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Original Article

Clinical relevance of tag single nucleotide polymorphisms within the CAT gene in patients with PTSD in the Chongqing Han population

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Abstract: Background: Free radical-induced oxidative damage of the brain has been implicated in a number of psychiatric disorders, including post-traumatic stress disorder (PTSD). Catalase (CAT) is a major antioxidant enzyme and a number of polymorphisms in CAT have been shown to be associated with several diseases, including hypertension, diabetes mellitus, Alzheimer's disease, and vitiligo. The aim of this study was to evaluate the association of CAT gene polymorphisms with PTSD in a case-control study. Materials and methods: A total of 460 unrelated adult Chinese Han adults, including 287 healthy volunteers and 173 patients with PTSD. Six tag single-nucleotide polymorphisms (tSNPs) were selected from the entire CAT gene through construction of haplotype bins, and they were genotyped using an improved multiplex ligation detection reaction (iMLDR) technique. Allelic frequencies and clinical characteristics were compared in two independent Chinese Han populations. Results: Six tag SNPs were identified in the Chinese Han population and all were common SNPs. However, we could detect no evidence of genetic association between six tag SNPs in the CAT gene and PTSD in the Chinese Han population. Conclusions: This result suggests that six tag SNPs of the CAT gene may not be associated with PTSD, and that CAT gene might not influence the development of PTSD in patients following exposure to a traumatic event, also may be the sample sizes too small to allow a meaningful test.

Keywords: Catalase, tag single nucleotide polymorphisms, post-traumatic stress disorder, case-control study

Introduction

Posttraumatic stress disorder (PTSD) is classified as an anxiety disorder within the DSM-IV. It is defined as the development of symptoms following exposure to an extreme traumatic event (criterion A). These symptoms are characterized by the following three separate but interrelated symptom clusters: re-experiencing, avoidance and numbing, and hyper arousal (DSM 1994). Exposure to a traumatic event is the essential element in the development of PTSD. Approximately 50-70% of the U.S. population is exposed to a traumatic event sometime during their lifetime. However, only 5-12% develops PTSD [1, 2]. The reasons for these differences in prevalence are not completely clear, but likely relate to the heterogeneity in the populations studied in terms of severity and type of trauma, pre-existing traumatic episodes, and criteria used for diagnosis. The importance of genetic effects on PTSD risk have been recognized for half a century, and twin studies have suggested a heritability of 70% [3, 4], i.e., approximately one third to up to 70% of the entire variance in the pathogenesis of PTSD can be attributed to genetic factors with the remainder of the variance being explained by environmental factors. These results suggest that certain individuals have an underlying vulnerability to developing this disorder in the aftermath of trauma. Identifying vulnerable individuals may allow for early and targeted intervention to prevent or reduce the symptoms and functional impairment associated with PTSD.

Table 1. The primer sequence and concentration in PCR mixture1

Primer Name	Primer Sequence	Annealing Temperature
rs10836233 F	TGCTATGGCCATCACCAGACAC	54.6°C
rs10836233 R	CCACAGCTCCATGACTCCTGTTC	56.5°C
rs208679 F	GCCCACTTTGTCACAGGCAGAA	54.6°C
rs208679 R	GGCTCTACACTGAGGGATTTTCCAATA	44.4°C
rs2300182 F	GTTGAAGCTTTCCTGCCCCACT	54.6°C
rs2300182 R	CCTCCTGCCCAATGAAGGAATC	54.6°C
rs7104301 F	TCAGCACTGATTTCACAACAGATCA	48.0°C
rs7104301 R	TGGAGTCCTCGAGATACTGGCATTT	49.0°C
rs769217 F	CCTTTTTGCCTATCCTGACACTCAC	48.0°C
rs769217 R	AGGGGGAGCCCAACGTCTTTAG	59.0°C
rs7949972 F	GCTGGTCTTTGGTTACCCTGGTATT	48.0°C
rs7949972 R	CATTCCCCAGGGATCACTCTGA	54.6°C

