Association of T-box gene polymorphisms with the risk of Wolff–Parkinson–White syndrome in a Han Chinese population

Bing Han, MDa,b,c,d, Yongxiang Wang, MDa,c,d, Jing Zhao, MDa,c,d, Qingsu Lan, MD, Jin Zhang, MDa,c,d, Xiaoxue Meng, MDa,c,d, Jianjian Jin, MD, Ming Bai, MDa,b,c,d, Zheng Zhang, MDa,b,c,d

Abstract
Abnormal development of the atrioventricular (AV) ring can lead to the formation of a bypass pathway and the occurrence of Wolff–Parkinson–White (WPW) syndrome. The genetic mechanism underlying the sporadic form of WPW syndrome remains unclear. Existing evidence suggests that both T-box transcription factor 3 (TBX3) and T-box transcription factor 2 (TBX2) genes participate in regulating annulus fibrosus formation and atrioventricular canal development. Thus, we aimed to examine whether single-nucleotide polymorphisms (SNPs) in the TBX3 and TBX2 genes confer susceptibility to WPW syndrome in a Han Chinese Population. We applied a SNaPshot SNP assay to analyze 5 selected tagSNPs of TBX3 and TBX2 in 230 patients with sporadic WPW syndrome and 231 sex- and age-matched controls. Haplotype analysis was performed using Haploview software. Allele C of TBX3 rs1061657 was associated with a higher risk of WPW syndrome (odds ratio [OR] = 1.41, 95% confidence interval [CI]: 1.06–1.83, P = .011) and left-sided accessory pathways (OR = 1.40, 95% CI: 1.07–1.84, P = .016). However, allele C of TBX2 rs8853 was likely to reduce these risks (OR = 0.71, 95% CI: 0.54–0.92, P = .011; OR = 0.70, 95% CI: 0.53–0.92, P = .011, respectively). The data revealed no association between TBX3 rs77412687, TBX3 rs2242442, or TBX2 rs75743672 and WPW syndrome. TBX3 rs1061657 and rs8853 are significantly associated with sporadic WPW syndrome among a Han Chinese population. To verify our results, larger sample sizes are required in future studies.

Abbreviations: AV = atrioventricular, FDR = false discovery rate, LD = linkage disequilibrium, SNP = single-nucleotide polymorphism, TBX2 = T-box transcription factor 2, TBX3 = T-box transcription factor 3, UTR = untranslated region, WPW = Wolff–Parkinson–White.

Keywords: genetic susceptibility, single-nucleotide polymorphism (SNP), T-box transcription factor 2 (TBX2), T-box transcription factor 3 (TBX3), Wolff–Parkinson–White syndrome (WPW)

1. Introduction
Wolff–Parkinson–White (WPW) syndrome, an arrhythmogenic defect characterized by 1 or more accessory pathways that cause premature ventricular excitation, is present in some patients with accessory atrioventricular (AV) connections.[1] The prevalence of WPW syndrome is estimated to be 1 to 3/1000 individuals and is mostly isolated and sporadic.[2] AV reciprocating tachycardia attributed to accessory pathways is the second most common cause of paroxysmal supraventricular tachycardia.[3] Mutations in the protein kinase adenosine monophosphate (AMP)-activated noncatalytic subunit gamma 2 (PRKAG2) gene have been described in individuals with familial WPW syndrome associated with hypertrophic cardiomyopathy and/or AV block.[4,5] However, protein kinase AMP-activated noncatalytic subunit gamma 2 mutations are not commonly associated with sporadic WPW syndrome.[6] Hsu and colleagues[7] demonstrated that the distribution of bypass positions is dependent on age and sex, and they speculated that the formation of accessory connections does not occur randomly, but is partly due to malformation of the annulus fibrosus and developmental dysregulation of the AV canal myocardium.[8–10] One study using transgenic mice indicated that imperfect gene regulation and patterning within the AV canal myocardium might cause malformation of the annulus fibrosus, the formation of accessory pathways, and ventricular preexcitation.[11] Therefore, we hypothesized that the genes involved in annulus fibrosus formation and AV canal myocardium development in the stages of embryonic development would be related to sporadic WPW syndrome in humans.

