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RESEARCH ARTICLE

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SLC2A3 variants in familial and sporadic congenital heart diseases in a Chinese Yunnan population

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Abstract

Background: Solute carrier family 2 member 3 (*SLC2A3*), is a member of a superfamily of transport protein genes. *SLC2A3* played an important role in embryonic development. Previous research reported *SLC2A3* duplication was reportedly associated with congenital syndromic heart defects. However, it is not clear whether the gene is associated with non-syndromic congenital heart disease. Our study aimed to elucidate the relationship between its variation and congenital heart disease.

Methods: Genomic DNA extracted from the peripheral blood leukocytes of two families with CHD were sequenced with whole-exome sequencing to identify variations, used Sanger sequencing to investigate *SLC2A3* variants in 494 Chinese patients with CHD and 576 healthy unrelated individuals.

Results: In members from the two families, three with CHD had the *SLC2A3* (rs3931701) C > T variant. Of the 494 patients with CHD, 394 had gene variants (86 had the TT type and 308 had the CT type). Of the 576 healthy controls, 272 participants had gene variants (36 had the TT type and 236 had the CT type). The TT type [$p < 0.0001$, odds ratio (OR) =7.262, 95% confidence interval (CI) =4.631–11.388] and CT type ($p < 0.0001$, OR =3.967, 95% CI =2.991–5.263) of *SLC2A3* (rs3931701) significantly increased the risk of sporadic ASD in a Chinese Yunnan population.

Conclusions: Single nucleotide variations of *SLC2A3*, particularly, the *SLC2A3* (rs3931701) C > T variant increased the risk of CHD among the studied population.

KEYWORDS
congenital heart diseases, genetic mutation, *SLC2A3* variants, whole-exon sequencing

1 | INTRODUCTION

Congenital heart disease (CHD) is the most prevalent type of human birth defect. It occurs in approximately 16% and 14.7% of neonates in east China[1] and west of Iran,[2] respectively. CHD is a result of a complex interaction between genetic and environmental factors. This is the so-called polygenic model.[3]

Numerous genetic mutations are reportedly associated with CHD. For example, *GATA4*, *NKX2.5*, and *TBX5* are associated with atrial septal defect (ASD), ventral septal defect (VSD), and tetralogy of Fallot. *GATA4* mutations cause human congenital heart defects and demonstrate an interaction with *TBX5*.[4,5] Other than these transcription factors, numerous genes are implicated in various roles in the development of CHD. Among these is a loss-of-function mutation in *HYDIN*, which inhibits *GATA4* expression, thereby increasing the risk for ASD.[6] *ASXL3* is important in cardiac development and can affect the expression of mRNAs associated with cell apoptosis and cell proliferation.[7] Research on environmental factors has focused on folate deficiency, suggesting that normal folic acid levels reduce the risk for CHD.[8,9] Other environmental factors associated with CHD include pregestational diabetes, hypothyroidism, infection/influenza during early pregnancy, alcohol consumption, cigarette smoking, and maternal occupational exposure to mineral and organic dust as well as metal dust and fumes.[10–15]

With the development of the whole-exome/genome sequencing, more genes associated with CHD have been identified. This has increased our knowledge of the genetic causes of CHD. Based on the results of targeted deletion studies in mice, it has been suggested that there are more than 500 genes involved in CHD.[16] Numerous epidemiological studies based on twin studies and familial clustering have suggested that CHD is heritable.[17–19] Screening of suspected pathologic genes from the genealogy of CHD can improve the screening for susceptibility genes in CHD. *SLC2A3* encodes glucose transporter 3 (GLUT3) and is expressed in the brain, pharyngeal arches, and the left ventricular outflow tract during development.[20–22] A recent study showed that variants of the *SLC2A3* gene were associated with the risk of CHD.[23] GLUT3 facilitates the diffusion of glucose across the plasm so as to mediate glucose uptake for organogenesis.[20] Therefore, in the present study, we conducted whole-exon sequencing (WES) in two families with CHD. We further verified the gene mutations in the population with sporadic CHD and investigated whether Solute carrier family 2 member 3 (*SLC2A3*) was a susceptibility factor for CHD.

