The role of NKX2-5 gene polymorphisms in congenital heart disease (CHD): a systematic review and meta-analysis

Sana Ashiq1*, Kanwal Ashiq2,3 and Muhammad Farooq Sabar1

Abstract

Background: The gene NKX2-5 is a key transcription factor that plays an essential role in normal cardiac development. Although some recent studies have studied the role of polymorphisms in the NKX2-5 gene in congenital heart diseases (CHDs), the results were not consistent and remained uncertain. Therefore, we conduct a review of literature and investigate the association of genetic polymorphisms with CHDs.

Results: We selected seventeen studies regarding the association of NKX2-5 gene rs2277923 polymorphism with CHDs. Overall, in all the tested genetic models, the 63A > G polymorphism was not significantly associated with increased congenital heart defects risk. We used pooled odds ratios (OR) to calculate the association of CHDs with rs2277923 including allelic model: OR 1.00, 95% CI 0.82–1.21; homozygote model: OR 0.95, 95% CI 0.68–1.33, recessive model: OR 0.89 CI 0.70–1.13, heterozygote model: OR 1.09, 95% CI 0.87–1.37, dominant model: OR 1.08 CI 0.82–1.42 and overdominant model: OR 1.17 CI 1.01–1.35. In addition, our analysis suggests that no publication bias exists in this meta-analysis.

Conclusions: Our findings suggested that 63A > G polymorphism in the NKX2-5 gene was not significantly associated with congenital heart defects. However, in the future, more studies with increased sample size are required that may provide us more definite conclusions.

Keywords: NKX2-5, Polymorphisms, Congenital heart diseases, Meta-analysis, CHDs

Background

Congenital heart diseases (CHDs) or congenital heart defects are defects in great vessels or the heart that arise during cardiac development in the embryo [1, 2]. CHDs are considered one of the major causes of mortality and morbidity in infants. Globally, it is the most common disease with an estimated prevalence of six per thousand live births [3]. Every year, 1.35 million infants are born with these cardiac defects worldwide [4]. The 2020 classification system is being used nowadays to classify congenital heart defect patients into mild, moderate, and severe [5]. It can be further divided as isolated lesion and complex lesion in combination with various heart defects, or it may occur as syndromic CHDs [6]. The patient suffering from CHDs clinically presents with cough, difficulty in breathing, repeated chest infections, and mostly failure to thrive depending upon the type or subcategory of CHD [7]. It is a multifactorial disease that involves genetic, as well as environmental risk factors [8]. The extrinsic factors include abnormal embryonic development due to the lack of essential nutrients or use of an excessive toxic substance such as thalidomide, alcohol, smoking, hypoxia, anticonvulsants, and antidepressants, while the intrinsic factors include maternal diseases such as obesity, gestational diabetes, and maternal rubella virus infections which can cause CHD in 90% of cases [9]. The genetic variants in the genes encoding structural
proteins, signal transduction, and transcription factors including T-box factors (TBX), NKX2-5, and the GATA can cause perturbation in normal cardiac development [10]. The NKX2-5 gene is a key player in almost all phases of cardiac development including septation, regulation of cardiac progenitors cell numbers, valve formation, and conduction system development [11]. The NKX2-5 contains two exons and situated on chromosome 5q34 that encodes the 324 amino acid protein. The gene belongs to the family of homeodomain-containing transcription factors which interacts with DNA through its helix-turn-helix DNA-binding motif. Thus, a single-nucleotide polymorphism (SNP) can disrupt the gene function resulting in abnormal cardiac morphogenesis [12]. The rs2277923 is a synonymous variant in which arginine is replaced by guanine at position 63 on exon 1. This polymorphism affects the normal gene function that was first reported in 52 control subjects by Benson et al. in 199 [13].

Rationale
There are many inconsistencies in the published study results regarding the role of rs2277923 in the NKX2-5 gene, including Iranian [14] Asian [15] Moroccan [16], and Caucasian population [17]; hence, it is needed to analyze all the available published literature that provide us the most definite results for the role of rs2277923 polymorphism in congenital heart diseases.

Methods
Aims
The purpose of the present study was to determine the association of rs2277923 polymorphism in the NKX2-5 gene with congenital heart defects.

