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Associations and interaction effects of maternal smoking and genetic polymorphisms of cytochrome P450 genes with risk of congenital heart disease in offspring

A case–control study

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Abstract

To assess associations and interactions of maternal smoking and cytochrome P450 (CYP450) genetic variants with the developments of congenital heart disease (CHD) and specific subtypes.

A case–control study of 654 cases and 666 controls was conducted from November 2017 to March 2020. The exposures of interest were maternal active and passive smoking before/in the early pregnancy and CYP450 genetic polymorphisms. Data were analyzed using the Chi-square test and logistic regression analysis.

After adjusting for the potential confounding factors, our study showed maternal active (OR_{adj} = 2.34, 95%CI: 1.19–4.60) or passive (OR_{adj} = 1.76, 95%CI: 1.34–2.31) smoking before pregnancy, passive smoking in the early pregnancy (OR_{adj} = 3.05, 95%CI: 2.26–4.12), as well as polymorphisms of CYP450 at rs1065852 (G/A vs G/G: OR_{adj} = 1.46, 95%CI: 1.07–1.99; A/A vs G/G: OR_{adj} = 1.63, 95%CI: 1.15–2.33) and rs16947 (A/A vs G/G: OR_{adj} = 3.61, 95%CI: 2.09–6.23), were significantly associated with risk of total CHD in offspring. Similar results were also found for some subtypes of CHD. Additionally, significant interactions between maternal smoking and CYP450 genes on the risk of CHD were observed.

Maternal smoking and CYP450 genetic variants were associated with increased risk of CHD and specific subtypes in offspring. And the effects of CYP450 genes on CHD may be modified by maternal smoking.

Abbreviations: ASD = atrial septal defect, AVSD = atrioventricular septal defect, CHD = congenital heart disease, CI = confidence interval, CYP450 = cytochrome P450, HWE = Hardy–Weinberg equilibrium, OR = odds ratio, OR_{adj} = adjusted OR, OR_{unadj} = unadjusted OR, PDA = patent ductus arteriosus, SNPs = single nucleotide polymorphisms, VSD = ventricular septal defect.

Keywords: case–control study, congenital heart disease, CYP450, interaction, maternal smoking, single nucleotide polymorphism

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All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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1. Introduction

Congenital heart disease (CHD) is the leading cause of perinatal and infant mortality, with a birth prevalence of 9.41‰ worldwide[1] and 8.98‰ in China.[2] The etiology of CHD is multifactorial. Over the past decades, researchers have found that one-fifth of CHD can be attributed to exposure to teratogen, genetic syndromes, and maternal diabetes, while the remaining remains unclear.[1] Smoking during peri-conception is an important environmental factor that has been reported to have an obvious teratogenic effect.[3] A series of studies suggested that maternal smoking was significantly associated with the risk of CHD in offspring.[4,5] However, these published studies focused mainly on maternal active smoking and its effect on CHD, few researchers paid attention to maternal passive smoking. Maternal passive smoking, which is more common than active smoking, did not get enough attention.[6] Moreover, most studies did not assess the risk of specific CHD subtypes associated with maternal smoking. There may be some differences in risk factors of different subtypes of CHD. And attention to the differences for risk factors of different subtypes will be helpful for the accurate prevention and intervention of CHD.

As well, of note, available evidence showed that not all pregnant women who were exposed to smoking give birth to a child with CHD, which may be due to differences in individual genetic susceptibility. Cytochrome P450 (CYP450) superfamily takes part in the activation processes of carcinogens and teratogens, including tobacco compounds (eg, polycyclic aromatic hydrocarbons, dioxin).[7–9] It has been reported that single nucleotide polymorphisms (SNPs) of CYP450 genes had significant impacts on the biological activities of CYP450 enzymes, and result in different susceptibility to diseases.[10,11] To date there have been only 2 studies focused on the association between maternal CYP450 genes and birth defects, with inconsistent results.[12,13] However, the above-mentioned 2 studies did not assess the association of maternal CYP450 gene with the risk of CHD.

Considering that most of CHD were the result of interactions between genetic and environmental factors, we supposed that cigarette smoke may modify the effect of SNPs of CYP450 genes on CHD in offspring. However, this supposition has not been verified yet. Therefore, this study was conducted to fill these research gaps with the following objectives: (i) to examine the risk of CHD and its specific subtypes associated with maternal active and passive smoking before or in the early pregnancy; (ii) to assess whether the SNPs of maternal CYP450 genes were significantly associated with risk of CHD and its specific subtypes in offspring; and (iii) to analyze interactions between maternal active and passive smoking and CYP450 genetic variants for the risk of CHD in offspring.

2. Materials and methods

2.1. Recruitment of study participants

A hospital-based case–control study was reviewed and authorized by the ethics committee of the Xiangya School of Public Health, Central South University. Recruitment was conducted by the Hunan Children's Hospital from November 2017 to March 2020. The Hunan Children's Hospital, as a large specialized hospital for children in China, is responsible for the provincial prevention, treatment, and management of childhood illness, and receives about 1000 patients with CHD every year. Eligible children and their parents were recruited for the present study during health counseling, medical examination, or treatment. Children with CHD and their parents were recruited from the Department of Cardiothoracic Surgery in this hospital into the case group. Meanwhile, healthy children and their parents from the health examination clinic in this hospital were recruited into the control group after health counseling or a medical examination. To minimize potential recall bias of exposure by mothers during the pre-pregnancy to the early stage of this pregnancy, all cases and controls were recruited when their children were less than 1-year old. The convenience sample, driven mainly by the number of respondents, was used for this study. All parents have given written informed consent before recruitment. Additionally, this study has been registered in Chinese Clinical Trial Center (registration number: ChiCTR1800016635).

2.2. Inclusion and exclusion criteria

In this study, the exposures of interest were maternal active and passive smoking before this pregnancy or during the early pregnancy as well as SNPs of maternal CYP450 genes. And outcomes of interest were CHD including the following subtypes: atrial septal defect (ASD), ventricular septal defect (VSD), atrioventricular septal defect (AVSD), patent ductus arteriosus (PDA), aortopulmonary window, tetralogy of Fallot, and complete transposition of great arteries. Patients with CHD were diagnosed using echocardiography or confirmed by surgery.

All participants were required to complete the same questionnaire in the same way by some professionally trained investigators. Eligible parents need to provide informed consent, belonged to singleton pregnancies for this pregnancy, were of Han Chinese descent, had a complete record of the questionnaire, and provided the blood sample. We only concerned non-syndromic CHD, and patients with structural malformations involving another organ system or known chromosomal abnormalities were excluded. Participants who reported a history of depression or other psychiatric disorders or were diagnosed with depression or a psychiatric illness were also excluded when they were recruited into the study. Besides, mothers who achieved pregnancy by assisted reproductive technology including in vitro fertilization and intracytoplasmic sperm injection were further excluded from case and control groups.

