Protective Effect of CRHR1 Gene Variants on the Development of Adult Depression Following Childhood Maltreatment

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Context: A previous study reported a gene × environment interaction in which a haplotype in the corticotropin-releasing hormone receptor 1 gene (CRHR1) was associated with protection against adult depressive symptoms in individuals who were maltreated as children (as assessed by the Childhood Trauma Questionnaire [CTQ]).

Objective: To replicate the interaction between childhood maltreatment and a TAT haplotype formed by rs7209436, rs110402, and rs242924 in CRHR1, predicting adult depression.

Design: Two prospective longitudinal cohort studies.

Setting: England and New Zealand.

Participants: Participants in the first sample were women in the E-Risk Study (N=1116), followed up to age 40 years with 96% retention. Participants in the second sample were men and women in the Dunedin Study (N=1037), followed up to age 32 years with 96% retention.

Main Outcome Measure: Research diagnoses of past-year and recurrent major depressive disorder.

Results: In the E-Risk Study, the TAT haplotype was associated with a significant protective effect. In this effect, women who reported childhood maltreatment on the CTQ were protected against depression. In the Dunedin Study, which used a different type of measure of maltreatment, this finding was not replicated.

Conclusions: A haplotype in CRHR1 has been suggested to exert a protective effect against adult depression among research participants who reported maltreatment on the CTQ, a measure that elicits emotional memories. This suggests the hypothesis that CRHR1’s protective effect may relate to its function in the consolidation of memories of emotionally arousing experiences.

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Major depression is one of the most common and disabling medical disorders and is associated with excess of morbidity, mortality, and costs to society. Research documents the contribution of both genetic and environmental risk factors, particularly maltreatment occurring early in life, to the development of depression.

Molecular genetic studies have evaluated the association between a wide range of candidate polymorphisms in different genes and major depressive disorder (MDD). Although positive associations have been detected, replications are scarce. One possible reason for the lack of consistency across genetic association studies of depression is that these studies focus on genetic main effects on disease rather than on genetic effects on vulnerability to environmental causes of disease. Early-life stress has been substantially associated with neurobiological modifications and increased risk of depression. However, there are substantial differences in the way that individuals respond to the same stressful event, and such response variability may be under genetic influence. Several studies have reported that specific polymorphisms exert genetic control of sensitivity to stressful experiences in early life, and it is likely that there are more polymorphisms involved.

The concept that genes may moderate the causal effect of environmental stressors on depression has direct implications for the selection of candidate genes for the disorder. Genes associated with the physiological response to environmental stressors, particularly in the hypothalamic-pituitary-adrenal (HPA) axis, would be
natural candidates. Accordingly, Bradley et al\(^\text{15}\) investigated the interaction between child abuse and 10 single-nucleotide polymorphisms (SNPs) in the corticotropin-releasing hormone receptor 1 gene (\textit{CRHR1}), and haplotypes formed by selected SNPs, in relation to depressive symptoms. The \textit{CRHR1} is a G protein–coupled receptor localized in frontal cortical areas, forebrain, brainstem, amygdala, cerebellum, and the anterior pituitary.\(^\text{16}\) This receptor plays a key role in the regulation of the HPA axis in response to stressful events, mediating the action of corticotropin-releasing hormone (CRH) on the pituitary to release adrenocorticotropic hormone that stimulates the production of cortisol in the adrenal cortex.\(^\text{9,17}\) Moreover, CRH acts as a neurotransmitter via \textit{CRHR1} eliciting anxiety-related behavior and influencing arousal, attention, executive functions, the conscious experience of emotions, and learning and memory consolidation related to these emotions.\(^\text{16,18}\) Bradley et al\(^\text{15}\) reported that a TAT haplotype formed by the 3 most significant \textit{CRHR1} SNPs investigated (rs7209436, rs110402, and rs242924) was associated with a protective effect in terms of reduced depression symptoms among individuals exposed to moderate to severe child abuse, as assessed by the Childhood Trauma Questionnaire (CTQ).\(^\text{15}\) The effect was also detected for a TCA haplotype formed by rs7209436, rs4792887, and rs110402.\(^\text{15}\)

Since replication is a sine qua non for accepting a genetic hypothesis,\(^\text{19}\) we aimed to test the gene \(\times\) environment interaction (G \(\times\) E) between childhood maltreatment and \textit{CRHR1} haplotypes in the development of depression. In this study, we extend the original finding by offering 4 complementary methodological features: (1) We evaluated 2 representative community cohorts to complement the samples selected in hospital and clinical settings in the original report. (2) We assessed exposure to childhood maltreatment using 2 different measures, the CTQ, as used by Bradley et al\(^\text{15}\), and a combined prospective-retrospective measure, to complement the exclusive retrospective assessment originally used. (3) We report DSM diagnoses of depression assessed through standardized interviews to complement the self-completed symptom scales originally used. (4) We assessed individuals longitudinally to evaluate the effect of the investigated interaction on recurrent episodes of MDD to complement the cross-sectional assessment of depressive symptoms originally performed.

