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FLT4 gene polymorphisms influence isolated ventricular septal defect predisposition in a Southwest China population

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Abstract

Background Ventricular septal defect (VSD) is the most common congenital heart disease. Although a small number of genes associated with VSD have been found, the genetic factors of VSD remain unclear. In this study, we evaluated the association of 10 candidate single nucleotide polymorphisms (SNPs) with isolated VSD in a population from Southwest China.

Methods Based on the results of 34 congenital heart disease whole-exome sequencing and 1000 Genomes databases, 10 candidate SNPs were selected. A total of 618 samples were collected from the population of Southwest China, including 285 VSD samples and 333 normal samples. Ten SNPs in the case group and the control group were identified by SNaPshot genotyping. The chi-square (χ^2) test was used to evaluate the relationship between VSD and each candidate SNP. The SNPs that had significant P value in the initial stage were further analysed using linkage disequilibrium, and haplotypes were assessed in 34 congenital heart disease whole-exome sequencing samples using Haploview software. The bins of SNPs that were in very strong linkage disequilibrium were further used to predict haplotypes by Arlequin software. ViennaRNA v2.5.1 predicted the haplotype mRNA secondary structure. We evaluated the correlation between mRNA secondary structure changes and ventricular septal defects.

Results The χ^2 results showed that the allele frequency of *FLT4* rs383985 ($P=0.040$) was different between the control group and the case group ($P<0.05$). *FLT4* rs3736061 ($r^2=1$), rs3736062 ($r^2=0.84$), rs3736063 ($r^2=0.84$) and *FLT4* rs383985 were in high linkage disequilibrium ($r^2>0.8$). Among them, rs3736061 and rs3736062 SNPs in the *FLT4* gene led to synonymous variations of amino acids, but predicting the secondary structure of mRNA might change the secondary structure of mRNA and reduce the free energy.

Conclusions These findings suggest a possible molecular pathogenesis associated with isolated VSD, which warrants investigation in future studies.

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Keywords Ventricular septal defect, *FLT4*, Polymorphism, mRNA secondary structure

Introduction

Congenital heart diseases (CHDs) are abnormalities in the structure of the heart and blood vessels, cardiovascular malformations are mainly caused by aberrant cardiac development during foetal development [1, 2]. CHDs are the most frequent birth defects [3]. Globally, the incidence is approximately 1 in 80 to 110 newborns and accounts for 30–50% of all foetal losses [4, 5]. Not all CHD patients can receive an early diagnosis, and the true frequency may be greater than previously thought [6]. Our knowledge of the underlying aetiology of CHDs is still unclear. A lot of data indicates that hereditary factors play a significant role in CHDs, despite the longstanding belief that the interaction between genetic and environmental factors have a substantial impact on the development of CHDs, despite the long-held belief that the interplay and correlation between genetic and environmental variables significantly influences the development of CHDs [2, 7, 8]. Therefore, it is increasingly important to discover the genetic pathogenesis of CHDs. The most frequent type of CHD is VSD, which can occur alone, in conjunction with other heart defects, or as a component of more complicated combinations including functionally univentricular hearts, tetralogy of Fallot (TOF), double outlet right ventricles, transposition, or other structural abnormalities [9], which are characterized by a hole or defect in the septum that separates the heart's left and right ventricles [10]. VSD occurs in approximately 1.5 to 3.5 per 1000 live births [11], and accounts for approximately 34% of all CHDs [12]. In Asia, it is estimated that 2.63 per 1000 children are born with VSD [13]. However, despite efforts to uncover the mechanism of VSD formation [14, 15], the details remain largely unknown.

