Association of NKX2-5, GATA4, and TBX5 polymorphisms with congenital heart disease in Egyptian children

Eman G. Behiry1 | Mahmoud A. Al-Azzouny1 | Dina Sabry2 | Ola G. Behairy3 | Nessrine E. Salem4

1Clinical and Chemical Pathology Department, Benha University, Benha, Egypt
2Biochemistry Department, Cairo University, Cairo, Egypt
3Pediatrics Department, Benha University, Benha, Egypt
4Histology Department, Benha University, Benha, Egypt

Correspondence
Eman G. Behiry, Clinical and chemical pathology Department, Benha University, Benha, Egypt.
Email: emangamal24@yahoo.com

Funding information
Benha University

Abstract

Background: Several genes encoding transcription factors are known to be the primary cause of congenital heart disease. NKX2-5 and GATA4 were the first congenital heart disease–causing genes identified by linkage analysis. This study designed to study the association of five single–nucleotide variants of NKX2-5, GATA4, and TBX5 genes with sporadic nonsyndromic cases of a congenital cardiac septal defect in Egyptian children.

Methods: Venous blood samples from 150 congenital heart disease children (including a ventricular septal defect, atrial septal defect, tetralogy of Fallot, and patent ductus arteriosus) and 90 apparently healthy of matched age and sex were studied by polymerase chain reaction followed by direct sequencing in order to study two single–nucleotide variants of NKX2-5 (rs2277923, rs28936670), two single–nucleotide variants of GATA4 (rs368418329, rs56166237) and one single–nucleotide variant TBX5 (rs6489957). The distribution of genotype and allele frequency in the congenital heart diseases (CHD) group and control group were analyzed.

Results: We found different genotype frequencies of the two variants of NKX2-5, as CT genotype of rs2277923 was present in 58% and 36% in cases and control respectively, and TT genotype present in 6% of the cases. Also regarding missense variant rs28936670, heterozygous AG presented in 82% of the cases. Also, we observed a five prime UTR variant rs368418329, GT (42% of the cases) and GG (46% of the cases) genotypes showed the most frequent presentation in cases. While regarding a synonymous variant rs56166237, GT and GG were the most presented in cases (41.4%, 56% respectively) in contrast to control group (20%, 1.7% respectively). Also, a synonymous variant in TBX5, the distribution of genotype frequency was significantly different between the CHD group and control group. CT genotype of TBX5 -rs6489957 was found in 12 ASD, 24 VSD, six PDA, three aortic coarctation and nine fallot that represent 42% of the cases.

Conclusions: Significantly higher frequency of different allele of five variants was observed in cases when compared to the control group, with significant risky effect for the development of septal defect. In addition to two polymorphisms of NKX2-5


1 | BACKGROUND

Congenital heart diseases (CHD) could be a major cause of morbidity and mortality in children, a previous study revealed that CHD occurred in 80.8% as a solitary lesion, and in 19.2% of the cases they were combined with other cardiac lesions (Bassili et al., 2000). The prevalence increased over time in total CHD birth, from 0.6 to 9.1 per 1,000 live births from 1930 to 1934. Stabilization occurred all over the last 15 years, reaching 1.35 million newborns with CHD every year (van der Linde et al., 2011).

Several studies have been made to identify genes that could be responsible for syndromic and nonsyndromic forms of CHD, by identifying the human gene mutations associated, it has been estimated that \( NKX2-5 \) (OMIM: 600,584), mutations account for at least 4% of fallot’s tetralogy cases. Also authors found that nonsyndromic cardiac septal defects have been linked to mutations in \( GATA4 \) (OMIM: 600,576) (McDermott, Basson, & Hatcher, 2006). Nineteen mutations in \( GATA4 \) have been studied in patients with atrial septal defect (ASD), ventricular septal defect (VSD), and Fallot’s tetralogy (Mattapally, Nizamuddin, Murthy, Thangaraj, and Banerjee (2015)).

Mutations in genes known to be resposible for cardiac development such as \( NKX2-5 \), \( TBX20 \), \( GATA4 \), \( GATA6 \), and \( MYH6 \) have been studied in families with isolated, nonsyndromic cardiovascualr deformities, these genes are resposible for cardiac development in animal models. \( GATA4, \ NKX2-5, \) and \( TBX5 \) (the gene causing Holt-Oram syndrome) may function in a complex to regulate a group of genes required for formation of cardiac septa (Ware & Jefferies, 2012).

