

Research Article

ADCYAP1R1 GENOTYPE, POSTTRAUMATIC STRESS DISORDER, AND DEPRESSION AMONG WOMEN EXPOSED TO CHILDHOOD MALTREATMENT

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Background: *A growing literature indicates that genetic variation, in combination with adverse early life experiences, shapes risk for later mental illness. Recent work also suggests that molecular variation at the ADCYAP1R1 locus is associated with posttraumatic stress disorder (PTSD) in women. We sought to test whether childhood maltreatment (CM) interacts with ADCYAP1R1 genotype to predict PTSD in women. Methods:* Data were obtained from 495 adult female participants from the Detroit Neighborhood Health Study. Genotyping of rs2267735, an ADCYAP1R1 variant, was conducted via TaqMan assay. PTSD, depression, and CM exposure were assessed via structured interviews. Main and interacting effects of ADCYAP1R1 and CM levels on past month PTSD and post-traumatic stress (PTS) severity were examined using logistic regression and a general linear model, respectively. As a secondary analysis, we also assessed main and interacting effects of ADCYAP1R1 and CM variation on risk of past-month depression diagnosis and symptom severity. **Results:** No significant main effects were observed for ADCYAP1R1 genotype on either PTSD/PTS severity. In contrast, a significant ADCYAP1R1 × CM interaction was observed for both past month PTSD and PTS severity, with carriers of the “C” allele showing enhanced risk for these outcomes among women exposed to CM. No significant main or interaction effects were observed for past month depression/depression severity. **Conclusions:** Genetic variation at the ADCYAP1R1 locus interacts with CM to shape risk of later PTSD, but not depression, among women. The molecular mechanisms contributing to this interaction require further investigation. *Depression and Anxiety 30:251–258, 2013.* © 2012 Wiley Periodicals, Inc.

Key words: PTSD; depression; childhood maltreatment; candidate gene; replication; gene-environment interaction

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INTRODUCTION

Posttraumatic stress disorder (PTSD) is an anxiety disorder that emerges following exposure to trauma. Although a substantial proportion (50–90%;^[1,2]) of Americans are exposed to traumatic events in their lifetime, only a minority (8–20%) go on to develop PTSD.^[1] Trauma exposure characteristics such as type or severity do not fully explain differences in risk of PTSD, suggesting that additional factors, both genetic and environmental,^[3] contribute to risk of PTSD following trauma exposure.

One of the most potent predictors of PTSD is childhood maltreatment (CM). For example, meta-analyses indicate that CM exposure is one of the most robust risk factors for PTSD among trauma-exposed adults.^[4,5] Similarly, prospective studies of PTSD risk show that among adults with documented cases of childhood abuse, the odds of current and lifetime PTSD are 1.86 and 1.75 greater, respectively, compared to non-CM-exposed controls.^[6] Notably, across all types of prospectively assessed abuse, women are more than twice as likely as men to develop PTSD.^[7]

Recently, Ressler et al.^[8] reported that rs2267735, a variant in the pituitary adenylate cyclase activating polypeptide 1 receptor type I gene (*ADCYAP1R1*) predicted PTSD, but not depression, diagnosis, and symptoms in women only. *ADCYAP1R1* serves as a receptor to pituitary adenylate cyclase-activating polypeptide (PACAP), a neuropeptide critical to the regulation of prolonged stress circuit activation in both the central and peripheral nervous systems.^[9] Although the functional significance of the rs2267735 *ADCYAP1R1* variant is not currently known, its location within a predicted estrogen response element,^[8] combined with evidence indicating that estrogen influences *ADCYAP1R1* gene expression,^[8,10] suggests the plausibility of potential sex-specific effects of rs2267735. Nevertheless, recently, Chang et al.^[11] failed to replicate the initial report of an rs2267735-PTSD association in two independent samples, although the direction of the reported effects for females was in the expected direction.