The process of cardiac development is intricate and multifaceted. The formation of the ventricular septum involves intricate interactions between cells derived from various lineages, as well as the regulation of apoptosis, specification, migration, differentiation, and proliferation of these cells [16]. For example, dysregulated vascular endothelial growth factor (*VEGF*), which regulates cell proliferation, plays an important role in the pathogenesis of VSD. Studies have shown that it is associated with an increased risk for isolated VSD [17]. Additionally, the varying levels of gene expression related to energy metabolism, cell cycle regulation and growth, cytoskeletal organization, and cell adhesion are significant factors influencing the progression of VSD [18, 19]. Given the studies that have already been reported, it is reasonable to investigate the genetic factors contributing to VSD. Prior research has indicated that genetic alterations associated with the regulation of cell growth, skeletal formation, and cell adhesion could impact cardiac development. Numerous genes implicated in susceptibility to VSD are known to participate in these biological pathways [16, 20, 21]. Based on 34 congenital heart disease whole-exome sequencing and gene function candidate strategies, we selected 10 SNPs in 9 genes for genotyping. *FN1* encodes fibronectin, a protein that plays a crucial role in cellular adhesion and migration mechanisms, particularly during embryonic development [22, 23]. *DNAH5* encodes dynein, which is part of the microtubule-associated dynein complex [24]. The *FLT4* gene is responsible for encoding tyrosine kinase receptors that bind to vascular endothelial growth factors [25]. Polymorphisms in *FLT4* have been linked to TOF [26]. *LAMC3* belongs to laminin, which is involved in cell adhesion, differentiation, migration, signal transduction, neurite growth and metastasis [27]. *IQGAP1* encodes scaffold proteins and is involved in cytoskeletal rearrangement, cell adhesion, cell proliferation gene transcription and cell polarization [28]. *HYDIN* encodes an axonal and cilial protein found primarily in the foetal heart and bronchial ciliated epithelium [29]. *B9D1* is involved in cilia formation [30]. Subsequently, 10 selected SNPs were validated in 618 samples (285 VSD patients and 333 normal controls) from Southwest China to identify the genetic association with VSD.

Methods and materials

Subjects

A total of 285 non-consanguineous individuals with isolated VSD were selected for a case-control study from patients treated at Fuwai Yunnan Cardiovascular Hospital from 2017 to 2021. The clinical diagnosis was performed by a cardiologist based on the clinical and echocardiography findings with the surgical notes, and these people were confirmed as VSD patients after undergoing surgery. The control group comprised 333 non-consanguineous healthy subjects with no history of congenital heart disease. The participants in this study were exclusively sourced from Yunnan Province in southwestern China. Individuals with additional CHDs, hypertension, coronary heart disease, cardiac valve disease, tachyarrhythmia, Alzheimer's disease, acute viral myocarditis, or systemic illnesses were deliberately excluded from the research cohort. The study complied with the Declaration of Helsinki and was approved by the Ethics Committee of Fuwai Yunnan Cardiovascular Hospital (No. 2017-BG006). Prior to their involvement in the trial, the subjects provided written informed consent.

SNP selection and genotyping

This research conducted a preliminary screening of potential SNPs within the population of Southwest China by utilizing whole-exome sequencing data from 34 individuals diagnosed with congenital heart disease (novogene, Beijing City, China), The 34 individuals with congenital heart disease include 20 individuals with VSD and 14 individuals with atrial septal defects (ASD). Genomic DNA was extracted by the AxyPrep Blood Genomic DNA MiniPrep Kit (Axygen, Hangzhou City, China), 0.4 μg genomic DNA from blood were used to construct liberty by Agilent SureSelect Human All Exon V6 (Agilent USA), and sequenced on Illumina platforms with PE150 strategy in Novogene Bioinformatics Technology Co., Ltd (Beijing, China). The sequencing results were converted into fastq format, and the adapter and low-quality reads were removed to obtain clean reads. Clean data was mapped to the reference genome GRCh37 (Homo sapiens) by Burrows Wheeler Aligner (BWA) software [31]. SAMtools [32] was used to call germline SNPs and annotated by ANNOVAR [33]. The 1000 Genomes database was used as controls. The allele frequencies between the VSDs & controls, ASDs & controls, and all 34 patients & controls were assessed through the χ2 test, the False Discovery Rate (FDR) was employed for the purpose of correcting P value. Then selected the positive SNPs (FDR < 0.05) for making an intersection analysis of three comparisons. A total of 10 SNPs from the nine genes (rs6707530 in *FN1*, rs12659700 in *DNAH5*, rs383985 in *FLT4*, rs710074 in *LAMC3*, rs3124309 in *COL5A1*, rs598893 in *COL4A1*, rs2589941 in *IQGAP1*, rs7198975 and rs1774266 in *HYDIN*, rs11650112 in *B9D1*) were selected. Following this, individual genotyping was conducted on patients with VSD and control groups to validate the correlation with VSD. A total of ten candidate SNPs were genotyped using the SNaPshot method [34, 35].

