ISSN 2220-3206 (online)

World Journal of *Psychiatry*

World J Psychiatry 2023 July 19; 13(7): 402-494

Published by Baishideng Publishing Group Inc

World Journal of Psychiatry

Contents

Monthly Volume 13 Number 7 July 19, 2023

OPINION REVIEW

MINIREVIEWS

ORIGINAL ARTICLE

Case Control Study

Retrospective Study

Observational Study

World Journal of Psychiatry

Monthly Volume 13 Number 7 July 19, 2023

Contents

NAME OF JOURNAL
World Journal of Psychiatry

ISSN
ISSN 2220-3206 (online)

LAUNCH DATE
December 31, 2011

FREQUENCY
Monthly

EDITORS-IN-CHIEF
Rajesh R Tampi, Ting-Shao Zhu, Panteleimon Giannakopoulos

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/2220-3206/editorialboard.htm

PUBLICATION DATE
July 19, 2023

INSTRUCTIONS TO AUTHORS
https://www.wjgnet.com/bpg/gerinfo/204

GUIDELINES FOR ETHICS DOCUMENTS
https://www.wjgnet.com/bpg/GerInfo/287

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH
https://www.wjgnet.com/bpg/gerinfo/240

PUBLICATION ETHICS
https://www.wjgnet.com/bpg/GerInfo/288

PUBLICATION MISCONDUCT
https://www.wjgnet.com/bpg/gerinfo/208

ARTICLE PROCESSING CHARGE
https://www.wjgnet.com/bpg/gerinfo/242

STEPS FOR SUBMITTING MANUSCRIPTS
https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION
https://www.f6publishing.com

E-mail: bpgoffice@wjgnet.com https://www.wjgnet.com

World Journal of Psychiatry

Submit a Manuscript: https://www.f6publishing.com

World J Psychiatry 2023 July 19; 13(7): 435-443

DOI: 10.5498/wjp.v13.i7.435

ISSN 2220-3206 (online)

ORIGINAL ARTICLE

Case Control Study

Glutamate decarboxylase 1 gene polymorphisms are associated with respiratory symptoms in panic disorder

Zhi-Li Zou, Jian Qiu, Xiao-Bo Zhou, Yu-Lan Huang, Jin-Yu Wang, Bo Zhou, Yuan Zhang

Specialty type: Psychiatry

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Hosak L, Czech Republic; Sobanski T, Germany

Received: April 12, 2023
Peer-review started: April 12, 2023
First decision: May 12, 2023
Revised: May 18, 2023
Accepted: May 31, 2023
Article in press: May 31, 2023
Published online: July 19, 2023

Zhi-Li Zou, Jian Qiu, Xiao-Bo Zhou, Yu-Lan Huang, Jin-Yu Wang, Bo Zhou, Sichuan Provincial Center for Mental Health, Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital, Chengdu 610000, Sichuan Province, China

Yuan Zhang, Personalized Drug Therapy Key Laboratory of Sichuan Province, Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital, Chengdu 610072, Sichuan Province, China

Corresponding author: Yuan Zhang, MS, Assistant Professor, Personalized Drug Therapy Key Laboratory of Sichuan Province, Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital, No. 32 West Second Section First Ring Road, Chengdu 610072, Sichuan Province, China. 447415054@qq.com

Abstract

BACKGROUND

Genetic factors play an important role in the pathogenesis of panic disorder (PD). However, the effect of genetic variants on PD remains controversial.

AIM

To evaluate the associations between *glutamate decarboxylase 1 (GAD1)* gene polymorphisms and PD risk and assess the effect of *GAD1* gene polymorphisms on the severity of clinical symptoms in PD.

METHODS

We recruited 230 PD patients and 224 healthy controls in this study. All participants were assessed for anxiety and panic symptom severity using the Hamilton Anxiety Rating Scale (HAM-A) and Panic Disorder Severity Scale (PDSS). *GAD1* gene polymorphisms (rs1978340 and rs3749034) were genotyped and assessed for allele frequencies.

RESULTS

There were no significant differences between cases and controls in the genotype distributions or allele frequencies of *GAD1* (rs1978340 and rs3749034). In addition, the effect of *GAD1* (rs1978340 and rs3749034) on PD severity was not significant. However, regarding respiratory symptoms, patients with the *GAD1* rs1978340 A/A genotype had significantly higher scores than those with the A/G or G/G genotype.

CONCLUSION
Here, we showed that the A/A genotype of *GAD1* rs1978340 was associated with increased severity of respiratory symptoms in patients with PD.

Key Words: Panic disorder; Gene polymorphisms; Respiratory symptoms; Allele frequencies; Pathogenesis; Chinese population

Core Tip: The study found that the A/A genotype of *glutamate decarboxylase 1 (GAD1)* rs1978340 was associated with increased severity of respiratory symptoms in patients with panic disorder (PD). However, there were no significant differences between cases and controls in the genotype distributions or allele frequencies of *GAD1* (rs1978340 and rs3749034), and neither did *GAD1* (rs1978340 and rs3749034) have a significant effect on the severity of PD symptoms. These findings suggest that genetic factors may play a role in the pathogenesis of PD, particularly in respiratory symptoms, but further studies with larger sample sizes are needed to confirm these results.

Citation: Zou ZL, Qiu J, Zhou XB, Huang YL, Wang JY, Zhou B, Zhang Y. *Glutamate decarboxylase 1* gene polymorphisms are associated with respiratory symptoms in panic disorder. *World J Psychiatry* 2023; 13(7): 435-443
URL: https://www.wjgnet.com/2220-3206/full/v13/i7/435.htm
DOI: https://dx.doi.org/10.5498/wjp.v13.i7.435

INTRODUCTION

Panic disorder (PD), the most common anxiety disorder, is characterized by recurrent and unexpected panic attacks and has an estimated 12-mo and lifetime prevalence rates of 2.4% and 3.8%, respectively[1,2]; the lifetime prevalence rate of panic attacks is 13.2%[3]. PD typically occurs in young adults, and women are more likely to be affected than men. However, the etiology of PD is multifactorial and complex, involving genetic, environmental, psychological and neurobiological factors[2,3]. Recent studies examining twins and family shows that the heritability of panic disorder is 30%-40%, suggesting strong evidence for a genetic etiology[4]. To date, genetic studies have reported several susceptibility genes for PD such as *neuropeptide Y*, *catechol-O-methyltransferase* and particularly 5-HT system-related genes[5,6]. For example, a previous study found that patients with PD were characterized by significantly higher frequencies of the LL genotype and L allele variant of the 5-HT transporter-linked promoter region (5-HTTLPR)[7]. However, few of these findings have been replicated by other researchers, and the pathogenesis of PD remains unclear[8-12]. Therefore, other candidate gene polymorphisms in PD should be explored.

