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ORIGINAL RESEARCH

Rs7219 Regulates the Expression of *GRB2* by Affecting miR-1288-Mediated Inhibition and Contributes to the Risk of Schizophrenia in the Chinese Han Population

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Abstract

In the present study, we examined a potential genetic association between the variant rs7219 within the 3′-UTR of *GRB2* and the susceptibility to schizophrenia (SCZ) and bipolar disorder (BD) in the Chinese Han population. A genetic association study, including 548 SCZ patients, 512 BD patients, and 598 normal controls, was conducted in the Chinese Han population. Genotyping was performed through the Sequenom MassARRAY technology platform. The expression of *GRB2* was detected using quantitative real-time polymerase chain reaction (qRT-PCR). A dual-luciferase reporter assay was performed to determine whether miR-1288 could bind to the 3′-UTR region of *GRB2* containing rs7219. We found that rs7219 was significantly associated with the susceptibility to SCZ under different genetic models, including additive [OR (95% CI) = 1.24 (1.02–1.49), $P = 0.027$], dominant [OR (95% CI) = 1.31 (1.04–1.66), $P = 0.025$], and allelic models[OR (95% CI) = 1.24 (1.03–1.49), $P = 0.027$]. However, no significant associations were found between rs7219 and the risk for BD (all $P > 0.05$). Moreover, we observed that the expression of *GRB2* significantly decreased in SCZ patients compared with the controls ($P = 0.004$). The dual-luciferase reporter assay showed that the minor allele C of rs7219 significantly decreased the luciferase activity by binding miR-1288 ($P < 0.001$). In summary, we are the first to reveal that rs7219 is significantly associated with the susceptibility to SCZ in the Chinese Han population. Moreover, the minor allele C of rs7219 is identified as a risk allele for SCZ because it generates a binding site for miR-1288, thereby resulting in decreased expression of *GRB2* and ultimately increasing the risk of SCZ.

Keywords Rs7219 · GRB2 · Dual-luciferase reporter assay · Schizophrenia · Bipolar disorder

Introduction

Schizophrenia (SCZ) and bipolar disorder (BD) are two common neuropsychiatric disorders that are characterized by relatively high morbidity and heritability and affect approximately 3% of the world's population (Perala et al. 2007; Craddock and Jones 1999; Jablensky et al. 1987). SCZ is considered a severe mental disease and has profound social and economic impacts. The main characteristics of SCZ include hallucinations, delusions, and cognitive dysfunction, and its individual heritability is approximately 80% (Cardno and Gottesman 2000; Burmeister et al. 2008). BD is another severe psychiatric disorder accounting for 7% of disability-adjusted life years (DALY) worldwide (Whiteford et al. 2013). With an individual heritability estimated at 65–75%, BD is mainly characterized by alternating manic and depressive episodes (Burmeister et al. 2008). It has been reported that the lifetime prevalence of SCZ and BD in mainland China is approximately 0.54% and 0.11%, respectively (Long et al. 2014; Zhang et al. 2017). Numerous family, twin, and adoption studies highlight a complex interaction of genetic components with environmental factors in the pathogenesis of SCZ and BD (Lichtenstein et al. 2009). Although the full pathogenesis of the two mental disorders

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still remains elusive (Insel 2010), the genetic component has been confirmed as a vital etiological factor in the two psychiatric diseases (Tandon et al. 2008).

In humans, *Growth factor receptor bound protein 2* (*GRB2*) spans an approximately 8.7 kb region on the long arm of chromosome 17 (17q25) and encodes the GRB2 protein, which is ubiquitously present in all tissues throughout development (Law et al. 1999; Asada et al. 1999). GRB2 is generally known as an important adaptor protein that comprises three protein-binding modules, including a central SH2 domain flanked by two SH3 domains (Lowenstein et al. 1992). Studies have revealed that GRB2 exerts its biological function mainly by interacting with various growth factor receptors and then transmitting growth factor signal to downstream signal cascades (Jang et al. 2009; Tups et al. 2012; Feller and Lewitzky 2006). For instance, the nerve growth factor (NGF) initially forms a complex by interacting with the specific receptor nerve growth factor receptor tyrosine kinase A (TrkA) on the neuronal membrane; then, the complex recruits GRB2 to transmit signals to Ras and, finally, activates the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) signaling cascade to regulate neuronal growth and development (Qian et al. 1998; Colledge and Froehner 1998). In addition, Easton et al. indicated that brain-derived neurotrophic factor (BDNF) enhances neuronal survival by inducing the formation of a complex containing FRS2, GRB2, and SOS and thereby activates the MAPK/ERK pathway (Easton et al. 1999). The above evidence demonstrates that GRB2 is a crucial link between growth factor signaling and the activation of the MAPK/ERK pathway. The ERK pathway is the most studied and best characterized MAPK signaling cascade and is an important regulator of neuronal function (Mazzucchelli and Brambilla 2000; Sweatt 2001; Adams and Sweatt 2002). ERKs are diffusely expressed in the central nervous system (CNS) and necessary for the appropriate development of the CNS (Fukunaga and Miyamoto 1998). Evidence from postmortem tissue suggests that the MAPK/ERK signaling cascade is abnormally activated in the cerebellum of SCZ patients (Kyosseva et al. 1999; Kyosseva 2004), providing a preliminary basis to investigate an association between the MAPK/ERK signaling cascade and the pathogenesis of SCZ. In addition, the MAPK/ERK pathway has been reported to play a key role in the regulation of neuronal plasticity and survival (Di Daniel et al. 2005; Klesse and Parada 1999). One study suggested that GRB2 is also involved in the regulation of neuregulin (NRG)-induced synapse formation and synaptic plasticity (Ma et al. 2003). These neural characteristics, including neuronal plasticity, neuronal survival, synapse formation, and synaptic plasticity, are reportedly associated with the pathogenesis of SCZ and BD; this association indirectly implies that GRB2 may contribute to the pathogenesis of SCZ and BD (Nanou and Catterall 2018; Rudenko 2017; Sorg et al. 2016; Lindberg et al. 2015).

