Promoter Variant in the *Catalase* Gene Is Associated with Vitiligo in Chinese People

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Vitiligo is an acquired depigmentation disorder, and reactive oxygen species have an important role in the physiology of cell damage. Reduced catalase enzyme activity and accumulation of excessive hydrogen peroxide have been observed in vitiligo. In a hospital-based case–control study of vitiligo patients ($n = 749$) and age- and sex-matched healthy controls ($n = 763$), we investigated three *catalase* (*CAT*) gene polymorphisms ($-89A > T$, $389C > T$, and $419C > T$) to examine whether *CAT* gene polymorphisms are associated with vitiligo susceptibility in the Chinese population. The case–control analysis revealed a 1.54-fold (95% confidence interval (CI) 1.25–1.91) increased risk of developing vitiligo for $-89A > T$ genotype carriers. No evidence for any association between $389C > T$ and $419C > T$ polymorphisms in the *catalase* gene and vitiligo susceptibility was found. An analysis of haplotypes showed increased risk for $T_{-89}C_{389}$ (odds ratio (OR) 1.90, 95% CI 1.26–2.86) and $T_{-89}T_{389}$ (OR 2.80, 95% CI 1.24–6.30). Logistic regression analysis of catalase activity also showed a dose–response relationship between increased risk and decreased activity in *CAT* $-89A > T$ variant genotype carriers, especially in vitiligo patients ($P_{trend} < 0.001$). Our molecular epidemiologic findings suggest that the *CAT* $-89A > T$ variant genotypes were associated with a significant decrease in catalase enzyme activity and a genetic predisposition for vitiligo in Chinese people.

INTRODUCTION

Vitiligo is a common, chronic skin disorder resulting from selective destruction of melanocytes. The disease affects individuals of both sexes, with a prevalence ranging from 0.1 to 8.8% worldwide (Handa and Kaur, 1999; Herane, 2003; Gautam et al., 2006). In China, the incidence rate of vitiligo is approximately 0.09%, without a propensity for male or female, and has increased in the past decade (Gautam et al., 2006; Lu et al., 2007). There is much debate over the precise pathogenesis of the depigmentation, including autoimmune, biochemical, neural, self-destructive, and genetic hypotheses (Castanet and Ortonne, 1997; Shaffrali and Gawkrodger, 2000). In recent years, oxidative stress has attracted much attention, and some findings have suggested that oxidative stress may be the triggering event in the melanocyte degeneration of vitiligo (Passi et al., 1998; Jimbow et al., 2001; Boisseau-Garsaud et al., 2002; Rokos et al., 2002; Yildirim et al., 2003; Kocaturk et al., 2004; Ines et al., 2006; Arican and Kurutas, 2008).

Oxidative stress is the condition of prooxidant/antioxidant disequilibrium (Sies, 1991). Reactive oxygen species can be generated by mitochondria through enzyme complexes during normal processes of oxidative phosphorylation and can cause cell injury and death (Arita et al., 2006; Rezvani et al., 2006). Previous studies of vitiliginous melanocytes showed that an imbalance between oxidative and anti-oxidative patterns, such as the accumulation of hydrogen peroxide (H_2O_2) and low catalase (CAT) level/activity, may induce the destruction of melanocytes (Schallreuter, 1999; Rokos et al., 2002; Hasse et al., 2004; Schallreuter et al., 2004; Maresca et al., 2007).