In light of these recent findings, and the extensive literature confirming the potent effect of CM on risk of mental illness during adulthood (reviewed in^[12,13]), we genotyped rs2267735 specifically in females to explore possible main and interacting effects of *ADCYAP1R1* and CM variation on risk of PTSD. In addition, due to the substantial genetic overlap between PTSD and depression,^[14] and the possibility of previously undetected gene–environment interactions for depression at this locus,^[8] we also assessed main and interacting effects of *ADCYAP1R1* and CM variation on risk of depression as a secondary analysis.

METHODS

PARTICIPANTS

Samples for this study were drawn from the Detroit Neighborhood Health Study (DNHS). The DNHS is a population-representative cohort study of adult residents residing in Detroit and has been described.^[15,16] In brief, 1,547 adult participants (age 18 years or older) were selected from the Detroit population to participate in wave 1 of the DNHS via a telephone survey by choosing a probability sample of households within the city limits of Detroit and then randomly selecting one adult from each household. A dual-frame probability sample design was employed to draw a sample of residential addresses, obtaining telephone numbers from two sources: (1) US Postal Service Delivery Sequence File (DSF), which consists of the entire Detroit population and includes nontelephone and cellular phone-only households and (2) a list-assisted sampling random-digit-dial frame, covering Detroit households that are not residential directory-listed numbers (the unlisted number frame). Telephone numbers in these two databases were matched to identify the sample addresses that have at least one listed landline telephone number; these people were then contacted by telephone to participate in the telephone survey. A postal mail effort was also initiated in order to invite the other part of the sample with no listed landline, telephone, or cell phone to participate in the survey. The overall response rate among those eligible was 53.0%, which compares very favorably with other true population-based samples that have collected both interview and biologic data.^[17] The DNHS was approved by the Institutional Review Board at the University of Michigan. All participants provided informed consent prior to participating in this study.

DNA SAMPLES AND GENOTYPING

Previously extracted DNA samples from 514 available female participants in waves 1 and 2 were selected for inclusion in this study. DNA samples were isolated from either whole blood or saliva in the Wildman laboratory. Whole blood samples were isolated using Qiamp Mini kits (Qiagen, Valencia, CA) or the QuickGene DNA whole blood kit (Fujifilm Life Sciences, Tokyo, Japan) following the manufacturers' recommended protocol. Saliva samples were isolated using Oragene OG-500 (DNAgenotek, Kanata, Canada) kit and following the manufacturer's recommended protocol.

Genotyping of rs2267735, located in an intronic region of the *ADCYAP1R1* locus, was genotyped in the Ressler laboratory using a TaqMan[®] assay (Life Technologies Corporation, Carlsbad, CA) as previously described.^[8] Genotyping was conducted by individuals who were blinded to participants' PTSD and depression status.

ASSESSMENT OF PTSD AND DEPRESSION

Structured telephone interviews assessed participants' PTSD and depression symptoms as previously described.^[15,18] Symptoms were assessed as occurring within the past month in order to approximate the time frame used in the Ressler et al study.^[8] Briefly, PTSD diagnosis was assessed via telephone using a structured diagnostic interview validated against the Clinician-Administered PTSD Scale for DSM-IV (CAPS). Participants were initially asked to identify potentially traumatic events (PTEs) that they experienced in the past from a list of 19 events.^[19] PTSD symptoms were then assessed by referencing two traumatic events that the respondent may have experienced: one that the participant regarded as the worst and one randomly selected event from the remaining PTEs a respondent may have experienced. Respondents were considered affected by past month PTSD if all six DSM-IV criteria were met in reference to either the worst or the random event *and* they reported being affected by the symptoms within

the past month. PTS severity was assessed by summing participants' ratings of the 17 posttraumatic symptoms on a scale indicating the degree to which the respondent had been bothered by a particular symptom as a result of the worst trauma, ranging from 1 (not at all) to 5 (extremely); total PTS scores could thus range from 17 to 85. Past month PTS severity equated to the total PTS score if participants reported being affected by the symptoms within the past month; otherwise, past month PTS severity was set to 0.