Following the patient's informed consent, the DNA from the peripheral venous blood sample was extracted in order to conduct targeted amplification of the candidate SNPs, supplementary Table 1 shows the primer sequences.

Linkage disequilibrium and haplotype blocks

Based on these genotype results, SNPs of positive genes were selected from the 34 congenital heart disease whole-exome sequencing results, and SNP information was retrieved from the NCBI dbSNP database (https://www.ncbi.nlm.nih.gov/snp/). The selected SNPs were analysed using linkage disequilibrium (LD). The haplotype reconstruction results were calculated by Haploview v3.32. Haploview used the confidence interval method to perform LD assessment. We used an r^2 threshold of 0.8. SNPs were selected as the markers for our study and further plotted using a bioinformatics online tool (http://www.bioinformatics.com.cn). The ELB algorithm of Arlequin V3.5.2.2 predicted haplotypes of the bins of SNPs that were in very strong linkage disequilibrium with a specified r^2 threshold. ViennaRNA v2.5.1 [36] performed mRNA secondary structure prediction for these haplotypes.

Statistical analysis

The Statistical Package for Social Sciences (SPSS version 19, IBM Corporation, Armonk, NY) was utilized for conducting statistical analysis in this study. The age data are presented as the median (25% Percentile, 75% Percentile), categorical data are presented as the number (%), other quantitative data are presented as the mean ± standard deviation (SD), and comparisons between the two groups were carried out using the Student's t-test. Qualitative data and allele frequency were compared using the χ^2 test and Mann-Whitney test, and the genetic analysis model (dominant, recessive and additive) was employed to assess the relationship between candidate SNPs and the susceptibility to congenital heart disease. The odds ratio (OR) and 95% confidence interval (95% CI) were used to express the relative risk of disease. All statistical analyses were two-tailed and were conducted using Plink 1.9. A significance level of $P < 0.05$ was considered statistically significant.

Results

Basic characteristics of the study subjects

A total of 617 subjects were recruited, including 285 VSD individuals and 333 healthy individuals. VSD was diagnosed by cardiologists according to echocardiographic results and surgical records. The final patients' parents included in the study are: have no history of alcohol abuse, mothers have no history of smoking and obvious symptoms of infection during pregnancy and no history of gestational diabetes, no history of gestational hypertension. There were 148 males and 137 females with VSD, and the median age was 7 years (IQR 3–10 years), there are 83.51% for perimembranous VSD, 3.16% for muscular VSD, and 13.33% for subpulmonary VSD. There were 121 males and 212 females in the control group, and the median age was 48 years (IQR 38–61 years) (Table 1). The levels of blood glucose, haemoglobin, red blood cells, white blood cells, platelets, and neutrophils did not significantly differ between the two groups.

