Association Between Single-Nucleotide Polymorphisms of NKX2.5 and Congenital Heart Disease in Chinese Population: A Meta-Analysis

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Abstract

Background: *NKX2.5* is a transcription factor that plays a key role in cardiovascular growth and development. Many independent studies have been conducted to investigate the association between the single nucleotide polymorphism 606G>C (rs3729753) in the coding region of *NKX2.5* and congenital heart disease (CHD), although the results were inconsistent. This study aimed to reveal as much as possible the relationship between *NKX2.5* single nucleotide polymorphism 606G>C and the risk of congenital heart disease in the Chinese population through meta-analysis.

Methods and Results: After retrieving related articles in PubMed, MEDLINE, EMBASE, Web of science, Coherane, China National Knowledge Infrastructure (CNKI), Wanfang DATA, VIP database until Aug 2021, a total of 8 studies were finally included. Then, we merged the qualified research data into allele model, dominant model, recessive model, heterozygous model, homozygous model, additive model respectively. Overall meta-analysis results showed that 606G>C was not associated with congenital heart disease of the Chinese population in any model. Also, subgroup analysis based on congenital heart disease type gave the same negative result. Sensitivity analysis showed that there was no significant correlation after the deletion of each study. The results were negative and the heterogeneity was not significant.

Conclusion: Our results show that *NKX2-5* single nucleotide polymorphism 606G>C may not lead to the risk of congenital heart disease in Chinese.

Introduction

Congenital heart disease (CHD) refers to a severe structural abnormality of the heart or intrathoracic blood vessels that occurs at birth[1]. Common types of CHD include atrial septal defect (ASD), ventricular septal defect (VSD), and patent ductus arteriosus (PDA). CHD is one of the most common types of deformities in humans[2] and the leading cause of neonatal death[3]. Worldwide, the incidence of CHD is estimated to be 9.1 per 1,000 live births[4], while in China, about 800,000 to 1.2 million children are born with birth defects each year, including about 220,000 cases of CHD[5]. At present, the diagnosis of congenital fetal abnormalities mainly depends on prenatal ultrasound screening and laboratory tests[6], while computed tomography (CT), magnetic resonance imaging (MRI) and echocardiography are also common imaging methods used to diagnose CHD[7]. Laboratory methods for prenatal diagnosis include cytogenetics, the biochemical examination of amniotic fluid, etc. The gold standard for invasive prenatal diagnosis is classic cytogenetic analysis[8]. With the advancement of medical technology, the survival rate of patients with CHD has increased[9]. CHD is a multifactorial disease related to genetic and environmental factors[10]. The causes of birth defects have been widely discussed, but have not been fully elucidated[12]. By identifying the source of the defect, the defect rate could be reduced or eliminated[12].

The human homeobox gene *NKX2.5* is the earliest discovered gene related to human heart development. It locates at the far end of chromosome 5, that is, the 5q34-q35 region. It is abundantly expressed in the human fetal heart, suggesting that it plays a key role in human heart development[13]. In animal model studies, the deletion of *NKX2.5* could cause abnormalities in the morphology of the embryonic heart, growth retardation, embryo death in mice[14], and malformations in Xenopus laevis hearts[15]. These further confirmed the important role of *NKX2.5* on cardiac development.
Several studies have found that the \textit{NKX2.5} mutation was associated with variety of human heart malformations\cite{16–25}. So far, a total of about 105 \textit{NKX2.5} mutations has been discovered, included synonymous mutations, missense mutations, insertion mutations, and deletion mutations. The evaluation of the \textit{NKX2.5} mutation could not only provide a basis for the early diagnosis of CHD, but also identify family members who may be at risk. \cite{26}. \textit{NKX2.5} single nucleotide polymorphism (SNP) 63A>G (rs2277923, Glu21Glu) and 606 G>C (rs3729753, Leu202Leu) are two SNPs that have been studied more frequently in CHD. The latest meta-analysis found that there was no correlation between the \textit{NKX2.5} gene 63 A>G (rs2277923, Glu21Glu) polymorphism and CHD susceptibility in Chinese and non-Chinese populations\cite{27}. 606 G>C (rs3729753, Leu202Leu) is a synonymous mutation of \textit{NKX2.5}, a large number of independent studies have explored its relationship with CHD, but the results are still controversial\cite{28–45}. Because the statistical power of individual research was not enough, in order to better assess the relationship between the \textit{NKX2.5} gene 606 G>C polymorphism and the risk of Chinese CHD, we conducted this meta-analysis.