\( TBX5 \) changes cause both skeletal and cardiovascular phenotypes. It has been revealed that major cardiovascular yet milder skeletal abnormalities are because of missense alterations at the amino terminal of the DNA binding domain (Basson et al., 1999). Human \( NKX2-5 \) is imparted in the cardiovascular primordia from day 7 and is an early sign of both embryonic heart fields development (Stanley et al., 2004). Though alterations in \( NKX2-5 \) are linked to an extensive variety of CHDs and thyroid dysgenesis, \( NKX2-5 \) codes for a homeodomain–containing transcription factor with an important role in heart development, and mutations affecting this gene in individuals with congenital heart disease were reported (Dentice et al., 2006).

\( NKX2-5 \) has a role in nearly almost all phases of heart development, including the regulation of number of cardiac progenitor cells, conduction system development, valve formation, and septation. Interestingly, \( NKX2-5 \) acts in combination with other transcription factors that are highly-conserved to organize cardiogenesis. Genome–wide expression analysis has begun to investigate \( NKX2-5 \) dependent genes (Elliott, Kirk, Schaft, & Harvey, 2010).

Previous authors have identified an unknown mutation in the \( TBX5 \) (OMIM: 601,620) leading to an amino acid change at position 85 from proline to threonine. The mutation had dramatically reduced biological activity that lead to clinical HOS phenotype (Dreßen et al., 2016).

This study planned to study two single–nucleotide variants of \( NKX2-5 \) (rs2277923, rs28936670), two single–nucleotide variants of \( GATA4 \) (rs368418329, rs56166237) and one single–nucleotide variant \( TBX5 \) (rs6489957) in sporadic non-syndromic CHD cases in Egyptian children with congenital cardiac septal deformity from Qalubeya.

2 | METHODS

This research was accepted by the Research Ethics Committee of Benha University according to the “World Medical Association Declaration of Helsinki 1964” (Idänpään-Heikkilä, 2001). Written informed consents were obtained from the Guardians of all participants.

2.1 | Subjects

In this case-controlled study, we recruited 150 unrelated affected children with non-syndromic isolated and non-isolated cardiac septal defects from Benha University Hospital, they were 84 females and 66 males with age ranged from days to 4 years with a mean age (7.8 ± 2.2) months during the period from March 2017 to May 2018. Ninety healthy children with matched age and sex and without a family history of cardiac diseases served as a control group. Patients were diagnosed according to ESC Guidelines for congenital heart disease (Baumgartner et al., 2010). Syndromic CHD cases diagnosed by clinical examination and karyotyping or cases with congenital heart malformations without septation defects were excluded.
2.2 | Methods

2.2.1 | Clinical evaluation

For each case, three-generation pedigree constructions, complete history of patients obtained from their parents or medical records and detailed clinical examination were conducted for all participants. Echocardiography, electrocardiogram and Plain chest X-rays were carried out in all cases.

2.2.2 | Cytogenetic studies

Conventional cytogenetic analysis using GTG banding technique and Fluorescence In Situ Hybridization was done for all participants to exclude chromosomal aberration syndromes.

2.2.3 | Molecular studies

All subjects were genotyped for five single–nucleotide variants of *NKX2-5* (rs2277923, rs28936670), *GATA4* (rs368418329, rs56166237) and *TBX5* (rs6489957) using direct Sanger sequencing technique. Five SNPs were selected from the dbSNP database (https://www.ncbi.nlm.nih.gov/projects/SNP/) in the following steps:

DNA extraction
Three millilitres of venous blood samples was collected under a complete aseptic condition in vacutainers containing Na₂EDTA as an anticoagulant. Genomic DNA was extracted from the peripheral blood sample according to the procedures of the DNA isolation kit iNtRON G-spin Total DNA extraction kit, catalogue number 17045, Korea (https://www.intronbio.com/eg/).