2.3. Information collection

A structured questionnaire (test-retest reliability = 0.833; Cronbach alpha = 0.782), designed according to the results of the literature search and suggestions of several experts, was used to interview all participants by professionally trained investigators. All cases and controls were interviewed face-to-face after obtaining their informed consent. We collected the status of maternal active and passive smoking during 3 months before this pregnancy to the early stage of this pregnancy. Active smoking was defined as smoking for more than 6 months continuously or cumulatively, and the smoking index in the past 6 months is more than 100 (smoking index: cigarette/day*year). Passive smoking was defined as there are smokers in the maternal immediate family or other close contacts (smoking index > 100), or the time of exposure to smoke is more than 15 minutes/d in a week. Furthermore, we obtained corresponding information on polymorphisms of CYP450 genes, which were described below.

To control the potential confounding factors as much as possible when evaluating the association of maternal smoking

and genetic variants of maternal CYP450 genes with risk of CHD in offspring, we further collected the following information: maternal demographic characteristics (ie, child-bearing age, education level, annual income, and residence); abnormal pregnancy history (ie, spontaneous abortion, induced abortion, stillbirth, preterm birth, low birth weight, and gestational diabetes and hypertension); family history (ie, consanguineous marriages and congenital malformations); personal medical history before or during this pregnancy (ie, pre-gestational diabetes mellitus, congenital malformations, cold or fever, and folate supplementation); personal lifestyle and habit in the 3 months before this pregnancy (ie, drinking, drinking tea, drinking coffee, cosmetics use, and dyeing or perming hair experiences); exposure history to environmental hazardous substance (ie, exposure to environmentally harmful substances near place of residence, noise pollution exposure, and history of decorating housing); and spouse's baseline characteristics (ie, age, education level, smoking history, and drinking history). The above-mentioned information was further confirmed by consulting their Maternal and Child Health Manual and medical records.

2.4. Genotyping

All mothers were requested to provide 3 to 5 mL of peripheral venous blood for genotyping after completing the questionnaires. Genomic DNA was extracted from a peripheral venous blood sample using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's standard protocol and dissolved in sterile tris-borate-EDTA buffer. Presently, considering the fact that there were few studies on the association between CYP450 genes and risk of CHD, we selected candidate loci of CYP450 genes mainly based on previous similar studies that assessed the association of smoking or other harmful environmental factors, CYP450 genes, and their interactions with risk of cancers.[14–16] As a result, these genetic loci including rs1048943 and rs4646903 of CYP1A1 gene as well as rs1065852 and rs16947 of CYP2D6 gene were selected as candidate loci for this study. CYP1A1 and CYP2D6 are the 2 main members of the CYP450 superfamily. According to the theory of linkage disequilibrium, we used rs4646421 to replace rs4646903 (r^2 = 1.000), rs5751210 to replace rs1065852 (r^2 = 0.900), and rs4147641 to replace rs16947 (r^2 = 0.965). The polymorphisms of CYP450 genes were genotyped using the matrix-assisted laser desorption and ionization time-of-flight mass spectrometry Mass Array system (Agena iPLEXassay, San Diego, CA, USA). The laboratory technician, who performed the genotyping, retyped and double-checked each sample, and recorded the genotype data, was blinded to whether the samples were from cases or controls. The error rate of genotyping was lower than 5%.

Finally, for the CYP1A1 gene, 3 genotypes were identified: homozygous wild-type TT, heterozygous variant TC, and homozygous variant CC at rs1048943; and homozygous wild-type AA, heterozygous variant GA, and homozygous variant GG at rs4646903. For the CYP2D6 gene, 3 genotypes were identified: homozygous wild-type GG, heterozygous variant GA, and homozygous variant AA at rs1065852; and homozygous wild-type GG, heterozygous variant AG, and homozygous variant AA at rs16947.

2.5. Statistical analysis

Categorical variables were described using frequencies and percentages. Differences of unordered categorical variables between 2 groups were calculated by Chi-square test or Fisher exact test. Wilcoxon rank-sum test was used to compare the difference in ordinal categorical variables. Hardy–Weinberg equilibrium (HWE) was tested for the control group (significance level at $P < 0.01$). Odds ratios (ORs) and their 95% confidence intervals (CIs) were used to measure the level of association of maternal smoking and CYP 450 genes with the risk of CHD. Unadjusted ORs (OR_{unadj}) were calculated by univariate logistic regression. Adjusted ORs (OR_{adj}) were calculated by multivariable logistic regression. We used logistic regression and controlled for the potential confounding factors to examine the main effects and interactive effects of the gene-environment interaction of CYP450 genes and maternal smoking for the risk of CHD in offspring.

We referred to a method described by Wallace[17] to build and explain models of gene-environment interactions. Interaction coefficient (γ) was calculated by regression coefficient (β) from logistic regression analysis (eg, $\gamma_1 = \beta_{e^*g}/\beta_e$ and $\gamma_2 = \beta_{e^*g}/\beta_g$) and was used to evaluate the interaction. When all γ values were more than 1, there was a positive interaction; when all γ values were less than 1, there was a negative interaction; and when the γ values were equal to 1, there was no interaction. Significance was set at a P value less than 0.05 (two-tailed).

In the present study, we focused not only on the risk of total CHD associated with maternal smoking and genetic variants of CYP450 genes but also on the risk of specific CHD subtypes including ASD, VSD, AVSD, and PDA. However, we did not assess the remaining CHD subtypes, such as aortopulmonary window, tetralogy of Fallot, and complete transposition of great arteries because of the limited sample size for these subtypes. Moreover, for the same reason, we only focus on the risk of total CHD when assessing the impact of gene-environment interactions on CHD in offspring. All analyses were performed using SAS 9.1 (SAS Institute, Cary, NC, USA).

3. Results

3.1. Respondent baseline characteristics

From November 2017 to March 2020, total of 654 CHD cases, 666 controls, and their corresponding parents were recruited for the present study according to eligibility criteria. Among 654 CHD cases, there were 110 children with ASD, 401 with VSD, 71 with AVSD, 185 with PDA, 10 with aortopulmonary window, 37 with tetralogy of Fallot, and 2 with complete transposition of great arteries. It should be noted that some cases have multiple CHD subtypes at the same time, so the sum of the subtypes does not equal 654.