As a member of the ancient T-box gene family, TBX3 is conserved in a wide spectrum of species. TBX3 is also a crucial...
developmental regulator of the heart.\textsuperscript{[12–15]} TBX2 and TBX3 are highly related members in the T-box gene family, as they contain some identical domains, namely a T-box DNA binding domain, 2 repression domains, and an activation domain. Both TBX2 and TBX3 are required for development of the AV canal.\textsuperscript{[11,16]} Previous studies have demonstrated the role of TBX3 and TBX2 deficiency in the development of heart defects, including preexcitation.\textsuperscript{[11,17]} However, there is still lack of a systematic research on their roles in the more common sporadic form of WPW syndrome in humans. In this study, we hypothesized that TBX3 and TBX2 polymorphisms are associated with the formation of accessory AV connections in isolated WPW syndrome. We used SNaPshot analysis to detect TBX3 and TBX2 single nucleotide polymorphisms (SNPs). In addition, the relationship between TBX3 and TBX2 genetic variants and the risk of WPW syndrome in the Han Chinese population was analyzed.

\section{Materials and Methods}

\subsection{Study population}

This study population included 230 patients with sporadic WPW syndrome (148 men and 82 women, age = 46.0 ± 15.2 years). These patients were recruited from our electrophysiology laboratory registry of patients with paroxysmal supraventricular tachycardia and were involved in an electrophysiological study from January 2013 to April 2020. No patient had a clear family history of WPW syndrome, associated hypertrophic cardiomyopathy, or other cardiac lesions, as confirmed by transthoracic echocardiography on all patients. Healthy controls (143 men and 88 women, age = 47.6 ± 14.4 years) were recruited from the population of patients admitted to The First Hospital of Lanzhou University for physical examination from January 2013 to March 2020. These controls did not have a history of arrhythmia and had normal electrocardiogram and echocardiography results. All participants signed an informed consent form for genetic screening. The protocol of the study conformed with the Declaration of Helsinki. The Ethics Committee of The First Hospital of Lanzhou University issued the ethical approval for this study.

WPW syndrome was first diagnosed based on the appearance of a short PR interval on the electrocardiogram (PR < 120 ms) and the existence of a delta wave. Then, patients with manifest and concealed accessory AV connections were confirmed by a clinically indicated invasive electrophysiological study. Therefore, the diagnostic criteria for the inclusion of patients were as follows: during transesophageal atrial pacing before the electrophysiological study, supraventricular tachycardia was induced with a ventricular–atrial interval of >70 ms; at the time of electrophysiological study, supraventricular tachycardia was induced with preexcitation of the atria by ventricular extrastimulus during refractoriness of the His bundle. Anterograde or retrograde conduction over an accessory pathway was revealed at the time of the study. The location of the bypass was also determined during the cardiac electrophysiological examination. All individuals underwent radiofrequency ablation of an accessory pathway, and all ablations were successful.

\subsection{SNP selection and genotyping}

Information on the genetic variants present in the population for TBX3 and TBX2 genes was obtained from the dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/). Candidate tagSNPs were filtered as dbSNP of TBX3 and TBX2 from the 1000 genomes database into Haplovie. Furthermore, tagSNPs were selected if they had a minor allele frequency of ≥ 0.05 and the setting \( r^2 \) exceeding 0.8 in the Han Chinese population of Beijing. Then, the tagSNPs were preferentially selected in potentially functional regions like exons, promoters, untranslated regions (UTRs), and introns.

