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An association study between the genetic polymorphisms within *TBX1* and schizophrenia in the Chinese population

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Abstract

The strong association between common psychiatric disorders and the 22q11.2 microdeletion suggests that haploinsufficiency of one or more genes in the region confers susceptibility to these disorders. Recent mouse studies have shown that the T-box 1 (*TBX1*) gene in the 22q11.2 region can cause prepulse inhibition (PPI) impairment in the heterozygous state. A study has also shown that phenotypic features of 22q11 deletion syndrome (22q11DS) were segregated with an inactivating mutation of *TBX1* in one family, suggesting that the *TBX1* gene plays a role in the pathogenesis of some psychiatric disorders. We performed an association study between three single nucleotide polymorphisms (SNPs) in the *TBX1* gene and schizophrenia. However, we found no significant difference in the genotype or allele distributions between the 328 schizophrenics and 288 controls for any of the polymorphisms, nor was there any haplotype association. Our data suggest that the genetic polymorphisms within *TBX1* do not confer an increased susceptibility to schizophrenia in the Chinese population.

Keywords: Schizophrenia; *TBX1*; Genetic polymorphisms; Association; Chinese

Schizophrenia is a severe mental illness, which affects as much as 1% of the world's population and typically causes severe functional decline, significant suffering, and lifelong disability [10]. The multifactorial polygenic model provides the most widely accepted explanation the mode of inheritance responsible for the familial distribution of schizophrenia. It is likely therefore that a variety of genetic, environmental and stochastic factors are involved in its etiology. However, it is also possible that specific genes play a major role in susceptibility to schizophrenia, and efforts to identify such genes have indicated that those involved in 22q11.2 deletion syndrome substantially increases susceptibility to schizophrenia and that 22q11DS-schizophrenia is a true genetic subtype of schizophrenia [4,15].

Caused by a heterozygous multigene deletion, 22q11DS is a relatively common genetic disorder, occurring in one in every 4000 births [6,29]. 22q11DS presents a variable phenotype that can include specific congenital heart defects, thymic hypoplasia, hypocalcemia, velopharyngeal defects and it is associated with several diagnostic labels including DiGeorge syndrome (DGS), velocardiofacial (or Shprintzen) syndrome (VCFS), conotruncal anomaly face, Cayler syndrome and Opitz GBBB syndrome [22]. 22q11DS is also associated with profound neurodevelopmental, cognitive, behavioral and psychiatric symptoms [12,23,27], Several studies have suggested linkage between 22q11 and schizophrenia [18,28,31], An initial study and a follow-up replication study both estimated that 25–31% of patients with the 22q11 microdeletion met diagnostic criteria for schizophrenia or schizoaffective disorder [17,21]. This

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suggests that the risk of schizophrenia for a patient with a 22q11 microdeletion may be approximately 25–31 times the 1% chance for the general population. Some genes, for example, the Catechol-*O*-methytransferase (*COMT*), Proline dehydrogenase (*PRODH*), Zinc finger DHHC domain containing 8 (*ZDHHC8*), Claudin-5 (*CLDN5*), DiGeorge syndrome critical region protein 14 (*DGCR14*), DiGeorge syndrome critical region protein 2 (*DGCR2*) and so on, from the region deleted in 22q11DS have been reported as candidate genes for association with schizophrenia. Of these, *COMT* has been the most extensively studied.

TBX1, a 22q11.2 gene encoding a T-box transcription factor, is located in the 1.5-Mb region deleted in 22q11DS. *TBX1* point mutations have been identified in five individuals with classic 22q11DS, but without the common chromosomal deletion [33], suggesting that *TBX1* is responsible, at least in part, for physical deficiency of 22q11DS. The contribution of *TBX1* haploinsufficiency to psychiatric disease has been suggested by mouse models of *TBX1* and the identification of a family with VCFS in a mother and her two sons. Paylor et al. [19] mapped PPI deficits in a panel of mouse mutants that carry deletions and defined a PPI critical region comprising four genes. This study also revealed that either *Tbx1*± or *Gnb1l*± mice had reduced PPI because of single-gene mutants. Expression of *Tbx1* is limited to the vasculature in brains of full-term mouse embryos and adult mice [19]. A role for the microvasculature in the pathophysiology of schizophrenia has been proposed on theoretical grounds because microvascular damage is consistent with both the developmental and degenerative models of schizophrenia [11]. In a family with VCFS, all three patients had a null mutation of *TBX1*. A diagnosis of Asperger syndrome in one of the sons was made after psychiatric assessment [19], suggesting that in humans, haploinsufficiency of *TBX1* may be related to behavioral abnormalities.