The baseline characteristics of different groups are summarized in Table 1. There were statistically significant differences in baseline characteristics, such as maternal education level, annual income, residence, abnormal pregnancy history, family history, personal medical history, personal lifestyle and habit, and exposure history to environmental hazardous substance as well as paternal education level, and smoking and drinking history between total CHD and control groups ($P < 0.05$ for all comparisons). Additionally, the baseline characteristics of different CHD subtypes were also compared with those of the control group. These variables that were significantly different across groups were controlled in subsequent multivariate logistic analyses.

Table 1

Comparison of baseline characteristics in case and control groups.

	Controls	Total CHD		ASD		VSD		AVSD		PDA	
Baseline characteristics	**N (%)**	**N (%)**	***P***	**N (%)**	***P***	**N (%)**	***P***	**N (%)**	***P***	**N (%)**	***P***
Demographic characteristics											
Child-bearing age (years)											
<35	571 (85.7)	566 (86.5)	0.671	88 (80.0)	0.119	351 (87.5)	0.407	58 (81.7)	0.360	157 (84.9)	0.766
≥35	95 (14.3)	88 (13.5)		22 (20.0)		50 (12.5)		13 (18.3)		28 (15.1)	
Education level											
Less than primary or primary	9 (1.4)	95 (14.5)	0.000	34 (30.9)	0.000	40 (10.0)	0.000	8 (11.3)	0.000	16 (8.6)	0.000
Junior high school	134 (20.1)	273 (41.7)		43 (39.1)		184 (45.9)		23 (32.4)		84 (45.4)	
Senior middle school	224 (33.6)	185 (28.3)		19 (17.3)		114 (28.4)		23 (32.4)		59 (31.9)	
College or above	299 (44.9)	101 (15.4)		14 (12.7)		63 (15.7)		17 (23.9)		26 (14.1)	
Annual household income in the past 1 year (RMB)											
<50,000	182 (27.3)	530 (81.0)	0.000	99 (90.0)	0.000	316 (78.8)	0.000	61 (85.9)	0.000	150 (81.1)	0.000
50,000–100,000	292 (43.8)	92 (14.1)		5 (4.5)		59 (14.7)		10 (14.1)		31 (16.8)	
100,001–150,000	65 (9.8)	14 (2.1)		4 (3.6)		10 (2.5)		0		2 (1.1)	
>150,000	127 (19.1)	18 (2.8)		2 (1.8)		16 (4.0)		0		2 (1.1)	
Residence											
Rural	366 (55.0)	495 (75.7)	0.000	86 (78.2)	0.000	301 (75.1)	0.000	51 (71.8)	0.006	140 (75.7)	0.000
Urban	300 (45.0)	159 (24.3)		24 (21.8)		100 (24.9)		20 (28.2)		45 (24.3)	
Abnormal pregnancy history											
History of spontaneous abortion											
No	610 (91.6)	577 (88.2)	0.042	101 (91.8)	0.937	353 (88.0)	0.057	60 (84.5)	0.048	158 (85.4)	0.012
Yes	56 (8.4)	77 (11.8)		9 (8.2)		48 (12.0)		11 (15.5)		27 (14.6)	
History of induced abortion											
No	441 (66.2)	371 (56.7)	0.000	57 (51.8)	0.004	237 (59.1)	0.019	35 (49.3)	0.005	107 (57.8)	0.035
Yes	225 (33.8)	283 (43.3)		53 (48.2)		164 (40.9)		36 (50.7)		78 (42.2)	
History of stillbirth											
No	663 (99.5)	630 (96.3)	0.000	107 (97.3)	0.040	388 (96.8)	0.000	68 (95.8)	0.014	177 (95.7)	0.000
Yes	3 (0.5)	24 (3.7)		3 (0.5)		13 (3.2)		3 (4.2)		8 (4.3)	
History of preterm birth											
No	662 (99.4)	645 (98.6)	0.154	104 (94.5)	0.001	398 (99.3)	1.000	71 (100.0)	1.000	182 (98.4)	0.178
Yes	4 (0.6)	9 (1.4)		6 (5.5)		3 (0.7)		0		3 (1.6)	
History of low birth weight											
No	664 (99.7)	648 (99.1)	0.175	107 (97.3)	0.022	398 (99.3)	0.370	71 (100.0)	1.000	182 (98.4)	0.072
Yes	2 (0.3)	6 (0.9)		3 (2.7)		3 (0.7)		0		3 (1.6)	
History of gestational diabetes											
No	644 (96.7)	594 (90.8)	0.000	102 (92.7)	0.059	357 (89.0)	0.000	65 (91.5)	0.044	167 (90.3)	0.000
Yes	22 (3.3)	60 (9.2)		8 (7.3)		44 (11.0)		6 (8.5)		18 (9.7)	
History of gestational hypertension											
No	654 (98.2)	610 (93.3)	0.000	107 (97.3)	0.458	367 (91.5)	0.000	68 (95.8)	0.168	163 (88.1)	0.000
Yes	12 (1.8)	44 (6.7)		3 (2.7)		34 (8.5)		3 (4.2)		22 (11.9)	
Family history											
Consanguineous marriages											
No	664 (99.7)	627 (95.9)	0.000	102 (92.7)	0.000	386 (96.3)	0.000	67 (94.4)	0.001	177 (95.7)	0.000
Yes	2 (0.3)	27 (4.1)		8 (7.3)		15 (3.7)		4 (5.6)		8 (4.3)	
Congenital malformations											
No	661 (99.2)	614 (93.9)	0.000	93 (84.5)	0.000	380 (94.8)	0.000	69 (97.2)	0.140	173 (93.5)	0.000
Yes	5 (0.8)	40 (6.1)		17 (15.5)		21 (5.2)		2 (2.8)		12 (6.5)	
Personal medical history											
Pre-gestational diabetes mellitus											
No	637 (95.6)	561 (85.8)	0.000	91 (82.7)	0.000	351 (87.5)	0.000	56 (78.9)	0.000	156 (84.3)	0.000
Yes	29 (4.4)	93 (14.2)		19 (17.3)		50 (12.5)		15 (21.1)		29 (15.7)	
Congenital malformations											
No	664 (99.7)	648 (99.1)	0.175	107 (97.3)	0.022	398 (99.3)	0.370	71 (100.0)	1.000	185 (100.0)	1.000
Yes	2 (0.3)	6 (0.9)		3 (2.7)		3 (0.7)		0		0	
Cold history in the early pregnancy											
No	530 (79.6)	437 (66.8)	0.000	68 (61.8)	0.000	260 (64.8)	0.000	54 (76.1)	0.487	124 (67.0)	0.000
Yes	136 (20.4)	217 (33.2)		42 (38.2)		141 (35.2)		17 (23.9)		61 (33.0)	
Fever history in the early pregnancy											
No	643 (96.5)	591 (90.4)	0.000	104 (94.5)	0.283	356 (88.8)	0.000	59 (83.1)	0.000	161 (87.0)	0.000
Yes	23 (3.5)	63 (9.6)		6 (5.5)		45 (11.2)		12 (16.9)		24 (13.0)	
Folate intake											
No	621 (93.2)	541 (82.7)	0.000	77 (70.0)	0.000	349 (87.0)	0.001	61 (85.9)	0.026	158 (85.4)	0.001

(*continued*)

Table 1
(continued).