Genomic DNA was obtained from 2 mL of blood in EDTA anticoagulation tubes using the phenol–chloroform–isopropyl alcohol method. The SNaPshot (Applied Biosystems, Genesky Biotechnologies Inc., Shanghai) technique was used for SNP genotyping. Polymerase chain reaction and extension primers were designed using the online Primer3 software (http://bioinfo.ut.ee/primer3-0.4.0/). Shrimp alkaline phosphatase (Promega) and exonuclease I (Epicentre) were used to purify the polymerase chain reaction products, which were subsequently extended using the ABI SNaPshot Multiplex kit (Applied Biosystems). The results were analyzed using GeneMapper 4.0 (Applied Biosystems). As a quality control, 5% of genotyped samples were randomly selected to undergo repeated genotype testing for each locus. The results matched perfectly with the initial analysis. SNP details and Primer sequences are listed in Table 1.

\begin{table}[h]
\centering
\caption{Details of SNPs and primer sequences used in this study.}
\begin{tabular}{|l|l|l|l|l|l|l|l|l|l|}
\hline
\textbf{Gene} & \textbf{SNP ID} & \textbf{Alt} & \textbf{Ch} & \textbf{Region} & \textbf{Position} & \textbf{Primer} & \textbf{Sequence} \\
\hline
\textit{TBX3} & rs1061657 & T>C & 12 & 3'UTR & 115,108,136 & Forward & ACCCTCCCTGATCCTACATCTTGGCACCCTTCGCTACCTATCT \hline
& rs8853 & T>C & 12 & 3'UTR & 115,108,907 & Forward & CCACTCTTTTGGGCCTCAATCAGAAGCGCCAGAG \hline
& rs77412687 & A>G & 12 & 3'UTR & 115,108,334 & Forward & CCACTCTTTTGGGCCTCAATCAGAAGCGCCAGAG \hline
& rs2242482 & G>A & 12 & 5'UTR & 115,121,189 & Forward & TCCCCCTGGTACGGTACATATTTGACGTCCTACGATGCAGAC \hline
\textit{TBX2} & rs75743672 & C>A & 17 & Upstream & 59,477,903 & Forward & TCCCCCTGGTACGGTACATATTTGACGTCCTACGATGCAGAC \hline
\hline
\end{tabular}
\end{table}

Ch = chromosome, SNP = single-nucleotide polymorphism.

\subsection{Statistical analysis}

The SPSS 26.0 software package (SPSS Inc., Chicago, IL) and the Plink software (http://zzz.bwh.harvard.edu/plink/) were used to conduct the entire statistical analysis. A Student \( t \) test was applied to examine the differences in age between
the cases and controls. In addition, the discrepancies in sex distribution were analyzed by a chi-square test between the 2 groups. Hardy–Weinberg equilibrium was tested for both groups for each SNP employing Plink software with Fisher exact tests, and a P value of >.05 indicated a balanced genetic and Mendelian population. Logistic regression analysis was used to assess the risk of WPW syndrome associated with each SNP, and genetic models, such as dominant and recessive models, were applied. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to measure the association with each SNP. A P value of <.05 was considered significant. The Benjamini–Hochberg (1995) step up false discovery rate (FDR) control method was employed as an adjustment for multiple comparisons with Plink software. The FDR cutoff was set at 0.1. Haploview version 4.2 software (http://sourceforge.net/projects/haploview/) was used to perform the linkage disequilibrium (LD) analysis as well as haplotype detection and analysis.

3. Results

3.1. Demographic characteristics of the study population

In this study, we analyzed 230 patients with sporadic WPW syndrome and 231 healthy controls (Table 2). The average age of patients was 46.0 ± 15.2 (range, 12–76), while the mean age of healthy controls was 47.6 ± 14.4 (range, 15–77). Age and sex were well matched between cases and controls (P = .250 and .587, respectively).

3.2. Details of the chosen tagSNPs

Four tagSNPs of the TBX3 gene, corresponding to rs1061657, rs8853, rs77412687, and rs2242442, as well as rs75743672 of the TBX2 gene, were selected to analyze the association between TBX3 and TBX2 polymorphisms and the risk of WPW syndrome. Rs1061657, rs8853, and rs77412687 are localized to the 3′-UTR region, and rs2242442 is in the 5′-UTR region of TBX3. Rs75743672 resides in the upstream region of TBX2. Additional data are shown in Table 1.