As suggested by these findings, the *TBX1* gene may play a role in the pathogenesis of schizophrenia and in this study, we screened genetic polymorphisms within *TBX1* to investigate its potential association with the disease.

A total of 328 schizophrenic patients (203 males and 125 females) with a mean age of 47.05 years (S.D. = 11.31 years) were recruited from the Shanghai Mental Health Center. Diagnosis was made by two qualified psychiatrists with more than 10 years clinical experience, on the basis of the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) criteria for schizophrenia [1]. Structured Clinical Interview for DSM-IV Axis I disorders (SCID-I) was used to assess the patients [9]. Medical records and all other available information were also referred for diagnosis. All patients were unrelated. A total of 288 unrelated control individuals (159 males and 129 females) were selected from attendees at hematology clinics in Shanghai (with a mean age of 35.02, S.D. = 8.16). They showed good social functioning and reported themselves to be in good health and the subjects with a personal or family history of mental illness were excluded by psychiatric colleagues. A standard informed consent, which was reviewed and approved by the Shanghai Ethical Committee of Human Genetic Resources, was given by all the participants after the nature of study had been fully explained. All subjects recruited for this study were Han Chinese in origin.

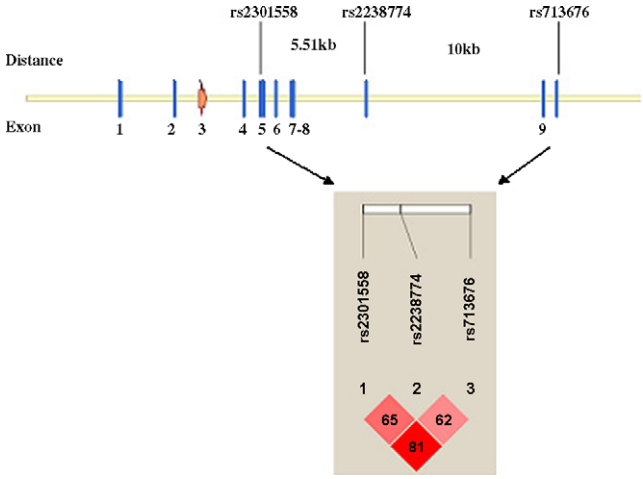

Fig. 1. Genomic structure of TBX1 and the location of SNPs included in the present study. The distance and the pair-wise linkage disequilibrium (LD) between adjacent markers are also shown in the figure.

Each patient and control agreed to provide 5 ml of venous blood for the study. Genomic DNA was prepared from the venous blood using the standard phenol chloroform extraction method. Polymerase chain reaction (PCR) was performed on GeneAmp PCR system 9700 (Applied Biosystems). The genomic structure of TBX1 and location of the SNPs genotyped in the present study are show in Fig. 1. We examined three tagging single nucleotide polymorphisms (tag-SNPs), rs2301558, rs2238774 and rs713676 in the *TBX1* gene using Haploview available at http://www.broad.mit.edu/mpg/haploview/index.php [3] and $r^2 > 0.8$. With these three high polymorphism tag-SNPs from our Han Chinese sample, we were able to cover the whole linkage disequilibrium (LD) structure of *TBX1*. All SNPs were genotyped by ligase detection reaction.

We used SHEsis (http://www.bio-x.cn/analysis/) to perform Hardy–Weinberg equilibrium tests, pairwise linkage disequilibrium estimation, haplotype reconstruction and case–control studies [25]. This program implemented a Monte Carlo simulation strategy [24]. A chi-square test and odds ratio test were used to compare the discrepancies of allele and genotype frequencies on single locus and multi-loci haplotypes between cases and controls. Pair-wise linkage disequilibrium of all pairs of SNPs was estimated using the SHEsis software and the extent of LD was measured by the standardized D'. This software uses an EM algorithm in haplotype reconstruction and frequency estimation. Those haplotypes with a frequency under 3% were excluded.

P values were two tailed and the statistical significance was set at $P < 0.05$. The G*Power program [8] was used to estimate the statistical power of our sample size.

A total of 328 cases and 288 controls were studied. The statistical power of rs2301558, rs2238774 and rs713676 were of 0.722, 0.947 and 0.930, respectively, assuming OR = 1.5 (95% CI) in our sample. The frequencies of alleles and genotypes are listed in Table 1. The genotypic distributions in both cases and controls were all in Hardy–Weinberg equilibrium. For the