ORIGINAL RESEARCH

First-Trimester Maternal Folic Acid Supplementation Reduced Risks of Severe and Most Congenital Heart Diseases in Offspring: A Large Case-Control Study

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BACKGROUND: Maternal folic acid supplementation (FAS) reduces the risk of neural tube defects in offspring. However, its effect on congenital heart disease (CHDs), especially on the severe ones remains uncertain. This study aimed to assess the individual and joint effect of first-trimester maternal FAS and multivitamin use on CHDs in offspring.

METHODS AND RESULTS: This is a case-control study including 8379 confirmed CHD cases and 6918 controls from 40 healthcare centers of 21 cities in Guangdong Province, China. Adjusted odds ratios (aORs) of FAS and multivitamin use between CHD cases (overall and specific CHD phenotypes) and controls were calculated by controlling for parental confounders. The multiplicative interaction effect of FAS and multivitamin use on CHDs was estimated. A significantly protective association was detected between first-trimester maternal FAS and CHDs among offspring (aOR, 0.69; 95% CI, 0.62–0.76), but not for multivitamin use alone (aOR, 1.42; 95% CI, 0.73–2.78). There was no interaction between FAS and multivitamin use on CHDs (P = 0.292). Most CHD phenotypes benefited from FAS (aORs ranged from 0.03–0.85), especially the most severe categories (ie, multiple critical CHDs [aOR, 0.16; 95% CI, 0.12–0.22]) and phenotypes (ie, single ventricle [aOR, 0.03; 95% CI, 0.004–0.21]).

CONCLUSIONS: First-trimester maternal FAS, but not multivitamin use, was substantially associated with lower risk of CHDs, and the association was strongest for the most severe CHD phenotypes. We recommend that women of childbearing age should supplement with folic acid as early as possible, ensuring coverage of the critical window for fetal heart development to prevent CHDs.

Key Words: congenital heart disease ■ folate ■ multivitamin ■ pregnancy ■ prevention

Although periconceptional folic acid supplementation (FAS) is recommended to women of childbearing age to prevent neural tube defects (NTDs) in their offspring worldwide,1,2 whether a similar protective effect of FAS can be achieved to prevent other major congenital defects, such as congenital heart diseases (CHDs), remains unknown.2 CHDs are the most common class of major congenital malformations, with a prevalence of 1% in live births, which is an ≈10-fold higher prevalence than NTDs.3 CHDs represent an important cause of infant morbidity and mortality worldwide.4 About 25% of CHD infants are the most severe multiple critical CHD cases, generally requiring surgical intervention during the first year of life.5 Without intervention, more than 60% of critical patients with CHDs die within 2 years.6

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Although the diagnosis and treatment of CHDs has improved significantly during the past decades, with a concomitant increase in life expectancy, sequelae of selected severe CHDs may even emerge after treatment in childhood and adulthood.7 Accordingly, CHDs bring substantial economic burdens worldwide. In the United States, hospital costs of CHDs exceeded $6 billion during 2013, 26.7% attributed to hospitalization for critical CHDs.8,9 In China, CHD costs exceeded $1.8 billion in 2002, 60-fold higher than direct economic loss attributable to NTDs.10 In addition to the economic burden, CHDs affected families also face a heavy psychological burden.11 Unfortunately, despite more than 5 decades of research, the etiology of CHDs remains unclear, and a primary prevention strategy is unavailable.