In the past several decades, genetic association studies have identified numerous valuable susceptibility loci for neuropsychiatric disorders, broadening our understanding of mental diseases (Zeng et al. 2018; Yu et al. 2018; Polushina et al. 2017). Many single-nucleotide polymorphisms (SNPs) in the *NGF* or *BDNF* genes were reported to be significantly associated with susceptibility to SCZ or BD (Ament et al. 2015; Misiak et al. 2018; Saravani et al. 2017; Zakharyan et al. 2014). Considering the above close relationship of the major neurotrophins (*NGF* and *BDNF*) with *GRB2*, polymorphisms within *GRB2* are presumed to be significantly associated with the susceptibility to SCZ and BD. Among the currently identified diseased-related SNPs, a special type of SNP located in the 3′ untranslated region (UTR) of candidate genes has garnered increasing attention due to its potential to regulate the expression of these genes. Although the 3′-UTR is located downstream of the protein coding sequence and does not contain a coding sequence, it plays a crucial role in mRNA regulatory processes, including mRNA stabilization, translation, and localization (Steri et al. 2018). One such regulatory process is 3′-UTR facilitation of mRNA decay or translational repression of candidate genes through binding with miRNAs (Djuranovic et al. 2012; Pasquinelli 2012). MiRNAs are a kind of small noncoding RNA with approximately 21 nucleotides and can reduce stability and/or translation of the target mRNA by interacting with the RNA-induced silencing complex (Tang 2005). The inhibitory regulation is dependent on the specific binding sequence of miRNAs; thus, any mutation within the binding region has the potential to alter binding and abolish the regulation of gene expression. For example, rs1130354(G) within the 3′-UTR of *human dopamine receptor D2* (*DRD2*) upregulates the expression of *DRD2* by abolishing miR-326-mediated inhibition (Shi et al. 2014). Rs550067317(A), which is located in the 3′-UTR of *ephrin B2* (*EFNB2*), independently reverses the miR-137-mediated repression of *EFNB2* expression, thereby affecting the progression of SCZ (Wu et al. 2016). Rs7219 is a gene variant located in the 3′-UTR of *GRB2*. To the best of our knowledge, the previous study of a genetic association between rs7219 and mental disorders was only assessed in the Japanese population (including 364 SCZ patients and 342 healthy controls), and the study demonstrated a negative association ($P_{\text{allele}} = 0.125$, $P_{\text{genotype}} = 0.239$) (Ikeda et al. 2008). However, similar studies assessing the genetic association between rs7219 and the susceptibility to SCZ and BD have not been reported in the Chinese Han population.

Therefore, in the present study, we conducted a genetic association study to determine the association of *GRB2* variant rs7219 with the risk of SCZ and BD in the Chinese Han

population. To further explore the potential mechanisms by which rs7219 contributes to the pathogenesis of SCZ and BD, we also investigated the expression of *GRB2* and conducted a dual-luciferase reporter assay.

Materials and Methods

Participants

Our sample set consisted of 548 schizophrenia cases (273 males and 275 females; mean age ± standard deviation [SD] = 35.08 ± 11.11 years), 512 bipolar disorder cases (237 males and 275 females; mean age ± SD = 35.08 ± 13.33 yea rs), and 598 normal controls (293 males and 305 females; mean age ± SD = 34.24 ± 8.80 years). No distribution differences in gender or age were detected between SCZ or BD patients and controls (all $P > 0.05$).