Genotyping by sanger sequencing method
Genotypic analysis The analysis was performed by conventional polymerase chain reaction (PCR) followed by DNA sequencing to examine two single nucleotide polymorphisms in *NKX2-5*, in comparison with sequence number NM_004387.3 for genome, first synonymous variant rs2277923 (NM_004387.3:c.63T/C) and second is missense variant rs28936670 (NM_004387.3:c.73 G/A), and this study examined two single nucleotide polymorphisms in *GATA4* according to sequence number NM_002052.4, first 5 prime UTR variant rs368418329 (NM_002052.4:c.-294G/T) and synonymous variant rs56166237 (NM_002052.4:c.99 G/T), and synonymous variant rs6489957 in *TBX5* according to sequence number NM_000192.3 (NM_000192.3:c.1281C/T).

Polymerase chain reaction (PCR) was carried out using a total of ten PCR primers that were designed using primer design software (Premier Biosoft Inc., Palo Alto). The amplification was performed in a reaction mixture of 50 μl containing approximately 2 μl genomic DNA, 25 μl PCR Master mix (2x) containing Taq DNA polymerase, dNTPs, 10Mm buffer containing 2mM MgCl₂ (iNtRON-Korea), 10 picomole from each primer and 22 μl Distilled Water (Table 1).

The amplified products were subjected to electrophoresis on a 2% agarose gel containing ethidium bromide and visualized using ultraviolet (UV) light trans-illumination. The PCR products were purified with wash steps and then the DNA was eluted in a low salt buffer. DNA was allowed to adsorb specifically to the silica membrane of a MEGA quick spin column and then sequenced directly by the ABI3730XL sequencer in LGC genomic GmbH, 12,459 Berlin/Germany (WWW.igcgroup.com).

2.3 | Statistical analysis

The data were organized using SPSS version 16 software (SPSS Inc, Chicago). Quantitative data were designed in the form of Mean and standard deviation, the significance of difference was tested using: -Student’s t-test and Mann–Whitney Test (*U* test) that was used to compare the mean of two groups of quantitative data. Categorical data were presented as number and percentages. Odds ratios (ORs) and the corresponding 95% CI were calculated. Regression analysis with the adjusted Odds Ratios was used to detect the significant predictors of congenital heart disease. *p* < 0.05 was considered significant.

3 | RESULTS

The present study was conducted on 150 congenital heart disease (CHD) cases, their mean age was 7.8 ± 2.2 months;

| SNP        | Site       | Forward primer       | Reverse primer       | Size  |
|------------|------------|----------------------|----------------------|-------|
| NKX2-5-    | (rs2277923) c.63C/T | tgacacgaactgctcatcg | gtaggcctctggcttgaagg | 416 bp |
| NKX2-5-    | (rs28936670) c.73G/A | ctggcgctgtgagactgg  | agtttcttggggacgaaagc | 422 bp |
| GATA4-(rs368418329) c.-294G/T | gttggtctgaagctgtgg | cctcggtgctctctctcc | 497 bp |
| GATA4 (rs56166237) c.99G/T | cgacagtaatattcgg | gccttgagggtaggacgaagc | 267 bp |
| TBX5-(rs6489957) c.1281C/T | ttggccaataactgtgctc | gccttgagggtaggacgaagc | 465 bp |
there were 66 males (44%) and 84 females (56%). In addition to 90 healthy control subjects of matched age and gender. Thirty three congenital heart disease children (22%) had positive first degree consanguinity, while nine control subjects (10%) had positive first degree consanguinity.

Applying Hardy Weinberg equation revealed that all studied single–nucleotide polymorphisms (SNPs) in control as well as in cases; groups were in HW equilibrium. Clinical and cardiac findings are summarized (Table 2). Five single–nucleotide polymorphisms were reported: a synonymous variant rs2277923, and a missense variant rs28936670 in NKX2-5 and 5 prime UTR variant rs368418329 and a synonymous variant rs56166237 in GATA4, and a synonymous variant rs6489957 in TBX5.

There were significant different genotype frequencies in cases with CHD than control group regarding five variants (Table 3). Regarding synonymous variant rs2277923 in NKX2-5, CT and TT genotypes were presented more frequent in CHD cases (15 ASD, 24 VSD, six PDA, six aortic coarctation and nine Fallot’s tetralogy) than control group, as CT genotype was present in 58% and 36% in cases and control respectively. TT genotype was present in 6% of the cases (three ASD patients) and not present in control (Figure 1), also regarding missense variant rs28936670, heterozygous AG shows that it was most frequently presented in 82% of the cases (27 ASD, 44 VSD, nine PDA and six Fallot’s tetralogy) than the control group (36.7%), while the AA genotype was present in 8% of the CHD cases (nine VSD and six Fallot’s tetralogy) (Table 4).