γ-Aminobutyric acid (GABA) is an important inhibitory neurotransmitter in the mammalian brain, and abnormalities in the GABAergic system have long been implicated in the pathophysiology of PD[13-15]. For example, a significant decrease in *GABA* has been detected in the anterior cingulate and medial prefrontal cortices of patients with PD[16]. The *GAD1* gene encodes the 67-kDa glutamic acid decarboxylase isoform (GAD67) and is the rate-limiting enzyme responsible for GABA biosynthesis from glutamic acid. The *GAD1* gene might play an important role in the GABAergic system. A previous study found a significant effect of rs1978340 on cingulate cortex GABA concentrations[17]. In addition, previous studies have indicated that *GAD1* rs3749034 is associated with mRNA expression[18]. Therefore, *GAD1* may be an important candidate gene in PD. Incidentally, previous reports have suggested that the *GAD1* single nucleotide polymorphisms (SNPs) rs3749034 or rs1978340 are significantly related to several psychiatric disorders such as bipolar disorder[19], schizophrenia[20], attention-deficit/hyperactivity disorder[21], and heroin dependence[22]. For instance, the allelic or genotypic frequencies of the rs1978340 polymorphism in heroin addicts significantly differ from those in normal controls[23]. However, few studies have examined the relationship between *GAD1* and PD, particularly in Chinese populations.

Previous genetic and chromosomal studies have yielded inconsistent results. It is likely that most cases of PD have a complex genetic basis. In addition, current data suggest that the genetic architecture underlying PD is heterogeneous and differs among cases[24]. PD is accompanied by various symptoms, including palpitations, accelerated heart rate, dyspnea, sweating, and chest pain. These symptoms may be linked to distinct genetic mechanisms, and genetic polymorphisms have been speculated to be linked to the discrete symptoms of PD. Hence, to test the hypothesis that the GAD1 polymorphism could be associated with PD, we have conducted a case-control study comparing the frequency of these SNPs (rs1978340 and rs3749034) in PD patients and healthy controls. Additionally, we examined the relationship between the presence of PD symptoms and these polymorphisms.

MATERIALS AND METHODS

Participants

A total of 230 patients with PD were recruited as in- and outpatients at the Department of Psychosomatics, Sichuan Provincial People's Hospital, from July 2012 to January 2016. Patients were qualified based on the following criteria: A primary diagnosis of PD performed by professional psychiatrists according to the standardized structured clinical interview of the diagnostic and statistical manual of mental disorders, fourth edition axis I disorders (SCID-I)[25], and no episodes of other psychiatric disorders in the past or at present. Additionally, 224 healthy controls (HCs) among community volunteers were recruited for the study during the same period. SCID-I was also performed by a trained clinical psychiatrist, and the HCs had no history of any psychiatric disorder or major psychiatric condition in their first-degree relatives. All participants in this study were Han Chinese, aged 18–60 years. None of the patients had acute or chronic somatic disorders, head trauma, or neurological illnesses. The study was approved by the Ethics Committee of the Sichuan Provincial People's Hospital [reference number: (2016) Ethics Review (29)]. All participants provided written informed consent before the initiation of study procedures.

Measures

PDSS: The 7-item PDSS was used to assess the severity of panic symptoms for all patients, and participants were instructed to rate each item from 0 to 4 based on the severity of each symptom, with possible responses ranging from "none" to "extremely severe"[26]. The scale was translated into Chinese by Xiong[27], and the PDSS-Chinese version had good internal consistency (Cronbach's alpha) with an overall score of (0.83).

Hamilton anxiety rating scale

The Hamilton anxiety rating scale (HAM-A) scale comprises 14 items (anxious mood, tension, fear, insomnia, cognitive function, depressed mood, somatic anxiety (muscular system), somatic anxiety (sensory system), cardiovascular symptoms, respiratory symptoms, gastrointestinal symptoms, genitourinary symptoms, autonomic symptoms, and behavior at interview) with 5-level responses for each item, *i.e.*, scores 0, 1, 2, 3, and 4 indicating not present, mild, moderate, severe, and very severe, respectively[28]. A total score > 17 indicates mild anxiety symptoms; 18-24, mild-to-moderate anxiety symptoms; and 25-30, moderate-to-severe anxiety symptoms.

DNA extraction and SNP genotyping

For each participant, 3 mL of peripheral blood was collected in EDTA tubes. An automatic nucleic acid extractor (TGuide M16; Tiangen Biotech, Beijing, China) was used to extract genomic DNA. SNP genotyping was performed using an improved multiplex ligation detection reaction (iMLDR) technique developed by Genesky Bio-Tech (Shanghai, China). The multiplex polymerase chain reaction (PCR) reaction volume included 1 μL GC-I buffer (Takara Bio Inc., Shiga, Japan), 3.0 mmol/L Mg^{2+}, 0.3 mmol/L dNTP, 1 U HotStar Taq Polymerase (Qiagen, Hilden, Germany), 1 μL genomic DNA (5–10 ng/μL), and 1 μL Multiplex-PCR primer mix. The cycling program for PCR was 95 °C for 120 s, followed by 11 cycles of 94 °C for 20 s, 65 °C for 40 s, and 72 °C for 90 s, and each cycle decreased by 0.5 °C. The third step comprised 24 cycles at 94 °C for 20 s, 59 °C for 30 s, and finally, 72 °C for 2 min, and a hold at 4 °C. The PCR product was purified with 5 U SAP and 2 U Exonuclease I at 37 °C for 1 h and then inactivated at 75 °C for 15 min. The primer and probe information is provided in Supplementary Tables 1 and 2, respectively. The ligation reaction included 1 μL of 10× ligation buffer, 0.4 μL 3′ ligation primer (2 μM), 0.25 μL Tag DNA ligase, 6 μL ddH_2O mixture, 0.4 μL 5′ ligation primer (1 μM), and 2μL purified multiplex PCR product. The ligation cycling program comprised 38 cycles at 94 °C for 60 s, 56 °C for 4 min, and a hold at 4 °C. Sequencing was conducted in 0.5 μL 500 LIZ Size Standard, 0.5 μL ligation product, and 9 μL Hi-Di mixture (ABI3730XL; Applied Biosystems, Waltham, MA, United States). Raw data were analyzed using GeneMapper v4.1 software (Applied Biosystems). A random sample accounting for approximately 5% of the total DNA samples was directly sequenced using Big Dye-terminator version 3.1 and an ABI3730XL automated sequencer (Applied Biosystems) to confirm the iMLDR results.

