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Original Article

Familial congenital heart disease: data collection and preliminary analysis

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Abstract The aim of this study was to explore genetic mechanisms of congenital heart disease by analysing family data. Families with two or more affected members were studied, and information on family history and risk factors was collected. A total of 25 families with congenital heart disease were identified, and among them the condition was confirmed in 57. The prevalence of congenital heart disease in first-degree relatives was 43.0%, that is 46 out of 107, significantly higher than that in second-degree relatives, that is, 4.4%, 11 out of 252) ($\chi^2 = 83.897$, $P < 0.01$). The prevalence difference between twins (90%) and siblings (62.2%) ($\chi^2 = 4.983$, $P < 0.05$) was also significant among first-degree relatives. Eleven families were found to have the same phenotype (44%), including ventricular septal defect in six families, atrial septal defect in two families, conotruncal defects in two families, and hypoplastic left heart syndrome in one family. Both twins were diagnosed with congenital heart disease in 8 out of 10 twin families – all eight twins were monozygotic. The cardiac phenotype of the twins was consistent in three families (37.5%). The cardiac phenotype of first- and second-degree relatives was not fully consistent with their probands. There was an increased incidence of threatened abortion in early pregnancy in patients with familial congenital heart disease when compared with sporadic congenital heart disease ($\chi^2 = 8.704$, $P < 0.05$). Morbidity in relatives was related to blood relationship, with a closer relationship increasing the risk of congenital heart disease. Genetic factors appear to play an important role in congenital heart disease.

Keywords: Family collection; pedigree; genetics; risk factors

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CONGENITAL HEART DISEASE IS THE MOST COMMON congenital birth defect and a leading cause of infant morbidity and disability, creating a tremendous toll on families, caregivers, and health-care systems. The incidence of congenital heart disease is between 6 out of 1000 and 8 out of 1000 live births and increased year by year.[1] The pathogenesis of congenital heart disease remains unclear. It is thought that the condition results from abnormal cardiac vascular development caused by the interaction of genes and the environment. Research shows that the current occurrence of congenital heart disease is associated with human disease genes, such as *TBX5*, *NKX2.5*, *GATA-4*, which have been confirmed in the pedigree of congenital heart disease by genetic testing.[2–4] In recent years, with the successful completion of the human genome project, experimental methods and techniques of identifying genetic mutations have progressed rapidly. It becomes much easier for researchers to find the genetic causes of congenital heart disease in patients with a family history. Furthermore, the knowledge gained about the causes of congenital heart disease could help us

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diagnose patients with sporadic congenital heart disease in order to improve clinical care. We already had data on patients with congenital heart disease at our disposal, and thus the aim of this study was to use this information to establish the pedigree, which in turn could potentially provide the basis for the exploration of the underlying mechanisms.

Subjects and methods

Patients

All congenital heart disease probands were diagnosed in Shanghai Children's Medical Center or Xinhua hospital between January, 2009 and July, 2011. Families in which two or more members had congenital heart disease were recruited as subjects, following which a detailed assessment of family history and risk factors was conducted. First-degree relatives were defined as parents or siblings, including monozygotic twins, dizygotic twins, or brothers and sisters, whereas second-degree relatives were defined as grandparents, uncle and aunts, or cousins.

Methods

Trained staff conducted a survey to obtain information on personal and family histories of those with congenital heart disease. Individuals who were reported as having congenital heart disease had the diagnosis confirmed by clinical evaluation, including detailed medical history, comprehensive physical examination, echocardiography, chest X-ray, electrocardiogram, cardiac catheterisation, or surgery.

Family genetic data were obtained from the probands. Pedigree trees were constructed on the basis of these data. Written informed consent was obtained from each participant before data collection. All protocols were approved by the human ethics committee of both hospitals.

A case–control study was conducted to find differences in risk factors between familial and sporadic congenital heart disease. Sporadic control individuals were recruited in the hospitals at the same time, matched by age, gender, and the cardiac phenotype. A standard questionnaire was administered by trained staff to collect lifestyle risk factors, information on parents and second-degree relatives of the probands, as well as on parents of controls. The questionnaire was designed and included the following risk factors: **A.** Move into a new house or the purchase of new non-wood furniture during the 6 months before pregnancy to child birth; **B.** Noise in working and living environment during pregnancy; **C.** Living in a room warmed directly by charcoal or coal cinder or with a high concentration of CO_2 and SO_2 for a significant length of time during pregnancy; **D.** Smoking or passive smoking; **E.** Drinking alcohol; **F.** Suffering from respiratory infections during the first trimester of pregnancy; and **G.** Threatened abortion in early pregnancy.