According to GATA4 genotypes, a 5-prime UTR variant rs368418329. GT (42% of the cases) and GG (46% of the cases) genotypes showed the most frequent presentation in cases (24 ASD, 48 VSD, six PDA, six aortic coarctation and 12 Fallot) (Table 5). While regarding a synonymous variant rs56166237, GT and GG were the most presented in cases (41.4%, 56% respectively) in contrast to the control group (20%, 1.7% respectively) (Figure 2). GT genotype was present in 15 ASD,18 VSD, three PDA, three aortic coarctation and nine Fallot’s tetralogy.

CT genotype of TBX5 –rs6489957 was found in 12 ASD, 24 VSD, six PDA, three aortic coarctation and nine fallot that represent 42% of cases, while CC genotype present in 58% of the cases in contrast to 0.03% of control group (Table 6).

### TABLE 2 Clinical presentation and cardiac findings of all studied cases

| Cases | N = 150 |
|-------|---------|
| LV enlargement | 3 | 2 |
| RV enlargement | 60 | 40 |
| Biventricular enlargement | 6 | 4 |
| ASD | 27 | 18 |
| VSD | 51 | 34 |
| PDA | 9 | 6 |
| TGA | 12 | 8 |
| Aortic coarctation | 6 | 4 |
| Epstein anomaly | 6 | 4 |
| Fallot tetralogy | 12 | 8 |
| complete AV canal | 6 | 4 |
| atrial fibrillation | 9 | 6 |
| heart block | 9 | 6 |
| LV strain pattern | 3 | 2 |
| p pulmonal,rt axis deviation | 33 | 22 |
| sinus tachcardia | 15 | 10 |
| RV stress pattern | 6 | 4 |
| Mean | 57 | 12 |

Note. ASD: atrial septal defect; VSD: ventricular septal defect; PDA: patent ductus arteriosus; TGA: transposition of great arteries; EF: ejection fraction.
nonsynonymous, 13 synonymous, 3′-UTR and two intronic mutations. Three mutations (Arg25Cys, Thr178Met and Ala219Val) are recognized, also detected NCBI dbSNPs rs2277923 (A239G, Glu21Glu) was found in patients. Only heterozygous genotypes were attained. They obtained the genotypic frequency 22 AA: 17 AG: 6 GG for dbSNP rs2277923 in 45 samples of lymphocytic DNA (Reamon-Buettner et al., 2004).

A new heterozygous DNA sequence variant (DSV), 1433A>G, was recognized in one tetralogy of Fallot (TOF) patient and one persistent left superior vena cava (PLSVC) patient, but none in controls. In agreement with our results, the occurrence of rs2277923 in CHD group was significantly increased than the control group. The allele and genotype were associated with the existence of CHD (Cao et al., 2015).

Amplicon libraries for 16 CHD-strictly related genes were produced and sequenced from 68 CHD patients. Fourteen variants were existing in community databases with very rare allele incidence. One variant (p.Arg25Cys in NKX2-5) has been formerly related to CHD (Pulignani et al., 2018).

**FIGURE 1** Electrophoretograms showing NKX2-5 polymorphism rs2277923 (NM_004387.3:c.63 C/T) identified in homozygous samples (TT)
Three nonsynonymous variants in NKX2-5 were recognized in the heterozygous state by sequence investigation: p. Glu21Gln was established in single ASD-II patient; and three patients had the p.Arg25Cys (R25C) variant. Contrary to our results, the p.Glu21Gln was also recognized in 0.88% of the controls. Their findings also support that variant of NKX2-5 is a polymorphism, as it was not significantly altered among DS patients with CHD and controls (Alcántara-Ortigoza et al., 2015).

Nine new and 19 possibly pathogenic variants were established using Sanger sequencing. Analyses completed by sex discovered dissimilar variants related to bicuspid aortic valve (BAV) in men (EGFR rs533525993 and TEX26 rs12857479) and females (NKX2-5 rs2277923) (Dargis et al., 2016).

