Int J Clin Exp Med 2016;9(8):16568-16577
www.ijcem.com /ISSN:1940-5901/IJCEM0029795

Original Article

Relative factors for congenital heart disease: a case-control study

Zhenyu Liu[1*], Shuyuan Yan[2*]

[1]Department of Pediatrics, Hunan Provincial People's Hospital, Changsha, People's Republic of China; [2]Child Health Care Center, Changsha Hospital for Maternal & Child Health Care, Changsha, People's Republic of China. [*]Co-first authors.

Received April 4, 2016; Accepted July 3, 2016; Epub August 15, 2016; Published August 30, 2016

Abstract: Background and objective: The congenital heart disease (CHD) is the leading cause of infant deaths due to birth defects in China. The prevention of CHD is very important. Most CHD are caused by several factors. We investigated relative (risk and protective) factors for CHD in order to explore the etiology of CHD and improve the prevention of CHD. Methods: A retrospective 1:2 matched case-control study of 80 CHD patients and 160 healthy controls was designed. All data analyses (univariate and multivariate) were conducted with SPSS 18.0 to identify relative factors for CHD, and the statistical significance was set at $P<0.05$. Results: Multivariate Logistic regression analysis revealed that the risk factors related to CHD were rural registered permanent residence (odds ratio [OR] = 2.827, 95% confidence interval [CI]: 1.161-6.883), father is a farmer (OR = 5.303, 95% CI: 1.944-14.465), low income parents (less than 2000 Chinese Yuan/month) (OR = 2.469, 95% CI: 1.004-6.070), maternal cold and fever (OR = 6.411, 95% CI: 2.477-16.592), and father smoked half a year before pregnancy (OR = 4.321, 95% CI: 1.802-10.362). Pregnant women often eat eggs (OR = 0.402, 95% CI: 0.184-0.878) is a protective factor for CHD. Conclusions: These findings provide evidence for the etiology and prevention of CHD. Improving healthcare of rural registered permanent residence and low income families, preventing maternal cold and fever, limiting father smoking before pregnancy, and increasing the ingestion of eggs during pregnancy might be helpful to lower the incidence of CHD.

Keywords: Congenital heart disease, relative factors, case-control study

Introduction

Congenital heart disease (CHD) is the most common cause of birth defects [1, 2] and the leading cause of infant deaths due to birth defects in China [3]. CHD is considered to be a severe condition because of the risk of resulting in abortion, stillbirth, neonatal death, and permanent disability. CHD threatens human health and reduces the birth population quality, and it is becoming one of the most important public health problems.

The mechanism of CHD is still unclear. In recent few years, there have been progresses in the research of inherited causes of CHD by identifying specific genetic abnormalities for some types of CHD [4]. Genetic and environmental factors are both involved in the aetiology of CHD, but only approximately 15% can be attributed to a known reason [5]. Less is known about noninherited factors for CHD.

Noninherited environmental relative factors might have an adverse effect on the fetal heart [6]. According to literature, the environmental risk factors of CHD in the part area of China including mother's previous pregnancies, mother's mental stress in the first trimester, mother's educational level, and maternal upper respiratory tract infection, *et al.* [7]. However, there are regional and population differences in the environmental risk factors of CHD. It is important to investigate the contribution of potential noninherited factors in different regional and population to reduce the local growing health burden of CHD. We initiated a retrospective case-control study evaluating the possible environmental risk factors and protective factors for CHD in order to explore the etiology of CHD and improve the local prevention of CHD.

Materials and methods

Subjects

Eighty cases of diagnosed CHD in the Department of Pediatric Cardiovascular and Thoracic Surgery, Hunan Provincial People's Hospital, People's Republic of China, from March 2014 to February 2015 were identified. Many patients with various CHD are transferred to this hospital which is the best diagnosis and treatment unit of childhood diseases in Hunan province.

CHD patients were diagnosed using echocardiography, computed tomographic angiography (CTA), cardiac catheterization, or surgeries by senior pediatric cardiologists. Patients over 12 years old, with abnormalities in other organs, or mental disorder diagnosed in birth parents were excluded. One hundred and sixty healthy cases were assigned to the control group in a 2:1 ratio with the matching criteria: 1) normal children without genetic disease and birth defects; 2) same sex; and 3) same birth region. A control with the diagnosis of CHD or other birth defect was excluded. They were recruited from children undergoing health checkups at the same hospital during the same period. Informed consent was obtained from each participant, and the study was approved by the Institutional Review Board.