Statistical analysis

Variables were summarised as percentages and compared using Pearson's chi-square test or Fisher's exact test. Statistical tests were based on a two-tailed probability, and values of $P < 0.05$ were considered statistically significant. All analyses were performed using the SPSS 13.0 software package (SPSS, Chicago, IL, USA).

Results

The prevalence in first- and second-degree relatives

The prevalence rates for all family relatives, first-degree relatives, and second-degree relatives were 15.9% (57/359), 43.0% (46/107), and 4.4% (11/252), respectively (Table 1).

In 25 family pedigree, five families in which one of their parents of the proband were suffered from congenital heart disease, the prevalence is 10.0% (5/50). We found 10 twin family pedigrees with congenital heart disease. Twins of the probands suffered from congenital heart disease in eight families, and the prevalence was 80% (8 out of 10). Siblings of the probands – excluding twins – suffered from congenital heart disease in seven families, and the prevalence was 62.2% (23 out of 37). The difference in prevalence between twin siblings and non-twin siblings was statistically significant ($\chi^2 = 4.983$, $P < 0.05$).

The prevalence in second-degree relatives was 4.4% (11 out of 252). The difference in the prevalence between first- and second-degree relatives was significant ($\chi^2 = 83.897$, $P < 0.01$). Congenital heart disease therefore has the potential to aggregate in families. The incidence of congenital heart disease in

Table 1. Prevalence of congenital heart disease in the relatives of probands.

Relationship	Total number	Patients	Prevalence (%)
First-degree relatives	107	46	43.0
Parents	50	5	10.0
Twins	20	18	90
Siblings (excluding twins)	37	23	62.2*
Second-degree relatives	252	11	4.4**
Grandparents	98	1	1.0
Uncles/aunts	102	4	3.9
Cousins	52	6	11.5
Total	359	57	15.9

*Compared with twins, $\chi^2 = 4.983$, $p < 0.05$

**Compared with first-degree relatives, $\chi^2 = 83.897$, $p < 0.01$

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the relatives of a proband was related to the degree of blood relationship, with the closer the relationship, the higher prevalence.

Distribution of the congenital heart disease phenotype

We collected 359 individuals data on 25 families with congenital heart disease, of whom 57 people had congenital heart disease. However, seven patients had died by the time of our survey. The probands, 11 males and 14 females, were between 27 weeks of pregnancy and 14 years of age, with an average age of 2.22 ± 2.79 years. The following were the most frequent congenital heart defects in first- and second-degree relatives: ventricular septal defect in 22 out of 57, 38.5%; atrial septal defect in 10 out of 57, 17.5%; and tetralogy of Fallot in 6 out of 57, 10.5%.

The cardiac phenotypes of first-degree relatives were consistent with the probands in 11 families (44%), including ventricular septal defect in six families, atrial septal defect in two families, conotruncal defects in two families, and hypoplastic left heart syndrome in one family. One of the parents' phenotype was consistent with the proband in 4 out of the 11 families and the phenotype was ventricular septal defect in all. Both twins suffered from congenital heart disease in 8 out of the 10 twin families – all the eight twins were monozygotic. There were three families (37.5%) that showed consistent cardiac phenotype. Their diseases comprised ventricular septal defect/patent ductus arteriosus/pulmonary hypertension, ventricular septal defect; tetralogy of Fallot, tetralogy of Fallot; and hypoplastic left heart syndrome, hypoplastic left heart syndrome, respectively. The echocardiogram of one of the twins in the monozygotic twin families with ventricular septal defect alone confirmed that the ventricular septal defect naturally closed and returned to normal during follow-up visits, whereas cardiac phenotypes of the other one included ventricular septal defect and patent ductus arteriosus/pulmonary hypertension requiring surgical treatment. Cardiac phenotypes and clinical symptoms of monozygotic twin families with tetralogy of Fallot and hypoplastic left heart syndrome were completely consistent. There were five twin families that showed inconsistent phenotypes (62.5%). In four families, one of the twins had tetralogy of Fallot or pulmonaryatresia pulmonary atresia/ventricular septal defect, the other had ventricular septal defect – echocardiogram indicated perimembranous ventricular septal defect. In one family, one of the twins had coarctation of the aorta and the other had patent ductus arteriosus. In two twin families – one monozygotic twin family and one dizygotic twin family – one of the twins was sick, and the other showed normal cardiac phenotype. In the monozygotic twin family, one suffered from pulmonary stenosis/right ventricular outflow tract obstruction, and the other showed normal cardiac phenotype. In the dizygotic twin family, the younger brother suffered from atrial septal defect/moderate regurgitation of the mitral valve/porosity of myocardial tissue and the sister showed normal cardiac phenotype; however, in the family the older sib brother of the twins died from congenital heart disease. Sibling phenotype – excluding twins – was consistent with that of the probands in four families and inconsistent in three. The cardiac phenotypes of second-degree relatives were consistent with the probands in three families, including ventricular septal defect in two families and aortic stenosis in one family. We consider that the congenital heart disease phenotype of relatives was not entirely consistent if more than one relative of the proband suffered from congenital heart disease.