As for NTDs, FAS might offer a cost-effective prevention strategy to reduce the risk of CHDs and lower associated healthcare costs. However, there are still gaps in knowledge to determine the role of maternal FAS in CHD prevention.12 The effect of FAS on CHDs remains controversial. Some studies reported a lower risk of CHDs among the offspring of mothers supplemented with folic acid (FA) alone, multivitamins with FA, or after FA food fortification,13–15 whereas others reported no associations between maternal FAS or use of FA-containing multivitamins with CHDs in offspring.16–18 There is also ongoing debate about the relative importance of FAS alone versus use of multivitamins, including FA to prevent birth defects.19 The interaction between FAS and multivitamin use on CHDs has not been studied previously. In addition, the optimal time window for FAS to prevent CHDs is also unclear. Authorities recommend FAS beginning from 2 or 3 months before conception until the end of the first trimester of pregnancy to prevent NTDs.1,2 However, preconceptional maternal FAS is difficult in practice since more than half of pregnancies are unplanned.20 First-trimester-initiated FAS would serve as a surrogate, but its effect on CHDs has not been well studied. Moreover, the impact of maternal FAS on the most severe phenotypes of CHDs is unclear. Previous studies of FAS and CHDs were limited by nonspecific outcomes, pooling of CHD phenotypes together with potentially different etiologies, and small case numbers.13–15 Furthermore, the possible impact of CHD prevention by universal FAS has not been estimated. Predicting the potential clinical and financial benefits from FAS according to major CHD phenotypes is essential when considering primary prevention of CHDs.

To fill these knowledge gaps, the current study aimed to (1) assess the association between first-trimester maternal FAS and CHDs in offspring; (2) estimate the individual and joint effects of FAS and multivitamin use on CHD risk; (3) assess the associations between FAS and individual CHD categories and phenotypes; and (4) predict the annual number of CHDs that could be prevented, as well as associated financial treatment costs saved from universal maternal FAS in China. The results of this study will help to advance development of primary prevention strategies to decrease the burden of CHDs worldwide.

**METHODS**

The data that support the findings of this study are available from the corresponding author upon reasonable request.
Study Design and Participants
This was a frequency-matched case-control study. Cases and controls were identified from the GRCHD (Guangdong Registry of Congenital Heart Disease) study. GRCHD is an ongoing, province-wide CHD registry study involving 40 centers from 21 cities across Guangdong Province in southern China.21,22

All fetuses and infants with confirmed CHD diagnoses registered in the GRCHD from 2004 to 2016 were included. Controls were randomly chosen from congenital malformation-free fetuses and infants and frequency matched to the cases on enrollment hospitals and year. Mothers of cases or controls who had been living in the study area for at least 6 months were eligible to participate.

Cases were defined using a modified code from the International Classification of Diseases, Tenth Revision (ICD-10) (Q20.000–Q28.000). For each individual, a primary CHD phenotype was assigned according to hemodynamics. All CHD phenotypes were categorized into main categories based on etiology as follows: conotruncal defects, atriopulmonary septal defect, anomalous pulmonary venous return, left ventricular outflow tract obstruction, right ventricular outflow tract obstruction, septal defect, other specified CHD, and unspecified CHD.23,24 CHD cases were further categorized according to severity as “critical CHDs” if prenatal structural malformations of the heart were present that usually require intervention during the first year of life (including anomalous pulmonary venous return, atriopulmonary septal defect, coarctation of aorta, double-outlet right ventricle, hypoplastic left heart syndrome, hypoplastic right heart syndrome, interrupted aortic arch, left ventricular outflow tract obstruction, right ventricular outflow tract obstruction, d-transposition of the great arteries, tetralogy of Fallot, valvular aortic stenosis, and valvular pulmonary stenosis),25 or as “minor CHDs” (including atrial septal defect and ventricular septal defect). Finally, cases were grouped into categories according to the plurality of CHD lesions, as “multiple CHDs” if at least 2 CHD phenotypes were present, or “single CHDs” if only 1 CHD phenotype was present.

We excluded CHD cases associated with chromosomal abnormalities (syndromes), genetic mutations, chromosomal microarray analysis abnormalities, and having extracardiac malformations. Preterm infants (<37 weeks’ gestation at birth) with only patent ductus arteriosus as a heart defect were also excluded as they would close spontaneously by 44 weeks postmenstrual age.26

Data Collection
Physicians at each participating clinical center actively screened all CHD cases and controls in the GRCHD.