Oxidative stress is a biological condition that is characterized by production of excessive amounts of oxidants, decreased levels of antioxidants or both. Excessive amount of free radicals may chemically react with cell membrane lipids, proteins and nucleic acids that consequently have the potential to damage the structure of neuronal cells [5, 6]. In contrast, oxidative stress results from impaired oxidative defense mechanisms, such as depletion of enzymatic (e.g., superoxide dismutase, catalase and glutathione peroxidase, GPX) and nonenzymatic (e.g., glutathione, GSH, vitamins A, C, and E, and selenium) antioxidants. Either way, the consequence of oxidative stress is increased damage to all major groups of cellular macromolecules including proteins, lipids, carbohydrates, and nucleic acids, which may result in apoptosis or necrosis [7, 8]. The theory of oxidative stress in mental disorders is based on vulnerability of the brain to oxidative damage [9]. Recently, several studies have suggested an association between oxidative stress and psychopathology in anxiety disorders including panic disorder, obsessive-compulsive disorder and PTSD [5]. However, to the best of our knowledge there has been no study published yet investigating the oxidative stress markers in PTSD.

Catalase is a ubiquitous enzyme found in all known organisms. It is most abundant in the liver, kidneys, and erythrocytes. Together with superoxide dismutase (SOD) and GPX, catalase constitutes a primary defense against oxidative stress. Catalase has a very high turnover number, decomposing H_2O_2 into O_2 and H_2O at an exceedingly high rate. It is considered to be the most important regulator of H_2O_2 metabolism. H_2O_2 in high concentrations could be toxic to cells and it modulates some physiological processes, such as cell proliferation, apoptosis, and platelet activation at low concentrations [10]. Catalase gene polymorphism in the promoter region (–262C>T) affects transcription factor binding and thus decreases catalase enzymatic activity leading to increased formation of hydroxyl radicals and elevated risk of breast cancer development, asbestosis, and arsenic-induced hyperkeratosis [11]. Recently, the association of the CAT gene polymorphisms with hypertension, angle closure glaucoma, Alzheimer's disease and vitiligo has been investigated [12-14]. However, to our knowledge, its genetic effects on PTSD have not been studied yet, despite the presumptively important role of CAT in anxiety disorders including PTSD. Therefore, we investigated the genetic effects of the entire CAT gene on the risk of PTSD.

In this study, we hypothesized that the genetic variations in the CAT gene might affect the morbidity of PTSD in patients following exposure to a traumatic event. To assess the association of common genetic variants within the entire CAT gene with PTSD susceptibility comprehensively, we selected a set of tag SNPs (tSNPs) within the entire CAT gene and investigated their clinical relevance in relation to the development of PTSD in patients with exposure to traumatic events.

Materials and methods

Study design and data collection

A total of 460 unrelated Han Chinese adults, including 287 healthy volunteers and 173 patients with PTSD, were recruited from the Chongqing district for this study. The protocol of this study was approved by the Ethical and Protocol Review Committee of the Third Military Medical University. The benefits and risks of the study participation were fully explained to each subject and written informed consent was obtained from the participants or their next of kin.

Table 2. The probe sequence and concentration in probe mixture

Probe Name	Target Allele	Probe Concentration	PROBE SEQUENCE (5' PHOSPHORATED)	Annealing Temperature
rs10836233 FG	G	1 μM	TCTCTCGGGTCAATTCGTCCTTGCCAAATAGGCAGAGCAGAAGGTATCTG	69.3°C
rs10836233 FA	A	1 μM	TGTTCGTGGGCCGGATTAGTGCCAAATAGGCAGAGCAGAAGGTATCTA	67.2°C
rs10836233 FP		2 μM	CTGCTACTGCATATAACACCACTGCTACTTTTTT	63.4°C
rs208679 FG	G	1 μM	TTCCGCGTTCGGACTGATATTTAGTGCCCTCAGATGTAAAGTGAAGGTAGGAG	66.4°C
rs208679 FA	A	1 μM	TACGGTTATTCGGGCTCCTGTTTAGTGCCCTCAGATGTAAAGTGAAGGTAGGAA	66.6°C
rs208679 FP		2 μM	SAGTCCATTCCCYCTTAYAGGATTGTTATAATTTTTTTTTTTTTTT	63.0°C
rs2300182 RA	A	1 μM	TCTCTCGGGTCAATTCGTCCTTTCCATTTCCCCACTCTAACAGTTAGATGAA	67.4°C
rs2300182 RT	T	1 μM	TGTTCGTGGGCCGGATTAGTTCCATTTCCCCACTCTAACAGTTAGATGAT	66.6°C
rs2300182 RP		2 μM	TTGTCCTCATTTATATAACAGCTTTCCCCTTTTTT	65.2°C
rs7104301 RG	G	1 μM	TCTCTCGGGTCAATTCGTCCTTTCAAATTAAGAGTCTGGTAGYAGTTTACAGTTAGAC	66.2°C
rs7104301 RA	A	1 μM	TGTTCGTGGGCCGGATTAGTTCAAATTAAGAGTCTGGTAGYAGTTTACAGTTAGAT	65.8°C
rs7104301 RP		2 μM	RTGGAGTCGAAAGAAAGAAAATACCAYCTTTTTTTTTTTTTTT	63.9°C
rs769217 FC	C	1 μM	TTCCGCGTTCGGACTGATATGAGTGGCCAACTACCAGCGTCAC	67.4°C
rs769217 FT	T	1 μM	TACGGTTATTCGGGCTCCTGTGAGTGGCCAACTACCAGCGTCAT	66.8°C
rs769217 FP		2 μM	GRCCCGATRTGCATGCAGGATTTTTTTTTTTTTTTTTTTTTTTTTTT	72.1°C
rs7949972 RC	C	1 μM	TTCCGCGTTCGGACTGATATCACGTTTTGAGCTGAGAATGGTCAG	66.3°C
rs7949972 RT	T	1 μM	TACGGTTATTCGGGCTCCTGTCACGTTTTGAGCTGAGAATGGTCAA	66.6°C
rs7949972 RP		2 μM	GTTCAGTCTTCAAGATGTCTGGGAATTTTTTTTT	63.0°C

