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ORIGINAL ARTICLE

Influence of Genetic Polymorphisms Involved in the Hypothalamic–Pituitary–Adrenal Axis and their Interactions with Environmental Factors on Antidepressant Response

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SUMMARY

Aims: To investigate the role of genetic polymorphisms in candidate genes associated with the HPA axis and their interactions with environmental stressors in antidepressant response. **Methods:** The remission of depressive symptoms after 8 weeks of antidepressant treatment was tested against 21 single nucleotide polymorphisms (SNPs) in five candidate genes associated with the HPA axis in a Chinese Han sample suffering from unipolar depression (n = 273). Any history of childhood trauma and recent negative life events were measured using the Childhood Trauma Questionnaire-Short Form (CTQ-SF) (n = 206) and the Life Event Scale (48 item, LES) (n = 207), respectively. Reporter gene assays were used to evaluate the possible effects of the most significant SNP on gene expression. **Results:** A functional polymorphism at 3′UTR of the corticotropin-releasing hormone receptor 1 (CRHR1) gene (rs28364032) and three haplotypes containing it showed significant relationships with antidepressant remission. Further laboratory-based genomic studies showed that the G-to-A change of rs28364032 resulted in a 10–12% decrease in the intensity of luciferase activity. However, we failed to find association of environments and their interaction with HPA system-related genes with antidepressant remission. **Conclusions:** Our results support a definite role for CRHR1 in the pharmacogenetics of antidepressant drugs. This may contribute to interpatient differences in their responses to antidepressant drugs.

Introduction

Major depressive disorder (MDD) is a common and serious psychiatric disorder. Up to now, the poor response and individual variation in drug response are major limitations of current antidepressant treatment [1]. There has been increasing evidence that stress and the impaired regulation of the hypothalamic–pituitary–adrenocortical (HPA) axis play a crucial role in the pathophysiology of depression and its response to antidepressant treatment [2].

It is widely accepted that stress, divided both from childhood trauma and recent negative life events, is involved in the pathophysiology of depression [3,4], and the key link of the stress response is the activation of HPA axis [5]. This notion opens a broad range of potential targets related to the HPA system for therapeutic interventions in MDD [6]. Increasing evidence also suggests that after several weeks of classical antidepressant treatment, tricyclic antidepressants [7], selective serotonin reuptake inhibitors (SSRIs) such as paroxetine [8], and reuptake-inhibiting antidepressants such as reboxetine [9] gradually increase the glucocorticoid receptor mRNA levels and hormone-binding activities [10], reduce the corticotropin-releasing hormone (CRH) gene expression and CRH protein synthesis [11], and thus normalize HPA hyperactivity, recovering the disturbed feedback control in depressed patients [12]. This view is also supported by genetic studies. The STAR*D study found that rs4713916 and rs1360780 in FKBP5 showed a nominally significant association with the treatment response in a white, non-Hispanic subsample [13]. Another candidate gene, CRHR1, was found to contain a three-SNP haplotype (composed of the alleles rs1876828, rs242939, and rs242941) that predicted the response to the antidepressant treatment [14,15].

Growing evidence also highlights the importance of gene-by-environment (G*E) interactions to the field. Zimmermann et al. [16] explored the interactions between FKBP5 gene variants and adverse life events in predicting the first occurrence of a major depressive episode in 884 Caucasians. The influence of trauma on incident major depressive episodes was significant among subjects homozygous for the minor alleles of five SNPs (rs3800373, rs1360780, rs4713916, rs9296158, rs9470080) but

not subjects with other genotypes. Several studies focused on the TAT haplotype (composed of the alleles rs7209436, rs110402, and rs242924) of CRHR1, which is associated with adverse childhood experiences, and found that it had a moderate effect on the risk for depression in independent samples [17–19]. However, there has been relatively little research on the interactions between genetic polymorphisms related to HPA and environmental factors influencing the efficacy of antidepressant treatments.

In this study, we aimed to test whether variants in the genes involved in the HPA system would affect patient response to antidepressants. Second, we assessed both childhood trauma and recent negative life events to further investigate the effect of genetic polymorphisms and environmental factor interactions on antidepressant efficacy. In addition, functional studies were performed to explore the functional implication of a certain polymorphism (CRHR1 rs28364032) that appeared to be associated with antidepressant response.