Baseline characteristics	Controls N (%)	Total CHD N (%)	P	ASD N (%)	P	VSD N (%)	P	AVSD N (%)	P	PDA N (%)	P
Yes	45 (6.8)	113 (17.3)		33 (30.0)		52 (13.0)		10 (14.1)		27 (14.6)	
Personal lifestyle and habit in the 3 months before this pregnancy											
Drinking history											
No	617 (92.6)	561 (85.8)	0.000	94 (85.5)	0.012	344 (85.8)	0.000	55 (77.5)	0.000	158 (85.4)	0.002
Yes	49 (7.4)	93 (14.2)		16 (14.5)		57 (14.2)		16 (22.5)		27 (14.6)	
History of drinking tea											
No	529 (79.4)	568 (86.9)	0.000	94 (85.5)	0.141	359 (89.5)	0.000	60 (84.5)	0.310	154 (83.2)	0.249
Yes	137 (20.6)	86 (13.1)		16 (14.5)		42 (10.5)		11 (15.5)		31 (16.8)	
History of drinking coffee											
No	633 (95.0)	588 (89.9)	0.000	106 (96.4)	0.548	363 (90.5)	0.004	54 (76.1)	0.000	160 (86.5)	0.000
Yes	33 (5.0)	66 (10.1)		4 (3.6)		38 (9.5)		17 (23.9)		25 (13.5)	
Frequency of cosmetics use											
Never	416 (62.5)	476 (22.8)	0.002	83 (75.5)	0.019	300 (74.8)	0.001	53 (74.6)	0.197	130 (70.3)	0.350
Sometime	165 (24.8)	80 (12.2)		15 (13.6)		45 (11.2)		5 (7.0)		16 (8.6)	
Often	35 (5.3)	42 (6.4)		3 (2.7)		30 (7.5)		2 (2.8)		18 (9.7)	
Every day	50 (7.5)	56 (8.6)		9 (8.2)		26 (6.5)		11 (15.5)		21 (11.4)	
Regular dyeing or perming hair											
No	631 (94.7)	572 (87.5)	0.000	94 (85.5)	0.000	354 (88.3)	0.000	60 (84.5)	0.003	172 (93.0)	0.355
Yes	35 (5.3)	82 (12.5)		16 (14.5)		47 (11.7)		11 (15.5)		13 (7.0)	
Exposure history to environmental hazardous substance											
Was there a factory discharging environmentally harmful substances near place of residence?											
No	622 (93.4)	522 (79.8)	0.000	86 (78.2)	0.000	316 (78.8)	0.000	62 (87.3)	0.060	151 (81.6)	0.000
Yes	44 (6.6)	132 (20.2)		24 (21.8)		85 (21.2)		9 (12.7)		34 (18.4)	
Was there a traffic road or a noisy factory near where you live?											
No	546 (82.0)	481 (73.5)	0.000	85 (77.3)	0.240	302 (75.3)	0.009	41 (57.7)	0.000	137 (74.1)	0.017
Yes	120 (18.0)	173 (26.5)		25 (22.7)		99 (24.7)		30 (42.3)		48 (25.9)	
Was your house newly renovated in the 3 months before this pregnancy?											
No	614 (92.2)	616 (94.2)	0.150	110 (100.0)	0.002	366 (91.3)	0.595	71 (100.0)	0.015	185 (100.0)	0.000
Yes	52 (7.8)	38 (5.8)		0		35 (8.7)		0		0	
Spouse's baseline characteristics											
Child-bearing age (years)											
<35	444 (66.7)	429 (65.6)	0.681	58 (52.7)	0.005	279 (69.6)	0.325	45 (63.4)	0.577	117 (63.2)	0.385
≥35	222 (33.3)	225 (34.4)		52 (47.3)		122 (30.4)		26 (36.6)		68 (36.8)	
Education level											
Less than primary or primary	17 (2.6)	82 (12.5)	0.000	31 (28.2)	0.000	29 (7.2)	0.000	13 (18.3)	0.000	12 (6.5)	0.000
Junior high school	140 (21.0)	307 (46.9)		47 (42.7)		199 (49.6)		26 (36.6)		86 (46.5)	
Senior middle school	237 (35.6)	161 (24.6)		18 (16.4)		100 (24.9)		25 (35.2)		63 (34.1)	
College or above	272 (40.8)	104 (15.9)		14 (12.7)		73 (18.2)		7 (9.9)		24 (13.0)	
Smoking history											
No	284 (42.6)	219 (33.5)	0.001	37 (33.6)	0.076	132 (32.9)	0.002	26 (36.6)	0.328	46 (24.9)	0.000
Yes	382 (57.4)	435 (66.5)		73 (66.4)		269 (67.1)		45 (63.4)		139 (75.1)	
Drinking history											
No	357 (53.6)	294 (45.0)	0.002	56 (50.9)	0.600	170 (42.4)	0.000	40 (56.3)	0.660	76 (41.1)	0.003
Yes	309 (46.4)	360 (55.0)		54 (49.1)		231 (57.6)		31 (43.7)		109 (58.9)	

ASD = atrial septal defect, AVSD = atrioventricular septal defect, CHD = congenital heart defect, PDA = patent ductus arteriosus, VSD = ventricular septal defect.