2 | SUBJECTS AND METHODS

2.1 | Participants

The study protocol was approved by the local medical ethics committee of the First People's Hospital of the Yunnan Province, China. Written informed consent was obtained from all participants. The patients' clinical information and medical histories were collected at the hospital. A total of 14 members from two families with a history of CHD were enrolled in our study (Table 1). To further verify the involvement of *SLC2A3* in patients with sporadic CHD, 494 patients with CHD (233 males and 261 females) and 576 healthy unrelated individuals (284 males and 292 females) were recruited. There was no significant difference in age and sex between patients and healthy controls (Table 2). All participants underwent detailed physical examination, including auscultation of precordial murmurs and inspection for the presence of cyanosis of the lips and tongue and other physical abnormalities. B-ultrasound examination was also performed to obtain the diagnosis of CHD.

2.2 | DNA extraction

Genomic DNA was isolated from the peripheral blood of the participants using the ReliaPrep Blood gDNA Miniprep System, No. A5082 (Promega). DNA concentration was measured using the Nanodrop 2000 (Thermo Fisher Scientific).

2.3 | WES study

A 1% agarose gel was used to analyze the degree of degradation of DNA and the contamination of RNAs and proteins. DNA concentration was measured using the Qubit® DNA Assay Kit in Qubit® 3.0 Fluorometer (Invitrogen). DNA samples with a content of more than 1.5 g were used to build the database. DNA samples with a concentration of at least 20 ng/μl and a total of more than 0.4 g were used for library construction. Clustering of the index-coded samples was performed on a cBot Cluster Generation System using the Illumina PE Cluster Kit (Illumina), according to the manufacturer's instructions. After cluster generation, DNA libraries were sequenced on an Illumina platform, and 150-base pair paired-end reads were generated.

2.4 | Mutation selection process and method

Qualified sequencing files were compared with the reference genome to obtain the mutation sites of each individual through bioinformatics processing. There were several to dozens of mutations in each of the two families that showed co-segregation with the disease. To identify potential harmful mutations in each individual, the SNP/InDel information detected by basic analysis was screened for mutation sites, and the specific screening process was as follows:

(1) The 1000 genome database was filtered, and the mutation loci with frequency lower than 0.005 and frequency lower than 0.01 in the NHLBI (national lung, heart and blood database) in 1000G were reserved. The aim is to remove the diversity of loci between individuals and get the rare mutations (rare) that can actually cause disease. (2) Variation of exonic or splicing sites (upper and lower 10 bp). (3) Remove synonymous mutations (mutations that do not

TABLE 1 Characteristics of family members and genotypes

Case number	Sex	Age	Symptoms	Defect size (cm)	Cardiac structure	Cardiac Shunts	Gene type of *SLC2A3* (rs3931701)
Family A I-1	Male	65	-	-	Normal	No	CT
Family A I-2	Female	63	Died due to heart disease	-	Normal	No	-
Family A II-1	Male	40	secundum ASD	1.53/1.85	Right heart enlargement with pulmonary hypertension	Left to right	TT
Family A II-2	Female	36	-	-	Normal	No	CT
Family A II-3	Male	32	-	-	Normal	No	CT
Family A II-4	Female	32	-	-	Normal		CC
Family A III-1	Male	14	multi-hole PmVSD	0.26/0.17/0.12	Tricuspid regurgitation	Left to right	TT
Family A III-2	Male	10	-	-	Normal	No	CT
Family A III-3	Female	13	-	-	Normal	No	CC
Family B I-1	Male	62	Died due to heart disease	-	Normal	No	-
Family B I-2	Female	59	-	-	Normal	No	CC
Family B II-1	Female	31	secundum ASD	1.5/2	Right heart enlargement with pulmonary hypertension	Left to right	CT
Family B II-2	Male	30	-	-	Normal	No	CT
Family B III-1	Male	4	secundum ASD	0.3/0.35	Normal	Left to right	TT

Abbreviations: ASD, atrial septal defect; VSD, ventricular septal defect.

TABLE 2 Characteristics of patients and controls

Parameters	CHD	Control	*p* Value
Total subjects	494	576	
Age (years)	11.57 ± 6.51	10.86 ± 5.55	0.055
Sex			
Female	233 (47.17%)	284 (49.31%)	0.485
Male	261 (52.83%)	292 (50.69%)	