3.3. Hardy–Weinberg equilibrium

Hardy–Weinberg equilibrium was tested for both groups for each SNP employing Plink software with Fisher exact test. This revealed that the control group was in genetic equilibrium for genotypes of rs1061657, rs8853, rs77412687, rs2242442, and rs75743672 polymorphisms (P > .05). Additional data are provided in Table 3.

3.4. Association of TBX3 and TBX2 polymorphisms and WPW syndrome

The association between TBX3 and TBX2 polymorphisms and WPW syndrome was investigated by logistic regression analysis (Table 4). The frequencies of allele C and genotype CC of rs1061657 were higher in patients than controls (OR = 1.41, 95% CI: 1.09–1.83, P = .010; OR = 2.24, 95% CI: 1.25–3.99, P = .006, respectively), whereas allele C and genotype CC of rs8853 were more frequent among controls (OR = 0.70, 95% CI: 0.54–0.92, P = .010; OR = 0.44, 95% CI: 0.23–0.83, P = .011, respectively). Subjects with dominant and recessive models of rs1061657 had a higher risk of WPW syndrome (TC + CC vs TT, OR = 1.54, 95% CI: 1.03–2.31, P = .035; CC vs TC + TT, OR = 1.81, 95% CI: 1.09–3.02, P = .022). Further, rs8853 was more likely to be protective for WPW syndrome under the dominant model (TC + CC vs TT, OR = 0.63, 95% CI: 0.43–0.92, P = .018). These associations persisted after adjusting for sex and age. However, after correcting for multiple testing, the differences between the 2 groups in dominant and/or recessive models disappeared. No significant differences were found in allelic or genotypic frequencies of other SNPs including TBX2 rs75743672 between the cases and controls.

3.5. TBX3 and TBX2 SNPs genotype frequencies in healthy controls and patients with different types and locations of accessory AV connections

Genotypes and allelic frequencies of TBX3 and TBX2 SNPs in patients with different accessory pathway types and locations and in healthy controls are shown in Tables 5 and 6. The association between TBX3 and TBX2 SNPs and accessory AV connection types and locations was examined by logistic regression analysis. As shown in Table 5, regardless of the accessory AV connection types, subjects with the CC genotype or carrying the C allele of rs1061657 had a significantly higher risk of WPW syndrome in comparison with that in individuals with the TT genotype or T allele (for the manifest type, OR = 2.39, 95% CI: 1.17–4.87, P = .017; OR = 1.45, 95% CI: 1.05–2.02, P = .025, respectively; for the concealed type, OR = 2.12, 95% CI: 1.07–4.19, P = .031; OR = 1.38, 95% CI: 1.01–1.88, P = .046, respectively). Subjects with the CC genotype or who were carrying the C allele of rs8853 had a decreased risk of manifest type

Table 2

Demographic characteristics of the study population.

| Gene | SNP     | Total N = 461, n (%) | Control N = 231, n (%) | Case N = 230, n (%) | P value |
|------|---------|----------------------|------------------------|---------------------|---------|
| Gender | Male    | 291 (63.12%) | 143 (61.9%) | 148 (64.35%) | .587    |
|       | Female  | 170 (36.88%) | 88 (38.1%) | 82 (35.65%)  |         |
| Age   | 46.8 ± 14.8 | 47.6 ± 14.4 | 46.0 ± 15.2 | .250    |

Gender was examined by chi-square test. Age was examined by Student t test.

Table 3

HWE of the case and control groups.

| Gene | SNP     | Control MAF | Control P value | Case MAF | Case P value |
|------|---------|-------------|----------------|----------|--------------|
| TBX3 | rs1061657 | 0.390        | .071           | 0.474    | .148         |
|      | rs8853  | 0.422        | .060           | 0.339    | .077         |
|      | rs77412687 | 0.065       | .607           | 0.074    | .024         |
|      | rs2242442 | 0.457        | .353           | 0.461    | .895         |
|      | rs75743672 | 0.325       | .072           | 0.278    | .625         |

