Original Article

Association between Maternal Drug Use and Cytochrome P450 Genetic Polymorphisms and the Risk of Congenital Heart Defects in Offspring*

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Abstract

Objective This study aimed to assess the associations between maternal drug use, cytochrome P450 (*CYP450*) genetic polymorphisms, and their interactions with the risk of congenital heart defects (CHDs) in offspring.

Methods A case-control study involving 569 mothers of CHD cases and 652 controls was conducted from November 2017 to January 2020.

Results After adjusting for potential confounding factors, the results show that mothers who used ovulatory drugs (adjusted odds ratio [a*OR*] = 2.12; 95% confidence interval [*CI*]: 1.08–4.16), antidepressants (a*OR* = 2.56; 95% *CI*: 1.36–4.82), antiabortifacients (a*OR* = 1.55; 95% *CI*: 1.00–2.40), or traditional Chinese drugs (a*OR* = 1.97; 95% *CI*: 1.26–3.09) during pregnancy were at a significantly higher risk of CHDs in offspring. Maternal *CYP450* genetic polymorphisms at rs1065852 (A/T *vs*. A/A: *OR* = 1.53, 95% *CI*: 1.10–2.14; T/T *vs.* A/A: *OR* = 1.57, 95% *CI*: 1.07–2.31) and rs16947 (G/G *vs.* C/C: *OR* = 3.41, 95% *CI*: 1.82–6.39) were also significantly associated with the risk of CHDs in offspring. Additionally, significant interactions were observed between the *CYP450* genetic variants and drug use on the development of CHDs.

Conclusions In those of Chinese descent, ovulatory drugs, antidepressants, antiabortifacients, and traditional Chinese medicines may be associated with the risk of CHDs in offspring. Maternal *CYP450* genes may regulate the effects of maternal drug exposure on fetal heart development.

Key words: Congenital heart defect; Maternal drug use; Cytochrome P450 genes; Case-control study

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INTRODUCTION

Congenital heart defects (CHDs) are the most common type of congenital malformation, representing a major global health problem. The global prevalence of CHDs is approximately 8.22 per 1,000 live births[1]. The highest prevalence has been reported in Asia (9.3‰), followed by Europe (8.2‰), and the lowest occurs in Africa (1.9‰)[2]. Presently, the prevalence of CHD in China is 8.98‰[3]. As CHDs are the main cause of non-infectious death for infants aged < 5-year-old, all cardiac defects were responsible for 46% of infant deaths caused by congenital malformations[4]. Additionally, children with a CHD are at significantly higher risk of cardiovascular diseases compared with healthy controls, and they achieve significantly lower height, weight, and linguistic competence in later life[5]. Thus, further etiological studies are needed for more targeted planning and decision-making in controlling and managing these diseases.

It is generally accepted that most CHD cases result from a combination of environmental and genetic factors, indicating that the complex interactions between environmental teratogens and genetic factors should be the direction of etiological research on CHDs. Many environmental factors have been identified as risk factors of CHDs[6-8], but the association between maternal drug use and the risk of CHDs remains controversial. It is difficult for pregnant women to completely avoid exposure to some drugs such as oral contraceptives, ovulatory drugs, antibiotics, antidepressants, and antiabortifacients, indicating that more attention should be paid to the potential effects of these drugs on embryonic heart development. Particularly in China, few studies have considered the relationship between maternal drug intake and CHDs.

The effects of environmental factors may be modified by the genes that are responsible for activating and detoxifying exogenous toxins, which leads to increased resistance or sensitivity of the embryonic heart to these environmental factors[9,10]. It is well known that phase I metabolic enzymes are involved in the metabolic transformation of exogenous compounds, and their activation effects are directly related to the sensitivity of the body to environmental toxins. Cytochrome P450 (CYP450) enzymes are important phase I metabolic enzymes with a strong catalytic effect that promotes the metabolism and activation of various drugs, lipids, carcinogens, and teratogens; thus, impacting their possible carcinogenic and teratogenic effects[11,12]. Single nucleotide polymorphisms (SNPs) of *CYP450* genes can cause variability in CYP450 activity[13]. Previous studies have considered the role of *CYP450* genes in the metabolism of drugs and the relationship between *CYP450* SNPs and cancer susceptibility in adults[14,15]. However, the relationship between maternal *CYP450* SNPs and CHDs in offspring has not been explored. Thus, we hypothesized that genetic variations in *CYP450* genes might increase the susceptibility to drug intake-related fetal diseases, including CHDs.

We conducted this study with the following objectives: (i) to examine whether maternal drug exposure was significantly associated with risk of CHDs in offspring; (ii) to assess the association between genetic variations in maternal *CYP450* genes and the risk of CHDs in offspring; and (iii) to analyze the interactions between maternal drug exposure and *CYP450* genetic variants on CHDs.