Patients and Methods

Study Design and Subjects

The project was conceived as a part-randomized pharmacogenetic study of two types of antidepressants, selective serotonin reuptake inhibitor (SSRI) and norepinephrine reuptake inhibitor (SNRI), among the Chinese. It was designed to investigate the relationship among genes, environment and antidepressant treatment response. The methods, design, and rationale of this study have been detailed elsewhere [20,21]. In brief, Chinese men and women aged between 18 and 60 were recruited. All of the patients were diagnosed with major depressive disorder (MDD) by two independent senior psychiatrists and were confirmed by a third psychiatrist according to the criteria of the DSM-IV. All of the subjects were new or recently relapsed depression patients who had been drug-free for over 2 weeks and scored >18 on the 17-item Hamilton Depression Rating Scale (HAMD-17). The exclusion criteria have been reported previously [21].

Finally, a total of 273 patients were treated with single antidepressant drugs according to current clinical practice (SSRI: n = 151, SNRI: n = 122). The HAMD-17 was used by a trained senior psychiatrist to assess the severity of the depressive symptoms at baseline and after 2, 4, 6, and 8 weeks of treatment. 'Remission' was defined as a total HAMD-17 score of ≤7 after 8 weeks of treatment [22]. All of the subjects gave informed consent for participation in the study, which had been approved by the Ethical Committee of ZhongDa Hospital, Southeast University.

Assessment of Environment Factors

Two retrospective self-report questionnaires were used to assess environmental factors. The Childhood Trauma Questionnaire (28-item short form, CTQ-SF) was used to assess the occurrence and severity of any childhood trauma that took place before the age of 16 years [23]. The life event scale (48 item, LES) was used to evaluate stressful life events that occurred during the previous year of the current depression episode [24]. Finally, the total CTQ-SF scores and negative life event score (NLES) scores were used and dichotomized in further gene–environment interaction analyses, the details have been described previously [21].

Gene Selection, Genotyping Methods, and Quality Control

Five candidate genes in the HPA axis (CRHR1, CRHR2, AVPR1A, FKBP5, NR3C1) were selected for analysis. A total of 26SNPs were selected. The HapMap data and SNP Tagger program on the Chinese Han, Beijing (CHB) population were used to select 23 tagging SNPs that had a minor allele frequency of 0.05 or more with a pair-wise $r^2 > 0.80$ within the genes. Hot SNPs from previous studies were added or used to replace tagging SNPs if they had high linkages. Three polymorphisms within the microRNA-binding sites of these genes have also been selected. Putative microRNA-binding sites within the 3′UTR of each gene were identified by means of specialized algorithms, including MicroSNiPer databases (http://cbdb.nimh. nih.gov/microsniper/) [25], PolymiRTS databases (http://compbio.uthsc.edu/miRSNP/) [26,27], Patrocles databases (http://www.patrocles.org/) [28], and TargetScan (http://www.targetscan.org/) [29].

Quality-verified DNA samples were genotyped with the Multiplex SNaPshot System using an ABI fluorescence-based assay discrimination method (Applied Biosystems, Foster City, CA, USA). For quality control, a negative control was used, and 5% of the samples were genotyped as duplicates. No discordance was recorded.

From the 26SNPs, rs1876828 (CRHR1) with minor allele frequency (MAF) <5%, rs242924 (CRHR1) with the percentage of nonmissing genotypes for the marker (%gene) <97% and rs2240403 (CRHR2), rs975537 (CRHR2), and rs1042615 (AVPR1A) with Hardy–Weinberg equilibrium (HWE) $P < 0.001$ [30] were excluded from further analysis after being tested by Haploview 4.0 [31]. Finally, a total of 21SNPs were included in the analyses. The details of all 21SNPs are provided in Table 1.

Statistical Analysis

The differences in the clinical variables between the remitting and nonremitting patients were evaluated by the Pearson's χ^2-test or Student's *t*-test using SPSS 13.0 (SPSS Inc., Chicago, IL, USA). UNPHASED 3.0.13 [32] was used to compare the allele, genotype and haplotype distributions between the remitters and nonremitters. The odds ratios (ORs) were assessed for significant associations and expressed with 95% confidence intervals (95% CIs). A total of 10,000 permutation tests were performed to correct the *P*-values in the allelic, genotypic and haplotype association analyses among all of the SNPs tested in this study. Logistic regression was used to analyze the interactions between genes and the environment in the associations with treatment outcome, age, HAMD-17 baseline score, sex, and drug type, which were treated as covariates.