HWE was tested for both groups for each SNP employing Plink software with Fisher exact test. HWE = Hardy–Weinberg equilibrium, MAF = minor allele frequency, SNP = single-nucleotide polymorphism.
of accessory AV connections compared with that in individuals with the TT genotype or T allele (OR = 0.35, 95% CI: 0.15–0.82, P = .016; OR = 0.64, 95% CI: 0.45–0.90, P = .010, respectively). However, after correcting for multiple testing, all these differences disappeared. As illustrated in Table 6, for left-sided accessory pathways, subjects with the CC genotype or who were carrying the C allele of rs1061657 and rs8853 had a significantly higher risk of accessory AV connections compared with that in individuals with the TT genotype or T allele (OR = 0.45, 95% CI: 0.24–0.87, P = .017; OR = 0.70, 95% CI: 0.43–1.09–1.03, P = .011, respectively). After FDR-BH was adjusted for multiple testing, the differences remained in subjects with the CC genotype or C allele of rs8853. Subjects with the CA genotype or the C allele of rs1061657 and rs8853 had a decreased risk of left-sided accessory AV connections as compared to subjects with the CC genotype (OR = 0.67, 95% CI: 0.44–0.98, P = .039; OR = 0.68, 95% CI: 0.46–0.99, P = .046, respectively). However, the differences disappeared after multiple testing. There were no significant differences in the risk of right-sided pathways between the 2 groups.

3.6. LD analysis

Figure 1 shows D* and r^2 values for all pairwise combinations of the 4 studied TBX3 SNPs. R^2 refers to the statistical correlation between the 2 sites. The LD analysis of the 4 polymorphic loci of TBX3 (rs1061657, rs77412687, rs8853, and rs2242442) by Haploview version 4.2 demonstrated that rs1061657, rs77412687, and rs8853 were in 1 LD block.

3.7. Haplotype analysis

According to the defined block in 3.6, haplotype analysis was performed using Plink HAP-PHASE software. We finally considered haplotypes for the loci TBX3 rs1061657 T/C, rs77412687 A/G, and rs8853 T/C and found that the frequency of haplotype TAC was significantly lower in patients than that in controls (OR = 0.65, 95% CI: 0.48–0.89, P = .007; (Table 7).
4. Discussion

Several studies to date have linked WPW syndrome to genetic mutations. The implicated gene variants and their possible pathogenetic roles in the pathogenesis of accessory AV connections are as follows: NK2 homeobox 5 (NKX2-5) c.224T>A (C82S), c.839C>T (P280L), c.73C>T (R25C). [19–23] NKX2-5 is known as a pivotal cardiac transcription factor gene, causating anatomic hypoplasia of the cardiac conduction system; lysosomal associated membrane protein 2 c.755T>G (I252S), c.586A>T (T196S), c.1117_1119delGAC (373delD); [19] the dominant model (TC + CC vs TT) 152/79 91/32 1.48 (0.91–2.40) .115 .340 81/26 1.62 (0.96–2.72) .069 .412

