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

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Abstract

Abnormal development of the atrioventricular 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]: 1.08–1.83, *P* = .011) and left-sided accessory pathways (OR = 1.40, 95% CI: 1.07–1.84, *P* = .016). However, allele C of *TBX3* 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

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

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developmental regulator of the heart.[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.[11,16] Previous studies have demonstrated the role of *TBX3* and *TBX2* deficiency in the development of heart defects, including preexcitation.[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.

2. Materials and Methods

2.1. 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.

2.2. 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 Haploview. 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.

2.3. Statistical analysis

The SPSS 26.0 software package (SPSS Inc., Chicago, IL) and the Plink software (https://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

Table 1

Details of SNPs and primer sequences used in this study.

Gene	SNP ID	Alt	Ch	Region	Position	Primer	Sequence
TBX3	rs1061657	T>C	12	3′UTR	115,108,136	Forward	ACCCTCCCCTGACTGTCCATCT
						Reverse	GTGCCACTTTCCAGCTCCACTT
						Extension	TTTTTTTTTTTTTGAAGGTATATTTTGTGTTATAGTTGTTGAT
	rs8853	T>C	12	3′UTR	115,108,907	Forward	CGACATGAATCCTGCTACAGAGC
						Reverse	CCAATCCTTTGCCCTCAAATCA
						Extension	TTTTTTTTTTTTTTGCCCTCAAATCAGTGACCCA
	rs77412687	A>G	12	3′UTR	115,108,334	Forward	ACCCTCCCCTGACTGTCCATCT
						Reverse	GTGCCACTTTCCAGCTCCACTT
						Extension	TTTTTTTTTTTTTTTTTCAGAGGCTGCGGTTTGACTT
	rs2242442	G>A	12	5′UTR	115,121,189	Forward	AAAGGAGGCAGAAATCACAACTAATG
						Reverse	TCGCTTCTGAAACCGACGTTC
						Extension	TTTTTTTTTTTTTTTTTTTTTGCACTTCAAAGGGAGGAGGG
TBX2	rs75743672	C>A	17	Upstream	59,477,903	Forward	GAGGACGAGGTGGAGGACGAC
						Reverse	CTACCTCCCGGACTTGGTGATG
						Extension	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGGACCAGTTCCACAAGCTAGG

Ch = chromosome, SNP = single-nucleotide polymorphism.

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,[18] 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.

Table 2

Demographic characteristics of the study population.

	Total N = 461, n (%)	Control N = 231, n (%)	Case N = 230, n (%)	*P* value
Gender				.587
Male	291 (63.12%)	143 (61.9%)	148 (64.35%)	
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.

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 3

HWE of the case and control groups.

		HWE			
		Control		Case	
Gene	SNP	MAF	*P* value	MAF	*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
TBX2	rs75743672	0.325	.072	0.278	.625

HWE was tested for both groups for each SNP employing Plink software by Fisher exact test.

HWE = Hardy–Weinberg equilibrium, MAF = minor allele frequency, SNP = single-nucleotide polymorphism.

Table 4

Association of *TBX3* and *TBX2* polymorphisms with accessory AV connections.