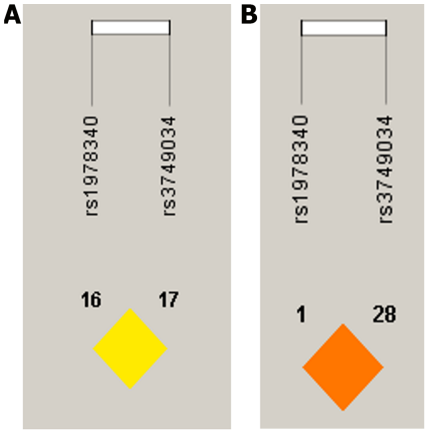
Statistical analysis

SPSS version 13.0 software (SPSS Inc., Chicago, IL, United States) was used to analyze the data. Student's *t*-test was used for intergroup comparisons of continuous variables, and Pearson's chi-square test was used for categorical variables. The Hardy-Weinberg equilibrium (HWE) *P* values were tested using Pearson's chi-square test. Associations between SNPs and PD were determined based on the distribution of allelic frequencies and genetic models (additive, dominant, and recessive models). Odds ratios and 95% confidence intervals were calculated by unconditional logistic regression analysis using PLINK v1.07. Analysis of variance was performed to compare the clinical variables with different *GAD1* SNPs (rs1978340 and rs3749034). Bonferroni's correction was used to avoid Type I errors. For all analyses, statistical tests were two-tailed, and an alpha level of 0.05 was used to define statistical significance.

RESULTS

Demographic data and clinical manifestations

The analyzed sample comprised 230 PD cases (92 men and 138 women; mean age, 35.38 ± 9.55 years) and 224 controls (100 men and 124 women; mean age, 36.57 ± 8.43 years). Of these patients, 54% (n = 124) resided in urban locations, and



DOI: 10.5498/wjp.v13.i7.435

Figure 1 Linkage disequilibrium in *glutamate decarboxylase 1* polymorphisms (rs1978340 and rs3749034). A: Data from this study; B: Data from 1000 genomes.

46% (n = 106) resided in rural locations. No statistically significant differences were found between the cases and controls in terms of sex, age, or residential location (P > 0.05). For the PD patients, the mean course of PD was 2.80 ± 1.68 years, the mean PDSS score was 14.13 ± 3.74, and the mean HAM-A score was 22.07 ± 6.86 (Table 1).

Association of GAD1 (rs1978340 and rs3749034) polymorphisms with PD risk

HWE was measured in all genotyped individuals. *GAD1* (rs1978340 and rs3749034) polymorphisms fulfilled the HWE (P > 0.05) in both patients and HCs. The linkage disequilibrium evaluated in patients with PD and HCs for variants rs1978340 and rs3749034 of *GAD1* is shown in Figure 1 (R^2 > 0.9). The genotype and allele distributions of *GAD1* (rs1978340 and rs3749034) did not significantly differ between PD patients and HCs (P > 0.05) (Table 2).

Association of GAD1 (rs1978340 and rs3749034) polymorphisms with clinical manifestations in PD patients

There were no statistically significant differences in the total PDSS and sub-item scores among the three genotype groups of *GAD1* polymorphisms (rs1978340 and rs3749034; all P > 0.05) (Table 3).

However, there was a significant difference among the three groups with different *GAD1* rs1978340 genotypes in item 10 of the HAM-A score for PD (P < 0.01). In addition, post hoc analyses indicated that patients with the *GAD1* rs1978340 A/A genotype had significantly higher scores than those with the A/G or G/G genotypes (all P < 0.001), and the results remained significant after Bonferroni's multiple comparison adjustment (P < 0.01), reflecting a higher score for respiratory symptoms in patients with the *GAD1* rs1978340 A/A genotype than in those with the A/G or G/G genotype. However, there was no statistically significant difference among the three groups with different *GAD1* rs1978340 genotypes for the remaining items or HAM-A total scores (P > 0.05). Moreover, there was no significant association between *GAD1* rs3749034 and anxiety severity in PD patients (all P > 0.05) (Table 4).

DISCUSSION

In this study, regarding respiratory symptoms, which include chest tightness, choking, and breathing difficulty, we found that patients with the *GAD1* rs1978340 A/A genotype had significantly higher scores than those with the A/G or G/G genotypes. In other words, the present study showed that the *GAD1* rs1978340 A/A genotype was associated with increased severity of respiratory symptoms in patients with PD and demonstrated that the *GAD1* genotype might be related to symptomatic profiles rather than vulnerability to developing PD. In addition, our findings imply that different clinical features in PD patients are closely related to the heterogeneity of heredity. Compared with PD patients of the non-respiratory subtype (non-RS), previous studies have shown that patients with the RS subtype have a more extensive family history of PD[29]. Moreover, experimental animal research has provided evidence of the important role of GABAergic neurotransmission in the amygdala in modulating anxiety-related behaviors. For example, diminished GAD67 expression in the amygdala blunts the anxiolytic-like effects of diazepam in adult mice[30]. Furthermore, *GAD1* SNP rs1978340 is potentially functional because it affects GABA concentrations in the cingulate cortex[22]. In addition, the presence of *GAD1* rs1978340 allele A has been associated with a higher Glu/GABA ratio[31]. Clinical trials have shown that patients with PD and RS have a more rapid response to antidepressants and benzodiazepines than that of non-RS PD patients[32]. These findings contribute to our understanding of the mechanism linking *GAD1* rs1978340 with respiratory-related symptoms.

