



# Sequence variations in *GATA4* and *CITED2* gene among patients with cardiac septation defects from Xinjiang, China

## Original Article

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### Abstract

Studies have shown that genetic factors play an important role in CHD's development. The mutations in *GATA4* and *CITED2* genes result in the failure of the heart to develop normally, thereby leading to septal defects. The present study investigated the underlying molecular aetiology of patients with cardiac septation defects from Xinjiang. We investigated variants of the *GATA4* and *CITED2* gene coding regions in 172 patients with cardiac septation defects by sequencing. Healthy controls (n = 200) were included. Three heterozygous variations (p.V380M, p.P394T, and p.P407Q) of the *GATA4* gene were identified in three patients. p.V380M was discovered in a patient with atrial septal defect. p.P394T was noted in a patient with atrial septal defect. p.V380M and p.P407Q of the *GATA4* gene were detected in one patient with ventricular septal defect. A novel homozygous variation (p.S192G) of the *CITED2* gene was found in one patient with ventricular septal defect. Other patients and healthy individuals were normal. The limited prevalence of genetic variations observed in individuals with cardiac septal defects from Xinjiang provides evidence in favour of the hypothesis that CHD is a polygenic hereditary disorder. It is plausible that mutations in the *GATA4* and *CITED2* genes could potentially underlie the occurrence of idiopathic CHD in affected patients.

CHDs are the most common developmental anomaly, with incidence rates ranging from eight to 12 per 1000 live births.<sup>1</sup> Atrial septal defects, ventricular septal defects, and atrioventricular septal defects are major contributors to CHD. Of these, 11.6% are atrial septal defect, 30.7% are ventricular septal defects, and 2% are atrioventricular septal defects.<sup>2</sup> In recent years, the aetiology of certain CHDs has increased significantly. The majority of the septal defects seen in the human population are the result of genetic modulation by epigenetic factors. Advancements in the understanding of the molecular basis of normal heart development demonstrate the necessity of numerous genes that are predominantly expressed during heart morphogenesis.<sup>3</sup> The causes of cardiac septation defects in most cases are unknown, but the molecular evolution and gene analysis of the heart have led to the identification of some mutations associated with cardiac septation defects. Many cohort studies have shown that susceptible genes *GATA4* and *CITED2* are important during heart development.<sup>4,5</sup> The mutations of these genes result in the failure of normal development of the heart, thereby leading to septal defects.<sup>6,7</sup> However, few studies have focused on the Xinjiang region. This study aimed to identify the nucleotide sequence variation of the *GATA4* and *CITED2* genes in patients with cardiac septation defects from Xinjiang, China.

## Materials and methods

### Subjects

A total of 172 patients with cardiac septation defects were recruited from the People's Hospital of the Xinjiang Uygur Autonomous Region, Xinjiang, China. There were 81 males and 91 females, ranging in age from 3 months to 61 years. These patients with CHD included 120 cases of atrial septal defect, 40 cases of ventricular septal defect, 10 cases of atrial septal defect complicated with ventricular septal defect, and two cases of atrioventricular septal defect. Among them, four cases had a family history of atrial septal defect. A summary of the clinical characteristics of the subjects is shown in Table 1. The evaluation of all subjects included their clinical history, physical examination, electrocardiogram, chest X-ray examination, and echocardiography. Several of them underwent cardiac catheterisation examination. All patients diagnosed with CHD underwent surgery. Syndromic CHDs, such as Noonan, DiGeorge, Holt–Oram, Marfan, Alagille, and Char syndromes, were excluded from this study. In addition, 200 unrelated healthy individuals (94 males and 106 females; age range, 3 months–60 years) were recruited as controls.

**Table 1.** Baseline clinical and demographic characteristics of the patients affected with CHDs (n = 172)

Variable	n or mean with SD	% or range
<b>Gender</b>		
Male	81	47.09
Female	91	52.91
<b>Mean Age (years)</b>	29	3 months 61 years
<b>Positive family history of CHDs</b>	4	2.32
<b>CHDs</b>	3	1.74
ASD	120	69.77
VSD	40	23.26
ASD + VSD	10	5.81
AVSD	2	1.16
<b>Cardiac surgery</b>	172	100

CHD = congenital heart defect; ASD = atrial septal defect; VSD = ventricular septal defect; AVSD = atrioventricular septal defect.

