Archival Report

Biological Psychiatry

Expression of the *PPM1F* Gene Is Regulated by Stress and Associated With Anxiety and Depression

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ABSTRACT

BACKGROUND: Molecular mechanisms underlying psychological sequelae of exposure to stressful experiences, such as posttraumatic stress disorder (PTSD) and depression, are not well understood.

METHODS: Using convergent evidence from animal and human transcriptomic and genomic studies, we aimed to identify genetic mechanisms underlying depression and anxiety after traumatic experiences.

RESULTS: From a transcriptome-wide analysis in mice, we found the *Ppm1f* gene to be differentially expressed in the amygdala and medial prefrontal cortex (mPFC) a week after immobilization stress. Next, we found that *PPM1F* messenger RNA levels in human blood were downregulated in cases with symptoms of comorbid PTSD and depression and consistently in cases with anxiety symptoms in a separate human dataset. Furthermore, we showed that a genetic variant of *PPM1F*, rs17759843, was associated with comorbid PTSD and depression and with *PPM1F* expression in both human brain and blood. Given prior reported mechanistic links between *PPM1F* and *CAMK2 (CAMKII)*, we examined blood messenger RNA level of *CAMK2G* in humans and found it to be lower in cases with comorbid PTSD and depression. We also found that PPM1F protein levels and colocalization with CAMK2G were altered in amygdala and mPFC of male mice. Additionally, we found that a systemic dose of corticosterone blocked the depressive-like phenotype elicited by stress in female mice. Lastly, corticosterone rescued the anxiety-like phenotype and messenger RNA levels of *Ppm1f* in amygdala and mPFC in male mice and in mPFC of female mice.

CONCLUSIONS: Taken together, our data suggest a mechanistic pathway involving PPM1F and CAMK2G in stress- and trauma-related manifestation of anxiety and depression across species.

Keywords: Anxiety, *CAMK2*, Depression, *PPM1F*, PTSD, Stress

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Exposure to stressful or traumatic life experiences increases risks of developing depressive disorders, anxiety disorders, or posttraumatic stress disorder (PTSD) (1). Anxiety and depressive disorders rank at the top of disabling mental illnesses, causing tremendous economic costs to society (2). However, effectiveness of pharmacological treatments for anxiety and depressive disorders still remains rather limited with small effect sizes (3,4). To facilitate efforts in developing novel treatments for these disorders, better insights into molecular mechanisms underlying anxiety and depression are needed.

With the goal of elucidating molecular mechanisms underlying anxiety and depression after traumatic experiences, we used convergent evidence from both animal and human studies. We note that among susceptible individuals, PTSD and depression are often comorbid (5). The shared symptoms and relatively high prevalence of PTSD and depression comorbidity suggest that examining their comorbidity in the aftermath of trauma may be a more powerful approach than examining either outcome alone. In addition, animal stress models leading to similar PTSD-like or depression-like behaviors can facilitate our efforts in uncovering their neurobiological mechanisms (6,7).

METHODS AND MATERIALS

Animals

All experiments were performed on adult wild-type C57BL/6J mice obtained from Jackson Labs (Bar Harbor, ME) and Parc Tecnològic del Vallès (Barcelona, Spain). Male and female mice were group-housed in a temperature-controlled vivarium with ad libitum access to food and water. See Supplemental Methods for ethics protocols. Female mice were used only when explicitly stated; otherwise, only male mice were used.

Immobilization Stress

Immobilization (IMO) stress procedures, as previously described (8–10), were conducted in a room separate from housing and behavioral paradigms (11). Mice were habituated to the fear conditioning chambers before IMO stress and received exposure to handling and the training context

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completely separate and distinct from the IMO context (9). We chose the time point of 6 to 8 days post–IMO stress because we wanted to compare these results with our previous findings studying gene regulation at this same time point (8,9,11,12).

Microarray Hybridization and Analysis

Mice were sacrificed under basal conditions (home cage control group) at 6 or 8 days after a 2-hour IMO (IMO stress group). The reason for having two amygdala microarrays was to replicate the findings from day 6 on day 8 and get more robust results. Amygdala and medial prefrontal cortex (mPFC) tissue from both hemispheres was extracted by 1-mm micropunch. Total RNA was extracted from the tissue and purified with the RNeasy Mini Kit (Qiagen, Valencia, CA) following the manufacturer's instructions. Illumina Mouse WG-6 v2 Expression BeadChip microarray (Illumina, Inc., San Diego, CA) was assayed for 45,281 transcripts. These microarrays are available at Gene Expression Omnibus GSE100084, GSE100085, and GSE100086. Plots of principal variance component analysis and volcano plots are shown in Supplemental Figure S1.

Bioinformatics of Microarray Analysis

The top genes regulated after stress in the amygdala and mPFC were further analyzed with Database for Annotation, Visualization and Integrated Discovery Bioinformatics Resources 6.8 to understand their functions. Note that *Ppm1f* was the only top gene present in all the microarrays (Figure 1A–E).

Reverse Transcription and Polymerase Chain Reaction Quantification

The protocol for reverse transcription and polymerase chain reaction (PCR) quantification was followed as previously described (8).

Reverse Transcriptase PCR Arrays

RNA was isolated and reverse transcribed as previously described (8). We used a custom plate format 24 × 4 (Applied Biosystems, Carlsbad, CA).

Elevated Plus Maze

The elevated plus maze (EPM) was performed as previously described (12).

Tail Suspension Test

For the tail suspension test (TST), mice were suspended with duct tape approximately 2 cm at the end of the tail. The session lasted for 6 minutes. Behavior was recorded by a video camera, and a researcher (RA, ERV, or AF) blinded to the experimental groups analyzed the immobility time.

Mouse Fear Conditioning

Mice were fear conditioned in standard rodent modular test chambers (ENV-008-VP; Med Associates Inc., Fairfax, VT) with an inside area of 30.5 cm (length) × 24.1 cm (width) × 21.0 cm (height). Mice received five trials of a conditioned stimulus (30-second tone, 6 kHz, 70 dB) co-terminating with a footshock (500 ms, 0.6 mA) unconditioned stimulus.

Gene Expression Profiles in Human Participants in Grady Trauma Project

Participants were recruited from Atlanta inner-city residents by the Grady Trauma Project (GTP) (13–15). This study was approved by the Institutional Review Board of Emory University School of Medicine and Grady Memorial Hospital.

PTSD symptoms were assessed with the modified PTSD Symptom Scale (PSS) (16). Depressive symptoms were measured with the Beck Depression Inventory (BDI) (17). Cases with current symptoms of comorbid PTSD and depression were defined as having a PSS score ≥14 and a BDI score ≥14. Control subjects were defined as not having either PTSD or depressive symptoms, as reflected by a PSS score ≤ 7 and BDI score ≤ 7, despite being exposed to trauma. See Supplemental Methods for rationale behind these cutoff scores. Of note, these participants were not part of a rigorously assessed clinical sample.

RNA was extracted from blood collected in the morning. All samples had RNA integrity number ≥ 6. Raw probe intensities were generated on Illumina HumanHT-12 v3 or v4 BeadChip arrays. We performed normalization of probe intensities using the supervised normalization of microarray algorithm (18), removing the effects of batch and RNA integrity number. Association between messenger RNA (mRNA) level of a gene of interest and comorbid PTSD and depression status was examined using multiple linear regression adjusting for gender, age, and population substructure.