Design
This study was done according to the standard Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2009 guidelines, and it is also registered with PROSPERO (PROSPERO registration number CRD42020207952). A PRISMA checklist is also given as a Additional file 1.

Literature search
Electronic databases of Ovid, PubMed, Web of Science, Cochrane Library, Medline, and EMBASE were searched till March 30, 2021, by using the following keywords and MeSH terms: ‘CHD,’ ‘NKX2-5,’ ‘worldwide,’ ‘congenital heart disease,’ ‘gene polymorphism,’ ‘variant,’ ‘genotype,’ and ‘mutation.’ Further identification of each potentially eligible article was conducted based on a manual search of the individual article reference list. In the final analysis, all the duplicate research articles were not included.

The characteristics of participants
For the selection of studies, the following criteria were used (1) The full-length published research studies that investigated the relationship of NKX2-5 gene polymorphism (rs2277923) with congenital heart diseases (2) The retrospective case–control studies (3) Adequate data were available for the genetic statistical analysis. The review articles either narrative or systematic, meta-analysis, doctoral thesis, not published in the English language, not designed as a case–control study, or research articles that not provided enough data for the statistical analysis were excluded from the present study.

Process
Data extraction
A predesigned data extraction table was used to minimize the selection bias. All three authors individually assessed and extracted the required detailed information from each included study. The following details were abstracted from each selected article: author names, country, publication year, the sample size of both case and control subjects, baseline characteristics, genotypic method, distribution of alleles and genotypes, and evidence confirming the Hardy–Weinberg equilibrium (HWE). Each study’s detailed characteristics are also summarized in Tables 1 and 2.

Quality evaluation
Each author evaluated the quality of published studies, and any discrepancies were solved by discussion to achieve a consensus. According to the Newcastle–Ottawa Scale (NOS), the quality of each original study was evaluated. The Newcastle–Ottawa Scale ranges from Worst (0) to Best (9).

Statistical analysis
The role of NKX2-5 polymorphism (rs2277923) in the pathogenesis of congenital heart disease was checked by calculating the pooled odds ratio (ORs) and 95% confidence interval (CI). The chi-squared test and I² statistic were used to calculate the heterogeneity between each included study. In the presence of significant heterogeneity, we used a random-effects model (DerSimonian–Laird). While in the absence of heterogeneity, a fixed-effect model (Mantel–Haenszel) was used. The genetic models used for rs2277923 were allelic model, heterozygote and homozygote model, overdominant, dominant, and recessive model. To check the stability of
### Table 1  Characteristics of each study included in the final analysis

| Serial no | Authors | Year | Country | Gender wise distribution (M/F) | SOC | Sample size | Genotyping | NOS score |
|-----------|---------|------|---------|---------------------------------|-----|-------------|------------|-----------|
| 1         | Behiry  | 2019 | Egypt   | 44% = M 56% = F                 | PB  | 150 90      | PCR-Sequencing | 8         |
| 2         | Peng    | 2010 | China   | NA                              | PB  | 135 114     | PCR-Sequencing | 7         |
| 3         | Cao     | 2015 | China   | 29 = M 41 = F                   | HB  | 70 136      | PCR-Sequencing | 7         |
| 4         | Ketharnathan | 2015  | India   | 26 = M 24 = F                   | PB  | 50 50       | PCR-Sequencing/RT-PCR | 8         |
| 5         | Pang    | 2012 | China   | 110 = M 103 = F                 | HB  | 213 194     | PCR-Sequencing | 8         |
| 6         | Dinesh  | 2010 | India   | NA                              | PB  | 150 70      | PCR-Sequencing | 7         |
| 7         | Ouyang  | 2011 | China   | NA                              | PB  | 125 105     | PCR-Sequencing | 7         |
| 8         | Wang et al. | 2019  | China   | 157 = M 282 = F                 | PB  | 439 567     | Multiplex PCR, qPCR, Sequencing | 8         |
| 9         | Liu [1] | 2009 | China   | NA                              | PB  | 160 200     | PCR-Sequencing | 7         |
| 10        | Liu [2] | 2009 | China   | NA                              | PB  | 180 200     | PCR-Sequencing | 7         |
| 11        | Han     | 2011 | China   | NA                              |     | 81 52       |             |           |
| 12        | Xie     | 2013 | China   | 71 = M 65 = F                   | PB  | 136 200     | PCR-Sequencing |           |
| 13        | Xiong   | 2013 | China   | 166 = M 58 = F                  | PB  | 224 121     | PCR, DHPLC-Sequencing | 7         |
| 14        | Yin     | 2018 | China   | 59 = M 39 = F                   | PB  | 98 200      | PCR-direct sequence analysis | 8         |
| 15        | Zhang   | 2009 | China   | 132 = M 98 = F                  | PB  | 230 200     | PCR, denaturing high-performance liquid chromatography, and sequencing | 8         |
| 16        | Zhao    | 2020 | China   | NA                              | HB  | 620 620     | PCR, MassARRAY system | 8         |
| 17        | Shi     | 2005 | China   | NA                              | NA  | 110 110     |             |           |