Note: S, Y, R, W were degeneracy bases of IUB CODES; S: G/C, Y: C/T, R: G/A, W: A/T.

Healthy control group

The healthy volunteers control group consisted of 175 men and 112 women with a median age of 35 (18-55) years. All volunteers were nursing staff, medical staff, and university staff and students in the Daping Hospital. Formal screening for psychological disorders was not undertaken in the control population. Therefore, the control subjects represented an unselected control group. Controls were recruited in the Chongqing region and all belonged to the Han population.

PTSD patients

A total of 173 unrelated Chongqing Han patients (103 men and 70 women) meeting the Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV) criteria for PTSD were recruited for the study. Patients were assessed for PTSD by a consultant psychiatrist or a senior psychiatric registrar using DSM-IV criteria. Furthermore, every patient exceeded the clinical cutoff score of 94 on the Mississippi Scale for combat-related PTSD [15]. The patients' clinical history and demographic data including the ethnic background were also obtained. None of the patients had received treatment for a psychiatric disorder or had been treated with psychotropic medication. Following PTSD assessment with a psychiatrist, treatment was then commenced. Patients were only excluded if other major Axis I diagnoses (excepting major depression) were present, such as bipolar disorder, schizophrenia or other psychotic disorders, and alcohol or substance abuse during the past 6 months. Patients were also excluded if there were any medical or psychiatric conditions, including pregnancy and lactation that could pose health risks during the course of the study. The patients had a mean age of 36 years (± 6.9). All patients had sufficient comprehension of Chinese and could understand the administered questionnaires. Patients were assessed for PTSD by a consultant psychiatrist or a senior psychiatric registrar using DSM-IV (American Psychiatric Association, 1994) criteria for a primary diagnosis of PTSD during the previous 6 months. All these patients were admitted to the Clinical Psychology Department of Southwest Hospital of Chongqing, Department of Psychiatry of the Mental Health Center of Chongqing, and outpatient in the Department of Psychiatry of the Chongqing Medical University, between the 1st of January 2011 and the 1st of June 2013.

Table 3. Frequencies of CAT gene polymorphisms in healthy volunteers and patients with PTSD

NCBI rs#	Location	Position	Variant	MAF (Hapmap-HCB)	healthy volunteers					patients with PTSD				
					Genotype, N (287)			MAF	HWE	Genotype, N (173)			MAF	HWE
rs208679	5' flanking	-6411	A/G	0.22	AA 170	AG 100	GG 17	0.23	0.65	AA 93	AG 65	GG 15	0.27	0.45
rs10836233	5' flanking	-2900	G/A	0.30	GG 137	GA 118	AA 32	0.32	0.39	GG 94	GA 60	AA 19	0.28	0.06
rs2300182	Intron1	7376	T/A	0.23	TT 201	TA 81	AA 5	0.16	0.33	TT 121	TA 46	AA 6	0.17	0.53
rs769217	exon 9 (synon)	22436	T/C	0.49	TT 50	TC 157	CC 80	0.55	0.07	TT 37	TC 79	CC 57	0.56	0.33
rs7104301	3' flanking	33166	A/G	0.23	AA 153	AG 113	GG 21	0.27	0.98	AA 101	AG 60	GG 12	0.24	0.46
rs7949972	3' flanking	41570	C/T	0.45	CC 96	CT 138	TT 53	0.43	0.78	CC 61	CT 79	TT 32	0.42	0.47