Typing and analyses of SNPs

In the all 34 samples, the Q30 (quality scores greater than 30) was 95.49 ± 1.32%. Total 265,763 SNPs were identified. The χ2 tests were performed between the VSDs & controls, ASDs & controls, and all 34 patients & controls, and adjusted P (FDR) were got. The positive SNPs

Table 1 Comparison of clinical characteristics between patients with normal people

Characteristic	Overall (*n* = 618)	VSD samples (*n* = 285)	Normal samples (*n* = 333)	*P* value
Demographic characteristics				
Age (years)	33 (7, 51.3)	7 (3, 10)	48 (38, 61)	**< 0.0001**
Male (%)	269 (43.53%)	148 (51.93%)	121 (36.34%)	**< 0.0001**
Weight (kg)	50.03 ± 27.83	26.24 ± 16.53	70.39 ± 15.03	**< 0.0001**
BMI (kg/m^2)	21.83 ± 13.21	17.29 ± 7.86	26.03 ± 16.55	**< 0.0001**
Laboratory examination				
GLU (mmol/L)	4.97 ± 0.72	4.93 ± 0.51	5.01 ± 0.86	0.166
Hb (g/L)	135.41 ± 13.55	134.67 ± 13.70	136.03 ± 13.42	0.214
RBC (10^{12}/L)	4.85 ± 0.45	4.88 ± 0.45	4.83 ± 0.45	0.266
WBC (10^9/L)	7.59 ± 2.61	7.67 ± 2.62	7.53 ± 2.60	0.486
PLT (10^9/L)	306.37 ± 83.44	309 ± 83.43	303.59 ± 83.47	0.372
NEUT (10^9/L)	3.28 ± 1.60	3.22 ± 1.62	3.34 ± 1.58	0.321
Clinical classification of surgery findings				
Perimembranous VSD	238 (38.51%)	238 (83.51%)	0 (0.00%)	NA
Muscular VSD	9 (1.46%)	9 (3.16%)	0 (0.00%)	NA
Subpulmonary VSD	38 (6.15%)	38 (13.33%)	0 (0.00%)	NA

The age data are presented as the median (25% Percentile, 75% Percentile), categorical data are presented as the number (%), other data are presented as mean (SD). P value in boldface indicates statistical significance ($P < 0.05$), VSD, ventricular septal defect; BMI, body mass index; GLU, blood glucose; Hb, haemoglobin; RBC, red blood cell; WBC, white blood cell; PLT, platelet; NEUT, Neutrophil

(FDR < 0.05) in these three comparisons were intersected. SNPs on genes that are potentially functionally related to heart development are selected. At last, 10 SNPs in 9 genes were found. All participants underwent genotyping using the SNaPshot method, achieving a 100% call rate. In both the control and case cohorts, all 10 selected SNPs adhered to Hardy-Weinberg equilibrium, with minor allele frequencies exceeding 0.05. The χ^2 test was employed to compare the allele frequencies of the 10 SNPs between the 285 cases and 333 controls, with statistical significance set at $P < 0.05$. The findings in Table 2, revealed a notable distinction in *FLT4* rs383985 between the VSD and control groups. In the broader population, alleles with low frequencies are often considered to arise through mutations, thus, the low-frequency alleles C and T in the *FLT4* rs383985 (C, T, G) were combined for correlation analysis [37], and the minor allele frequency (MAF) was statistically lower than that in the control group. Although the difference disappeared after Bonferroni correction, congenital heart disease is a complex condition caused by multiple genes, and this complexity can cause some risk alleles to be statistically borderline between positive and negative. Therefore, if there is other evidence suggesting that the *FLT4* rs383985 may be associated with the development of the ventricular septum, the effects of these mutations should still be considered.

Analysis of genetic models examining the association between candidate SNPs and VSD

The study assessed the relationship between the identified significant SNPs mentioned above and the risk of VSD using three genetic inheritance models. These models included a dominant model (MM + MW vs. WW), a recessive model (MM vs. WW + MW) and an additive model (MM vs. MW vs. WW), where M represents a low-frequency allele. The findings are presented in Table 3. According to our dominant model analysis, *FLT4* rs383985 showed a significant association with VSD ($P = 0.029$), with an odds ratio of 0.69 (95% CI: 0.50–0.90). This indicates a protective effect of *FLT4* rs383985 in relation to the rare allele, reducing susceptibility to VSD.