The two complete coding exon and partial flanking intron sequences of NKX2-5 gene were screened using DNA sequencing in 107 ASD patients and 391 VSD patients as well as 487 healthy individuals (control) from the Yunnan area in China. The results showed that single–nucleotide polymorphism (rs2277923) was identified. The incidence of nucleotide polymorphism (rs2277923) was significantly greater in the ASD group, and the allele and genotype were related to the occurrence of ASD. rs2277923 SNP may contribute to the danger of sporadic ASD in Chinese people (Cao et al., 2016).

The previously stated variant (rs2277923) was present at significantly greater levels in the CHD population than in the control group. NKX2-5 mutations may be mosaic in nature, therefore deserving investigation in both blood and tissue samples (Ketharnathan, Koshy, Sethuratnam, Paul, & Venkatesan, 2015).

The authors examined mutations of the NKX2-5 coding region in 230 nonsyndromic Chinese Han CHD patients using denaturing HPLC and sequencing. Two recognized single–nucleotide polymorphisms (rs2277923 and rs3729753) were identified, contrary to our results, there were insignificant differences in the allele and genotype frequencies between CHD and the controls (p > 0.05) (Zhang et al., 2009).

Altered heterozygous CSX/NKX2-5 mutations were established in congenital heart defects patients in an autosomal dominant fashion. All missense mutations in the Home
domain had decreased DNA binding and slight transcriptional role. While their study does not describe the genotype–phenotype association of the ten human mutations, they identify specific deformities of CSX/NKX2-5 function important for transactivation of target genes (Kasahara et al., 2000).

Previous study investigated the incidence and prevalence of GATA4, NKX2-5, gene mutations in a large group of individuals with controtruncal defect (CTD), including 178 patients with tetralogy of Fallot, 13 patients with double-outlet right ventricle (DORV), and 11 patients with truncus arteriosus. The mutation study identified one recognized missense variant (Arg25Cys) in the NKX2-5 in two (1.1%) sporadic patients with TOF. These sequence alterations were absent in 500 matched controls (De Luca et al., 2011).

Heterozygous mutations in transcription factor gene NKX2-5 are connected to either isolated or combined

| TABLE 5 | Comparison of cardiac data according to GATA4 (rs368418329 and rs56166237) genotypes in all studied cases |
|------------------------|------------------------|------------------------|------------------------|
| GATA4 (rs368418329) genotypes in all studied cases |
| GG | N = 69 | GT | N = 63 | TT | N = 18 |
| N | % | N | % | N | % |
| ASD | 15 | 21.7 | 9 | 14.2* | 3 | 16.7*# |
| VSD | 27 | 39 | 21 | 33* | 3 | 16.7*# |
| PDA | 3 | 4.3 | 3 | 4.7 | 3 | 16.7*# |
| Aortic coarctation | 0 | 0 | 6 | 9.5* | 0 | 0*# |
| Fallot tetralogy | 6 | 8.6 | 6 | 9.5 | 0 | 0*# |
| Mean SD | Mean SD | Mean SD |
| EF | 55 | 9 | 58 | 10 | 60 | 6 |

Note. GenBank reference sequence: variant rs368418329 in GATA4 (NM_002052.4:c.-294G/T) and synonymous variant rs56166237 in GATA4 (NM_002052.4:c.99 G/T).

| FIGURE 2 | Electrophoretograms showing GATA4 polymorphism rs56166237 (NM_002052.4:c.99 G/T) identified in homozygous samples (GG) |

| TABLE 6 | Comparison of cardiac data according to TBX5 (rs6489957) genotypes in all studied cases |
|------------------------|------------------------|------------------------|------------------------|
| CC | N = 87 | CT | N = 58 |
| N | % | N | % | P |
| ASD | 15 | 17 | 12 | 20 | 0.01 |
| VSD | 27 | 31 | 24 | 41 | 0.005 |
| PDA | 3 | 3.4 | 6 | 10.3 | 0.00 |
| Aortic coarctation | 3 | 3.4 | 3 | 6 | >0.05 |
| Fallot tetralogy | 3 | 3.4 | 9 | 15.5 | 0.00 |
| Mean SD | Mean SD |
| EF | 57 | 0.11 | 55 | 0.13 | >0.05 |

Note. GenBank reference sequence: variant rs6489957 in TBX5 to sequence number NM_000192.3 (NM_000192.3:c.1281C/T).
congenital heart disease (CHD), primarily secundum atrial septal defect-II (ASD-II), ventricular septal defect (VSD) or tetralogy of Fallot (TOF). Importantly, sporadic cases of CHD that share phenotypic features of NKK2-5 mutation carriers were negative on genetic investigation. Thus, even significant for cardiac development, germline mutations in NKK2-5 are infrequent in patients with sporadic CHD and genetic heterogeneity is likely for sporadic forms of CHD (Stallmeyer, Fenge, Nowak-Göttl, & Schulze-Bahr, 2010).

