Negative life events and corticotropin-releasing-hormone receptor1 gene in recurrent major depressive disorder

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Major depressive disorder (MDD) is a long-term, recurrent condition that often takes a chronic course. It seems imperative that research should be focused on gaining a better understanding of what predicts recurrent MDD. As a major mediator of the stress response, corticotropin-releasing-hormone receptor 1 (CRHR1) has been demonstrated to be an important contributor to the pathogenesis of MDD. In this study, we show a significant increase in the G-allele (rs242939) of the CRHR1 gene in the recurrent MDD group compared with the control group, and an overrepresentation of G-G-T haplotype of the CRHR1 gene in recurrent MDD. We also demonstrate the interaction of the CRHR1 gene and negative life events in recurrent MDD. These results suggest that the CRHR1 gene could modify the susceptibility to developing recurrent MDD following negative life events in adulthood.
The most established environmental risk factors for MDD are stressful life events. Both human and nonhuman primate studies have provided consistent evidence that variability in individuals’ behavioral responses to life events causes17,18. There is circumstantial evidence to link exposure to a range of specific psychosocial environmental pathogens with the development of MDD. Negative life events, such as divorce, serious illness, housing, relationships and social difficulties, are commonly studied in MDD19. Several findings have confirmed that negative life events are associated with the onset of MDD, and have revealed that the level of threat posed by a life event is strongly related to the risk of subsequent depressive onsets, negative life events have a positive correlation with the severity of depression20–22. A meta-analysis also confirmed the association between negative life events and MDD23.

Most behavioral geneticists would concur that the traditional idea of ‘nature’ and ‘nurture’ as distinct entities is outdated, with neither genes nor environment likely to act in isolation to increase susceptibility. Gene × environment (G × E) studies suggest that by moderating the effects of the environment, genetic variation explains why some individuals are vulnerable and others are resistant to the effects of adversity. G × E research has revealed several replicated findings, including polymorphisms in the serotonin transporter, brain-derived neurotrophic factor (BDNF), and HPA axis-related genes24–26. One of the most important notions that has arisen in MDD research is that genetic polymorphisms in stress-related genes (the CRHR1 gene) can modify the susceptibility to developing depression following negative life events. Two studies have reported that the CRHR1 moderates the effects of childhood maltreatment on HPA reactivity in later life27,28. In line with these findings, the same allele has been shown to be protective against the effects of childhood maltreatment on the development of several behavioral phenotypes, including MDD29,30.

However, the evidence for interaction between the polymorphisms and stressful life events (SLEs) in adulthood has been contradictory31–33. One potential reason for this inconsistency is that the phenotype examined has been too broadly defined34 by utilizing individuals with nonclinical depression3 or those with a history of only one depressive episode35. Given that depression is a long-term, recurrent condition and that individuals with persistent depression place a large burden on health services and the greater economy36, it seems imperative that research should focus on gaining a better understanding of what predicts recurrent depression to aid the prevention of this long-term disabling disorder.

Based on the initial findings mentioned above, this study was designed to examine the relationships between the CRHR1 gene, negative life events and adult recurrent MDD in the Han Chinese population.

## Results

Based on our total sample of 528 individuals, a power analysis for case-control samples was carried out by the G* Power program. The sample size had a post-hoc power of 0.99 to detect an effect size of 0.5 (moderate) at the 0.05 significance level. The MDD group experienced a significantly higher number of life events than the control group, with 18.7% of the 252 MDD patients and 4.4% of the 272 controls having increased experience with negative life events. The genotypic distributions of the three SNPs all conformed to Hardy-Weinberg equilibrium in both the recurrent MDD and control groups. The results from single marker analysis of the three SNPs are presented in Table 1. An allelic association between CRHR1 rs242939 and recurrent MDD was found in our sample (allelic: p = 0.0069, genotypic: p = 0.0066) with an odds ratio of 0.5271 (95% CI 0.3491–0.7958), which was reflected by a significant increase in the G-allele of 242939 in the recurrent MDD group compared with the control group. Two alleles (rs1876828 and rs242941 of CRHR1) showed no association with the risk of recurrent MDD in the present sample (p = 0.1125 and 0.2676, respectively).

Haplotype frequencies in the recurrent MDD and control groups were estimated using the expectation maximization (EM) algorithm embedded. Four common haplotypes (G-A-G, G-A-T, A-A-T, G-G-T respectively SNPs of rs1876828, rs242939, rs242941) were found to be present in the sample. Using the chi-2 test, a global test of these four haplotypes revealed a significantly different distribution between the recurrent MDD group and the control group (chi-2 = 12.590, df = 3, p = 0.017) (Figure 1).