Investigation content and data collection

Information collected by unified standardized questionnaire including: 1) Demographic characteristics of children: name, gender, birth date, birth region, ethnicity, registered permanent residence (rural or urban), address, guardian, regular physical examination or not, height, weight, and head circumference (<3 years old); 2) Demographic characteristics of birth parents: age, ethnicity, occupation, education level, chronic disease, family history, inherited disease, consanguineous marriage, and average monthly income, and mother's abnormal reproductive history; 3) Relative factors before and during pregnancy: parents' age at this pregnancy, distance from room to road (<50 meters), new house repair (<1 year), maternal cold and fever, maternal virus infection, maternal chemical substances exposure, maternal medication, parents wine-drinking, parents cigarette smoking, maternal passive smoking, morning sickness, maternal nutritional deficiency, maternal mental stress, maternal folic acid intake, maternal diet, and maternal electronics using (cell phones and computers); and 4) Examination: abnormal symptoms or signs, cardiac ultrasound examination, magnetic resonance imaging (MRI) and CTA, *et al.*. All the birth parents of participants were face-to-face interviewed by uniformly trained interviewers. The questionnaire data were checked on consistency and completeness by the researcher, and the database was established using SPSS 18.0 software (SPSS Inc., USA) after carefully checking.

Variables definitions

Maternal cold and fever: nasal congestion, runny nose, sneeze, cough, pharynx malaise or sore throat symptoms with or without headache, fever, and fatigue; Maternal virus infection: maternal blood test showed existence of cytomegalovirus (CMV), rubella, hepatitis B, or Coxsackie virus infections; Mother's abnormal reproductive history: miscarriage, stillbirth, or fetal malformation; Wine-drinking history: wine-drinking more than once a week, alcohol consumption ≥ 50 ml/time and last ≥ 6 months; Smoked half a year before pregnancy: smoking ≥ 1 cigarette/day; Mother's passive smoking during pregnancy: passive cigarette smoking ≥ 3 times/week; Chronic disease: including: heart disease, hepatitis, encephalopathy, pneumonopathy, nephritis, hypertension, diabetes, hematonosis, hyperthyroidism or hypothyroidism, or other severe disease affecting their daily work and life.

Statistical analysis

This trial was performed as a retrospectively case-control study. All of the data analyses were conducted with the use of SPSS 18.0 software (SPSS Inc., USA). Statistical significance was set at $P<0.05$. Data were analyzed for homogeneity of variance with the Levene test and for normal distribution with the Kolmogorov-Smirnov test. If the variables met assumptions of normality and homogeneity of variance, an independent samples t test for continuous variables analysis was used, and if not, a non-parametric test (Mann-Whitney Test) was used. The chi-square (χ^2) tests were used to evaluate the bivariate associations of the categoric data. The affecting and confounding

Table 1. Children and parents' characteristics and demographics among cases and controls

Variable	Cases (n = 80)	Controls (n = 160)	χ^2 (t)	*P* value
Children's ethnicity				
Han	77 (96.250)	149 (93.125)		
Minority	3 (3.750)	11 (6.875)	0.948	0.330[a]
Children's registered permanent residence				
Rural	22 (27.500)	101 (63.125)		
Urban	58 (72.500)	59 (36.875)	27.092	0.000[a]
Children's regular physical examination				
Yes	50 (62.500)	121 (75.625)		
No	30 (37.500)	39 (24.375)	4.485	0.034[a]
Children's guardian				
Parents	60 (75.000)	132 (82.500)		
Grandparents	18 (22.500)	26 (16.250)		
Other	2 (2.500)	2 (1.250)	-1.387	0.166[a]
Paternal age	30.26±5.951	28.56±5.067	2.197	0.030[b]
Father's ethnicity				
Han	77 (96.250)	151 (94.375)		
Minority	3 (3.750)	9 (5.625)	0.395	0.530[a]
Father's occupation				
Farmer	33 (41.250)	15 (9.375)		
Businessman	11 (13.750)	44 (27.500)		
Civil servant/technician	6 (7.500)	63 (39.375)		
Other	30 (37.500)	38 (23.750)	-2.208	0.043[a]
Father's education level				
Primary or junior high school	42 (52.500)	31 (19.375)		
Senior high school/junior college	30 (37.500)	81 (50.625)		
Bachelor degree or higher	8 (10.000)	48 (30.000)	-5.595	0.000[a]
Father's chronic disease				
No	62 (77.500)	151 (94.375)		
Yes	18 (22.500)	9 (5.625)	15.211	0.000[a]
Father's family history				
No	78 (97.500)	158 (98.750)		
Yes	2 (2.500)	2 (1.250)	0.528	0.468[a]
Maternal age	27.06±6.101	27.81±6.436	-0.391	0.696[b]
Mother's ethnicity				
Han	77 (96.250)	152 (95.000)		
Minority	3 (3.750)	8 (5.000)	0.119	0.662[a]
Mother's occupation				
Farmer	32 (40.00)	17 (10.625)		
Businessman	7 (8.750)	29 (18.125)		
Civil servant/technician	4 (5.000)	38 (23.750)		
Other	37 (46.250)	76 (47.500)	-2.385	0.017[a]
Mother's education level				
Primary or junior high school	38 (47.500)	29 (18.125)		
Senior high school/junior college	37 (46.250)	85 (53.125)		
Bachelor degree or higher	5 (6.250)	46 (28.750)	-5.428	0.000[a]
Mother's chronic disease				
No	73 (91.250)	149 (93.125)		
Yes	7 (8.750)	11 (6.875)	0.270	0.603[a]
Mother's family history				
No	79 (98.750)	159 (99.375)		