FAS and Multivitamin Use
We defined first-trimester maternal FAS as “yes” if mothers reported taking at least 0.4 mg of FA daily for >5 days per week continuously during the first trimester of pregnancy. This included FA tablets freely distributed by the government, prescribed, or purchased from other sources. Similarly, we defined first-trimester maternal multivitamin use as “yes” if mothers reported taking a multivitamin at least 5 days per week continuously during the first trimester of pregnancy. We adopted FAS with or without multivitamin use as the main exposure of interest. We further classified all study participants into no supplementation versus FAS only versus only multivitamin use versus both FAS and multivitamin use to assess the individual and joint effects of FAS and multivitamin use on CHDs.

Statistical Analysis
We described the distribution of covariates in CHD cases and controls and tested their unadjusted differences using chi-square tests. We investigated the multivariate associations between covariates of interest and FAS by incorporating all covariates with P≤0.10 in the univariate analysis into a multivariable unconditional logistic regression model predicting FAS.

We built a directed acyclic graph based on the results above and the literature, to make explicit
assumptions and identify minimally sufficient sets of variables to adjust for confounding when assessing the associations between FAS and CHDs. Based on the directed acyclic graph (Figure S1), we adjusted for maternal sociodemographics (age, education, and migrant worker), maternal disease in the first trimester of pregnancy (fever, flu, and threatened abortion), maternal traditional Chinese medicine use in the first trimester of pregnancy, reproductive history (previous pregnancy with stillbirth and spontaneous/ elective abortion history), maternal lifestyle factors and environmental exposures during the first trimester of pregnancy (manual worker and cigarette smoking) and during periconceptional periods (living in newly renovated room and residential proximity to a main road [<50 m]), and paternal factors during periconceptional periods (flu, cigarette smoking, and chemical agent contact). We excluded household income, gravidity, antimicrobial medication use, and paternal manual worker from the multivariable regression models because of their significant collinearities with maternal education, maternal age, threatened abortion, and maternal manual worker, respectively. We performed multivariable unconditional logistic regression adjusting for the 17 remaining confounding covariates to assess the effect of FAS and multivitamin use on total CHDs. The multiplicative interaction between FAS and multivitamin use was evaluated by including their cross-product term in the multivariable regression model. Unconditional logistic regression with the same algorithm was also used to assess the effects of FAS on CHD categories and specific CHD phenotypes. We calculated odds ratios and their 95% CIs by exponentiating regression coefficients. For specific CHD phenotypes, we corrected for multiple comparisons by introducing a false discovery rate in the confounder-adjusted models individually. The false discovery rate quantifies the likelihood for a false-positive result among the positive findings and is expressed as a P value–analogous Q value. We conducted sensitivity analysis by matching individual cases and controls on the enrollment hospitals, date of conception (±3 months), and sex of infant. Conditional logistic regression was performed to assess the associations of FAS and CHDs in the sensitivity analysis including the individually matched cases and controls.

We calculated the attributable risk percentage of CHDs, and its categories and phenotypes, associated with FAS based on the adjusted odds ratios (aORs). We used these results to predict the annual difference in numbers of FAS-associated CHD cases in China based on the prevalence of CHDs and the number of live births in 2017. We estimated the annual total number of CHD births as total birth number in 2017×prevalence of CHD×births attributable risk percentage of CHDs by FAS; and the remaining number of CHD live births as annual total number of CHD births—number of CHD cases prevented by FAS. We used R 3.6.1 and SPSS 22.0 (IBM Co. Ltd) for statistical analyses.

**Ethical Approval**

The GRCHD project was approved by the Ethics Committee of Guangdong Provincial People’s Hospital (No. GDREC2011135H(R1)). Informed consent was obtained from the mothers of all CHD cases and controls before data collection.

**RESULTS**

As shown in Figure S2, 15 297 participants (8379 cases and 6918 controls) were included in the current study. The distribution of parental sociodemographics, lifestyle factors, and environmental exposures were shown according to CHD status in Table S1. Case mothers were older, had lower educational attainment, and lower household income than controls, and were more likely to be manual workers, minorities, and migrants compared with controls (Table 1).