METHODS

Recruitment of the Study Participants

A hospital-based case-control study was performed from November 2017 to January 2020 to assess the association between maternal drug use, *CYP450* gene polymorphisms, and their interactions with the risk of CHDs in offspring. This study was approved by the Ethics Committee of the Xiangya School of Public Health of Central South University, and written informed consent was obtained from all mothers. This study was registered at the Chinese Clinical Trial Registry Center (registration number: ChiCTR1800016635). The research procedure and design have been described in our previous studies[16,17]. The study participants were recruited from two Hunan Children's Hospital clinics. The case group was recruited from the Department of Cardiothoracic Surgery, which provides diagnosis, treatment, surgery, and management for CHDs, and the control group was recruited from the Department of Child Healthcare after health counseling or a medical examination. The controls were selected from the same hospital during the same study period as the cases.

Inclusion and Exclusion Criteria

The outcome of interest was CHDs. Children with CHD and their mothers were identified as the case group. Children without any congenital malformations after a medical examination and their

mothers were identified as the control group. The CHD cases included the following subtypes: atrial septal defect (ASD), ventricular septal defect (VSD), atrioventricular septal defect (AVSD), patent ductus arteriosus (PDA), aortopulmonary septal defect (APSD), tetralogy of Fallot (TOF), and complete transposition of the great arteries (CTOGA). Non-syndromic CHD was of interest, but patients with other organ malformations or known abnormalities were excluded. All CHD patients were diagnosed by ultrasonography. Similarly, the control children were confirmed to have no malformations by ultrasonography. The exposures of interest were maternal drug use and genetic variants in the *CYP450* genes. To minimize recall bias of exposure by mothers, all cases and controls were recruited when the children were < 1-year-old. Additionally, this study was aimed at mothers of Han Chinese descent with singleton pregnancies. Eligible mothers completed a questionnaire and provided informed consent and blood samples. We excluded mothers who achieved pregnancy by assisted reproductive technology, including *in vitro* fertilization or intracytoplasmic sperm injection.

Information Collection

A standardized questionnaire was used to collect the corresponding maternal drug use information by specially trained investigators. As the critical stage of fetal heart development is the early stage of pregnancy, we investigated the maternal drug use information from 3 months before pregnancy to the first trimester of pregnancy. In our study, the drugs of interest were oral contraceptives, ovulatory drugs (e.g., clomiphene, luteinizing hormone-releasing hormone, and bromocriptine), macrolide antibiotics (e.g., erythromycin, azithromycin, clarithromycin, roxithromycin, dierythromycin, and fluoroerythromycin), antidepressants (e.g., clomipramine, citalopram, sympathomimetic decongestants, paroxetine, and phenylpropanolamine), traditional Chinese medicines, and antiabortifacients (e.g., progesterone preparations, chorionic gonadotropin, human blood immunoglobulin, and indomethacin). Additionally, we also collected data regarding the mothers' sociodemographic characteristics (i.e., child-bearing age, education level, annual family income in the past year, residential area); obstetric history (i.e., adverse pregnancy outcomes and pregnancy-related complications); family history (i.e., consanguineous marriage and congenital malformations); personal medical history (i.e., congenital malformations, and cold or fever history in the 3 months before this pregnancy); personal lifestyle and habits (i.e., history of active and passive smoking in the 3 months before this pregnancy, and drinking history in the 3 months before this pregnancy); exposure history to environmentally hazardous substances (i.e., radioactive substances, house decorations, and harmful chemicals); and folate supplementation. After completing the questionnaire, we consulted the perinatal health care handbook (PHCH) and maternal medical records to further confirm the corresponding information. Notably, not all the information we needed was recorded by the PHCH. Therefore, we used face-to-face interviews and consulted the PHCH to ensure that the relevant information was accurately collected. When the information provided by these two methods was inconsistent, we relied on the information provided by the PHCH, considering that there may be recall bias during a face-to-face interview. These measures may have helped to reduce recall bias.

Genotyping

After completing the questionnaire, all mothers were required to provide blood samples after consent. Three to five milliliters of peripheral venous blood were collected by a qualified phlebotomist from all participants using a 21-gage needle. Plasma and blood cells were separated by centrifugation and stored at −80 °C for later laboratory testing. The genomic DNA was extracted from blood cells using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol and dissolved in sterile TBE buffer.

We chose four SNPs of the *CYP450* genes (*CYP1A1* at rs1048943 and rs4646903, *CYP2D6* at rs1065852 and rs16947), which have been associated with adverse pregnancy outcomes and metabolism of drugs of interest[18,19], as candidate loci. Because the related primers were unavailable, we used rs4646421 instead of rs4646903 (r^2 = 1.000), rs5751210 instead of rs1065852 (r^2 = 0.900), and rs4147641 instead of rs16947 (r^2 = 0.965) according to linkage disequilibrium analysis. All SNPs (rs1048943, rs4646421, rs5751210, and rs4147641) were genotyped using the matrix-assisted laser desorption and ionization time-of-flight mass spectrometry Mass Array system (Agena iPLEXassay, San Diego, CA, USA). Different cycling conditions were used to optimally amplify the target sequences. The details of polymerase chain reaction primers are described in Supplementary Table S1 (available in www.besjournal.com). The laboratory technician,

who performed the genotyping, retyped and double-checked each sample, and recorded the genotype data, was blinded to the status of the control and case groups. The genotyping error rate was < 5%.