Note: MAF indicates minor allele frequency; HWE indicates Hardy-Weinberg Equilibrium.

Table 4. Analyses of association of CAT gene polymorphisms with the risk of PTSD between healthy volunteers and patients with PTSD

NCBI rs#	Location	Variant	Genotype	Frequency		Codominant		Dominant*		Recessive	
				healthy volunteers	patients with PTSD (+)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)
rs208679	5' flanking	A/G	AA	170 (59.20)	93 (53.76)	0.38	0.80 (0.59-1.09)	0.29	0.80 (0.55~1.17)	0.26	0.66 (0.32~1.37)
			AG	100 (34.80)	65 (37.57)						
			GG	17 (6.00)	15 (8.67)						
rs10836233	5' flanking	G/A	GG	137 (47.70)	94 (54.34)	0.35	0.85 (0.64-1.14)	0.18	1.30 (0.89~1.90)	1.00	1.02 (0.56~1.86)
			GA	118 (41.20)	60 (34.68)						
			AA	32 (11.10)	19 (10.98)						
rs2300182	Intron1	T/A	TT	201 (70.00)	121 (69.94)	0.49	1.07 (0.75-1.53)	1.00	1.00 (0.66~1.50)	0.35	0.49 (0.15~1.64)
			TA	81 (28.20)	46 (26.59)						
			AA	5 (1.80)	6 (3.47)						
rs769217	exon 9 (synon)	T/C	TT	50 (17.40)	37 (21.39)	0.17	1.02 (0.78-1.34)	0.25	1.27 (0.85~1.91)	0.33	0.78 (0.48~1.25)
			TC	157 (54.70)	79 (45.66)						
			CC	80 (27.90)	57 (32.95)						
rs7104301	3' flanking	A/G	AA	153 (53.30)	101 (58.38)	0.56	1.15 (0.85-1.57)	0.33	1.23 (0.84~1.80)	1.00	1.06 (0.51~2.21)
			AG	113 (39.40)	60 (34.68)						
			GG	21 (7.30)	12 (6.94)						
rs7949972	3' flanking	C/T	CC	96 (33.40)	61 (35.47)	0.88	1.02 (0.78-1.34)	0.69	1.09 (0.74~1.63)	0.90	0.95 (0.59~1.54)
			CT	138 (48.10)	79 (45.93)						
			TT	53 (18.50)	32 (18.60)						

*Note: recessive effect = variant homozygotes versus heterozygotes + wild-type homozygotes; dominant effect = variant homozygotes + heterozygotes versus wild-type homozygotes.

Selections of SNPs

The full sequence of the human CAT gene (Accession Number: NC-000011.9 Reference GRCh37.p5 Primary Assembly) observed in the current study included 10 kb upstream of the transcription start site all exons and introns and 10 kb downstream of the stop codon (33.235 kb total), which was pinpointed to chromosome 11, position 34460472-3449 3607 (data retrieved from Genbank in the Web site of NCBI). Genetic variation data for the entire CAT gene was obtained from the HapMap project for 45 healthy Chinese Han Beijing (CHB) adults (www.hapmap.org). From this database, a total of 105 SNPs were identified, of which, 44 SNPs with minor allele frequency (MAF) of more than or equal to 0.10 were selected for the analysis of htSNPs. Haplotype blocks were constructed throughout the entire CAT gene, using Haploview, version 4.0 (Broad Institute of MIT and Harvard, Cambridge, MA), a software package that provides computation of LD statistics and population haplotype patterns from genotype data [16]. Haplotype blocks represent regions inherited without substantial recombination in the ancestors of the current population. The history of recombination between a pair of SNPs can be estimated with the use of the normalized measure of allelic association D' (value of D prime between the two loci) [17, 18]. The criteria for the selected SNPs to construct a haplotype block is that all SNPs in one region must be in strong LD with D' >0.98 for the upper 95% confidence bound, and >0.7 for the lower bound. A maximally informative htSNP was then selected from each block using the software Tagger program (http://www.broad.mit.edu/mpg/haploview). This algorithm selects a subset of variants that capture all known common genetic variations in a gene, based on an LD threshold of $r^2 \geq 0.8$. The inverse of r^2 represents the ratio of sample size needed to detect an indirect association with an un-analyzed SNP to direct association at the same power.