Similar findings suggest that patients with PD carrying the 5-HTTLPR s-allele experience the most severe panic and depressive symptoms[33]. Another study showed higher anxiety levels among A/G carriers than those among A/A carriers in patients[34]. It is evident that molecular genetics showed inconsistent results across different studies. This may be due to different sample sizes and ethnic differences. In addition, different clinical symptoms may be partly attributed

Table 1 Demographic and clinical characteristics in patients with panic disorder and controls, *n* (%)

Variable	PD (*n* = 230)	Controls (*n* = 224)	t/χ^2	*P* value
Sex				
Male	92 (40.0)	100 (44.6)	1.002	0.317
Female	138 (60.0)	124 (55.4)		
Age, yr	35.38 ± 9.55	36.57 ± 8.43	1.410	0.159
Educational level				
< Junior high school	49 (21.3)	43 (19.2)		
High school	95 (41.3)	92 (41.1)	0.412	0.814
College and above	86 (37.4)	89 (39.7)		
Resident location				
Urban	124 (53.9)	126 (56.3)		
Rural	106 (46.1)	98 (43.7)	0.250	0.617
Total duration of PD, yr	2.80 ± 1.68			
PDSS score	14.13 ± 3.74			
HAMA score	22.07 ± 6.86			

PDSS: Panic Disorder Severity Scale; HAMA: Hamilton Anxiety Rating Scale; PD: Panic disorder.

Table 2 *Glutamate decarboxylase 1* gene polymorphisms of patients with panic disorder *vs* controls in the Chinese population, *n* (%)

SNP	Alleles and genotypes	PD (*n* = 230)	Controls (*n* = 224)	Model	OR (95%CI)	*P* value
rs1978340	A	122 (26.5)	116 (25.9)	Allele[a]	1.003 (0.769-1.389)	0.829
	G	338 (73.5)	332 (74.1)			
	A/A	17 (7.4)	20 (8.9)	Additive[b]	1.031 (0.774-1.373)	0.835
	A/G	88 (38.3)	76 (34.8)	Dominant[b]	1.120 (0.773-1.622)	0.549
	G/G	125 (54.3)	128 (57.1)	Recessive[b]	0.814 (0.415-1.598)	0.550
rs3749034	A	131 (28.5)	129 (28.8)	Allele	0.985 (0.738-1.313)	0.916
	G	329 (71.5)	319 (71.2)			
	A/A	17 (7.4)	15 (6.7)	Additive	0.984 (0.732-1.323)	0.914
	A/G	97 (42.2)	99 (44.2)	Dominant	0.948 (0.656-1.370)	0.777
	G/G	116 (50.4)	110 (49.1)	Recessive	1.112 (0.541-2.286)	0.773

[a]Chi-square test.

[b]Logistic regression analyses.

OR: Odds ratios; CI: Confidence interval; SNP: Single Nucleotide Polymorphism, "A" represent wild type and "a" represent mutant type: allele, a *vs* A; additive, aa *vs* Aa *vs* AA; dominant, aa + Aa *vs* AA, recessive, aa *vs* Aa +AA; PD: Panic disorder.

to different genetic backgrounds, leading to difficulties in reaching a consensus on the etiology of PD. Further studies with larger populations are needed to obtain precise results based on different symptom subtypes.

In this case-control study, we examined two SNPs (rs1978340 and rs3749034) in a Chinese population. The results revealed that there was no association between *GAD1* and PD. In addition, we did not observe a modulatory effect of *GAD1* (rs1978340 and rs3749034) on PD severity. Much evidence has indicated that *GAD1* gene polymorphisms may be involved in the etiology of several psychiatric disorders. However, only one study has found that *GAD1* variation is associated with PD in females[35]. The different results of these studies might be partly attributable to differences in sample size and sex. Moreover, samples from different ethnicities and meta-analyses are required to further test this association. The SNP coverage in the present study was limited, and other gene polymorphisms should be considered. In addition, the pathogenesis of PD may involve the interaction of multiple genes and signal pathway regulation, which may incorporate the combined effects of genetic and environmental factors. For example, a previous study suggested the effect of the interaction between 5-HTTLPR and separate life events on PD[36]. Finally, epigenetic mechanisms have been

Table 3 Panic disorder severity scale subdimension scores of panic disorder with different *glutamate decarboxylase 1* rs1978340 and rs3749034 polymorphisms

Variable	rs1978340			*F*	*P* value	rs3749034			*F*	*P* value
	A/A (*n* = 17)	A/G (*n* = 88)	G/G (*n* = 125)			A/A (*n* = 17)	A/G (*n* = 97)	G/G (*n* = 116)		
PDSS1	2.12 ± 0.93	2.11 ± 1.09	2.14 ± 1.08	0.022	0.978	1.94 ± 0.97	2.06 ± 1.04	2.22 ± 1.10	0.837	0.434
PDSS2	2.41 ± 1.00	2.32 ± 1.00	2.44 ± 1.10	0.349	0.706	2.35 ± 1.41	2.28 ± 1.07	2.49 ± 0.97	1.099	0.335
PDSS3	2.41 ± 1.18	2.08 ± 0.89	2.14 ± 1.05	0.786	0.457	2.18 ± 1.13	1.99 ± 1.00	2.26 ± 0.98	1.934	0.147
PDSS4	1.59 ± 1.28	1.57 ± 1.04	1.66 ± 1.06	0.218	0.804	1.41 ± 1.12	1.76 ± 1.12	1.53 ± 1.00	1.590	0.206
PDSS5	1.71 ± 1.26	1.91 ± 1.01	1.76 ± 1.10	0.581	0.560	2.12 ± 1.22	1.67 ± 0.93	1.89 ± 1.63	1.818	0.165
PDSS6	2.33 ± 1.22	2.15 ± 0.97	2.11 ± 1.03	0.417	0.660	2.18 ± 1.33	2.09 ± 1.03	2.18 ± 0.97	0.206	0.814
PDSS7	1.59 ± 0.94	1.83 ± 1.01	1.97 ± 1.18	1.087	0.339	2.00 ± 1.06	1.85 ± 1.05	1.91 ± 1.15	0.174	0.841
PDSS total	14.18 ± 4.68	13.97 ± 3.39	14.23 ± 3.86	0.132	0.877	14.18 ± 4.99	13.70 ± 3.54	14.47 ± 3.69	1.134	0.323

PDSS1: Panic attack frequency, PDSS2: Panic distress, PDSS3: Severity of anticipatory anxiety, PDSS4: Agoraphobic fear/avoidance, PDSS5: Fear/avoidance of panic-related sensations, PDSS6: Work impairment, PDSS7: Social impairment, PDSS: Panic Disorder Severity Scale.