Center for Health Discovery and Well Being Replication Sample and Gene Expression Profiles

The Center for Health Discovery and Well Being (CHDWB) project evaluated effectiveness of a health-focused and prevention-focused approach (19). Anxiety symptoms were assessed with the Generalized Anxiety Disorder-7 (GAD-7) scale (20), a well-validated and efficient tool for screening anxiety symptoms. Scoring of the GAD-7 is as follows: a score of 5 to 9 indicates mild anxiety symptoms; 10 to 14, moderate anxiety symptoms; and 15 to 21, severe anxiety symptoms (20). We categorized participants with GAD-7 scores ≥ 5 as having anxiety symptoms, and participants with GAD-7 scores ≤ 1 served as control subjects.

RNA was extracted from whole blood, and all samples had RNA integrity number > 7. Probe intensities were generated on Illumina HT12 v3 or v4 BeadChip arrays. We performed normalization using the supervised normalization of microarrays (18). Human *PPM1F* microarrays from CHWDB are available at Gene Expression Omnibus GSE61672.

PPM1F Genotypes and Principal Components Among GTP Participants

DNA was extracted from saliva or blood, and genotyping was conducted using Illumina HumanOmni1-Quad BeadChip. Standard quality control of the genotyping was performed using PLINK (21), removing individuals with >2% missing data and removing one in each pair of related individuals with an identity by descent proportion >0.12 (indicating cousins or a closer relation). We used principal component analysis to infer axes of ancestry and remove outlier subjects following methods previously described (22).

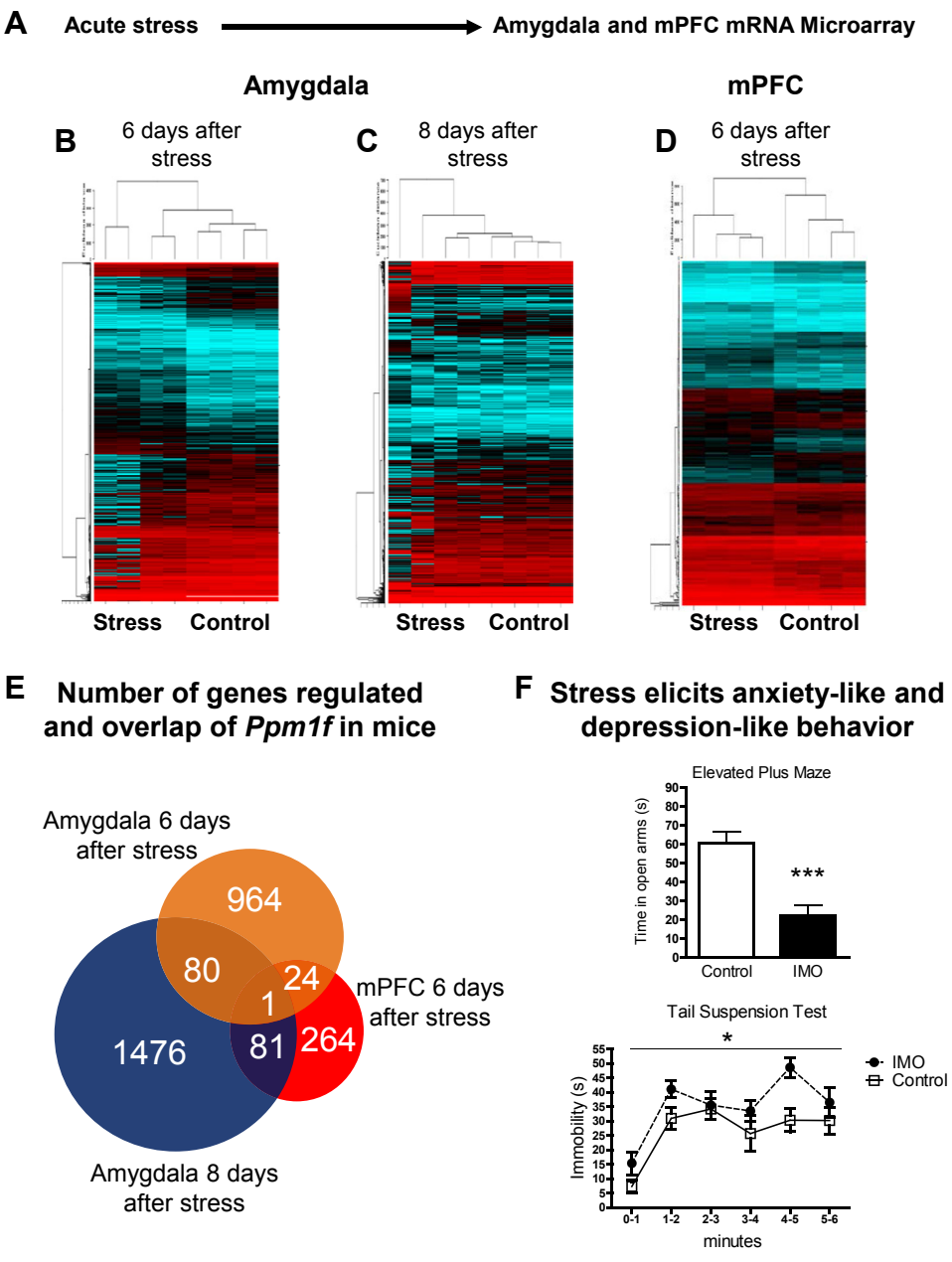


Figure 1. *Ppm1f* brain regulation after stress, anxiety-like, and depression-like behaviors. **(A)** Schematic of the experimental protocol ($n = 4$ per group). **(B)** Amygdala microarray 6 days after acute immobilization (IMO) stress. **(C)** Amygdala microarray 8 days after IMO stress. **(D)** Medial prefrontal cortex (mPFC) microarray 6 days after IMO stress. **(E)** Gene regulation of the microarrays show overlap in the *Ppm1f* gene. **(F)** (Top panel) Six days after stress, mice spend less time in the open arms in the elevated plus maze, indicating enhanced anxiety-like behavior ($p = .0004$; $n = 8$ per group). (Bottom panel) Six days after stress, mice present with enhanced levels of immobility in the tail suspension test, indicating enhanced depressive-like behavior ($p = .0361$; $n = 8$ per group). mRNA, messenger RNA. $^{***}p < .001$; $^{*}p < .05$.

Association between *PPM1F* single nucleotide polymorphisms (SNPs) and comorbid PTSD and depression was examined using PLINK, adjusting for gender and 10 principal components. Multiple testing was addressed with permutation (23).

Expression Quantitative Trait Locus Analysis

Association between rs17759843 and blood mRNA level of *PPM1F* was provided by the blood expression quantitative trait locus (eQTL) browser (genenetwork.nl/bloodeqtlbrowser) (24). Quality control for this dataset was described in detail by Westra *et al.* (24).

Postmortem eQTL Analysis

Association between rs17759843 and brain mRNA level of *PPM1F* was provided by BrainCloud, which has gene expression profiles from the dorsolateral PFC of 269 subjects without neuropathological and neuropsychiatric diagnosis (25).