\section*{Methods And Materials}

\subsection*{Literature Search Strategy}

Use the following keywords: “Homeobox Protein Nkx 2.5”, “Nkx-2.5, Homeobox Protein”, “NK2 Homeobox 5 Protein”, “Homeobox Transcription Factor Csx-Nkx2-5”, “Homeobox Transcription Factor Csx Nkx2 5”, “Homeobox Protein Csx-Nkx2.5”, “Csx-Nkx2.5, Homeobox Protein”, “Homeobox Protein Csx Nkx2.5”, “Cardiac-Specific Homeobox Protein”, “Cardiac Specific Homeobox Protein”, “Homeobox Protein, Cardiac-Specific”, “Transcription Factor Nkx-2.5”, “Nkx-2.5, Transcription Factor”, “Transcription Factor Nkx 2.5”, “Nucleotide Polymorphism, Single”, “Nucleotide Polymorphisms, Single”, “Polymorphisms, Single Nucleotide”, “Single Nucleotide Polymorphisms”, “SNPs”, “Single Nucleotide Polymorphism” to carry out an unlimited search of electronic databases of PubMed, MEDLINE, EMBASE, Web of science, Coherane, China National Knowledge Infrastructure (CNKI), Wanfang DATA, VIP database. The initial search was conducted in Sep 1, 2021, identified relevant articles published as of Aug 31, 2021. We conducted the meta-analysis and report the results based on the preferred reporting items stated by System Review and Meta-Analysis (PRISMA).

The inclusion criteria for this study were formulated before the literature search. Eligible studies met the following conditions: (1) Articles that have been published electronically; (2) Case-control studies of unrelated CHD patients and healthy controls; (3) Evaluation of the correlation between \textit{NKX2.5} 606G>C polymorphism (rs3729753) and the risk of CHD; (4) Provided sufficient genotype data to calculate odds ratios (ORs), corresponding 95\% confidence intervals (CIs) and could be able to establish various genetic models (5) All texts are available in English or Chinese; (6) Histological or pathological confirmation Of non-syndromic CHD; All clinical types, such as atrial septal defect (ASD) and ventricular septal defect (VSD), were included in this meta-analysis. Pedigree studies, case reports, case series, reviews, editorials, the meta-analysis, animal studies, expert opinions, and studies that consider CHD as part of any known genetic disease or multiple congenital abnormalities syndrome were excluded.

\subsection*{Data Extraction}

The two reviewers independently extracted the following information from the included studies: first author, year of publication, country, type of study design, types of CHD, genotype and gene frequency in patients with CHD
and control group, and whether the *NKX2.5* gene polymorphism was in accordance with Hardy-Weinberg equilibrium (HWE) in the control group. Using the classic assessment tool, the Newcastle-Ottawa Quality Assessment Scale (NOS), to evaluate the quality of non-randomized studies from the perspectives of selection, comparability, exposure, and evaluate the effectiveness of all case-control studies. Two reviewers (HC and XW) independently performed data extraction and quality evaluation. When necessary, the reviewer wrote to the author to obtain additional information or original data.

**Statistical Analysis**

All data were analyzed by Stata software version 16.0 (Stata Corp LP, TX, USA). If the P-value <0.05, the difference was considered statistically significant. Chi-square test was used to calculate the P value of the control group HWE. The association between SNP 606G>C and susceptibility to CHD was estimated by combined odds ratios (ORs) and 95% confidence intervals (CIs) under different genetic models. Cochran’s Q statistical test was used to assess heterogeneity between studies. If the probability value (P-value) was less than 0.1 or I² is greater than 50%, the random effect model was used because of the significant heterogeneity; otherwise, the fixed-effect model was applied. Then the subgroup analysis based on the type of CHD was performed. Sensitivity analysis was performed to assess the impact of each individual study on the overall estimate. Begg and Egger’s tests were used to assess potential publication bias.

**Results And Discussion**

**Literature inclusion**

Figure 1 shows the flow chart of document retrieval and selection. A total of 53 documents were found in the initial search, after excluding irrelevant or duplicate articles by reading the title and abstract, selected 24 articles for further evaluation. After reviewing the full text, 16 more publications were excluded for the following reasons: 2 studies had no 606G>C data; 9 studies were conducted among non-Chinese, 8 of these studies were unable to provide complete data to build various genetic models; complete allele data was not obtained in the other 5 studies based on Chinese population. Finally, including 4 English and 4 Chinese, a total of 8 studies (1361 cases and 2030 controls) were included. The HWE test of each control group included in the study was consistent with HWE. Among these studies, 2 studies explored only one type of CHD, 3 studies explored only ASD and VSD, and used the same population as a control group, and the remaining studies involved multiple types of CHD. The characteristics of the included studies are shown in Table 1 below.
Table 1

