Effects of GPX3 and GSTA1 Polymorphisms on the Risk of Schizophrenia in Chinese Han Population

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Research article

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Abstract

Background: Several lines of evidence support the fact that the presence of oxidative stress plays an important role in the pathophysiological mechanisms of schizophrenia (SCZ). The glutathione peroxidases (GPXs) and glutathione S-transferases (GSTs) are the major antioxidant enzymes. Polymorphic variants of GPX and GST can affect the antioxidant activities of their encoded enzymes. This study explored the possible associations of the GPX3 and GSTA1 gene polymorphisms and schizophrenia in the Chinese Han population. Methods: DNA from 316 healthy controls and 303 schizophrenic patients was genotyped for single nucleotide polymorphisms (SNPs) rs736775 in GPX3 and rs3957357 in GSTA1 using a PCR-LDR genotyping assay. The χ^2 test compared differences in genetic distributions between the two groups in a case-control study. Results: No significant differences in allelic or genotypic frequencies of GPX3 rs736775 or GSTA1 rs3957357 were detected between cases and controls (GPX3 rs736775: $\chi^2 = 0.036$, $P = 0.982$ by genotype, $\chi^2 = 0.020$, $P = 0.888$, odds ratio = 1.017, 95% confidence interval = 0.801-1.292 by allele; GSTA1 rs3957357: $\chi^2 = 1.100$, $P = 0.577$ by genotype, $\chi^2 = 0.924$, $P = 0.336$, odds ratio = 1.176, 95% confidence interval = 0.845-1.637 by allele). Conclusions: Our results suggest that GPX3 rs736775 and GSTA1 rs3957357 SNPs are unlikely to be a candidate gene for susceptibility to SCZ in at least Chinese Han population. However, these results should be validated by replication in different populations.

Background

Schizophrenia (SCZ) is a complex and severe mental disorder and is the most common among psychotic illness, with a prevalence of around 1% in the worldwide population [1-3]. Although the detailed mechanisms underlying the pathophysiology of SCZ is unknown for certain, multiple pieces of evidence illustrated that a complex interaction between genetic background and environmental factors was likely to be involved in the development of SCZ [4, 5].

Dopamine (DA) is the principal neurotransmitter in the brain and play a significant role in the functions of neurons. However, the oxidative metabolites of dopamine are hydrogen peroxide (H_2O_2) and DA quinones which is a principal source of reactive oxygen species (ROS) in the brain [6]. Oxidative stress arises when the balance between antioxidant activity and the formation of ROS is disrupted, and ROS can cause neuronal inflammation as a consequence of oxidative stress. Interestingly, several studies suggested that neuronal inflammation induced by oxidative stress play an important role in SCZ pathophysiological mechanisms [7-9].

Recently, glutathione (GSH) levels were reported to be decreased in the cerebrospinal fluid and in the prefrontal cortex of SCZ patients[10-12]. Furthermore, some studies showed changes in antioxidant enzyme activity in the plasma and postmortem brain of schizophrenia patients[7, 13]. Taking all these factors into consideration, we had to focus our attention on the role of oxidative stress in the pathophysiology of SCZ through investigating two important antioxidant enzymes: glutathione peroxidases (GPXs) and glutathione S-transferases(GSTs).

GPX family belongs to selenium-dependent peroxidases and plays important role in protecting cells from oxidative damage by reducing free hydrogen peroxide to water. The GPX3, a member of GPX family, was found to be highly expressed in the prefrontal cortex suggests that *GPX3* may be involved in antioxidant activity in brain[14]. Human *GPX3* is located on chromosome 5q33.1 and has a common single nucleotide polymorphism(SNP), *GPX3* rs736775. Several studies have suggested the effect of this variant on GPX3 activity and many disorders[15-18].

GSTs consist of phase II detoxication enzyme and can catalyze the conjugation of the reduced form of glutathione (GSH) to xenobiotic substrates for the purpose of detoxification[19]. The GST alpha, a member of GSTs family, are located in chromosome 6 and shows an important detoxifying activity that protects the cell from ROS. GST alpha 1 (GSTA1) represents one of the most abundant alpha- class GST isoenzymes. Besides its through conjugation of reduced form glutathione GSH, GSTA1 can also inactivates quinones[20]. SNP rs3957357 in *GSTA1* is just located in the promoter region of *GSTA1* genes, and several studies suggested the pathogenic effects of this variant in many disorders[21, 22].