Statistical Analysis

Unordered categorical variables are described using percentages and compared between two groups using Pearson's chi-square test or Fisher's exact test, as appropriate. The Wilcoxon rank-sum test was used for ordinal categorical variables. Hardy-Weinberg equilibrium (HWE) was tested in the control group (significance level at $P < 0.05$). Odds ratios (*OR*s) and their 95% confidence intervals (*CI*s) were used to assess the level of the associations between maternal drug exposure and CYP450 genetic variants with the risk of CHDs in offspring. The crude *OR*s were calculated by univariate logistic regression. Adjusted *OR*s (a*OR*s) were calculated by multivariate logistic regression. Moreover, all SNPs were estimated under the three genetic models (dominant, recessive, and additive).

Additionally, we used logistic regression analysis and controlled for other influencing factors to examine the effects of the environment-gene interaction of the maternal drug exposure and the *CYP450* genes for the risk of CHDs in offspring. We determined the patterns and implications of the environment-gene interactions according to a method introduced by Wallace[20]. Interactions were determined using interaction coefficients (γ). The γ values were calculated using the regression coefficient (β) from logistic regression analysis (i.e., $\gamma_1 = \beta_{e*g}/\beta_e$ and $\gamma_2 = \beta_{e*g}/\beta_g$ for *the* gene-environment interaction). γ values > 1 indicated a positive interaction; and γ values < 1 indicated a negative interaction; when the γ value = 1, there was no interaction. To minimize type I error, the false discovery rate *P*-value (FDR_*P*), which was adjusted for multiple testing, was estimated to obtain a more precise *P*-value[16]. All tests were performed for a two-sided *P*-value not exceeding 0.05. All analyses were performed using SAS 9.1 software (SAS Institute, Cary, NC, USA).

RESULTS

Baseline Characteristics

A total of 569 eligible mothers were included in the case group, and 652 were included in the control group from November 2017 to January 2020. Among the 569 CHD cases, 95 (16.7%) were diagnosed with ASD, 353 (62.0%) with VSD, 60 (10.5%) with AVSD, 170 (29.9%) with PDA, 8 (1.4%) with APSD, 32 (5.6%) with TOF, and 2 (0.4%) with CTOGA. Some cases were diagnosed with multiple CHD subtypes. Therefore, the sum of the various subtypes was not equal to 569. Maternal baseline characteristics in the case and control groups are summarized in Table 1. Significant differences in education level, annual family income, residential area, history of adverse pregnancy outcomes, pregnancy-related complications, consanguineous marriage, and congenital malformation history, drinking history before pregnancy, active or passive smoking history before pregnancy, cold and fever history before this pregnancy, and folate supplementation were observed between the two groups (all $P < 0.05$). These factors were adjusted when assessing the associations between maternal drug use, the *CYP450* gene polymorphisms, and their interactions with the risk of CHDs in offspring.

Maternal Drug Use and the Risk of CHDs in Offspring

The associations between maternal drug use and the risk of CHDs in offspring are summarized in Table 2. After adjusting for other influencing factors, the results show that mothers who used ovulatory drugs (a*OR* = 2.12, 95% *CI*: 1.08–4.16), antidepressants (a*OR* = 2.56, 95% *CI*: 1.36–4.82), antiabortifacients (a*OR* = 1.55, 95% *CI*: 1.00–2.40), or traditional Chinese medicines (a*OR* = 1.97, 95% *CI*: 1.26–3.09) during this pregnancy were at a significantly higher risk of CHDs in offspring compared with the reference group. However, we did not observe a significant association between oral contraceptive or macrolide antibiotic use and the risk of CHDs.

Maternal CYP450 Genetic Polymorphisms and the Risk of CHDs in Offspring

The genotype frequencies for each SNP of the maternal *CYP450* genes and *P*-values for the HWE test in the control group are summarized in Table 3. The genotype distributions in the control group were within HWE (all $P > 0.05$). The associations between maternal *CYP450* genetic variants and the risk of CHDs in offspring based on logistic regression analyses are summarized in Table 4. After adjusting for influencing factors, the results suggested that maternal *CYP450* genetic polymorphisms at rs1065852 and rs16947 were significantly associated with the risk of CHDs in offspring. Mothers with the A/T (a*OR* = 1.53, 95% *CI*: 1.10–2.14) and T/T (a*OR* = 1.57, 95% *CI*:

1.07–2.31) genotype for rs1065852 compared with those with the A/A genotype experienced a significantly increased risk of CHD in offspring. The dominant (a*OR* = 1.55, 95% *CI*: 1.13–2.12) and additive models (a*OR* = 1.25; 95% *CI*: 1.03–1.52) were significantly associated with an increased risk of CHDs in offspring.

Mothers with the G/G genotype for rs16947 were at a significantly higher risk of CHDs in offspring than those with the C/C genotype (a*OR* = 3.41, 95% *CI*: 1.82–6.39); and the dominant (a*OR* = 1.47, 95% *CI*: 1.10–1.95), recessive (a*OR* = 3.23, 95% *CI*: 1.73–6.02), and additive models (a*OR* = 1.51, 95% *CI*: 1.20–1.90) were significantly associated with an increased risk of CHDs in offspring.