lead to changes in amino acid coding), and obtain mutations that affect gene expression products. (4) Mutation sites were screened according to the rating prediction of SIFT, Polyphen, MutationTaster and CADD. It was required that at least half of the four software with scores supported that the site might be harmful before it was reserved. In order to better predict the harmfulness of variation, the classification system of the American College of Medical Genetics and Genomics (ACMG) was used. The variations are classified into pathogenic, likely pathogenic, uncertain significance, likely benign and benign. And finally, we identified 1,471 Deleterious. From these suspected deleterious mutations, we searched for mutations that fit certain genetic patterns and those that were co-isolated with CHD states. Among the co-segregation of potentially deleterious mutations, we did not identified the known pathogenic genes of CHD. If the mutations were not located in a known CHD pathogenic gene, we wanted by looking for common variants in different families to better identify susceptibility genes in this study. So the following considerations were taken into account: (1) Whether the gene was mutated in another family, (2) whether the gene was expressed in the heart, (3) whether the gene was abnormally expressed in patients, and (4) whether the gene was verified in an independent population. Through these steps, we found that *SLC2A3* (rs3931701) C > T coexist in the two CHD families we studied and the gene *SLC2A3* was expressed in the heart. The function of variations was uncertain significance classified by ACMG (https://github.com/maryicecream/SLC2A3.git).

2.5 | Primers and Sanger sequencing

According to the detection information, the reference sequence was searched and Primer Premier 5 software was used to design the primers. The primer information of *SLC2A3* was as follows: F:ATGTTGGTCAGGCTGGTCTT, R:CTTCCTCTAGGGTGTGGGGC. The specificity and efficiency of the primers were validated using polymerase chain reaction (PCR). The amplification system and experimental procedure for PCR are described in Table 3. Approximately 3 μl of PCR products were tested with 1.0% agarose gel to observe the band traits. DNA fragments with ligated adapter molecules on both ends were selectively enriched in a PCR reaction. After the PCR reaction, DNA libraries were prepared with liquid phase hybridized biotin-labeled probes. Magnetic beads with streptomycin were used to capture the exons of genes. Captured libraries were enriched in a

TABLE 3 Amplification system for polymerase chain reaction

Reagent component	Volume
Super Mix	15 μl
Primer F	1 μl
Primer R	1 μl
Template DNA	1 μl
ddH_2O	12 μl

PCR reaction to add index tags to prepare for sequencing. Products were purified using AMPure XP system (Beckman Coulter). Purified PCR products were detected on a computer. When the data were downloaded from the system, the software phred\phrap was used for single nucleotide polymorphism (SNP) analysis. Finally, the analysis results were derived.

2.6 | Statistical analysis

Comparisons of genotype and allele frequencies were evaluated using the χ^2 test. The association of *SLC2A3* (rs3931701) polymorphisms with CHD risk was estimated by computing the odds ratios (ORs) and 95% confidence intervals (CIs) using the multivariate logistic regression analysis. All statistical analyses were performed using the SPSS software, version 17.0 (SPSS Inc.).

3 | RESULTS

3.1 | Clinical features

We identified four patients, one female and three males, from two Chinese families (Figure 1). Two elderly patients in the two families died due to heart disease, which was heart failure. In family A, one patient had an ASD and another had a VSD. In family B, two patients had ASDs. Table 1 shows the characteristics of the family members and their *SLC2A3* genotypes (four affected, eight unaffected, and two uncertain).

3.2 | Genetic features

3.2.1 | Family members

We speculated that the pedigrees represented autosomal dominant inheritance. To elucidate the underlying genetic causes, genomic DNA of patients and healthy individuals from these two families was investigated using WES. We analyzed the sequencing data and found that the *SLC2A3* (rs3931701) C > T gene variant was present in both families. The *SLC2A3* gene was identified using Sanger sequencing in the two families. The II-1 and III-1 samples from family A and III-1 sample from family B with CHD demonstrated the *SLC2A3* (rs3931701) C > T variant and TT type mutation. The II-1 sample of family B with