| Genotype and allele | Controls (n = 231) | Accessory AV connections type (n = 230) | Manifest (n = 107) |
|---------------------|-------------------|--------------------------------------|------------------|
| rs1061657 (T>C)     |                   |                                      |                  |
| TT                  | 79                | 32                                   | 1 (reference)    |
| TC                  | 124               | 67                                   | 1.33 (0.80–2.22) |
| CC                  | 29                | 24                                   | 2.12 (1.07–4.19) |
| Dominant model (TC + CC vs TT) | 152/79 91/32 1.48 (0.91–2.40) .115 .340 81/26 1.62 (0.96–2.72) .069 .412 |
| recessive model (CC vs TT + CT) | 28/203 24/99 1.76 (0.97–3.19) .064 .460 22/85 1.88 (1.02–3.46) .044 .196 |
| Allele C            | 180               | 115                                  | 1.38 (1.01–1.88) |
| Allele T            | 282               | 131                                  | 1.11               |
| rs8833 (T>G)        |                   |                                      |                  |
| TT                  | 70                | 47                                   | 1 (reference)    |
| TC                  | 127               | 64                                   | 0.75 (0.47–1.21) |
| CC                  | 34                | 12                                   | 0.53 (0.25–1.12) |
| Dominant model (TC + CC vs TT) | 161/70 76/47 0.70 (0.44–1.11) .133 .340 60/47 0.56 (0.35–0.89) .015 .179 |
| recessive model (CC vs TT + CT) | 34/197 12/111 0.63 (0.31–1.26) .189 .460 8/99 0.47 (0.21–1.05) .065 .196 |
| Allele C            | 195               | 88                                   | 0.76 (0.55–1.05) |
| Allele T            | 267               | 158                                  | 146               |
| rs77412687 (A>G)    |                   |                                      |                  |
| AA                  | 201               | 111                                  | 1 (reference)    |
| AG                  | 30                | 11                                   | NA               |
| GG                  | 0                 | 0                                    | NA               |
| Dominant model (AG + GG vs AA) | 201/30 111/12 0.72 (0.36–1.47) .372 .447 89/18 1.36 (0.72–2.56) .349 .699 |
| recessive model (GG vs AA + GA) | 0/231 1/122 NA NA 3/104 NA NA |
| Allele G            | 30                | 13                                   | 0.80 (0.41–1.57) |
| Allele A            | 432               | 233                                  | 193               |
| rs2244242 (G>A)     |                   |                                      |                  |
| GG                  | 72                | 38                                   | 1 (reference)    |
| GA                  | 107               | 64                                   | 1.13 (0.69–1.87) |
| AA                  | 52                | 21                                   | 0.77 (0.40–1.45) |
| Dominant model (GA + AA vs GG) | 159/72 85/38 1.01 (0.63–1.63) .958 .958 79/28 1.28 (0.77–2.13) .349 .699 |
| recessive model (AA vs GG + GA) | 52/179 21/102 0.71 (0.40–1.24) .230 .460 27/80 1.16 (0.68–1.98) .582 .874 |
| Allele A            | 211               | 106                                  | 0.90 (0.66–1.23) |
| Allele G            | 251               | 140                                  | 108               |
| rs75743672 (C>A)    |                   |                                      |                  |
| CA                  | 99                | 62                                   | 1 (reference)    |
| AA                  | 114               | 53                                   | 0.74 (0.47–1.17) |
| Dominant model (CA + AA vs CC) | 132/99 61/62 0.74 (0.48–1.15) .175 .340 99/88 0.68 (0.43–1.08) .105 .418 |
| recessive model (AA vs CC + CA) | 18/213 8/115 0.82 (0.35–1.95) .659 .810 8/99 0.90 (0.40–2.27) .919 .991 |
| Allele A            | 150               | 69                                   | 0.81 (0.59–1.14) |
| Allele C            | 312               | 177                                  | 155               |

ORs and P values are estimated by logistic regression. Values in bold-italic font were statistically significant. BH (1995) (step up FDR control method was employed as adjustment for multiple comparisons by Plink software. FDR cutoff was set at 0.1.)