A power analysis to detect associations was performed using the program Power and Sample Size Calculation [33]. The sample had 80% power to detect a risk allele with a 5% frequency and a relative risk of 2.5 at the 0.05 significance level.

Table 1 General characteristics of polymorphisms genotyped

Gene	SNP ID	Location on chr	Location in gene	%gene	HWpval	MAF	Alleles
CRHR1	rs7209436	17:43870142	Intron	100	0.880	0.112	A>G
	rs110402	43880047	Intron	100	1	0.110	A>G
	rs242948	43913544	3′-gene	100	0.848	0.172	A>C
	rs28364026	43912294	3′-UTR	97.1	0.438	0.172	G>A
	rs28364032	43912342	3′-UTR	97.1	0.931	0.115	G>A
CRHR2	rs2270007	7:30699972	Intron	100	0.952	0.430	C>G
	rs2190242	30709475	Intron	99.6	0.573	0.428	C>A
	rs3779250	30694260	Intron	100	0.364	0.438	G>A
AVPR1A	rs10877969	12:63547239	5′-gene	100	0.706	0.147	A>G
	rs3759292	63547313	5′-gene	99.3	0.809	0.387	A>G
	rs11174816	63547648	5′-gene	98.9	1	0.113	G>A
NR3C1	rs33388	5:142697295	Intron	98.9	0.224	0.237	T>A
	rs6196	142661490	Exon	100	1	0.064	A>G
	rs41423247	142778575	Intron	97.1	0.329	0.215	G>C
	rs852977	142687494	Intron	100	0.898	0.095	A>G
FKBP5	rs7753746	6:35565422	Intron	99.3	0.756	0.186	A>G
	rs1360780	35607571	Intron	100	1	0.282	G>A
	rs9296158	35567082	Intron	98.9	1	0.328	G>A
	rs4713916	35669983	Intron	98.2	0.856	0.229	G>A
	rs2817035	35696363	5′-gene	99.3	0.917	0.245	G>A
	rs3800373	35542476	3′-UTR	97.1	0.580	0.274	A>C

HWpval, the Hardy–Weinberg equilibrium *P* value; %gene, the percentage nonmissing for this marker; MAF, the minor allele frequency for this marker; Alleles, the major and minor alleles for this marker.

Functional Genetic Analyses

Plasmid Construction

Using the pEGFP-C1 vector and pmirGLO Dual-Luciferase miRNA Target Expression vector, we constructed two plasmids for the qualitative and quantitative characterization of the 3′UTR SNP rs28364032. A 1022-bp fragment from the 3′UTR of the CRHR1 gene containing rs28364032-G was amplified using PCR and then cloned into the HindIII/KpnI site of the pEGFP-C1 vector and the NheI/SalI site of the pmirGLO vector (Promega, Madison, WI, USA). A modification of the Quikchange Site-Directed Mutagenesis method was used to obtain the mutated rs28364032-A construct [34]. The direction and sequence of the above constructs were verified with direct sequencing. The negative control (NC) constructs were blank pEGFP-C1 or pmirGLO vectors (lacking any 3′UTR sequences).

Oligonucleotide Synthesis

An hsa-miR-296-5p mimic was artificially synthesized by the Shanghai Integrated Biotech Solutions Company (Pudong New Area, Shanghai, China). The sequences were as follows: hsa-miR-296-5p 5′-AGGGCCCCCCCUCAAU-CCUGU-3′ and miRNA negative control (miR-NC) 5′-UUC UCCGAACGUGUCACGUUU-3′.

Reporter Gene Assay

The human glioblastoma U251 cell line and the AD-293 cell line were maintained in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (Si Ji Qing, Tian Hang Biological Technology, Zhe Jiang) and antibiotics at 37°C with 5% CO_2.

Cells in 24-well plates were transiently transfected with 1 μg of the rs28364032-G construct, rs28364032-A construct or the NC plasmids using PolyJetTM (Signagen, Rockville, MD, USA). Following a 24 h transfection period, the cells transfected with pEGFP-C1 vectors were collected for assay using a fluorescence microscope (Olympus, Shinjuku, Tokyo, Japan). The cells transfected with the pmirGLO vectors were used to measure luciferase activity with the Dual-Luciferase Reporter Assay System (Promega).