In one of the 25 families, the first child had died from congenital heart disease 9 months after birth. The second child died from biliary atresia at 1 year of age. Termination of the third pregnancy was conducted at week 24, and at week 27 in the fourth pregnancy, owing to foetal echocardiography findings of complex congenital heart disease and hypoplastic left heart syndrome, respectively (Table 2).

The results of the risk factor questionnaires

We have collected 42 completed questionnaires of familial congenital heart disease, and 84 completed questionnaires of sporadic congenital heart disease. We found that the incidence of threatened abortion during early pregnancy in familial congenital heart disease was higher than that observed in the control group ($\chi^2 = 8.704$, $P < 0.05$), using univariate Chi-square analysis. There were no significant differences in the other six factors (Table 3).

In the questionnaire of congenital heart disease families, we found that threatened abortion in early pregnancy occurred in seven families, including four twin families. Their diseases comprised tetralogy of Fallot, ventricular septal defect; tetralogy of Fallot, tetralogy of Fallot; hypoplastic left heart syndrome, hypoplastic left heart syndrome; and pulmonary atresia/ventricular septal defect/pulmonary stenosis, ventricular septal defect/patent ductus arteriosus/pulmonary hypertension, respectively. The other three families were sib ones, showing symptoms of threatened abortion in early pregnancy of each parity. Their diseases comprised pulmonary atresia/ventricular septal defect, tetralogy of Fallot; single atrium/single ventricle/complete atrioventricular

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Table 2. The phenotype of congenital heart disease probands and their first- and second-degree relatives.

Family	Phenotype of proband	Sex of proband	Phenotype of the first- and second-degree relatives	Relationship with proband
1	ASD	Female	VSD	Mother
2	ASD	Male	ASD	Mother
3	VSD	Female	VSD	Mother
4	VSD/ASD/PDA	Female	VSD	Mother
5	VSD	Male	VSD	Father
6	VSD/PDA/PH	Female	VSD	Identical twins's elder sister
7	TOF	Female	VSD	Identical twins's elder sister
8	TOF	Female	VSD	Identical twins's elder sister
9	TOF	Female	TOF	Identical twins's elder sister
10	TOF	Female	VSD	Identical twins's younger sister
11	HLHS	Male	HLHS	Identical twins's younger brother
12	COA	Male	PDA	Identical twins's older brother
13	PA/VSD/PS	Male	VSD/PDA/PH	Identical twins's younger brother
14	PS/RVOTO	Male	#CHD (dead, no details)	#Uncle
			##Normal	##Identical twins's elder brother
15	ASD/MRMV/PMT	Male	#CHD (dead, no details)	#Elder brother
			##Normal	##Fraternal twins of the elder sister
16	VSD	Male	VSD	Elder sister
17	PA/VSD	Female	TOF (dead)	Elder sister
18	SA/SV/CAVC/HLHS (termination of pregnancy at week 27)	Female	#CHD (dead)	#Eldest sister
			##CHD (dead)	##Three elder sisters
19	VSD	Male	VSD/ASD/PS (dead)	Older sister
20	ASD	Male	#ASD	#Older sister
			##PDA	##Elder female cousin
21	PDA	Male	VSD	Brother
22	CAF	Male	#ASD	#Father's sister
			##TAPVD	##Elder cousin
			###ASD	###Younger cousin
			####VSD	####Father's younger male cousin (## and ### are siblings)
23	VSD/RVOTO/COA	Male	#VSD/RVOTO	#Aunt
			##CHD (dead, no exact details)	##Grandfather
24	VSD	Female	VSD	Younger female cousin
25	AS	Female	AS	Younger male cousin