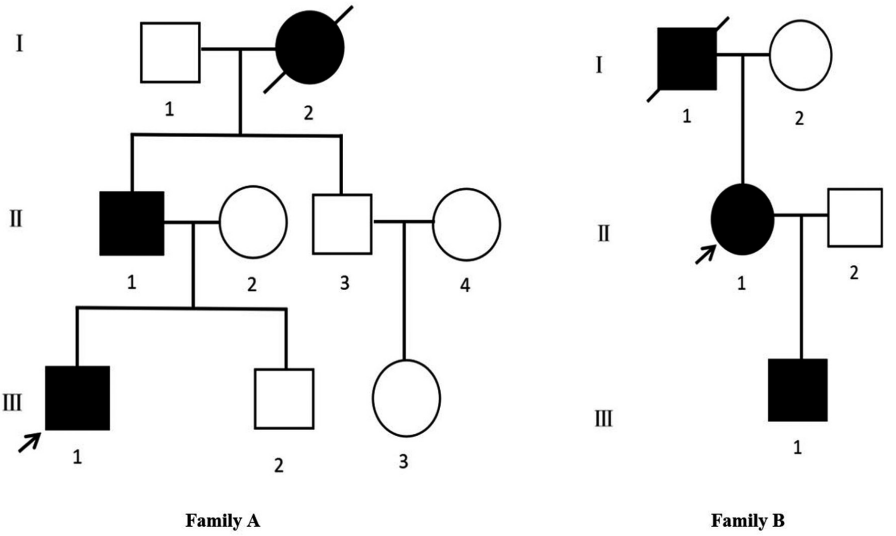

FIGURE 1 Family tree of the patients with congenital heart disease. Black symbols represent patients with CHD, white symbols represent healthy people. In family A, II-1 with ASD and III-1 with VSD. I-2 died due to heart disease. In family B, II-1 and III-1 all with ASD. I-1 died due to heart disease. ASD, atrial septal defect; VSD, ventricular septal defect

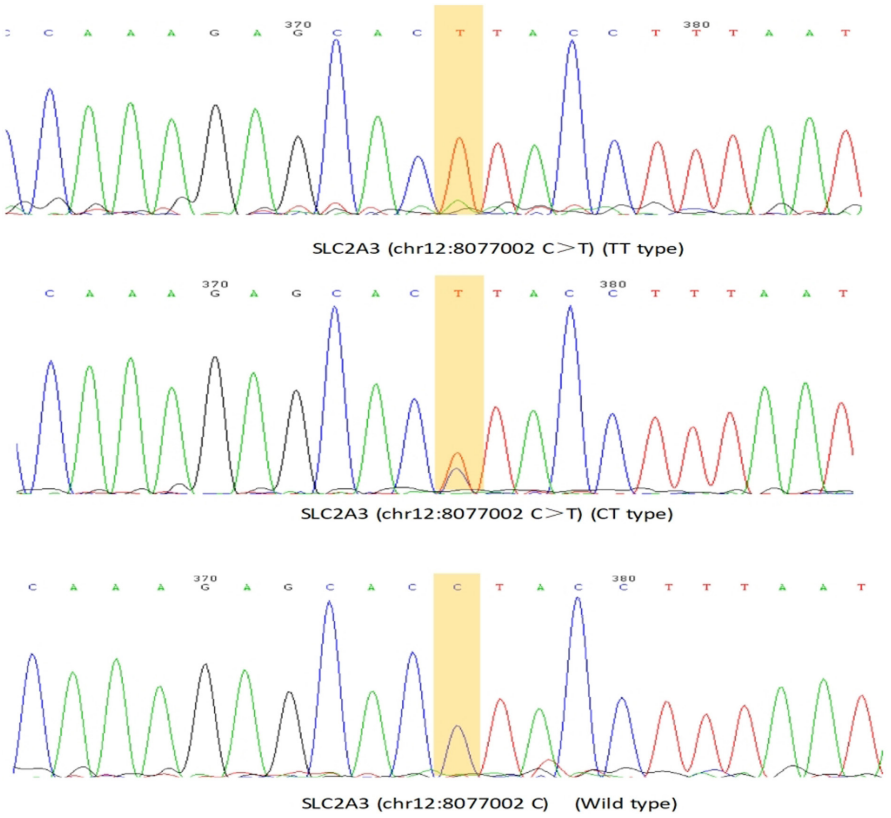

FIGURE 2 Gene sequencing map

CHD; II-2 sample of family B with no evident phenotype; and II-2, II-3, and III-2 samples of family A with no evident phenotype demonstrated the *SLC2A3* (rs3931701) C > T variant and CT type mutation (Figure 2, Table 1, https://github.com/maryicecream/SLC2A3.git.)

3.2.2 | Sporadic CHD samples verification

To investigate the possible association between *SLC2A3* (rs3931701) polymorphisms and susceptibility to the development of CHD, we compared the distribution genotypes among healthy controls and patients with CHD. As shown in Table 4, among the 494 cases of CHD, 86 cases had the TT type, 308 had the CT type, and 100 had the CC type. Among the 576 cases of healthy controls, 36 had the TT type, 236 had the CT type, and 304 had the CC type. Heterozygous (CT) and homozygous (TT) mutants for the *SLC2A3* polymorphism were more frequent in patients with CHD than in healthy controls (CT: $p < 0.0001$, OR =3.967, 95% CI =2.991–5.263; TT: $p < 0.0001$, OR =7.262, 95% CI =4.631–11.388).