The cause of low CAT level/activity in vitiligo patients has not been determined. In addition to substrate inhibition of CAT activity by high H_2O_2 levels in the epidermis of vitiligo patients, allelic variants in the *CAT* gene may have deleterious effects on the expression or function of CAT (Góth et al., 2004). The human *CAT* gene is located on chromosome 11p13, consisting of 13 exons and 12 introns. A number of *CAT* gene single-nucleotide polymorphisms (SNPs) and mutations have been associated with disease manifestations such as catalasemia/hypocatalasemia, hypertension, and type 2 diabetes mellitus in various races (Góth and Eaton, 2000; Forsberg et al., 2001; Góth et al., 2001; Jiang et al., 2001). Single polymorphisms located in the promoter region could influence rates of transcription, resulting in low CAT expression (Góth and Vitai, 1997; Park et al., 2006). Although the *C > T* SNP in codon 389 of exon 9 (rs769217) is a silent substitution, it has been associated with susceptibility to vitiligo in a North American population and

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Abbreviations: CAT, catalase; CI, confidence interval; H_2O_2, hydrogen peroxide; SNP, single-nucleotide polymorphism; OR, odds ratio

an English population in the United Kingdom (Casp *et al.*, 2002; Gavalas *et al.*, 2006). However, no difference in allelic and genotypic occurrences was observed in Korean people in a study with a relatively small sample size (Park *et al.*, 2006). The *C>T* SNP in codon 419 of exon 10 (rs11032709) is also a silent substitution, whose distribution of alleles and genotypes differs among races, and the variant genotype could be associated with susceptibility to vitiligo (Casp *et al.*, 2002).

Although there have been some reports of an association between *CAT* polymorphisms and risk of various diseases, the genotype distributions of the *CAT* polymorphisms vary with ethnicity, and until now, no study has been carried out on the association between the three *CAT* polymorphisms and vitiligo risk in the Chinese population. Therefore, we hypothesized that the three *CAT* gene polymorphisms (−89A>T, 389C>T, and 419C>T) were associated with the risk of vitiligo (Figure 1a) and tested this hypothesis in our hospital-based case–control study of 749 vitiligo patients and 763 vitiligo-free control subjects.

RESULTS
Characteristics of study subjects
This analysis included 749 Han Chinese patients with vitiligo and 763 age- and sex-matched controls. The demographic and clinical characteristics and the frequencies of alleles and genotypes in control and vitiligo patients were provided as we have described previously. The mean age was 24.7 ± 13.6 years for cases and 26.4 ± 13.3 years for controls ($P = 0.667$). The frequency distributions in men and women were 55.3% ($n = 414$) and 44.7% ($n = 335$), respectively, in the cases and 54.1% ($n = 413$) and 45.9% ($n = 350$) in the controls ($P = 0.655$) (Liu *et al.*, 2009).