3.2. Maternal smoking and risk of total CHD and its subtypes in offspring

Reported frequencies of maternal smoking among the different groups are summarized in Supplemental Table 1, http://links.lww.com/MD/G191. Associations of maternal smoking with risk of total CHD and its subtypes in offspring based on univariate and multivariable analyses are summarized in Table 2. After using multivariable logistic regression analyses to control potential confounding factors that were presented in Table 1, the present study suggested that maternal active smoking before pregnancy was significantly associated with increased risks of total CHD (OR_{adj} = 2.34; 95%CI: 1.19–4.60) and its 2 subtypes including ASD (OR_{adj} = 4.57; 95%CI: 1.75–11.97) and VSD (OR_{adj} = 2.60; 95%CI: 1.26–5.37) in offspring. Risks of total CHD (OR_{adj} = 1.76; 95%CI: 1.34–2.31) and its 2 subtypes including VSD (OR_{adj} = 1.98; 95%CI: 1.46–2.68) and PDA (OR_{adj} = 1.65; 95%CI: 1.11–2.44) in offspring were significantly increased among mothers reporting a history of passive smoking before pregnancy. Besides, maternal passive smoking in the early pregnancy was significantly associated with higher risks of total CHD (OR_{adj} = 3.05; 95%CI: 2.26–4.12) and its 4 subtypes including ASD (OR_{adj} = 1.85; 95%CI: 1.09–3.15), VSD (OR_{adj} = 2.92; 95%CI: 2.10–4.05), AVSD (OR_{adj} = 3.04; 95%CI: 1.67–5.54), and PDA (OR_{adj} = 3.67; 95%CI: 2.44–5.51) in offspring.

Table 2

Maternal smoking associated with risks of CHD and its subtypes in offspring.

Maternal smoking	Total CHD		ASD		VSD		AVSD		PDA	
	OR_{unadj} (95% CI)	OR_{adj} (95% CI)*	OR_{unadj} (95% CI)	OR_{adj} (95% CI)*	OR_{unadj} (95% CI)	OR_{adj} (95% CI)*	OR_{unadj} (95% CI)	OR_{adj} (95% CI)*	OR_{unadj} (95% CI)	OR_{adj} (95% CI)*
Active smoking before pregnancy										
No	1	1	1	1	1	1	1	1	1	1
Yes	3.44 (1.87–6.33)†	2.34 (1.19–4.60)†	6.24 (2.85–13.68)†	4.57 (1.75–11.97)†	3.50 (1.82–6.72)†	2.60 (1.26–5.37)†	None	None	1.83 (0.73–4.61)	1.03 (0.34–2.96)
Passive smoking before pregnancy										
No	1	1	1	1	1	1	1	1	1	1
Yes	1.84 (1.48–2.29)†	1.76 (1.34–2.31)†	1.07 (0.71–1.62)	1.02 (0.68–1.50)	2.15 (1.67–2.76)†	1.98 (1.46–2.68)†	2.11 (1.29–3.45)†	1.21 (0.66–2.22)	2.08 (1.50–2.89)†	1.65 (1.11–2.44)†
Passive smoking in the early pregnancy										
No	1	1	1	1	1	1	1	1	1	1
Yes	3.05 (2.38–3.92)†	3.05 (2.26–4.12)†	2.31 (1.49–3.58)†	1.85 (1.09–3.15)†	3.18 (2.41–4.21)†	2.92 (2.10–4.05)†	3.39 (2.04–5.63)†	3.04 (1.67–5.54)†	3.88 (2.74–5.50)†	3.67 (2.44–5.51)†

ASD = atrial septal defect, AVSD = atrioventricular septal defect, CHD = congenital heart defect, CI = confidence interval, OR_{adj} = adjusted odds ratio, PDA = patent ductus arteriosus, VSD = ventricular septal defect.

* Adjusted for maternal education level, income, residence, abnormal pregnancy history, family history of inbreeding and congenital malformations, personal medical history before or during this pregnancy, personal lifestyle and habit before this pregnancy and exposure history to environmental hazardous substance as well as spouse's education level, and smoking and drinking history, which were presented in Supplemental Table 1, http://links.lww.com/MD/G191.

† Statistically significant ($a = 0.05$).

3.3. SNPs of maternal CYP450 genes and risk of total CHD and its subtypes in offspring

Genotype distribution and allele frequencies of maternal CYP450 genes in the different groups are summarized in Supplemental Table 2, http://links.lww.com/MD/G192. The genotype distributions in the control group were within HWE ($\chi^2 = 0.054$–6.167; $P = 0.013$–0.817).

Genetic polymorphisms of maternal CYP450 genes associated with risks of CHD and its subtypes in offspring based on univariate and multivariable logistic regression analysis are summarized in Table 3. After adjusting for potential confounding factors, the data suggested that mothers with the G/G genotype at rs4646903 had significantly higher risks of VSD ($OR_{adj} = 1.79$; 95%CI: 1.17–2.72) and PDA ($OR_{adj} = 1.82$; 95%CI: 1.07–3.08) in offspring compared with those with the A/A genotype. For rs1065852, compared with mothers with the G/G genotype, those with the A/A genotype were at significantly higher risks of total CHD ($OR_{adj} = 1.63$; 95%CI: 1.15–2.33) and VSD ($OR_{adj} = 1.96$; 95%CI: 1.31–2.94) in offspring; and those with G/A genotype had significantly higher risks of total CHD ($OR_{adj} = 1.46$; 95%CI: 1.07–1.99) and ASD ($OR_{adj} = 1.94$; 95%CI: 1.04–3.60) in offspring. For rs16947, mothers with the A/A genotype experienced significantly increased risks of total CHD ($OR_{adj} = 3.61$; 95%CI: 2.09–6.23), VSD ($OR_{adj} = 3.35$; 95%CI: 1.79–6.24), AVSD ($OR_{adj} = 13.67$; 95%CI: 6.03–30.97), and PDA ($OR_{adj} = 3.84$; 95%CI: 1.71–8.62) compared with those with the G/G genotype; additionally, the A/G genotype also significantly increased the risk of ASD ($OR_{adj} = 1.99$; 95%CI: 1.18–3.38).

3.4. Interactions of maternal CYP450 genes and smoking associated with risk of total CHD

Gene-environment interactions between maternal CYP450 genes and smoking for the risk of total CHD in offspring are summarized in Table 4. For rs4646903, there were significant interactions for risk of total CHD in offspring between the variant genotype (G/A + G/G) and passive smoking before pregnancy ($OR_{adj} = 1.98$, 95%CI: 1.28–3.07; $P = 0.002$).

For rs1065852, significant interactions were found between the variant genotype (G/A + A/A) and smoking experiences including active ($OR_{adj} = 3.57$, 95%CI: 1.45–8.82; $P = 0.006$) and passive ($OR_{adj} = 2.19$, 95%CI: 1.41–3.39; $P = 0.000$) smoking before pregnancy, and passive smoking ($OR_{adj} = 3.42$, 95%CI: 2.17–5.40; $P = 0.000$) during early pregnancy.