Twelve distinct mutations in the NKK2-5 coding region were identified in 18 of 608 patients (3%) including 9 of 201 with Fallot tetralogy, 3 of 71 with ASD secundum, one with truncus arteriosus, one with double–outlet right ventricle. In one out of nine patients with the aortic and mitral valve and a small VSD identified heterozygosity for a C to T transition at nucleotide 73 (c.73C>T) of the NKK2-5 sequence. The identified nucleotide transition led to the substitution of an arginine at amino acid 25 to a cysteine (p.R25C). They did not find this gene variation in the parents or in the other 120 patients of the study and also in a control group of 380 healthy Caucasian (McElhinney, Geiger, Blinder, Benson, & Goldmuntz, 2003).

They found one formerly known NKK2-5 missense variation, heterozygous c.73C>T (p.Arg25Cys), in a 10-year-old child with Fallot tetralogy. The same heterozygous alteration was found also in two unrelated persons in the healthy control group. The study shows the presence of p.Arg25Cys in healthy control children other than African Americans, and in vitro study suggested a structural/functional change in the altered protein region (Akcaboy et al., 2008).

Four heterozygous mutations were recognized in six unrelated TOF patients, comprising three with pulmonary atresia and five with right aortic arch; none had ECG evidence of PR interval prolongation. Three of four mutations (Arg216Cys, Glu214Lhn and Ala219Val) changed highly–conserved amino acids, of which two mapped in the conserved NK2 domain. The fourth mutation (Arg25Cys) was identified in three unrelated patients and has been previously reported (Goldmuntz, Geiger, & Benson, 2001).

Previous study recognized two diverse mutations in the NKK2-5 coding region among the 159 (1.26%) individuals. An Arg25Cys mutation was recognized in a Tetralogy of Fallot patient (Soheili et al., 2016). Moreover, a single–nucleotide polymorphism c.99G>T was detected in GATA4. Though, the polymorphic frequency in ASD cases was like that in healthy controls (for genotype GT, p = 0.3847; for allel T, p = 0.3950) (Liu et al., 2010).

### 5 | CONCLUSION

In addition to reporting different genotype frequencies of two polymorphisms of NKK2-5 (rs2277923, rs28936670) in CHD cases, two variants in GATA4 (rs368418329, rs56166237) and one synonymous variant in TBX5 (rs6489957) seem to have a role in the pathogenesis of CHD. Our results propose the importance of numerous gene variants investigation of CHD children in Egypt.

### REFERENCES

Akcaboy, M. I., Cengiz, F. B., Inceöğlu, B., Ucar, T., Atalay, S., Tutar, E., & Tekin, M. (2008). The effect of p. Arg25Cys alteration in NKK2-5 on conotruncal heart anomalies: mutation or polymorphism? *Pediatric Cardiology*, 29(1), 126–129.

Alcántara-Ortigoza, M. A., De Rubens-Figueroa, J., Reyna-Fabian, M. E., Estandia-Ortega, B., González-del Angel, A., Molina-Álvarez, B., … García-Díaz, L. (2015). Germline mutations in NKK2-5, GATA4, and CRELD1 are rare in a Mexican sample of Down syndrome patients with endocardial cushion and septal heart defects. *Pediatric Cardiology*, 36(4), 802–808. https://doi.org/10.1007/s00246-014-1091-3

Bassili, A., Mokhtar, S. A., Dabous, N. I., Zaher, S. R., Mokhtar, M. M., & Zaki, A. (2000). Risk factors for congenital heart diseases in Alexandria, Egypt. *European Journal of Epidemiology*, 16(9), 805–814.