### Table 2  The allele frequencies and genotypes distributions of NKX2-5 polymorphism rs2277923

| Serial no | Authors | Year | Genotypes distribution | Alleles distribution |
|-----------|---------|------|------------------------|---------------------|
|           |         |      | AA AG GG               | Cases Controls      |
|           |         |      |                        | A G Cases Controls  |
| 1         | Behiry  | 2019 | 09 87 54               |                   |
| 2         | Peng    | 2010 | - - -                  |                   |
| 3         | Cao     | 2015 | 20 37 13               |                   |
| 4         | Ketharnathan | 2015  | 20 19 11               |                   |
| 5         | Pang    | 2012 | 27 49 24               |                   |
| 6         | Dinesh  | 2010 | 77 49 24               |                   |
| 7         | Ouyang  | 2011 | - - -                  |                   |
| 8         | Wang et al. | 2019  | - - -                  |                   |
| 9         | Liu [1] | 2009 | 30 70 60               |                   |
| 10        | Liu [2] | 2009 | 32 85 63               |                   |
| 11        | Han     | 2011 | 08 43 30               |                   |
| 12        | Xie     | 2013 | - - -                  |                   |
| 13        | Xiong   | 2013 | - - -                  |                   |
| 14        | Yin     | 2018 | 112 50 388             |                   |
| 15        | Zhang   | 2009 | 30 107 93              |                   |
| 16        | Zhao    | 2020 | 93 310 217             |                   |
| 17        | Shi     | 2005 | 7 27 76                |                   |

|           |         |      |                        |                   |
|           |         |      | A G Cases Controls      |                   |
the results, we performed the sensitivity test. Publication bias was assessed with the funnel plot. We performed the Begg’s and Egger’s test to evaluate the publication bias, and publication bias was considered present when $p \leq 0.05$. The MetaGenyo tool was used for performing the meta-analysis.

**Results**

**Characteristics of final included studies**

Initially, 334 published studies were selected, of which 217 articles were not included as they did not study the rs2277923 association with congenital heart diseases, thus 60 articles were selected for further evaluation. Among these, 17 original articles met our inclusion criteria; therefore, these were selected further for final analysis. The complete screening method for the literature search is given in Fig. 1. Of these seventeen studies, one study included the Egyptian population, fourteen studies included the Asian population, whereas two studies were performed in Caucasian ethnicity. The controls of three included studies were based on hospital-based population (HB), and the other fourteen were from the general
population-based (PB) [11, 12, 15, 18–31]. The quality of all included studies was ranged from 7–8. The detailed baseline characteristics are explained in Table 1. The controls included in seventeen studies were following the Hardy–Weinberg equilibrium. The allele frequencies and genotype distribution are given in Table 2.
Association of rs2277923 with congenital heart diseases

The pooled results of rs2277923 in the NKX2-5 gene show a non-significant association with CHDs. We selected the random-effects models to combine all information. Overall, the rs2277923 SNP not increased the risk of congenital heart diseases in each tested genetic model (allelic model: OR 1.00, 95% CI 0.82–1.21; over-dominant model: OR 1.17 CI 1.01–1.35; dominant model: OR 1.08 CI 0.82–1.42; recessive model: OR 0.89 CI 0.70–1.13). The meta-analysis of these four models is shown in Fig. 2. The results of homozygote and heterozygote models were given as, respectively: OR 0.95, 95%CI 0.68–1.33 and OR: 1.09, 95%CI 0.87–1.37 (Fig. 3).