Yes	1 (1.250)	1 (0.625)	none[c]	1.000[a]
Abnormal pregnancy history				
No	65 (81.250)	143 (89.375)		
Yes	15 (18.750)	17 (10.625)	3.047	0.081[a]
Parents' average monthly income (CNY[d])				
<2000	14 (0.175)	7 (0.044)		
≥2000	66 (0.825)	153 (0.956)	11.507	0.001[a]

Note: [a]*P* value is from Pearson's chi-square (χ^2) tests. [b]P value is from t tests. [c]χ^2 value is from Fisher' exact test. [d]CNY, Chinese Yuan.

Table 2. Univariate analysis in case group versus control group before and during pregnancy, n (%)

Variable	Cases (n = 80)	Controls (n = 160)	OR (95% CI)	*P*[a] value
Paternal wine-drinking history				
No	52 (65.000)	107 (66.875)		
Yes	28 (35.000)	53 (33.125)	1.087 (0.618-1.913)	0.772
Paternal wine-drinking half a year before pregnancy				
No	63 (78.750)	144 (90.000)		
Yes	17 (21.250)	16 (10.000)	2.429 (1.154-5.111)	0.017
Maternal wine-drinking history				
No	76 (95.000)	157 (98.125)		
Yes	4 (5.000)	3 (1.875)	2.754 (0.601-12.616)	0.175
Maternal wine-drinking half a year before pregnancy				
No	78 (97.500)	159 (99.375)		
Yes	2 (2.500)	1 (0.625)	4.077 (0.364-45.652)	0.218
Father's smoking history				
No	39 (48.750)	115 (71.875)		
Yes	41 (51.250)	45 (28.125)	2.687 (1.538-4.692)	0.000
Father smoked half a year before pregnancy				
No	48 (60.000)	130 (81.250)		
Yes	32 (40.000)	30 (18.750)	2.889 (1.589-5.254)	0.000
Mother's smoking history				
No	79 (98.750)	158 (98.750)		
Yes	1 (1.250)	2 (1.250)	1.000 (0.089-11.196)	1.000
Mother's passive smoking during pregnancy				
No	56 (70.000)	137 (85.625)		
Yes	24 (30.000)	23 (14.375)	2.553 (1.331-4.895)	0.004
House near road (<50 meters)				
No	56 (70.000)	131 (81.875)		
Yes	24 (30.000)	29 (18.125)	1.936 (1.036-3.616)	0.037
New house repair<1 year				
No	72 (90.000)	146 (91.250)		
Yes	8 (10.000)	14 (8.750)	1.159 (0.465-2.888)	0.752
Maternal chemical substances exposure				
No	70 (87.500)	159 (99.375)		
Yes	10 (12.500)	1 (0.625)	22.714 (2.853-180.866)	0.000
Maternal medication				
No	61 (76.250)	152 (95.000)		