Linkage disequilibrium and haplotype blocks

A total of 75 SNPs in the *FLT4* gene were selected from 34 congenital heart disease whole-exome sequencing results as the markers for our study. The detailed haplotype block information and linkage disequilibrium plot

Table 2 Comparison of the gene frequency of 10 SNPs in the VSD population and normal population

Gene	SNP	Minor/Major	MAF (VSD)	MAF (control)	Alle	HWE-*P*
					P value	
FN1	rs6707530	T/G	0.21	0.26	0.060	0.249
DNAH5	rs12659700	T/C	0.11	0.13	0.271	0.742
FLT4	rs383985	(C + T)/G	0.19	0.24	**0.040**	0.444
LAMC3	rs710074	C/A	0.35	0.36	0.515	0.730
COL5A1	rs3124309	T/C	0.50	0.49	0.778	0.872
COL4A1	rs598893	C/T	0.20	0.21	0.812	0.310
IQGAP1	rs2589941	C/T	0.17	0.15	0.342	0.305
HYDIN	rs1774266	A/G	0.43	0.42	0.686	0.265
	rs7198975	A/G	0.43	0.42	0.726	0.237
B9D1	rs11650112	T/C	0.17	0.13	0.087	0.103

P value in boldface indicates statistical significance ($P < 0.05$), SNP, single nucleotide polymorphism; MAF(VSD), Minor allele frequency in VSD patients; MAF (control), Minor allele frequency in normal controls; HWE-P, P value of Hardy–Weinberg equilibrium

Table 3 Genetic model analyses of the candidate SNPs in VSD and normal populations

Gene (SNP)	Genotype	VSD Freq	Control Freq	*P* value	OR (95% CI)
FLT4(rs383985)					
Dominant	MM + MW	0.34	0.43	**0.029**	0.69(0.50–0.90)
	WW	0.66	0.57		
Recessive	MM	0.04	0.05	0.531	
	MW + WW	0.96	0.95		
Additive	MM vs.	0.04 vs.	0.05vs.	0.355	
	MW vs.	0.30 vs.	0.38vs.		
	WW	0.66	0.57		

P value in boldface indicates statistical significance ($P < 0.05$). SNP, single nucleotide polymorphism; Freq, frequency; OR, odds ratio; CI, confidence interval

Table 4 The SNP markers selected in the 34 CHD samples

Gene Symbol	dbSNP ID	Genomic Position (GRCh37)	Function
FLT4	rs3736061	Chr5:180057231	Exon 4
FLT4	rs383985	Chr5:180055862	Intron 8
FLT4	rs3736062	Chr5:180052946	Exon 10
FLT4	rs3736063	Chr5:180052817	Intron 11

are shown in Fig. 1A. *FLT4* rs383985 was strongly linked with rs3736061, rs3736062 and rs3736063 ($r^2 > 0.8$). A total of 4 SNPs were selected as the markers for our study. Figure 1B; Table 4 depict the characteristics of the SNPs in *FLT4*, including dbSNP ID, genomic position and genomic function. The *FLT4* rs3736061 and rs3736062, which cause a synonymous change in the amino acid, were not in the protein domain structures (Fig. 1C).

mRNA analysis

Based on 34 congenital heart disease whole-exome sequencing results, *FLT4* rs383985 and its strongly linked loci were selected for haplotype analysis. In the haplotype analysis, which was predicted using the ELB algorithm based on Arlequin V3.5.2.2, the four-locus haplotype consisted of *FLT4* rs383985, rs3736063, rs3736062, and rs3736061. The four-locus haplotypes in *FLT4* were obtained: CGGG, ACAA, ACGG, and CTGG. Since only rs3736061 and rs3736062 are located on the exon, only the different sites on the mRNA can be obtained. Three two-locus haplotypes consisted of rs3736061 and rs3736062: CG (carrier frequency=89.7%), AA (carrier frequency=8.8%), and AG (carrier frequency=1.5%). Among them, the CG haplotype with the highest frequency is consistent with the reference sequence in the database and carries *FLT4*-positive polymorphism linkage sites in the AG haplotype and AA haplotype. The first 1600 nucleotides of *FLT4* were selected, and the secondary structure of mRNA was predicted using ViennaRNA V2.5.1 software. Detailed secondary structure information of mRNA (the centroid secondary structure) is shown in Fig. 2. The secondary structure of mRNA carrying the highest frequency of the CG haplotype (Fig. 2A) was used as the control, with a minimum free energy of -550.44 kcal/mol. In the AG haplotype