RESULTS

Behavior and Gene Expression Profiles in Amygdala and mPFC of Stressed Mice

We performed transcriptome-wide differential expression analysis of genes in the amygdala and mPFC in mice 6 and

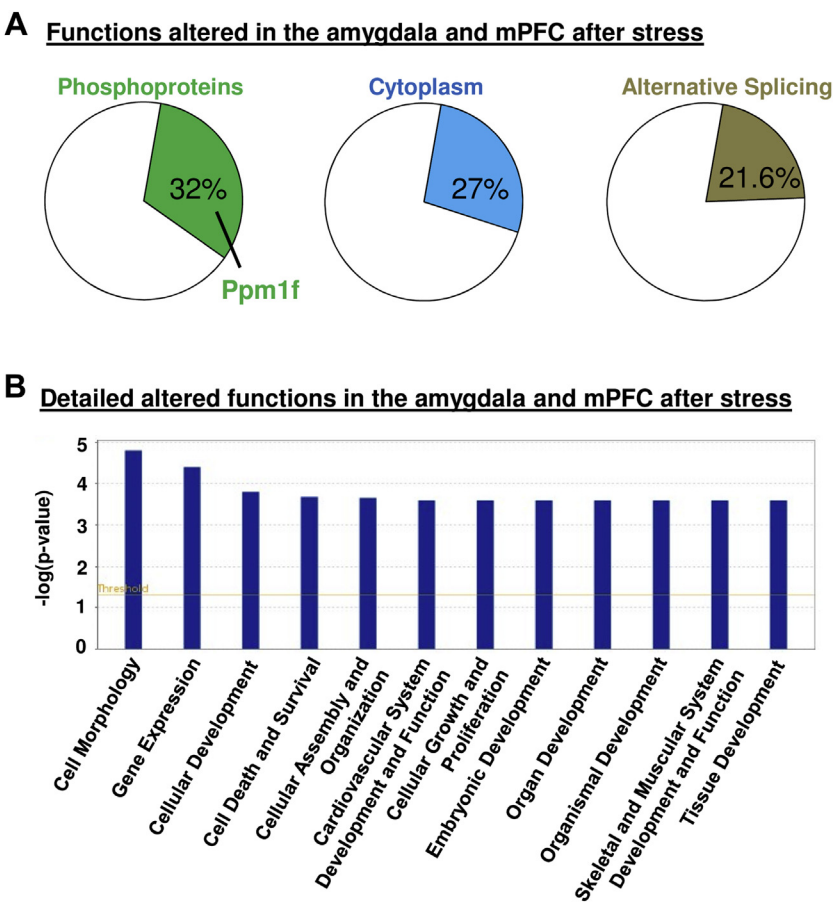


Figure 2. Bioinformatic analysis of the altered functions in amygdala and medial prefrontal cortex (mPFC) after exposure to stress in mice. **(A)** Analysis with DAVID version 6.8 revealed general altered functions 6–8 days after acute stress in both amygdala and mPFC. Phosphoproteins (including PPM1F), cytoplasm, and alternative splicing were the more frequently affected functions in this analysis. **(B)** Ingenuity pathway analysis showed more detailed functions altered after a single exposure to 2 hours of stress immobilization.

8 days after IMO stress versus control mice. We examined gene expression profiles in the amygdala at 6 days and 8 days after IMO stress to determine if the long-term gene expression changes found at 6 days were sustained at 8 days. Figure 1A–D shows the heatmaps revealing differences in the gene expression in stressed versus control mice groups. Of the probes that were differentially expressed at unadjusted $p < .05$ in the amygdala, we used the following criteria to select probes for follow-up: 1) probe differentially expressed at unadjusted p value $< .05$ at both 6 days and 8 days after stress, 2) probe with >1.5-fold change between cases and controls, and 3) probe is expressed at moderate to high levels in the amygdala (through search in the Allen Mouse Brain Atlas). Supplemental Table S1 lists the 24 probes that met these criteria. Of note, without adjusting for multiple testing, there is an increased risk of false-positive probes. Therefore, we performed replication of these probes in different mouse cohorts to determine if they were differentially expressed between cases and controls using reverse transcriptase PCR. Of these 24 probes, 21 had available primers for the reverse transcriptase PCR replication (Supplemental Table S2). Supplemental Table S3 lists the results of the PCR replication in the amygdala. We found five probes whose altered mRNA levels were replicated with PCR; *Ppm1f* was one of them. Along with our replication studies, we showed that these gene expression changes, which occurred 6 to 8 days after IMO stress, may need an incubation period because no significant changes in gene expression were found at 1 day after exposure to stress (Supplemental Table S3). Interestingly, although *Ppm1f* is significantly regulated 6 to 8 days poststress, it is not regulated in the amygdala 30 minutes or 2 hours after fear conditioning or in the mPFC 2 hours after fear conditioning (Supplemental Figure S2). Thus, *Ppm1f* appears to have a dynamic expression after stress in the brain. Moreover, only five of these genes were significantly regulated after stress—*Hbp1, Ssbp4, Fbxl5, Ppm1f,* and *Fhl1*—at three different time points: 6, 7, and 8 days after IMO (Supplemental Table S3). Thus, we examined expression of these five genes in humans. Among them, only *PPM1F* was differentially expressed between cases with symptoms of comorbid PTSD and depression versus control subjects (Supplemental Table S4; more detailed description follows).

We followed similar criteria to select probes with unadjusted p value $< .05$ from the mPFC transcriptome-wide differential analysis for further replication in a mouse microarray (more details in Supplemental Methods). See Supplemental Table S5 for the list of genes that survived the filtering criteria and were followed up with reverse transcriptase PCR replication. The number of top-regulated genes within these microarrays and their overlap are represented in Figure 1E. *Ppm1f* was the only gene highly regulated in all three mouse microarray conditions.