Genotype distribution of *CAT* polymorphisms between cases and controls
The distributions of *CAT* −89A>T and *CAT* 389C>T genotypes among cases and controls are shown in Table 1. The distributions of all genotypes among control subjects were in agreement with the Hardy–Weinberg equilibrium ($\chi^2 = 0.473$, $P = 0.492$ for −89A>T; and $\chi^2 = 0.006$,
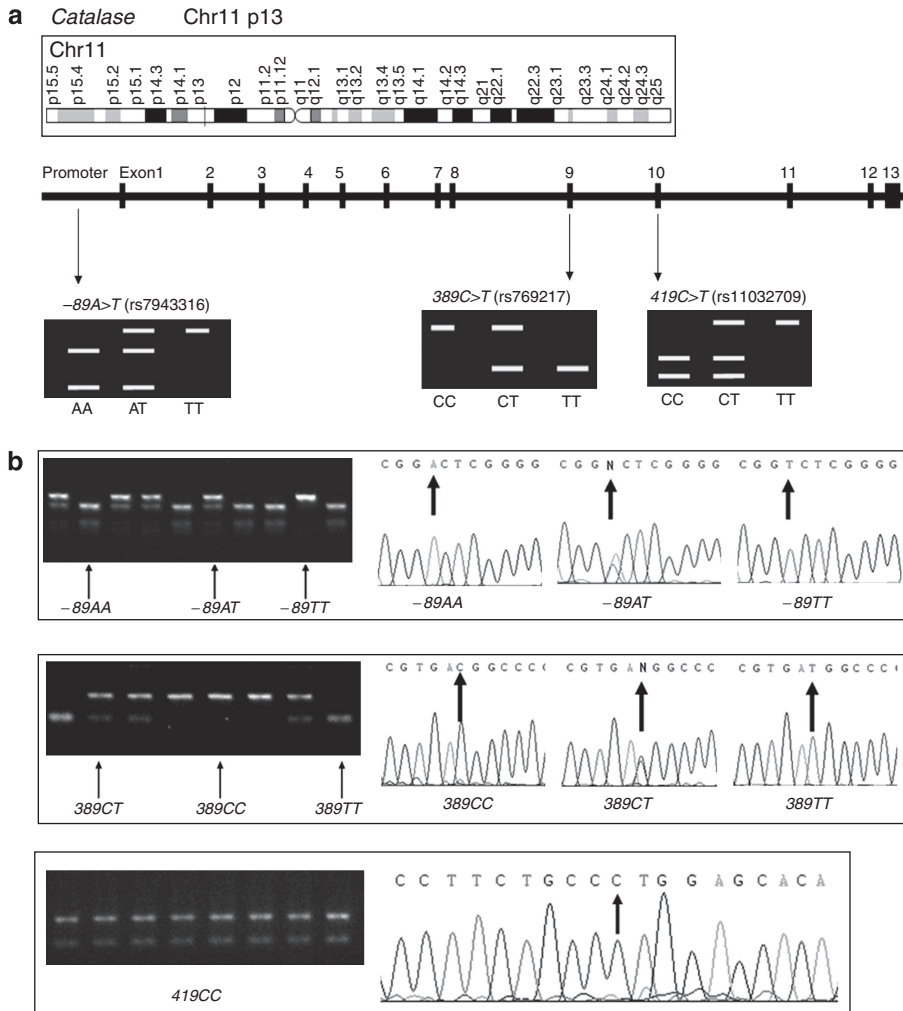
Figure 1. Location and detection of catalse (CAT) and single-nucleotide polymorphisms (SNPs). (**a**) *CAT* gene structure and the location of selected SNPs. The human *CAT* gene is mapped to chromosome 11p13. The rs7943316 is an A-to-T transition at −89 base pairs from the transcription site. The rs769217 is a C-to-T substitution in exon 9 and rs11032709 is a C-to-T substitution in exon 10. (**b**) Sequence analyses of the *CAT* PCR products using an ABI PRISM 3700 (Carlsbad, CA) automatic sequencer.

$P=0.937$ for *389C>T*). The *CAT* -89 variant *T* allele frequency was significantly higher among the cases than among the controls ($P=0.001$). Consistent with the *T* allele distribution, the frequencies of variant genotypes (*AT* and *TT*) were significantly higher among the cases than among the controls (48.5 vs. 39.2% and 9.2 vs. 8.2%, $P<0.001$). When we used the *AA* genotype as the reference, we found that the *AT* genotype was associated with a higher risk of vitiligo (adjusted odds ratio (OR) 1.54; 95% confidence interval (CI) 1.25–1.91), whereas the *TT* genotype was associated with a borderline increased vitiligo risk (adjusted OR 1.40; 95% CI 0.96–2.03). We found that the trend test was also significant ($P_{trend} < 0.001$). There was no evidence that *CAT 389C>T* was associated with susceptibility to vitiligo ($P=0.604$ for variant *T* allele frequency). For *CAT 419C>T*, the *T* allele could not be detected in any of the control subjects or the vitiligo patients; thus all individuals recruited in this study were homozygous for the *C* allele.