There was no statistically significant difference in gender ratio or mean age between the CHD and control groups ( $p > 0.05$ ). The study protocol was approved by the Institutional Research Ethics Committee of People's Hospital of the Xinjiang Uygur Autonomous Region (KY2021101304). Peripheral blood samples from all participants were obtained after informed consent.

Blood samples from participants were stored in 2 mL vacuum tubes containing Ethylenediaminetetraacetic Acid. The blood samples were immediately transferred to the freezing tubes and stored at  $-80^{\circ}\text{C}$ .

### Deoxyribonucleic acid extraction

Whole blood genomic deoxyribonucleic acid extraction kit (Tiangen Biochemical Science and Technology Co. Ltd, Beijing, China) was utilised to extract genomic deoxyribonucleic acid among white blood cells. After extraction, an ultramicro UV spectrophotometer was used to detect the concentration and purity of the deoxyribonucleic acid samples, which were then stored in a  $-20^{\circ}\text{C}$  refrigerator.

### Polymerase chain reaction amplification and deoxyribonucleic acid sequencing

Primers were designed using Primer premier 5.0 for the amplification of the coding exons of the *GATA4* (NM\_002052.3)

and *CITED2* (NM\_006079.4) genes (Table 2). Polymerase Chain Reaction was performed in 20  $\mu\text{L}$  of standard Polymerase Chain Reaction buffer, containing 2  $\mu\text{L}$  of genomic deoxyribonucleic acid, 1  $\mu\text{L}$  of forward and reverse primers, 10  $\mu\text{L}$  of Goldstar Best MasterMix, and 6  $\mu\text{L}$  of water. The amplification programme was one cycle of an initial denaturation step at  $95^{\circ}\text{C}$  for 10 min; followed by 35 cycles at  $95^{\circ}\text{C}$  for 30 s,  $64^{\circ}\text{C}$  for 30 s, and  $72^{\circ}\text{C}$  for 30 min; and a final extension at  $72^{\circ}\text{C}$  for 10 min. Reactions were performed on an ABI 9700 Polymerase Chain Reaction machine (Applied Biosystems Inc., Carlsbad, CA, USA). Products were run on 1% agarose gel to verify amplification, and purified products were sequenced in the sense direction with an ABI 3730XL deoxyribonucleic acid Analyzer (Applied Biosystems, Inc., Foster City, CA, USA). Deoxyribonucleic acid sequence files were analysed using Chromas and DNASTar software. The pathogenicity of the identified missense variants was assessed in silico using Sorting Intolerant from Tolerant (<http://sift.jcvi.org/>), Polyphen2 (<http://genetics.bwh.harvard.edu/pph2/>), and Mutation Taster ([www.mutationtaster.org](http://www.mutationtaster.org)) software.

### Genetic and bioinformatics analysis

The protein sequences of *GATA4* and *CITED2* from other species were obtained from the National Center for Biotechnology Information database. All sequences were aligned using the MUSCLE programme ([www.ebi.ac.uk/Tools/msa/muscle/](http://www.ebi.ac.uk/Tools/msa/muscle/)). Sorting Intolerant from Tolerant (<http://sift.jcvi.org/>), PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2/>), and MutationTaster ([www.mutationtaster.org](http://www.mutationtaster.org)) programmes were utilised to validate the mutation prediction of single-nucleotide variants. The structural stability of resultant proteins was predicted by I-Mutant 2.0 (<https://folding.biofold.org/i-mutant/i-mutant2.0.html>).

### Data analysis

The data analysis was performed using SPSS 24.0. The comparison of categorical variables between two groups was conducted using either the chi-square ( $\chi^2$ ) test or the Fisher exact test. Statistical significance was determined at a two-tailed  $p$  value of  $< 0.05$ .

