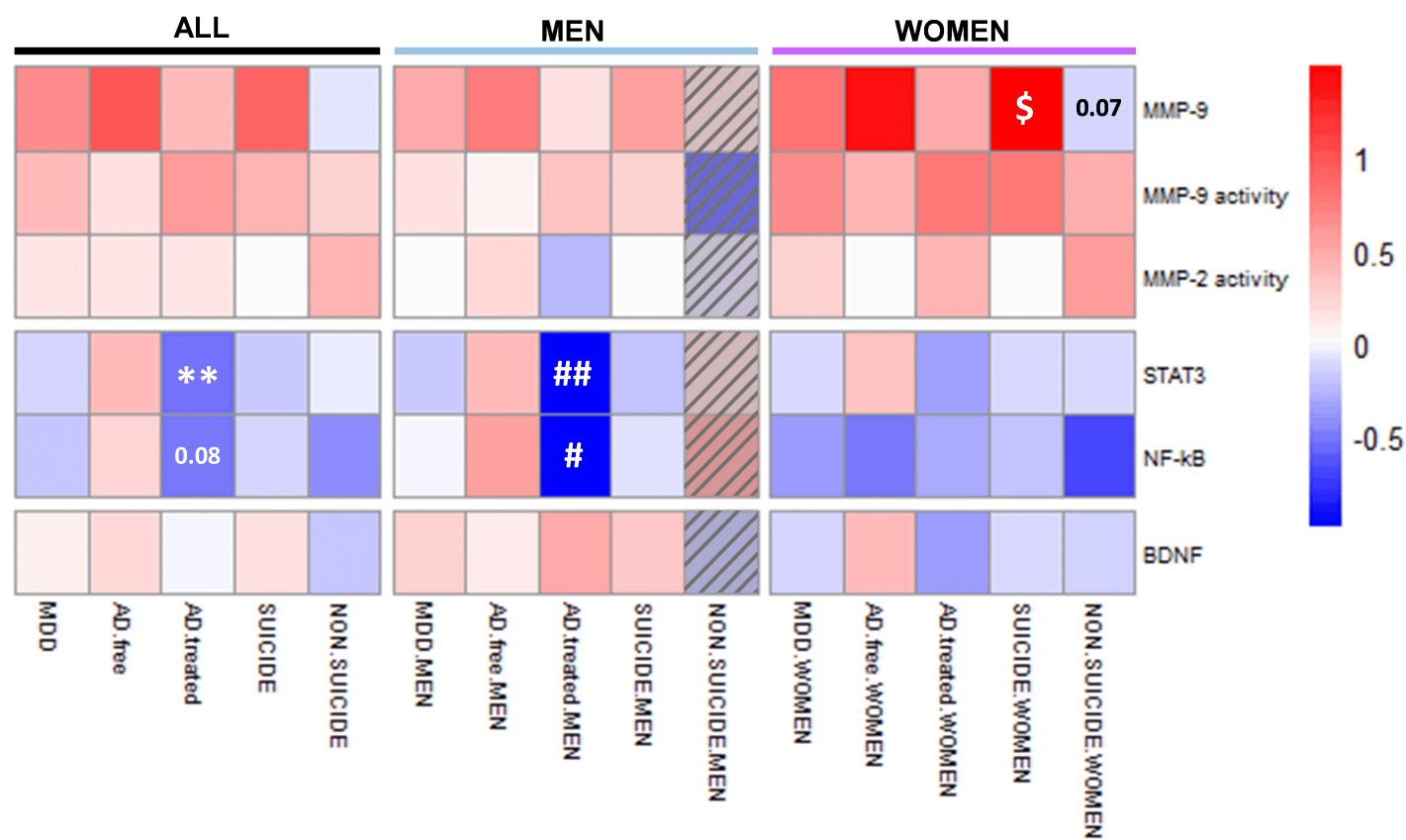


Figure 6

Heatmap of the relative enrichment of protein levels and MMP-9 and MMP-2 activity in the prefrontal cortex. Results are displayed as the mean of log₂ of the ratio of each patient to their matched control (control mean = 0). Red boxes indicate protein/activity upregulation, blue boxes indicate protein/activity downregulation, and white boxes indicate no differences in the log₂ ratio scale. The data for the values shown in this heatmap can be found in Table S2. Unpaired Student's *t*-test ***p*<0.01 vs AD-free group. Two-way ANOVA followed by a Fisher's LSD *post hoc* test #*p*<0.05, ##*p*<0.01 vs AD-free men group. Unpaired Student's *t*-test \$*p*<0.05 vs suicide men group. n = 23-26 samples per MDD group.

Supplementary Files

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