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

Jingyi Diao, MDα, Lijuan Zhao, MDα, Liu Luo, MDα, Jinqi Li, MDα, Yihuan Li, MDα, Senmao Zhang, PHDα, Tingting Wang, PhDα, Letao Chen, PhDα, Peng Huang, MDβ,*, Jiabi Qin, PhDα,c,d,e,∗

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 (ORadj = 2.34, 95%CI: 1.19–4.60) or passive (ORadj = 1.76, 95%CI: 1.34–2.31) smoking before pregnancy, passive smoking in the early pregnancy (ORadj = 3.05, 95%CI: 2.26–4.12), as well as polymorphisms of CYP450 at rs1065852 (G/A vs G/G: ORadj = 1.46, 95%CI: 1.07–1.99; A/A vs G/G: ORadj = 1.63, 95%CI: 1.15–2.33) and rs16947 (A/A vs G/G: ORadj = 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 = atroventricular septal defect, CHD = congenital heart disease, CI = confidence interval, CYP450 = cytochrome P450, HWE = Hardy–Weinberg equilibrium, OR = odds ratio, ORadj = adjusted OR, ORunadj = 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
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 association 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, and tobacco compounds (eg, polycyclic aromatic hydrocarbons, dioxins).[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 susceptibilities 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 confirmed by some professionally trained investigators. Eligible women were recruited when their children were less than 1-year old. The recall bias of exposure by mothers during the pre-pregnancy was defined as smoking for more than 6 months continuously or defined as there are smokers in the maternal immediate household, 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/day. 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. As a result, these genetic loci including rs1048943 and rs4646903 of CYP1A1 gene as well as rs1063852 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 (r² = 1.00), rs3751210 to replace rs1063852 (r² = 0.900), and rs4147641 to replace rs16947 (r² = 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 rs1063852; 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 (ORunadj) were calculated by univariate logistic regression. Adjusted ORs (ORadj) 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 to build and explain models of gene-environment interactions. Interaction coefficient \( (\gamma) \) was calculated by regression coefficient \( (\beta) \) from logistic regression analysis \( (\gamma_1 = \beta_0 + \beta_1X) \) and \( (\gamma_2 = \beta_0 + \beta_2X) \) and was used to evaluate the interaction. When all \( \gamma \) values were more than 1, there was a positive interaction; when all \( \gamma \) values were less than 1, there was a negative interaction; and when the \( \gamma \) 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.