WPW syndrome and the features of Alagille syndrome. [25] As shown, there are only a few studies referring to the gene polymorphism associated with accessory AV connections and each SNP seems to explain a small number of WPW cases, suggesting that more polymorphisms need to be discovered. In the present study, 4 SNPs of TBX3, rs1061657, rs8853, rs77412687, and rs2244242, as well as rs75743672 of TBX2, were investigated to determine their association with susceptibility to sporadic WPW syndrome in the Han Chinese population. To the best of our knowledge, this is the first study to investigate the influence of TBX polymorphisms on sporadic WPW susceptibility. Our data revealed that the C allele of TBX3 rs1061657 could be a risk factor for WPW syndrome, with the C allele of rs8853 being a protective factor. The TBX3 rs1061657 and rs8853 were significantly associated with WPW syndrome susceptibility, particularly for left-sided accessory pathways. There was no distinct association between TBX2 rs75743672 and WPW syndrome susceptibility in the overall study population. The TBX3...
There is increasing evidence that both genetic and epigenetic alterations cooperate in TBX3 deregulation in cells. A recent study revealed that TBX3-deficient mice may both suppress abnormal accessory AV pathways elsewhere and specify the development of the normal AV conduction system. In our study, all patients had normal sinus and AV node function, whereas TBX3 rs1061657 and rs8853 were associated with sporadic WPW syndrome, which corresponds to some extent to the ventricular preexcitation phenotypes described in WPW in human hearts directly; however, their results provided more evidence and further supported the important role of TBX3 on the mechanism of AV bypass formation. The mechanism underlying ventricular preexcitation formation is partly the abnormal expression of TBX3 in the AV canal myocardium, where it inhibits the continuation of accessory pathways. In the early stages of embryonic development, the atria and ventricles show electrical continuity around the AV canal. Subsequently, myocytes in the AV canal separate from each other to form an insulating fibrous annulus, leaving the His bundle as the single AV connection. TBX3-deficient mice have histologically confirmed AV myofibers attributed to anatomically incomplete myocyte separation. A recent study revealed that TBX3 may both suppress abnormal accessory AV pathways elsewhere and specify the development of the normal AV conduction system. In our study, all patients had normal sinus and AV node function, whereas TBX3 rs1061657 and rs8853 were associated with sporadic WPW syndrome, which corresponds to some extent to the ventricular preexcitation phenotypes described in TBX3-deficient mice.

rs1061657 T/C, rs77412687 A/G, and rs8853 T/C SNPs were in perfect LD, and the distribution of haplotype TAC was significantly different between the cases and controls. The human TBX3 gene, which is located on the reverse strand of chromosome 12 at position 12q23-24.1 and consists of 7 exons within a region of 4.7 kb, has been implicated in susceptibility to many human diseases, such as ulnar mammary syndrome, rheumatoid arthritis, cancer, and obesity. TBX3 expression is particularly important for the developmental formation of the pacemaker cardiomyocytes of the sinoatrial node, right atrium, and the ventricular septum of the heart. The abnormal expression of TBX3 in the AV canal myocardium, where it inhibits the continuation of accessory pathways. In the early stages of embryonic development, the atria and ventricles show electrical continuity around the AV canal. Subsequently, myocytes in the AV canal separate from each other to form an insulating fibrous annulus, leaving the His bundle as the single AV connection. TBX3-deficient mice have histologically confirmed AV myofibers attributed to anatomically incomplete myocyte separation. A recent study revealed that TBX3 may both suppress abnormal accessory AV pathways elsewhere and specify the development of the normal AV conduction system. In our study, all patients had normal sinus and AV node function, whereas TBX3 rs1061657 and rs8853 were associated with sporadic WPW syndrome, which corresponds to some extent to the ventricular preexcitation phenotypes described in TBX3-deficient mice.

There is increasing evidence that both genetic and epigenetic alterations cooperate in TBX3 dysregulation in cells. TBX3 expression is regulated by an important upstream modulator, the...
Recent studies have revealed that miR-137 and miR-93 can bind the 3'UTR region of Tbx3, resulting in the suppression of Tbx3 levels, thus preventing the self-renewal of embryonic stem cells and mouse adipocyte precursor cells. The specific variants rs1061657 (3'UTR) and rs8853 (3'UTR), which are associated with accessory AV pathways, mainly affect TBX3 gene transcription. SNPs in the UTRs, although not resulting in changes to the protein sequence directly, could influence the function of the gene by affecting its expression. Therefore, we speculate that the TBX3 mutations in the 3'UTR region might lead to an abnormal combination between miRNA and this region, resulting in anomalous regulation of the TBX3 gene and different levels of the TBX3 protein. This would further result in differences in the suppression of abnormal accessory AV pathways, causing the existence of muscular bundles in the AV annulus, and eventually resulting in WPW syndrome. The regulatory mechanisms leading to WPW syndrome need to be further elucidated in future studies.