Yes	19 (23.750)	8 (5.000)	5.918 (2.460-14.237)	0.000
Maternal frequent use of electronics				
No	45 (56.250)	87 (54.375)		
Yes	35 (43.750)	73 (45.625)	0.927 (0.540-1.591)	0.783
Maternal cold and fever				
No	51 (63.750)	141 (88.125)		
Yes	29 (36.250)	19 (11.875)	4.220 (2.178-8.174)	0.000
Maternal intrauterine virus infection				
No	73 (91.250)	159 (99.375)		
Yes	7 (8.750)	1 (0.625)	15.247 (1.842-126.203)	0.002
Maternal severe mental stress				
No	68 (85.000)	156 (97.500)		
Yes	12 (15.000)	4 (2.500)	6.882 (2.143-22.106)	0.000
Maternal severe morning sickness				
No	46 (57.500)	103 (64.375)		
Yes	34 (42.500)	57 (35.625)	1.429 (0.821-2.487)	0.206
Maternal nutritional deficiency				
No	69 (86.250)	155 (96.875)		
Yes	11 (13.750)	5 (3.125)	4.942 (1.654-14.766)	0.002
Taking folic acid in first trimester				
No	42 (52.500)	40 (25.000)		
Yes	38 (47.500)	120 (75.000)	0.302 (0.171-0.531)	0.000
Maternal intake more meat				
No	34 (42.500)	44 (27.500)		
Yes	46 (57.500)	116 (72.500)	0.513 (0.292-0.901)	0.019
Maternal intake more eggs				
No	48 (60.000)	57 (35.625)		
Yes	32 (40.000)	103 (64.375)	0.369 (0.212-0.641)	0.000
Maternal intake more beans				
No	54 (67.500)	108 (67.500)		
Yes	26 (32.500)	52 (32.500)	1.000 (0.564-1.774)	1.000
Maternal intake more milk				
No	58 (72.500)	104 (65.000)		
Yes	22 (27.500)	56 (35.000)	0.704 (0.391-1.269)	0.242
Maternal intake more vegetables				
No	18 (22.500)	38 (23.750)		
Yes	62 (77.500)	122 (76.250)	1.073 (0.566-2.032)	0.829
Maternal intake more fruits				
No	31 (38.750)	45 (28.125)		
Yes	49 (61.250)	115 (71.875)	0.619 (0.351-1.090)	0.095

Note: OR: odds ratio. CI: confidence interval. [a]*P* value is from Pearson's chi-square (χ^2) tests.

factors were assessed by stratified analyses. Odds radio (OR)>1 describes a risk factor in the case group compared with the control group, and OR<1 describes a protective factor. A multivariate conditional stepwise logistic regression model construction was completed using the significant variables.

Results

Characteristics of cases and controls

Among the 80 CHD patients, 45 (56.250%) were boys and 35 (43.750%) were girls. Seventy-seven patients were Han nationality,

Table 3. Results of logistic regression analysis

Variable	*B*	*Wald*	*P*	OR (95% CI)
Rural registered permanent residence	1.039	5.238	0.022	2.827 (1.161-6.883)
Father is a farmer	1.668	10.617	0.001	5.303 (1.944-14.465)
Low income parents (<2000 CNY/M)	0.904	3.878	0.049	2.469 (1.004-6.070)
Father smoked in first trimester	1.463	10.753	0.001	4.321 (1.802-10.362)
Maternal cold and fever	1.858	14.669	0.000	6.411 (2.477-16.592)
Maternal intake more eggs	-0.912	5.219	0.022	0.402 (0.184-0.878)

Note: Wald test for entire group, *P* value for specific categories refers to significant difference present among the categories (constant: -3.512).

and 3 patients were minorities. The age range was 0.25 to 11 years, and the average age was 5.8 years. Among the 160 controls, only eleven children were minorities.

There was no significant difference between the two groups regarding ethnicity (χ^2 = 0.948, P = 0.330) and guardian (χ^2 = -1.387, P = 0.166). There was statistical difference between the two groups regarding registered permanent residence (rural or urban) (χ^2 = 27.092, P = 0.000), regular physical examination (χ^2 = 4.485, P = 0.034) (**Table 1**).

The types of CHD were various

Among the 80 CHD patients, six (7.500%) cases had three types of CHD, and 23 (28.750%) cases had two types of CHD. Forty-seven (58.750%) had ventricular septal defect (VSD), 24 (30.000%) cases had atrial septal defect (ASD), 15 (18.750%) cases had patent ductus arteriosus (PDA), 8 (10.000%) cases had patent foramen ovale (PFO), 7 (8.750%) cases had pulmonary stenosis (PS), 2 (2.500%) cases had double outlet of right ventricle (DORV), 1 (1.250%) case had tetralogy of Fallot (TOF), 1 (1.250%) cases had transposition of the great arteries (TGA), and 11 (13.750%) cases had other types of CHD.