Depressive symptoms were evaluated with a structured interview based on a modified version of Patient Health Questionnaire (PHQ-9).^[20] Each of the nine questions was scored as 0 (not at all) to 3 (nearly every day), such that scores ranged from 0 to 27.^[20] Past month depression cases were participants who reported depressed mood or anhedonia and at least one additional symptom for "more than half the days" for two or more weeks, whose symptoms were present during the past month and occurred together. One symptom, "thoughts that you would be better off dead or of hurting yourself in some way," counted if present at all, regardless of duration. Past month depression severity was the sum of symptoms reported as being present within the past month, such that scores could range from 0 to 27.

PTSD and depression diagnoses obtained from the telephone interview responses has been validated in a random subsample of participants via in-person clinical interview using the CAPS and Structured Clinical Interview for DSM-IV Disorders, which has been described previously.^[15,21] The comparison showed high internal consistency and concordance.

ASSESSMENT OF CM

Exposure to CM was assessed in the wave 2 survey and was scored on a continuous scale following the method employed in.^[22] Briefly, CM questions were drawn from the Conflict Tactics Scale (CTS),^[23] the Childhood Trauma Scale (CTQ),^[24] and Wyatt's eight-item interview guide^[25] as implemented by the Nurse's Health Study II.^[26] CTQ items assessed physical abuse (e.g. "People in my family hit me so hard that it left me with bruises and marks"), and emotional abuse (e.g. "People in my family said hurtful or insulting things to me") assessed before age 11. Response options were rated on a 5-point scale and ranged from "never true" (1) to "very often true" (5). CTS items assessed physical abuse before age 18 (e.g. "Did your parent, step-parent, or adult guardian ever push, grab, or shove you") with response options ranging from "never" to "more than a few times." Sexual abuse before age 18 was assessed with two items regarding sexual assault and rape used in the Nurse's Health Study II (NHSII)^[26] (e.g. "Were you ever touched in a sexual way by an adult or older child") which was coded as occurring once, multiple times, or never. CTQ physical abuse and emotional abuse questions, CTS physical questions, and NHSII-modified Wyatt's sexual abuse questions were recoded into three-level variables indicating whether each abuse type occurred: (1) never, (2) rarely or sometimes, or (3) often. Scores were summed to create a continuous variable ranging from 0 to 22. In addition, number of CM types (i.e. the presence of exposure to physical, emotional, and sexual abuse) was assessed in order to provide a visual representation of the association among CM, rs2267735 genotype, and past month PTSD.

STATISTICAL ANALYSIS

The distribution of the primary study variables (past month PTSD, past month depression, CM) was calculated using means with standard deviations for continuous variables, and frequencies and percentages for categorical variables. Bivariate associations were assessed for each variable with respect to rs2267735 genotype. Chi-square tests were performed for categorical variable comparisons; for continu-

ous variable comparisons, Kruskal-Wallis tests were used. Hardy-Weinberg equilibrium of rs2267735 genotypes was assessed chi-square tests as applied in the online Hardy-Weinberg Equilibrium calculator.^[27]

Past month PTSD and past month depression were modeled using logistic regression. To assess possible subthreshold effects of *ADCYAP1R1* variation on these mental illnesses, past month PTS severity and past month depression severity were modeled using a general linear model (GLM), with the severity measure log transformed to improve normality. We assessed main effects of each outcome using the continuous (0–22) CM measure and rs2267735 genotype as the main predictors, controlling for race and age as covariates. In addition, in order to isolate the effects of these main predictors (and their interaction) on each disorder, net of the effects of the other disorder, we controlled for comorbid past month depression/depression severity (in PTSD/PTS severity models) and comorbid past month PTSD/PTS severity (in depression/depression severity models). Following main effect analyses, we assessed the presence/absence of CM × rs2267735 genotype interactions by including an interaction term in each of the main effects models. Effect estimates were accepted as significant if $P < .05$. An additive effect of rs2267735 genotype was assumed in all models, with GG designated as the reference genotype.