Gene-environment Interaction Associated with the Risk of CHDs in Offspring

The gene-environment interactions between the maternal *CYP450* genetic variants and drug use for the risk of CHDs in offspring are summarized in Table 5. In the interaction analyses, we only considered those drugs and SNPs that were significantly associated with the risk of CHDs in the above analyses. Significant interactive effects were observed between the maternal genetic variants at rs1065852 and drug use for the development of CHDs in offspring. Overall, when mothers had a risk genotype (A/T or T/T) at rs1065852, the risk of CHDs in offspring increased significantly if they used an

Table 1. Maternal baseline characteristics in the case and control groups

Baseline characteristics	Control group, *n* (%) (*n* = 652)	Case group, *n* (%) (*n* = 569)	*P* values
Child-bearing age (years) (≥ 35)	92 (14.1)	75 (13.2%)	0.637
Education level			
Less than primary or primary	9 (1.4)	85 (14.9)	< 0.001
Junior high school	127 (19.5)	231 (40.6)	
Senior middle school	210 (32.2)	162 (28.5)	
College or above	306 (46.9)	91 (16.0)	
Annual income in the past 1 year (RMB)			
< 50,000	187 (28.7)	463 (81.4)	< 0.001
50,000–100,000	275 (42.2)	77 (13.5)	
100,001–150,000	59 (9.0)	11 (1.9)	
> 150,000	131 (20.1)	18 (3.2)	
Residence areas (rural areas)	349 (53.5)	428 (75.2)	< 0.001
History of adverse pregnancy outcomes (yes)	73 (11.2)	96 (16.9)	0.004
History of pregnancy related complications (yes)	37 (5.7)	95 (16.7)	< 0.001
Family consanguineous marriages (yes)	2 (0.3)	24 (4.2)	< 0.001
Family congenital malformations history (yes)	4 (0.6)	40 (7.0)	< 0.001
Individual congenital malformations (yes)	2 (0.3)	5 (0.9)	0.261*
Alcohol use before pregnancy (yes)	45 (6.9)	78 (13.7)	< 0.001
Active smoking before pregnancy (yes)	12 (1.8)	46 (8.1)	< 0.001
Passive smoking before pregnancy (yes)	249 (38.2)	293 (51.5)	< 0.001
Exposure history of radioactive substance (yes)	12 (1.8)	19 (3.3)	0.097
History of house decoration (yes)	37 (5.7)	44 (7.7)	0.149
Exposure history to harmful chemicals (yes)	24 (3.7)	27 (4.7)	0.354
Cold history (yes)	76 (11.7)	122 (21.4)	< 0.001
Fever history (yes)	13 (2.0)	47 (8.3)	< 0.001
Folate supplementation (no)	44 (6.7)	95 (16.7)	< 0.001

Note. *Fisher's exact probability test.

antidepressant (a*OR* = 8.32, 95% *CI*: 3.56–19.45) or an antiabortifacient (a*OR* = 2.47, 95% *CI*: 1.48–4.10).

There were also statistically significant interactive effects between maternal genetic variants at rs16947 and drug use for the development of CHDs in offspring. Overall, mothers with a risk genotype (C/G or G/G) at rs16947 had a significantly higher risk of CHDs in offspring if they used ovulatory drugs (a*OR* = 6.49, 95% *CI*: 1.82–23.14) or traditional Chinese medicines (a*OR* = 3.55, 95% *CI*: 1.72–7.29).

DISCUSSION

The past few years have seen an increased interest in exploring the etiology of CHDs. Although some genetic and environmental factors have been identified, the etiologies of most non-syndromic CHDs are still unknown. In the present study, we examined whether maternal drug use before or during early pregnancy would increase the risk of CHDs in offspring. We assessed the associations between maternal CYP450 genetic variants and the risk of CHDs in offspring and analyzed the interactions between maternal drug use and the CYP450 genetic variants on the risk of CHDs. To our knowledge, this is the first exhaustive exploration of the association between maternal drug use and CYP450 genetic polymorphisms, and their interactions with the risk of CHDs in offspring, which provides new insight into the etiology of CHDs, and guides pregnant women with a high risk of CHDs to

Table 2. Maternal drug use and risk of congenital heart defect in offspring

Maternal drug use	Control group, *n* (%) (*n* = 652)	Case group, *n* (%) (*n* = 569)	Crude *OR*		Adjusted *OR*[*]	
			OR (95% *CI*)	*P* values	*OR* (95% *CI*)	*P* values
Oral contraceptives (yes)	17 (2.6)	20 (3.5)	1.36 (0.71-2.62)	0.358	1.48 (0.70-3.16)	0.306
Ovulatory drugs (yes)	17 (2.6)	36 (6.3)	2.52 (1.40-4.54)	0.002	2.12 (1.08-4.16)	0.029
Macrolides antibiotics (yes)	31 (4.6)	57 (10.0)	2.23 (1.42-3.51)	0.001	1.45 (0.77-2.71)	0.251
Antidepressant drugs (yes)	21 (3.2)	45 (7.9)	2.58 (1.52-4.39)	0.000	2.56 (1.36-4.82)	0.004
Traditional Chinese drugs (yes)	75 (11.5)	103 (18.1)	1.70 (1.23-2.35)	0.001	1.97 (1.26-3.09)	0.003
Antiabortifacients (yes)	81 (12.4)	120 (21.1)	1.88 (1.39-2.56)	0.000	1.55 (1.00-2.40)	0.048

Note. [*]Adjusted for maternal education level, family annual income, residence areas, history of adverse pregnancy outcomes, history of pregnancy related complications, family consanguineous marriages, family history of congenital malformation, alcohol use before pregnancy, active or passive smoking before pregnancy, cold or fever history for this pregnancy, folate supplementation and the remaining drugs. *OR*: odds ratio; *CI*: confidence interval.