All participants were of Chinese Han origin and recruited from Guangxi, China. SCZ and BD patients were diagnosed by at least two experienced psychiatrists using strict International Classification of Diseases 10 (ICD10) criteria before being enrolled into this study. The exclusion criteria for patients were as follows: serious physical diseases that could impair brain function, substance-induced psychotic disorders, or mental retardation. All patients were recruited from six psychiatric specialist hospitals in Guangxi. The corresponding controls were recruited from the Health Examination Center of comprehensive hospitals in Guangxi, China. None of the controls presented with a family history of psychiatric disorders and/or a severe medical illness. All participants were not biologically related to one another. Written informed consent was obtained from all participants, and our study was reviewed and approved by the Institutional Ethical Committee of Guangxi Medical University.

DNA Extraction and Genotyping

The Genomic DNA Extraction Kit (Aidlab Biotechnologies Co., Ltd.) was used to extract genomic DNA from the peripheral blood of participants according to the manufacturer's instructions. The specific primers were designed for the genotyping of rs7219 using AssayDesigner 3.1 software, and primer sequences were as follows: 5′-ACG TTGGATGAGTAGGAGACAAATTGGCTG-3′ (forward primer) and 5′-ACGTTGGATGGGTTGCTTCTGAGT GGTGTT-3′ (reverse primer). Genotyping was performed at Bomiao Biological Co., Ltd. (Beijing, China) by using the Sequenom MassARRAY technology platform (Sequenom, San Diego, CA, USA). To confirm the reliability of the genotyping method, 5% random samples were regenotyped, and the concordance rate of the genotyping data reached 100%.

Determination of *GRB2* Expression Level

GRB2 expression was detected in a portion of the participants, including 48 SCZ patients (25 males and 23 females; mean age: 29.38 ± 6.08 years), 48 BD patients (25 males and 23 females; mean age: 27.46 ± 8.09 years), and 48 controls (26 males and 22 females; mean age: 29.19 ± 4.16 years). No distribution differences in gender or age were recorded between the 48 SCZ patients or 48 BD patients and the 48 controls (all $P > 0.05$). Total RNA was extracted and purified from the peripheral blood of the 144 participants by using TRIzol™ Reagent (Invitrogen™, California, USA) following the manufacturer's protocol. cDNA synthesis was performed by a PrimeScriptTM RT reagent Kit with gDNA Eraser (Takara Bio Inc.) and stored at − 80 °C until use. Quantitative real-time polymerase chain reaction (qRT-PCR) was used to determine the relative expression of *GRB2* using the SYBR® Premix Ex Taq™ II kit (Takara Bio Inc.). The specific primer sequences of *GRB2* were as follows: 5′-AAGCCATCG CCAAATATGACTTC-3′ (forward primer) and 5′-TTT CCATTAAGCTCTGCCTTGTACC-3′ (reverse primer). The primer sequences of GAPDH (housekeeping gene) were as follows: 5′-GGTGGTCTCCTCTGACTTCAACA-3′ (forward primer) and 5′-GTTGCTGTAGCCAAATTC GTTGT-3′ (reverse primer). The above primers were synthesized by Sangon Biotech Co., Ltd, Shanghai, China. We utilized the $2^{-\Delta CT}$ method to calculate the relative expression of *GRB2*.

Prediction of miRNA Binding to Rs7219

Two available databases, SNPinfo Web Server (https://snpinfo.niehs.nih.gov/snpinfo/snpfunc.html) and miRanda (http://www.microrna.org), were used to search for the putative miRNA that can bind with the seed region containing the *GRB2* variant rs7219. After the predictive score (score in SNPinfo Web Server and mirSVR score in miRanda) and the location of miRNA-binding region were considered comprehensively, the results showed that miR-1288 satisfied the requirements and can bind with the minor allele C of rs7219 but not with the major allele T of rs7219 in the 3′-UTR of *GRB2*. In addition, by analyzing two miRNA profiles, GSE65367 and GSE54578, in the GEO database (https://www.ncbi.nlm.nih.gov/geo/), we found that miR-1288 was expressed in induced pluripotent

stem cells and blood samples from SCZ patients. Therefore, miR-1288 was selected to test whether rs7219 can influence *GRB2* expression by regulating the putative binding of miRNAs at the posttranscriptional level.

Cell Culture

Human embryonic kidney 293T cells (HEK-293T cells) (Chinese Academy of Sciences, Shanghai), which are generally used in the dual-luciferase reporter assay system, were selected to assess the regulatory role of miR-1288 on expression of the exogenous reporter gene. Cell lines were grown in Dulbecco's modified Eagle's medium (DMEM) (from Corning) supplemented with 10% fetal bovine serum (FBS) (from Ausbian) at 37 °C under 5% CO_2.