SNPs genotyping

The genomic DNA was extracted from peripheral blood leukocytes using a Wizard Genomic DNA Purification Kit (Promega, Madison, Wisconsin, USA) following the manufacturer's instructions. A multiplex polymerase chain reaction (PCR) -ligation detection reaction method was used for genotyping the selected 6 tag SNPs. For each SNP, the alleles were distinguished by different fluorescent labels of allele specific oligonucleotide probe pairs. Different SNPs were further distinguished by different extended lengths at the 3' end. The primer and probe information in two mixtures are described in **Tables 1** and **2**, respectively. Genotyping was performed in a blinded fashion without knowledge of the patients' clinical data, and approximately 10% of the samples were genotyped in duplicate to monitor genotyping quality.

Statistical analyses

Allele frequencies for each tSNP were determined by gene counting. The genotype distribution of each tSNP was analyzed for deviations from the Hardy-Weinberg equilibrium using χ^2 analyses. The extent of pairwise LD (r^2-value) between polymorphisms was determined using the Haploview software version 4.2. Three genetic models were used (allele-dose, dominant, and recessive). A Fisher's exact test was performed to identify statistical associations between the genotype and disease status. A Pearson's χ^2 test was performed to identify statistical associations between the allele and disease status. ORs with 95% confidence intervals were calculated by using multivariate logistic regression models to estimate the relative risk of PTSD. Haplotypes were generated and analyzed for linkage disequilibrium (LD) measures (D' and r^2) using JLIN [19]. All *P*-values were two-sided, and $P<0.05$ after the Bonferroni correction for multiple testing was defined as statistically significant. All statistical analyses were carried out using the SPSS version 17.0 statistical software package (SPSS, Inc, Chicago, IL, USA).

Results

Construction of haplotype bins and selection of tag SNPs

Six tag SNPs with a minor allele frequency of more than or equal to 10% were found in the CHB population, which constructed four haplotype bins. Based on the analysis of tagging threshold of r^2 of SNPs in each bin, one htSNP was selected from each bin for genotyping. A pairwise analysis of linkage disequilibrium (LD), based on r^2, was conducted among the 44

Table 5. Pairwise LD tests among six tag SNPs in CAT gene between healthy volunteers and patients with PTSD

		D'					
r^2	SNPs	rs1	rs2	rs3	rs4	rs5	rs6
	rs208679 (rs1)	-	1.00	1.00	1.00	0.93	1.00
	rs10836233 (rs2)	0.45	-	1.00	1.00	1.00	1.00
	rs2300182 (rs3)	0.32	0.82	-	1.00	1.00	1.00
	rs769217 (rs4)	0.14	0.15	0.41	-	1.00	0.99
	rs7104301 (rs5)	0.89	0.07	0.35	0.06	-	1.00
	rs7949972 (rs6)	0.48	0.44	0.16	0.44	0.15	-

SNPs with a minor allele frequency (MAF) ≥10% within the CAT gene and the 10-kb up- and downstream regions. The selected six tag SNPs (tSNPs) are indicated by trigones. A LD plot of the 44 SNPs in the 33.235-kb region is displayed by using an r^2 black-and-white color scheme. Black represents very high LD correlation between SNPs (r^2 = 0.8 to 1), and white indicates the absence of correlation between SNPs (r^2 = 0 to 0.2).

Allele frequencies and genotype distribution of the CAT gene polymorphisms among participants with potentially traumatic events

The genotyping success rates of the six tSNPs by an improved multiplex ligation detection reaction (iMLDR) technique ranged from 99.6% to 100% in our study cohort. The MAFs among the 287 healthy volunteers were 31.70% (rs10836233), 23.30% (rs208679), 15.90% (rs2300182), 27.00% (rs7104301), 44.80% (rs769217) and 42.50% (rs7949972). Among the 173 patients with PTSD they were 28.30% (rs10836233), 27.50% (rs208679), 16.80% (rs2300182), 24.30% (rs7104301), 44.20% (rs769217), and 41.90% (rs7949972), respectively, which were quite similar to those observed in the 45 unrelated CHB cohorts in the HapMap database. The genotype distribution of all six tSNPs was in agreement with the Hardy-Weinberg equilibrium (*P*>0.05) (**Table 3**), indicating that the allele and genotype frequencies of these tSNPs in the population remain constant, i.e., they are maintained from generation to generation.