	Control	Case	OR (95% CI)	*P* value	AOR*(95% CI)	*P* value*	BH adjusted *P* value*
rs1061657 (T>C)							
TT	79	58	1 (reference)		1 (reference)		
TC	124	126	1.38 (0.91–2.11)	.129	1.37 (0.90–2.09)	.146	***.086***
CC	28	46	2.24 (1.25–3.99)	***.006***	2.24 (1.25–4.00)	***.007***	***.086***
Dominant model (TC + CC vs TT)	152/79	172/58	1.54 (1.03–2.31)	***.035***	1.53 (1.02–2.30)	***.042***	.166
Recessive model (CC vs TC + TT)	28/203	46/184	1.81 (1.09–3.02)	***.022***	1.82 (1.09–3.04)	***.022***	.179
Allele T frequency	282	242	1.41 (1.09–1.83)	***.010***	1.41 (1.08–1.83)	***.011***	***.067***
Allele C frequency	180	218					
rs8853 (T>C)							
TT	70	94	1 (reference)		1 (reference)		
TC	127	116	0.68 (0.46–1.01)	.058	0.68 (0.45–1.01)	.057	***.086***
CC	34	20	0.44 (0.23–0.83)	***.011***	0.44 (0.23–0.84)	***.012***	***.086***
Dominant model (TC + CC vs TT)	161/70	136/94	0.63 (0.43–0.92)	***.018***	0.63 (0.43–0.93)	***.019***	.166
Recessive model (CC vs TT + CT)	34/197	20/210	0.55 (0.31–0.99)	***.047***	0.56 (0.31–1.01)	.054	.179
Allele T frequency	267	304	0.70 (0.54–0.92)	***.010***	0.71 (0.54–0.92)	***.011***	***.067***
Allele C frequency	195	156					
rs77412687 (A>G)							
AA	201	200	1 (reference)		1 (reference)		
AG	30	26	NA	NA	NA	NA	NA
GG	0	4	NA	NA	NA	NA	NA
Dominant model (AG + GG vs AA)	201/30	200/30	1.01 (0.58–1.73)	.986	1.02 (0.59–1.76)	.944	.944
Recessive model (GG vs AA + AG)	231/0	226/4	NA	NA	NA	NA	NA
Allele A frequency	432	426	1.15 (0.69–1.91)	.592	1.17 (0.70–1.94)	.552	.792
Allele G frequency	30	34					
rs2242442 (G>A)							
GG	72	66	1 (reference)		1 (reference)		
GA	107	116	1.18 (0.77–1.81)	.439	1.18 (0.77–0.81)	.437	.707
AA	52	48	1.01 (0.60–1.69)	.979	1.04 (0.62–1.74)	.890	.707
Dominant model (GA + AA vs GG)	72/159	66/164	1.13 (0.76–1.68)	.562	1.14 (0.76–1.70)	.529	.635
Recessive model (AA vs GG + GA)	52/179	48/182	0.91 (0.58–1.41)	.669	0.94 (0.60–1.46)	.767	.852
Allele G frequency	251	248	1.02 (0.79–1.32)	.900	1.03 (0.80–1.34)	.813	.887
Allele A frequency	211	212					
rs75743672 (C>A)							
CC	99	118	1 (reference)		1 (reference)		
CA	114	96	0.71 (0.48–1.03)	.074	0.71 (0.48–1.03)	.072	.314
AA	18	16	0.75 (0.36–1.54)	.427	0.75 (0.36–1.55)	.434	.314
Dominant model (CA + AA vs CC)	99/132	118/112	0.71 (0.49–1.03)	.070	0.71 (0.49–1.03)	.069	.206
Recessive model (AA vs CC + CA)	18/213	16/214	0.89 (0.44–1.78)	.732	0.89 (0.44–1.80)	.741	.852
Allele C frequency	312	332	0.80 (0.61–1.06)	.125	0.80 (0.60–1.06)	.125	.374
Allele A frequency	150	128					

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

AOR = adjusted odds ratio, AV = atrioventricular, BH = Benjamini–Hochberg, CI = confidence interval, FDR = false discovery rate, NA = the value was not available where the number of individuals of the risk genotype was 0, OR = odds ratio, SNP = single-nucleotide polymorphism, TBX2 = T-box transcription factor 2, TBX3 = T-box transcription factor 3.

*Adjusted for age and gender.

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 had a significantly higher risk of accessory AV connections compared to individuals with the TT genotype or T allele (OR = 2.21, 95% CI: 1.22 –4.02, *P* = .009; OR = 1.40, 95% CI: 1.07–1.84, *P* = .016, respectively). Subjects with the CC genotype or C allele of rs8853 had a decreased risk of left-sided accessory AV pathways compared to subjects with the TT genotype or carrying the T allele (OR = 0.45, 95% CI: 0.24–0.87, *P* = .017; OR = 0.70, 95% CI: 0.53–0.92, *P* = .011, respectively). After FDR-BH was adjusted for multiple testing, the differences remained in subjects with the C allele of rs1061657 and rs8853. Subjects with the CA genotype and dominant model of *TBX2* rs75743672 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 rs2242422) 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).