Table 4 Hamilton Anxiety Rating Scale subdimension scores of panic disorder with different *glutamate decarboxylase 1* rs1978340 and rs3749034 polymorphisms

Variable	rs1978340			*F*	*P* value	rs3749034			*F*	*P* value
	A/A (*n* = 17)	A/G (*n* = 88)	G/G (*n* = 125)			A/A (*n* = 17)	A/G (*n* = 97)	G/G (*n* = 116)		
HAMA1	1.59 ± 1.06	1.88 ± 1.16	1.81 ± 1.18	0.439	0.645	1.82 ± 1.33	1.81 ± 1.14	1.82 ± 1.17	0.001	0.999
HAMA2	2.00 ± 1.00	2.06 ± 0.94	2.17 ± 1.11	0.402	0.669	2.24 ± 1.30	2.07 ± 1.01	2.13 ± 1.03	0.206	0.814
HAMA3	1.94 ± 1.09	1.66 ± 1.07	1.98 ± 1.08	2.295	0.103	2.24 ± 1.25	1.78 ± 1.01	1.85 ± 1.11	1.260	0.286
HAMA4	1.71 ± 1.11	1.43 ± 1.11	1.58 ± 1.02	0.728	0.484	1.47 ± 0.87	1.48 ± 1.02	1.58 ± 1.12	0.231	0.794
HAMA5	1.59 ± 1.06	1.58 ± 1.04	1.65 ± 1.03	0.121	0.886	1.65 ± 1.17	1.55 ± 1.06	1.67 ± 0.99	0.402	0.670
HAMA6	1.53 ± 1.01	1.25 ± 1.05	1.54 ± 0.99	2.255	0.107	1.65 ± 1.32	1.38 ± 1.07	1.44 ± 0.94	0.498	0.747
HAMA7	1.76 ± 1.03	1.34 ± 0.99	1.62 ± 1.01	2.459	0.088	1.59 ± 1.06	1.46 ± 1.01	1.56 ± 1.02	0.277	0.759
HAMA8	1.06 ± 0.83	1.38 ± 1.10	1.37 ± 1.12	0.644	0.526	1.41 ± 0.94	1.34 ± 1.10	1.34 ± 1.11	0.032	0.969
HAMA9	2.06 ± 1.20	1.91 ± 0.99	1.94 ± 1.03	0.154	0.858	1.82 ± 1.13	2.08 ± 1.07	1.84 ± 0.97	1.647	0.195
HAMA10	2.88 ± 0.93	1.94 ± 0.98	1.90 ± 1.02	7.445	0.001	2.41 ± 1.00	1.94 ± 0.96	1.97 ± 1.08	1.601	0.204
HAMA11	0.76 ± 0.66	0.86 ± 0.79	0.97 ± 0.91	0.672	0.512	1.00 ± 0.94	0.89 ± 0.87	0.92 ± 0.82	0.143	0.867
HAMA12	1.00 ± 1.00	1.20 ± 0.96	1.34 ± 0.92	1.214	0.299	1.41 ± 1.00	1.22 ± 0.93	1.28 ± 0.95	0.340	0.712
HAMA13	1.12 ± 0.93	1.35 ± 0.87	1.30 ± 1.03	0.427	0.653	1.29 ± 1.11	1.25 ± 0.99	1.36 ± 0.92	0.377	0.687
HAMA14	1.59 ± 1.06	1.48 ± 0.98	1.35 ± 0.98	0.693	0.501	1.53 ± 1.28	1.44 ± 0.99	1.38 ± 0.94	0.229	0.796
HAMA total	22.59 ± 5.22	21.34 ± 6.94	22.50 ± 6.97	0.799	0.451	23.53 ± 7.13	21.70 ± 6.93	22.16 ± 6.75	0.535	0.587

HAMA1: Anxious mood; HAMA2: Tension; HAMA3: Fears; HAMA4: Insomnia; HAMA5: Intellectual; HAMA6: Depressed mood; HAMA7: Somatic complaints muscular; HAMA8: Somatic complaints sensory; HAMA9: Cardiovascular symptoms; HAMA10: Respiratory symptoms; HAMA11: Gastrointestinal symptoms; HAMA12: Genitourinary symptoms; HAMA13: Autonomic symptoms; HAMA14: Behavior at interview; HAMA: Hamilton Anxiety Rating Scale.

suggested to play important roles at the intersection of genetic and environmental factors[37]. Environmental factors may influence biological processes through epigenetic mechanisms, particularly DNA methylation[38]. For instance, patients with PD exhibit significantly lower average *GAD1* methylation levels than those of HCs[39]. Another study showed that patients had significantly lower methylation of the *GAD1* promoter region on cytosine-phosphate-guanine 7 than that of HCs, and a significant negative association was found between the cg171674146 site and clinical severity[40]. Therefore,

epigenetic modifications may play an important role and should be further investigated in future studies.

CONCLUSION

In conclusion, the present study showed that the A/A genotype of GAD1 rs1978340 is associated with increased severity of respiratory symptoms in patients with PD. However, the results of our study should be considered in light of the following limitations: Since this was a small sample investigating the associations between *GAD1* gene polymorphisms and PD, it would be valuable to replicate our findings in a larger cohort. In addition, SNP coverage in the present study was limited, and other gene polymorphisms should be considered.

ARTICLE HIGHLIGHTS

Research background

Genetic factors are known to play a significant role in the development of panic disorder (PD). However, the impact of genetic variants on PD is still a subject of controversy.