Association between *CAT* haplotypes and risk of vitiligo
The interaction of multiple SNPs within a haplotype can affect biological phenotype (Drysdale *et al.*, 2000). Hence, we analyzed the effect of *CAT* $-89A>T$ and *CAT 389C>T* on the risk of vitiligo in the context of haplotype, using SAS software (version 10.0; SAS Institute, Cary, NC). We observed

that the two *CAT* SNPs were in linkage disequilibrium ($D=0.652$, $P<0.001$), suggesting that a joint effect between the two SNPs may exist. Four possible *CAT* haplotype alleles were derived from the known genotypes (i.e., $A_{-89}C_{389}$, $A_{-89}T_{389}$, $T_{-89}C_{389}$, and $T_{-89}T_{389}$ alleles), and the distributions of the four haplotypes between the cases and the controls were statistically significantly different ($P<0.001$; Table 2). Compared with the $A_{-89}C_{389}$ haplotype, the haplotypes containing the $-89T$ allele were associated with an increased risk of vitiligo (for $T_{-89}C_{389}$, adjusted OR 1.90, 95% CI 1.26–2.86; and for $T_{-89}T_{389}$, adjusted OR 2.80, 95% CI 1.24–6.30.)

Risk of vitiligo associated with *CAT* $-89A>T$ genotype by *389C>T* genotype
We also examined whether there was a statistical interaction between *CAT* $-89A>T$ and *389C>T* (Table 3). Participants carrying only one risk genotype of $-89TT$ or *389TT* had an OR of 1.80 (95% CI 1.08–3.00) or 1.38 (95% CI 0.91–2.10) for developing vitiligo, respectively, compared with those who were homozygous wild type for both SNPs. It was observed that the patients carrying the *CAT* $-89AT$ genotype were more likely to carry the *CAT 389TT* genotype than the controls. Patients carrying two risk genotypes of $-89AT$ and *389TT* had an OR of 3.00 (95% CI 1.59–5.64), which is larger than the product ($1.80 \times 1.38 = 2.48$) of the OR for participants carrying only one risk genotype ($P<0.01$, test for overall interaction), compared with those who were homozygous wild type for both SNPs. These results suggest an interaction between the *CAT* $-89A>T$ and the *CAT 389C>T* genotypes intensifying the risk of developing vitiligo.

Logistic regression analysis of CAT activity in vitiligo patients and controls
We tested serum CAT activity in 244 vitiligo patients and 259 age- and sex-matched controls whose demographic and clinical characteristics and frequencies of alleles and genotypes were in concordance with the overall samples (744 cases and

Table 1. Genotype and allele frequencies of the *CAT* polymorphism among the cases and controls and associations with the risk of vitiligo

Genotype	Cases (N=749)		Controls (N=763)[1]		*P*-value[2]	OR (95% CI)[3]
	N	%	N	%		
$-89A>T$						
AA	317	42.3	401	52.6	<0.001*	1.00 (Reference)
AT	363	48.5	299	39.2		1.54 (1.25–1.91)
TT	69	9.2	63	8.2		1.40 (0.96–2.03)
T allele	—	33.4	—	27.9	0.001	—
						$P_{trend} <0.001$[4]
$389C>T$						
CC	240	32.0	256	33.6	0.822	1.00 (Reference)
CT	372	49.7	371	48.6		1.07 (0.85–1.34)
TT	137	18.3	136	17.8		1.07 (0.80–1.44)
T allele	—	43.1	—	42.1	0.604	—

Abbreviations: CAT, catalase; CI, confidence interval; OR, odds ratio.
[1]The observed genotype frequencies among the controls were in agreement with the Hardy–Weinberg equilibrium ($\chi^2=0.473$, $P=0.492$ for $-89A>T$; and $\chi^2=0.006$, $P=0.937$ for *389C>T*).
[2]Two-tailed χ^2 test for either genotype distributions or allele frequencies between the cases and controls: * for distribution of three genotypes.
[3]ORs were obtained from a logistic regression model with adjustment for age and sex.
[4]*P*-values were obtained in a logistic regression model adjusted by age and sex.