Table 5

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

	Controls (n = 231)	Accessory AV connections type (n = 230)							
		Concealed (n = 123)				Manifest (n = 107)			
Genotype and allele	No.	No.	OR (95% CI)	*P* value	BH adjusted *P* value	No.	OR (95% CI)	*P* value	BH adjusted *P* value
rs1061657 (T>C)									
TT	79	32	1 (reference)			26	1 (reference)		
TC	124	67	1.33 (0.80–2.22)	.265	.471	59	1.45 (0.84–2.48)	.182	.170
CC	28	24	2.12 (1.07–4.19)	***.031***	.471	22	2.39 (1.17–4.87)	***.017***	.170
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)	***.046***	.238	103	1.45 (1.05–2.02)	***.025***	.150
Allele T	282	131				111			
rs8853 (T>C)									
TT	70	47	1 (reference)			47	1 (reference)		
TC	127	64	0.75 (0.47–1.21)	.238	.471	52	0.61 (0.37–1.00)	***.048***	.170
CC	34	12	0.53 (0.25–1.12)	.095	.471	8	0.35 (0.15–0.82)	***.016***	.170
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)	.096	.238		0.64 (0.45–0.90)	***.010***	.119
Allele T	267	158				146			
rs77412687(A>G)									
AA	201	111	1 (reference)			89	1 (reference)		
AG	30	11	NA	NA	NA	15	NA	NA	NA
GG	0	1	NA	NA	NA	3	NA	NA	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	NA	3/104	NA	NA	NA
Allele G	30	13	0.80 (0.41–1.57)	0.522	.625	21	1.57 (0.88–2.81)	.131	.524
Allele A	432	233				193			
rs2242442 (G>A)									
GG	72	38	1 (reference)			28	1 (reference)		
GA	107	64	1.13 (0.69–1.87)	.624	.471	52	1.25 (0.72–2.16)	.425	.808
AA	52	21	0.77 (0.40–1.45)	.413	.471	27	1.34 (0.71–2.53)	.374	.808
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)	.511	.625	106	1.17 (0.84–1.62)	.350	.620
Allele G	251	140				108			
rs75743672(C>A)									
CC	99	62	1 (reference)			56	1 (reference)		
CA	114	53	0.74 (0.47–1.17)	.199	.471	43	0.67 (0.41–1.08)	.098	.455
AA	18	8	0.71 (0.29–1.73)	.451	.471	8	0.79 (0.32–1.92)	.597	.455
Dominant model (CA + AA vs CC)	132/99	61/62	0.74 (0.48–1.15)	.175	.340	99/8	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	0.96 (0.40–2.27)	.919	.991
Allele A	150	69	0.81 (0.58–1.14)	.226	.452	59	0.79 (0.55–1.13)	.201	.601
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.

AV = atrioventricular, BH = Benjamini–Hochberg, CI = confidence interval, FDR = false discovery rate, NA = the value was not available where the number of individuals of the risk genotype was 0, OR = odds ratio, TBX2 = T-box transcription factor 2, TBX3 = T-box transcription factor 3.

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),[19] c.73C>T (R25C).[20–23] *NKX2-5* is known as a pivotal cardiac transcription factor gene, causing 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 reason why the detected lysosomal associated membrane protein 2 variants might cause accessory AV connections is still not understood; an R225W mutation in protein kinase AMP-activated noncatalytic subunit gamma 3 (*PRKAG3)* results in glycogen accumulation in the AV annular region,[24] leading to the formation of AV bypass; and the microdeletion of the bone morphogenetic protein 2 region within 20p12.3 is involved in conduction abnormalities of the heart and contributes to both 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 rs2242442, 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*

Table 6

***TBX3* and *TBX2* SNPs genotype and allele frequencies in healthy controls and in patients with differentlocations of accessory AV connections.**