For rs16947, the data suggested significant interactions for risk of total CHD in offspring between the variant genotype (A/G + A/A) and passive smoking before pregnancy ($OR_{adj} = 2.72$, 95%CI: 1.72–4.32; $P = 0.000$) or in early pregnancy ($OR_{adj} = 3.91$; 95% CI: 2.31–6.63; $P = 0.002$).

4. Discussion

Owing to the growing prevalence and the large disease burden, the past few years have seen a rapidly growing interest in exploring the etiology of CHD. Although more and more researchers supported that CHD is a result of multiple factors and caused by genetic and environmental factors, the exact pathogeny remains not elucidated. Our study aimed to examine whether maternal active and passive smoking, as well as CYP450 genetic variants, were significantly associated with the risk of CHD and its specific subtypes in offspring, and assess the interaction effects between maternal smoking and CYP450 genetic variants for the risk of developing CHD in offspring. As we know, this study is the first time to assess the association of maternal smoking, CYP450 genes, and their interactions with the risk of CHD and specific subtypes, which will help to provide a new clue for etiological exploration and prevention of CHD.

Findings from the present study suggested that maternal smoking was significantly associated with the risk of CHD in offspring, with an increased risk of 134% for active smoking before pregnancy, 76% for passive smoking before pregnancy, and 205% for passive smoking in the early pregnancy. Additionally, maternal active and passive smoking were also significantly associated with the risk of specific CHD subtypes including ASD, VSD, AVSD, and PDA. Overall, the results in our study were consistent with previous studies on this topic.[18–20]

Table 3

Genetic variants of maternal CYP450 genes associated with risks of CHD and its subtypes in offspring.

	Total CHD		ASD		VSD		AVSD		PDA	
Maternal CYP450 genes	OR_{unadj} (95% CI)	OR_{adj} (95% CI)*	OR_{unadj} (95% CI)	OR_{adj} (95% CI)*	OR_{unadj} (95% CI)	OR_{adj} (95% CI)*	OR_{unadj} (95% CI)	OR_{adj} (95% CI)*	OR_{unadj} (95% CI)	OR_{adj} (95% CI)*
rs1048943										
T/T	1	1	1	1	1	1	1	1	1	1
T/C	1.20 (0.96–1.51)	1.06 (0.82–1.37)	1.02 (0.67–1.54)	0.90 (0.53–1.53)	1.24 (0.98–1.69)	1.10 (0.82–1.47)	1.00 (0.61–1.67)	0.78 (0.44–1.39)	1.07 (0.77–1.50)	1.03 (0.70–1.53)
C/C	0.55 (0.32–0.95)†	0.76 (0.38–1.55)	-	-	0.85 (0.48–1.51)	0.65 (0.34–1.25)	0.44 (0.10–1.90)	0.47 (0.10–2.10)	0.44 (0.17–1.13)	0.64 (0.21–2.19)
Dominant	1.11 (0.90–1.38)	0.97 (0.76–1.25)	0.88 (0.58–1.32)	0.73 (0.43–1.24)	1.25 (0.97–1.60)	1.03 (0.78–1.37)	0.93 (0.56–1.52)	0.74 (0.42–1.31)	0.99 (0.71–1.37)	0.93 (0.63–1.36)
Recessive	0.51 (0.30–0.87)†	0.74 (0.33–1.59)	-	-	0.76 (0.43–1.33)	0.63 (0.33–1.19)	0.44 (0.11–1.87)	0.52 (0.12–2.30)	0.42 (0.17–1.09)	0.52 (0.17–1.97)
Additive	0.99 (0.83–1.19)	0.89 (0.72–1.09)	0.76 (0.53–1.08)	0.63 (0.40–1.01)	1.12 (0.91–1.37)	0.96 (0.76–1.21)	0.86 (0.57–1.31)	0.74 (0.46–1.21)	0.90 (0.68–1.18)	0.83 (0.60–1.15)
rs4646903										
A/A	1	1	1	1	1	1	1	1	1	1
G/A	1.09 (0.85–1.39)	1.14 (0.86–1.51)	0.76 (0.50–1.17)	0.99 (0.58–1.70)	1.45 (1.09–1.95)†	1.35 (0.97–1.89)	0.83 (0.48–1.42)	0.72 (0.40–1.32)	0.89 (0.61–1.30)	0.90 (0.58–1.41)
G/G	1.24 (0.91–1.71)	1.33 (0.92–1.92)	0.42 (0.20–1.24)	0.43 (0.17–1.11)	1.65 (1.14–2.39)†	1.79 (1.17–2.72)†	0.89 (0.43–1.83)	0.89 (0.40–2.00)	1.60 (1.02–2.49)†	1.82 (1.07–3.08)†
Dominant	1.13 (0.90–1.42)	1.19 (0.91–1.55)	0.68 (0.45–1.02)	0.85 (0.50–1.43)	1.51 (1.14–1.99)†	1.46 (1.06–2.01)†	0.84 (0.51–1.40)	0.76 (0.43–1.34)	1.07 (0.75–1.51)	1.12 (0.74–1.69)
Recessive	1.18 (0.89–1.56)	1.22 (0.88–1.69)	0.49 (0.25–1.57)	0.43 (0.17–1.07)	1.30 (0.95–1.78)	1.48 (1.03–2.12)†	1.00 (0.52–1.91)	1.07 (0.51–2.24)	1.72 (1.17–2.52)†	1.93 (1.22–3.05)†
Additive	1.11 (0.95–1.30)	1.15 (0.96–1.38)	0.69 (0.51–1.08)	0.76 (0.52–1.12)	1.30 (1.08–1.56)†	1.34 (1.09–1.65)†	0.92 (0.64–1.32)	0.89 (0.59–1.33)	1.23 (0.98–1.55)	1.31 (0.99–1.72)
rs1065852										
G/G	1	1	1	1	1	1	1	1	1	1
G/A	1.60 (1.23–2.08)†	1.46 (1.07–1.99)†	2.28 (1.35–3.86)†	1.94 (1.04–3.60)†	1.62 (1.18–2.21)†	1.38 (0.96–1.97)	1.61 (0.85–3.06)	1.14 (0.56–2.33)	1.30 (0.86–1.94)	0.99 (0.62–1.58)
A/A	1.59 (1.16–2.17)†	1.63 (1.15–2.33)†	1.14 (0.58–2.24)	0.60 (0.25–1.46)	1.90 (1.33–2.72)†	1.96 (1.31–2.94)†	2.00 (0.99–4.07)	1.97 (0.91–4.31)	1.65 (1.05–2.59)†	1.42 (0.84–2.40)
Dominant	1.59 (1.24–2.05)†	1.52 (1.13–2.03)†	1.92 (1.15–3.20)†	1.50 (0.82–2.75)	1.71 (1.27–2.30)†	1.56 (1.12–2.19)†	1.74 (0.85–3.19)	1.37 (0.70–2.68)	1.41 (0.96–2.06)	1.12 (0.72–1.73)
Recessive	1.16 (0.90–1.50)	1.27 (0.95–1.70)	0.63 (0.37–1.10)	0.69 (0.42–1.17)	1.38 (1.04–1.83)†	1.59 (1.15–2.19)†	1.46 (0.85–2.50)	1.80 (0.99–3.26)	1.39 (0.97–2.01)	1.43 (0.93–2.20)
Additive	1.26 (1.08–1.48)†	1.28 (1.07–1.52)†	1.10 (0.83–1.47)	0.88 (0.60–1.28)	1.37 (1.15–1.64)†	1.40 (1.15–1.72)†	1.40 (0.99–1.97)	1.44 (0.96–2.14)	1.28 (1.02–1.61)†	1.19 (0.91–1.56)
rs16947										
G/G	1	1	1	1	1	1	1	1	1	1
A/G	1.46 (1.14–1.86)†	1.19 (0.90–1.59)	2.30 (1.51–3.50)†	1.99 (1.18–3.38)†	1.42 (1.07–1.87)†	1.02 (0.74–1.42)	0.57 (0.27–1.19)	0.44 (0.13–1.03)	1.34 (0.93–1.93)	0.95 (0.62–1.47)
A/A	2.12 (1.31–3.42)†	3.61 (2.09–6.23)†	1.16 (0.39–3.41)	2.88 (0.74–11.32)	1.90 (1.10–3.28)†	3.35 (1.79–6.24)†	6.85 (3.51–13.36)†	13.67 (6.03–30.97)†	1.88 (0.94–3.74)	3.84 (1.71–8.62)†
Dominant	1.55 (1.23–1.95)†	1.46 (1.12–1.90)†	2.13 (1.42–3.22)†	2.05 (1.23–3.43)†	1.48 (1.14–1.93)†	1.25 (0.93–1.70)	1.46 (0.88–2.43)	1.18 (0.67–2.07)	1.40 (0.99–1.99)	1.18 (0.79–1.77)
Recessive	1.89 (1.17–3.04)†	3.44 (2.00–5.91)†	0.86 (0.30–2.50)	2.27 (0.58–8.86)	1.71 (0.99–2.93)	3.33 (1.79–6.17)†	7.74 (4.02–14.90)†	16.52 (7.37–37.00)†	1.72 (0.87–3.40)	3.88 (1.74–8.66)†
Additive	1.46 (1.21–1.75)†	1.53 (1.24–1.89)†	1.63 (1.18–2.27)†	1.88 (1.21–2.93)†	1.40 (1.13–1.72)†	1.38 (1.09–1.76)†	1.98 (1.40–2.81)†	2.06 (1.37–3.08)†	1.36 (0.85–1.78)	1.37 (0.98–1.90)