Plasmid Construction and Transfection

A 3′-UTR fragment of the *GRB2* gene 200 bp in length containing the putative miR-1288 binding region was chemically synthesized by Genechem Co., Ltd., Shanghai. Then, the sequences were inserted into the downstream multiple cloning site (MCS) of the firefly luciferase coding region in the GV272 vector (Shanghai Genechem Co., Ltd.) at the XbaI/XbaI site. The vector carrying the major allele T of rs7219 was referred to as the wild type, and the mutated type vector carried the minor allele C of rs7219. For the miR-1288 overexpression vector, the inserted sequence was generated by PCR and cloned into the MCS region of the GV268 vector (Shanghai Genechem Co., Ltd.) between the XhoI/KpnI sites. The primers used to generate the miR-1288 overexpression fragment were as follows: forward primer: 5′-ACGGGCCCTCTAGACTCGAGAAGACCCATCCTC AGACATTC-3′, reverse primer: 5′-AGTCCAGTGTGG TGGAATTCGCAAAGCTGAAGGAAATTTGG-3′. All constructs were further confirmed by sequencing (Shanghai Genechem Co., Ltd).

Transfections with *GRB2* 3′-UTR luciferase vectors [wild type (WT), mutated type (MU), and negative control (NC)], miR-1288 vectors [overexpression type and NC], positive control vectors (*TRAF6* 3′-UTR, miR-146b-NC, and miR-146b), and Renilla luciferase vectors were performed using X-tremegene HP reagent (Roche), according to the manufacturer's instructions. We conducted eight co-transfection groups in this study, including group 1: 3′-UTR-NC + miR-1288-NC; group 2: 3′-UTR-NC + miR-1288; group 3: 3′-UTR-WT + miR-1288-NC; group 4: 3′-UTR-WT + miR-1288; group 5: 3′-UTR-MU + miR-1288-NC; group 6: 3′-UTR-MU + miR-1288; group 7: *TRAF6* 3′-UTR + miR-146b-NC; and group 8: *TRAF6* 3′-UTR + miR-146b. To verify that the transfection efficiency was acceptable, we transfected the green fluorescent plasmid (GFP) into the HEK-293T cells in the same batch. After transfecting these vectors for 48 h, cells were harvested for further detection.

Luciferase Activity Assays

After these vectors were transfected into HEK-293T cells using X-tremegene HP reagent for 5 h, the complete medium containing 10% FBS was used for further incubation. Luciferase activity was assayed using the Dual-Luciferase® Reporter Assay System (Promega, E2910) according to the manufacturer's instructions. Renilla luciferase activity was used to normalize the corresponding firefly luciferase activity.

Statistical Analysis

A genetic association analysis of rs7219 with the risk of SCZ and BD was carried out using PLINK software (http://pngu.mgh.harvard.edu/~purcell/plink/). Hardy–Weinberg equilibrium was evaluated using the Chi-square goodness-of-fit test. The Chi-square test was used to evaluate the distribution difference of genotype between the patients and controls. The association between rs7219 and susceptibility to SCZ and BD under different genetic models, including additive, dominant, recessive, and allelic models, was analyzed by multivariate logistic regression analysis. Statistical tests involving mRNA expression, receiver operating characteristic (ROC) analyses, and luciferase activity were conducted using SPSS software (17.0). For all analyses, we set the statistical significance at a threshold of 0.05 and adapted a two-tailed test.

Results

GRB2 Variants rs7219 is Significantly Associated with the Susceptibility to SCZ

No deviation from Hardy–Weinberg equilibrium was observed in the controls ($P = 0.655$), and the genotype distribution of *GRB2* variant rs7219 in SCZ patients, BD patients, and controls are listed in Table 1. The results revealed no significant distribution differences in the rs7219 genotype between SCZ patients and controls ($\chi^2 = 5.188$, $P = 0.075$). The same negative result was observed between BD patients and the controls ($\chi^2 = 0.792$, $P = 0.673$). After gender stratification analysis, the negative result remained (all $P > 0.05$). Moreover, a genetic association analysis under four genetic models was further conducted to evaluate the potential association of rs7219 with the risk of SCZ or BD. For SCZ patients, rs7219 was significantly associated with the risk of SCZ under additive [OR (95% CI) = 1.24 (1.02–1.49), $P = 0.027$], dominant [OR (95% CI) = 1.31 (1.04–1.66),

Table 1 Genotype distribution and Hardy–Weinberg equilibrium test of rs7219 in participants

Group	Total						Male					
	CC	CT	TT	$\chi 2$	P	P_H	CC	CT	TT	χ^2	P	P_H
SCZ	42	220	268	5.188	0.075	0.832	14	112	134	1.263	0.532	0.156
Control	37	212	334			0.655	13	113	162			0.262
BD	25	177	287	0.792	0.673	0.800	9	85	137	0.523	0.770	0.448
Control	37	212	334			0.655	13	113	162			0.262