| Baseline characteristics                          | Controls Total CHD | Controls ASD | Controls VSD | Controls AVSD | Controls PDA |
|----------------------------------------------------|--------------------|--------------|--------------|---------------|--------------|
| Demographic characteristics                       | N (%)              | N (%)        | N (%)        | N (%)         | N (%)        |
| Child-bearing age (years)                          |                    |              |              |               |              |
| <35                                                | 571 (85.7)         | 566 (86.5)   | 0.671        | 88 (80.0)     | 0.119        |
| ≥35                                                | 95 (14.3)          | 88 (13.5)    | 22 (20.0)    | 50 (12.5)     | 13 (18.3)    |
| Education level                                    |                    |              |              |               |              |
| Less than primary or primary                       | 9 (1.4)            | 95 (14.5)    | 0.000        | 34 (30.9)     | 0.000        |
| Junior high school                                 | 134 (20.1)         | 273 (41.7)   | 43 (39.1)    | 184 (45.9)    | 23 (32.4)    |
| Senior middle school                               | 224 (33.6)         | 185 (28.3)   | 19 (17.3)    | 114 (28.4)    | 23 (32.4)    |
| College or above                                   | 299 (44.9)         | 101 (15.4)   | 14 (12.7)    | 63 (15.7)     | 17 (23.9)    |
| Annual household income in the past 1 year (RMB)  |                    |              |              |               |              |
| <50,000                                            | 182 (27.3)         | 530 (81.0)   | 0.000        | 99 (90.0)     | 0.000        |
| 50,000–100,000                                     | 292 (43.8)         | 92 (14.1)    | 5 (4.5)      | 59 (14.7)     | 10 (14.1)    |
| 100,000–150,000                                    | 65 (9.8)           | 14 (2.1)     | 4 (3.6)      | 10 (2.5)      | 0            |
| >150,000                                           | 127 (19.1)         | 18 (2.8)     | 2 (1.8)      | 16 (4.0)      | 0            |
| Residence                                          |                    |              |              |               |              |
| Rural                                              | 366 (55.0)         | 495 (75.7)   | 0.000        | 86 (78.2)     | 0.000        |
| Urban                                              | 300 (45.0)         | 159 (24.3)   | 24 (21.8)    | 100 (24.9)    | 0.000        |
| Abnormal pregnancy history                         |                    |              |              |               |              |
| History of spontaneous abortion                    |                    |              |              |               |              |
| No                                                 | 610 (91.6)         | 577 (88.2)   | 0.042        | 101 (91.8)    | 0.937        |
| Yes                                                | 56 (8.4)           | 77 (11.8)    | 9 (8.2)      | 48 (12.0)     | 11 (15.5)    |
| History of induced abortion                        |                    |              |              |               |              |
| No                                                 | 441 (66.2)         | 371 (56.7)   | 0.000        | 57 (51.8)     | 0.004        |
| Yes                                                | 225 (33.8)         | 283 (43.3)   | 53 (48.2)    | 164 (40.9)    | 36 (50.7)    |
| History of stillbirth                              |                    |              |              |               |              |
| No                                                 | 663 (99.5)         | 630 (96.3)   | 0.000        | 107 (97.3)    | 0.040        |
| Yes                                                | 3 (0.5)            | 24 (3.7)     | 3 (0.5)      | 13 (3.2)      | 3 (4.2)      |
| History of preterm birth                           |                    |              |              |               |              |
| No                                                 | 662 (99.4)         | 645 (98.6)   | 0.154        | 104 (94.5)    | 0.001        |
| Yes                                                | 4 (0.6)            | 9 (1.4)      | 6 (5.5)      | 3 (0.7)       | 0            |
| History of low birth weight                        |                    |              |              |               |              |
| No                                                 | 664 (99.7)         | 648 (99.1)   | 0.175        | 107 (97.3)    | 0.022        |
| Yes                                                | 2 (0.3)            | 6 (0.9)      | 3 (2.7)      | 3 (0.7)       | 0            |
| History of gestational diabetes                    |                    |              |              |               |              |
| No                                                 | 644 (96.7)         | 594 (90.8)   | 0.000        | 102 (92.7)    | 0.059        |
| Yes                                                | 22 (3.3)           | 60 (9.2)     | 8 (7.3)      | 44 (11.0)     | 6 (8.5)      |
| History of gestational hypertension                |                    |              |              |               |              |
| No                                                 | 654 (98.2)         | 610 (93.3)   | 0.000        | 107 (97.3)    | 0.458        |
| Yes                                                | 12 (1.8)           | 44 (6.7)     | 3 (2.7)      | 34 (8.5)      | 3 (4.2)      |
| Family history                                     |                    |              |              |               |              |
| Consanguineous marriages                           |                    |              |              |               |              |
| No                                                 | 664 (99.7)         | 627 (95.9)   | 0.000        | 102 (92.7)    | 0.000        |
| Yes                                                | 2 (0.3)            | 27 (4.1)     | 8 (7.3)      | 15 (3.7)      | 4 (5.6)      |
| Congenital malformations                           |                    |              |              |               |              |
| No                                                 | 661 (99.2)         | 614 (93.9)   | 0.000        | 93 (84.5)     | 0.000        |
| Yes                                                | 5 (0.8)            | 40 (6.1)     | 17 (15.5)    | 21 (5.2)      | 2 (2.8)      |
| Personal medical history                           |                    |              |              |               |              |
| Pre-gestational diabetes mellitus                  |                    |              |              |               |              |
| No                                                 | 637 (95.6)         | 561 (85.8)   | 0.000        | 91 (82.7)     | 0.000        |
| Yes                                                | 29 (4.4)           | 93 (14.2)    | 19 (17.3)    | 50 (12.5)     | 15 (21.1)    |
| Congenital malformations                           |                    |              |              |               |              |
| No                                                 | 664 (99.7)         | 648 (99.1)   | 0.175        | 107 (97.3)    | 0.022        |
| Yes                                                | 2 (0.3)            | 6 (0.9)      | 3 (2.7)      | 3 (0.7)       | 0            |
| Cold history in the early pregnancy                |                    |              |              |               |              |
| No                                                 | 530 (79.6)         | 437 (66.8)   | 0.000        | 68 (61.8)     | 0.000        |
| Yes                                                | 136 (20.4)         | 217 (33.2)   | 42 (38.2)    | 141 (35.2)    | 17 (23.9)    |
| Fever history in the early pregnancy               |                    |              |              |               |              |
| No                                                 | 643 (96.5)         | 591 (90.4)   | 0.000        | 104 (94.5)    | 0.283        |
| Yes                                                | 23 (3.5)           | 63 (9.6)     | 6 (5.5)      | 45 (11.2)     | 12 (16.9)    |
| Folate intake                                      |                    |              |              |               |              |
| No                                                 | 621 (93.2)         | 541 (82.7)   | 0.000        | 77 (70.0)     | 0.000        |
| Yes                                                | 39 (6.8)           | 126 (17.3)   | 14 (12.7)    | 40 (11.2)     | 3 (4.2)      |
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 (ORadj = 2.34; 95%CI: 1.19–4.60) and its 2 subtypes including ASD (ORadj = 4.57; 95%CI: 1.75–11.97) and VSD (ORadj = 2.60; 95%CI: 1.26–5.37) in offspring. Risks of total CHD (ORadj = 1.76; 95%CI: 1.34–2.31) and its 2 subtypes including ASD (ORadj = 1.88; 95%CI: 1.46–2.68) and PDA (ORadj = 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 (ORadj = 3.05; 95%CI: 2.26–4.12) and its 4 subtypes including ASD (ORadj = 1.88; 95%CI: 1.09–3.15), VSD (ORadj = 2.92; 95%CI: 2.10–4.05), AVSD (ORadj = 3.04; 95%CI: 1.67–5.54), and PDA (ORadj = 3.67; 95%CI: 2.44–5.51) in offspring.