For cotransfection experiments, the pmirGLO rs28364032_G-allele or mutated A-allele constructs were mixed with the synthetic miR-296-5p mimics or miR-NC and transfected into AD-293 and U251 cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). The luciferase assay was performed using the Dual-Luciferase Reporter Assay System (Promega) 48 h after the transfection.

Results

Demographics and Clinical Characteristics of the Antidepressant-Treated Patients

A total of 273 patients who completed 8 weeks of treatment were successfully genotyped, and 150 of these patients met the criteria for remission. There were no significant differences between the remission and nonremission subgroups with regard to age, age of onset, years of education, family history of mood disorder, or baseline HAMD-17 scores. However, gender was significantly

associated with antidepressant response ($\chi^2 = 6.034$, $P = 0.014$), and was used as a covariate in further analyses. The demographics and clinical characteristics of the patients in the two subgroups are shown in Table 2.

Significant Influence of CRHR1 Variations on Antidepressant Treatment

The remission rate in the study was 54.9% at 8 weeks. Among the 21SNPs analyzed, one SNP, rs28364032, located in the 3′UTR of the CRHR1 gene showed a significant association with remission (Table 3). In a post hoc analysis, this effect was observed in both SSRI and SNRI subgroups (allelic association $P = 0.024$ and 0.010, respectively), although these results did not withstand permutation testing. When compared to the G/G genotype and the G allele, the A/A genotype and the A allele were associated with an increased likelihood of better response to treatment in the total group. The haplotype analysis showed that three haplotypes of the CRHR1 gene have significant differences between remitters and nonremitters. In the two SNP (rs242948 andrs28364032) haplotype analysis, C-A haplotype was significantly associated with antidepressant remission compared with C–G haplotype (OR = 7.424, 95% CI = 2.854–19.31). Moreover, the G-A of CRHR1 (rs28364026 and rs28364032) was significantly associated with remission compared with the G–G haplotype (OR = 3.018, 95% CI = 1.486–6.128). In the three SNPs (rs242948, rs28364026, and rs28364032) haplotype, the C-G-A haplotype was associated with an increased likelihood of remission compared with C–G–G (OR = 7.032, 95% CI = 2.484–19.91) (Table 3).

Table 2 Demographic and clinical characteristics of the remitter and nonremitter groups

	Remitters (n = 150)	Nonremitters (n = 123)	χ^2/t	*P*
Sex (male/female)	50/100	59/64	6.034	0.014
Age (mean ± SD)	39.2 ± 12.0	39.0 ± 14.1	−0.079	0.937
Age of onset (mean ± SD)	35.4 ± 11.5	34.1 ± 13.8	−0.837	0.404
Years of education (mean ± SD)	11.0 ± 3.80	11.5 ± 4.27	1.068	0.287
Family history of MDD (yes/no)	22/128	23/100	0.798	0.372
HAMD 0w (mean ± SD)	27.2 ± 5.60	28.2 ± 5.34	1.450	0.148

MDD, major depressive disorder; HAMD, Hamilton Rating Scale for Depression.

Gene–Environment Interactions

In this study, the total CTQ-SF and NLES scores between remitters and nonremitters showed no significant differences ($P > 0.05$) (Table 4). Among the 273 participants, 206 patients completed the CTQ-SF, and 207 patients completed the LES. Of these, 70 and 74 patients were categorized as high adversity based on their total CTQ-SF scores or NLES scores, respectively.

No significant interactions were found between the SNPs and CTQ ($P > 0.05$), and similar results were found in the interaction model of SNPs and NLES scores, including rs28364032 in CRHR1 (Table 5). A significant drug effect was apparent in this analysis.