## Results

### The frequencies of *GATA4* and *CITED2* variants

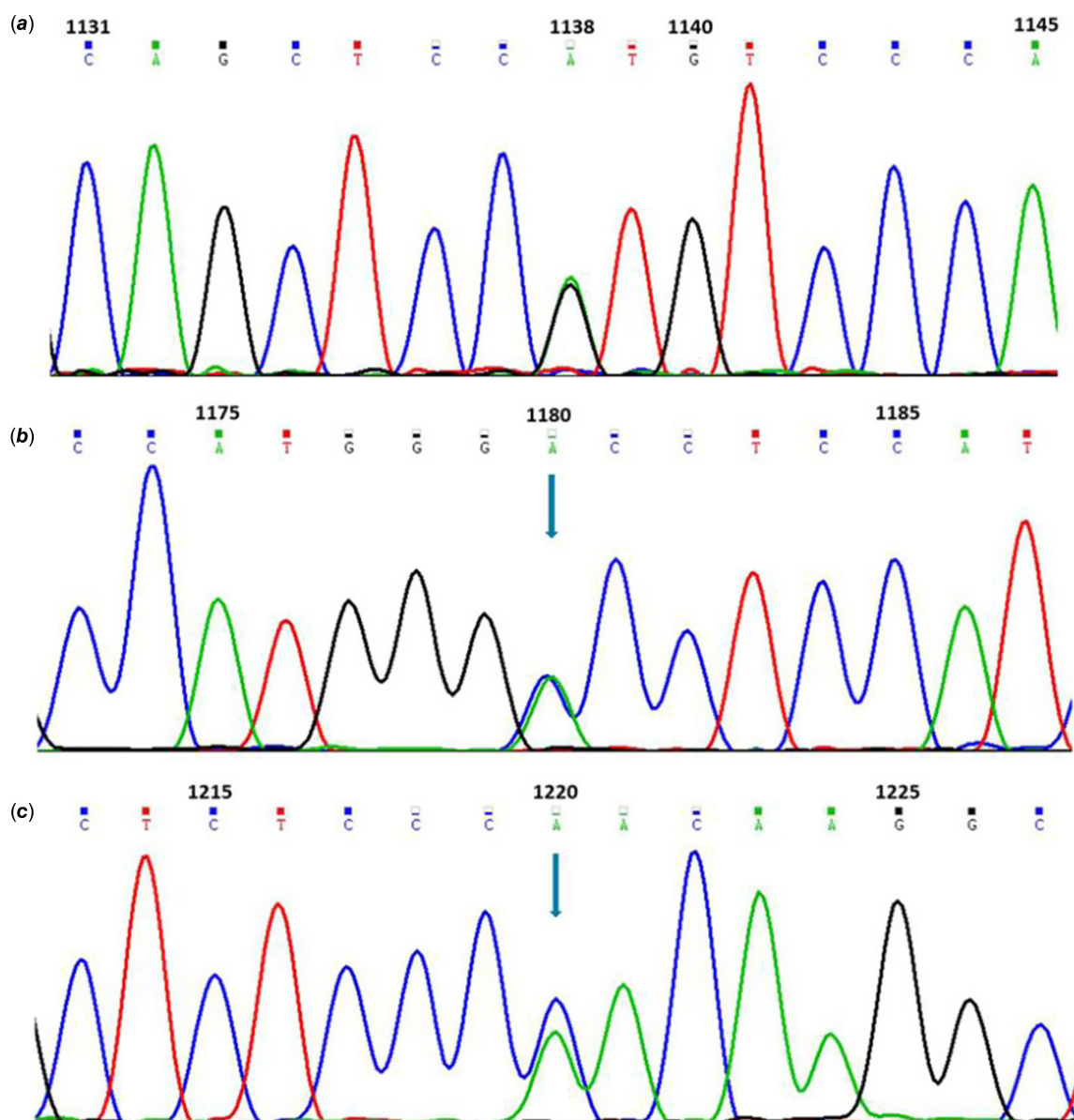
Four nonsynonymous variants of *GATA4* and *CITED2* were identified in the four CHD patients, but not in the healthy control group, resulting in an overall detection frequency of 2.33% in the patient cohort. Among the CHD patients, three reported *GATA4* variants were observed in three individuals, accounting for 1.74% of the patient group. Additionally, one novel *CITED2* variant was