Although polymorphic variants of oxidative stress-related candidate genes including *GSTP1*, *GSTT1*, *GSTM1*, *GPX1* and *GSTA1* have been shown to be risk factors for SCZ[23-25], genetic polymorphism vary by race considerably and we therefore estimated the possible associations of the *GPX3* rs736775 and *GSTA1* rs3957357 gene polymorphisms and

schizophrenia in the Chinese Han population for the purpose of identifying potential prognostic or predictive tools for the individuals at risk of SCZ.

Methods

Population

The study was approved by the Ethical Committees of Jining Medical University in accordance with the Code of Ethics of the Declaration of Helsinki. The participants were recruited from the Rizhao Mental Health Center and Affiliated Hospital of Jining Medical University and they were original northern Han Chinese individuals. The sample consisted of 303 patients with SCZ (140 men and 163 women, mean age 47.2 ± 4.3 years) and 316 healthy controls (145 men and 171 women, mean age: 46.9 ± 4.4 years) living in the same geographic area. The patients with SCZ were interviewed by two board-certified psychiatrists according to the Diagnostic and Statistical Manual of Mental Disorders, 4th ed. (DSM-IV) criteria. The normal controls were confirmed to be free from any mental illness by two board-certified psychiatrists. All participants gave written informed consent to participate in the study.

Genetic studies

Total Genomic DNA was extracted from whole blood using TIANamp Genomic DNA Kit (TIANGEN, China), according to the manufacturer's instructions. Genotyping for SNPs *GPX3* rs736775 and *GSTA1* rs3957357 was performed using the polymerase chain reaction-ligase detection reaction (PCR-LDR) method. The sequences of primers are listed in Table 1. PCR was performed in a volume of 15 μ l reaction system, containing 7.5 μ l 2 $\times$ PCR Master Mix, 2 μ l Primer mix, and 2 μ l genomic DNA and DNase-free water. Multiplex PCR amplifications were performed under the following conditions: an initial denaturation at 94°C for 3min, followed by 35 cycles at 94 °C for 30 s, 55 °C for 30 s, 7 °C for 30 s, and a terminal extension 72 °C for 3min. After multiplex PCR amplification, the LDR was performed in a volume of 10 μ l reaction system, including 3 μ l PCR product, 1 μ l 10 $\times$ Taq DNA ligase buffer, 0.125 μ l Taq DNA ligase(40 U/ μ l), 2 μ l Probe mix, and ddH₂O,

followed by 30 cycles at 94 °C for 30 s, 56 °C for 3min. The sequences of probes are listed in Table 2. Hence, the final reaction system containing 1 µl LDR product and 9 µl highly deionized formamide were performed under denaturation at 95°C for 3min and the genotypes were analyzed by ABI 3730XL sequencer and Genemapper software.

Table 1 The information of primer of *GPX3* rs736775 and *GSTA1* rs3957357 polymorphism

Primer name	Sequence (5'-3')	PCR length
rs736775	F TAAACCCAAGTCCCCTGAGT	96bp
	R CTCTTGAGTAATGGTGACGTATCA	
rs3957357	F ACAACTGAATTCCAGGTCCTAATG	114bp
	R GCCATGAAATGTGTGGGAGT	

Table 2 The information of probe of *GPX3* rs736775 and *GSTA1* rs3957357 polymorphism

Probe name	Sequence (5'-3')	LDR length
Rs736775_modify	P- TGCCCTACCCTCAGGGAGGCCTGAC-FAM	
rs736775_C	CCTAGGCTCCTTCCACTGCCCCGGC	50
rs736775_T	CTGCCTAGGCTCCTTCCACTGCCCCGGT	53
Rs3957357_modify	P- GTCAAGTTAGGGAAAAGCCCTGACTGAC -FAM	
rs3957357_A	CTGAATAGTTCTCTCCCACTGAAAGAAGA	57
rs3957357_G	CTGACTGATAGTTCTCTCCCACTGAAAGAAGG	60

Statistical analysis

The χ^2 test was used to test the Hardy–Weinberg equilibrium (HWE) of the genotype distribution and to compare the differences in genotypic and allelic frequencies of *GPX3* rs736775 and *GSTA1* rs3957357 between cases and controls. $P<0.05$ was considered to denote statistical significance. The degree of relative risk was estimated by Odds ratios (ORs) and 95% confidence intervals (CIs). Statistical analysis in this study was carried out using the Statistical Package (version 21.0 for Windows; SPSS Inc., Chicago, Illinois, USA).