	Controls (n = 231)	Accessory AV connections location (n = 230)							
		Left (n = 196)				Right (n = 34)			
Genotype and allele	No.	No.	OR (95% CI)	*P* value	BH Adjusted *P* value	No.	OR (95% CI)	*P* value	BH adjusted *P* value
rs1061657 (T>C)									
TT	79	51	1 (reference)			7	1 (reference)		
TC	124	105	1.31 (0.85–2.03)	.224	.112	21	1.91 (0.78–4.70)	.159	.690
CC	28	40	2.21 (1.22–4.02)	***.009***	.112	6	2.42 (0.75–7.81)	.140	.690
Dominant model (TC + CC vs TT)	152/79	145/51	1.48 (0.97–2.25)	.068	.205	27/7	2.15 (0.84–4.81)	.119	.715
Recessive model (CC vs TT + CT)	28/203	40/156	1.86 (1.10–3.15)	***.021***	.209	6/28	1.55 (0.59–4.08)	.372	.565
Allele C	180	185	1.40 (1.07–1.84)	***.016***	***.093***	33	1.48 (0.89–2.46)	.135	.562
Allele T	282	207				35			
rs8853 (T>C)									
TT	70	82	1 (reference)			12	1 (reference)		
TC	127	96	0.65 (0.43–0.98)	***.038***	.112	20	0.92 (0.42–1.99)	.830	.690
CC	34	18	0.45 (0.24–0.87)	***.017***	.112	2	0.34 (0.07–1.62)	.177	.690
Dominant model (TC + CC vs TT)	161/70	114/82	0.61 (0.41–0.90)	***.013***	.161	22/12	0.80 (0.37–1.70)	.557	.854
Recessive model (CC vs TT + CT)	34/197	18/178	0.59 (0.32–1.07)	.084	.280	2/32	0.36 (0.08–1.58)	.177	.531
Allele C	195	132	0.70 (0.53–0.92)	***.011***	***.093***	24	0.75 (0.44–1.27)	.281	.562
Allele T	267	260				44			
rs77412687(A>G)									
AA	201	171	1 (reference)			29	1 (reference)		
AG	30	21	NA	NA	NA	5	NA	NA	NA
GG	0	4	NA	NA	NA	0	NA	NA	NA
Dominant model (AG + GG vs AA)	201/30	25/171	0.98 (0.56–1.73)	.943	.943	29/5	1.16 (0.42–3.22)	.782	.854
Recessive model (GG vs AA + GA)	0/231	4/192	NA	NA	NA	0/34	NA	NA	NA
Allele G	30	29	1.15 (0.68–1.95)	.604	.725	5	1.14 (0.43–3.05)	.790	.900
Allele A	432	363				63			
rs2242442 (G>A)									
GG	72	58	1 (reference)			8	1 (reference)		
GA	107	100	1.16 (0.75–1.80)	.508	.593	16	1.35 (0.55–3.31)	.518	.690
AA	52	38	0.91 (0.53–1.56)	.725	.593	10	1.73 (0.64–4.69)	.280	.690
Dominant model (GA + AA vs GG)	159/72	138/58	1.09 (0.71–1.63)	.724	.790	26/8	1.47 (0.64–3.41)	.367	.749
Recessive model (AA vs GG + GA)	52/179	38/158	0.83 (0.52–1.32)	.431	.615	10/24	1.43 (0.65–3.19)	.377	.565
Allele A	211	176	0.97 (0.74–1.27)	.821	.896	36	1.34 (0.80–2.23)	.263	.562
Allele G	251	216				32			
rs75743672(C>A)									
CC	99	103	1 (reference)			15	1 (reference)		
CA	114	78	0.67 (0.44–0.98)	***.039***	.271	18	1.04 (0.50–2.18)	.913	.690
AA	18	15	0.80 (0.38–1.68)	.556	.271	1	0.37 (0.05–2.95)	.346	.690
Dominant model (CA + AA vs CC)	132/99	93/103	0.68 (0.46–0.99)	***.046***	.205	19/15	0.95 (0.46–1.96)	.890	.890
Recessive model (AA vs CC + CA)	18/213	15/181	0.98 (0.48–2.00)	.957	.957	1/33	0.36 (0.05–2.78)	.326	.565
Allele A	150	108	0.79 (0.59–1.06)	.119	.358	20	0.87 (0.50–1.51)	.615	.819
Allele C	312	284				48			

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

AOR = adjusted odds ratio, AV = atrioventricular, BH = Benjamini–Hochberg, CI = confidence interval, FDR = false discovery rate, NA = the value was not available where the number of individuals of the risk genotype was 0, OR = odds ratio, SNP = single-nucleotide polymorphism, TBX2 = T-box transcription factor 2, TBX3 = T-box transcription factor 3.