To further confirm our results obtained by logistic regression analyses, which included a relatively small number of past month PTSD and depression cases, we performed permutation tests. We used the logregperm package^[28] in R v2.13.0 to carry out 100,000 permutations to test the primary study variables (i.e. single nucleotide polymorphism or SNP, CM, and SNP × CM in interaction models) with its residuals from a linear regression on the other independent variables (age, race, etc). The residuals were subjected to random permutation, binary logistic regression was reperformed, and the permutation P -value (i.e. the fraction of the permutations that have a likelihood-based P -value less than or equal to that for the unpermuted data) was calculated.

To further confirm that our results obtained by linear regression did not depend on normal distributional assumptions, we used the ape package^[29] in R v2.13.0 to conduct 100,000 permutation tests. We performed linear regression (least squares) to obtain a t -value as a reference t -value (t_{ref}) for key independent variables (SNP, CA, and SNP × CA), randomly permuted our dependent variables, and then reconducted linear regression to obtain t -values for the same key independent variables. The permutation P -value was then computed as the proportion of t -values greater than or equal to the reference t -value (t_{ref}).

To assess possible genotype-dependent correlations between past month PTS or depression severity and CM exposure, partial correlations, controlling for age, were assessed between: (i) past month PTS severity and CM and (ii) past month depression severity and CM. Partial correlations were determined using the Spearman rank method due to nonnormality of the data. Z -scores were employed to test for the presence of significant differences in correlations between genotypes.

Analyses of past month PTSD/PTS severity and past month depression/depression severity were restricted to participants with available CM data; for PTSD/PTS severity, analyses were further restricted to trauma-exposed participants. In addition, due to the race-specific genotype × environment interactions that have been reported in the child abuse literature,^[30,31] main effect and interaction analyses were restricted to participants who self-identified as either white or African-American, that is, those self-reported race categories that had sufficient members to be included in analyses. In addition, logistic regression and GLM analyses were run using African-American participants only.

TABLE 1. Distribution of past month PTSD, depression, CM, and *ADCYAP1R1* genotype ($n = 401$ for PTSD, $n = 430$ for others)

| Genotype | PTSD | | Depression | | CM exposure 0–22 | Number of CM types | | |
|------------|--------------------|-----|------------|-----|---------------------|--------------------|----|-----|
| | No | Yes | No | Yes | | 0 | 1 | 2+ |
| GG | 51 | 2 | 54 | 6 | 4.68 ± 4.03 | 37 | 7 | 16 |
| CG | 192 | 10 | 195 | 21 | 4.98 ± 4.45 | 117 | 54 | 45 |
| CC | 135 | 11 | 134 | 20 | 5.55 ± 5.52 | 80 | 34 | 40 |
| Total | 378 | 23 | 383 | 47 | 5.14 ± 4.81 | 234 | 95 | 101 |
| Chi-square | 1.481 ^a | | 1.046 | | 0.198 ^b | 0.969 | | |
| P-value | 0.477 | | 0.593 | | 0.906 | 0.616 | | |

CM, childhood maltreatment.

^aChi-square test.

^bKruskal–Wallis Test.

RESULTS

Genotypes at the rs2267735 locus were successfully determined for 495 of 514 participants (success rate 96.3%). *ADCYAP1R1* genotypes at the rs2267735 were in Hardy–Weinberg Equilibrium in the full sample ($P = .31$). The frequency of the common “C” allele was 60.2%.

