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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 hyplotype 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.

Major depressive disorder (MDD) is one of the most prevalent, complex, and costly brain diseases, which affects 7–11% of the general population[1]. Three-quarters of the MDD patients experience more than one episode of depression, and the risk of recurrence is higher if either the first episode occurs at a young age or there is a family history of depression[2]. The risk of recurrence increases with each new episode and as the number of depressive episodes increases, the influence of life stress on recurrence decreases[3]. Despite significant progress in our understanding of the probable causes of MDD, however, we are still far from elucidating the etiological risks of depression and understanding the mechanisms behind the action. Genetic factors account for 40–70% of the risk of developing MDD[4], and environmental factors, such as life events, have been demonstrated to play important roles in the pathogenesis of illness[5].

The stress response is the most important etiological hypothesis about depression[6]. The hypothalamic-pituitary-adrenal (HPA) axis is the final common pathway in the mediation of the stress response. Dysfunction of the HPA system is one of the most robust findings in many (up to 70%) patients with MDD[7]. As a major mediator of the stress response in the central nervous system, corticotropin-releasing-hormone (CRH) has been demonstrated to be an important contributor to the pathogenesis of MDD. CRH neurons are strongly activated in depression; specifically, a significant percentage of MDD patients have increased levels of CRH in the cerebrospinal fluid (CSF), plasma, and urine and an increased size (as well as activity) of the pituitary and adrenal glands[7,8]. The expression of CRH is markedly increased in the hypothalamic paraventricular nucleus of MDD patients compared with controls[9].

The biological action of CRH is mediated through binding to two receptors, namely CRH receptors types 1 and 2 (CRHR1 and CRHR2), each of which has a distinct expression profile and physiological function[10]. CRHR1 is considered to play a key role in mediating CRH-elicited effects on depression and anxiety[11]. The CRHR1 gene is located on chromosome 17q21–22, spanning 20 kb of genomic DNA and containing 14 exons, and it belongs to the family of Gs protein coupled receptors[12]. CRHR1 has three known isoforms, which arise from alternative splicing[13]. As CRHR1 may play a significant role in the etiology and treatment of depression, it has been suggested that CRHR1 is a relevant candidate gene for MDD. In the Mexican-Americans population, a significant association has been reported between CRHR1 gene and a greater response to treatment with selective serotonin reuptake inhibitors (SSRI) in highly anxious MDD patients, but the distribution of the CRHR1 gene among MDD

patients was similar to that among healthy controls[14]. We have already reported that rs242939 AG carriers of the CRHR1 gene have a greater risk of MDD in the Han Chinese population, and that the CRHR1 gene is likely to be involved in the antidepressant response of MDD patients[15,16]. The geographical and ethnic variability of MDD may be one interpretation of these results, but the etiological contributions of some environmental factors to MDD should be taken into account.

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 causes[17,18]. 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 MDD[19]. 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 depression[20–22]. A meta-analysis also confirmed the association between negative life events and MDD[23].

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 genes[24–26]. One of the most important notions that has arisen in MDD research in recent years 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 life[27,28]. 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 MDD[26,29,30].

However, the evidence for interaction between the polymorphisms and stressful life events (SLEs) in adulthood has been contradictory[31–33]. One potential reason for this inconsistency is that the phenotype examined has been too broadly defined[34] by utilizing individuals with nonclinical depression[35] or those with a history of only one depressive episode[36]. 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 economy[6], 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 ($p = 0.1225$ 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

		Genotype				Allele			
SNP ID	Position	AA	AG	GG	P	A	G	P	Odds Ratio (95%CI)
rs1876828	chr17:43911525 MDD	210	43	3	0.1224	463	49	0.1224	1.373
	CON	208	59	5		475	69		(0.9312–2.023)
rs242939	chr17:43895579 MDD	192	61	3	**0.0066**	445	67	**0.0069**	0.5271
	CON	236	32	4		504	40		(0.3491–0.7958)
		GG	GT	TT		G	T		
rs242941	chr17:43892520 MDD	174	66	16	0.2922	414	98	0.2676	1.247
	CON	168	84	20		420	124		(0.9261–1.680)

[a]SNP, single nucleotide polymorphism.
[b]Bold numerals p-values after Bonferroni correction.

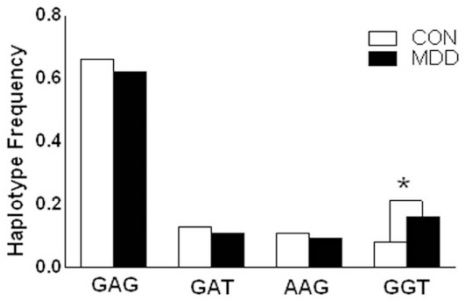

Figure 1 | Haplotype frequencies (%) estimation using the EM algorithm from Arlequin. All haplotypes estimated to occur with a frequency of at least 1% in recurrent MDD patients or controls are represented. (a) Haplotype frequencies (%) estimation using the EM algorithm from Arlequin. (b) All haplotypes estimated to occur with a frequency of at least 1% in recurrent MDD patients or controls are represented.

G × E interaction between the CRHR1 gene and negative life events (Table 2).