AS = aortic stenosis; ASD = atrial septal defect; CAF = coronary arterial fistula; CAVC = complete atrioventricular canal; COA = coarctation of the aorta; HLHS = hypoplastic left heart syndrome; PA = pulmonary atresia; PDA = patent ductus arteriosus; PH = pulmonary hypertension; PMT = porosity of myocardial tissue; PS = pulmonary stenosis; MRMV = moderate regurgitation of the mitral valve; RVOTO = right ventricular outflow tract obstruction; SA = single atrium; SV = single ventricle; TAPVD = total anomalous pulmonary venous drainage; TOF = tetralogy of Fallot; VSD = ventricular septal defect; # = the cardiac phenotype of the relatives and their corresponding relationship with the probands.

canal/hypoplastic left heart syndrome, congenital heart disease, congenital heart disease; and total anomalous pulmonary venous drainage, atrial septal defect, respectively. The survey found that threatened abortion in early pregnancy mainly occurred in complex congenital heart disease, but according to existing data, specific complex congenital heart disease threatened abortion in early pregnancy could not be determined. Meanwhile, in 84 questionnaires of sporadic congenital heart diseases, six patients combined with threatened abortion in early pregnancy, and their cardiac phenotypes showed complex congenital heart disease (data not shown) (Table 4).

Discussion

The pathogenesis of congenital heart disease remains unclear. Studies suggest that congenital heart disease is a heterogeneous multifactorial disorder resulting from a combination of environmental and genetic factors. Previous studies have shown that a small number of those with congenital heart disease have the potential for familial aggregation, with the risk in relatives higher than in the normal population and correlated with the closeness of the blood relationship.[5,6] Our study revealed similar results, by demonstrating the potential of congenital heart disease aggregation in families, with the risk of congenital heart disease

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Table 3. The univariate Chi-square analysis of sporadic and familial.

	Congenital heart disease			
Factor	Sporadic	Familial	χ^2	p
A*				
No	77 (91.7)	40 (95.2)	0.538	0.463
Yes	7 (8.3)	2 (4.8)		
B*				
No	77 (91.7)	42 (100.0)	3.706	0.054
Yes	7 (8.3)	0 (0.0)		
C*				
No	82 (97.6)	42 (100.0)	1.016	0.313
Yes	2 (2.4)	0 (0.0)		
D*				
No	68 (81.0)	37 (88.1)	1.029	0.310
Yes	16 (19.0)	5 (11.9)		
E*				
No	79 (94.0)	41 (97.6)	0.788	0.375
Yes	5 (6.0)	1 (2.4)		
F*				
No	52 (61.8)	30 (71.4)	1.118	0.290
Yes	32 (38.2)	12 (28.6)		
G*				
No	78 (92.9)	31 (73.8)	8.704	0.003
Yes	6 (7.1)	11 (26.2)		

A*, moving into a new house or buying new non-wood furniture during half a year before pregnancy to birth of child; **B***, noise in working and living environment during the pregnancy; **C***, living in a room warmed directly by charcoal or coal cinder or with a high concentration of CO_2 and SO_2 for a significant length during the pregnancy; **D***, smoking or passive smoking; **E***, drinking alcohol; **F***, suffering from respiratory infections during the first trimester of pregnancy; **G***, threatened abortion in early pregnancy.

Table 4. Threatened abortion in early pregnancy families.