Among all genotyped participants, 11.7% were white, 83.1% were African American, and 5.2% reported belonging to another race/ethnic group. Table 1 shows the distribution of the main study variables of interest by *ADCYAP1R1* genotype in our analytic sample. Among trauma-exposed participants, 23 (6%) reported symptoms consistent with past month PTSD. A larger proportion (11%) of participants reported symptoms of past month depression. Past month PTSD, past month depression, and average CM scores (Mean = 5.14, $SD = 4.81$) did not vary by *ADCYAP1R1* genotype.

Table 2 shows the results of main and interaction logistic regression analyses of the effect of *ADCYAP1R1* genotype and CM on PTSD, controlling for age, race, and past month depression. In main effect analyses, the odds of past month PTSD were significantly associated with exposure to CM ($P < .002$, permutation $P = .0021$): for every one-unit increase in CM score,

there was a 15% increase in odds of past month PTSD. In contrast, there was no significant association between *ADCYAP1R1* genotype and past month PTSD ($P = .60$). However, results from interaction effect analyses showed that participants with one versus no “C” alleles at the *ADCYAP1R1* rs2267735 locus showed a 17% increased odds of past month PTSD for every one-unit increase in CM score ($P = .029$, permutation $P = .030$). Analyses based on African–American participants only showed similar results (Table S1).

Results from our secondary analyses assessing the joint and interacting effects of *ADCYAP1R1* genotype and CM exposure on depression are presented in Table 3. Similar to the PTSD results, the odds of past month depression were significantly associated with exposure to CM (OR = 1.12, 95% CI = 1.05, 1.20, $P = .001$, permutation $P = .0013$), but not *ADCYAP1R1* genotype, in main effect analyses (Table 3A). In contrast, no significant interaction was observed between *ADCYAP1R1* genotype and CM ($P = .226$; Table 3B). Analyses based on African–American participants only showed similar results (Table S2). Results were similar when PTS severity (Table 4) and depression severity (data not shown) were used as outcomes.

Correlations between CM and past month PTS and past month depression severity stratified by genotype are reported in Table 5. Significant partial correlations,

TABLE 2. Main (A) and interaction (B) effect logistic regression model results predicting past month PTSD ($n = 380$)

| | A. Main effect | | | | B. Interaction effect | | | |
|-----------------------------|----------------|--------|-------|-------|-----------------------|--------|-------|------|
| | Adjusted OR | 95% CI | | P | Adjusted OR | 95% CI | | P |
| Age | 0.97 | 0.93 | 1.00 | .045 | 0.96 | 0.93 | 1.00 | .034 |
| African-American | 0.17 | 0.05 | 0.57 | .004 | 0.15 | 0.04 | 0.51 | .003 |
| Past month depression | 6.42 | 2.26 | 18.19 | <.001 | 5.62 | 1.90 | 16.63 | .002 |
| CM | 1.15 | 1.06 | 1.26 | <.002 | 0.79 | 0.56 | 1.12 | .192 |
| <i>ADCYAP1R1</i> “C” allele | 1.23 | 0.57 | 2.64 | .596 | 0.38 | 0.11 | 1.33 | .130 |
| <i>ADCYAP1R1</i> × CM | — | — | — | — | 1.17 | 1.02 | 1.34 | .029 |
| –2LogL | 127.87 | | | | 123.05 | | | |

CM, childhood maltreatment measured on a scale of 0–22.

Comparing the main effect and interaction models, Chi-square = 4.82 $P = .028$.

TABLE 3. Main (A) and interaction (B) effect logistic regression model results predicting past month depression (*n* = 409)