### Table 2 (continued).

| Baseline characteristics | Controls | Total CHD | ASD | VSD | AVSD | PDA |
|--------------------------|----------|----------|-----|-----|------|-----|
| N (%)                    | N (%)    | N (%)    | N (%)| N (%)| N (%)| N (%)|
| 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 (73.3) | 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) | 0.000 | 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 |

Diao et al. Medicine (2021) 100:23 www.md-journal.com

ASD = atrial septal defect, AVSD = atrioventricular septal defect, CHD = congenital heart defect, PDA = patent ductus arteriosus, VSD = ventricular septal defect.
Table 4

| Maternal smoking | OR (95% CI) | OR adj (95% CI) |
|------------------|------------|----------------|
| Active smoking before pregnancy | | |
| Yes | 3.44 | 2.31 |
| No | 1.00 | 1.00 |
| Passive smoking before pregnancy | | |
| Yes | 1.84 | 1.76 |
| No | 1.00 | 1.00 |

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 (p = 0.05).

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 pathogenesis 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 etiologic 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.13–20

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 (χ² = 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 had 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 the 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).
### Table 3

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

| Maternal CYP450 genes | Total CHD (95% CI) | ASD (95% CI) | AVS (95% CI) | VSD (95% CI) | AVD (95% CI) | PDA (95% CI) |
|-----------------------|-------------------|-------------|-------------|-------------|-------------|-------------|
| rs1048943             |                   |             |             |             |             |             |
| T/G                   |                   |             |             |             |             |             |
| rs1065852             |                   |             |             |             |             |             |
| G/A                   |                   |             |             |             |             |             |
| rs16947               |                   |             |             |             |             |             |