Research motivation

γ-Aminobutyric acid (GABA) is an important neurotransmitter that inhibits brain activity. Previous reports have linked the *glutamate decarboxylase 1 (GAD1)* genetic variants to various psychiatric disorders, including bipolar disorder, schizophrenia, attention-deficit/hyperactivity disorder, and heroin dependence. However, few studies have examined the relationship between *GAD1* and PD, particularly in Chinese populations.

Research objectives

The main objectives of this study were to examine the associations between *GAD1* gene polymorphisms (rs1978340 and rs3749034) and PD risk, and to determine the effect of these polymorphisms on the severity of clinical symptoms, specifically respiratory symptoms, in individuals with PD.

Research methods

The study included a total of 230 PD patients and 224 healthy controls. All participants underwent assessments for anxiety and panic symptom severity using the Hamilton Anxiety Rating Scale (HAM-A) and Panic Disorder Severity Scale (PDSS). The GAD1 gene polymorphisms (rs1978340 and rs3749034) were genotyped, and allele frequencies were analyzed.

Research results

The study findings revealed no significant differences in the genotype distributions or allele frequencies of *GAD1* (rs1978340 and rs3749034) between the PD cases and the control group. Furthermore, the *GAD1* gene polymorphisms (rs1978340 and rs3749034) did not exhibit a significant effect on the overall severity of PD. However, in relation to respiratory symptoms, PD patients with the *GAD*1 rs1978340 A/A genotype demonstrated significantly higher scores compared to those with the A/G or G/G genotype.

Research conclusions

In conclusion, this study demonstrated that the A/A genotype of *GAD1* rs1978340 is associated with increased severity of respiratory symptoms in individuals with PD. However, no significant associations were found between *GAD1* gene polymorphisms and the risk of developing PD or the overall severity of the disorder.

Research perspectives

Further research is needed to explore other potential genetic factors involved in the development and severity of PD. Additionally, investigating the underlying mechanisms through which *GAD1* gene polymorphisms affect respiratory symptoms in PD patients could provide valuable insights for future studies.

FOOTNOTES

Author contributions: Zou ZL contributed to study design, manuscript preparation; Qiu J contributed to experiment performance and data collection; Zhou XB, Huang YL, Wang JY contributed to data collection, analysis and inspection; Zhou B contributed to fund acquisition; Zhang Y contributed to manuscript preparation, inspection and revision.

Institutional review board statement: The study was approved by the Ethics Committee of the Sichuan Provincial People's Hospital [reference number: (2016) Ethics Review (29)]. All participants provided written informed consent before the initiation of study procedures.

Conflict-of-interest statement: The authors report no conflict of interest.

Data sharing statement: Data is available on request due to privacy/ethical restrictions.

STROBE statement: The authors have read the STROBE Statement–checklist of items, and the manuscript was prepared and revised according to the STROBE Statement – checklist of items.

Country/Territory of origin: China

ORCID number: Xiao-Bo Zhou 0000-0003-0139-8893; Yuan Zhang 0000-0001-8840-7531.