Table 3. Maternal cytochrome P450 genotype frequencies and *P* values of HWE test

SNPs	Major allele	Minor allele	MAF	Group	Genotype frequencies[†], *n* (%)			HWE test *P*
					AA	AB	BB	
rs1048943	T	C	0.2514	Control	359 (55.1)	256 (39.3)	37 (5.7)	0.3244
				Case	305 (53.6)	244 (42.9)	20 (3.5)	
rs4646903	G	A	0.4423	Control	205 (31.4)	331 (50.8)	116 (17.8)	0.3768
				Case	168 (29.5)	285 (50.1)	116 (20.4)	
rs1065852	A	T	0.4906	Control	192 (29.4)	313 (48.0)	147 (22.5)	0.3676
				Case	121 (21.3)	305 (53.6)	143 (25.1)	
rs16947	C	G	0.1937	Control	462 (70.9)	166 (25.5)	24 (3.7)	0.0660
				Case	353 (62.0)	173 (30.4)	43 (7.6)	

Note. [†]AA = homozygous wild-type; AB = heterozygous variant type; BB = homozygous variant type. SNPs, single nucleotide polymorphisms; HWE, Hardy-Weinberg equilibrium; MAF, minimum allele frequency.

use drugs reasonably during pregnancy.

The findings from the present study indicate that using ovulatory drugs, antidepressants, antiabortifacients, or traditional Chinese medicines before or during the early stage of pregnancy was significantly associated with the risk of CHDs in offspring. However, our study showed that using oral contraceptives and macrolide antibiotics was not associated with the risk of CHDs in offspring. Mothers who used ovulatory drugs for this pregnancy compared with those who did not were at a significantly increased risk of CHDs in offspring (*OR* = 2.12), which is generally consistent with previous studies[21,22]. Clomiphene, which is often used as a first-line infertility drug, is associated with the risk of congenital defects, including CHDs. For

Table 4. Maternal cytochrome P450 genetic polymorphisms and risk of congenital heart defect in offspring

SNPs	Crude *OR*		Adjusted *OR**		
	OR (95% *CI*)	*P* values	*OR* (95% *CI*)	*P* value	*FDR_P* values
*CYP1A*1 at rs1048943					
T/T	1		1		
T/C	1.12 (0.89–1.42)	0.332	1.03 (0.78–1.37)	0.802	0.802
C/C	0.64 (0.36–1.12)	0.117	0.88 (0.46–1.71)	0.716	0.818
Dominant	1.06 (0.85–1.33)	0.610	1.02 (0.78–1.34)	0.887	0.887
Recessive	0.61 (0.35–1.06)	0.077	0.87 (0.45–1.67)	0.677	0.677
Additive	0.98 (0.81–1.19)	0.835	1.00 (0.79–1.26)	0.978	0.978
CYP1A1 at rs4646903					
G/G	1		1		
G/A	1.05 (0.81–1.36)	0.708	1.18 (0.87–1.59)	0.287	0.383
A/A	1.22 (0.88–1.70)	0.235	1.43 (0.98–2.10)	0.067	0.134
Dominant	1.10 (0.86–1.40)	0.468	1.22 (0.91–1.64)	0.175	0.233
Recessive	1.18 (0.89–1.58)	0.249	1.40 (0.99–1.99)	0.057	0.114
Additive	1.10 (0.93–1.29)	0.258	1.19 (0.99–1.44)	0.067	0.089
CYP2D6 at rs1065852					
A/A	1				
A/T	1.55 (1.17–2.04)	0.002	1.53 (1.10–2.14)	0.011	0.044
T/T	1.54 (1.12–2.13)	0.009	1.57 (1.07–2.31)	0.023	0.061
Dominant	1.55 (1.19–2.01)	0.001	1.55 (1.13–2.12)	0.007	0.028
Recessive	1.15 (0.89–1.50)	0.290	1.17 (0.86–1.61)	0.316	0.421
Additive	1.25 (1.06–1.46)	0.008	1.25 (1.03–1.52)	0.022	0.044
CYP2D6 at rs16947					
C/C	1				
C/G	1.36 (1.06–1.76)	0.017	1.24 (0.91–1.68)	0.179	0.286
G/G	2.35 (1.40–3.94)	0.001	3.41 (1.82–6.39)	< 0.001	< 0.001
Dominant	1.49 (1.17–1.89)	0.001	1.47 (1.10–1.95)	0.009	0.018
Recessive	2.14 (1.28–3.57)	0.004	3.23 (1.73–6.02)	< 0.001	< 0.001
Additive	1.44 (1.19–1.75)	< 0.001	1.51 (1.20–1.90)	< 0.001	< 0.001

Note. *Adjusted for maternal education level, family annual income, residence areas, history of adverse pregnancy outcomes, history of pregnancy related complications, family consanguineous marriages, family history of congenital malformation, alcohol use before pregnancy, active or passive smoking before pregnancy, cold or fever history for this pregnancy, folate. *OR*, odds ratio; *CI*, confidence interval; SNPs, single nucleotide polymorphisms; *FDR_P*, false discovery rate *P*. supplementation and the remaining SNPs.