Basson, C. T., Huang, T., Lin, R. C., Bachinsky, D. R., Weremowicz, S., Vaglio, A., … Seidman, C. E. (1999). Different TBX5 interactions in heart and limb defined by Holt-Oram syndrome mutations. *Proceedings of the National Academy of Sciences*, 96(6), 2919–2924. https://doi.org/10.1073/pnas.96.6.2919

Baumgartner, H., Bonhoeffer, P., De Groot, N. M. S., de Haan, F., Deanfield, J. E., Galie, N., … Westby, J. (2010). ESC Guidelines for the management of grown-up congenital heart disease (new version 2010) The Task Force on the Management of Grown-up Congenital Heart Disease of the European Society of Cardiology (ESC). *European Heart Journal*, 31(23), 2915–2957. https://doi.org/10.1093/eurheartj/ehq249

Cao, Y., Lan, W., Li, Y., Wei, C., Zou, H., & Jiang, L. (2015). Single nucleotide polymorphism of NKK2-5 gene with sporadic congenital heart disease in Chinese Bai population. *International Journal of Clinical and Experimental Pathology*, 8(11), 14917.

Cao, Y. u., Wang, J., Wei, C., Hou, Z., Li, Y., Zou, H., … Jiang, L. (2016). Genetic variations of NKK2-5 in sporadic atrial septal defect and ventricular septal defect in Chinese Yunnan population. *Gene*, 575(1), 29–33. https://doi.org/10.1016/j.gene.2015.08.033

Dargis, N., Lamontagne, M., Gaudreault, N., Sharrar, L., Henry, C., Pibarot, P., … Bossé, Y. (2016). Identification of gender-specific genetic variants in patients with bicuspid aortic valve. *The American Journal of Cardiology*, 117(3), 420–426. https://doi.org/10.1016/j.amjcard.2015.10.058

De Luca, A., Sarkozy, A., Ferese, R., Consoli, F., Lepri, F., Dentici, M. L., … Dallapiccola, B. (2011). New mutations in ZFPM2/FOG2 gene in tetralogy of Fallot and double outlet right ventricle. *Clinical Genetics*, 80(2), 184–190. https://doi.org/10.1111/j.1399-0004.2010.01523.x

Dentici, M., Cordeddu, V., Rosica, A., Ferrara, A. M., Santarpia, L., Salvatore, D., … Macchia, P. E. (2006). Missense mutation in the transcription factor NKK2-5: A novel molecular event in the pathogenesis of thyroid dysgenesis. *The Journal of Clinical
Endocrinology & Metabolism, 91(4), 1428–1433. https://doi.org/10.1210/je.2005-1350

Dreßen, M., Lahm, H., Lahm, A., Wolf, K., Doppler, S., Deutsch, M. A., … Malcic, I. (2016). A novel de novo TBX5 mutation in a patient with Holt-Oram syndrome leading to a dramatically reduced biological function. Molecular Genetics and Genomic Medicine, 4(5), 557–567.

Elliott, D. A., Kirk, E. P., Schaft, D., & Harvey, R. P. (2010). NK-2 class homeodomain proteins: conserved regulators of cardiogenesis. In D. J. Amack, B. A. Amendt, R. H. Anderson (Eds.), Heart Development and Regeneration (pp. 569–597). Amsterdam: Academic Press.

Goldmuntz, E., Geiger, E., & Benson, D. W. (2001). NKX2.5 mutations in patients with tetralogy of fallot. Circulation, 104(21), 2565–2568. https://doi.org/10.1161/hc4601.098427

Idänpään-Heikkilä, J. E. (2001). Ethical principles for the guidance of physicians in medical research: The declaration of Helsinki. Bulletin of the World Health Organization, 1, 279–279.

Kasahara, H., Lee, B., Schott, J. J., Benson, D. W., Seidman, J. G., Seidman, C. E., & Izumo, S. (2000). Loss of function and inhibitory effects of human CSX/NKX2.5 homeoprotein mutations associated with congenital heart disease. The Journal of Clinical Investigation, 106(2), 299–308.

Ketharnathan, S., Koshy, T., Sethuratnam, R., Paul, S., & Venkatesan, V. (2015). Investigation of NKX2.5 gene mutations in congenital heart defects in an Indian population. Genetic testing and molecular Biomarkers, 19(10), 579–583.

Liu, X. Y., Yang, Y. Q., Ma, J., Lin, X. P., Zheng, J. H., Bai, K., & Chen, Y. H. (2010). Novel GATA4 mutations identified in patients with congenital atrial septal defects. Zhonghua Xin Xue Guan Bing Za Zhi, 38(8), 724–727.