| | A. Main effect | | | | B. Interaction effect | | | |
|-----------------------------|----------------|--------|-------|----------|-----------------------|--------|-------|----------|
| | Adjusted OR | 95% CI | | <i>P</i> | Adjusted OR | 95% CI | | <i>P</i> |
| Age | 0.99 | 0.97 | 1.01 | .356 | 0.99 | 0.97 | 1.01 | .345 |
| African-American | 1.33 | 0.44 | 4.01 | .619 | 1.25 | 0.41 | 3.80 | .693 |
| Past month PTSD | 5.41 | 1.96 | 14.96 | .001 | 4.67 | 1.63 | 13.37 | .004 |
| CM | 1.12 | 1.05 | 1.20 | <.001 | 0.98 | 0.77 | 1.23 | .836 |
| <i>ADCYAP1R1</i> “C” allele | 0.90 | 0.54 | 1.49 | .681 | 0.61 | 0.27 | 1.36 | .223 |
| <i>ADCYAP1R1</i> × CM | — | — | — | — | 1.06 | 0.96 | 1.17 | .226 |
| −2LogL | 242.68 | | | | 241.21 | | | |

CM, childhood maltreatment measured on a scale of 0–22.
 Comparing the main effect and interaction models, Chi-square = 1.47 *P* = .225.

controlling for age, were observed between CM score and PTS severity in the full analytic sample (*r* = .290, *P* < .001); however, stratification by *ADCYAP1R1* genotype showed that these correlations were significant only among genotypes containing the “C” allele. Moreover, correlation coefficients were significantly higher in the CC versus GG group (*z*-value for difference, 2.071, *P* < .05) and in the CC versus CG group (*z*-value for difference, 2.868, *P* < .01). In contrast, CM score and past month depression severity were significantly

correlated in both the full analytic sample (*r* = .306, *P* < .001, *n* = 429) and in the three subsamples defined by *ADCYAP1R1* genotype, and none of the three correlation coefficients differed significantly from one another.

Figure 1 provides a visual summary of the *ADCYAP1R1* × CM interactions detected in the PTSD-related analyses. Among participants exposed to two or more CM types, those with two “C” alleles show a markedly increased prevalence of past month PTSD

TABLE 4. Adjusted main (A) and interaction (B) effect linear regression model results predicting past month PTS severity (*n* = 380)

| | A. Main effect | | | | B. Interaction effect | | | |
|-----------------------------|----------------|--------|--------|----------|-----------------------|--------|-------|----------|
| | b | 95% CI | | <i>P</i> | b | 95% CI | | <i>P</i> |
| Age | −0.002 | −0.004 | 0.000 | .582 | −0.001 | −0.003 | 0.000 | .677 |
| African-American | −0.188 | −0.375 | −0.001 | .200 | −0.185 | −0.370 | 0.000 | .205 |
| Depression severity | 0.645 | 0.000 | 1.289 | <.0001 | 0.649 | 0.000 | 1.297 | <.0001 |
| CM | 0.047 | 0.000 | 0.093 | <.0001 | −0.031 | −0.062 | 0.000 | .425 |
| <i>ADCYAP1R1</i> “C” allele | −0.018 | −0.036 | 0.000 | .805 | −0.188 | −0.377 | 0.000 | .083 |
| <i>ADCYAP1R1</i> × CM | — | — | — | — | 0.033 | 0.000 | 0.066 | .037 |
| Adj <i>R</i> ² | .175 | | | | .182 | | | |

CM, childhood maltreatment measured on a scale of 0–22.

TABLE 5. Correlation^a between childhood maltreatment and past month PTSD (A) and depression (B)

| Genotype | GG | CG | CC |
|--|---------------|-----------------|----------------|
| A. Correlation between childhood maltreatment and past month PTS severity (<i>n</i> = 401) | | | |
| Number of participants | 53 | 202 | 146 |
| Spearman correlation <i>R</i> -value | .162 | .187 | .465 |
| <i>P</i> -value | .251 | .008 | <.0001 |
| <i>Z</i> -value for difference (ΔZ) | GG/CG = 0.163 | CG/CC = 2.868** | CC/GG = 2.071* |
| B. Correlation between childhood maltreatment and past month depression symptom severity (<i>n</i> = 429) | | | |
| Number of participants | 60 | 215 | 154 |
| Spearman correlation <i>R</i> -value | .315 | .289 | .346 |
| <i>P</i> -value | .015 | <.001 | .001 |
| <i>Z</i> -value for difference (ΔZ) | GG/CG = 0.192 | CG/CC = 0.596 | CC/GG = 0.224 |

^aPartial correlation controlling for age.
 *Significant at *P* < 0.05; **significant at *P* < 0.01.