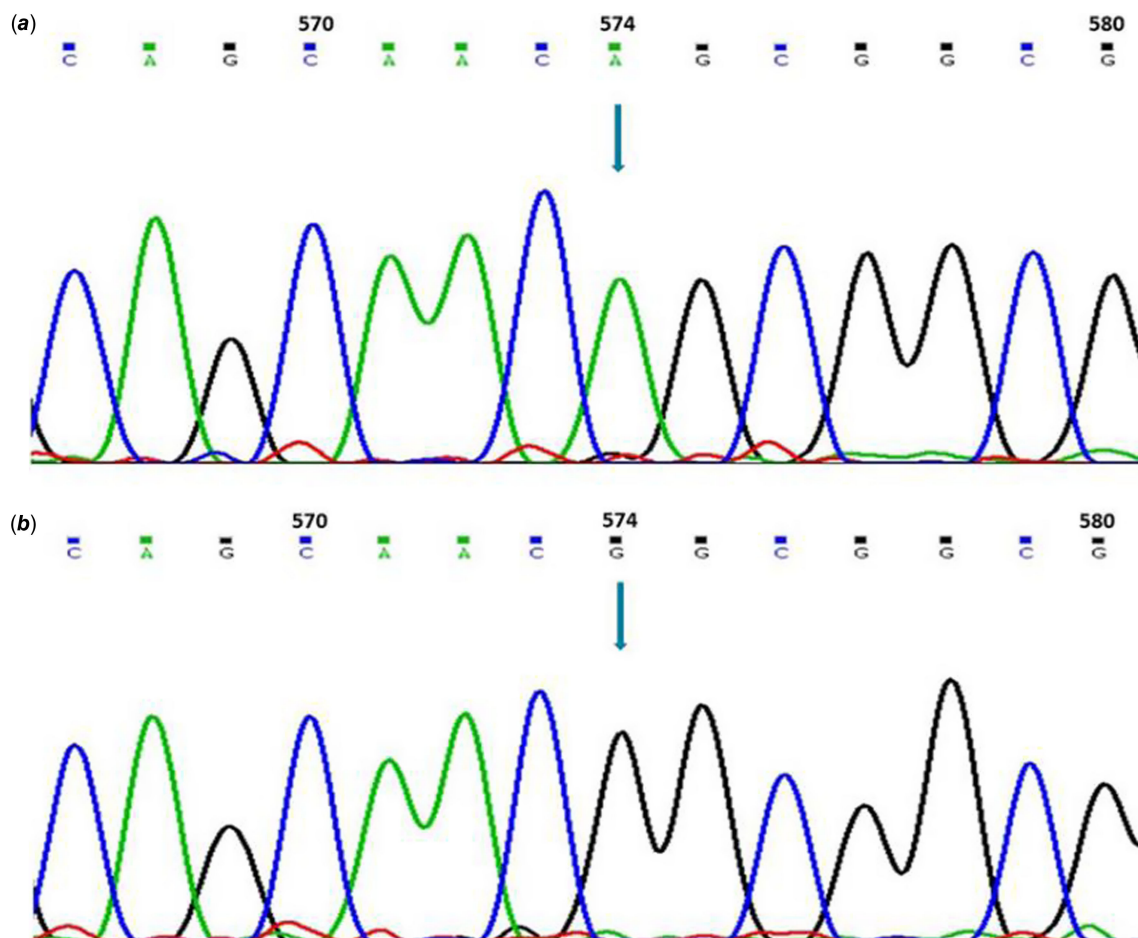
**Table 2.** Summary of the primers and size of the *GATA4* and *CITED2* genes

Amplicon	Forward Primer	Reverse Primer	Size(bp)
<i>GATA4</i> -E2	ACAGTTCCTCCACGCATATT	TGCCTTCCCTAGAACTCGAG	869
<i>GATA4</i> -E3	GTGCTGGGATTAGGGAGCAG	CACTTCTGGGCTTTGGGATTT	792
<i>GATA4</i> -E4	TTTGACACCGTGATTCCTCA	CCACTGAATCGAAACGTACC	773
<i>GATA4</i> -E5	CCCCAGGACAGAGTTTGTGT	CCAAAGATGAAAGGACCGAG	758
<i>GATA4</i> -E6	AGTATTATCTCAGGCCCTGCTG	TGATGGCATAGAGGAGCTGTT	767
<i>GATA4</i> -E7	TCACCGGGATCAGGAGAAAC	TCCAGGTACATGGCAAACAGA	770
<i>CITED2</i> -E2	AGGCTGTTAGTGGGATCTTGG	TGGGTGAATGTCAAGGCTACA	1189