A/G: Adjusted for maternal education level, income, residence, abnormal pregnancy history, family history of inbreeding and consanguineous marriages, 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/MG/G191.

a Statistically significant (p < 0.05).
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 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. 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. The other hypothesis is that changes in related genes may increase the risk of CHD. An animal experiment indicated that nicotine could suppress the expression of cardiac development-related genes, TNFα and GATA4, by promoting DNA hypermethylation, and then the differentiation of myocardia was inhibited. 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. Some evidence suggests that side-stream smoke, caused by passive smoking, can inhibit the expression of GATA4 at a non-cytotoxic concentration. 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 rs106497 were positively associated with susceptibility of congenital heart defect, while rs1065852 and rs16947 were negatively associated with susceptibility toward CHD and adverse pregnancy outcomes. A case-control study presented that mutant alleles of maternal CYP450 genes can increase the risk of preterm birth. However, different from previous studies, our study was focused not only on maternal active smoking (ORadj = 2.24 vs 1.25) but also on maternal passive smoking (ORadj = 1.43 vs 0.57) and passive smoking in early pregnancy (ORadj = 2.77 vs 1.12).

### Table 4

| Maternal CYP450 genes | Status | Active smoking before pregnancy | Passive smoking before pregnancy | Passive smoking in early pregnancy |
|-----------------------|--------|---------------------------------|----------------------------------|----------------------------------|
| **ORadj**(95%CI)      | **P**  | **β**                           | **ORadj**(95%CI)                | **P**                           | **β**                           |
| rs1048943             |        |                                 |                                  |                                 |                                 |
| w                     | No     | 1.05 (0.79–1.40)                | 0.746                            | 0.047 (β)                       | No                              | 1.19 (0.81–1.74)                | 0.382                            | 0.171 (β)                       | No                              | 0.81 (0.58–1.33)                | 0.215                            | 0.213 (β)                       |
| v                     | No     | 3.81 (1.98–7.03)                | 0.021                            | 1.126 (β)                       | Yes                             | 1.75 (1.19–2.59)                | 0.005                            | 0.561 (β)                       | Yes                             | 1.63 (1.09–2.44)                | 0.168                            | 0.487 (β)                       |
| v                     | Yes    | 1.02 (0.28–3.76)                | 0.973                            | 0.022 (β)                       | Yes                             | 1.55 (1.00–2.41)                | 0.049                            | 0.439 (β)                       | Yes                             | 3.94 (2.26–6.86)                | 0.000                            | 1.370 (β)                       |
| rs4646903             |        |                                 |                                  |                                 |                                 |                                 |                                  |                                 |                                 |                                 |                                 |                                 |
| w                     | No     | 1.38 (1.02–1.88)                | 0.040                            | 0.324 (β)                       | No                              | 1.26 (0.83–1.91)                | 0.276                            | 0.231 (β)                       | No                              | 1.00 (0.69–1.44)                | 0.991                            | 0.002 (β)                       |
| v                     | Yes    | 15.34 (2.24–85.17)              | 0.005                            | 2.731 (β)                       | Yes                             | 1.50 (0.90–2.49)                | 0.117                            | 0.405 (β)                       | Yes                             | 1.31 (0.76–2.32)                | 0.329                            | 0.267 (β)                       |
| v                     | Yes    | 1.43 (0.57–3.63)                | 0.450                            | 0.359 (β)                       | Yes                             | 1.98 (1.28–3.07)                | 0.002                            | 0.684 (β)                       | Yes                             | 3.51 (2.18–5.65)                | 0.000                            | 1.255 (β)                       |
| rs1065852             |        |                                 |                                  |                                 |                                 |                                 |                                  |                                 |                                 |                                 |                                 |                                 |
| w                     | No     | 1.47 (1.06–2.06)                | 0.023                            | 0.387 (β)                       | No                              | 1.50 (0.97–2.32)                | 0.067                            | 0.407 (β)                       | No                              | 1.60 (1.08–2.35)                | 0.018                            | 0.468 (β)                       |
| v                     | Yes    | 1.25 (0.82–5.59)                | 0.773                            | 0.221 (β)                       | Yes                             | 1.57 (0.87–2.86)                | 0.136                            | 0.454 (β)                       | Yes                             | 3.15 (1.59–6.22)                | 0.001                            | 1.147 (β)                       |
| v                     | Yes    | 3.57 (1.45–8.82)                | 0.006                            | 1.273 (β)                       | Yes                             | 2.19 (1.41–3.39)                | 0.000                            | 0.783 (β)                       | Yes                             | 3.42 (2.17–5.40)                | 0.000                            | 1.231 (β)                       |
| rs16947               |        |                                 |                                  |                                 |                                 |                                 |                                  |                                 |                                 |                                 |                                 |                                 |
| w                     | No     | 1.58 (1.16–2.14)                | 0.003                            | 0.456 (β)                       | No                              | 1.23 (0.82–1.85)                | 0.318                            | 0.207 (β)                       | No                              | 1.44 (1.01–2.06)                | 0.046                            | 0.364 (β)                       |
| v                     | Yes    | 2.77 (1.12–6.85)                | 0.027                            | 1.019 (β)                       | Yes                             | 1.31 (0.91–1.87)                | 0.146                            | 0.267 (β)                       | Yes                             | 2.23 (1.51–3.31)                | 0.000                            | 0.803 (β)                       |
| v                     | Yes    | 1.89 (0.49–7.29)                | 0.358                            | 0.634 (β)                       | Yes                             | 2.72 (1.72–4.42)                | 0.000                            | 1.001 (β)                       | Yes                             | 3.91 (2.31–6.63)                | 0.000                            | 1.364 (β)                       |