Table 2. Frequencies of the *CAT* haplotypes among the cases and controls and associations with the risk of vitiligo

Haplotype allele[1]	Cases (N=1,498)		Controls (N=1,526)		OR (95% CI)[2]
	N	%	N	%	
$A_{-89}C_{389}$	433	28.9	520	34.1	1.00 (Reference)
$A_{-89}T_{389}$	565	37.7	581	38.1	1.29 (0.89–1.86)
$T_{-89}C_{389}$	419	28.0	363	23.8	1.90 (1.26–2.86)
$T_{-89}T_{389}$	81	5.4	62	4.0	2.80 (1.24–6.30)

Abbreviations: CAT, catalase; CI, confidence interval; OR, odds ratio.
[1]LD (linkage disequilibrium) analysis: $D=0.652$, $P<0.001$ (for the differences in haplotype allele distributions between cases and controls).
[2]ORs were obtained from a logistic regression model with adjustment for age and sex.

Table 3. Risk of vitiligo associated with *CAT Hin*fI genotypes by *Bst*XI genotype

	−89A>T genotype					
389C>T genotype	*AA*[1]	OR (95% CI)[2]	*AT*[1]	OR (95% CI)[2]	*TT*[1]	OR (95% CI)[2]
CC	61/98	1.00 (Reference)	127/112	1.82 (1.21–2.75)	52/46	1.80 (1.08–3.00)
CT	158/190	1.33 (0.90–1.95)	199/167	1.90 (1.30–2.78)	15/14	1.78 (0.80–3.95)
TT	98/113	1.38 (0.91–2.10)	37/20	3.00 (1.59–5.64)	2/3	1.08 (0.18–6.62)

Abbreviations: *CAT*, catalase; CI, confidence interval; OR, odds ratio.
[1]Number of cases/number of controls.
[2]ORs were obtained from a logistic regression model with adjustment for age and sex.
$P<0.001$, test for overall interaction.

Table 4. Logistic regression analysis of CAT activity (nmol min^{-1} ml^{-1}) in vitiligo patients and controls

	Cases (*N*=244)		Controls (*N*=259)		
Activity	N	%	N	%	OR (95% CI)[1]
By median					
<76.15	165	66.0	150	50.0	1.00 (Reference)
⩾76.15	85	34.0	150	50.0	0.52 (0.37–0.74)
By tertile					
<65.60	134	53.6	99	33.0	1.00 (Reference)
65.60–86.97	61	24.4	101	33.7	0.46 (0.30–0.69)
⩾86.97	55	22.0	100	33.3	0.42 (0.28–0.65)
Trend test	—	—	—	—	$P<0.001$[2]

Abbreviations: *CAT*, catalase; CI, confidence interval; OR, odds ratio.
[1]ORs were obtained from a logistic regression model with adjustment for age and sex.
[2]Adjusted for age and sex.

Table 5. Risk of vitiligo associated with *CAT Hin*fI genotypes by CAT activity

	CAT -89A>T (cases/controls)			
Activity	1 And 2 risk genotypes	OR (95% CI)[1]	0 Risk genotype	OR (95% CI)[1]
By median				
<76.15	80/77	1.00 (Reference)	85/73	1.12 (0.72–1.74)
⩾76.15	67/66	0.98 (0.62–1.56)	18/84	0.21 (0.12–0.38)
By tertile				
<65.60	62/52	1.00 (Reference)	72/48	1.26 (0.75–2.12)
86.97–65.60	38/43	0.75 (0.42–1.33)	23/58	0.34 (0.18–0.62)
⩾86.97	47/48	0.83 (0.48–1.44)	8/51	0.14 (0.06–0.31)
Trend test	—	—	—	$P<0.001$[2]

Abbreviations: *CAT*, catalase; CI, confidence interval; OR, odds ratio.
[1]ORs were obtained from a logistic regression model with adjustment for age and sex.
[2]Adjusted for age and sex.