**Table 3.** Frequencies of genotypes and alleles in CHD patients and controls

Gene	Nucleotide change	Frequency (%) of genotypes			Frequency (%) of alleles		
<i>GATA4</i>	c.1138G > A	GG	GA	AA	G	A	
	CHD	0.988(170)	0.012(2)	0	0.994	0.006	
	Control	1(200)	0	0	1	0	
	c.1180C > A	CC	CA	AA	C	A	
	CHD	0.994(171)	0.006(1)	0	0.997	0.003	
	Control	1(200)	0	0	1	0	
<i>GATA4</i>	c.1220C > A	CC	CA	AA	C	A	
	CHD	0.994(171)	0.006(1)	0	0.997	0.003	
	Control	1(200)	0	0	1	0	
	<i>CITED2</i>	c.574A > G	AA	AG	GG	A	G
		CHD	0.994(171)	0	0.006(1)	0.994	0.006
		Control	1(200)	0	0	1	0

**Figure 1.** Sequencing results of *GATA4* variations. **a:** Heterozygous c.1138 G > A variation. **b:** Heterozygous c.1180 C > A variation. **c:** Heterozygous c.1220 C > A variation.



**Figure 2.** Sequencing result of *CITED2* variation. Homozygous c.574 A > G variation. **a:** Homozygous A/A. **b:** Homozygous G/G.

detected in one patient, with a detection frequency of 0.58%. The frequencies of genotypes and alleles for the four missense variants in both CHD patients and controls are presented in Table 3.

#### Phenotypes of *GATA4* and *CITED2* variants

Our analysis of these patients with CHD identified three reported variants (c.1138G > A, p.V380M; c.1180C > A, p.P394T; and c.1220C > A, p.P407Q) in the *GATA4* gene (Fig. 1). The p.V380M variant was discovered in a patient with atrial septal defect. The p.P394T variant was noted in a patient with atrial septal defect. One patient with ventricular septal defect carried both p.V380M and p.P407Q variants. A homozygous variant in the *CITED2* gene, c.574A > G (p. S192G); was found in a patient suffering from ventricular septal defect and validated as the first reported variant (Fig. 2). A detailed description of the specific sequence variants of the *GATA4* and *CITED2* genes and the cardiac phenotype of patients is summarised in Table 4.

#### Identification of novel variant

We have identified a previously unreported variant in the *CITED2* gene. This variant, specifically the homozygous c.574A > G variant, resulted in the p. S192G variant. This variant was observed in a patient with ventricular septal defect and has been confirmed as the first reported variant of its kind (Fig. 2). The alignment of *CITED2* sequences, depicted in Figure 3, demonstrates

evolutionary conservation across multiple species. The p. S192G variant of *CITED2* was predicted to have deleterious effects, although it was also found to be tolerated and polymorphic. The stability of the resulting proteins was assessed using I-Mutant 2.0. This particular variant has been verified to induce a reduction in the stability of the resulting protein, as indicated in Table 4.

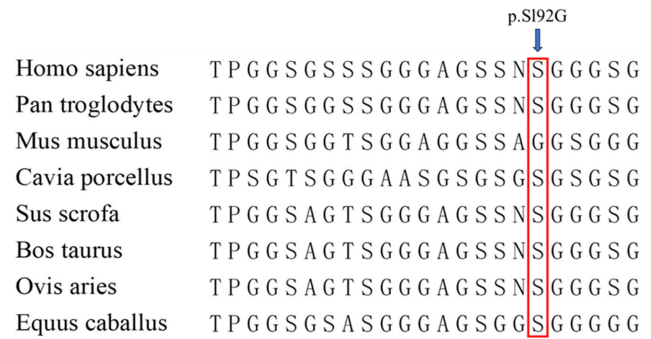
#### Discussion

*GATA4* is a master regulator required for ventral morphogenesis and heart tube formation.<sup>8</sup> The coding region of *GATA4* includes two transactivation domains, two forms of type IV zinc-finger motif, and a nuclear localisation signal. The highly preserved N-terminal zinc finger interacted with *Fog2*, and the C-terminal zinc finger interacted with transcription factors to regulate the expression of cardiac genes. Evidence from these studies confirmed that *GATA4* plays an important role embryonic heart development and associated mutations are linked with various cardiac abnormalities.<sup>9</sup> *GATA4* has also been identified as a potential aetiology of human dilated cardiomyopathy. It has been observed that *GATA4* mutants demonstrate a noteworthy decrease in transcriptional activity, thereby contributing to the pathogenesis of pathological cardiac hypertrophy.<sup>10</sup> Furthermore, a significant correlation has been noted between *GATA4* mutations and the prevalence of atrial fibrillation.<sup>11</sup> There have been more than 100 mutations of *GATA4* reported over the past decades.<sup>12</sup> Its mutation