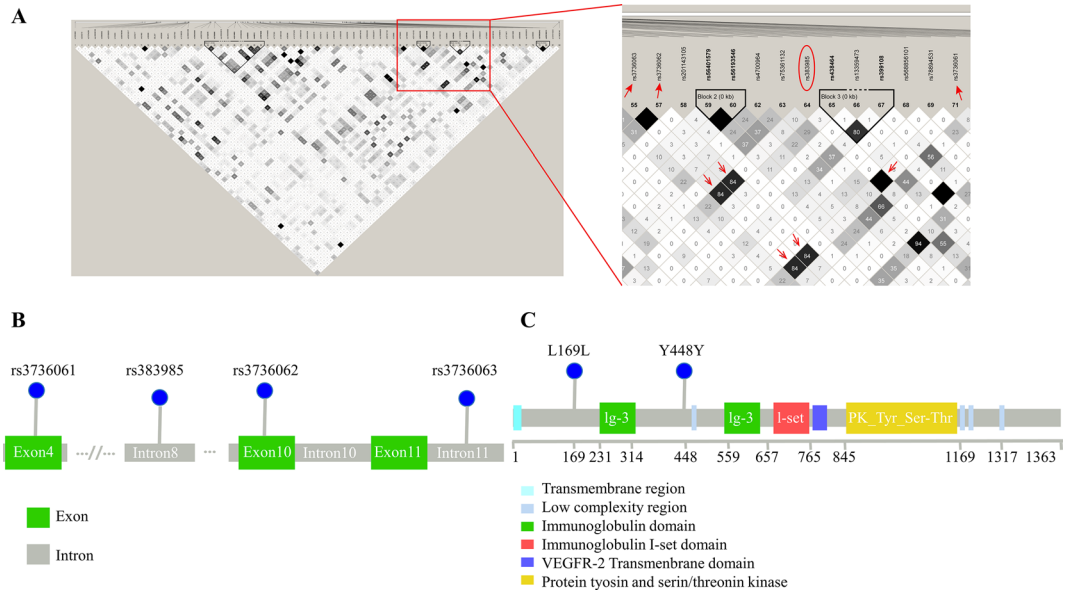

Fig. 1 Haplotype structure of *FLT4* and genomic position of *FLT4*. (**A**) Greyscale indication: black represents D ' = 1; white represents D ' = 0; 0 < D ' < 1, the darker the colour, the bigger D ' is. r^2 values times 100 are shown in the square. The red circle marks the location of *FLT4* rs383985, and the red arrow marks the strong linkage r^2 value and corresponding site location. (**B**) The positions of the high linkage disequilibrium SNPs annotated using the NCBI dbSNP database and lollipop labels show that *FLT4* rs3736061, rs383985, rs3736062 and rs3736063 were discovered in the GRCh37 assembly. (**C**) Protein polymorphism map. Lollipop labels show that *FLT4* rs3736061 and rs3736062 SNPs, which cause a synonymous change in the amino acid, were located at 169 and 448