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.[26–29] TBX3 expression is particularly important for the developmental formation of the pacemaker cardiomyocytes of the sinoatrial node,[30,31] cardiac conduction system, and the ventricular septum of the heart,[12–15] and mutations in the *TBX3* gene result in sinus node dysfunction, widening of the QRS interval, high-grade AV block, and ventricular preexcitation.[17] The studies above did not mention the relationship between *TBX3* and 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.[32] *Tbx3*-deficient mice have histologically confirmed AV myofibers attributed to anatomically incomplete AV myocytes separation.[33] A recent study revealed that *TBX3* may both suppress abnormal accessory AV pathways elsewhere and specify the development of the normal AV conduction system.[17] 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

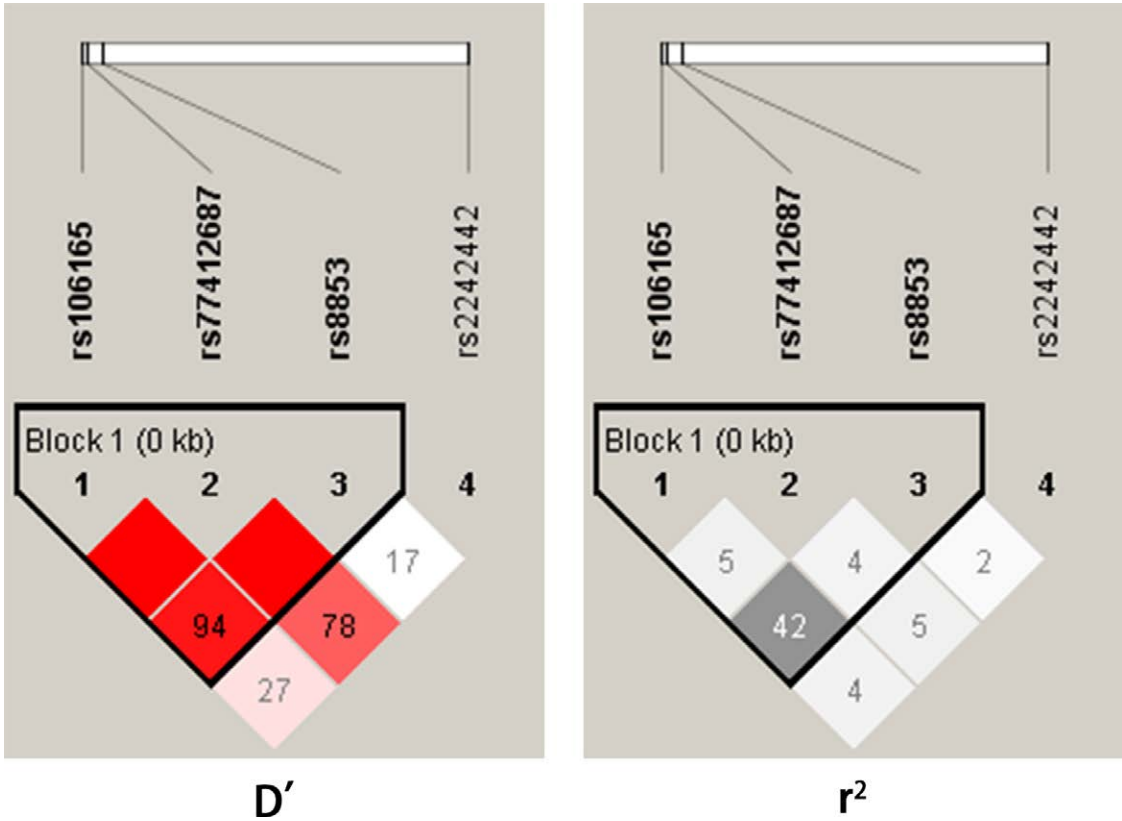

Figure 1. LD plot of the TBX3 rs1061657, rs77412687, rs8853, and rs2242442. Strong LD is represented by a higher percentage and a darker square. LD = linkage disequilibrium.