Functional Genetic Studies

To explore the possible functional impact of rs28364032 in the CRHR1 gene, we examined the regulatory activity of each

Table 3 Distributions of rs28364032 genotypes/alleles and haplotype analysis in the CRHR1 genes of remitters and nonremitters

		Remitters	Nonremittes			
Gene/rs#	Haplotype	Total group (n = 273)		*P*	*P**	OR (95% CI)
CRHR1/rs28364032	A	46	15	3.53×10^{-4}	0.0027	2.926 (1.59–5.38)
	G[a]	240	229	3.53×10^{-4}		
	AA+AG	42	15	7.47×10^{-4}	0.0045	2.966 (1.550–5.678)
	GG[a]	101	107	7.47×10^{-4}		
CRHR1/rs242948, rs28364032	A-A	4.17	2.26	0	0.0012	7.424 (2.854–19.31)
	A-G	228.8	203.7	0.2837		
	C-A	41.8	12.7	4.27×10^{-4}		
	C-G[a]	11.2	25.3	0.0039		
CRHR1/rs28364026, rs28364032	A-G	46	44.5	0.4730	0.0014	3.018 (1.486–6.128)
	G-A	46	14.5	3.53×10^{-4}		
	G-G[a]	194	184.5	0.0545		
CRHR1/rs242948, rs28364026, rs28364032	A-A-G	45.7	42.2	0.5747	0.0019	7.032 (2.484–19.91)
	A-G-A	4.18	1.51	0		
	A-G-G	183.1	161.5	0.6588		
	C-A-G	0.33	2.01	0		
	C-G-A	41.8	12.7	4.28×10^{-4}		
	C-G-G[a]	10.9	23.3	0.0052		

OR, odds ratios; CI, confidence interval. aReference haplotype. *Adjusted *P*-value from 10,000 permutation tests.

Table 4 CTQ-SF and NLES scores of remitters and nonremitters

	Remitters	Nonremitters	t	*P*
CTQ-SF (mean ± SD)	41.0 ± 10.6	41.7 ± 11.4	0.492	0.623
NLES (mean ± SD)	49.2 ± 58.3	43.8 ± 53.0	−0.688	0.493

CTQ-SF, the Childhood Trauma Questionnaire (28-item Short Form); NLES, the negative Life Events Scale.

Table 5 Results of the interactions of CTQ-SF/NLES and rs28364032 on antidepressant responses

	β	SE	*P*	Odds ratio	95% CI
Age	0.343	0.300	0.253	1.409	0.783–2.535
Sex	0.009	0.012	0.459	1.009	0.986–1.032
Drug type	0.622	0.312	0.046	1.863	1.011–3.432
Baseline HAMD-17 score	−0.016	0.029	0.570	0.984	0.930–1.041
CTQ-SF	−1.877	1.535	0.222	0.153	0.008–3.104
rs28364032	−2.637	1.305	0.043	0.072	0.006–0.924
rs28364032 by CTQ-SF interaction	0.960	0.824	0.244	2.613	0.520–13.14
Age	0.411	0.303	0.175	1.509	0.832–2.734
Sex	0.012	0.012	0.302	1.012	0.989–1.036
Drug type	0.660	0.319	0.038	1.935	1.036–3.614
Baseline HAMD-17 score	−0.033	0.028	0.239	0.967	0.915–1.022
NLES	0.668	1.609	0.678	1.950	0.083–45.63
rs28364032	−0.812	1.207	0.501	0.444	0.042–4.730
rs28364032 by NLES interaction	−0.296	0.858	0.730	0.744	0.139–3.998

HAMD-17, 17-item Hamilton Depression Rating Scale; CTQ-SF, total score of Childhood Trauma Questionnaire; NLES, score of negative Life Events Scale. Logistic regression analysis with age, sex, drug type, and baseline HAMD-17 score as covariates.

genotype using a green fluorescent protein reporter gene and a luciferase reporter gene assay. Plasmids constructed with either green fluorescent protein or luciferase as the reporter genes were transfected in two different cultured cell lines. First, the green fluorescent protein reporter gene assays were performed. As shown in Figure 1A, in both the AD-293 and U251 cell lines, the blank pEGFP-C1 plasmid showed the greatest green fluorescence intensity. Greater green fluorescence intensity was obtained using the construct with the rs28364032_G-allele when compared with the rs28364032_A-allele. Next, luciferase reporter gene studies were performed. The blank pmirGLO plasmid showed the highest luciferase activity (AD-293: 0.382 ± 0.0531; U251: 0.2801 ± 0.0323), but the G-to-A change of rs28364032 resulted in a 10–12% decrease in the intensity of luciferase activity in both the AD-293 (rs28364032_G: 0.2368 ± 0.0368; rs28364032_A: 0.2078 ± 0.0291; $P = 0.047$) and U251 cells (rs28364032_G: 0.1213 ± 0.0113; rs28364032_A: 0.1093 ± 0.004; $P = 0.038$). The results suggested that the mutated A allele had lower protein expression levels than the wild-type G allele.