763 controls; data not shown). We performed a logistic regression analysis of CAT activity in vitiligo patients and controls (Table 4). When the CAT activity was dichotomized by the median activity of the controls, higher activity was associated with a 0.52-fold reduced risk for vitiligo (95% CI 0.37–0.74). Furthermore, when the CAT activity was divided into quartiles according to the controls' activity, a dose–response relationship between decreased risk and increased activity was evident: suboptimal (upper tertile), intermediate (midtertile), and efficient (lower tertile) activities were associated with the adjusted ORs of 1.00, 0.46 (95% CI 0.30–0.69), and 0.42 (95% CI 0.28–0.65; P_{trend} <0.001), respectively.

Risk of vitiligo associated with *CAT* −89A>T genotypes by CAT activity

We further estimated the risk of vitiligo associated with *CAT* −89A>T genotypes by CAT activity (Table 5). *CAT* −89A>T genotypes were divided into two categories: 1 and 2 risk genotypes (−89AT or −89TT) and 0 risk genotype (−89AA). When we used 1 and 2 risk genotypes as the reference (−89AT or −89TT), the individuals with 0 risk genotype and higher

CAT activity (⩾76.15) showed a larger decrease in the risk of vitiligo (adjusted OR 0.21, 95% CI 0.12–0.38). Consistent with the preceding results, when the CAT activity was divided into quartiles according to the controls' activity, a dose–response relationship between decreased risk and increased activity was obvious (P_{trend} <0.001). The −89AA genotype with higher CAT activity was associated with increased protection against vitiligo (86.97–65.6, adjusted OR 0.34, 95% CI 0.18–0.62; ⩾86.97, adjusted OR 0.14, 95% CI 0.06–0.31).

DISCUSSION

The past two decades have seen reports of increased epidermal oxidative stress in the cutaneous epidermis of vitiligo patients and suggesting that increased prooxidant activities and reduced antioxidant activities can lead to elevated levels of H_2O_2 (Hazneci *et al.*, 2005; Spencer *et al.*, 2007). CAT has an important role in protecting cells against severe oxidative stress, and allelic variants in the *CAT* gene could have deleterious effects on the expression or function

of CAT, which may result in high sensitivity to H_2O_2 (Góth *et al.*, 2004).

We investigated whether genetic polymorphisms in *CAT* contribute to the risk of developing vitiligo. In the hospital-based case–control study of 749 vitiligo patients and 763 vitiligo-free controls, we demonstrated a statistically significant increased risk of vitiligo associated with the variant *CAT −89A>T* genotypes, but no evident risk was associated with the *389C>T* or *419C>T* variant genotypes. In the combined analysis of the variant alleles of *CAT*, the genotypes with more variant alleles were associated with a higher risk of vitiligo. Moreover, we observed a significant interaction between the *CAT −89AT* genotype and the *CAT 389C>T* variant genotype. The results suggested that the *CAT* promoter region polymorphisms may affect CAT expression and the risk of vitiligo in the Chinese population.

The variant promoter sequence region can bind different transcription factors, resulting in observed differences in promoter activity, which may provide a clue for future epidemiological association studies (Forsberg *et al.*, 2001). The association of *CAT −89A>T* SNP polymorphisms with vitiligo risk has been studied in the Korean population (Park *et al.*, 2006). But in the small sample size study of 118 cases and 200 controls, no evidence was found that the *−89A>T* variant genotype was associated with a significantly increased risk of vitiligo. In contrast, our study showed that the *−89AT* genotype was associated with a significantly increased risk of vitiligo in Chinese people, whereas the *−89TT* genotype was associated with a borderline increased vitiligo risk. Compared with homozygous carriers of the *T* allele, there seemed to be a disparity in the increased risk for heterozygotes. We inferred that there might be unknown polymorphisms nearby that interfered with the phenotype, and hence the heterozygotes showed a higher risk.