The total FAS prevalence in our study was 12%, and it varied over time (Table 2). The prevalence of FAS increased significantly from 0.2% before 2013 to 33.3% after 2013 (P<0.001), when the folic acid was freely distributed by the Chinese government, as part of the National Free Preconception Health Examination Project launched in 2010 and a substantially increasing proportion of FAS in pregnant women was observed nationwide. FAS practice varied by parental sociodemographic characteristics, lifestyle factors, and environmental exposures as described in Table S2. After adjusting for covariates with P<0.1 in univariate analysis, we detected a lower prevalence of FAS among mothers with <12 years of education (Table 2).

As described in Table 3, we detected a significant protective effect of first trimester FAS on total CHDs, with or without multivitamin use (aOR, 0.69; 95% CI, 0.62–0.76) after adjustment for confounders. We also found a protective effect for multivitamin use with or without FAS on CHDs (aOR, 0.78; 95% CI, 0.66–0.93). We further classified participants as no supplement use (FAS=0; multivitamin=0; as reference), only FAS (FAS=1; multivitamin=0), only multivitamin use (FAS=0; multivitamin=1), and both FA and multivitamin use (FAS=1; multivitamin=1) to assess the individual and joint effects of FAS and multivitamin use. We found that compared with no users, FAS only was associated with 31% lower CHD risk (aOR, 0.69; 95% CI, 0.61–0.78), but multivitamin use only
was not associated with CHDs (aOR, 1.42; 95% CI, 0.71–0.96), although without an association with single minor CHDs (aOR, 0.92; 95% CI, 0.8–1.05).

We found a significantly lower risk associated with FAS for all CHD etiologic categories, except unspecified CHDs (Table 4). For specific phenotypes, most of them benefited from FAS. The protective effect of FAS was strongest on SV (aOR, 0.03; 95% CI, 0.004–0.21), followed by hypoplastic right heart syndrome, double-outlet right ventricle, d-transposition of the great arteries, and pulmonary atresia, for which risks were about 90% lower. Most associations remained statistically significant when adjusted for multiple comparisons using the false discovery rate.

In the sensitivity analysis including 4726 matched cases and controls, we observed even stronger protective associations between first-trimester FAS and CHDs and its major categories (Table S3).

According to our estimates shown in Table 5, first-trimester FAS was associated with 31% fewer overall CHD fetuses and infants without chromosomal or noncardiac anomalies. Annual births with CHDs could decrease from >190,000 to <132,000 in China with universal implementation of first-trimester FAS. For the most severe CHD category, multiple critical CHDs, annual births could decrease by 84% from >45,000 to only 7250 in China. For most severe CHD phenotypes, such as SV, with which FAS had the strongest association, a 97% reduction could be achieved, with only 72 live SV births annually in China.

**DISCUSSION**

**Principal Findings**

We found that first-trimester maternal FAS, even after conception, was associated with a statistically significant lower prevalence of overall CHDs and most specific CHD phenotypes in offspring. We detected the strongest protective effects from FAS on the most severe CHDs. However, multivitamin use did not protect against CHDs in the absence of FAS and did not interact with FAS on CHDs. Our results also suggest that at least 60,000 live births with CHDs could be prevented annually in China.

**Strengths and Limitations of This Study**

Our study offers notable strengths. We used individual data from a registry of CHDs with >8000 cases and 6000 controls, making this one of the largest studies of CHDs to date. Controls were frequency matched to CHD cases to ensure representativeness of the sampling frame and to facilitate adjustment for confounding. The comprehensive definition and classification of CHDs in severity, plurality, etiology, and of specific phenotypes minimized outcome
Qu et al. CHDs Benefited From First-Trimester Maternal FAS misclassification and enabled us to assess associations between FAS and CHDs resulting from disparate etiologies, including the rarer and most severe phenotypes. Case diagnosis was standardized using common diagnostic rules for all hospitals and physicians participating in the CHD registry system. CHD diagnoses were reviewed by 2 experienced pediatric cardiologists.