Clinical association of CAT gene polymorphisms with development of PTSD in patients with potentially traumatic events

A total of 460 unrelated Han Chinese adults, including 287 healthy volunteers and 173 patients with PTSD were recruited from the Chongqing district for this study. The patient cohort met the Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV) criteria for PTSD consisted of 173 consecutive Han Chinese patients, 103 male and 70 female, with a mean ± SD age of 35.6 ± 11.4 years. There were no significant differences in age and sex ratio among patients stratified according to the different genotypes of each tSNP. The *P* values of each polymorphism were analyzed with respect to a comparison between PTSD patients and the healthy volunteers' controls by logistic analysis. We found that none of six tag SNPs of the CAT gene were significantly associated with the risk of PTSD in all alternative analysis models (*P*>0.05) (**Table 4**).

Combination effects of the six tag SNPs

Because LD is highly structured as conserved blocks of sequences separated by hotspots of recombination, the final function of a conserved haplotype may be the result of interaction among polymorphisms within the block. The LD coefficient between polymorphisms was calculated, which showed that all genotyped polymorphisms are in strong LD (**Tables 5**, **6**). Six tagging SNPs in the LD block were selected, haplotypes were reconstructed and the haplotype frequencies were compared between the healthy volunteers and PTSD patients. Haplotype analysis showed that the haplotype ht2 (A-A-T-C-A-C) and ht4 (A-G-T-T-A-T) was significantly higher in patients than healthy volunteers, suggesting that haplotype ht2 and ht4 had a risk effect on PTSD development (**Table 6**, *P*=0.01, odds ratio (95% CI) is 1.85 (1.15-2.97) for ht2, and *P*=0.000, odds ratio (95% CI) is 2.25 (1.45-3.49) for ht4). However, haplotype association analysis did not provide a higher OR value or a more significant result than the OR and *P* values of single locus SNPs.

These results suggest that gene polymorphisms of the CAT gene, which is a major antioxidant enzyme, might not be associated with an increased susceptibility to PTSD in the Chongqing Han population.

Discussion

PTSD is associated with exposure to a traumatic event outside the range of usual human experience, e.g., severe accidents, intense

Table 6. Analyses of association of CAT gene haplotypes with the risk of PTSD between healthy volunteers and patients with PTSD (+)

Loci	Genotype	Frequency		Chi^2	Pearson's p	Odds Ratio (95% CI)
		Case (173)	Control (287)			
ht1	A A A C A C	58.00 (0.17)	91.00 (0.16)	0.09	0.77	1.06 [0.74~1.51]
ht2	A A T C A C	38.51 (0.11)	36.09 (0.06)	6.55	0.01	1.85 [1.15~2.97]
ht3	A G T C A C	96.49 (0.28)	186.76 (0.33)	2.54	0.11	0.79 [0.59~1.06]
ht4	A G T T A T	49.87 (0.14)	39.67 (0.07)	13.45	0.00	2.25 [1.45~3.49]
ht5	G G T T G T	84.00 (0.24)	134.00 (0.23)	0.05	0.81	1.04 [0.76~1.42]

Note: Haplotypes were ignored if the haplotype frequency was less than 3%.

physical or sexual abuse, life-threatening medical conditions, combats, and natural disasters [20]. Common life events such as bereavement, chronic illness, and divorce rarely produce PTSD. Twin studies have shown that genetic factors can influence the risk of developing PTSD. As in combat veterans, the development of PTSD symptoms after non-combat trauma also seems to be moderately heritable [21]. Moreover, many of the same genes that influence exposure to assault trauma appear to influence susceptibility to PTSD symptoms in their wake.