S-Editor: Ma YJ
L-Editor: A
P-Editor: Cai YX

REFERENCES

1 **Kessler RC**, Chiu WT, Jin R, Ruscio AM, Shear K, Walters EE. The epidemiology of panic attacks, panic disorder, and agoraphobia in the National Comorbidity Survey Replication. *Arch Gen Psychiatry* 2006; **63**: 415-424 [PMID: 16585471 DOI: 10.1001/archpsyc.63.4.415]
2 **Kessler RC**, Petukhova M, Sampson NA, Zaslavsky AM, Wittchen H -U. Twelve-month and lifetime prevalence and lifetime morbid risk of anxiety and mood disorders in the United States. *Int J Methods Psychiatr Res* 2012; **21**: 169-184 [PMID: 22865617 DOI: 10.1002/mpr.1359]
3 **de Jonge P**, Roest AM, Lim CC, Florescu SE, Bromet EJ, Stein DJ, Harris M, Nakov V, Caldas-de-Almeida JM, Levinson D, Al-Hamzawi AO, Haro JM, Viana MC, Borges G, O'Neill S, de Girolamo G, Demyttenaere K, Gureje O, Iwata N, Lee S, Hu C, Karam A, Moskalewicz J, Kovess-Masfety V, Navarro-Mateu F, Browne MO, Piazza M, Posada-Villa J, Torres Y, Ten Have ML, Kessler RC, Scott KM. Cross-national epidemiology of panic disorder and panic attacks in the world mental health surveys. *Depress Anxiety* 2016; **33**: 1155-1177 [PMID: 27775828 DOI: 10.1002/da.22572]
4 **Middeldorp CM**, Birley AJ, Cath DC, Gillespie NA, Willemsen G, Statham DJ, de Geus EJ, Andrews JG, van Dyck R, Beem AL, Sullivan PF, Martin NG, Boomsma DI. Familial clustering of major depression and anxiety disorders in Australian and Dutch twins and siblings. *Twin Res Hum Genet* 2005; **8**: 609-615 [PMID: 16354503 DOI: 10.1375/183242705774860123]
5 **Maron E**, Hettema JM, Shlik J. Advances in molecular genetics of panic disorder. *Mol Psychiatry* 2010; **15**: 681-701 [PMID: 20048750 DOI: 10.1038/mp.2009.145]
6 **Kim EJ**, Kim YK. Panic disorders: The role of genetics and epigenetics. *AIMS Genet* 2018; 5: 177-190 [PMID: 31435520 DOI: 10.3934/genet.2018.3.177]
7 **Maron E**, Lang A, Tasa G, Liivlaid L, Tõru I, Must A, Vasar V, Shlik J. Associations between serotonin-related gene polymorphisms and panic disorder. *Int J Neuropsychopharmacol* 2005; **8**: 261-266 [PMID: 15670397 DOI: 10.1017/S1461145704004985]
8 **Xia DS**, Lu CZ, Guo QY, Song YQ, Li C, Xu JQ, Zhang F. [Association of tryptophan hydroxylase gene A218C and serotonin transporter gene polymorphism with essential hypertension in Chinese northern Han population]. *Zhonghua Xinxueguanbing Zazhi* 2009; **37**: 610-614 [PMID: 19961731]
9 **Strug LJ**, Suresh R, Fyer AJ, Talati A, Adams PB, Li W, Hodge SE, Gilliam TC, Weissman MM. Panic disorder is associated with the serotonin transporter gene (SLC6A4) but not the promoter region (5-HTTLPR). *Mol Psychiatry* 2010; **15**: 166-176 [PMID: 18663369 DOI: 10.1038/mp.2008.79]
10 **Annerbrink K**, Westberg L, Olsson M, Allgulander C, Andersch S, Sjödin I, Holm G, Eriksson E. Association between the catechol-O-methyltransferase Val158Met polymorphism and panic disorder: a replication. *Psychiatry Res* 2010; **178**: 196-198 [PMID: 20457471 DOI: 10.1016/j.psychres.2009.11.022]
11 **Karacetin G**, Bayoglu B, Cengiz M, Demir T, Kocabasoglu N, Uysal O, Bayar R, Balcioglu I. Serotonin-2A receptor and catechol-O-methyltransferase polymorphisms in panic disorder. *Prog Neuropsychopharmacol Biol Psychiatry* 2012; **36**: 5-10 [PMID: 22036916 DOI: 10.1016/j.pnpbp.2011.10.010]
12 **Rothe C**, Koszycki D, Bradwejn J, King N, Deluca V, Tharmalingam S, Macciardi F, Deckert J, Kennedy JL. Association of the Val158Met catechol O-methyltransferase genetic polymorphism with panic disorder. *Neuropsychopharmacology* 2006; **31**: 2237-2242 [PMID: 16525418 DOI: 10.1038/sj.npp.1301048]
13 **Goddard AW**, Mason GF, Almai A, Rothman DL, Behar KL, Petroff OA, Charney DS, Krystal JH. Reductions in occipital cortex GABA levels in panic disorder detected with 1h-magnetic resonance spectroscopy. *Arch Gen Psychiatry* 2001; **58**: 556-561 [PMID: 11386984 DOI: 10.1001/archpsyc.58.6.556]
14 **Goddard AW**, Mason GF, Rothman DL, Behar KL, Petroff OA, Krystal JH. Family psychopathology and magnitude of reductions in occipital cortex GABA levels in panic disorder. *Neuropsychopharmacology* 2004; **29**: 639-640 [PMID: 14973435 DOI: 10.1038/sj.npp.1300374]
15 **Ham BJ**, Sung Y, Kim N, Kim SJ, Kim JE, Kim DJ, Lee JY, Kim JH, Yoon SJ, Lyoo IK. Decreased GABA levels in anterior cingulate and basal ganglia in medicated subjects with panic disorder: a proton magnetic resonance spectroscopy (1H-MRS) study. *Prog Neuropsychopharmacol Biol Psychiatry* 2007; **31**: 403-411 [PMID: 17141385 DOI: 10.1016/j.pnpbp.2006.10.011]
16 **Long Z**, Medlock C, Dzemidzic M, Shin YW, Goddard AW, Dydak U. Decreased GABA levels in anterior cingulate cortex/medial prefrontal cortex in panic disorder. *Prog Neuropsychopharmacol Biol Psychiatry* 2013; **44**: 131-135 [PMID: 23391588 DOI: 10.1016/j.pnpbp.2013.01.020]

17 **Marenco S**, Savostyanova AA, van der Veen JW, Geramita M, Stern A, Barnett AS, Kolachana B, Radulescu E, Zhang F, Callicott JH, Straub RE, Shen J, Weinberger DR. Genetic modulation of GABA levels in the anterior cingulate cortex by GAD1 and COMT. *Neuropsychopharmacology* 2010; **35**: 1708-1717 [PMID: 20357758 DOI: 10.1038/npp.2010.35]

18 **Straub RE**, Lipska BK, Egan MF, Goldberg TE, Callicott JH, Mayhew MB, Vakkalanka RK, Kolachana BS, Kleinman JE, Weinberger DR. Allelic variation in GAD1 (GAD67) is associated with schizophrenia and influences cortical function and gene expression. *Mol Psychiatry* 2007; **12**: 854-869 [PMID: 17767149 DOI: 10.1038/sj.mp.4001988]

19 **Chung YE**, Chen SC, Chuang LC, Shih WL, Chiu YH, Lu ML, Chen HC, Kuo PH. Evaluation of the interaction between genetic variants of GAD1 and miRNA in bipolar disorders. *J Affect Disord* 2017; **223**: 1-7 [PMID: 28710909 DOI: 10.1016/j.jad.2017.07.024]

20 **Kirenskaya AV**, Storozheva ZI, Gruden MA, Sewell RDE. COMT and GAD1 gene polymorphisms are associated with impaired antisaccade task performance in schizophrenic patients. *Eur Arch Psychiatry Clin Neurosci* 2018; **268**: 571-584 [PMID: 29429137 DOI: 10.1007/s00406-018-0881-7]

21 **Bruxel EM**, Akutagava-Martins GC, Salatino-Oliveira A, Genro JP, Zeni CP, Polanczyk GV, Chazan R, Schmitz M, Rohde LA, Hutz MH. GAD1 gene polymorphisms are associated with hyperactivity in Attention-Deficit/Hyperactivity Disorder. *Am J Med Genet B Neuropsychiatr Genet* 2016; **171**: 1099-1104 [PMID: 27530595 DOI: 10.1002/ajmg.b.32489]

22 **Levran O**, Peles E, Randesi M, Correa da Rosa J, Ott J, Rotrosen J, Adelson M, Kreek MJ. Glutamatergic and GABAergic susceptibility loci for heroin and cocaine addiction in subjects of African and European ancestry. *Prog Neuropsychopharmacol Biol Psychiatry* 2016; **64**: 118-123 [PMID: 26277529 DOI: 10.1016/j.pnpbp.2015.08.003]

23 **Wu W**, Zhu YS, Li SB. Polymorphisms in the glutamate decarboxylase 1 gene associated with heroin dependence. *Biochem Biophys Res Commun* 2012; **422**: 91-96 [PMID: 22564729 DOI: 10.1016/j.bbrc.2012.04.112]