Table 5. Interactions between maternal cytochrome P450 gene and drug use for risk of congenital heart defect in offspring

Maternal drug use	Maternal cytochrome P450 genotype	Number of control	Number of case	Adjusted *OR* (95% *CI*)*	*P*	Regression coefficient	Interactive coefficient	
							γ_1	γ_2
Ovulatory drugs	rs1065852							
No	Wild genotype (A/A)	188	109	1				
No	Variant genotype (A/T or T/T)	447	424	1.68 (1.24–2.27)	0.001	0.519 (β_g)		
Yes	Wild genotype (A/A)	4	12	5.65 (1.59–20.11)	0.008	1.732 (β_e)		
Yes	Variant genotype (A/T or T/T)	13	24	3.84 (1.74–8.49)	0.001	1.345 (β_{g*e})	2.592	0.777
Antidepressant drugs	rs1065852							
No	Wild genotype (A/A)	186	114	1				
No	Variant genotype (A/T or T/T)	445	410	1.59 (1.15–2.20)	0.005	0.462 (β_g)		
Yes	Wild genotype (A/A)	6	7	8.23 (2.22–30.44)	0.002	2.108 (β_e)		
Yes	Variant genotype (A/T or T/T)	15	38	8.32 (3.56–19.45)	0.000	2.118 (β_{g*e})	4.584	1.005
Traditional Chinese drugs	rs1065852							
No	Wild genotype (A/A)	176	97	1				
No	Variant genotype (A/T or T/T)	401	369	1.83 (1.29–2.57)	0.001	0.602 (β_g)		
Yes	Wild genotype (A/A)	16	24	6.63 (2.80–15.71)	0.000	1.892 (β_e)		
Yes	Variant genotype (A/T or T/T)	59	79	3.25 (1.94–5.44)	0.000	1.179 (β_{g*e})	1.958	0.623
Antiabortifacients	rs1065852							
No	Wild genotype (A/A)	171	96	1				
No	Variant genotype (A/T or T/T)	400	353	1.58 (1.12–2.22)	0.009	0.457 (β_g)		
Yes	Wild genotype (A/A)	21	25	1.66 (0.74–3.70)	0.216	0.507 (β_e)		
Yes	Variant genotype (A/T or T/T)	60	95	2.47 (1.48–4.10)	0.001	0.902 (β_{g*e})	1.974	1.779

Continued

Maternal drug use	Maternal cytochrome P450 genotype	Number of control	Number of case	Adjusted *OR* (95% *CI*)*	*P*	Regression coefficient	Interactive coefficient	
							γ_1	γ_2
Ovulatory drugs	rs16947							
No	Wild genotype (C/C)	449	334	1				
No	Variant genotype (C/G or G/G)	186	199	1.52 (1.13–2.05)	0.006	0.420 (β_g)		
Yes	Wild genotype (C/C)	13	19	2.87 (1.13–7.28)	0.027	1.054 (β_e)		
Yes	Variant genotype (C/G or G/G)	4	17	6.49 (1.82–23.14)	0.004	1.871 (β_{g*e})	4.455	1.775
Antidepressant drugs	rs16947							
No	Wild genotype (C/C)	441	329	1				
No	Variant genotype (C/G or G/G)	190	195	1.49 (1.10–2.01)	0.010	0.397 (β_g)		
Yes	Wild genotype (C/C)	21	24	3.81 (1.72–8.44)	0.001	1.338 (β_e)		
Yes	Variant genotype (C/G or G/G)	0	21	–	0.998	21.672 (β_{g*e})		
Traditional Chinese drugs	rs16947							
No	Wild genotype (C/C)	405	283	1				
No	Variant genotype (C/G or G/G)	172	183	1.65 (1.20–2.26)	0.002	0.500 (β_g)		
Yes	Wild genotype (C/C)	57	70	2.59 (1.61–4.18)	0.000	0.952 (β_e)		
Yes	Variant genotype (C/G or G/G)	18	33	3.55 (1.72–7.29)	0.001	1.265 (β_{g*e})	2.530	1.329
Antiabortifacients	rs16947							
No	Wild genotype (C/C)	408	270	1				
No	Variant genotype (C/G or G/G)	163	179	1.76 (1.28–2.41)	0.000	0.563 (β_g)		
Yes	Wild genotype (C/C)	54	83	2.05 (1.28–3.30)	0.003	0.720 (β_e)		
Yes	Variant genotype (C/G or G/G)	27	37	1.67 (0.87–3.21)	0.125	0.512 (β_{g*e})	0.909	0.711