Group	Female					
	CC	CT	TT	$\chi^2 2$	P	P_H
SCZ	28	108	134	4.320	0.115	0.388
Control	24	99	172			0.086
BD	16	92	150	0.888	0.641	0.733
Control	24	99	172			0.086

P_HHWE for participants

Table 2 Association analysis of *GRB2* variant rs7219 with SCZ or BD risk

Disease	Allele (minor/major)	Genetic model	Crude OR (95% CI)	Crude P	Adjusted OR (95% CI)	P_{adj}
SCZ	C/T	Additive model	1.24(1.02 ~ 1.49)	0.027	1.24(1.03 ~ 1.50)	0.026
		Dominant model	1.31(1.04 ~ 1.66)	0.025	1.32(1.04 ~ 1.67)	0.023
		Recessive model	1.27(0.80 ~ 2.01)	0.307	1.27(0.80 ~ 2.01)	0.309
		Allelic model	1.24(1.03 ~ 1.49)	0.027		
BD	C/T	Additive model	0.93(0.76 ~ 1.14)	0.477	0.92(0.76 ~ 1.13)	0.442
		Dominant model	0.94(0.74 ~ 1.20)	0.643	0.94(0.74 ~ 1.20)	0.613
		Recessive model	0.80(0.47 ~ 1.34)	0.390	0.78(0.46 ~ 1.32)	0.357
		Allelic model	0.93(0.76 ~ 1.14)	0.476		

P_{adj}adjusted by age, sex

$P = 0.025$], and allelic models [OR (95% CI) = 1.24 (1.03–1.49), $P = 0.027$]. After adjustment for age and sex, the significant association remained [additive model: OR (95% CI) = 1.24 (1.03–1.50), P_{adj}=0.026; dominant model: OR (95% CI) = 1.32 (1.04–1.67), P_{adj} =0 .023]. However, no significant associations between rs7219 and risk of BD were observed under the four genetic models (all $P > 0.05$). The results are summarized in Table 2.

The Expression of *GRB2* is Significantly Decreased in SCZ Patients

The qRT-PCR results showed that the expression of *GRB2* in the SCZ patients was significantly decreased compared to the controls ($P = 0.004$). Nonetheless, no significant difference in *GRB2* expression was found between BD patients and normal controls ($P = 0.228$) (Fig. 1). To evaluate the potential diagnostic value of *GRB2* for SCZ, we plotted the ROC curve. The values above the cut-off are defined as negative; otherwise, the values are positive. We found that the area under the ROC curve (AUC) was 0.657 and the cut-off value was 0.300. The sensitivity and specificity were 0.542 and 0.750, respectively (Fig. 2).

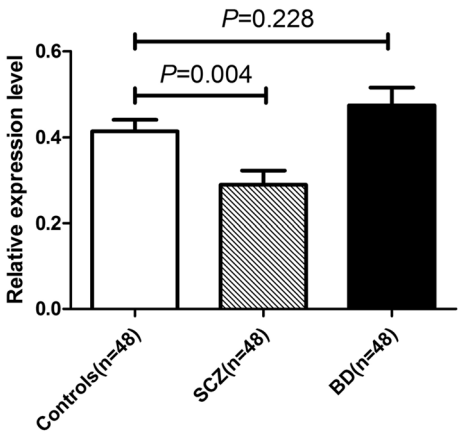

Fig. 1 Relative expression level of *GRB2* in SCZ patients, BD patients and healthy controls

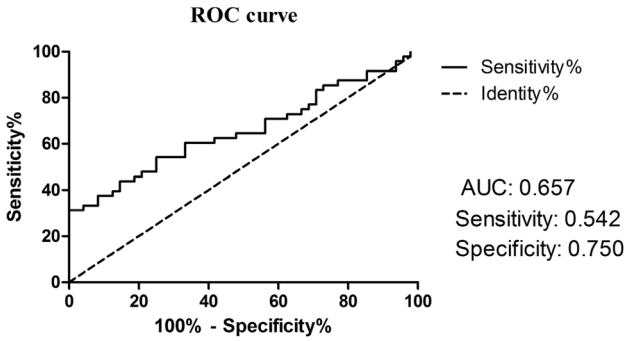

Fig. 2 Receiver operating characteristic(ROC) curve of the relative expression of *GRB2* in SCZ patients and controls

The Minor Allele C of rs7219 Generates the miR-1288 Binding Site and Inhibits Luciferase Activity