TABLE 4 The type of *SLC2A3* (rs3931701) variants in the family with congenital heart disease (CHD) versus healthy controls

	CHD		Control			95% CI		
Gene type	Number (%)	Frequency (%)	Number (%)	Frequency (%)	OR	Lower limit	Upper limit	*p* Value
CC	100	20.24	304	52.78	1	_	_	_
TT	86	17.41	36	6.25	7.262	4.631	11.388	<0.001
CT	308	62.35	236	40.97	3.967	2.991	5.263	<0.001
CT + TT	394	79.76	272	47.22	4.404	3.349	5.790	<0.001

4 | DISCUSSION

Epidemiological data showed that environmental factors increased the risk for CHD; genetic causes were also associated with CHD.[24] Several studies on families with patients who have CHD highlighted the fact that screening high-risk patients with multiple affected family members resulted in the discovery of novel pathogenic gene variants.[25–30] Here, we used WES for patients with CHD from two families. We found that these families shared the *SLC2A3* (rs3931701) C > T mutation. We further investigated sporadic cases of CHD and found that the *SLC2A3* (rs3931701) C > T variant increased the susceptibility for CHD.

The results showed that the frequency of the *SLC2A3* (rs3931701) C > T variant was increased in patients with CHD, which provides new information for the study of the pathogenic genes of CHD and a new basis for the prevention and diagnosis of CHD.

SLC2A3, encoding GLUT3, is located on chromosome 12p13.3 and has 10 exons.[31] *SLC2A3* is not only expressed in the brain and pharyngeal arches but also in the left ventricular outflow tract during development. Its knockdown in mouse and zebrafish orthologs caused early embryonic death.[20–22] More recently, the GLUT3 expression in human placental tissue was reportedly present throughout gestation, with a predominance in the placenta during the first trimester.[32] In rodents, changes in GLUT3 expression occurred concurrently with organ maturation of the central nervous system[33] and heart.[34]

In the human heart, GLUT3 expression increased from the tenth to fifteenth week in fetal cardiomyocytes and subsequently decreased until the twenty-first week of gestation.[34] This suggested that GLUT3 plays an important role in cardiogenesis. A recent study found that *SLC2A3* mRNA expression in fetal myocardium was 15.6-fold higher than those in the myocardium of newborns.[35] GLUT3 facilitates diffusion of glucose across the plasma membranes. Glucose, the primary nutrient of cells, is a critical regulator of growth in rapidly developing embryos.[36] As one of the highest energy consuming organs in mammals, the heart has to be provided with a high amount of energy as soon as it is functional in utero; during perinatal development, cellular energy metabolism of the cardiac tissue, including progenitors, switched from prenatal anaerobic glycolysis to postnatal fatty acid oxidation, which contributed to cardiac maturation.[37] *SLC2A3* knockout mouse models were developed by several working groups.[38,39] Heterozygosity resulted in early pregnancy loss in approximately 25% of affected mouse embryos, whereas a bi-allelic deletion of *SLC2A3* was deleterious, leading to abortion on the twelfth day. It was, therefore, suggested that GLUT3-mediated glucose uptake was pivotal for organogenesis.[20] Recent studies showed that variants of the *SLC2A3* gene were associated with the risk of various clinical diseases, such as attention-deficit/hyperactivity disorder,[40,41] rheumatoid arthritis,[42] cancer,[23] and CHD.[43,44] In the human heart, GLUT4, GLUT1, and GLUT3 are expressed.[35] *SLC2A3* variants were reportedly associated with congenital syndromic heart defects, including Turner syndrome[45] and 22q11.2 deletion syndrome.[43] Cardiogenesis requires strict regulation of energy for normal cell guidance and proliferation,[46] and *SLC2A3*, encoding the high-affinity glucose transporter GLUT3, plays an essential role in providing energetic fuel for cardiac consumption; therefore, it was suggested that impaired glucose metabolism may contribute to cardiac developmental abnormalities. An alternative hypothesis for the markedly increased frequency of the *SLC2A3* variation was the CHD variant ion as a functional compensation due to improved energy supply that may have promoted survival in fatal cardiac dysgenesis.