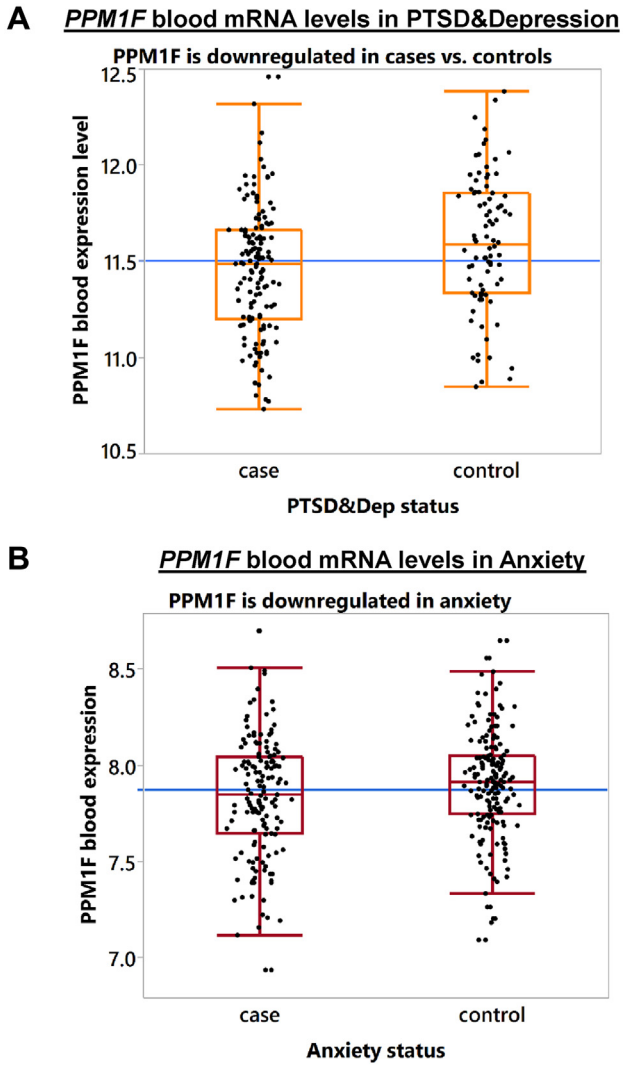


Figure 3. *PPM1F* blood messenger RNA (mRNA) levels in comorbid posttraumatic stress disorder and depression (PTSD&Depression, PTSD&Dep) and in anxiety. **(A)** Box plot showing that *PPM1F* mRNA level is lower in cases with symptoms of comorbid PTSD and depression vs. control subjects after adjusting for gender, age, and population substructure ($p = .005$; $n = 230$). The black dots represent the *PPM1F* expression level of each sample. The blue horizontal line represents the mean of *PPM1F* level in the overall sample. **(B)** *PPM1F* mRNA level is significantly downregulated in cases with anxiety vs. control subjects after adjusting for gender, age, and ethnicity ($p = .044$; $n = 316$).

Behaviorally, two groups of mice were exposed to IMO stress; 6 days later they were tested in the EPM (anxiety-like behavior) or the TST (depressive-like behavior). The EPM results showed that stress reduced the time mice spent in the open arms ($t_{14} = 4.597$, $p = .0004$) (Figure 1F, top panel). Moreover, the TST analysis revealed that stress increased the time mice spent in immobility ($t_7 = 2.280$, $p = .0361$) (Figure 1F, bottom panel).

Brain Functions Altered After Acute Stress, Network Analysis, and Predicted Interactions With Drugs

We performed bioinformatics analyses of the shared genes that had altered expression in the microarray. The genes studied with Database for Annotation, Visualization and Integrated Discovery Bioinformatics Resources are listed in Supplemental Tables S1 and S5 and Supplemental Figure S3. We found that the top three functions altered in both amygdala and mPFC were phosphoproteins (including *Ppm1f*), alternative splicing, and splice variants (Figure 2A). A more detailed functional analysis with ingenuity pathway analysis showed that the biological pathways that were most different between stress and control animals included cell morphology, gene expression, and cellular development (Figure 2B). Supplemental Figure S4 shows that PPM1F has direct relationships with the calcium/calmodulin-dependent protein kinase 2 (CAMK2) family and indirect relationships with brain-derived neurotrophic factor, cyclic adenosine monophosphate response element-binding, extracellular signal-regulated kinase 1/2, and mitogen-activated protein kinase kinase.

Examination of Expression of Five Genes in Comorbid PTSD and Depression

We next examined expression of the five genes identified to be significantly differentially regulated in mouse amygdala after exposure to stress in 230 GTP human participants who were exposed to at least one traumatic experience. The sociodemographic characteristics of the participants are presented in Supplemental Table S6. Of these participants, 142 were cases with comorbid PTSD and depression symptoms, and

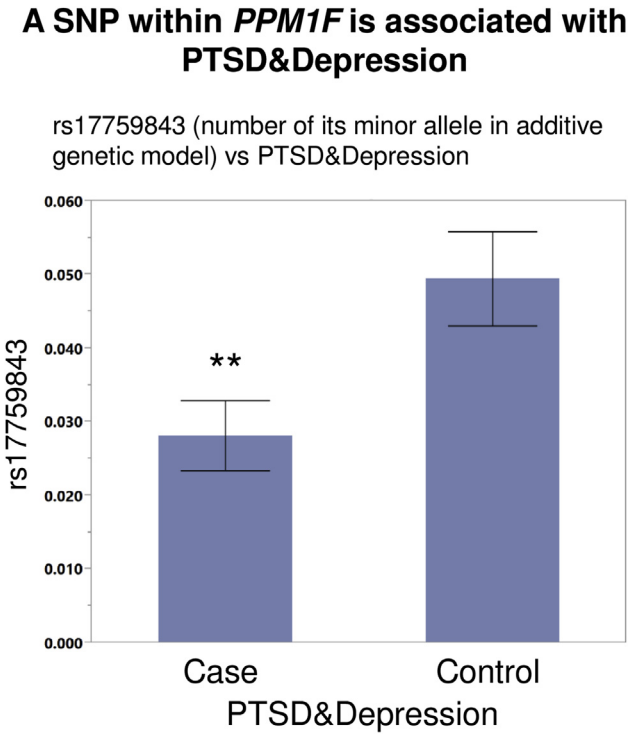


Figure 4. A single nucleotide polymorphism (SNP) within the *PPM1F* gene is associated with comorbid posttraumatic stress disorder and depression (PTSD&Depression). The minor allele of rs17759843, a *PPM1F* 3′UTR SNP, is significantly associated with lower odds for comorbid PTSD and depression after adjusting for gender and population substructure (odds ratio = 0.53; $p = .005$; $^{**}p < .01$; permuted $p = .046$; $n = 2361$). Each error bar is constructed using 1 standard error from the mean.