However, several potential limitations should be considered when interpreting our results. First, selection bias may be a concern for this study because not all CHD cases in the study area were included. However, we registered CHD cases from a geographically diverse representation, which covered each city of the study area. Controls were frequency matched by hospital and year of enrollment to the cases to ensure representativeness of the sampling frame. The response rates were similar for cases and controls with over 98%. Second, misclassification of FAS and multivitamin use could be another concern. However, we adopted the standardized questionnaire with detailed questions about FAS and multivitamin use, including the product name and frequency of use during the first trimester. This information was collected and recognized by obstetricians who were familiar with the local FAS and multivitamin products. That enabled us to define first-trimester FAS and multivitamin use and isolate them accurately. Although we did not capture specific

### Table 2. Associations Between First-Trimester Maternal Folic Acid Supplementation and Maternal Sociodemographic Characteristics, Guangdong Registry of Congenital Heart Disease, 2004–2016, China (n=15297)

| Characteristics | FAS=1, n (%) | FAS=0, n (%) | cORs (95% CI) | aORs (95% CI)* |
|-----------------|-------------|-------------|---------------|----------------|
| Total           | 1877 (12.3) | 13420 (87.7)| ...           | ...            |
| Calendar year   |             |             |               |                |
| After 2013      | 1853 (33.3) | 3714 (66.7) | 201.65 (134.57–302.16) | 115.16 (76.40–173.60) |
| Before 2013     | 24 (0.2)    | 9700 (99.8) | 1.00 (Reference) | 1.00 (Reference) |
| Age, y          |             |             |               |                |
| >35             | 178 (14.1)  | 1084 (85.9) | 1.00 (0.83–1.21) | 0.90 (0.71–1.13) |
| 15–29           | 1262 (11.5) | 9672 (88.5) | 0.80 (0.71–0.90) | 0.94 (0.82–1.09) |
| 30–35           | 437 (14.1)  | 2664 (85.9) | 1.00 (Reference) | 1.00 (Reference) |
| Education attainment |         |             |               |                |
| <12 y           | 52 (5.0)    | 985 (95.0)  | 0.36 (0.27–0.48) | 0.62 (0.45–0.88) |
| ≥12 y           | 1799 (12.8) | 12206 (87.2)| 1.00 (Reference) | 1.00 (Reference) |
| Ethnicity       |             |             |               |                |
| Minorities      | 25 (13.4)   | 161 (86.6)  | 1.11 (0.73–1.70) | 0.91 (0.53–1.57) |
| Han             | 1852 (12.3) | 13259 (87.7)| 1.00 (Reference) | 1.00 (Reference) |
| Residence       |             |             |               |                |
| Rural           | 651 (10.8)  | 5369 (89.2) | 0.80 (0.72–0.88) | 0.99 (0.87–1.13) |
| City            | 1226 (13.2) | 8051 (86.8) | 1.00 (Reference) | 1.00 (Reference) |
| Migrants†       |             |             |               |                |
| Yes             | 389 (10.0)  | 3483 (90.0) | 0.75 (0.66–0.84) | 0.93 (0.80–1.08) |
| No              | 1488 (13.0) | 9937 (87.0) | 1.00 (Reference) | 1.00 (Reference) |
| Household income, CNY/month/person |         |             |               |                |
| <3000           | 590 (7.5)   | 7317 (92.5) | 0.39 (0.35–0.43) | NA             |
| ≥3000           | 1235 (17.2) | 5939 (82.8) | 1.00 (Reference) | 1.00 (Reference) |
| Manual worker‡§ |             |             |               |                |
| Yes             | 168 (9.9)   | 1535 (90.1) | 0.76 (0.64–0.90) | 0.90 (0.72–1.11) |
| No              | 1709 (12.6) | 11885 (87.4)| 1.00 (Reference) | 1.00 (Reference) |

aORs indicates adjusted odds ratios based on multivariable logistic regression; BMI, body mass index; cORs, crude odds ratios based on univariable analysis; FAS, folic acid supplementation; and NA, not available.