characteristics of the included documents

| First author | Year | Country | CHD case | Control | phenotype |
|--------------|------|---------|----------|---------|-----------|
|              |      |         | Genotypes | Alleles | Genotypes | Alleles | Genotypes | Alleles |
|              |      |         | n | GG/GC/CC | G/C (%) | n | GG/GC/CC | G/C (%) |
| Yin, J.      | 2019 | China   | 98 | 92/6/0 | 96.9/3.1 | 200 | 189/11/0 | 97.3/2.7 | Multiple |
| Cao, Y.a     | 2016 | China   | 107 | 101/6/0 | 97.2/2.8 | 487 | 465/22/0 | 97.7/2.3 | ASD |
| Cao, Y.a     | 2016 | China   | 385 | 367/18/0 | 95.3/4.7 | 487 | 465/22/0 | 97.7/2.3 | VSD |
| Zhang, W.    | 2016 | China   | 120 | 116/4/0 | 98.3/1.7 | 120 | 117/3/0 | 98.7/1.3 | ASD |
| Cao, Y.      | 2015 | China   | 70  | 68/2/0 | 98.6/1.4 | 136 | 131/5/0 | 98.2/1.8 | Multiple |
| Tang, J. a   | 2015 | China   | 50  | 48/2/0 | 98/2 | 50 | 47/3/0 | 97/3 | VSD |
| Tang, J. a   | 2015 | China   | 51  | 49/2/0 | 98/2 | 50 | 47/3/0 | 97/3 | ASD |
| Zhao a       | 2014 | China   | 40  | 37/3/0 | 96.2/3.8 | 50 | 45/5/0 | 95/5 | ASD |
| Zhao a       | 2014 | China   | 50  | 47/3/0 | 96.9/3.1 | 50 | 45/5/0 | 95/5 | VSD |
| Zhang, W.    | 2009 | China   | 230 | 219/11/0 | 97.6/2.4 | 130 | 118/12/0 | 97/3 | Multiple |
| Liu, X. Y.   | 2009 | China   | 160 | 145/15/0 | 95.3/4.7 | 200 | 191/9/0 | 97.8/2.2 | VSD |

a: These three studies shared identical control subjects

Overall Meta-analysis

Figure 2 shows the combined results of the 606G> C dominant model. Since heterogeneity was not significant (p= 0.884), a fixed-effects model was used. In the heterozygous gene, allele gene, dominant gene, and additive gene models, NKX2.5 606G> C SNP was not significantly related to the occurrence of CHD. Table2 shows the results of various models. Because the CC data of 606G> C was zero, the recessive gene and homozygous gene models could not be analyzed.

Table 2 Overall analysis results of various models
### Subgroup Analysis

For the *NKX2.5* 606G>C polymorphism and CHD risk, a subgroup analysis of existing data was performed based on the type of CHD in the study population. In multiple, VSD and ASD subgroups (Figure 3), we failed to find any significant correlation between *NKX2.5* 606G>C polymorphism and CHD in any model. (Table 3).

#### Table 3 Subgroup analysis results of various models

| Model  | OR    | 95% CI    | P   | I²   | Ph  |
|--------|-------|-----------|-----|------|-----|
| Heterozygote | 1.062 | 0.772-1.461 | 0.884 | 0.0% |
| Allele | 1.061 | 0.774-1.452 | 0.896 | 0.0% |
| Dominant | 1.062 | 0.772-1.461 | 0.884 | 0.0% |
| Additive | 0.941 | 0.684-1.295 | 0.884 | 0.0% |

### Sensitivity Analysis

Although there was no significant heterogeneity between studies, we still conducted a sensitivity analysis to assess the impact of each study on the overall estimate. Sensitivity analysis was performed by removing one individual study at each time. For the subgroup 606G>C polymorphism, deleting any study did not affect the overall results. Sensitivity analysis shows relatively robust results.

### Publication Bias
Using Begg's Test and Egger's test to detect publication bias, no publication bias was found for NKX2.5, 606G>C (Pr>|z| = 0.350). As shown in the funnel diagram and egger diagram (Figure 4&5).

**Discussion**

In most societies, heart disease-related deaths are the most common, and CHD accounts for a large proportion, especially among infants and children[46]. Genetic factors play an important role in its development[47]. Prenatal diagnosis is an important means to prevent CHD[48]. Through genetic diagnosis, accurate genetic counseling and prenatal examination could be provided [49], thereby reducing the rate of birth defects.