Next, we further investigated how this SNP was involved in the regulation of gene expression. According to TargetScan (http://www.targetscan.org/) [29], rs28364032 was predicted to be located within the seed sequence of hsa-miR-296-5p (Figure 1B). The interaction of miR-296 and the CRHR1 mRNA 3′UTR was assessed using a 3′UTR luciferase assay. Neither the pmirGLO rs28364032_G nor the mutated A-allele constructs, which were each cotransfected with miR-296-5p, led to a significant decrease of the reporter activity when compared with the negative control ($P > 0.05$). It was further validated that miR-296-5p does not recognize the 3′-UTR of CRHR1 transcripts and rs28364032 is not found in the seed region of miR-296-5p.

Discussion

This study investigated the association of genetic variation in HPA system-related genes and their interactions with childhood trauma or recent negative life events on the efficiency of antidepressant treatment. The results supported a major effect of CRHR1 rs28364032 on the outcome of antidepressant treatment. Three haplotypes of CRHR1 genes containing rs28364032 showed a significant relationship with antidepressant remission. Additionally, functional assays showed that the G-to-A change of rs28364032 does indeed influence the protein expression of CRHR1. However, no association of negative life events, childhood adversity or their interactions with HPA system-related genes with antidepressant remission was identified, although a significant drug effect indicative of drug stratification was apparent.

We found only one SNP in CRHR1 (rs28364032) that withstood the correction for multiple testing in the pharmacogenetic study and showed an effect on the overall response to treatment during the 8 weeks. Additionally, three haplotypes containing the A allele of rs28364032 and two polymorphisms (rs242948, rs28364026) were strongly associated with a better response, all with a frequency above 5% in the remitter and nonremitter groups. CRHR1 is a major regulator of the HPA axis, through which CRH stimulates the stress response [35]. Studies have reported that CRH1 receptor antagonist could improve the depression and anxiety symptoms in both animal [36] and major depressive episode patients [37], although these results need to be validated in further clinical trials. Several genetic studies also have shown that antidepressant treatment can be moderated by variations in CRHR1. Papiol et al. [38] found that the CRHR1 gene (SNP rs110402) was associated with an increased risk for presenting with an early age of onset for the first depressive episode, but there was no association with the response to the antidepressant citalopram. Licinio et al. [14] and Liu et al. [15] found that the GAG haplotype (rs1876828, rs242939, and rs242941) of CRHR1 increased the response to antidepressants in highly anxious Mexican-American and Chinese MDD patients. In this study, we were unable to replicate significant results with rs110402. This result may be due to differences in the study populations, as rs110402 has never been replicated in Chinese samples [17,18]. Moreover, due to the low MAF (0.002) in our sample, rs1876828 was excluded from further analysis in this study.

The most significant SNP (rs28364032) identified in our study is located in the 3′ untranslated region of CRHR1 mRNA. Further functional genomic studies of this polymorphism showed that the