To the best of our knowledge, we were the first to show that the *SLC2A3* variant was associated with an increased susceptibility for CHD. This variation located in the eighth intron of the *SLC2A3* and its function is not yet clear. From our research, we speculate it has the potential and ability to cause CHD for the following reasons: First, compared with healthy people, patients with CHD had a higher frequency and odds for the *SLC2A3* (rs3931701) C > T mutation; second, as a crucial glucose transporter, the regulatory functions of *SLC2A3* depend on its quality and abundance. Single nucleotide variants (SNVs) might influence the gene at the RNA level, giving rise to changes in the abundance and distribution of *SLC2A3* transcription, which subsequently disrupts the downstream targets of *SLC2A3*. The population in our study is limited to Yunnan, China, which may lead to biased results. In order to better explain the relationship between this gene variation and congenital heart disease, it is necessary to further expand the study population. And how the *SLC2A3* (rs3931701) C > T mutation leads to CHD requires further study in future.

5 | CONCLUSIONS

In conclusion, the novel SNV of the *SLC2A3* gene may contribute to the increased risk for CHD in a Chinese Yunnan population. Therefore, future studies with a larger sample size are needed to determine the association between *SLC2A3* and CHD and to determine the pathogenic mechanism of the *SLC2A3* (rs3931701) C > T variant.

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CONFLICT OF INTEREST

The authors declare no relationships that could be construed as a conflict of interest.

AUTHOR CONTRIBUTIONS

Lihong Jiang, Jie Liu conceived and designed the study; Lijing Ma did the experiments, analyzed the data, and wrote the first draft of the paper; Jiaxin Xu, Yu Cao did the clinical diagnosis, analyzed the data, and participated in the writing up; Qisheng Tang did the experiments, analyzed the data; Yu Cao and Ruize Kong did the recruitment and participated in the analysis of data; Kunlin Li analyzed the data, participated in the writing of the paper.

DATA AVAILABILITY STATEMENT

The datasets during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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REFERENCES