Results

The genotype distributions of the two tested alleles were in accordance with Hardy–Weinberga equilibrium (for rs736775, $P=0.071$; rs3957357, $P=0.157$). The allelic and genotypic frequencies of *GPX3* rs736775 and *GSTA1* rs3957357 in SCZ and control groups are shown in Table3. No significant differences in allelic or genotypic frequencies of *GPX3*

rs736775 and *GSTA1* rs3957357 were observed between schizophrenic and control groups. (*GPX3* rs736775: $\chi^2 = 0.036$, $P = 0.982$ by genotype, $\chi^2 = 0.020$, $P = 0.888$, odds ratio = 1.017, 95% confidence interval = 0.801- 1.292 by allele; *GSTA1* rs3957357: $\chi^2 = 1.100$, $P = 0.577$ by genotype, $\chi^2 = 0.924$, $P = 0.336$, odds ratio = 1.176, 95% confidence interval = 0.845-1.637 by allele).

Table 3 The comparison of genotypic and allelic frequencies between SCZ and control groups

SNP	Allelic test					Genotypic test			
	Allele	SCZ/control	p	χ^2	OR(95% CI)	Genotype	SCZ/control	p	χ^2
rs3957357	G	521/555	0.336	0.924	1.176 (0.845-1.637)	GG	220/241	0.577	1.100
	A	85/77				AG	81/73		
						AA	2/2		
rs736775	T	411/431	0.888	0.020	1.017 (0.801-1.292)	TT	132/140	0.982	0.036
	C	195/201				CT	147/151		
						CC	24/25		

Furthermore, in order to ensure sufficient statistical power for the detection of disease susceptibility loci, additive, dominant and recessive genetic models were used to analyze genotype frequencies of *GPX3* rs736775 and *GSTA1* rs3957357. Table 4 shows that rs736775 and rs3957357 were not the risk factors for SCZ on the basis of these models (all $P > 0.05$).

Table 4 Analysis of the two SNPs based on three genetic models

SNP	Genotype	SCZ/control	Additive model		Dominant model		Recessive model	
			p	OR(95% CI)	p	OR(95% CI)	p	OR(95% CI)
rs3957357	GG	220/241	0.93	1.10 (0.15-7.84)	0.29	1.21 (0.84-1.74)	0.97	1.04 (0.15-7.45)
	AG	81/73						
	AA	2/2						
rs736775	TT	132/140	0.95	1.02 (0.55-1.87)	0.85	1.03 (0.75-1.42)	0.99	1.00 (0.56-1.80)
	CT	147/151						
	CC	24/25						

Discussion

It is well known that the oxygen consumption of brain is the highest in our body and hence generation of ROS increase. Moreover, the oxidative metabolites of dopamine are hydrogen peroxide (H_2O_2) and DA quinones, which is a principal source of ROS in the brain[6].

Therefore, the brain is considered particularly vulnerable to ROS. Normally, ROS can be eliminated by the antioxidant system. When the balance between the formation of ROS and intrinsic antioxidant capacity is upset, oxidative metabolite damage to neurons arises. Increasing evidence indicates that oxidative injury to neurons can play important roles in the pathophysiology of neuropsychiatric disorders including schizophrenia[7-9].

The antioxidant system comprises of enzymatic and nonenzymatic antioxidants, and enzymes involved in the antioxidant systems comprise of glutathione peroxidase (GPx), glutathione S transferase (GST), and so on. Polymorphic variants of *GPX* and *GST* may affect their antioxidant activities, contributing to the imbalance of ROS production and antioxidant capacity in SCZ patients[23, 24, 26].