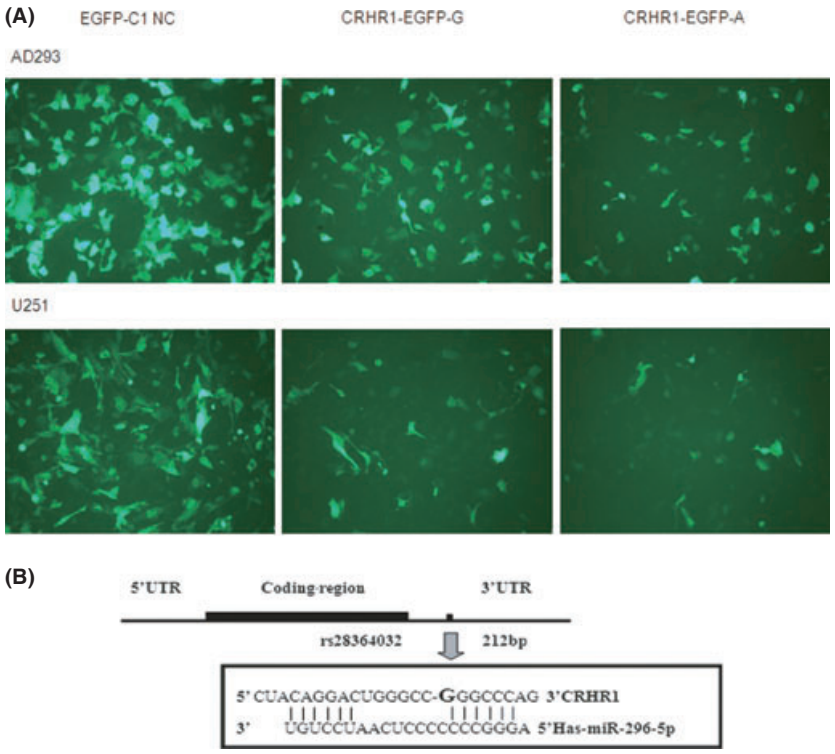

Figure 1 Expression of rs28364032-NC, rs28364032-G, and the mutated A constructs in transiently transfected AD-293 and U251 cells. (**A**) The rs28364032-EGFP constructs scanned through a fluorescence microscope. The images were acquired using the same exposure time. (**B**) Schematic view of the position of rs28364032 and the predicted target sites for hsa-miR-296-5p in the 3′UTR of CRHR1 mRNA.

mutated A allele leads to lower luciferase activity than the G allele and is associated with the down-regulation of transcription, potentially resulting in decreased CRHR1 activity and thus a better antidepressant response. Unfortunately, we failed to locate rs28364032 at the microRNA-296-5p binding site, as predicted via TargetScan, but we found that this SNP is located in the transcription factor binding sites of HDBPs (Huntington's disease gene regulatory region binding proteins). This finding hints that this SNP may be involved in a more complex mechanism regulating the process of transcription. Furthermore, Keck et al. [39] genotyped 18 SNPs spanning 57 kb of CRHR1 in a German sample and found that an SNP, rs878886, located in the 3′UTR of CRHR1 was nominally associated with panic disorders. This finding may reveal a possible mechanism of the CRHR1 3′UTR in regulating psychiatric and psychosomatic diseases and their treatment. However, further experiments will be needed to clarify this hypothesis.

Substantial evidence has suggested that both childhood trauma and recent negative life events exert deleterious effects on depressive episodes and symptom severity, but relatively few studies have tested the direct association of early or recent life adverse events and the response to antidepressants in MDD. Klein et al. [40] suggested that among 808 MDD patients completing the 12-week pharmacotherapy, 32% patients with a history of clinically significant abuse achieved remission, a percentage that was lower than those without such a history. Johnstone et al. [41] reported that paternal neglect and maternal overprotection was more predictive of response to treatment for depression than sexual, physical, or psychological abuse. Inconsistent results have been reported about life events that are considered other major environmental factors of depression. A study containing 126 MDD patients showed a significant interaction between specific types of nonsevere life events and medication in a 3-year randomized maintenance protocol [42]. However, Mandelli et al. [43] failed to find a significant impact of adverse life events on current treatment response. In present study, we found no associations of environment factors and treatment responses with or without of HPA-system-related genetic variants. To the best of our knowledge, there have been few clinical studies on the HPA system that have related gene–environment interaction with antidepressant response. The reported findings should be viewed as preliminary results that must be replicated in larger independent samples.

There are some limitations to our investigation. First, the sample size should be augmented to achieve greater power, and the results of this study should be replicated in other independent samples. Moreover, the absence of a placebo or control group limited our consideration of treatment specificity, which could have confounded the specific effects of the antidepressant treatment with the natural disease course in patients. Another limitation was the variety of antidepressant treatment types used in the sample, although we did adhere to a more naturalistic process that may be extrapolated to routine treatment environments. While we did observe an effect of drug type that suggested drug stratification, we also found that the genotype effect was apparent in each drug subgroup (SSRI and SNRI). A combined analysis across various antidepressant treatments is justifiable, given that most of the antidepressants involved in this study are likely related to effects on HPA system remodeling [12].

Conclusions

In conclusion, we have identified one polymorphism located in the 3′UTR of CRHR1 to be associated with antidepressant

response. Further laboratory-based genomic studies provided support for functional effects of this polymorphism on gene expression. Our results provide evidence to support a definite role for CRHR1 in the pharmacogenetics of antidepressant drugs. An independent replication extended to a larger sample size and further functional studies will be needed to confirm our preliminary findings.

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Conflict of Interest

The authors declare no conflict of interest.

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