Sensitivity analysis

After sequentially excluding each study, the overall changes in OR with a 95% confidence interval were not statistically significant, suggesting the reliability and stability of current meta-analysis results.

Publication bias

In the current study, funnel plot analysis does not explain any apparent asymmetry in each tested genetic model as illustrated in Fig. 4. Moreover, the Eggers test also confirmed no statistically significant effect. For the rs2277923, the p value for each genetic model was given as: allelic: 0.19, overdominant: 0.96, dominant: 0.82, recessive: 0.32, homozygote: 0.66 and heterozygote: 0.89.

Discussion

To date, few systematic reviews and meta-analyses have been done to find the association of rs2277923 association with congenital heart defects [13, 32], but the present analysis was the most comprehensive assessment of rs2277923 role in CHDs. In addition, our analysis includes recent studies conducted (till 2020). The NKX2-5 gene is the vital gene that interacts with other transcriptional factors, including the T-box transcription factor (TBX5) and GATA4; thus, it plays a crucial role in cardiac development. Hence, a single-nucleotide polymorphism can alter the gene function that ultimately affects the growth and heart structural morphogenesis [33].

In the current study, we analyzed all the available literature on rs2277923 polymorphism and congenital heart defects, and the pooled results suggest a non-significant relationship between congenital heart diseases and the selected polymorphism. Our analysis showed...
that in different ethnic groups, minor allele was not associated with CHDs.

Our results are in accordance with those performed by the Kalayinia et al. as they showed the overall percentage of the mutant and wild allele was 2.5%, and 65.8%, respectively [14], but in contrast to those reported by the Cao et al., they found that genotypic frequency distribution significantly differed.
between the control group and patients with atrial septal defects as \( p = 0.009 \) [34]. Xie et al. also conducted a meta-analysis of thirteen original studies and reported the non-significant association (\( p = 0.39 \), OR = 1.10, 95% CI = 0.88–1.38) [32]. Similarly, Liang et al. meta-analysis results are consistent with our findings. They include eight studies for final analysis and reported \( p = 0.73 \) for the allelic model [35]. Similar conclusions were reported in Caucasians [17]. Wang et al. revealed a significant association for rs2277923 with CHDs in the Chinese population [13]. We did the Begg and Egger test on all included studies so that any false-positive result due to publication bias can be eliminated in the current analysis [36]. We found no publication bias in the current meta-analysis, which further increases the reliability of our included studies.

**Limitations**

Although the cumulative results of this study are quite comprehensive, however, certain limitations also exist in this meta-analysis. First, we only chose one single-nucleotide polymorphism of the selected gene that may be influenced by gene–environment and gene–gene interactions. Second, we cannot exclude the possibility of publication bias because, in this study, we selected only English language published literature.

**Conclusions**

We concluded that \( NKX2-5 \) rs2277923 single-nucleotide polymorphism was not significantly associated with congenital heart defects. It is suggested that there is a need for further meta-analysis with a larger cohort size in various subgroups that may provide us the more definite conclusions. Moreover, in the future, more genetic variants should be included in the analysis that may help us in better understanding the genetic mechanism involved in the pathogenesis of CHDs.

**Abbreviations**

CHDs: Congenital heart diseases; TBX5: T-box transcription factors; SNP: Single-nucleotide polymorphism; PRISMA: Preferred reporting items for systematic reviews and meta-analyses; HWE: Hardy–Weinberg equilibrium; NOS: Newcas-nucleotide polymorphism; PRISMA: Preferred reporting items for systematic

**Supplementary Information**

The online version contains supplementary material available at https://doi.org/10.1186/s43044-021-00199-w.

**Authors’ contributions**

SA contributed to study concept; SA, KA and MFS contributed to study design, data collection, data analysis and interpretation, literature review, write and critically review the manuscript. All the authors read and approved the final manuscript.

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**Declarations**

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

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**Competing interests**

The authors declare that they have no competing interests.

**Author details**
1 Centre for Applied Molecular Biology, University of the Punjab, 87-West Canal Bank Road, Thokar Niaz Baig, Lahore 53700, Pakistan. 2 Punjab University College of Pharmacy, University of the Punjab, Lahore, Pakistan. 3 Faculty of Pharmaceutical Sciences, Superior University, Lahore, Pakistan.

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