Relative factors univariate analysis

Twenty-three factors were statistically significant after univariate analysis of all 43 research factors (**Tables 1** and **2**), including: registered permanent residence (rural or urban) (χ^2 = 27.092, P = 0.000), regular physical examination (χ^2 = 4.485, P = 0.034), paternal age (t = 2.197, P = 0.030), father's occupation (farmer) (χ^2 = -2.208, P = 0.043), father's education level (χ^2 = -5.595, P = 0.000), father's chronic disease (χ^2 = 15.211, P = 0.000), mother's occupation (χ^2 = -2.385, P = 0.017), mother's education level (χ^2 = -5.428, P = 0.000), parents' average monthly income (CNY) (χ^2 = 11.507, P = 0.001), paternal wine-drinking half a year before pregnancy (χ^2 = 5.692, P = 0.017), father's smoking history (χ^2 = 12.404, P = 0.000), father smoked half a year before pregnancy (χ^2 = 12.570, P = 0.000), mother's passive smoking during pregnancy (χ^2 = 8.268, P = 0.004), house near road (<50 meters) (χ^2 = 4.371, P = 0.037), maternal chemical substances exposure (χ^2 = none, Fisher' exact test, P = 0.000), maternal medication (χ^2 = 18.779, P = 0.000), maternal cold and fever (χ^2 = 19.805, P = 0.000), maternal intrauterine virus infection (χ^2 = none, Fisher' exact test, P = 0.002), maternal severe mental stress (χ^2 = 13.393, P = 0.000), maternal nutritional deficiency (χ^2 = 9.696, P = 0.002), maternal folic acid intake in the first trimester (χ^2 = 17.951, P = 0.000), maternal intake more meat (χ^2 = 5.470, P = 0.019), and maternal intake more eggs (χ^2 = 12.876, P = 0.000). There was no significant difference between the two groups regarding other variables.

Logistic regression analysis

The association between CHD and potential relative factors was explored through multivariable stepwise logistic regression. The results of the logistic regression model are presented in **Table 3**. The final model included the variables of rural registered permanent residence, father is a farmer, low income parents (<2000 CNY/M), father smoked half a year before pregnancy, maternal cold and fever, and maternal intake more eggs. Pregnant women eat more eggs might be a protective factor for CHD, and other 5 variables might be risk factors for CHD.

Discussion

CHD is common and costly, and is an important cause of infant disability and mortality. There are about 1.5 million CHD children in China. It brings heavy mental and economic burden to patients, families and whole society. About 30% of CHD might be preventable through maternal behavior and environmental modifications [8]. CHD is associated with genetic and environmental factors, and the precise pathogenic mechanisms remain unclear.

The present research showed that the proportion of mother suffered cold and fever during pregnancy was significantly increased in patients with CHD compared with those healthy controls (OR = 6.411, 95% CI 2.477-16.592). This finding suggests that maternal cold and fever is probably a risk factor for CHD. With limited research, the precise mechanism of maternal febrile illnesses during pregnancy resulting in offspring with CHD is still unclear.

Many studies suggested that maternal febrile illnesses during pregnancy might be associated with an increased risk for certain heart defects. The febrile illness was often characterized as respiratory infections (flu-associated fever or influenza, common cold). Botto *et al.* [9] reported that febrile illness around the time of conception or in early pregnancy was positively associated with the occurrence of CHD in the offspring (OR = 1.8), and there was association between influenza like illness and CHD (OR = 2.1). Bao *et al.* [10] reported a 3.7-fold increase in the risk of offspring with CHD in maternal cold and fever compared with normal pregnant woman.

Most respiratory tract infections are caused by various viruses, including: respiratory syncytial virus, influenza virus, parainfluenza virus, adenovirus, rhinovirus, Coxsackie virus, coronavirus, and Bocavirus, *et al.*. Distinguish influenza and intrauterine virus infection sometimes difficult. Wang *et al.* [11] reported that 24.1% CHD cases' mother might exist intrauterine infection. Begic *et al.* [12] observed that CHD is associated with maternal rubella virus, toxoplasma gondii, cytomegalovirus, and parvovirus infections during early pregnancy. As we all known, maternal rubella infection in the first trimester is one of the important reasons of birth defects. It could result in offspring with various types of CHD [13].

According to previous researches, the cardiovascular embryogenesis occurs early in the first trimester of pregnancy. Maternal virus infection during this period might be result in a heterogeneous group of malformations affecting the cardiac chambers, vascular connections, valves, and other cardiac structures. At last, increase in the risk for the occurrence of CHD. One important mechanism is that CHD might be related to altered apoptosis. Apoptosis is known to be involved in cardiac morphogenesis in the development of the cardiac outflow tract. Watanabe *et al.* [14] found that the apoptosis is one mechanism by which the outflow tract myocardium remodels to form the proper connection between the appropriate arterial trunks and the ventricular chambers. Apoptosis might be altered by both fever and infection. Infection by most viruses triggers apoptosis of the infected cell, and some viruses seem to use apoptosis as a mechanism of cell killing and virus spread. Apoptosis might be changed if pregnant woman suffered cold and fever in the process of embryonic heart development, resulting in the occurrence of CHD.