1. Zhang X, Sun Y, Zhu J, Zhu Y, Qiu L. Epidemiology, prenatal diagnosis, and neonatal outcomes of congenital heart defects in eastern China: a hospital-based multicenter study. *BMC Pediatr*. 2020;20(1):416. doi:10.1186/s12887-020-02313-4
2. Nezami A, Heidari G, Tarhani F, Kariminia M. Prevalence of congenital heart disease among children in Khorramabad (West of Iran). *Cardiovasc Hematol Disord Drug Targets*. 2021;21(1):61-65. doi:10.2174/1871529X20999201231205746
3. Nora JJ. Multifactorial inheritance hypothesis for the etiology of congenital heart diseases. The genetic-environmental interaction. *Circulation*. 1968;38(3):604-617. doi:10.1161/01.cir.38.3.604
4. Chung IM, Rajakumar G. Genetics of congenital heart defects: the NKX2-5 gene, a key player. *Genes (Basel)*. 2016;7(2):6. doi:10.3390/genes7020006
5. Behiry EG, Al-Azzouny MA, Sabry D, Behairy OG, Salem NE. Association of NKX2-5, GATA4, and TBX5 polymorphisms with congenital heart disease in Egyptian children. *Mol Genet Genomic Med*. 2019;7(5):e612. doi:10.1002/mgg3.612
6. Cao Y, Guo J, Zhang J, et al. HYDIN loss-of-function inhibits GATA4 expression and enhances atrial septal defect risk. *Mech Dev*. 2020;162:103611. doi:10.1016/j.mod.2020.103611
7. Fu F, Li R, Lei TY, et al. Compound heterozygous mutation of the ASXL3 gene causes autosomal recessive congenital heart disease. *Hum Genet*. 2021;140(2):333-348. doi:10.1007/s00439-020-02200-z
8. Czeizel AE. Periconceptional folic acid containing multivitamin supplementation. *Eur J Obstet Gynecol Reprod Biol*. 1998;78(2):151-161. doi:10.1016/s0301-2115(98)00061-x
9. Øyen N, Olsen SF, Basit S, et al. Association between maternal folic acid supplementation and congenital heart defects in offspring in birth cohorts from Denmark and Norway. *J Am Heart Assoc*. 2019;8(6):e011615. doi:10.1161/JAHA.118.011615
10. Leirgul E, Brodwall K, Greve G, et al. Maternal diabetes, birth weight, and neonatal risk of congenital heart defects in Norway, 1994–2009. *Obstet Gynecol*. 2016;128(5):1116-1125. doi:10.1097/AOG.0000000000001694
11. Grattan MJ, Thomas DS, Hornberger LK, Hamilton RM, Midodzi WK, Vohra S. Maternal hypothyroidism may be associated with CHD in offspring. *Cardiol Young*. 2015;25(7):1247-1253. doi:10.1017/S1047951114001887
12. Xia YQ, Zhao KN, Zhao AD, et al. Associations of maternal upper respiratory tract infection/influenza during early pregnancy with congenital heart disease in offspring: evidence from a case-control study and meta-analysis. *BMC Cardiovasc Disord*. 2019;19(1):277. doi:10.1186/s12872-019-1206-0
13. Webster WS, Germain MA, Lipson A, Walsh D. Alcohol and congenital heart defects: an experimental study in mice. *Cardiovasc Res*. 1984;18(6):335-338. doi:10.1093/cvr/18.6.335
14. Correa A, Levis DM, Tinker SC, Cragan JD. Maternal cigarette smoking and congenital heart defects. *J Pediatr*. 2015;166(4):801-804. doi:10.1016/j.jpeds.2015.01.013
15. Spinder N, Bergman JE, Kromhout H, et al. Maternal occupational exposure and congenital heart defects in offspring. *Scand J Work Environ Health*. 2020;46(6):599-608. doi:10.5271/sjweh.3912
16. Bult CJ, Blake JA, The Mouse Genome Database Group. Mouse Genome Database (MGD) 2019. *Nucleic Acids Res*. 2019;47(D1):D801-D806. doi:10.1093/nar/gky1056
17. Ling D, Dayan JG. In utero diagnoses of strikingly similar presentations of complete atrioventricular septal defects in a pair of dizygotic twins concordant for trisomy 21. *Case Rep Pediatr*. 2018;2018:6215675. doi:10.1155/2018/6215675
18. LaHaye S, Corsmeier D, Basu M, et al. Utilization of whole exome sequencing to identify causative mutations in familial congenital heart disease. *Circ Cardiovasc Genet*. 2016;9(4):320-329. doi:10.1161/CIRCGENETICS.115.001324
19. Caputo S, Capozzi G, Russo MG, et al. Familial recurrence of congenital heart disease in patients with ostium secundum atrial septal defect. *Eur Heart J*. 2005;26(20):2179-2184. doi:10.1093/eurheartj/ehi378
20. Ganguly A, McKnight RA, Raychaudhuri S, et al. Glucose transporter isoform-3 mutations cause early pregnancy loss and fetal growth restriction. *Am J Physiol Endocrinol Metab*. 2007;292(5):E1241-E1255. doi:10.1152/ajpendo.00344.2006
21. Ganguly A, Collis L, Devaskar SU. Placental glucose and amino acid transport in calorie-restricted wild-type and Glut3 null heterozygous mice. *Endocrinology*. 2012;153(8):3995-4007. doi:10.1210/en.2011-1973
22. Carayannopoulos MO, Xiong F, Jensen P, et al. GLUT3 gene expression is critical for embryonic growth, brain development and survival. *Mol Genet Metab*. 2014;111(4):477-483. doi:10.1016/j.ymgme.2014.01.013
23. Do SK, Choi SH, Lee SY, et al. Glucose transporter 3 gene variant is associated with survival outcome of patients with non-small cell lung cancer after surgical resection. *Gene*. 2019;703:58-64. doi:10.1016/j.gene.2019.04.013
24. Bruneau BG. The developmental genetics of congenital heart disease. *Nature*. 2008;451(7181):943-948. doi:10.1038/nature06801
25. Garg V, Kathiriya IS, Barnes R, et al. GATA4 mutations cause human congenital heart defects and reveal an interaction with TBX5. *Nature*. 2003;424(6947):443-447. doi:10.1038/nature01827
26. Schott JJ, Benson DW, Basson CT, et al. Congenital heart disease caused by mutations in the transcription factor NKX2-5. *Science*. 1998;281(5373):108-111. doi:10.1126/science.281.5373.108
27. Granados-Riveron JT, Ghosh TK, Pope M, et al. Alpha-cardiac Myosin Heavy Chain (MYH6) mutations affecting myofibril formation are associated with congenital heart defects. *Hum Mol Genet*. 2010;19(20):4007-4016. doi:10.1093/hmg/ddq315
28. Kodo K, Nishizawa T, Furutani M, et al. GATA6 mutations cause human cardiac outflow tract defects by disrupting semaphorin–plexin