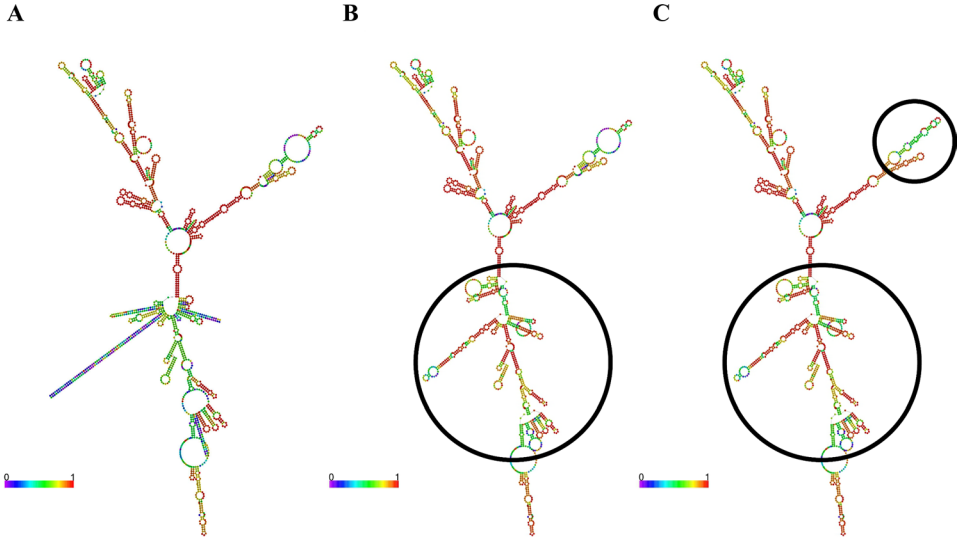

Fig. 2 mRNA secondary structure (centroid secondary structure). the structure is colored by base-pairing probabilities. (**A**) CG haplotype; (**B**) AG haplotype; (**C**) AA haplotype. Several differences (black circle) in the AG haplotype and AA haplotype compared with the mRNA secondary structure from the CG haplotype

with only one allele changed, the mRNA secondary structure was changed (Fig. 2B), and the minimum free energy decreased to -655.14 kcal/mol. In the AA haplotype with two changed alleles, the mRNA secondary structure changed even more (Fig. 2C) with a minimum free energy of -675.10 kcal/mol, and the decrease was more obvious. Compared with the reference haplotype (Fig. 2A), the more allele changes there are, the greater the change in the mRNA secondary structure of the corresponding haplotype, the more obvious the free energy decrease, and the more stable the mRNA structure.

Discussion

VSD is a prevalent type of CHDs, with research indicating that the occurrence of VSD may be influenced by genetic variation, genetic polymorphisms, and environmental factors [2, 16]. The present study aims to identify the genetic variation and aetiology of genetic polymorphisms involved in VSD. In our study, *FLT4* rs383985 was found to be associated with VSD in the population of southwest China. The human *FLT4* gene is comprised of 34 exons and is situated on chromosome 5q35.3. This gene is responsible for encoding the receptor for vascular endothelial growth factor 3 (*VEGFR3*), a crucial component of the *VEGF* signaling pathway [38]. Vascular endothelial growth factor receptors (*VEGFRs*) play a crucial role in regulating the formation and upkeep of the cardiovascular and lymphatic vascular systems. Anomalies in their expression or malfunction have been linked to various human ailments [39–41]. Early embryonic mouse hearts have been shown to exhibit the Vegfr3 protein throughout the heart at E12.5 and in the endocardium at E9.5 [42]. Moreover, mice with a complete knockout of *VEGFR3* exhibited cardiovascular failure at E9.5. Given the prevalence of this severe cardiovascular phenotype, the receptor plays a unique function in the development of the cardiovascular system in the early stages [43]. Moreover, it is possible that *VEGFR3* plays a role in controlling the expression of Vascular Endothelial Growth Factor Receptor 2 (*VEGFR2*), which is the main regulator of the process of angiogenesis [44]. Through promoting paracrine communication between endothelial cells and cardiomyocytes during normal cardiac development, *VEGFR2* plays a major role in the process of cardiomyocyte hypertrophy. Recent studies provide compelling evidence indicating that uncommon harmful polymorphisms in *FLT4* increase the likelihood of spontaneous, nonsyndromic TOF [45], the majority of *FLT4* variations associated with TOF tend to lead to the truncation of the protein-coding sequence. This truncation can occur through the introduction of stop codons, frame shift polymorphisms, or the disruption of conserved splice site regions responsible for removing intronic sequences from transcripts prior to translation [41]. Xie et al. [46] identified copy number variations (CNVs) of *FLT4* in individuals with pulmonary atresia within a cohort of patients with VSD. These results indicate that *FLT4* likely contributes significantly to the development of VSD.