**METHODS**

**SAMPLE**

Participants in the first sample were the mothers of the children in the Environmental Risk (E-Risk) Longitudinal Twin Study. The base sample of the E-Risk Study was formed in 1999-2000, when 1116 mothers with 5-year-old twins (93% of eligible families) participated in home-visit assessments. Details about the sample are reported elsewhere.\(^\text{20}\) Participants represent the full range of socioeconomic status in the general population of England and more than 90% are white. Four assessments were undertaken: women were on average 33 years of age at the initial assessment and 35, 38, and 40 years (range, 26-55 years; SD = 5.8) on the subsequent assessments. In the most recent assessment, undertaken in 2007-2008, we evaluated 96% of the women. Women gave written informed consent before participating. The Maudsley Hospital Ethics Committee approved each phase of the study.

Participants in the second sample were members of the Dunedin Multidisciplinary Health and Development Study.\(^\text{21}\) Of infants born in Dunedin, New Zealand, between April 1972 and March 1973, 1037 children (91% of eligible births; 52% male) participated in the first follow-up at age 3 years, constituting the base sample for the longitudinal study. Participants represented the full range of socioeconomic status in the general population of New Zealand’s South Island and more than 90% are white. Assessments were undertaken at ages 3, 5, 7, 9, 11, 13, 15, 18, 21, and 26 years and most recently at age 32 years when we assessed 96% of the 1015 study members still alive in 2004-2005. Study members gave written informed consent before participating. The Otago Ethics Committee approved each phase of the study.

**MEASURES**

**Childhood Maltreatment**

In the E-Risk Study, women completed the CTQ\(^\text{22}\) when they were on average 40 years of age. This is the same measure used in the 2 samples studied by Bradley et al.\(^\text{13}\) The CTQ inquires about the history of 5 categories of childhood maltreatment: emotional, physical, and sexual abuse and emotional and physical neglect. The validity of the instrument has been previously demonstrated in clinical and community samples.\(^\text{23}\) Bradley et al used an abbreviated 3-scale version of the CTQ; the E-Risk Study used all 5 scales. We used the score classification evaluated and recommended by the CTQ manual\(^\text{24}\) and considered a specific category of maltreatment present if the woman had a moderate to severe score. Subsequently, we derived a cumulative exposure index for each woman by counting the number of maltreatment categories present: 75.5% of women experienced no maltreatment, 16.8% experienced some maltreatment (1-2 categories), and 7.7% experienced severe maltreatment (≥3 categories).

In the Dunedin Study, we used the maltreatment measure described in our previous publications.\(^\text{25,25}\) Briefly, maltreatment between ages 3 to 11 years was evaluated. Evidence of childhood maltreatment was ascertained from 5 sources. Three sources were prospective: (1) behavioral observations by researchers of rejecting mother-child interactions at age 3 years; (2) parental reports of harsh disciplinary practices at ages 7 and 9 years; and (3) multiple changes in the person occupying the role of the child’s primary caregiver during the first decade of life. Two sources were retrospective: study members’ reports in adulthood of (4) physical and (5) sexual abuse. We derived a cumulative exposure index by counting the number of maltreatment experiences during the first decade of life: 64% of the cohort experienced no maltreatment, 27% experienced some maltreatment (1 indicator), and 9% experienced severe maltreatment (≥2 indicators).

**Major Depressive Disorder**

In the E-Risk Study, MDD was evaluated with the Diagnostic Interview Schedule,\(^\text{26}\) and diagnoses followed DSM-IV\(^\text{27}\) criteria at the 4 waves of assessment. In the Dunedin Study, MDD was also evaluated with the Diagnostic Interview Schedule.\(^\text{26,28}\) At ages 18 and 21 years, diagnoses followed the current DSM-III-R,\(^\text{29}\) and at ages 26 and 32 years, diagnoses followed DSM-IV.\(^\text{27}\) In both studies, (1) diagnostic interviews were conducted by mental health trainees or professionals, (2) func-
Table 1. Genotype Frequencies of the CRHR1 Polymorphisms Evaluated in the E-Risk and Dunedin Studies

<table>
<thead>
<tr>
<th>SNP Accession No.</th>
<th>E-Risk Study</th>
<th>Dunedin Study</th>
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<tr>
<td>rs7209436</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>46</td>
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</tr>
<tr>
<td>CC</td>
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<td>31.9</td>
</tr>
<tr>
<td>TT</td>
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<td>17.8</td>
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<td></td>
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<td>AG</td>
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</tr>
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<tr>
<td>GG</td>
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<tr>
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</tr>
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</table>

Abbreviation: SNP, single-nucleotide polymorphism.