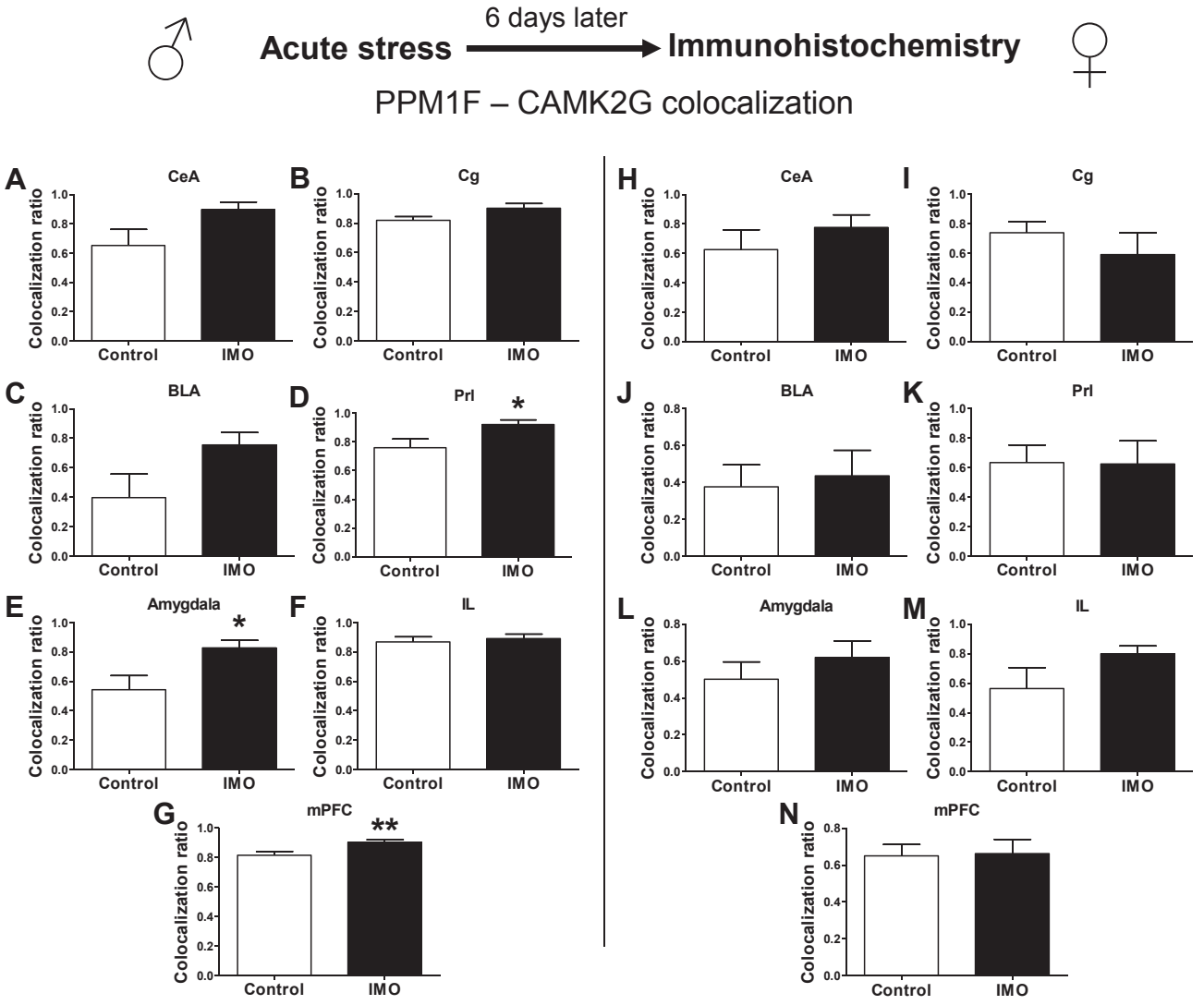


Figure 5. **(A–N)** Stress immobilization (IMO) and PPM1F and CAMK2G levels in amygdala and medial prefrontal cortex (mPFC) in both male and female mice: an immunohistochemistry study. Quantification of PPM1F and CAMK2G levels reveals that both are more densely colocalized after traumatic stress in male mice in the amygdala and mPFC. **(A, H)** Central amygdala (CeA). **(B, I)** Cingulate cortex (Cg). **(C, J)** Basolateral amygdala (BLA). **(D, K)** Prelimbic cortex (Prl). **(E, L)** Amygdala—CeA and BLA. **(F, M)** Infralimbic cortex (IL). **(G, N)** mPFC—Cg, Prl, and IL. $^{*}p \leq .05$, $^{**}p \leq .01$ ($n = 2$ per group).

88 were control subjects with no PTSD and no depressive symptoms. Among these five genes, *PPM1F* (ILMN_2059535) was significantly associated with comorbid PTSD and depression after adjusting for gender, age, and population substructure ($\beta = .135$; $p = .005$; Bonferroni adjusted $p = .025$; $n = 230$). Cases with comorbid PTSD and depression symptoms had lower *PPM1F* expression than control subjects (Figure 3A). Notably, *PPM1F* is highly expressed in the brain in both mice and humans (Supplemental Figure S5).

PPM1F in Anxiety in a Replication Sample

Given that cases with PTSD present with anxiety symptoms, we examined blood mRNA of *PPM1F* (ILMN_2059535) in cases in the CHWDB cohort; this included 151 cases with anxiety symptoms and 165 control subjects ($n = 316$). Their characteristics are presented in Supplemental Table S7. We found that *PPM1F* was significantly downregulated in cases with anxious symptomatology versus control subjects after adjusting for gender and ethnicity ($p = .044$; $n = 316$) (Figure 3B and Supplemental Table S7), consistent with the association found in the GTP sample.

Association Between *PPM1F* SNPs and Comorbid PTSD and Depression

Having identified *PPM1F* mRNA expression level to be significantly associated with PTSD, depression, and anxiety, we next investigated whether certain *PPM1F* SNPs influence this association. To this end, we examined the association between the *PPM1F* SNPs and comorbid PTSD and depression, and whether the identified significant SNPs influence *PPM1F*

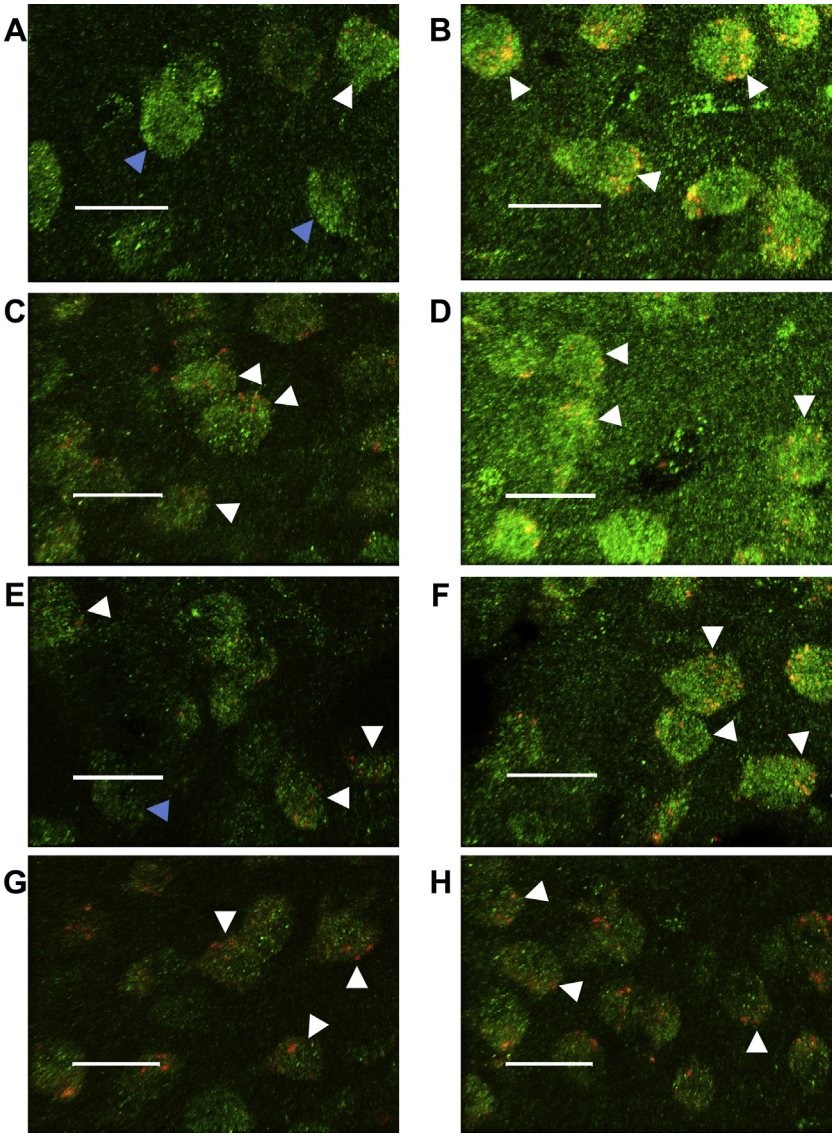
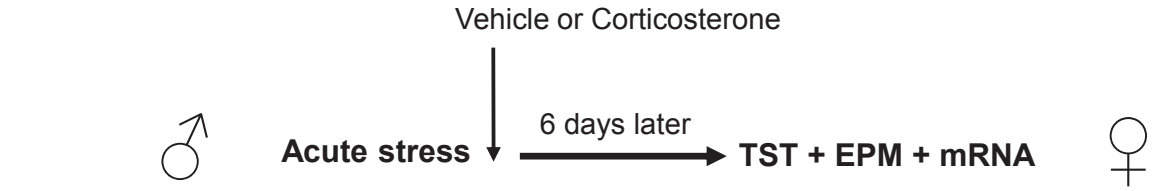