ASD = atrial septal defect, AVSD = atrioventricular septal defect, CHD = congenital heart defect, CI = confidence interval, CYP450 = cytochrome P450, OR_{adj} = adjusted odds ratio, PDA = patent ductus arteriosus, VSD = ventricular septal defect.

* Adjusted for maternal education level, income, residence, abnormal pregnancy history, family history of inbreeding and congenital malformations, personal medical history before or during this pregnancy, personal lifestyle and habit before this pregnancy and exposure history to environmental hazardous substance as well as spouse's education level, and smoking and drinking history, which were presented in Supplemental Table 1, http://links.lww.com/MD/G191.

† Statistically significant (a = 0.05).

Table 4

Interactions of maternal CYP450 genes and maternal smoking associated with risk of total CHD.

Maternal CYP450 genes*	Active smoking before pregnancy				Passive smoking before pregnancy				Passive smoking in early pregnancy			
	Status	OR_{adj} (95%CI)†	*P*	β	Status	OR_{adj} (95%CI)†	*P*	β	Status	OR_{adj} (95%CI)†	*P*	β
rs1048943												
w	No	1			No	1			No	1		
v	No	1.05 (0.79–1.40)	0.746	0.047 (β_g)	No	1.19 (0.81–1.74)	0.382	0.171 (β_g)	No	0.81 (0.58–1.13)	0.215	−0.213 (β_g)
w	Yes	3.08 (1.18–8.03)	0.021	1.126 (β_e)	Yes	1.75 (1.19–2.59)	0.005	0.561 (β_e)	Yes	1.63 (1.08–2.44)	0.019	0.487 (β_e)
v	Yes	1.02 (0.28–3.76)	0.973	0.022 (β_{g*e})	Yes	1.55 (1.00–2.41)	0.049	0.439 (β_{g*e})	Yes	3.94 (2.26–6.86)	0.000	1.370 (β_{g*e})
rs4646903												
w	No	1			No	1			No	1		
v	No	1.38 (1.02–1.88)	0.040	0.324 (β_g)	No	1.26 (0.83–1.91)	0.276	0.231 (β_g)	No	1.00 (0.69–1.44)	0.991	−0.002 (β_g)
w	Yes	15.34 (2.24–85.17)	0.005	2.731 (β_e)	Yes	1.50 (0.90–2.49)	0.117	0.405 (β_e)	Yes	1.31 (0.76–2.23)	0.329	0.267 (β_e)
v	Yes	1.43 (0.57–3.63)	0.450	0.359 (β_{g*e})	Yes	1.98 (1.28–3.07)*	0.002	0.684 (β_{g*e})	Yes	3.51 (2.18–5.65)	0.000	1.255 (β_{g*e})
rs1065852												
w	No	1			No	1			No	1		
v	No	1.47 (1.06–2.06)	0.023	0.387 (β_g)	No	1.50 (0.97–2.32)	0.067	0.407 (β_g)	No	1.60 (1.08–2.35)	0.018	0.468 (β_g)
w	Yes	1.25 (0.28–5.59)	0.773	0.221 (β_e)	Yes	1.57 (0.87–2.86)	0.136	0.454 (β_e)	Yes	3.15 (1.59–6.22)	0.001	1.147 (β_e)
v	Yes	3.57 (1.45–8.82)‡	0.006	1.273 (β_{g*e})	Yes	2.19 (1.41–3.39)‡	0.000	0.783 (β_{g*e})	Yes	3.42 (2.17–5.40)‡	0.000	1.231 (β_{g*e})
rs16947												
w	No	1			No	1			No	1		
v	No	1.58 (1.16–2.14)	0.003	0.456 (β_g)	No	1.23 (0.82–1.85)	0.318	0.207 (β_g)	No	1.44 (1.01–2.06)	0.046	0.364 (β_g)
w	Yes	2.77 (1.12–6.85)	0.027	1.019 (β_e)	Yes	1.31 (0.91–1.87)	0.146	0.267 (β_e)	Yes	2.23 (1.51–3.31)	0.000	0.803 (β_e)
v	Yes	1.89 (0.49–7.29)	0.358	0.634 (β_{g*e})	Yes	2.72 (1.72–4.32)‡	0.000	1.001 (β_{g*e})	Yes	3.91 (2.31–6.63)‡	0.000	1.364 (β_{g*e})

CHD = congenital heart defect, CI = confidence interval, CYP450 = cytochrome P450, OR_{adj} = adjusted odds ratio.