CHD = congenital heart defect, CI = confidence interval, CYP450 = cytochrome P450, ORadj = 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, smoking and drinking history, which were presented in Supplemental Table 1.
- There was a statistically significant interaction between maternal CYP450 gene and smoking experiences on the development of total CHD in offspring.
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. 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. 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 all statistical interactions, which cannot represent biological interactions. Further physiological researches are needed.

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. Interactions between maternal smoking and genetic variants of CYP450 genes with the risk of CHD and congenital heart defects in the offspring of smoking mothers: a population-based study. J Pediatr 2015;166:978–84.

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.

Acknowledgments
The authors would like to thank the editors and reviewers for their suggestions and all colleagues working in Maternal and Child Health Promotion and Birth Defect Prevention Group.

Investigation: Jingyi Diao, Lijuan Zhao, Liu Luo, Jinqi Li, Yihuan Li, Senmao Zhang, Tingting Wang, Letao Chen, Peng Huang, Jiabi Qin.
Methodology: Jingyi Diao, Lijuan Zhao, Jiabi Qin.
Project administration: Jingyi Diao, Lijuan Zhao, Peng Huang, Jiabi Qin.
Resources: Jingyi Diao, Peng Huang, Jiabi Qin.
Software: Liu Luo, Jinqi Li, Jiabi Qin.
Supervision: Jingyi Diao, Liu Luo, Jinqi Li, Yihuan Li, Peng Huang, Jiabi Qin.
Validation: Jingyi Diao, Peng Huang, Jiabi Qin.
Visualization: Jingyi Diao, Peng Huang, Jiabi Qin.
Writing – original draft: Jingyi Diao.
Writing – review & editing: Jingyi Diao, Peng Huang, Jiabi Qin.