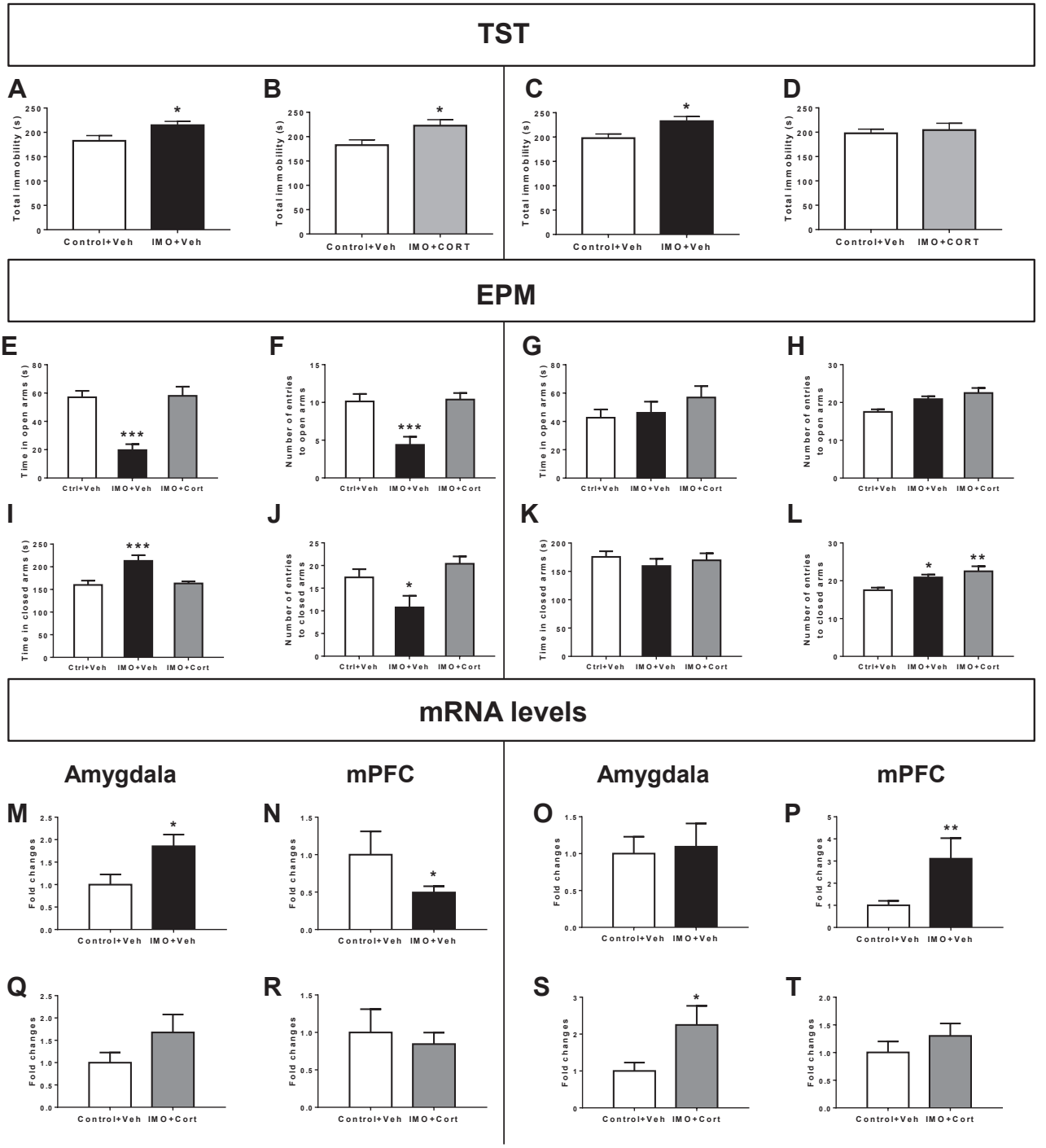
Figure 6. Representative images of the immunohistochemistry study evaluating colocalization of PPM1F (green) and CAMK2G (red) in the amygdala and medial prefrontal cortex. **(A)** Male basolateral amygdala (BLA), control group. **(B)** Male BLA, immobilization (IMO) stress group. **(C)** Male prelimbic cortex (PrL), control group. **(D)** Male PrL, IMO stress group. **(E)** Female BLA, control group. **(F)** Female BLA, IMO stress group. **(G)** Female PrL, control group. **(H)** Female PrL, IMO stress group. Blue arrow indicates no colocalization. White arrow indicates colocalization. Scale bar = 20 μm.

expression level in blood and brain. In the GTP human dataset, there were 14 tagging SNPs for *PPM1F*. Among these SNPs, rs17759843, located in the 3′UTR of *PPM1F*, was significantly associated with comorbid PTSD and depression after adjusting for sex and 10 genetic principal components (odds ratio = 0.53; p = .0046; permuted p = .0463; n = 2361) (Figure 4). The minor allele of rs17759843 was associated with lower odds for comorbid PTSD and depression (Figure 4). rs17759843 has a minor allele frequency of 0.018 and p value for departure from Hardy-Weinberg equilibrium of .6 in the unaffected population and 1 in affected population.