In recent years, more and more evidence has suggested that oxidative stress is involved in the pathogenesis of various neuropsychiatry diseases, including anxiety disorders, major depression, and PTSD [5]. Depletion of specific antioxidants and decreased activity of antioxidant enzymes, as observed in the blood, leads to an impaired defense against oxidative stress. Consequences of oxidative stress include damage to macromolecules, of which lipid peroxidation has been extensively studied. Several mechanisms are likely involved in the increased generation of reactive species and impairment of oxidative defenses, both of which contribute to increased oxidative stress and result in damage to cellular macromolecules. Eventually the consequences will be increased apoptosis, neuronal degeneration, and brain damage, which contribute to the manifestation of neuropsychiatry illness in susceptible individuals. However, the detailed mechanisms remain largely unknown.

CAT is an enzyme in the first line of antioxidant defense and might be contribute to the etiology and development of PTSD. To the best of our knowledge, we are the first investigators to identify the potential clinical relevance of the rs10836233, rs208679, rs2300182, rs769217, rs7104301 and rs7949972 polymorphisms, which are identified as tSNPs of the entire CAT gene in the Han Chinese population. tSNPs are a subset of all variants in a chromosome region within a disease study. The genetic effects of SNPs that are not genotyped in the study can be detected through LD with a tSNP [16]. In this case, the six tSNPs selected in the current study theoretically reflect the biological significance of all the genetic variations across the entire CAT gene because of their strong LD with other unassayed variants.

A case-control study is a common and convenient association study design for finding the genetic basis of a disease. However, a major limitation of this approach is the potential for population stratification when inappropriate patient-control matching occurs, such as using healthy blood donors as the control group. To avoid confounding associations, we only selected patients with exposures to traumatic events and followed them prospectively to determine whether those who had genetic variants had a lesser or higher risk of PTSD. In addition, we limited the patients recruited into this study cohort to Han Chinese to avoid an artifact due to population admixture. In the context of a biologically relevant phenotype and a racially uniform population, this might maximize the likelihood of finding a meaningful genetic association.

As shown by our clinical association study (**Tables 3**-**6**) none of the six tSNPs selected in our study cohort had clinical relevance in relation to the development of PTSD in patients with exposure to traumatic events, only two haplotype, ht2 (A-A-T-C-A-C) and ht4 (A-G-T-T-A-T) were significantly higher in PTSD patients than healthy volunteers. This conclusion essen-

tially applies to the late onset type of the disease, given the small number of early onset types in our PTSD population. However, as already mentioned, age stratification between or within populations did not affect our results. Our failure to assign significance to the six tag SNPs and haplotypes of CAT gene should be interpreted with caution for a number of reasons. One important reason might be due to polygenetic and multifactorial involvement in the pathogenesis of PTSD. In fact, any one gene might not determine the clinical phenotype of PTSD although the gene is a pivotal one. Therefore, a gene polymorphism should have much less power on the PTSD outcome. The susceptibility to PTSD might be the result of a combination of numerous genetic polymorphisms. Another possible reason might be that we do not know whether these particular polymorphisms have an upregulating effect in the brain and to what extent, and whether it increases the overall antioxidant capacity of the regions most affected by PTSD, especially given the relatively low levels of CAT in brain tissue. Even in the event that antioxidant capacity is affected significantly, the result could manifest itself only as an effect in disease progression, something that the present study was not designed to test. There are other antioxidant enzymes whose activity could be important in the human brain. The glutathione peroxidases constitute a family of enzymes invol GPX4 for its ability to reduce peroxidized phospholipids and cholesterol [22], are also promising candidates, provided that cved in hydrogen and lipid peroxide reduction, but the only common polymorphism characterized so far, the GPX1 593C/T, does not result in an alteration in the enzymatic activity. Other known peroxidases, and especiallyommon polymorphisms altering their activity or expression are identified in their genes. Therefore, additional studies of other antioxidant enzyme genes, with a variety of ethnic populations, will be needed before the significance of the various components of the antioxidant machinery of the brain is elucidated in the context of PTSD onset and/or progression.

Conclusions

Here, we investigated the clinical relevance of the genetic variations within the entire CAT gene by means of constructing haplotype bins in patients with PTSD after potentially traumatic events. We have demonstrated that none of the six tSNPs selected in our study cohort had clinical relevance in the development of PTSD in patients with exposure to potentially traumatic events. It suggests that the CAT gene might not affect the development of PTSD, also may be the sample size may also be too small to allow a meaningful test.

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Disclosure of conflict of interest

The authors do not have a conflict of interest to declare.

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