As shown in Fig. 3, the qualified transfection efficiency of GFP indicated that the reaction system of transfection satisfied the experimental requirements. In the present study, groups 1, 3, and 5 were used to calibrate the luciferase activity of groups 2, 4, and 6, respectively, by eliminating the interference of the miR-1288-NC vector. After constructing the luciferase reporter gene vector, we cotransferred the luciferase reporter plasmids and miRNA overexpression plasmids into HEK-293T cells and tested the relative luciferase activity after 48 h of culture. The result of the positive control group indicated that miR-146b significantly decreased the luciferase expression activity compared with miR-146b NC ($P < 0.001$), indicating that no problem exists in the transfection reaction system. Finally, the results showed that miR-1288 significantly decreased luciferase expression in the *GRB2* 3′-UTR-MU group compared to the *GRB2* 3′-UTR-NC ($P = 0.001$) or *GRB2* 3′-UTR-WT ($P < 0.001$) groups. However, no significant difference in the relative luciferase activity was found between *GRB2* 3′-UTR-NC and *GRB2* 3′-UTR-WT when co-transfected with miR-1288 ($P = 0.116$). Detailed information is shown in Fig. 4.

Discussion

We investigated the genetic association between *GRB2* variant rs7219 and the risk of SCZ and BD in the Chinese Han population. Our study is the first to identify rs7219 as a risk gene variant for SCZ in the Chinese Han population. However, we found no significant association between rs7219 and the risk of BD. Another major finding of our study is that the relative expression of *GRB2* was significantly decreased in SCZ patients compared with normal controls. In addition, the results of dual-luciferase reporter assay showed that the minor allele C of rs7219 significantly decreased luciferase activity by binding with miR-1288,

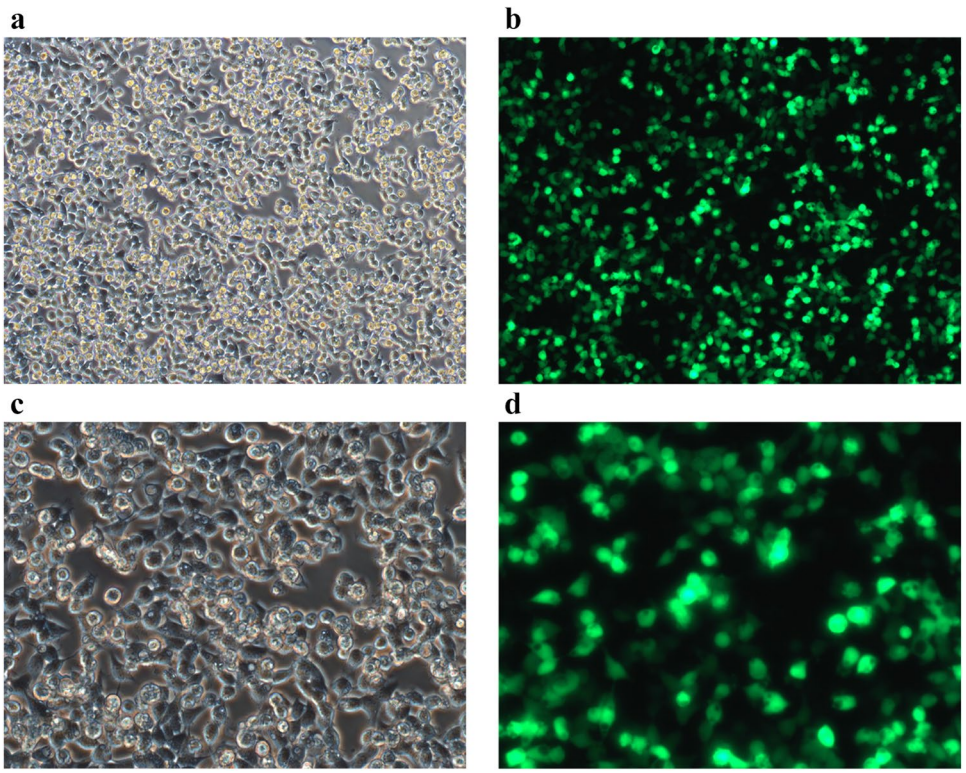

Fig. 3 The transfection efficiency of GFP in the same batch of co-transfection experiments. **a** GFP × 100 B, **b** GFP × 100 G, **c** GFP × 200 B, **d** × GFP G)