Another important possibility is that women often take medications for febrile illnesses. Some drugs are suspected of causing congenital defects, including CHD. Physicians and mothers should realize the adverse effects of medications in order to make correct decisions about medication use during pregnancy. In order to prevent CHD, various infections and harmful medication taking should be avoided during pregnancy, especially in the early pregnancy.

Our research revealed that association between the father's cigarette smoking half a year before pregnancy and CHD (OR = 4.321, 95% CI 1.802-10.362). This finding suggests that father smoked half a year before pregnancy is probably a risk factor for CHD. Our result is similar to that from Wasserman *et al.* [15]. They reported that elevated risk (OR = 1.9, 95% CI 1.2-3.1) was observed for conotruncal heart defects when both parents smoked compared to neither parent smoking.

Cigarette smoking is a well-known risk factor for birth defects. Birth defects are positively associated with maternal smoking or passive smoking (secondhand tobacco smoke) [16]. Cigarette smoke contains more than 4000 chemical substances including various toxic heavy metals, carcinogens, and other chemical substances which are harmful to human reproduction and development [17].

Cigarette smoking can be teratogenic and affect a number of developing structures. The relationship between cigarette smoking and CHD might be explained from the following aspects [18]: 1) Cigarette smoking might affect folic acid uptake, which might be associated with the occurrence of CHD and neural tube defects (NTD); 2) Nicotine could affect sperm activity greatly and lead to chromosome aberration, which might affect the growth and development of the fetus and result in the occurrence of cardiac malformations; and 3) Effects of tobacco exposure on placenta are critical and still not adequately clarified. Maternal smoking or passive smoking (for example from father's cigarette smoking) alters the blood flow to the placenta. The composition of the cigarette (such as nicotine and carbon monoxide) could rapidly cross the placenta and increase the adrenaline secretion to cause blood vessel contraction, which influence the placental blood supply, hinder placental oxygen transfer, and affect nutrient transportation. All the above might result in the abnormal cardiovascular development.

Cigarette smoking is a preventable cause of CHD. Stopping smoking and creating a suitable living environment might effectively reduce the incidence of CHD and other diseases. It is important and urgent to be included in public health educational materials to encourage more women and their husbands to quit before or during pregnancy.

This investigation showed that low socio-economic status (SES), including rural residence, father is a farmer, and low income parents (<2000 CNY/M) might be risk factors for CHD (OR = 2.827, 5.303 and 2.469, respectively). This result is similar to that from Carmichael *et al.* [19]. They found that low SES was associated with increased risk of TGA.

Low SES such as poverty, unemployment, poor living conditions, and poor education may be related to a variety of stressful life events and health problems. Liu *et al.* [7] and Carmichael *et al.* [20] both reported that women might be at increased risk of delivering CHD infants when they experienced stressful life events during the periconceptional period or early pregnancy.

One reasonable explanation is that medical resources in China are limited. According to recent studies on health care resources in China, 80% of health resources were concentrated in big cities [21]. Rural residents and low income parents might result in carelessness of health care around the time of conception or early gestation and pay no attention to prenatal nutrition. The lower SES is, the fewer medical resources will be available.

Another possible explanation is that rural parents might often expose to chemical substances, especially pesticide, which might increase the incidence of CHD. Lai *et al.* [22] reported 2356 cases of CHD, among them 1639 (69.57%) cases were come from the rural and remote areas, only 717 (30.43%) cases were from cities, and 135 (5.72%) cases had a history of long-term exposure to organophosphorus pesticides.

From here we see that improve the living conditions of low SES parents, including promoting the rural economic development, improving the medical care and treatment, improving the economic situation of rural residents, improving their living conditions and environment, avoiding toxic substances contact, spreading knowledge of CHD prevention, and caring for the health and education of rural families are important in reducing the occurrence of CHD.

We found that pregnant women eat more eggs is associated with a reduced occurrence of CHD (OR = 0.402, 95% CI 0.184-0.878). This finding reveals that maternal intake more eggs might be a protective factor for CHD.

Eggs are rich in various nourishments. The protein contains abundant nutritional elements including essential amino acids for human growth and development. Yolk contains a lot of trace elements such as calcium, phosphorus, iron, *et al.* It also provides a source of vitamin A, vitamin D and riboflavin.