Family	Cardiac phenotypes	Frequency of occurrence
7	TOF, VSD (twins)	1
9	TOF, TOF (twins)	1
11	HLHS, HLHS (twins)	1
13	PA/VSD/PS, VSD/PDA/PH (twins)	1
17	PA/VSD, TOF (siblings)	2
18	SA/SV/CAVC/HLHS, CHD, CHD (siblings)	3
22	TAPVD, ASD (siblings)	2

ASD = atrial septal defect; CAVC = complete atrioventricular canal; HLHS = hypoplastic left heart syndrome; PA = pulmonary atresia; PDA = patent ductus arteriosus; PH = pulmonary hypertension; PS = pulmonary stenosis; SA = single atrium; SV = single ventricle; TAPVD = total anomalous pulmonary venous drainage; TOF = tetralogy of Fallot; VSD = ventricular septal defect.

increasing as the closeness of the blood relationship of the relatives with the probands increased. Thus, genetic factors appear to play an important role in congenital heart disease.

The phenotypes of first-degree relatives were consistent with the probands in 11 families, including ventricular septal defect in six families, atrial septal defect in two families, conotruncal defects in two families, and hypoplastic left heart syndrome in one family. Speculating from the genetic perspective, the same phenotype may be caused by the same gene or multiple genes that can induce cardiac malformation. One parent of the probands suffered from congenital heart disease in five families, mothers in four and the father in one, suggesting that more congenital heart defects occurred in the offspring of affected women than in those of affected men, which is consistent with the findings of Burn et al.[7] The natural twin birth rate is 4–9%, and the rate increases with assisted reproductive technology, artificial ovulation drugs, and environmental changes. A twin pregnancy is considered to be one of the risk factors leading to congenital heart disease and the concordance rate of monozygotic twins is higher than that of dizygotic twins.[8–12] In this study, the ratio of twin pedigrees was higher in 25 family pedigrees and the prevalence of monozygotic twins was higher than that of siblings, excluding twins, suggesting that genetic factors may play an important role in acquiring congenital heart disease. In theory, the monozygotic twins have the same genetic background and embryonic development environment, and thus the phenotype of congenital heart disease should be the same. However, the cardiac phenotype of monozygotic twins was different in five families in our study. This inconsistency in phenotype indicates that, on the one hand, different phenotypes may be different manifestations of the same disease from the perspective of embryology, while on the other there may be other factors involved in the occurrence of congenital heart disease apart from genetic factors. It has been found that both intrauterine environmental and genetic factors may result in inconsistent phenotypes in the development process of monozygotic twins.[13–15] Foetal placental vascular anastomosis may exist in monochorionic/diamniotic twin gestations. If anastomosis occurs between arteries and veins, this may lead to the twin–twin transfusion syndrome. The connecting blood vessels within the placenta allow blood to pass from one twin to the other. Depending on the number, type, and direction of the interconnecting blood vessels, blood can be transferred disproportionately from one of the twins, the "donor", to the other, the "recipient". The transfusion causes the donor twin to suffer from a decreased blood volume, with a consequent decrease in the donor's development and growth, and urinary output, leading to a lower level of amniotic fluid than normal – oligohydramnios. The blood volume of the recipient twin increases, which

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can strain the foetal heart, leading to heart failure and a higher than normal urinary output with polyhydramnios. Twin–twin transfusion syndrome has been shown to be associated with congenital heart disease.[16]

The first 2–8 weeks of gestation is considered a critical period for embryo heart development, and thus this is the high-risk period for the development of cardiac malformations. If the pregnant woman is exposed to the risk factors for congenital heart disease during the first trimester of pregnancy and 3 months before pregnancy, the foetus may suffer from cardiovascular malformations.[17,18] Studies have shown that, in addition to genetic factors, many non-genetic factors, such as maternal infection, noise, air pollution, parental smoking or drinking, and threatened abortion, are also associated with congenital heart disease.[18,19] In our study, we found that the incidence of threatened abortion during early pregnancy in familial congenital heart disease was higher than that in sporadic congenital heart disease, which suggests that the occurrence of threatened abortion is predominantly affected by genetic factors.

Our analysis of familial congenital heart disease indicates that genetic factors play an important role in the occurrence and development of congenital heart disease. Therefore, through family pedigree collection, we may gain more genetic information that will enhance our understanding of the pathogenesis of congenital heart disease.[20,21] It is important to carry out routine foetal echocardiography in order to reduce birth defects and infant morbidity in congenital heart disease families. Congenital heart disease family data and specimen collection will help us in the identification of the molecular mechanisms of congenital heart disease, providing a theoretical basis for genetic counselling and a potentially effective approach for early intervention and gene therapy for congenital heart disease.

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