The G × E interactions were examined using the generalized multifactor dimensionality reduction (GMDR) method. The GMDR software provides a number of output parameters, including the cross-validation (CV) consistency (the CV consistency score is a measure of the degree of consistency with which the selected interaction is identified as the best model among all possibilities considered), the testing balanced accuracy, and empirical p values, to assess each selected interaction. The number of interacting factors was set at either 2 or 4 of complexity so that the meaning of biological information can be performed easily. The most significant model revealed by GMDR analysis gives the best value of the maximized CV and prediction error (PE). The interaction between single SNP of the CRHR1 gene (rs242939) and negative life events had a CV consistency of 10, PE of 0.4017 and p value of 0.023 after Bonferroni correction and, was considered the better of the two factors. The interaction between the combination of rs1876828-rs242939-rs242941 and negative life events had a CV consistency of 6, PE of 0.4367 and p value of 0.037 and, was considered the best of the four factors, indicating a potential

### Table 1 | Genotype distributions and allele frequencies of CRHR1 polymorphisms of recurrent MDD patients and controls

| SNP ID  | Position | Genotype | Allele | P    | A  | G  | P    | Odds Ratio (95% CI) |
|---------|----------|----------|--------|------|----|----|------|---------------------|
| rs1876828 | chr17:43911525 MDD | 210 43 3 | AA 463 49 0.1224 | 0.0066 | G | 0.5271 | (0.9312–2.023) |
| rs242939 | chr17:43895579 MDD | 192 61 3 | AG 475 69 0.0066 | CON 0.5271 | 0.2676 | (0.3491–0.7958) |
| rs242941 | chr17:43892520 MDD | 168 84 20 | GG 504 40 0.2922 | CON 0.5271 | 0.2676 | (0.9261–1.680) |

*SNP: single nucleotide polymorphism.

Bold numerals p-values after Bonferroni correction.
carrying the G-allele of 242939 or haplotype G-G-T may be highly susceptible to recurrent MDD when exposed to negative life events.

This is the first report on the effect of the CRHR1 gene on modifying the response to negative life events and conferring susceptibility to recurrent MDD. The present study extends previous knowledge of the interplay between the CRHR1 gene and childhood maltreatment in the onset of adult depression. Previous studies have reported that childhood maltreatment is strongly and directly related to persistent forms of adult depression\(^\text{20,30}\). In adulthood, stressful life events are strongly associated with the onset of major depressive episodes\(^\text{33}\). Evidence that an initial episode of depression is more likely to be immediately preceded by stressful life events than recurrent episodes is consistent with the hypothesis that people may become increasingly sensitized to life stress over successive recurrences of depression\(^\text{35}\). The present study extends this observation to suggest that CRHR1 confers sensitivity to high-negative life events in relation to the development persistent depression. This finding may help provide insight into the problem of "missing heritability" in psychiatric illness\(^\text{41,42}\).

The negative life events and genetic components that affect responses to harmful stimulating events may be two possible factors involved in the etiology of depression\(^\text{33}\). Severe stressors in adult life exert a myriad of psychopathological consequences, which, depending on an individual’s genetic vulnerability, may include depression. Most studies suggest that the HPA axis hyperactivity previously described in depression may not be the consequence of depression \textit{per se}, but rather the manifestation of persistent neurobiological abnormalities that predispose to depression\(^\text{43}\). Under the same conditions, the depression will be prior to the people with susceptible quality. Negative life events may induce recurrent MDD or result in personality disturbances or heightened sensitivity to negative life events. As a major mediator of the stress response in the central nervous system, CRHR1 has been demonstrated to be an important contributor to the pathogenesis of MDD. The present study provides preliminary evidence in support of the hypothesis that the effects of the CRHR1 gene may modify a patient’s response to negative life events with regard to the triggering of recurrent MDD.

Animal studies have demonstrated that separating neonatal rodents and non-human primates from their mothers for long periods elicits HPA axis changes that persist into adulthood and resemble those present in depressed adult individuals, including hyperactivity of the HPA axis and increased activity of CRH-containing circuits\(^\text{44}\). Clinical studies have also shown that women who are sexually or physically abused in childhood exhibit a markedly enhanced activation of the HPA axis as an adult\(^\text{44}\). Moreover, a recent study using the DEX/CRH test also revealed persistent HPA axis hyperactivity in men who had experienced early life trauma\(^\text{45}\). These findings suggest that the HPA axis hyperactivity previously described in depression may not be a consequence of depression per se, but rather the manifestation of persistent neurobiological abnormalities that predispose to depression. This hypothesis could also explain why previous studies that did not take into account early life stressors have been inconsistent in documenting the presence of HPA axis hyperactivity in depression\(^\text{45}\).