GPXs are encoded by the *GPX* gene family and consists of eight groups, GPx1-8. The GPX3, a member of GPX family, was found to be highly expressed in the prefrontal cortex suggests that *GPX* may be involved in antioxidant activity in brain. The human *GPX3* gene is located on chromosome 5q33.1 and multiple *GPX3* SNPs have been reported, with rs736775 being one of the most common. For instance, Noci et al. demonstrated that *GPX3* rs736775 was associated with overall survival in colorectal cancer patients[16]. Zhang et al. also suggested *GPX3* rs736775 was a prognostic markers in patients with gastric cancer[17]. Another study showed that the increased risk for cardiovascular toxicity among patients was associated with *GPX3* rs736775[27]. Taking these results into consideration, *GPX3* rs736775 might contribute to the altered antioxidant capacity in SCZ patients. In this study, we compared allelic and genotypic frequencies of rs736775 between 303 SCZ patients and 316 controls. However, we observed a TT genotype frequency of 43.56%, a CT genotype frequency of 48.51%, and a CC genotype frequency of 7.92% for patients with SCZ, compared with 44.30%, 47.79%, and 7.91%, respectively, in controls. No

significant differences in allelic or genotypic frequencies of *GPX3* rs736775 were observed between SCZ patients and controls in our study. This finding suggest that the *GPX3* rs736775 polymorphism may not be the genetic risk factor for SCZ patients.

GSTs are encoded by the *GST* family of genes located on different chromosomes and the cytosolic GST can be classified into four major groups: Alpha, Mu, Pi, and Theta. The *GST alpha*(*GSTA*) family are located in chromosome 6 and shows an important detoxifying activity that protects the cell from ROS. The *GSTA1* rs3957357 is one of five polymorphisms just located in the promoter region of *GSTA1* genes, resulting in decreased enzyme activity. In recent years, *GSTA1* rs3957357 has been reported to be associated with many disorders including bladder cancer, leukemia and gestational hypertension, as well as SCZ. For instance, Rossi et al. reported that *GSTA1* rs3957357 may associate with event-free survival in patients with diffuse large B-cell lymphoma[21]. Iorio et al. suggested that the *GSTA1* rs3957357 was significantly associated with gestational hypertension risk[28]. However, we are intrigued by the effects of *GSTA1* polymorphisms on the risk of SCZ. Gravina et al. found a higher frequency of the combined genotypes including *GSTA1* polymorphisms in Italy patients with SCZ[23]. Spalletta et al. suggested *GSTA1* polymorphisms was associated with the altered microstructure of the thalami in Italy patients with SCZ[24]. Because of several factors, such as population and sample, is necessary to test the gene polymorphisms on the risk of some disorders, we sought to analyze the Chinese Han population in the present study. We found GG, AG and AA genotype frequencies of 72.61% , 26.73%, and 0.66% in patients with SCZ, compared with 76.27, 23.10, and 0.63%, respectively, in controls. Consequently, no significant differences in allelic or genotypic frequencies of *GSTA1* rs3957357 between SCZ patients and controls in our study, and *GSTA1* rs3957357 does not appear to be a potential predictive tool for Chinese population at risk of SCZ.

Conclusions

In conclusion, this is the first report to investigate the effects of *GPX3* and *GSTA1* SNPs in SCZ in a Chinese population. Our results suggest that *GPX3* rs736775 and *GSTA1*

rs3957357 SNPs are unlikely to be a candidate gene for susceptibility to SCZ in at least Chinese Han population. However, most subjects were Han nationality in Shandong region in our study and the sample size is relatively small, larger-scale studies are necessary to validate this findings.

Abbreviations

SCZ: Schizophrenia; DA: Dopamine; ROS: Reactive oxygen species; GSH: Glutathione; GPX: Glutathione peroxidase; GST: Glutathione S-transferase; SNP: Single-nucleotide polymorphism; PCR-LDR: Polymerase chain reaction-ligase detection reaction; ORs: Odds ratios; CIs: Confidence intervals.

Declarations

Acknowledgements

Not applicable.

Authors' contributions

LC and GHJ designed the experiments. LC, SSJ, ZJK and LX collected the blood samples and extracted DNA from the blood samples. LC, ZJK and LX analyzed the raw data. LC and SSJ wrote the manuscript. GHJ participate in revising the manuscript. All authors reviewed and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study was approved by the Ethical Committees of Jining Medical University in accordance with the Code of Ethics of the Declaration of Helsinki. All participants gave written informed consent to participate in the study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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