We computed the OR (with 95% CI) values using SPSS software for Windows (version 11.5) to assess the set of risk factors selected by the GMDR method. The analysis of a single role the G × E interaction in contribution to recurrent MDD showed an OR value of 8.738 (95% CI 2.556–29.870) in the individuals carrying the A/G genetype of rs242939 combining with the high-negative life events. In the individuals carrying haplotype GGT, high-negative life events yielded an OR value of 7.849 (95% CI 2.275–27.076). However, among individuals carrying the other genotypes of rs242939, low-negative life events yielded an OR value of 2.346 (95% CI 1.470–3.475). Among the individuals carrying the other haplotypes, low-negative life events yielded an OR value of 1.517 (95% CI 1.333–2.802).

Discussion

Disturbances in family functioning, such as parental (particularly maternal) depression, severe marital conflict or divorce, or death of a parent, represent additional risk factors for MDD[37]. Controlled clinical studies and epidemiological investigations have shown that individuals with major depression have a significantly greater prevalence of negative life events compared to the general population[38]. However, the interplay between life stress and depression recurrence has only recently begun to receive theoretical and empirical attention. In this study, positive associations were found between the CRHR1 gene and recurrent MDD according to single SNP and haplotype evaluation, although this result is not the consistent with previously reported findings in the Mexican-Americans population[14]. Licinio et al. found no difference in the frequencies of alleles and haplotypes of the CRHR1 gene between the MDD and control groups, but they found a homozygous GAG haplotype that was associated with a greater response to SSRI treatment in highly anxious MDD patients. It is difficult to interpret these controversial results but geographical and ethnic variability may be one of the major reasons for the inconsistent findings. Furthermore, the G × E interactions may contribute to the pathophysiology of illness. A combined effect of negative life events and CRHR1 gene on recurrent MDD is posited by this study. The results show that individuals 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[29,30]. In adulthood, stressful life events are strongly associated with the onset of major depressive episodes[39]. 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[40]. 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[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[43]. 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 *per se*, but rather the manifestation of persistent neurobiological abnormalities that predispose to depression[44]. 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[45]. 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[44]. Moreover, a recent study using the DEX/CRH test also revealed persistent HPA axis hyperactivity in men who had experienced early life trauma[46]. 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[47].

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[48,49]. Second, our

Table 2 | GMDR analysis gene–environmental interactions in recurrent MDD patients and controls

No of factors considered	Best combination	Prediction error	Cross-validation consistency	P-value
2	Negative life event, rs242939	0.4017	10	**0.023**
4	Negative life event rs1876828, rs242939, 242941	0.4367	6	**0.037**

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	
Average onset times	3.75	
HAMD score	28.89 ± 5.66	

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[50]. The severity of depression was assessed using the 21-item Hamilton Rating Scale for Depression (HAMD-21) and the Clinical Global Impression Scale (CGI)[51,52]. The inter-rater reliability kappa value was 0.82 of SCID. Only subjects with a minimum score of 18 on the HAMD-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, concomitant 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[53]. 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 Malaysian groups with American populations, not including the Chinese. The life events scale (LES) created by Desen Yang and Yalin Zhang[54] classifies a total of 48 items into 3 aspects, including 28 items on family life, 13 items on working problems, and 7 items on social and other aspects, and has been assessed in a Chinese population. These events encompassed serious illness, housing, relationship, and social difficulties, relationship breakups, unemployment and financial crisis, etc. Each life event is given a score that indicates the amount of readjustment a person has to make as a result of the event. Not all of the events included in the scale are necessarily negative events. This scale indicates that change in one's life requires an effort to adapt and then an effort to regain stability. Event scores have demonstrated high reliability and validity. Negative life events were assessed using the LES. This scale assessed the following four aspects of negative life events: when the life events occurred (absent = 1, 1 year earlier = 2, within the last 1 year = 3, chronicity = 4), how the life events were characterized (good = 1, bad = 2), how greatly the respondent was affected by them (absent = 1, mild = 2, moderate = 3, severe = 4, extreme = 5), and how long the influence of the events lasted (≤3 months = 1, 3–6 months = 2, 6–12 months = 3, >12 months = 4). The 95% percentile (a score of 65) of the sample was used as a cutoff value for grouping the high- or low- level negative life events.

DNA extraction and genotyping. Genomic DNA was extracted from EDTA anticoagulated venous blood samples. In this study, three SNPs of the CRHR1 gene were assayed, corresponding to the following dbSNP identifiers: rs1876828, rs242939, and rs242941. SNPs were genotyped with TaqMan technology (Assay-by-Design) on an ABI 7900 system (Applied Biosystems). A standard PCR reaction was carried out using the TaqManR 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[55]. Haplotype frequencies in recurrent MDD patients and controls were estimated using the EM algorithm embedded in the program Arlequin[56]. 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 software[57], 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 acquisition of data, which were analyzed by C.Y., K.G. and H.W., Z.L., W.L. and Z.X. wrote the article, F.Z. and G.W. critically reviewed it. All authors reviewed the manuscript, and gave approval for the final version of the article to be published.

Additional information

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