**Table 4.** Detailed description of *GATA4* and *CITED2* nonsynonymous variations identified in this study

Gene	Nucleotide change	Amino acid alteration	Cardiac phenotype	Status	Minor allele frequency (GnomAD)	minor allele frequency (GnomAD East Asian)	SIFT	PolyPhen	Mutation-Taster	I-Mutant v2.0 (DDG)
<i>GATA4</i>	c.1138G > A	p.V380M	ASD(1),VSD(1)#	rs114868912	0.005689	0.003310	T	B	N	-1.56
	c.1180C > A	p.P394T	ASD(1)	rs200319078	0.002027	0.0001504	T	B	N	-2.58
	c.1220C > A	p.P407Q	VSD(1)#	rs115099192	0.0004667	0.003358	D	B	D	-2.11
<i>CITED2</i>	c.574A > G	p. S192G	VSD(1)	rs563655306	0.002938	0.001868	D	B	N	-0.87

SIFT, "D" meaning deleterious, "T" meaning tolerated; PolyPhen2, "D" meaning probably damaging, "P" meaning possibly damaging, "B" meaning benign; MutationTaster, "A" represents as disease-causing automatic meaning known deleterious reported in HGMD/ClinVar/dbSNP, "D" represents as disease-causing meaning likely deleterious, "N" represents polymorphism or likely harmless, "P" represents polymorphism automatic meaning known harmless. I-Mutant v2.0, DDG: DG(NewProtein)-DG(WildType) in Kcal/mol, DDG < 0: Decrease Stability, DDG > 0: Increase Stability.  
 #One patient with VSD carried both p.V380M and p.P407Q variants in *GATA4* gene.  
 DDG = Delta Delta G.



**Figure 3.** The alignment of the *CITED2* sequence. The analysis of amino acid conservation at the variation site indicates that the arrow represents the *CITED2* variation site (p.S192G), which demonstrates evolutionary conservation across most species.

is most widely studied in CHD with various clinical phenotypes, including atrial septal defect, ventricular septal defect, atrio-ventricular septal defect, tetralogy of Fallot, pulmonary stenosis, patent ductus arteriosus, double outlet right ventricle, and hypoplastic left-heart syndrome. Atrial septal defect is the most common clinical phenotype carrying the *GATA4* gene mutation in the CHD family.<sup>13,14</sup>

We identified three heterozygous variants of *GATA4* (p.V380M, p.P394T, and p.P407Q) in the CHD group. These variants have not been previously reported in previous studies. Tang et al. (2006) first found p.V380M variant in a patient with CHD, and they reported it as a disease-causing mutation in CHD.<sup>15</sup> The p.V380M variant has been implicated in a variety of CHDs, including ventricular septal defect, atrial septal defect, atrioventricular septal defect, and tetralogy of Fallot.<sup>15-20</sup> In this study, the p.V380M variant was discovered in a patient with atrial septal defect (1/172), but it was not found in our control cohort. The p.V380M variant of the *GATA4* gene is rare in cases with CHD in Chinese populations.<sup>15,20</sup> Liu et al. detected p.V380M variant in four patients (4/600) including tetralogy of Fallot, double outlet right ventricle, PA and ventricular septal defect, and transposition of the great arteries, as well as one in controls (1/300), in China.<sup>21</sup> Several studies have observed the p.V380M variant to be present at a very low frequency in populations from North America, Japan, and India. However, these studies did not establish a correlation between this single-nucleotide variant and CHD.<sup>22-26</sup> Notably, our analysis demonstrated a statistically significant higher occurrence of the SNP c.1138G > A (p.V380M) in individuals with CHD compared to the control group (Table 5).