Table 7

Haplotype analysis for *TBX3* gene in 230 patients with accessory AV connections and 231 healthy controls.

rs1061657	rs77412687	rs8853	Healthy controls, haplotype frequency	Patients haplotype, frequency	OR (95% CI)	*P* value
C	A	T	176 (0.595)	216 (0.635)	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.

bone morphogenetic protein pathway.[34,35] Recent studies have revealed that miR-137and 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.[36,37] 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.[38] 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.[11] 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

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.

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References

[1] Deal BJ, Keane JF, Gillette PC, et al. Wolff-Parkinson-White syndrome and supraventricular tachycardia during infancy: management and follow-up. J Am Coll Cardiol. 1985;5:130–5.
[2] Munger TM, Packer DL, Hammill SC, et al. A population study of the natural history of Wolff-Parkinson-White syndrome in Olmsted county, Minnesota, 1953-1989. Circulation. 1993;87:866–73.
[3] Wan Q, Wu N, Fan W, et al. Clinical manifestations and prevalence of different types of supraventricular tachycardia among Chinese. Chin Med J (Engl). 1992;105:284–8.
[4] Gollob MH, Seger JJ, Gollob TN, et al. Novel PRKAG2 mutation responsible for the genetic syndrome of ventricular preexcitation and conduction system disease with childhood onset and absence of cardiac hypertrophy. Circulation. 2001;104:3030–3.
[5] Gollob MH, Green MS, Tang AS, et al. Identification of a gene responsible for familial Wolff-Parkinson-White syndrome. N Engl J Med. 2001;344:1823–31.
[6] Vaughan CJ, Hom Y, Okin DA, et al. Molecular genetic analysis of PRKAG2 in sporadic Wolff-Parkinson-White syndrome. J Cardiovasc Electrophysiol. 2003;14:263–8.
[7] Hsu JC, Tanel RE, Lee BK, et al. Differences in accessory pathway location by sex and race. Heart Rhythm. 2010;7:52–6.
[8] Hahurij ND, Gittenberger-De Groot AC, Kolditz DP, et al. Accessory atrioventricular myocardial connections in the developing human heart: relevance for perinatal supraventricular tachycardias. Circulation. 2008;117:2850–8.
[9] Stroud DM, Gaussin V, Burch JBE, et al. Abnormal conduction and morphology in the atrioventricular node of mice with atrioventricular canal-targeted deletion of Alk3/Bmpr1a receptor. Circulation. 2007;116:2535–43.
[10] Anderson RH, Ho SY, Gillette PC, et al. Mahaim, kent and abnormal atrioventricular conduction. Cardiovasc Res. 1996;31:480–91.
[11] Aanhaanen WT, Boukens BJ, Sizarov A, et al. Defective Tbx2-dependent patterning of the atrioventricular canal myocardium causes accessory pathway formation in mice. J Clin Invest. 2011;121:534–44.
[12] Bakker ML, Boink GJ, Boukens BJ, et al. T-box transcription factor TBX3 reprogrammes mature cardiac myocytes into pacemaker-like cells. Cardiovasc Res. 2012;94:439–49.
[13] Mesbah K, Harrelson Z, Théveniau-Ruissy M, et al. Tbx3 is required for outflow tract development. Circ Res. 2008;103:743–50.
[14] Ribeiro I, Kawakami Y, Büscher D, et al. Tbx2 and Tbx3 regulate the dynamics of cell proliferation during heart remodeling. PLoS One. 2007;2:e398.
[15] Washkowitz AJ, Gavrilov S, Begum S, et al. Diverse functional networks of Tbx3 in development and disease. Wiley Interdiscip Rev Syst Biol Med. 2012;4:273–83.
[16] Singh R, Hoogaars WM, Barnett P, et al. Tbx2 and Tbx3 induce atrioventricular myocardial development and endocardial cushion formation. Cell Mol Life Sci. 2012;69:1377–89.
[17] Nguyen HH, Grange DK, Johnson MC, et al. Dichotomous roles of TBX3 in the establishment of atrioventricular conduction pathways in the human heart. HeartRhythm Case Rep. 2019;5:109–11.
[18] Shan GA. note on exact conditional and unconditional tests for Hardy-Weinberg equilibrium. Hum Hered. 2013;76:10–7.
[19] Esposito G, Grutter G, Drago F, et al. Molecular analysis of PRKAG2, LAMP2, and NKX2-5 genes in a cohort of 125 patients with accessory atrioventricular connection. Am J Med Genet A. 2009;149a:1574–7.
[20] Benson DW, Silberbach GM, Kavanaugh-McHugh A, et al. Mutations in the cardiac transcription factor NKX2.5 affect diverse cardiac developmental pathways. J Clin Invest. 1999;104:1567–73.
[21] Goldmuntz E, Geiger E, Benson DW. NKX2.5 mutations in patients with tetralogy of fallot. Circulation. 2001;104:2565–8.
[22] McElhinney DB, Geiger E, Blinder J, et al. NKX2.5 mutations in patients with congenital heart disease. J Am Coll Cardiol. 2003;42:1650–5.
[23] Dentice M, Cordeddu V, Rosica A, et al. Missense mutation in the transcription factor NKX2-5: a novel molecular event in the pathogenesis of thyroid dysgenesis. J Clin Endocrinol Metab. 2006;91:1428–33.
[24] Weng KP, Yuh YS, Huang SH, et al. PRKAG3 polymorphisms associated with sporadic Wolff-Parkinson-White syndrome among a Taiwanese population. J Chin Med Assoc. 2016;79:656–60.
[25] Lalani SR, Thakuria JV, Cox GF, et al. 20p12.3 microdeletion predisposes to Wolff-Parkinson-White syndrome with variable neurocognitive deficits. J Med Genet. 2009;46:168–75.
[26] Frank DU, Emechebe U, Thomas KR, et al. Mouse TBX3 mutants suggest novel molecular mechanisms for ulnar-mammary syndrome. PLoS One. 2013;8:e67841.
[27] Quarta C, Fisette A, Xu Y, et al. Functional identity of hypothalamic melanocortin neurons depends on Tbx3. Nat Metab. 2019;1:222–35.
[28] Sardar S, Kerr A, Vaartjes D, et al. The oncoprotein TBX3 is controlling severity in experimental arthritis. Arthritis Res Ther. 2019;21:16.
[29] Willmer T, Cooper A, Peres J, et al. The T-box transcription factor 3 in development and cancer. Biosci Trends. 2017;11:254–66.
[30] Eif VV, Protze SI, Bosada FM, et al. Genome-wide analysis identifies an essential human TBX3 pacemaker enhancer. Circ Res. 2020;127:1522–35.
[31] Yan Y, Liu F, Dang X, et al. TBX3 induces biased differentiation of human induced pluripotent stem cells into cardiac pacemaker-like cells. Gene Expr Patterns. 2021;40:119184.