Mattapally, S., Nizamuddin, S., Murthy, K. S., Thangaraj, K., & Banerjee, S. K. (2015). 620C>T mutation in GATA4 is associated with congenital heart disease in South India. BMC Medical Genetics, 16(1), 7.

Mattapally, S., Singh, M., Murthy, K. S., Asthana, S., & Banerjee, S. K. (2018). Computational modeling suggests impaired interactions between NKX2.5 and GATA4 in individuals carrying a novel pathogenic D16N NKX2.5 mutation. Oncotarget, 9(17), 13713.

McDermott, D. A., Basson, C. T., & Hatcher, C. J. (2006). Genetics of cardiac septation defects and their pre-implantation diagnosis. In Congenital heart disease (pp. 19–42). New York, NY: Humana Press.

McElhinney, D. B., Geiger, E., Blinder, J., Benson, D. W., & Goldmuntz, E. (2003). NKX2.5 mutations in patients with congenital heart disease. Journal of the American College of Cardiology, 42(9), 1650–1655.

Pulignani, S., Vecoli, C., Borghini, A., Foffa, I., Ait-Ali, L., & Andreassi, M. G. (2018). Targeted Next-Generation Sequencing in Patients with Non-syndromic Congenital Heart Disease. Pediatric Cardiology, 39(4), 682–689. https://doi.org/10.1007/s00246-018-1806-y

Rajagopal, S. K., Ma, Q., Obler, D., Shen, J., Manichaikul, A., Tomita-Mitchell, A., … Pu, W. T. (2007). Spectrum of heart disease associated with murine and human GATA4 mutation. Journal of Molecular and Cellular Cardiology, 43(6), 677–685. https://doi.org/10.1016/j.yjcc.2007.06.004

Reamou-Buettn, S. M., Hecker, H., Spanel-Borowski, K., Craatz, S., Kuenzel, E., & Borlak, J. (2004). Novel NKX2–5 mutations in diseased heart tissues of patients with cardiac malformations. The American Journal of Pathology, 164(6), 2117–2125. https://doi.org/10.1016/S0002-9440(10)63770-4

Soheili, F., Darabi, P., Dahnmardeh, F., Heidary, N., Jalili, Z., Fooladi, S., … Heidarizadeh, M. (2016). Nx2-5 mutations in patients with non-syndromic congenital heart disease. Zahedan Journal of Research in Medical Sciences, 18(11), 2–5. https://doi.org/10.17795/zjrms-5873

Stallmeyer, B., Fenge, H., Nowak-Göttl, U., & Schulze-Bahr, E. (2010). Mutational spectrum in the cardiac transcription factor gene NKX2.5 (CSX) associated with congenital heart disease. Clinical Genetics, 78(6), 533–540.

Stanley, E. G., Biben, C., Elefany, A., Barnett, L., Koentgen, F., Robb, L., & Harvey, R. P. (2004). Efficient Cre-mediated deletion in cardiac progenitor cells conferred by a 3'UTR-ires-Cre allele of the homeobox gene Nkx2-5. International Journal of Developmental Biology, 48(4), 431–439.

van der Linde, D., Konings, E. E., Slager, M. A., Witsenburg, M., Helbing, W. A., Takkenberg, J. J., & Roos-Hesselink, J. W. (2011). Birth prevalence of congenital heart disease worldwide: A systematic review and meta-analysis. Journal of the American College of Cardiology, 58(21), 2241–2247. https://doi.org/10.1016/j.jacc.2011.08.025

Ware, S. M., & Jeffries, J. L. (2012). New genetic insights into congenital heart disease. Journal of Clinical and Experimental Cardiology, 58(21). https://doi.org/10.4172/2155-9880.s8-003

Yin, J., Qian, J., Dai, G., Wang, C., Qin, Y., Xu, T., Yang, S. (2018). Search of Somatic Mutations of NKK2-5 and GATA4 Genes in Chinese Patients with Sporadic Congenital Heart Disease. Pediatric Cardiology, 40, 1–6.

Zhang, W., Li, X., Shen, A., Jiao, W., Guan, X., & Li, Z. (2009). Search of somatic mutations of NKX2-5, GATA4, and TBX5 polymorphisms with congenital heart disease in Egyptian children. BMC Medical Genetics, 10(2), 299–308.

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