The *CAT 389C>T* and *419C>T* genotypes result in silent substitution of aspartic acid and leucine residue, respectively. Compared with the previous data, our results indicate that the genotype distributions of the two *CAT* polymorphisms vary with ethnicity. *CAT 389C>T* was associated with susceptibility to vitiligo in a North American population, and similar results were found in an English population in the United Kingdom (Casp *et al.*, 2002; Gavalas *et al.*, 2006). *CAT 389C>T* may be linked to other CAT mutations that are deleterious to the expression or the activity of CAT, but no evidence showed that *CAT 389C>T* was associated with the susceptibility to vitiligo (adjusted OR 0.48, 95% CI 0.26–0.90). For *CAT 419C>T*, nearly all the subjects analyzed in a North American population were homozygous for the *T* allele (Casp *et al.*, 2002). Conversely, the *T* allele could not be detected in any of our vitiligo patients, indicating that all individuals recruited in our study were homozygous for the *C* allele. In the Chinese population, *CAT 389C>T* and *419C>T* have no influence on the risk of vitiligo.

The interaction of multiple SNPs within a haplotype can affect biological phenotype (Drysdale *et al.*, 2000). Although *CAT 389C>T* had no influence on the risk of vitiligo, we found that the *CAT −89A>T* and *389C>T* variants may have a joint effect on the risk for vitiligo when we analyzed the effects of these two *CAT* polymorphisms together in the context of haplotype. Compared with the wild-type haplotype, the haplotypes containing more variant alleles were associated with a higher risk of vitiligo. Patients with the $T_{−89}T_{389}$ haplotype were at nearly threefold increased risk, and the *−89AT+ TT389CT+ TT* combined genotype was significantly associated with susceptibility to vitiligo. The multiplicative interaction between *CAT −89AT* and *389C>T* genotypes suggested a statistical interaction between the two SNPs (Brennan, 2002). The biological phenotype is the result of the synergistic effect of *CAT −89A>T* and *389C>T* within a haplotype.

As early as in the 1990s, decreased CAT activity was found in the epidermis of vitiligo patients, resulting in more sensitivity of melanocytes to oxidative stress (Schallreuter *et al.*, 1991). Low CAT activity in blood was also proven to be associated with diabetes or dyslipidemia (Kosmidou *et al.*, 2009; Takemoto *et al.*, 2009). On one hand, low CAT levels reflect inactivation by high concentrations of H_2O_2; on the other, variants in the *CAT* gene have detrimental effects on the expression or function of CAT (Wood *et al.*, 2008). We evaluated serum CAT activity, and a dose–response relationship existed in the deleterious effect of low CAT levels. Comprehensive analysis of CAT activity and *CAT −89A>T* genotypes suggested that, much as with antioxidants, the *−89A>T* wild genotype played an important role in protection from oxidative damage. Individuals with higher CAT activity and the *−89A>T* wild genotype might have a minor risk for developing vitiligo compared with those with low CAT activity and a *−89A>T* variant genotype. High CAT activity and the *CAT 89A* allele indicate protection against oxidative damage in the Chinese population.

In summary, to our knowledge, this is the first report that *CAT* promoter region polymorphisms may decrease CAT expression and activity and affect the risk of vitiligo in the Chinese population. The *CAT −89A>T* variant genotypes were associated with susceptibility to vitiligo and had interactions with other polymorphisms in exons. There also exists a dose–response relationship between increased risk and decreased CAT activity. Because of uncontrolled biases in the selection of subjects and limited sample size, larger and population-based studies with inclusion of more SNPs in genes involved in oxidant stress are warranted to confirm these findings. Furthermore, studies are necessary to evaluate whether *CAT −89A>T* creates a transcriptional factor binding site or other potential transcriptional mechanisms behind it.