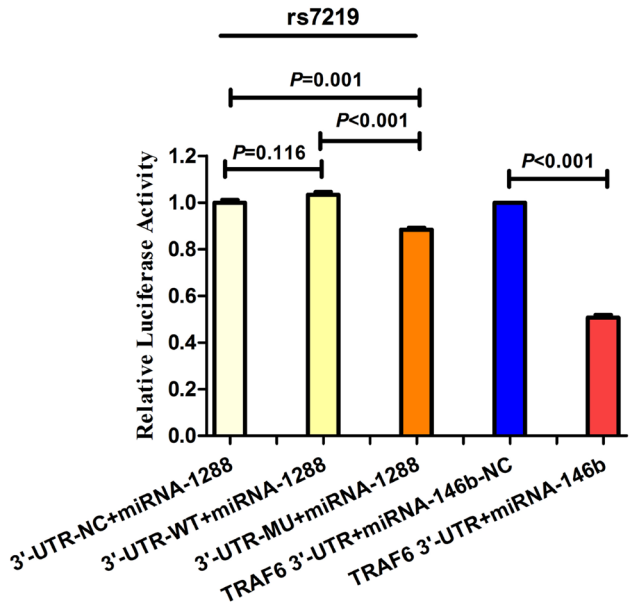

Fig. 4 Dual-luciferase reporter assay of miR-1288 co-transfected with construct of *GRB2* 3′-UTR and positive control groups in HEK-293T cells

whereas the major allele T of rs7219 can abolish the miR-1288-mediated inhibition.

As common psychiatric diseases, SCZ and BD have a profound social and economic impact. Previous studies have reported that the individual heritability of SCZ is extremely high, reaching approximately 80%, implying that genetic factors are core entities in the pathogenesis of SCZ (Cardno and Gottesman 2000; Burmeister et al. 2008). Moreover, Sun et al. (Sun et al. 2011) used a systems biology approach to identify *GRB2* as a susceptibility gene for SCZ. In the present study, the *GRB2* variant rs7219 was found to be significantly associated with the risk of SCZ in the Chinese Han population. Furthermore, the minor allele C of rs7219 is considered to be the risk allele for SCZ. Only one study has previously explored the association between rs7219 and the risk for SCZ; a negative association was detected in the Japanese population (Ikeda et al. 2008). Poor replication is a common problem for genetic association studies. For a specific disease, the risk gene polymorphisms identified in one population are not always replicated in another population. We speculate that two main reasons account for the inconsistency between our positive association and the previous negative association. On the one hand, the sample size of our study (including 548 SCZ patients and 598 healthy controls) is larger than that of the previous study in the Japanese population (which included 364 SCZ patients and 342 healthy controls). Thus, the present study had greater statistical power to uncover a weak genetic association between rs7219 and the risk for SCZ. On the other hand, increasing evidence shows that SCZ results from a complex interaction of genetic and environmental risk factors (Ghaemi 2006; Lichtenstein et al. 2009). In general, people living in diverse areas experience different living situations and customs, which also contribute to the inconsistency of results to a certain extent. In summary, in the present study, the *GRB2* variant rs7219 has been identified as a new susceptibility locus for SCZ in the Chinese Han population.

As a member of the GRB family adaptors, GRB2 plays an essential role in a variety of basic cellular functions (Gale et al. 1993; Egan et al. 1993). GRB2, which is firstly found in the nematode *C*. (Clark et al. 1992), is a multifunctional adaptor protein that transmits the signals of various neurotrophins, such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) (Mahata et al. 1999; Easton et al. 1999). Evidence indicates that NGF and BDNF are expressed in almost all areas of the adult brain (Pitts and Miller 2000; Anderson et al. 1995; Kumar et al. 2017). During neuronal development, NGF can regulate synaptic plasticity, the release of neurotransmitters, and cognitive function by directing the differentiation and formation of axons and dendrites (Isaev et al. 2017; Schinder and Poo 2000; Guo et al. 2012; Kumar et al. 2017). BDNF is another common neurotrophin and affects the activity of synapses by regulating synaptic connections, synapse structure, neurotransmitter release, and synaptic plasticity (Choo et al. 2017; Guo et al. 2018a; Song et al. 2017). These neurophysiological processes related to NGF and BDNF, including neural development, synaptic plasticity, and cognitive functions, have been shown to contribute to the pathogenesis of SCZ (Durany and Thome 2004; Negron-Oyarzo et al. 2016; Forsyth and Lewis 2017). Moreover, several studies have demonstrated that the expression of NGF or BDNF is significantly decreased in diverse brain regions and tissues of patients with SCZ, including cerebrospinal fluid, cortices, hippocampus, prefrontal cortex, serum, and plasma (Iritani et al. 2003; Kale et al. 2009; Pillai 2008; Weickert et al. 2003). The expression level of BDNF in blood is also considered to be a marker of various clinical characteristics in SCZ patients, including mental state, clinical symptoms, response to therapy, and outcome (Libman-Sokolowska et al. 2015; Kudlek Mikulic et al. 2017). In addition, treatment-related studies showed that the use of antipsychotics such as olanzapine and aripiprazole in patients with SCZ can increase NGF and BDNF levels (Martinotti et al. 2012; Nowakowska et al. 2014). This evidence suggests that the physiological functions of NGF and BDNF largely contribute to the pathogenesis of SCZ. Previous studies have indicated that GRB2 plays an indispensable role in NGF and BDNF signal transmission (Qian et al. 1998; Colledge and Froehner 1998; Easton et al. 1999), demonstrating that GRB2 has the potential to indirectly alter the pathological progression of SCZ by affecting the physiological functions of NGF and BDNF. In