One limitation of this study is its reliance on cross-sectional retrospective self-report of depression and SLEs. However, studies have demonstrated that depressive symptoms do not result in the exaggeration of retrospectively recalled stressful events\(^\text{46,47}\). Second, our
positive finding may be a result of type I error; however, we addressed this limitation with the Bonferroni correction. Third, despite the relatively small sample size, our study had a power of 0.99 to detect an effect size of 0.5 (moderate) at the 0.05 significance level (2-tailed). This study provides only preliminary evidence that the interaction of the CRHR1 gene and negative life events may be related to the pathogenesis of recurrent MDD. Thus the study should be replicated in large samples and in other ethnic populations.

**Methods**

**Subjects and clinical assessments.** The patient sample consisted of 256 unrelated Chinese recurrent MDD individuals (male/female: 98/158; mean age: 34.40 ± 11.01 years, range 18–60 years), which were outpatients and inpatients from the Psychiatric Department of Renmin Hospital at Wuhan University. A total of 272 (male/female: 102/170; mean age: 35.40 ± 12.81 years, range 18–60 years) age, gender, and ethnically matched healthy controls were selected from the general population. Patients were interviewed by trained psychiatrists using the Structured Clinical Interview for DSM-IV disorders (SCID-I) and diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria.4 The severity of depression was assessed using the 21-item Hamilton Rating Scale for Depression (HAM-D-21) and the Clinical Global Impression Scale (CGI).11,12 The inter-rater reliability kappa value was 0.82 of SCID. Only subjects with a minimum score of 18 on the HAM-D-21 were selected. There were no significant differences concerning in other investigated variables (age, clinical variables such as HAMD-21 scores) between males and females. Patients with pregnancy, significant medical conditions, major neurological diseases, unstable psychiatric features (e.g. suicidal, substance/alcohol dependence, comorbid Axis I psychiatric disorders were excluded (Table 3). All patients and controls were ethnically Han and from the same geographical region in China. The Medical Ethics Committees of Renmin Hospital of Wuhan University approved the research project. Patients were included in the study after they provided written informed consent.

**Assessment of negative life events.** The Holmes and Rahe stress scale was designed by Holmes and Rahe and, contains 43 life events.13 Patients were asked to rank a list of 43 life events based on a relative score. A positive correlation of 0.118 was found between their life events and their illnesses. The Holmes and Rahe stress scale was also assessed against different populations within the United States (with African-American, Hispanic-American, and Caucasian-American groups) and used to compare Japanese and Malay families with American populations, not including the Chinese. The life events scale (LES) created by Desen Yang and Yalin Zhang5 classifies a total of 48 items into 3 aspects, including 28 items on family life, 13 items on social and other aspects, and has been assessed in a Chinese. The life events scale (LES) created by Desen Yang and Yalin Zhang5 classifies a total of 48 items into 3 aspects, including 28 items on family life, 13 items on social and other aspects, and has been assessed in a Chinese. The life events scale (LES) created by Desen Yang and Yalin Zhang5, 3, severe

| Table 3 | Population characteristics of recurrent MDD patients and controls |
|----------|---------------------------------------------------------------|
|          | Recurrent MDD | Controls |
| N        | 256           | 272 |
| Age (mean ± S.D.) | 34.40 ± 11.01 | 35.40 ± 12.81 |
| Gender [males/females] | 98/158 | 102/170 |
| Age of onset [males/females] | 28.18 ± 9.01 | 28.18 ± 9.01 |
| Average onset times | 3.75 |
| HAMD score | 28.89 ± 5.66 |

using the TaqMan® Universal PCR Master Mix reagent kit. Fluorescence data files from each plate were analyzed by automated software (SDS 2.1; Applied Biosystems). All laboratory procedures were carried out with investigators blinded to the case-control status.

**Statistical analysis.** The GENEPOP program was used to compare the overall allele and genotype distributions for each SNP in MDD patients and controls and to test the Hardy–Weinberg equilibrium.14 Haplotype frequencies in recurrent MDD patients and controls were estimated using the EM algorithm embedded in the program Arlequin.15 A total of 10,000 permutation tests were performed in each analysis. The Bonferroni correction was used for multiple testing, with the total number of SNPs used as a correction factor. The G × E interactions were analyzed using the GMDR software16, which has the ability to classify and predict disease risk status using CV. The model with the combination of loci and/or discrete environmental factors that maximizes the CV consistency and minimizes the PE was selected. To narrow down the number of possible combinations, we analyzed dominant models only. To clarify the GMDR results, we computed the OR values (with 95% CI) using SPSS software for Windows (version 13.0) to determine the set of risk factors selected by GMDR analysis. We also corrected the p-value using the Bonferroni method. Power analysis for case-control samples was carried out by the G*Power program (with an alpha set at 0.05).
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Author contributions

Z.L. and Z.X. designed the study, Z.L., W.L., L.Y., L.X. and Q.W. participated in the collection of the data. F.Z. and G.W. critically reviewed it. All authors reviewed the manuscript, and approved the final version of the article to be published.

Additional information

Competing financial interests: The authors declare no competing financial interests.

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