* Single nucleotide polymorphisms were classified as wild type (w) and variant genotype (v).

† Adjusted for maternal education level, income, residence, abnormal pregnancy history, family history of inbreeding and congenital malformations, personal medical history before or during this pregnancy, personal lifestyle and habit before this pregnancy and exposure history to environmental hazardous substance as well as spouse's education level, and smoking and drinking history, which were presented in Supplemental Table 1, http://links.lww.com/MD/G191.

‡ There was a statistically significant interaction between maternal CYP450 gene and smoking experiences on the development of total CHD in offspring.

However, different from previous studies, our study focused not only on the risk of total CHD but also on the risk of specific subtypes that were not considered by previous studies. Again, most of the previous studies focused only on maternal active smoking and did not assess maternal passive smoking.

Although both the present study and previous studies[18–20] indicated that maternal tobacco exposure significantly increased the risk of developing CHD in offspring, the exact mechanisms are still unclear and warrant future research. According to epidemiological studies and animal experiences, 2 possible hypotheses were proposed. One hypothesis is that anomalous hemodynamics caused by tobacco compounds might influence the development of the fetal cardiovascular system.[21] For example, the vasoconstrictor action of nicotine can lead to embryo hypoxia, elevated fetal blood pressure, decreased placental blood flow, and then the function of aortic muscle and myocardia will be affected.[22] The other hypothesis is that changes in related genes may increase the risk of CHD. An animal experience indicated that nicotine could suppress the expressions of cardiac development-related genes, TBX5 and GATA4, by promoter DNA hypermethylation, and then the differentiation of myocardia was inhibited.[23–25] These evidences all indicated that maternal smoking could increase the risk of CHD, and this research supports these inferences.

The meta-analysis of Zhao et al reported that mothers exposed to passive smoking are at higher risk of CHD than those exposed to active smoking (OR: 2.24 vs 1.25), while our results are opposite.[26] Some evidence suggests that side-stream smoke, caused by passive smoking, can inhibit the expression of GATA4 at a non-cytotoxic concentration.[23] However, this hypothesis neglects differences in metabolic mechanism and pathway between side-stream and main-stream smoke. So the difference of harmfulness between active and passive smoking needs more epidemiological and physiological researches.

The results of this research suggested that polymorphisms of CYP1A1 at rs4646903 and CYP2D6 at rs1065852 and rs16947 were positively associated with susceptibility toward CHD and specific subtypes in offspring. It is remarkable that our study also found significant interactions between maternal smoking and polymorphisms of CYP450 genes in the development of CHD in offspring.

After literature retrieval, CYP450 genes are associated with kinds of cancers, including liver cancer,[27] gastric cancer,[28] cervical cancer,[29] and so on. Several other studies reported that CYP450 genetic polymorphisms were significantly related to adverse pregnancy outcomes. A case–control study presented that mutant alleles of maternal CYP450 genes can increase the risk of preterm birth.[30] Chen et al suggested that genetic polymorphisms of CYP1A1 may be correlated with susceptibility to low birth weight.[31] While, up to date, there has been a blank about the associations between maternal CYP450 genes and CHD in offspring and our research fills this gap. As for interactions between maternal smoking and CYP450 genes, interact effects were significantly associated with risk of oncohematological diseases,[32] spontaneous preterm delivery,[33]and so on. While the result of Wang et al was different in that they found an interaction between maternal passive smoking and CYP1A1 mutant gene was not significantly associated with birth defects, which might be because of their limited sample size and statistical power.[13] These interactions including our findings are

all statistical interactions, which cannot represent biological interactions. Further physiological researches are needed.

Teratogenesis is a multi-step and multifactorial process that indicated different genetic alterations and several biological pathways. Thus, it is believed that influencing factors of CHD interact with each other. As the most important phase I metabolic enzymes, the CYP450 superfamily exists in all kinds of cells. It has been confirmed that the activity of CYP450 enzymes encoded by mutational genotypes is several times higher than that encoded by wild types.[34] So that mutations in CYP450 genes can encode more enzymes to activate exogenous compounds. Based on this characteristic, we supposed that teratogenic intermediate metabolites of nicotine and other tobacco compounds accumulate in the maternal body and affect the development of the embryo.

Limitations also existed in this research. At first, because cases and controls in this study were recruited from different hospital departments, the balance of baseline characteristics between the 2 groups was influenced. However, we adjusted the baseline data when exploring the associations and interactions of maternal smoking and genetic variants of CYP450 genes with the development of CHD and specific subtypes in CHD. Secondly, because of the lack of primers, 3 SNPs were replaced according to the theory of linkage disequilibrium. This might cause confounding bias that overestimate the statistical correlation of our findings.[35] Thirdly, due to the restricted sample size, data of maternal active smoking in the early pregnancy and interactions on specific subtypes lacked. What is more, compared with the analyses of main effects, the sample size required for interaction is larger. Our limited sample size affected the statistical power of interactions, and increased the possibility of false-negative results in interaction analyses. Lastly, because of the recall bias, we could not evaluate the exact number of cigarettes that mothers were exposed to. The lack of a dose–response relationship limited the depth of the research.

5. Conclusions

This study presents that maternal active and passive smoking before or in the early pregnancy is positively associated with the development of CHD and specific subtypes in offspring. In addition, our study supports a significant association between maternal CYP450 genetic polymorphisms and the risk of CHD and specific subtypes in offspring. Interactions between maternal smoking and CYP450 genetic polymorphisms associated with CHD were observed, which suggested that the effects of CYP450 genes on the risk of CHD may be modified by maternal smoking. However, these findings need more population studies and in vitro and in vivo experiments to test and verify.

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