Note. *Adjusted for maternal education level, family annual income, residence areas, history of adverse pregnancy outcomes, history of pregnancy related complications, family consanguineous marriages, family history of congenital malformation, alcohol use before pregnancy, active or passive smoking before pregnancy, cold or fever history for this pregnancy, and folate supplementation. *OR*: odds ratio; *CI*: confidence interval.

example, one study that used data from the National Birth Defects Prevention Study 1997–2005 to assess the association between clomiphene use and birth defects showed that clomiphene use before pregnancy significantly increases the risk of several malformations, including anencephaly, Dandy-Walker malformation, septal heart defects, muscular ventricular septal defect, coarctation of the aorta, esophageal atresia, cloacal exstrophy, craniosynostosis, and omphalocele[21]. Källén et al. reported a similar result[22]. Additionally, our previous cohort study showed that preterm birth, low birth weight, and congenital defects increase significantly among mothers who use ovulatory drugs compared with a reference group[23].

One study showed no association between the use of most antibiotics and cardiovascular defects[22], which is consistent with our study. However, some studies have reported that using macrolides, such as erythromycin, during early pregnancy significantly increases the risk of cardiovascular defects in offspring[24-27]. Of note, as maternal viral infections, such as rubella virus and cytomegalovirus, have been confirmed to be significantly associated with the risk of CHDs[6], we cannot rule out the possibility that a teratogenic effect of an antibiotic may be due to a viral infection. The relationship between exposure to antidepressants and birth defects has always been the focus of attention. The present study showed that antidepressants were significantly associated with the risk of CHDs in offspring, which was supported by previous studies[22,28,29]. For example, the risk of CHDs increases significantly among mothers who use clomipramine during early pregnancy[22]. Additionally, several meta-analyses have confirmed the association between antidepressant use and the risk of CHDs[28,29].

During pregnancy, antiabortifacients, such as dydrogesterone and chorionic gonadotropin, are often used to prevent early miscarriage and preterm labor. Our study supports a positive association between antiabortifacients and the risk of CHDs, which is consistent with a previous study[30]. Dydrogesterone is a progestogen that is teratogenic, and some studies have demonstrated cardiac anomalies after progestin use during pregnancy[22,31]. For example, Zaqout et al. showed that taking dydrogesterone during early pregnancy is significantly associated with the risk of CHDs[31]. Källén et al. reported a significant positive association between the administration of chorionic gonadotropin and CHDs[22]. Although there are some different views, previous studies have generally found that maternal exposure to various drugs, including ovulatory drugs, antiabortifacients, and antidepressants, increases the risk of CHDs in offspring. However, whether these associations between maternal drug use and CHDs are due to the underlying diseases themselves, true drug effects, or combinations of underlying diseases and drugs remains uncertain and warrants further research.

In our study, we also assessed the SNPs of maternal *CYP450* genes (i.e., *CYP1A1* gene at rs1048943 and rs4646903 as well as the *CYP2D6* gene at rs1065852 and rs16947) associated with the risk of CHDs in offspring. The present results indicate that genetic variants of maternal *CYP450* genes could play an important role in the development of the fetal heart. After adjusting for other influencing factors, the results suggested that maternal *CYP450* genetic polymorphisms at rs1065852 (A/T *vs*. A/A: *OR* = 1.53; T/T *vs.* A/A: *OR* = 1.57) and rs16947 (G/G *vs.* C/C: *OR* = 3.41) were significantly associated with an increased risk of CHDs in offspring. The importance of these results depends on the finding that the *CYP450* genes might be potential CHD susceptibility genes. A specific CHD gene has not been found, although much research has been conducted on this topic.

CYP450 enzymes, as a superfamily of activation enzymes, exist in many cell types. According to the sequence homology of two-fifths or more, CYP450 enzymes are divided into 18 families, and according to homologies ≥ 55%, they are categorized into 44 subfamilies. CYP1A1 and CYP2D6, the enzymes included in our study, are involved in drug metabolism. When *CYP450* genes mutate, the activity of the CYP450 enzymes is higher than that coded by the wild-type, which may change susceptibility to disease[32]. Although many studies have assessed the associations between *CYP450* SNPs and the risk of various cancers[33-35] and specific adverse pregnancy outcomes[36,37], the results have often been inconsistent. For example, a meta-analysis showed that the *CYP1A1* rs1048943 and rs4646903 polymorphisms contribute to the risk of laryngeal cancer in the Asian population[38]. However, a study published in 2016 showed that the *CYP1A1* rs1048943 polymorphism is not significantly associated with the risk of gastric cancer[35]. Some differences in the results have been reported for other diseases[33,36-37]. In the present study, no significant positive associations were observed between the *CYP1A1* rs1048943 and rs4646903 polymorphisms and the risk of CHDs. However, our study showed that genetic polymorphisms at

rs1065852 and rs16947 were significantly associated with the risk of CHDs. No study has evaluated the association between *CYP450* genetic polymorphisms and the risk of CHDs, until now. Undoubtedly, our study will provide a new clue for screening candidate genes for CHDs.