MATERIALS AND METHODS
Study subjects
A total of 749 vitiligo patients and 763 control subjects were recruited from Xijing Hospital, Fourth Military Medical University. Only Han Chinese patients were included in this analysis to avoid the variance of genotype frequencies among different ethnic groups. None of the patients had had any therapy in the past 6 months. The vitiligo-free control subjects were individuals who had come to the

hospital for a health examination and had no blood relationship with the vitiligo patients. We used a questionnaire to obtain demographic and other information (stage, clinical type, onset age, and family history), and frequency matched the controls to the cases by age (± 5 years), sex, and ethnicity. Genomic DNA was extracted by standard procedure from the peripheral blood of each subject, collected after informed consent had been obtained. The research protocol was approved by the ethics review board of the Fourth Military University. The study was conducted according to the Declaration of Helsinki Principles.

Polymorphisms and genotyping

The *CAT* (−89A>T, 389C>T, and 419 C>T) polymorphisms were determined using the PCR–restriction fragment length polymorphism method. PCR amplications were generated using the following primers: for *CAT* −89A>T, forward 5'-AATCAGAAGGCAGTCC TCCC-3' and reverse 5'-TCGGGGAGCACAGAGTGTAC-3' (product of 250 bp); for *CAT 389C>T*, forward 5'-GCCGCCTTTTTGCCTA TCCT-3' and reverse 5'-TCCCGCCCATCTGCTCCAC-3' (product of 202 bp); for *CAT 419C>T*, forward 5'-CCTAAGTGCATCTGGGT GGT-3' and reverse 5'-TACATCAGACAGTTGGGGCA-3' (product of 230 bp). PCR was performed using 50 ng DNA as a template under the following conditions: 94 °C for 10 minutes, then 30 cycles of 94 °C for 55 seconds, annealing temperature (−89A>T at 66 °C, 389C>T at 69.5 °C, and 419C>T at 60.5 °C) for 55 seconds and 72 °C for 90 seconds, and final extension at 72 °C for 10 minutes. After affinity membrane purification, the PCR products were subjected to cycle sequencing with the respective forward and reverse primers. The *Hin*fI, *Bst*XI, and *Bst*NI restriction enzymes (New England Biolabs, Ipswich, MA, UK) were used to delineate the *CAT* −89A>T, 389C>T and 419C>T polymorphisms, respectively. Restriction fragment length polymorphism–PCR was used to analyze −89A>T, using *Hin*fI to digest the 250 bp PCR amplification products, which resulted in 177 and 73 bp fragments in the case of the *A* allele. In addition, *389C>T* was analyzed by restriction fragment length polymorphism–PCR with *Bst*XI, which cuts only the *T* allele, resulting in 108 and 94 bp fragments. The *419C>T* polymorphism was analyzed by restriction fragment length poly-morphism–PCR with *Bst*NI, which cuts only the *C* allele (Figure 1b). More than 10% of the samples were randomly selected and genotyped again with the same method to test the discrepancy rate, and the results were 100% concordant.

CAT activity

The blood of each subject was collected without anticoagulant and then clotted for 30 minutes at room temperature. Samples were centrifuged at 2,000*g* for 15 minutes at 4 °C. The top yellow serum layer was pipetted off and frozen at −80 °C. Serum activity of CAT was measured by peroxidatic function using the Cayman CAT assay kit (Ann Arbor, MI, catalog no. 707002). The detection was based on the reaction of the enzyme with methanol in the presence of an optimal concentration of H_2O_2.

Statistical analysis

We used the χ^2 test to evaluate the differences in frequency distributions of selected demographic variables, including each allele and genotype of the *CAT* polymorphisms and serum CAT activity between the cases and the controls. Because of the potential joint effects of the different *CAT* polymorphisms on vitiligo risk, the associations between the combined genotypes of the *CAT* −89A>T, *CAT* 389C>T, and *CAT* 419C>T polymorphisms and vitiligo risk were evaluated. Unconditional univariate and multivariate logistic regression analyses were performed to obtain the crude and adjusted ORs for the risk of vitiligo and their 95% CIs. The multivariate adjustment included age and sex. Two-tailed tests of statistical significance were performed using the SAS software (version 10.0; SAS Institute). *P*-values of <0.05 were considered statistically significant.

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