One patient with atrial septal defect presented with p.P394T variant for *GATA4* in this study. This nucleotide change was not found in the control population. Schluterman et al. identified the p.P394T variant in an individual of Asian ethnicity with CHD (the examined individuals were of variable ethnicity and composed of 76 European Americans, 53 Hispanics, 21 African Americans, and 7 Asians). The variant was not found in the original control population of 159 individuals. The allele frequency was 3.1% in 192 control individuals of Asian ethnicity, which likely represented an SNP in the Asian subpopulation (not specified).<sup>22</sup> The p.P394T variant was found in the affected and control populations and likely represented common SNPs in India.<sup>23,26</sup> The variant of the *GATA4* gene in Chinese and Japanese populations was rare in cases with CHD and was not identified in any of the controls.<sup>20,21,24,25,27,28</sup> Some reports did not detect an association

**Table 5.** A list of the three nonsynonymous variants of *GATA4* identified among CHD patients and controls in previous studies

Position	Nucleotide variation	Amino acid variation	Patient rate	CHD Phenotype	Control rate	Population	References
rs114868912	c.1138G > A	p.V380M	2/157	unspecified	3/159	North-American population	Schluterman MK (2007) <sup>22</sup>
			10/100	1ASD, 7VSD, 1COA, 1PHT	2/50	Indian	Dinesh SM (2011) <sup>23</sup>
			5/256	TOF 125, PAVSD 84, DORV 23, PTA 24	6/500	Japanese	Kodo K (2012) <sup>24</sup>
			2/111	1DORV+Hypoplastic LV, 1CoA+VSD	3/200	Japanese	Yoshida A (2016) <sup>25</sup>
			4/600	1TOF, 1DORV, 1PA+VSD, 1TGA	1/300	Chinese	Liu Y (2017) <sup>21</sup>
rs200319078	c.1180C > A	p.P394T	11/285	unspecified	6/200	Indian	Dixit R (2018) <sup>26</sup>
			1/157	unspecified	12/192	North-American population	Schluterman MK (2007) <sup>22</sup>
			8/100	1VSD, 3PDA, 1COA, 1TOF, 1AVSD, 1TOF+VSD	5/50	Indian	Dinesh SM (2011) <sup>23</sup>
rs115099192	c.1220C > A	p.P407Q	8/285	unspecified	8/200	Indian	Dixit R (2018) <sup>26</sup>
			8/256	unspecified	18/500	Japanese	Kodo K (2012) <sup>24</sup>
			4/111	1DORV+hypoplastic LV, 1CoA+VSD, 1VSD, 1SV+PS	2/200	Japanese	Yoshida A (2016) <sup>25</sup>
			4/600	1TOF, 1DORV, 1PA+VSD, 1TGA	1/300	Chinese	Liu Y (2017) <sup>21</sup>

ASD = atrial septal defect; AVSD = atrioventricular septal defect; COA = *coarctation of aorta*; DORV = double outlet right ventricle; LV = left ventricle; PA = pulmonary atresia; PAVSD = pulmonary atresia with ventricular septal defect; PDA = patent ductus arteriosus; PHT = pulmonary hypertension; PS = pulmonary stenosis; PTA = Persistent truncus arteriosus; SV = *single ventricle*; TGA = transposition of the great arteries; TOF = tetralogy of Fallot; VSD = ventricular septal defect.

between this single-nucleotide variant and CHD in an Indian population (Table 5).

The p.P407Q variant was reported to be pathogenic in the Human Gene Mutation Database and Clin-Var. The p.P407Q variant has been implicated in a variety of CHDs, including ventricular septal defect and tetralogy of Fallot.<sup>20,26–29</sup> In most Chinese studies, the p.P407Q variant was found only in patients with CHD.<sup>20,27–29</sup> Only Liu et al. detected p.P407Q variant in four patients (4/600) including tetralogy of Fallot, double outlet right ventricle, PA and ventricular septal defect, and transposition of the great arteries, as well as one in controls (1/300), in China.<sup>21</sup> However, the p.P407Q variant was previously reported as a single-nucleotide variant in Japanese, Canadian, and American populations.<sup>24,25</sup> These analyses revealed that p.P407Q variant was found significantly more frequently in CHD cases than in controls (Table 5). One patient with ventricular septal defect carried two variants (p.V380M and p.P407Q) in this study. Two nonsynonymous variations of the *GATA4* gene were rarely detected from one patient with CHD. Qian et al. found two variants of *GATA4* (p.V380M and p.P407Q) in two patients with tetralogy of Fallot and PFO.<sup>20</sup>

The identified variations in *GATA4* were exclusively observed in the cases group, while no occurrences were found in the control group within our study. The minor allele frequency of three *GATA4* variations was assessed using the GnomAD and GnomeAsia databases. Interestingly, in certain studies, these variations were detected in both cases and healthy controls at a significantly higher frequency (Table 5). This observation implies that the *GATA4* gene variant may exhibit racial and regional disparities.