In this study, we examined the proposition that variations in the *FLT4* gene may play a role in the predisposition to isolated VSD, *FLT4* rs383985 is associated with susceptibility to VSD in the southwest region, and carrying the low-frequency C or T allele is a protective factor for VSD (OR=0.69, P=0.029). *FLT4* rs383985 is located between exon 8 and exon 9 and close to exon 8. The LD analysis indicates a significant association between this

site and *FLT4* rs3736061, rs3736062, and rs3736063, in which *FLT4* rs3736061 and rs3736062 are located in exon 4 and exon 10, although both cause amino acid synonymous variations. Recent studies have shown that even the synonymous variation does not change the protein structure and affects protein expression by changing mRNA levels. Synonymous variations play nearly the same role in causing disease as nonsynonymous variations [47]. Therefore, the two-locus haplotypes in *FLT4* (rs3736061 and rs3736062) predicted the mRNA secondary structure. The AG haplotype, which accounted for approximately 1.5% of our sample, had altered mRNA secondary structure and decreased the minimum free energy relative to the reference haplotype CG. The AA haplotype had an estimated frequency of 8.8%, suggesting that the mRNA secondary structure changed more and that the minimum free energy decreased even more relative to the reference haplotype, CG. As the allele changes increased, the mRNA secondary structure changes increased, the minimum free energy decreased more, and the mRNA structure was more stable. This suggests that the synonymous variation of *FLT4* may make mRNA difficult to degrade and allow it to exist for a longer time, which plays a certain role in the prevention of VSD.

Congenital heart disease is a multifaceted genetic disorder with a polygenic nature, making the identification of its causative genes a challenge. Generally, gene association analysis is employed to investigate the impact of genetic variation on the susceptibility to complex diseases. The presence of genetic diversity among populations can pose challenges in replicating pathogenic genes or loci across different races or geographical regions. Therefore, it is significant to perform association an analysis of susceptibility to VSD in populations with diverse genetic backgrounds. This is the first reported association between *FLT4* SNPs and isolated VSD. It is important to identify the aetiology associated with genetic polymorphisms, and improving the understanding of their pathogenesis and supporting genetic causes may be important for designing prenatal screening and genetic counselling for high-risk families. In addition, this research will aid in the advancement of novel diagnostic and therapeutic approaches.

Conclusion

In this study, *FLT4* rs383985 and its strongly linked synonymous variations were found to be associated with the occurrence of isolated VSD. Hence, further investigation is necessary to validate the functional correlation between genetic variations and vulnerability to VSD, a potential avenue for enhancing the identification and treatment of congenital heart disease.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12920-024-01971-y.

Supplementary Material 1

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Author contributions
Hao Sun conceived the study, Yunhan Zhang, Xiaoli Dong and Jun Zhang analysed the data and wrote the manuscript, Miao Zhao and Jiang Wang diagnosed the patients, Jiayou Chu, Zhaoqing Yang and Shaohui Ma carried out the experiments. Keqin Lin and Zhiling Luo provided financial support and supervised the manuscript. All authors read and approved the final manuscript.

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Data availability
Data relevant to this study are available from the corresponding author (Hao Sun) upon reasonable request.

Declarations

Ethics approval and consent to participate
The study was approved by the Research Ethics Committee of Fuwai Yunnan Cardiovascular Hospital (No. 2017-BG006). All the patients enrolled in the study signed written informed consent. No animal studies were carried out by the authors for this article.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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