Table 2 provides information about the distribution of demographic characteristics, maltreatment exposure, and depression phenotypes, according to the estimated number of CRHR1 TAT haplotype copies, in each cohort.

STATISTICAL ANALYSES

We tested the haplotype x environment interaction in a logistic regression framework with the haplo.glm method. The haplo.glm method iteratively and simultaneously estimates both regression parameters and haplotype frequencies by maximizing the prospective likelihood, even when the phase of the genetic data are not known. Haplotypes with estimated frequencies of 10% or less are by default collapsed into a single category, “rare” haplotypes.

Using inferred haplotype frequencies to study G x E relies on an assumption of HWE in the pooled data. Although there was no indication of Hardy-Weinberg disequilibrium in any of the individual SNP genotypes in our data, this assumption is difficult to verify. Haplotype inference using the prospective likelihood is generally relatively robust to departures from HWE, and simulations suggest that the haplo.glm method is not affected by departures from HWE.

An interaction model including the haplotypes (with the most common haplotype CGG as the reference category), childhood maltreatment (treated as a continuous variable), and the interactions between haplotypes and childhood maltreatment was fitted to data from the E-Risk and Dunedin studies, under an additive genetic model assumption. Analyses for the Dunedin Study included sex as a covariate.

RESULTS

At mean age 40 years, 13.9% of women presented with past-year MDD. Between ages 33 and 40 years, 12.7% of the women had recurrent MDD. Of those with recurrent MDD, 66% were depressed at the most recent assessment at age 40 years.
Childhood maltreatment was significantly associated with adult depression. As the severity of reported maltreatment increased, so did rates of past-year ($\chi^2=71.90; P<.001$) and recurrent ($\chi^2=84.96; P<.001$) MDD. Among women with no exposure to childhood maltreatment, the rate of past-year MDD was 9.6%; among women with some maltreatment, the rate of past-year MDD was 19.8%; and among women with severe maltreatment, the rate of past-year MDD was 43.4%. The comparable rates of recurrent MDD were 13.5%, 20.3%, and 43.4%. In contrast, there was no significant association between number of TAT haplotype copies and either the rate of past-year ($\chi^2=2.64; P=.26$) or recurrent ($\chi^2=0.73; P=.69$) MDD (Table 2).

Logistic regression analysis demonstrated a significant interaction between maltreatment and number of CRHRI TAT haplotype copies in predicting past-year MDD ($P=.04$) (Table 3). The odds ratio of past-year MDD in women exposed to severe maltreatment in comparison with those exposed to no or some maltreatment was 8.61 (95% CI, 4.05-18.33) for those with 0 copies, 7.81 (95% CI, 3.54-17.21) for those with 1 copy, and 1.92 (95% CI, 0.38-9.61) for those with 2 copies of the TAT haplotype (Figure 1B).

**DUNEDIN STUDY**

At age 32 years, 16.5% of the participants presented with past-year MDD. Between ages 18 and 32 years, 16.9% of the participants had recurrent MDD. Of those with recurrent MDD, 60% were depressed at the most recent assessment at age 32 years.

As the severity of reported childhood maltreatment increased, so did rates of past-year ($\chi^2=19.09; P<.001$) and recurrent ($\chi^2=18.63; P<.001$) MDD. Among individuals with no exposure to maltreatment, the rate of past-year MDD was 14.1%; among individuals with some maltreatment, the rate of past-year MDD was 16.6%; and among individuals with exposure to severe maltreatment, the rate of past-year MDD was 33.8%. The comparable rates of recurrent MDD were 13.5%, 20.3%, and...
There was no significant association between number of TAT haplotype copies and either rate of past-year (χ² = 0.89; P = .63) or recurrent (χ² = 1.58; P = .45) MDD (Table 2).