PPM1F rs17759843 Influences Expression of *PPM1F* in Human Blood

As we found rs17759843 to be significantly associated with comorbid PTSD and depression, we examined whether this SNP is associated with human blood *PPM1F* mRNA level in 358 GTP participants. There was no significant association between this SNP and *PPM1F* mRNA level after adjusting for sex, age, and population substructure (p = .635; n = 358). However, when we looked at the blood eQTL browser (24) of 5300 human samples, there was a highly significant association between rs17759843 and *PPM1F* ($p = 6.1 \times 10^{-25}$, false discovery rate = 0.00; effect size Z score = 10.31). Specifically, the minor allele of rs17759843 was associated with higher expression of *PPM1F*. This directionality is consistent with our genetic and gene expression findings in the GTP sample, in which the minor allele of rs17759843 was significantly associated with lower probability of having comorbid PTSD and depression, as well as the finding that control subjects had higher expression of *PPM1F* compared with cases with comorbid PTSD and depression. With a larger sample


Vehicle or Corticosterone
Acute stress
6 days later
TST + EPM + mRNA


TST
A
B
C
D
Total immobility (s)
Control+Veh
IMO+Veh
IMO+CORT
EPM
E
F
G
H
Time in open arms (s)
Number of entries to open arms
I
J
K
L
Time in closed arms (s)
Number of entries to closed arms
Ctrl+Veh
IMO+Veh
IMO+Cort
mRNA levels
Amygdala
mPFC
M
N
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R
S
T
Fold changes
Control+Veh
IMO+Veh
IMO+Cort

size and thus more power, we conclude that rs17759843 is indeed a blood *cis*-eQTL for *PPM1F* mRNA expression in humans.

rs17759843 Influences Expression of *PPM1F* in Human Brain

As *Ppm1f* mRNA level was upregulated in the amygdala and downregulated in the mPFC of stressed mice, which is consistent with another published study (26), we examined whether rs17759843 is associated with brain mRNA level of *PPM1F* in the publicly available BrainCloud dataset, which contains and compiles human gene expression profiles of the PFC. There were three probes for *PPM1F* in this dataset. Of these, two probes had a significant association with *PPM1F*: probe 8896 (p = .0032, Bonferroni-adjusted p = .0097) and probe 16009 (p = .0004, Bonferroni-adjusted p = .0012). In summary, rs17759843 is significantly associated with *PPM1F* mRNA level in human FPC.

CAMK2

As PPM1F regulates CAMK2 in neuronal cells (27,28) and fibroblasts (29), we examined blood mRNA level of *CAMK2* in comorbid PTSD and depression. After quality control, the human dataset had three probes for *CAMK2* (one for *CAMK2D* and two for *CAMK2G*; none for *CAMK2A* or *CAMK2B*). Of these three probes, the *CAMK2G* probe had significantly lower expression in cases with comorbid PTSD and depression symptoms compared with control subjects after adjusting for gender, age, and population substructure (β = .072; p = .0158; Bonferroni corrected p = .0474, N = 230) (Supplemental Figure S6A and B).

We analyzed *Camk2g* in the mouse brain because this CAMK2 isoform was the most significantly regulated in our human dataset. After stress IMO, *Camk2g* mRNA was marginally upregulated in the mouse amygdala 6 days after IMO (t_8 = 2.213, p = .058) (Supplemental Figure S6C). However, *Camk2g* expression was not regulated in mouse amygdala 2 hours after fear conditioning in mice with or without a previous exposure to stress (Supplemental Figure S6D).

Immunohistochemistry of PPM1F and CAMK2G

PPM1F protein levels were upregulated in the central amygdala, basolateral amygdala (BLA), central amygdala plus BLA (total amygdala), and mPFC in male mice 6 days after stress IMO (Supplemental Figure S7). Moreover, PPM1F levels in the BLA were upregulated in female mice 6 days after IMO (Supplemental Figure S8). CAMK2G levels were upregulated in the central amygdala, BLA, total amygdala, and mPFC in male mice 6 days after IMO (Supplemental Figure S9). However, there were no changes in CAMK2G levels 6 days after IMO in female mice (Supplemental Figure S10). Figure 5 shows that stress IMO enhances the colocalization of PPM1F and CAMK2G in the prelimbic cortex (t_{12} = −2.308, p = .046), total amygdala (t_{24} = −2.598, p = .017), and mPFC in male mice (t_{41} = −2.814, p = .008) (Figure 5). See Figure 6 for representative images of these immunohistochemistry studies.

Corticosterone and *Ppm1f*

Vehicle (Veh) or corticosterone was given 1 hour after stress IMO in male and female mice. TST, EPM, and mRNA levels were evaluated 6 days later. In male mice, there was a significant increase in total immobility as measured by TST in the IMO + Veh group (t_{14} = −2.328, p = .037) (Figure 7A) and IMO + corticosterone group (t_{14} = −2.45, p = .029) (Figure 7B) compared with the control + Veh group. In female mice, there was a significant increase in total immobility as measured by a TST in the IMO + Veh group (t_8 = −2.394, p = .044) (Figure 7C) compared with the control + Veh group. Analysis of the EPM in male mice showed significant differences in the time in open arms ($F_{2,21}$ = 10.358, p = .001; post hoc IMO + Veh group p = .001 vs. other groups), entries to open arms ($F_{2,21}$ = 11.902, p = .000, post hoc p = .0001 vs. other groups), time in closed arms ($F_{2,21}$ = 9.691, p = .001, post hoc p = .001 vs. other groups), and entries to closed arms ($F_{2,21}$ = 5.762, p = .010, post hoc p = .033 vs. other groups). EPM results in female mice showed a significant effect for treatment on the total number of entries to closed arms ($F_{2,21}$ = 6.879, p = .005). Post hoc comparisons revealed an increased number of entries to closed arms in the IMO + Veh group (p = .023) and IMO + corticosterone group (p = .002) compared with the control + Veh group. Analysis of mRNA levels in male mice showed that the IMO + Veh group presented with enhanced levels of *Ppm1f* in the amygdala compared with the control group (t_{13} = 2.383, p = .0331) (Figure 7M) and decreased levels in the mPFC (t_{13} = 2.321, p = .0371) (Figure 7N) compared with the control group. mRNA levels in female mice were upregulated in the mPFC in the IMO + Veh group compared with the control + Veh group (t_{13} = 3.248, p = .0064) (Figure 7P) and in the amygdala in the IMO + corticosterone group compared with the control + Veh group (t_{14} = 2.579, p = .0218) (Figure 7S).

DISCUSSION

We found that *Ppm1f* mRNA levels were significantly altered in the amygdala and mPFC after stress exposure in a mouse model. Furthermore, we demonstrate that the human blood

Figure 7. **(A–T)** *Ppm1f* and corticosterone (Cort) in anxiety-like behavior and depressive-like behavior in both male and female mice (n = 5–8 per group). **(A, B)** Systemic corticosterone given 1 hour after stress did not rescue the depressive-like phenotype elicited by immobilization (IMO) stress in male mice. **(C, D)** However, corticosterone rescued depressive-like behavior induced by IMO stress in female mice (*$p \leq .05$). **(E, F, I, J)** Elevated plus maze (EPM) results showed that corticosterone rescued the anxiety-like behavior in male mice (*$p \leq .05$, **$p \leq .01$, ***$p \leq .001$ vs. other groups). **(G, H, K)** In contrast, IMO stress did not induce anxiety-like behavior in female mice. **(L)** The number of entries in closed arms was increased in the IMO + vehicle (Veh) group vs. control (Ctrl) + Veh group (*$p \leq .05$, **$p \leq .01$ vs. control + Veh). Analysis of messenger RNA (mRNA) levels in male mice showed that the IMO + Veh group presented with enhanced levels of *Ppm1f* compared with control group (p = .0331) **(M)** in amygdala and decreased levels in medial prefrontal cortex (mPFC) (p = .0371) **(N)**. Corticosterone rescued the *Ppm1f* gene changes induced by IMO stress in amygdala **(Q)** and mPFC **(R)**. mRNA levels in female mice were upregulated in mPFC in the IMO + Veh group vs. the control + Veh group **(P)** as well as in amygdala in the IMO + Cort group vs. the control + Veh group **(S)**. Corticosterone rescued the *Ppm1f* gene changes in mPFC **(T)**. TST, tail suspension test.