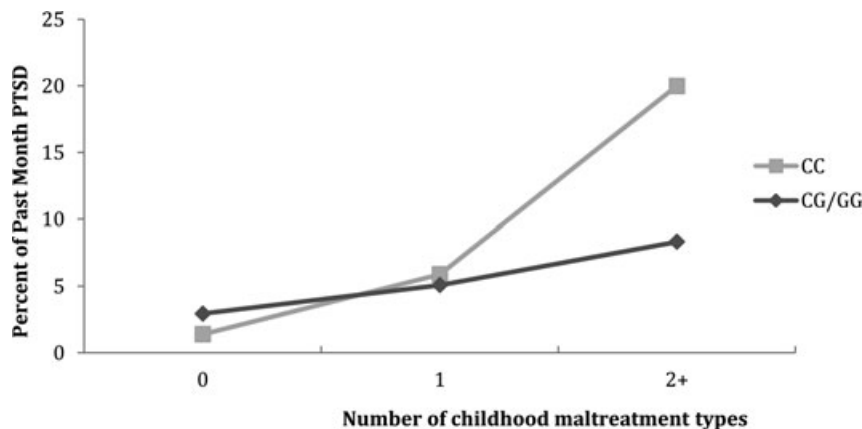


Figure 1. The effect of *ADCYAP1R1* genetic variation on risk of PTSD in women is moderated by exposure to CM. Shown is the percent of DNHS participants ($n = 401$; all female) with past month PTSD by *ADCYAP1R1* rs2267735 genotype (CC versus CG/GG genotypes) and number of CM types (physical, sexual, and emotional abuse).

compared to those with one or no “C” alleles. In contrast, among participants unexposed to CM, those with two “C” alleles show a slightly reduced prevalence of past month PTSD compared to those with one or no “C” alleles.

DISCUSSION

In this study, we sought to examine the joint and interacting effects of CM and *ADCYAP1R1* genetic variation on risk of PTSD in women. We also tested for evidence of these effects on depression as a secondary analysis. Results showed that CM had a significant main effect on both PTSD and depression, and that the magnitude of the CM effect size was similar in both cases. In addition, we observed a significant *ADCYAP1R1* \times CM interaction in predicting PTSD, whereby women exposed to CM carrying one or more *ADCYAP1R1* “C” alleles showed an increased risk of PTSD and PTS severity. These results were further corroborated by our detection of a significant PTS severity/CM correlation among C allele carriers only, with correlation coefficients that differed significantly between the “riskiest” CC genotype compared to G-carrying genotypes. This suggests CM is associated with increased risk of PTSD most strongly among persons with the “CC” genotype. Notably, significant interactions and differing correlations by genotype were not detected in our secondary analyses of depression, suggesting that PTSD is the primary phenotype influenced by genetic variation, in conjunction with CM exposure, at the *ADCYAP1R1* locus.

Our finding of a significant PTSD-related *ADCYAP1R1* \times CM interaction lends support to recent work reported by Ressler et al.,^[8] which found a significant association between *ADCYAP1R1* variation and risk of PTSD, but not depression and other mental illnesses. In the present report, the PTSD-specific association of *ADCYAP1R1* variation was revealed only in interaction analysis with CM, rather than in main

effect analyses as in the original work reported by Ressler et al.