The transcription factors NKX2.5, GATA 4, Myocardin, and Tbx20 play a vital role in cardiac morphogenesis and differentiation. During development, the expression of NKX2.5 precedes the expression of other known heart-specific genes[50]. Mice with the targeted disruption of the NKX2.5 gene showed cardiac developmental arrest during the circulatory stage and died in the uterus. Its gene mutation could lead to the key determinant of cardiac morphology-circular morphogenesis does not start [14]. The mutation of NKX2.5 Homeodomain seriously reduced the DNA binding activity, with little or no transcription activation function [51], resulting in atrial septal defect, Junctional atrial septal defects, other ventricular septal abnormalities, and mild conduction defects[52]. In embryonic, neonatal and adult mouse models, progressive and severe cardiac conduction defects and heart failure occurred [53, 54].

Up to now, there has not been any functional research on the 606G>C polymorphism (Leu202Leu). It locates in exon 2 of NKX2.5 gene. Although this SNP does not change the amino acid sequence and structure of the NKX2.5 domain, most studies have found that this polymorphism has no significant difference between sporadic congenital heart disease and healthy controls. However, some studies have reached the opposite conclusion, which suggests that this polymorphism may cause CHD [28, 30]. Interestingly, neither article provided complete genotype and allele numbers, so it was not included in the study. Both were published in 2018 and beyond. Therefore, we conducted the current meta-analysis to resolve the conflict and obtained a more conclusive result. Consistent with previous meta-analysis results [55, 56, 57], our overall results suggested that the NKX2.5 606G>C polymorphism may not be related to the risk of CHD. Although the results were consistent with previous meta-analysis results, considering our findings that NKX2.5 606G>C polymorphism was based on more qualified and relatively high-quality studies, the sample size of this analysis was significantly larger than previous studies (Table 4), and for the first time analyzing varies genetic models, which further confirmed this polymorphism has no obvious relationship with CHD.

| Author     | year | case number | control number | result  |
|------------|------|-------------|----------------|--------|
| Wang, Z.   | 2013 | 748         | 630            | negative |
| Xie, X.    | 2016 | 1330        | 1167           | negative |
| Chen, L. T.| 2018 | 978         | 937            | negative |
This study was not without limitations: First, the number of studies that study the relationship between \textit{NKX2.5} gene polymorphisms and risk of CHD was still limited, especially the number of studies that include genotype and allele results was limited, and only some studies the data could be integrated. Second, this meta-analysis was based on the Chinese population, also there was only one non-Chinese study that could provide enough data to establish various genetic models, so the role of this polymorphism in other ethnic groups could not be known. Third, all included studies were published in English or Chinese; therefore, some qualified articles in other languages might be missed. Fourth, the sample size was relatively small. In addition, all included studies were retrospective study designs. Therefore, other large-scale and well-designed studies are needed to further confirm our results. Fifth, it was impossible to obtain some basic data for comparison, such as the ratio of men to women, age, etc., which prevents further analysis.

**Conclusions**

In conclusion, the current meta-analysis shows that \textit{NKX2.5} 606 G>C polymorphism has no significant correlation with the pathogenesis of CHD. However, considering that the current results are based on a limited number of case-control studies, it is necessary to draw more credible conclusions in a multi-center, larger sample, high-quality case-control study. The homeobox transcription factor \textit{NKX2.5} is a key regulator of cardiac gene expression and cardiac development, and it is also the most studied cardiac development gene. Although this meta-analysis is negative, we look forward to more research in the future focusing on the role of this gene and its polymorphism in the pathogenesis of CHD.

**Declarations**

**Ethics statement**

Ethics approval was not required for this research.

**Contributorship statement**

Huan Chen wrote the manuscript. Huan Chen and Xin Wang participated in the search strategy development. Xi Wang, MingYuan Wang assisted in acquisition, analysis, or interpretation of data for the work. Yuqing Wu, Tianjiao Li prepared figures and tables. All authors reviewed the manuscript.

**Competing Interest**

None declared.

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**Data sharing**

No additional data available.
No patient involved.

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Figures
Figure 1

Flowchart of this study
Figure 2

Forest plot on association between NKX2-5 606G>C polymorphism and CHD risk (heterozygous gene model). NKX2.5 606G>C SNP was not significantly related to the occurrence of CHD.
The subgroup forest plot on association between NKX2.5 606G>C polymorphism and CHD risk (heterozygous gene model). There is no significant correlation between NKX2.5 606G>C polymorphism and CHD.
Figure 4

Begg's funnel plot
**Figure 5**

Egger's publication bias plot

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