mRNA level of *PPM1F* was significantly downregulated in cases with comorbid PTSD and depression. Moreover, we show that a genetic variant in the 3′UTR of *PPM1F*, rs17759843, was significantly associated with comorbid PTSD and depression and with mRNA levels of *PPM1F* in human blood as well as brain. Additionally, we found that one of the substrates of *PPM1F, CAMK2G*, had significantly downregulated blood mRNA levels in cases with comorbid PTSD and depression, which is consistent with CAMK2 being a substrate of PPM1F. Consistently, PPM1F protein levels and colocalization of PPM1F with CAMK2G were altered in the amygdala and mPFC of male mice. Taken together, our findings suggest a novel and critical role of *PPM1F* in association with stress, anxiety, and depressive symptoms following trauma exposure.

PPM1F is a protein phosphatase and a member of the protein serine/threonine phosphatase 2C family of Ser/Thr protein phosphatases, which are negative regulators of stress response pathways. CAMK2 subunit gamma is one of the substrates of this phosphatase (27–29). Interestingly, *Ppm1f* expression in mice was not altered at early time points after an aversive learning paradigm or 1 day after IMO stress. A potential interpretation is that these changes in basal expression of *Ppm1f* require about 1 week to develop in mice. Also, it is possible that *Ppm1f* changes may not be related to fear learning, per se, or are not detected at the time of the collection of the brain tissue in these experiments. In fact, our data suggest that it may be a marker of the chronic effects of prior trauma exposure.

PPM1F specifically dephosphorylates CAMK2 in rat neurons in vitro and in cells (30). This results in an inactive form of CAMK2. CAMK2 is critical in serotonergic regulation of PFC neuronal activity, which plays a major role in depression, suicide, anxiety, and schizophrenia (31–33) and which may explain the neuropsychiatric behavioral patterns seen in *Camk2* knockout mice (34). Ca^{2+}/CaMK levels in the brain have been previously associated with anxiety (35), depression (36), and suicide (37). Our present data suggest that *PPM1F* could be an upstream regulator—activated by traumatic stress—of CAMK, promoting the development of anxiety and depression-like symptoms. However, the consequences of regulation of *PPM1F* by stress could have broader implications than described here because of ubiquitous expression of *PPM1F* in the body and brain. For example, PTSD and depression have been associated with higher rates of coronary heart disease (38–40), and knowledge of the underlying mechanisms for this association is still limited. As CAMK2 plays an important role in the development and progression of cardiovascular disease (41–43), it is possible that the PPM1F/CAMK2 pathway may play a role in coronary diseases. Further study of *PPM1F* and *CAMK2* may shed light on the mechanisms linking PTSD and depression to elevated risk for heart disease.

Using a different traumatic model, previous studies have shown that exposing rats to a predator results in amygdala upregulation but mPFC downregulation of phosphorylated CAMK2 1 week later (44). Consistently, we found significant changes in mRNA levels of CAMK2G in blood in cases with comorbid PTSD and depression symptoms and in the amygdala of mice exposed to stress. Concordantly, we found that stress also regulated CAMK2G protein in the mouse brain. Moreover, PPM1F protein level in the amygdala is regulated by stress in male mice, suggesting that IMO alters PPM1F levels. Regarding directionality, the *Ppm1f* mRNA level and PPM1F protein level are in the same direction in the amygdala but in the opposite direction in the mPFC. Interestingly, PPM1F/CAMK2G colocalization is regulated by stress in the mouse brain. Thus, it is possible that PPM1F levels may be regulating CAMK2G after stress, promoting the development of depression and anxiety behaviors.

We also examined whether poststress manipulation, i.e., systemic administration of corticosterone, may decrease stress-related behavioral and molecular phenotypes (44–46). Systemic corticosterone did not rescue the depressive-like phenotype elicited by IMO in male mice. However, corticosterone rescued the depressive-like behavior induced by IMO in female mice. EPM results showed that corticosterone rescued the anxiety-like behavior in male mice. In contrast, IMO did not induce an anxiety-like behavior in female mice. Analysis of mRNA levels showed that corticosterone treatment shortly after stress rescued the genetic regulation of *Ppm1f* in the amygdala and mPFC in male mice and in the mPFC in female mice. Thus, there appear to be relevant sex differences in *Ppm1f* regulation in the brain, acute exposure to traumatic stress, and treatment with corticosterone.

Our study should be interpreted in light of its limitations. First, the data obtained with IMO should be interpreted with caution. Whereas most rodents develop anxiety and depression-like behavior after IMO, only a small percentage of the human population develops PTSD and/or depression. Second, rs17759843 is associated with blood expression of *PPM1F* in a sample of mostly European descent. rs17759843 has a minor allele frequency of 0.11 in Caucasians and 0.01 in African Americans per database of Genotypes and Phenotypes (dbGAP) HapMap populations. Whether this SNP is an eQTL in African Americans remains to be determined, as we were likely underpowered to detect this relationship in our GTP dataset. Third, the blood eQTL browser provides statistics on bivariate association between rs17759843 and blood *PPM1F* expression. Hence, rs17759843 may be associated with *PPM1F* owing to its linkage disequilibrium with other stronger eQTL SNPs for *PPM1F*. Fourth, we did not have access to human brain amygdala to examine association between rs17759843 and *PPM1F* expression. Fifth, we analyzed cases with significant current symptoms of comorbid PTSD and depression, but we did not have rigorous clinical assessment to determine if these cases had the diagnosis of PTSD or major depressive disorder. Additionally, our cutoff score of 14 for the PSS and BDI, though based on published psychometric studies, may be lenient and thus could increase the risk of false-positive results. Sixth, the CHDWB replication sample uses the GAD-7, which is a good screening tool but could provide a false-positive detection if not followed by an accurate clinical evaluation. Seventh, we found blood mRNA *PPM1F* level significantly downregulated in cases with comorbid PTSD and depression symptoms in the GTP sample and replicated this association in cases with anxiety symptoms in an independent CHDWB cohort. Our replication is only a quasi-replication, as anxiety symptoms can be PTSD symptoms but they can also

be symptoms of generalized anxiety disorder or social anxiety disorder. Lastly, only a subset of GTP participants with genetic data had gene expression data. However, the two datasets are comparable as reflected by the distribution of sex and BDI, and PSS scores (Supplemental Table S8).

In conclusion, mRNA and protein levels of phosphatase PPM1F and CAMK2G are regulated in the brain after exposure to traumatic stress in male and female mouse models and are associated with chronic anxiety-related (PTSD) and depression symptoms in humans who have been traumatized. Because of the widespread expression of PPM1F in the body and brain, these changes in expression of PPM1F induced by stress may possibly have other pathophysiological implications that should be examined in future studies. Insights into the role of *CAMK2* and *PPM1F* following trauma exposure may contribute to discovering novel approaches to treat or prevent the disabling conditions of PTSD and depression.

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