The most impressive findings of this study were the interactions between maternal drug use and the *CYP450* genetic polymorphisms in the development of CHDs. The interaction analyses showed that mothers with a *CYP450* gene risky genotype at rs1065852 and rs16947 had a significantly increased risk of CHDs in offspring if they had used ovulatory drugs (*OR* = 6.49), antidepressant (*OR* = 8.32), antiabortifacients (*OR* = 2.47), or traditional Chinese medicines (*OR* = 3.55), indicating that maternal *CYP450* genes may regulate the effects of the corresponding drugs on embryonic heart development. However, the interactions between the *CYP450* genetic polymorphisms and drug use on the development of CHD were different, which was not surprising given the differences in the teratogenic effects of different drugs on fetal CHDs, as well as the differences in the degradation of different drugs by CYP450 enzymes. Teratogenesis is a process accompanied by complex steps and various factors, indicating different gene mutations and several biological pathways. Therefore, interactive effects of different factors exist in the development of CHDs. One view suggests that genetic polymorphisms in metabolic enzymes play a pathogenic role by altering the effects of environmental factors rather than directly causing disease[39]. We supposed that the genetic variants in the *CYP450* genes promote metabolic activation of exogenous compounds, including drugs, by coding higher activity in the CYP450 enzymes. Then, teratogenic intermediate drug metabolites accumulate in the maternal body and influence the development of the embryo. Although previous studies have shown that genetic polymorphisms in *CYP450* genes may affect the metabolism of drugs[40,41], no study has assessed the interactions between maternal *CYP450* genes and drug use in the development of CHD until now. However, the specific mechanism of the combined effect between the *CYP450* genes and drug use on the risk of CHDs remains unclear and needs further research. There is increasing evidence that gene-environment interactions affect CHDs, so the effects of these interactions will be the future research direction for CHDs.

Some limitations in our study should be discussed. First, our study may have suffered from selection bias. For example, a convenience sample, driven mainly by the number of respondents, was used for our study. The target population did not include mothers whose pregnancy was terminated because of a CHD. This limitation could lead to subsequent problems, including sample representativeness and generalization of the findings. Second, we replaced three *CYP450* gene SNPs according to linkage disequilibrium, which may have overestimated the statistical correlation in our findings. Third, residual confounders are always a concern in observational studies. Although we adjusted for a wide range of factors influencing CHDs, we could not exclude the possibility that other unmeasured or inadequately measured factors confounded the true associations. Fourth, as this was a case-control study, we could not verify causality. Fifth, although some measures were adopted, recall bias was inevitable. Sixth, because of the limited sample size, we analyzed drugs in a wide range of categories rather than specific drugs, and we did not assess the risk of specific CHD phenotypes. Additionally, the frequency of drug use was not measured, and exposure to a drug was only dichotomized as yes or no independently. Finally, because the present study only included Han Chinese subjects, additional research in other populations is warranted to generalize the findings. These limitations highlight the need for larger samples, a prospective approach, and different ethnic populations.

In conclusion, our study indicates that using ovulatory drugs, antidepressants, antiabortifacients, or traditional Chinese medicines before or during the early stage of pregnancy may be significantly associated with an increased risk of CHDs in offspring of Chinese descent. Additionally, this study supports a significant positive association between maternal *CYP450* genetic variants at rs1065852 and rs16947 and the risk of fetal CHDs. Significant interactions were observed between the maternal *CHY450* genetic polymorphisms and drug use, suggesting that maternal *CYP450* genes may regulate the effects of maternal drug exposure on fetal heart development. However, the mechanism by which these factors affect the development of the fetal heart remains unknown, and more studies in different ethnic populations and with a larger sample are required to confirm these findings.

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DECLARATION OF INTEREST STATEMENT

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

JBQ, JYD, and PZ designed and supervised the project. LL, MTS, PH, TTW, JYD, SMZ, JQL, YHL, and LTC collected and analyzed the data. JBQ, JYD, and PZ conducted the experiments. JBQ and JYD wrote the manuscript. JBQ and PZ edited the manuscript.

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Supplementary Table S1. Primer sequences for cytochrome P450 genetic polymorphisms

Genes	SNPs	Linkage	r^2	Primer sequences
CYP1A1	**rs1048943**		–	
	1st-PCRP	No		ACGTTGGATGGGTGATTATCTTTGGCATGG
	2nd-PCRP			ACGTTGGATGGGTGATTATCTTTGGCATGG
	rs4646903	**rs4646421**	1.000	
		1st-PCRP		ACGTTGGATGAGACTCCTTAGGGACACTTC
		2nd-PCRP		ACGTTGGATGCATTGATCTGACCACTCTTC
CYP2D6	**rs1065852**	**rs5751210**	0.900	
		1st-PCRP		ACGTTGGATGATGGACAGAGTTTTCCGGAC
		2nd-PCRP		ACGTTGGATGACAGCACTGGTCGGTGCGG
	rs16947	**rs4147641**	0.965	
		1st-PCRP		ACGTTGGATGATGTCTGGACAATGAGGTGG
		2nd-PCRP		ACGTTGGATGGGTACCAGTTAACCACAGAG

Note. SNPs, single nucleotide polymorphisms.