In contrast to findings from the E-Risk Study, the interaction between childhood maltreatment and number of CRHR1 TAT haplotype copies in predicting past-year MDD was not significant (P = .94) (Table 3). The odds ratio of MDD in individuals exposed to severe maltreatment in comparison with those exposed to no or some maltreatment was 3.42 (95% CI, 1.49-7.82) for those with 0 copies, 2.75 (95% CI, 1.27-5.90) for those with 1 copy, and 2.36 (95% CI, 0.67-8.23) for those with 2 copies of the TAT haplotype (Figure 2A). Similarly, there was no significant G × E predicting recurrent MDD (P = .93) (Table 3). The odds ratio of recurrent MDD was 2.86 (95% CI, 1.24-6.62) for those with 0 copies, 2.21 (95% CI, 1.02-4.81) for those with 1 copy, and 2.34 (95% CI, 0.73-7.43) for those with 2 copies of the TAT haplotype (Figure 2B).

We considered the possibility that the interaction between maltreatment and CRHR1 may be female specific. In the original report, 61% of sample 1 were women and 100% of sample 2 and 100% of participants in the positive replication in the E-Risk Study. However, when we restricted the Dunedin analysis to women, the pattern of results did not differ from that in the full cohort. Among women with severe maltreatment, the rates of past-year MDD were 47.1%, 25%, and 37.5% for those with 0, 1, and 2 copies of TAT haplotype, respectively, and the interaction term was not significant (P = .46). The comparable rates of recurrent MDD were 47.1%, 28.6%, and 50%, with a nonsignificant interaction term (P = .96). This excluded the possibility that the Dunedin Study’s negative findings resulted from its mixed sex composition.

We also considered the possibility that the interaction between maltreatment and CRHR1 may depend on retrospective assessment of childhood maltreatment. In the original report and in the E-Risk Study, maltreatment...
ment was ascertained via retrospective reports (on the CTQ). In Dunedin, maltreatment was ascertained using a combination of prospective and retrospective indicators. To test this hypothesis, we disaggregated the original composite measure used in the Dunedin Study and repeated our analyses separately for the prospective and retrospective indicators of maltreatment. The rates of past-year MDD in individuals exposed to prospectively measured maltreatment were 20.9%, 19.6%, and 15.6% for those with 0, 1, and 2 copies of TAT haplotype, respectively, with a nonsignificant interaction term ($P = .98$). The comparable rates of recurrent MDD were 21.6%, 18.1%, and 23.5%, with a nonsignificant interaction term ($P = .81$). The rates of past-year MDD in individuals exposed to retrospectively measured maltreatment were 29.5%, 30.2%, and 20% for those with 0, 1, and 2 copies of TAT haplotype, respectively, with a nonsignificant interaction term ($P = .55$). The comparable rates of recurrent MDD were 31.8%, 31.3%, and 23.8%, with a nonsignificant interaction term ($P = .43$). This excluded the possibility that the occurrence of the G × E is a function of retrospective data collection.

We undertook a replication test of the G × E between childhood maltreatment and a haplotype formed by rs7209436, rs110402, and rs242924 in CRHRI in predicting the development of adult depression. In the E-Risk Study, the TAT haplotype was associated with significant protection of women exposed to severe maltreatment against developing past-year and recurrent MDD. In the Dunedin Study, we did not replicate this G × E finding.

The significant G × E in the E-Risk Study corroborates the original findings and extends them, considering that (1) a community sample (vs a clinical sample) was evaluated, (2) depression was diagnosed according to DSM-IV criteria (vs symptom ratings previously used), (3) depression was operationalized not only as past-year MDD, but also as recurrent MDD (during a multiyear follow-up period), and (4) an ethnically homogeneous European sample was studied (vs the original American samples).

Unexpectedly, results of the Dunedin Study did not corroborate the G × E finding originally reported in the 2 samples studied by Bradley et al and replicated here in the E-Risk Study. If this G × E is valid, what could explain the negative results from the Dunedin Study? The discrepant results between the E-Risk and Dunedin studies are unlikely to arise from sampling differences; the 2 samples were equally powered to detect the same effect sizes, both samples were of European ancestry and similar age, and analyses excluded the possibility that findings differed because the Dunedin cohort contained males. The discrepancy is unlikely to arise from phenotyping differences because the 2 cohorts shared the same DSM-based diagnostic interview for MDD. The discrepancy is unlikely to arise from genotyping differences because our 2 cohorts shared the same laboratory and technical procedures. It is unlikely that population stratification affected the results, since the linkage disequilibrium structure was very similar in the UK-European and New Zealand–European samples, and both samples differed from the original African American sample. In fact, the positive findings in the samples studied by Bradley et al and in the E-Risk Study, which presented different haplotype frequencies and different linkage disequilibrium structures, provide important transpopulational replication. This may suggest that the TAT haplotype has functional relevance, or tags functional variant(s) across populations of different geographic origins. Finally, the discrepancy is unlikely to arise from retrospective vs prospective measurement of maltreatment because, although the G × E was observed in studies using the retrospective CTQ, it was not observed when retrospective reports of maltreatment were analyzed in the Dunedin Study.