There are very few known protective factors for CHD, and better diet might help reduce the risk for CHD. Botto *et al.* [23] confirmed that better and improved maternal diet quality was associated with a reduction in certain CHD (septal and conotruncal heart defects). Dietary approaches have been applied to the risk of birth defect recently. Some studies suggest that periconceptional multivitamin use could decrease the risk for CHD. Botto *et al.* [24] found that nearly one in four major cardiac defects could be prevented by multivitamin supplements before conception and during early pregnancy.

As reported, vitamin A is a key micronutrient required during crucial stages of embryonic development and might be crucial for heart morphogenesis during pregnancy. Vitamin A deficiency could result in embryonic heart malformation [25]. As the active form of vitamin A, retinoic acid is an endogenous signal molecule and plays a specific role in the process of heart development because of activating many gene regulations. Either deficiency or excess of retinoic acid could induce certain CHD.

In order to reduce the occurrence of CHD, mother should pay attention to the balance of nutrition, increase the egg ingestion, and take vitamin supplements appropriately during pregnancy, especially in the first trimester.

Limitations of the study existed because the sample size was small, some important risk factors for CHD might be missed due to the multifactorial causes of CHD, and recall bias due to information was collected by questionnaire. *et al.* Because of above limitations, the findings need to be interpreted cautiously.

CHD is associated with many factors, and the precise pathogenic mechanisms are still indistinct. Our research suggests that improving healthcare of rural residence and low income families, preventing maternal cold and fever, limiting father smoking before pregnancy and increasing the ingestion of eggs during pregnancy might lower the occurrence of CHD. This study might contribute to the pathogenesis and local prevention of CHD, and some cases of CHD might potentially preventable through eliminating relevant risk factors. Parents and clinicians should be aware of the possible existence of potential risk factors so that careful measures can be taken to reduce the occurrence of CHD.

However, changes of Chinese natural and social environment, improvement of industrialization, and changes of lifestyle, all which imposes serious threatens on human living and affect growth and development of the offspring. It might increase the risk of CHD and other disease in China. It's important to provide good obstetrical care and health counseling for women. Women should avoid infection, carefully use medications, and comply with doctors' guidance and prescriptions. We consider that further longer follow-up, high-quality, multiple-center, and large-sample researches on the potential risk factors are important to prevent CHD.

Disclosure of conflict of interest

None.

Authors' contribution

ZYL and SYY designed the study, collected the participants' information, performed statistical analysis, and drafted the manuscript. All authors read and approved the final manuscript. These authors contributed equally to this work and should be considered co-first authors.

Abbreviations

ASD, atrial septal defect; CHD, congenital heart disease; CI, confidence interval; CMV, cytomegalovirus; CNY, Chinese Yuan; CTA, computed tomographic angiography; DORV, double outlet of right ventricle; MRI, magnetic resonance imaging; NTD, neural tube defects; OR, odds ratio; PDA, patent ductus arteriosus; PFO, patent foramen ovale; PS, pulmonary stenosis; SES, socio-economic status; TGA, transposition of the great arteries; TOF, tetralogy of Fallot; VSD, ventricular septal defect.

Address correspondence to: Shuyuan Yan, Child Health Care Center, Changsha Hospital for Maternal & Child Health Care, 416 Chengnan East Road, Changsha 410007, Hunan, People's Republic of China. Tel: 86-731-84196669; Fax: 86-731-84134539; E-mail: yanyisheng2016@163.com

References

[1] Cuneo BF, Curran LF, Davis N, Elrad H. Trends in prenatal diagnosis of critical cardiac defects in an integrated obstetric and pediatric cardiac imaging center. J Perinatol 2009; 24: 674-678.
[2] Parcharidis G. Adult Congenital Heart Disease in Greece: Need for a Step Forward. Hellenic J Caediol 2011; 52: 193-194.