The structural stability of the resultant proteins was assessed using I-Mutant 2.0, which provided an expression of the output in

terms of free energy change value (Delta Delta G). Among the three variants of *GATA4* that were examined, it was confirmed that they caused a decrease in stability of the resultant proteins. Based on a Delta Delta G value < -0.5, it can be inferred that these variants exert a substantial influence on the proteins. The stability of proteins plays a pivotal role in determining their biological activity and functionality, making it a crucial factor. Mutations that result in reduced protein stability could potentially contribute to the onset of CHD.

*CITED2* is a cAMP responsive element-binding protein/p300 transactivator, with an Glutamate Glu(E) Aspartate Asp(D) (Glu/Asp)-rich C-terminal domain that functions as an important modulator in heart development.<sup>30</sup> Both human genetic and model organism studies have shown that altered expression of *CITED2* causes various forms of CHD. *CITED2* mutations lead to a constellation of cardiac defects. Since Sperling et al. first uncovered the connection between CHDs and *CITED2* mutations in 2005,<sup>7</sup> a growing number of mutations have been reported to cause CHD.<sup>31–38</sup> Most of these mutations are located in the SRJ region, which indicates that this region might be a mutation hot spot in patients with CHD.<sup>7,31–34,37</sup> In this study, we have identified a homozygous variation (c.574 A > G, p. S192G) of *CITED2* in a patient with ventricular septal defect. This variant has been validated as the first reported variant and was also found in the SGJ region. To assess the sequence conservation of *CITED2*, we conducted multiple sequence alignment (Fig. 3), revealing its evolutionary conservation across most species. Furthermore, the mutation prediction results indicate that the p. S192G variant is likely to be disease-causing, as it is deemed deleterious in Sorting Intolerant from Tolerant (Table 4). The structural stability of resultant proteins was predicted by I-Mutant 2.0. The variants of *CITED2* were confirmed to cause decrease in stability to the

resultant proteins (Delta Delta G value  $< -0.87$ ), indicating its greater impact towards the proteins. Proteins with decreased stability due to mutation might give rise to CHD. The I-Mutant 2.0 tool was utilised to forecast the structural stability of the resulting proteins. It was determined that the variants of *CITED2* induce a reduction in stability of the resulting proteins, as evidenced by a Delta Delta G value below  $-0.5$ . This observation suggests that the impact of the variant on the proteins is significant. Consequently, the diminished stability of proteins resulting from mutations may contribute to the development of CHD.

The heterozygous variation (c.574A > G, p. S192G, and rs563655306) in *CITED2* was previously identified in patients with aortic stenosis<sup>32,33</sup> and ventricular septal defect (<https://www.hgmd.org>). Li et al. found that p. S192G in *CITED2* results in the loss of the ability to mediate the expression of VEGF by disabling competitive binding with HIF1- $\alpha$  to cAMP responsive element-binding protein/p300.<sup>32</sup> Therefore, p. S192G mutations affect the transcriptional regulatory properties of PITX2C and VEGF, and this effect is closely related to cardiac development. These data provide important evidence that the mutation of *CITED2* may play a causative role in the development of CHD in humans. Further investigation is warranted to explore the *in silico* and *in vitro* functional validation of variations in *CITED2*.

## Conclusion

Our study indicated that nonsynonymous sequence variants in the *GATA4* and *CITED2* genes are rare and do not appear to play any significant pathogenic role in some patients with cardiac septal defects. We found that the p.V380M, p.P394T, and p.P407Q variants of the *GATA4* gene differed greatly in many studies. This gene may differ among races and regions, and it may not be an important contributor to subjects with sporadic CHD in Xinjiang. Our findings also supported the notion that the p. S192G variant of the *CITED2* gene could be pathogenic. These findings show that most CHDs have a multifactorial aetiology. *GATA4* and *CITED2* gene mutations may be the causes of idiopathic CHD in several patients. Further studies are warranted to unravel the genetic basis of cardiac defects.

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**Competing interests.** None.

**Ethical standards.** The study was approved by the Ethics Committee of People's Hospital of the Xinjiang Uygur Autonomous Region.

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