The current study revealed that TBX3 rs1061657 and rs8853 are associated with left-sided AV pathways susceptibility rather than that of the right. However, the prevalence of right-sided AV pathways was considerably lower than that of the left in our study, which was similar to the research of Guize. Patients with left-sided accessory pathways accounted for 85.2% (196/230) in this study, while only 34 patients had right-sided AV connections. Differences in the SNP genotype frequency distribution could become apparent if the number of patients with right-sided AV connections were sufficiently large. Meanwhile, a broad FDR cutoff was set at 0.1 to screen out more SNPs to provide a foundation for basic research relevant to TBX gene polymorphisms.

TBX2, also a transcription factor that is essential for AV canal patterning, is functionally highly correlated with TBX3. Cardiac mutations in Tbx2 in mice have provided an example of the developmental mechanisms that can cause accessory pathways. Myocardium-specific inactivation of Tbx2 causes inaccurate gene expression as well as morphogenesis of the partial embryonic AV canal, resulting in malformation of the myocardium and acquired fast conducting properties that affect annulus fibrosus formation, which lead to

| rs1061657 | rs77412687 | rs8853 | Healthy controls, haplotype frequency | Patients haplotype, frequency | OR (95% CI) | P value |
|-----------|------------|--------|----------------------------------------|-----------------------------|-------------|---------|
| c         | a          | t      | 176 (0.595)                            | 216 (0.636)                 | 1           |         |
| t         | a          | c      | 191 (0.430)                            | 154 (0.347)                 | 0.65 (0.48–0.89) | .007    |
| t         | a          | t      | 61 (0.168)                             | 54 (0.134)                  | 0.75 (0.50–1.13) | .168    |
| t         | g          | t      | 30 (0.090)                             | 34 (0.093)                  | 1.04 (0.63–1.73) | .878    |

ORs and P values are estimated by logistic regression. Value in bold-italic font was statistically significant. AV = atrioventricular, CI = confidence interval, OR = odds ratio, TBX3 = T-box transcription factor 3.
ventricular preexcitation.\[11\] The TBX2 rs75743672 variation was not found in patients in our study, whereas previous results showed that mutations in and inactivation of Tbx2 led to bypass formation. This discrepancy could be due to the relatively small sample size or different TBX2 expression between humans and mice.

4.1. Limitations
This study had some limitations. First, it was a single-center study and did not include a large number of subjects. Second, we selected a limited number of SNPs for genotyping, and we cannot exclude the possibility of random association. For further studies, larger sample sizes will be used to increase the confidence of our findings.

4.2. Future directions
As for clinical practice, TBX3 rs1061657 and rs8853 variants may provide a basis for genetic diagnosis of sporadic WPW. In addition, further studies are needed to explain the functional roles of TBX3 SNPs rs1061657 and rs8853 in the specific pathogenesis of WPW syndrome.

5. Conclusion
We observed that the tested TBX3 SNPs from the case–control study were remarkably associated with sporadic WPW syndrome in a Han Chinese population. Our data showed that allele C of rs1061657 may be associated with a higher risk of WPW syndrome, whereas allele C of rs8853 was likely to reduce its incidence. TBX3 rs1061657 T/C, rs77412687 A/G, and rs8853 T/C SNPs were in perfect LD, and the frequency of haplotype TAC was significantly lower in patients than that in controls. Genotyping of TBX3 could potentially reveal the etiology of the formation of sporadic WPW syndrome from a molecular biology perspective. Further studies are needed to explain the functional roles of SNPs rs1061657 and rs8853 in the specific pathogenesis of WPW syndrome.

Author contributions
Conceptualization: Bing Han, Zheng Zhang
Data curation: Zheng Zhang
Investigation: Bing Han, Zheng Zhang
Methodology: Bing Han, Qingsu Lan, Jin Zhang, Jianjian Jin, Xiaoxue Meng, Ming Bai
Project administration: Bing Han
Resources: Zheng Zhang, Bing Han
Software: Jing Zhao
Supervision: Bing Han, Jing Zhao
Validation: Yongxiang Wang, Jing Zhao
Visualization: Bing Han

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