signaling. *Proc Natl Acad Sci USA*. 2009;106(33):13933-13938. doi:10.1073/pnas.0904744106
29. Kirk EP, Sunde M, Costa MW, et al. Mutations in cardiac T-box factor gene TBX20 are associated with diverse cardiac pathologies, including defects of septation and valvulogenesis and cardiomyopathy. *Am J Hum Genet*. 2007;81(2):280-291. doi:10.1086/519530
30. Ching YH, Ghosh TK, Cross SJ, et al. Mutation in myosin heavy chain 6 causes atrial septal defect. *Nat Genet*. 2005;37(4):423-428. doi:10.1038/ng1526
31. Joost HG, Bell GI, Best JD, et al. Nomenclature of the GLUT/SLC2A family of sugar/polyol transport facilitators. *Am J Physiol Endocrinol Metab*. 2002;282(4):E974-E976. doi:10.1152/ajpendo.00407.2001
32. Novakovic B, Gordon L, Robinson WP, Desoye G, Saffery R. Glucose as a fetal nutrient: dynamic regulation of several glucose transporter genes by DNA methylation in the human placenta across gestation. *J Nutr Biochem*. 2013;24(1):282-288. doi:10.1016/j.jnutbio.2012.06.006
33. Vannucci SJ, Clark RR, Koehler-Stec E, et al. Glucose transporter expression in brain: relationship to cerebral glucose utilization. *Dev Neurosci*. 1998;20(4–5):369-379. doi:10.1159/000017333
34. Grover-McKay M, Walsh SA, Thompson SA. Glucose Transporter 3 (GLUT3) protein is present in human myocardium. *Biochim Biophys Acta*. 1999;1416(1–2):145-154. doi:10.1016/s0005-2736(98)00216-8
35. Kong B, Liu YL, Lü XD. Microarray-bioinformatics analysis of altered genomic expression profiles between human fetal and infant myocardium. *Chin Med J (Engl)*. 2008;121(14):1257-1264.
36. Nakano H, Fajardo VM, Nakano A. The role of glucose in physiological and pathological heart formation. *Dev Biol*. 2021;475:222-233. doi:10.1016/j.ydbio.2021.01.020
37. Sayed A, Valente M, Sassoon D. Does cardiac development provide heart research with novel therapeutic approaches? *F1000Res*. 2018;7:1756. doi:10.12688/f1000research.15609.1
38. Schmidt S, Hommel A, Gawlik V, et al. Essential role of glucose transporter GLUT3 for post-implantation embryonic development. *J Endocrinol*. 2009;200(1):23-33. doi:10.1677/JOE-08-0262
39. Fidler TP, Campbell RA, Funari T, et al. Deletion of GLUT1 and GLUT3 reveals multiple roles for glucose metabolism in platelet and megakaryocyte function. *Cell Rep*. 2017;21(6):1705. doi:10.1016/j.celrep.2017.10.086
40. Lesch KP, Selch S, Renner TJ, et al. Genome-wide copy number variation analysis in attention-deficit/hyperactivity disorder: association with neuropeptide Y gene dosage in an extended pedigree. *Mol Psychiatry*. 2011;16(5):491-503. doi:10.1038/mp.2010.29
41. Merker S, Reif A, Ziegler GC, et al. SLC2A3 single-nucleotide polymorphism and duplication influence cognitive processing and population-specific risk for attention-deficit/hyperactivity disorder. *J Child Psychol Psychiatry*. 2017;58(7):798-809. doi:10.1111/jcpp.12702
42. Veal CD, Reekie KE, Lorentzen JC, Gregersen PK, Padyukov L, Brookes AJ. A 129-kb deletion on chromosome 12 confers substantial protection against rheumatoid arthritis, implicating the gene SLC2A3. *Hum Mutat*. 2014;35(2):248-256. doi:10.1002/humu.22471
43. Mlynarski EE, Sheridan MB, Xie M, et al. International chromosome 22q11.2 consortium. Copy-number variation of the glucose transporter gene SLC2A3 and congenital heart defects in the 22q11.2 deletion syndrome. *Am J Hum Genet*. 2015;96(5):753-764. doi:10.1016/j.ajhg.2015.03.007
44. Monteiro RAC, de Freitas ML, Vianna GS, et al. Major contribution of genomic copy number variation in syndromic congenital heart disease: the use of MLPA as the first genetic test. *Mol Syndromol*. 2017;8(5):227-235. doi:10.1159/000477226
45. Prakash SK, Bondy CA, Maslen CL, et al. Autosomal and X chromosome structural variants are associated with congenital heart defects in Turner syndrome: the NHLBI GenTAC registry. *Am J Med Genet A*. 2016;170(12):3157-3164. doi:10.1002/ajmg.a.37953
46. Kloesel B, DiNardo JA, Body SC. Cardiac embryology and molecular mechanisms of congenital heart disease: a primer for anesthesiologists. *Anesth Analg*. 2016;123(3):551-569. doi:10.1213/ANE.0000000000001451

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