A remaining explanation for the discrepant findings concerns differences in the nature of the measures of child-
Two lessons may be learned about stress exposure assessment. The first lesson relates to the importance of how stress exposures are measured. In the 3 studies replicating the $G \times E$ (the original 2 samples evaluated by Bradley et al15 and the E-Risk Study), childhood maltreatment was assessed using the same measure, the CTQ. To our knowledge, this is the first instance in the history of psychiatric $G \times E$ research that stress has been ascertained by more than 1 study using the same instrument. Although comparable characterization of genotypes and diagnostic phenotypes is now routinely attainable across studies, heterogeneity in the measurement of environmental stressors across studies has been and continues to be a major obstacle to replication in $G \times E$ research. For replication, it is important that similar measures of stress are used as much as possible. Failure to take differences in exposure measurement seriously may produce seemingly inconsistent findings. Moreover, studies with different environmental measures may not be comparable and, therefore, suitable for meta-analysis. We recommend that once enough studies have accumulated in the literature, meta-regression methods can be used to compare findings from studies that use different types of stress-exposure measurement. In this way, the effect of methodological factors on the heterogeneity of $G \times E$ results can be evaluated more rigorously, paving the road to meta-analytical strategies.

The second lesson relates to the important question of how adverse experiences in childhood can result in depression that only emerges years later, during adulthood. What mediating cognitive and biological processes ensue during the decades-long span separating cause from effect? A hypothesis suggests itself: the CTQ successfully identifies individuals who have sustained memories with emotional content since childhood, such emotional memories may be necessary to precipitate MDD, and such memories may be influenced by CRHR1.

A consistent body of evidence has implicated the stress hormones, including CRH, in mediating the effects of emotional arousal on memory consolidation.16,18 The basolateral complex of the amygdala is a key area in modulating the effects of CRH on memory32 and the consolidation of memories of emotionally arousing experiences through projections to several brain regions. The basolateral amygdala contains a high density of CRHR1 receptors,33 and experimental evidence shows their selective blockade after stressful training impairs the consolidation of fear memory34,35 which demonstrates the importance of this receptor in emotional memory consolidation. Although the role of the studied haplotype in the receptor functioning remains elusive, our results point in the same direction as experimental studies36 and might indicate that individuals with 2 copies of the TAT haplotype have impaired activation of fear memory consolidation processes, resulting in relatively unemotional cognitive processing of memories of childhood experiences. Such a cognitive mechanism could protect them from depression. If the hypothesis were correct, it could explain why the pattern of $G \times E$ was not found in the Dunedin Study.

The importance of negative and ruminative memories to the development of depression has long been recognized.36,37 Emotional arousal and memory consolidation, 2 processes intrinsically linked, are influenced by projections from a number of different systems, such as noradrenergic and HPA systems, that are integrated by the amygdala.16,32 The amygdala has been shown to modulate memories congruent with sad mood in individuals with a history of depression.38 Recently, amygdala activity during an emotionally loaded memory task was assessed with neuroimaging techniques.39 During the recall of negative memories, depressed individuals showed increased activity in the right amygdala, which was correlated with activity in caudate-putamen and hippocampus, demonstrating the importance of this area in the negative memories of individuals with depression and the activation of neural systems that encode affective material.39 Taken together, these findings suggest the hypothesis that the role of CRHR1 in the consolidation of aversive memories may be involved in the mediation of the pathway from childhood maltreatment to adulthood depression. This should be investigated using genetic neuroimaging designs. Moreover, the results presented herein indicate that retrospective measures can be of great value (contrary to what is commonly argued) when the aim is to measure not only the objective occurrence of events, but also the emotional memories associated with events, which may be important in the developmental etiology of depression. Unfortunately, because we do not have an objective measure of maltreatment and the CTQ in the same sample, we were unable to test this hypothesis.

Stressful experiences, especially those occurring during childhood, are linked to depression and other mental disorders. Better understanding of the processes by which adverse experiences produce distinctive patterns of response between individuals, from severe psychopathology to adaptive and even improved functioning, is...
fundamental to understanding and reducing mental illness. The identification of genes involved in these processes is likely to improve understanding, and to eventually improve treatment and prevention. Progress will be hastened by integrating the fields of epidemiology, genetics, neuroscience, and psychiatry.

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REFERENCES