the present study, the expression of *GRB2* was found to be significantly decreased in SCZ patients compared to controls. Therefore, the decreased expression of *GRB2* could affect the pathological processes of SCZ by interfering with the physiological functions of NGF and BDNF. In addition, our results suggest that circulating GRB2 could play a role in the diagnosis of SCZ (AUC = 0.657, Sensitivity = 0.542, and Specificity = 0.750). However, to the best of our knowledge, no other evidence has suggested that GRB2 has diagnostic value. Another SCZ-related gene, CTNNB1, has been suggested as a potential biomarker for the diagnosis of SCZ (AUC = 0.727, Sensitivity = 0.500, and Specificity = 0.958) (Guo et al. 2018b). Moreover, Noto et al. suggested that the combination of five cytokines (sTNF-R1, sTNF-R2, CCL11, IP-10, IL-4) could predict the diagnosis of SCZ (Sensitivity = 70%, Specificity = 89.4%) (Prata et al. 2017). Although many novel noncoding RNAs have been discovered and proposed as diagnostic biomarkers, few studies have focused on SCZ-associated genes that may function in diagnosis. Our future studies will consider the integration of SCZ-associated genes and explore their dependent and independent diagnostic value.

In the present study, we first observed that the *GRB2* variant rs7219 was significantly associated with the susceptibility to SCZ in the Chinese Han population, and the minor allele C of rs7219 was identified as the risk allele for SCZ. The rs7219 is a special gene variant that is located in the 3′-UTR of *GRB2*. Numerous gene variants located in this functional region have been reported to be involved in regulating the expression of the parental gene by influencing miRNA-mediated inhibition (Yu et al. 2015; Rossi et al. 2014; Shi et al. 2014). Using biological information analysis, we found that miR-1288 had the potential to bind the minor allele C of rs7219 and was expressed in the blood of SCZ patients. Previously, researchers demonstrated similar gene expression profiles in whole blood and brain tissue (Zhang et al. 2015; Sullivan et al. 2006). To explore whether rs7219 has a regulatory function involving miRNA, we used a dual-luciferase reporter assay system. The results showed that the minor allele C of rs7219 significantly decreased luciferase activity by binding with miR-1288. However, the major allele T of rs7219 abolished the miR-1288-mediated inhibition. These results imply that the *GRB2* transcript carrying the minor allele C of rs7219 generates a binding site for endogenous miR-1288, attenuating the transcript and leading to decreased expression of *GRB2*. However, the transcript of *GRB2* carrying the major allele T of rs7219 maintains the expression level of *GRB2* in vivo by preventing the binding inhibition of the endogenous miR-1288. The rs7219-mediated regulation of *GRB2* expression is consistent with the previous observation that the minor allele C of rs7219 is a risk allele for SCZ. In other words, the minor allele C of rs7219 decreases the expression of *GRB2* by generating a binding site for miR-1288 and ultimately increasing the risk for SCZ in the Chinese Han population. A similar regulatory mechanism whereby the minor allele generates the binding site for miRNA has also been reported in other studies (Wang et al. 2016; Hou et al. 2018).

Several limitations in this study should be noted. First, the sample size in the present study is not particularly large. Thus, a firm conclusion requires further verification. Second, we examined only rs7219-mediated regulation of *GRB2* expression, which includes possible combined functional effects from other variants located in *GRB2*. Finally, we enrolled only the Chinese Han population; thus, the conclusion should be extended to other populations with caution.

In summary, this is the first study to reveal that GRB2 variant rs7219 is strongly associated with susceptibility to SCZ in the Chinese Han population. Abnormal expression of *GRB2* could influence the pathogenesis of SCZ. In addition, the minor allele C of rs7219 is identified as a risk allele for SCZ because it generates a binding site for miR-1288, which results in decreased expression of *GRB2* and ultimately increases the risk of SCZ.

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Author Contribution Li Su designed the study protocol and conceived the framework of this article. Jialei Yang, Jiao Huang, Qiang Chen, and Runde Pan collected the study samples. Jialei Yang, Xiaojing Guo, Lulu Zhu, Jiao Huang, Zhaoxia Chen, and Xulong Wu performed experiments. Jiao Huang, Jianxiong Long, Zhaoxia Chen, and Xulong Wu undertook the statistical analysis. Jialei Yang, Xiaojing Guo, and Lulu Zhu wrote the first draft of this manuscript. All authors contributed to and have approved the final manuscript.

Compliance with Ethical Standards

Conflict of interest All of authors declare that they have no financial or other conflict of interests.

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