24 **Schumacher J**, Kristensen AS, Wendland JR, Nöthen MM, Mors O, McMahon FJ. The genetics of panic disorder. *J Med Genet* 2011; **48**: 361-368 [PMID: 21493958 DOI: 10.1136/jmg.2010.086876]

25 **Kübler U**. Structured Clinical Interview for DSM-IV (SCID). In: Gellman MD, Turner JR, editor. American Psychiatric Press Inc, New York: Springer, 2013

26 **Shear MK**, Brown TA, Barlow DH, Money R, Sholomskas DE, Woods SW, Gorman JM, Papp LA. Multicenter collaborative panic disorder severity scale. *Am J Psychiatry* 1997; **154**: 1571-1575 [PMID: 9356566 DOI: 10.1176/ajp.154.11.1571]

27 Xiong HF, Li ZJ, Han HY, Xu ZY, Guo ZH, Yao SM, Guo M, Jiang CQ. Panic disorder severity scale-chinese version: reliability and validity. *Chin J Psychiatry* 2012; **45**: 285-8. [DOI: 10.3760/CMA.J.ISSN.1006-7884.2012.05.009]

28 **Hamilton M**. The assessment of anxiety states by rating. *Br J Med Psychol* 1959; **32**: 50-55 [PMID: 13638508 DOI: 10.1111/j.2044-8341.1959.tb00467.x]

29 **Freire RC**, Perna G, Nardi AE. Panic disorder respiratory subtype: psychopathology, laboratory challenge tests, and response to treatment. *Harv Rev Psychiatry* 2010; **18**: 220-229 [PMID: 20597592 DOI: 10.3109/10673229.2010.493744]

30 **Heldt SA**, Mou L, Ressler KJ. In vivo knockdown of GAD67 in the amygdala disrupts fear extinction and the anxiolytic-like effect of diazepam in mice. *Transl Psychiatry* 2012; **2**: e181 [PMID: 23149445 DOI: 10.1038/tp.2012.101]

31 **Scotti-Muzzi E**, Chile T, Moreno R, Pastorello BF, da Costa Leite C, Henning A, Otaduy MCG, Vallada H, Soeiro-de-Souza MG. ACC Glu/GABA ratio is decreased in euthymic bipolar disorder I patients: possible in vivo neurometabolite explanation for mood stabilization. *Eur Arch Psychiatry Clin Neurosci* 2021; **271**: 537-547 [PMID: 31993746 DOI: 10.1007/s00406-020-01096-0]

32 **Zugliani MM**, Freire RC, Perna G, Crippa JA, Nardi AE. Laboratory, clinical and therapeutic features of respiratory panic disorder subtype. *CNS Neurol Disord Drug Targets* 2015; **14**: 627-635 [PMID: 25924997 DOI: 10.2174/1871527314666150430163142]

33 **Lonsdorf TB**, Rück C, Bergström J, Andersson G, Ohman A, Schalling M, Lindefors N. The symptomatic profile of panic disorder is shaped by the 5-HTTLPR polymorphism. *Prog Neuropsychopharmacol Biol Psychiatry* 2009; **33**: 1479-1483 [PMID: 19683026 DOI: 10.1016/j.pnpbp.2009.08.004]

34 **Sáiz PA**, Martínez-Barrondo S, García-Portilla MP, Corcoran P, Morales B, Bascarán MT, Paredes B, Álvarez V, Coto E, Fernández JM, Bousoño M, Bobes J. Role of serotonergic polymorphisms in the clinical severity of the panic disorder. *Revista de Psiquiatría y Salud Mental* 2009 [DOI: 10.1016/s2173-5050(09)70029-9]

35 **Weber H**, Scholz CJ, Domschke K, Baumann C, Klauke B, Jacob CP, Maier W, Fritze J, Bandelow B, Zwanzger PM, Lang T, Fehm L, Ströhle A, Hamm A, Gerlach AL, Alpers GW, Kircher T, Wittchen HU, Arolt V, Pauli P, Deckert J, Reif A. Gender differences in associations of glutamate decarboxylase 1 gene (GAD1) variants with panic disorder. *PLoS One* 2012; **7**: e37651 [PMID: 22662185 DOI: 10.1371/journal.pone.0037651]

36 **Choe AY**, Kim B, Lee KS, Lee JE, Lee JY, Choi TK, Lee SH. Serotonergic genes (5-HTT and HTR1A) and separation life events: gene-by-environment interaction for panic disorder. *Neuropsychobiology* 2013; **67**: 192-200 [PMID: 23635830 DOI: 10.1159/000347084]

37 **Ziegler C**, Grundner-Culemann F, Schiele MA, Schlosser P, Kollert L, Mahr M, Gajewska A, Lesch KP, Deckert J, Köttgen A, Domschke K. The DNA methylome in panic disorder: a case-control and longitudinal psychotherapy-epigenetic study. *Transl Psychiatry* 2019; **9**: 314 [PMID: 31754096 DOI: 10.1038/s41398-019-0648-6]

38 **Nöthling J**, Malan-Müller S, Abrahams N, Hemmings SMJ, Seedat S. Epigenetic alterations associated with childhood trauma and adult mental health outcomes: A systematic review. *World J Biol Psychiatry* 2020; **21**: 493-512 [PMID: 30806160 DOI: 10.1080/15622975.2019.1583369]

39 **Domschke K**, Tidow N, Schrempf M, Schwarte K, Klauke B, Reif A, Kersting A, Arolt V, Zwanzger P, Deckert J. Epigenetic signature of panic disorder: a role of glutamate decarboxylase 1 (GAD1) DNA hypomethylation? *Prog Neuropsychopharmacol Biol Psychiatry* 2013; **46**: 189-196 [PMID: 23906988 DOI: 10.1016/j.pnpbp.2013.07.014]

40 **Wu H**, Zhong Y, Xu H, Ding H, Yuan S, Wu Y, Liu G, Liu N, Wang C. Glutamic Acid Decarboxylase 1 Gene Methylation and Panic Disorder Severity: Making the Connection by Brain Gray Matter Volume. *Front Psychiatry* 2022; **13**: 853613 [PMID: 35686186 DOI: 10.3389/fpsyt.2022.853613]

Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: bpgoffice@wjgnet.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com