However, the Grady Trauma project cohort, on which the Ressler et al study was based, is a low-income study population recruited from an urban hospital setting in which prior trauma levels, possibly including CM, may be higher than those in the DNHS. Thus, *ADCYAP1R1* genetic effects observed in the Grady cohort may only be apparent in the DNHS when the latter is stratified according to degree of previous (i.e. CM) trauma exposure. Indeed, when the present DNHS study sample is limited to those in the upper quartile of CM exposure, we find that, controlling for similar covariates to the Ressler et al. study (i.e. age and number of previous trauma types), there is a significant ($P = 0.04$) main effect of *ADCYAP1R1* variation for PTSD; this effect is not observed for depression ($P = 0.1629$). Although it has been argued that environmental risk factors should be included in genetic studies only once robust genetic associations have been identified,^[32] findings from this study emphasize importance of assessing contextual factors that may influence the effect of genetic variation on outcomes of interest,^[33] in this case PTSD.

PACAP and its receptor *ADCYAP1R1* have a range of known functions with implications for mental health and illness, including regulation of the stress response,^[9,34] mediation of adult neurogenesis in the lateral ventricle and hippocampus,^[35] and transcriptional control of neurotrophic factors important to normal neural development (i.e. BDNF).^[36] In addition to its recent association with PTSD, genetic variation in and around *ADCYAP1R1* has also been previously implicated in other mental illnesses, including schizophrenia^[37] and major depression,^[38] although these associations have been at SNPs other than the one tested in this report. Notably, *PACAP* genetic variation was significantly associated with bilateral hippocampal volume in schizophrenics, but not controls, identifying an

intermediate phenotype for schizophrenia that is consistent with known PACAP-*ADCYAP1R1* function.^[37] Moreover, the SNP associated with MDD, which occurs ~4,200 bp downstream of *ADCYAP1R1*, also showed a significant male-specific association with the disorder.^[38] The SNP examined here, r2267735, is particularly interesting in its apparent increased association with female risk, in that the SNP lies within a putative estrogen response element and was shown to associate with symptoms in a female-specific manner.^[8] Thus, it remains possible that additional SNPs within *ADCYAP1R1* may show main or interacting effects with other mental illnesses, with some of these occurring in a sex-specific manner.

Our results should be evaluated in light of existing study limitations. First, CM was assessed via retrospective self-report during adulthood, which could have introduced recall bias into our CM measure. However, previous work has shown that retrospective self-report during adulthood among those with documented CM cases is associated with underreporting of physical^[39] and sexual^[40] abuse, suggesting that our estimates of CM are likely to be underestimates. Second, given the relatively small number of participants who reported belonging to non-African-American race/ethnic backgrounds, we were unable to assess the possible existence of race-specific *ADCYAP1R1* × CM interactions, as have recently been reported in other studies that include G × CM analyses.^[30,31] Future work in other cohorts with both genetic and CM measures should evaluate this possibility. Third, although we used largely the same dataset to test for the joint and interacting effects of *ADCYAP1R1* and CM on two outcomes—PTSD and depression—we did not correct our results for multiple hypothesis testing. However, our primary analyses focused on PTSD, with results that approached significance following multiple correction for testing two phenotypes (Table 2B; $P = .029$). Fourth, our CM measure included some traumas that were assessed for their occurrence prior to both ages 11 and age 18 (e.g. physical abuse via the CTQ and CTS questions), whereas others were assessed only prior to age 18 (e.g. sexual abuse via the NHSII questions). Thus, we were unable to determine whether there were specific developmental periods of CM exposure that may have been contributing to the observed interactions. Finally, our study was not able to directly assess whether participants' PTSD diagnosis and symptoms were due to a specific childhood abuse trauma. Thus, we cannot say with certainty that the conditional risk of PTSD is significantly higher among C allele carriers whose PTSD is attributable to a specific CM event; our conclusions must necessarily be limited to the observation that C allele carrying women previously exposed to CM are at increased risk of PTSD during adulthood.

Despite these limitations, our results provide novel evidence that the impact of CM on PTSD is moderated by genetic variation at *ADCYAP1R1*, a locus involved

in regulating the response to stress.^[34] Future investigations focused on identifying the molecular mechanisms contributing this moderation are warranted.

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