References
[1] Liu Y, Chen S, Zühlke L, et al. Global birth prevalence of congenital heart defects 1970–2017: updated systematic review and meta-analysis of 260 studies. Int J Epidemol 2019;48:435–43.
[2] Zhao QM, Liu F, Wu L, Ma XJ, Niu C, Huang GY. Prevalence of congenital heart disease at live birth in China. J Pediatr 2019;204:53–8.
[3] Alberman ED, Goldstein H. Possible teratogenic effect of cigarette smoking. Nature 1971;231:529–30.
[4] Alverson CJ, Strickland MJ, Gilboa SM, Correa A. Maternal smoking and congenital heart defects in the Baltimore-Washington Infant Study. Pediatrics 2011;127:e647–53.
[5] Sullivan PM, Dervan LA, Reiger S, Buddle S, Schwartz SM. Risk of congenital heart defects in the offspring of smoking mothers: a population-based study. J Pediatr 2015;166:978–84.
[6] Yang L, Mao ZZ, Elisa KT, et al. Studying on the status of pregnant women being exposed to smoking and influencing factors on related behaviors. Chin Health Serv Manag 2009;26:771–3.
[7] Santonj Y, Nishino H. Inhibition of the enzyme activity of cytochrome P450 1A1, 1A2 and 3A4 by fucosanthen, a marine carotenoid. Oncol Lett 2013;6:860–4.
[8] Pavek P, Dvorak Z. Xenobiotic-induced transcriptional regulation of xenobiotic metabolizing enzymes of the cytochrome P450 superfamily in human extrahepatic tissues. Curr Drug Metab 2008;9:129–43.
[9] Rodriguez-Antona C, Ingelman-Sundberg M. Cytochrome P450 pharmacogenetics and cancer. Oncogene 2006;25:1679–81.
[10] McGraw J, Waller D. Cytochrome P450 variations in different ethnic populations. Expert Opin Drug Metab Toxicol 2012;8:571–82.
[11] Taspinar M, Aydos SE, Gomez O, Elhan AH, Karabulut HG, Sunguroglu A. CYP1A1, GST gene polymorphisms and risk of chronic myeloid leukemia. Swiss Med Wkly 2008;138:12–7.
[12] Sommer A, Blanton SH, Weymouth K, et al. Smoking, the xenobiotic pathway, and clubfoot. Birth Defects Res A Clin Mol Teratol 2011;91:20–8.
[13] Wang HY. Research on correlation of pregnant woman smoking during pregnancy and CYP1A1, GST gene polymorphism with birth defects [dissertation]. China: Southern Medical University; 2014.
[14] Abbas M, Srivastava K, Imran M, et al. Association of CYP1A1 gene variants rs4646903 (T>C) and rs1048943 (A>G) with cervical cancer in a North Indian population. Eur J Obstet Gynecol Reprod Biol 2014;176:68–74.
[15] Li M, Li A, He R, et al. Gene polymorphism of cytochrome P450 significantly affects lung cancer susceptibility. Cancer Med 2019;8:4892–905.
[16] Zhou LP, Luan H, Dong XH, Jin GJ, Man DL, Shang H. Genetic variants of CYP2D6 gene and cancer risk: a HuGe systematic review and meta-analysis. Asian Pac J Cancer Prev 2012;13:3165–72.
[17] Wallace HM. A model of gene-gene and gene-environment interactions and their implications for targeting environmental interventions by genotype. Theor Biol Med Model 2006;3:35.
[18] Karatzas AA, Giannakopoulos I, Dassios TG, Belavgenis G, Mantagos SP, Varvarigou AA. Periconceptional tobacco smoking and isolated congenital heart defects in the neonatal period. Int J Cardiol 2011;148:295–9.
[19] Hobbis CA, James SJ, Jernigan S, et al. Congenital heart defects, maternal homocysteine, smoking, and the 677 C>T polymorphism in the

Author contributions
Conceptualization: Jingyi Diao, Peng Huang, Jiabi Qin.
Data curation: Senmao Zhang, Tingting Wang, Jingyi Diao.
Formal analysis: Jingyi Diao, Lijuan Zhao, Jiabi Qin.
Funding acquisition: Jingyi Diao, Jiabi Qin.

Software: Liu Luo, Jinqi Li, Jiabi Qin.
Supervision: Jingyi Diao, Liu Luo, Jinqi Li, Yihuan Li, Peng Huang, Jiabi Qin.
Validation: Jingyi Diao, Peng Huang, Jiabi Qin.
Visualization: Jingyi Diao, Peng Huang, Jiabi Qin.
Writing – original draft: Jingyi Diao.
Writing – review & editing: Jingyi Diao, Peng Huang, Jiabi Qin.

www.md-journal.com
methylenetetrahydrofolate reductase gene: evaluating gene-environment interactions. Pediatrics 2006;194:218–24.