[3] Ministry of Health of the People's Republic of China. China Health Statistics Yearbook 2009 [in Chinese]. Beijing: Peking Union Medical College Press 2009; 270-273.
[4] Pierpont ME, Basson CT, Benson DW Jr, Gelb BD, Giglia TM, Goldmuntz E, McGee G, Sable CA, Srivastava D, Webb CL. Genetic basis for congenital heart defects: current knowledge: a scientific statement from the American Heart Association Congenital Cardiac Defects Committee, Council on Cardiovascular Disease in the Young. Circulation 2007; 115: 3015-3038.
[5] Botto LD, Correa A. Decreasing the burden of congenital heart anomalies: an epidemiologic evaluation of risk factors and survival. Prog Pediatr Cardiol 2003; 18: 111-121.
[6] Jenkins KJ, Correa A, Feinstein JA, Botto L, Britt AE, Daniels SR, Elixson M, Warnes CA, Webb CL. Noninherited risk factors and congenital cardiovascular defects: current knowledge: a scientific statement from the American Heart Association Council on cardiovascular disease in the Young: endorsed by the American Academy of Pediatrics. Circulation 2007; 115: 2995-3014.
[7] Liu S, Liu J, Tang J, Ji J, Chen J, Liu C. Environmental risk factors for congenital heart disease in the Shandong Peninsula, China: a hospital-based case-control study. J Epidemiol 2009; 19: 122-130.
[8] Wilson PD, Loffredo CA, Correa-Villasenor A, Ferencz C. Attributable fraction for cardiac malformations. Am J Epidemiol 1998; 148: 414-423.
[9] Botto LD, Lynberg MC, Erickson JD. Congenital heart defects, maternal febrile illness, and multivitamin use: a population-based study. Epidemiology 2001; 12: 485-490.
[10] Bao YF, Kong XQ, Qian W, Wu XQ, Yang ZY, Wang RX, Qian DJ. Analysis on the risk factors of congenital heart disease in preschool children in Wuxi No.1 [in Chinese]. Journal of Chinese Microcirculation 2007; 11: 271-274.
[11] Wang XM, Zhang G, Liu F, Han M, Xu D, Zang Y. Prevalence of human parvovirus B19 DNA in cardiac tissues of patients with congenital heart diseases indicated by nested PCR and in situ hybridization. J Clin Virol 2004; 31: 20-24.
[12] Begić H, Tahirović HF, Dinarević S, Ferković V, Pranjić N. Risk factors for the development of congenital heart defects in children born in the Tuzla Canton. Med Arh 2002; 56: 73-77.
[13] Campbell M. Place of maternal rubella in the aetiology of congenital heart disease. BMJ 1961; 1: 691-696.
[14] Watanabe M, Choudhry A, Berlan M, Singal A, Siwik E, Mohr S, Fisher SA. Developmental remodeling and shortening of the cardiac outflow tract involves myocyte programmed cell death. Development 1998; 125: 3809-3820.
[15] Wasserman CR, Shaw GM, O'Malley CD, Tolarova MM, Lammer EJ. Parental cigarette smoking and risk for congenital anomalies of the heart, neural tube, or limb. Teratology 1996; 53: 261-267.
[16] Hackshaw A, Rodeck C, Boniface S. Maternal smoking in pregnancy and birth defects: a systematic review based on 173,687 malformed cases and 11.7 million controls. Hum Reprod Update 2010; 17: 589-604.
[17] Rogers JM. Tobacco and pregnancy. Reprod Toxicol 2009; 28: 152-160.
[18] Correa A, Levis DM, Tinker SC, Cragan JD. Maternal cigarette smoking and congenital heart defects. J Pediatr 2015; 166: 801-804.
[19] Carmichael SL, Nelson V, Shaw GM, Wasserman CR, Croen LA. Socio-economic status and risk of conotruncal heart defects and orofacial clefts. Paediatr Perinat Epidemiol 2003; 17: 264-271.
[20] Carmichael SL, Shaw GM. Maternal life event stress and congenital anomalies. Epidemiology 2000; 11: 30-35.
[21] Lu HX. Rational thoughts on imbalanced allocation of China's rural basic health resources [in Chinese]. Chin Health Econ 2009; 28: 38-42.
[22] Lai XJ, Li SS, Wei LZ, Liang Y, Song ZQ, Liao MS, Zhou XW, Zhang X, LiuBY, Wen ZL. Epidemiology analysis of correlative factor proportion of 2356 cases of congenital heart disease [in Chinese]. Medicial Journal of National Defending Forces in Southwest China 2002; 12: 298-301.
[23] Botto LD, Krikov S, Carmichael SL, Munger RG, Shaw GM, Feldkamp ML. Lower rate of selected congenital heart defects with better maternal diet quality: a population-based study. Arch Dis Child Fetal Neonatal Ed 2016; 101: 43-49.
[24] Botto LD, Mulinare J, Erickson JD. Occurence of congenitalheart defects in relation to maternal multivitamin use. Am J Epidemiol 2000; 151: 878-884.
[25] Feng Y, Yu YM, Yin MZ, Hong L, Cai W. Ectopic expression of retinoic acid receptors and change of myocardial structure in the offspring heart with vitamin A deficiency. J Nutr Sci Vitaminol (Tokyo) 2012; 58: 309-318.