[32] Argüello C, Alanís J, Pantoja O, et al. Electrophysiological and ultrastructural study of the atrioventricular canal during the development of the chick embryo. J Mol Cell Cardiol. 1986;18:499–510.
[33] Frank DU, Carter KL, Thomas KR, et al. Lethal arrhythmias in Tbx3-deficient mice reveal extreme dosage sensitivity of cardiac conduction system function and homeostasis. Proc Natl Acad Sci USA. 2012;109:E154–63.
[34] Yamada M, Revelli JP, Eichele G, et al. Expression of chick Tbx-2, Tbx-3, and Tbx-5 genes during early heart development: evidence for BMP2 induction of Tbx2. Dev Biol. 2000;228:95–105.
[35] Yang L, Cai CL, Lin L, et al. Isl1Cre reveals a common Bmp pathway in heart and limb development. Development. 2006;133:1575–85.
[36] Jiang K, Ren C, Nair VD. MicroRNA-137 represses Klf4 and Tbx3 during differentiation of mouse embryonic stem cells. Stem Cell Res. 2013;11:1299–313.
[37] Cioffi M, Vallespinos-Serrano M, Trabulo SM, et al. MiR-93 controls adiposity via inhibition of sirt7 and Tbx3. Cell Rep. 2015;12:1594–605.
[38] Guize L, Soria R, Chaouat JC, et al. Prevalence and course of Wolf-Parkinson-White syndrome in a population of 138,048 subjects. Ann Med Interne (Paris). 1985;136:474–8.