[20] Kuciene R, Dulskiene V. Maternal socioeconomic and lifestyle factors during pregnancy and the risk of congenital heart defects. Medicina (Kaunas) 2009;45:904–9.

[21] Piepoli M, Clark AL, Volterrani M, Adamopoulos S, Sleight P, Coats AJ. Contribution of muscle afferents to the hemodynamic, autonomic, and ventilatory responses to exercise in patients with chronic heart failure: effects of physical training. Circulation 1996;93:940–52.

[22] Cheng W, Zhou R, Feng Y, Wang Y. Mainstream smoke and sidestream smoke affect the cardiac differentiation of mouse embryonic stem cells discriminately. Toxicology 2016;357-358:1–10.

[23] Jiang XY, Feng YL, Ye LT, et al. Inhibition of Gata4 and Tbx5 by nicotine-mediated DNA methylation in myocardial differentiation. Stem Cell Rep 2017;8:290–304.

[24] Granados-Riveron JT, Pope M, Bu’Lock FA, et al. Combined mutation screening of NKX2-5, GATA4, and TBX5 in congenital heart disease: multiple heterozygosity and novel mutations. Congenit Heart Dis 2012;7:151–9.

[25] He A, Gu F, Hu Y, et al. Dynamic GATA4 enhancers shape the chromatin landscape central to heart development and disease. Nat Commun 2014;5:4907.

[26] Zhao L, Chen L, Yang T, et al. Parental smoking and the risk of congenital heart defects in offspring: an updated meta-analysis of observational studies. Eur J Prev Cardiol 2020;27:1284–93.

[27] Guo XG, Wang ZH, Dong W, He XD, Liu FC, Liu H. Specific CYP450 genotypes in the Chinese population affect sorafenib toxicity in HBV/HCV-associated hepatocellular carcinoma patients. Biomed Environ Sci 2018;31:586–95.

[28] Hidaka A, Sasazuki S, Matsuo K, et al. CYP1A1, GSTM1 and GSTT1 genetic polymorphisms and gastric cancer risk among Japanese: a case-control study within a large-scale population-based prospective study. Int J Cancer 2016;139:759–68.

[29] Li S, Li G, Kong F, et al. The association of CYP1A1 gene with cervical cancer and additional SNP-SNP interaction in Chinese Women. J Clin Lab Anal 2016;30:1220–5.

[30] Jin Y, Chen DF, Yang F, et al. Association of cytochrome P450 gene CYP1A1 polymorphism and risk of preterm delivery. Beijing Da Xue Xue Bao Yi Xue Ban 2004;36:595–9.

[31] Li S, Li G, Kong F, et al. The association of CYP1A1 gene with cervical cancer and additional SNP-SNP interaction in Chinese Women. J Clin Lab Anal 2016;30:1220–5.

[32] Chen LH, Li JH. Interactive effects of passive smoking and gene polymorphisms of CYP450 and GST on low birth weight. Maternal Child Health Care China 2012;27:2944–7.

[33] Cerliani MB, Pavicic W, Gili JA, Klein G, Saba S, Richard S. Cigarette smoking, dietary habits and genetic polymorphisms in GSTT1, GSTM1 and CYP1A1 metabolic genes: A case-control study in oncohematological diseases. World J Clin Oncol 2016;7:395–405.

[34] Luo YJ, Wen XZ, Ding P, et al. Interaction between maternal passive smoking during pregnancy and CYP1A1 and GSTs polymorphisms on spontaneous preterm delivery. PLoS One 2012;7:e49155.

[35] Leng S, Dai Y, Niu Y, et al. Effects of genetic polymorphisms of metabolic enzymes on cytokinesis-block micronucleus in peripheral blood lymphocyte among coke-oven workers. Cancer Epidemi Biomar 2004;13:1631.

[36] Ragsdale AP, Gravel S. Unbiased estimation of linkage disequilibrium from unphased data. Mol Biol Evol 2020;37:923–32.