*Adjusted for year (before vs after 2013), maternal demographic characteristics (age, ethnicity, education, residence, migrants, and manual worker), maternal disease (fever, flu, diabetes mellitus, threatened abortion, and thalassemia), maternal medication use (traditional Chinese medication), maternal lifestyle factors and environmental exposures (prepregnancy BMI, passive smoking, chemical agent contact, living in newly renovated room, and residential proximity to a main road <50 m), reproductive history (previous pregnancy with still birth, and spontaneous/elective abortion history), and paternal factors during periconceptional period (fever, flu, smoking, and chemical agent contact); household income, gravity, maternal antimiscarriage medication use, and paternal manual worker were excluded from the model because of their significant collinearity with maternal education, maternal age, threatened abortion, and maternal manual worker, respectively.

†Migrants: people living and working outside their origin.

‡Manual worker: working in handcraft industry, working by hand, or operating machine in manufactory.

§Exposure window: during periconceptional period (3 months before pregnancy to the end of the first trimester).
information on the dose of FAS, which may have misclassified exposure for some women, we expect the impact to be modest, as FA tablets in China are standardized exposure for some women, we expect the Disease in Offspring, Guangdong Registry of Congenital Heart Disease, 2004–2016, China (n=15 297) J Am Heart Assoc. 7

Table 3. Associations of First-Trimester Maternal Folic Acid Supplementation and Multivitamin Use and Congenital Heart Disease in Offspring, Guangdong Registry of Congenital Heart Disease, 2004–2016, China (n=15 297)

| First-Trimester Use | CHD cases, n (%) | Controls, n (%) | cORs (95% CI) | aORs (95% CI)* |
|---------------------|------------------|----------------|--------------|----------------|
| Total               | 8379             | 6918           | ...          | ...            |
| FAS with/without multivitamin use |                   |                |              |                |
| Yes                 | 928 (11.1)       | 949 (13.7)     | 0.78 (0.71–0.86) | 0.69 (0.62–0.76) |
| No                  | 7451 (88.9)      | 5969 (86.3)    | 1.00 (Reference) | 1.00 (Reference) |
| Multivitamin use with/without FAS |                   |                |              |                |
| Yes                 | 332 (4.0)        | 323 (4.7)      | 0.85 (0.73–0.99) | 0.78 (0.66–0.93) |
| No                  | 8047 (96.0)      | 6595 (95.3)    | 1.00 (Reference) | 1.00 (Reference) |
| Both FAS and multivitamin† | 301 (3.6)        | 310 (4.5)      | 0.78 (0.66–0.92) | 0.68 (0.57–0.81) |
| Only FAS            | 627 (7.5)        | 639 (9.2)      | 0.79 (0.70–0.88) | 0.69 (0.61–0.78) |
| Only multivitamin   | 31 (0.4)         | 13 (0.2)       | 1.91 (1.00–3.86) | 1.42 (0.73–2.77) |
| No FAS or multivitamin | 7420 (88.6)     | 5956 (86.1)    | 1.00 (Reference) | 1.00 (Reference) |

aORs indicates adjusted odds ratios based on multivariable conditional logistic regression model; CHD, congenital heart disease; cORs, crude odds ratios based on univariate analysis; and FAS, folic acid supplementation.

†Adjusted for year (before vs after 2013), maternal demographics (age, education, migrants, and manual worker), maternal disease (fever, flu, and threatened abortion), maternal medication use (Chinese medication use), reproductive history (previous pregnancy with stillbirth and spontaneous/elective abortion history), maternal lifestyle factors and environmental exposures (smoking, living in newly renovated room, and residential proximity to a main road (<50 m), paternal factors (flu, smoking, and chemical agent contact); household income, gravidity, maternal antmiscarriage medication use, and paternal manual worker were excluded from the model because of their significant collinearity with maternal education, maternal age, threatened abortion, and maternal manual worker, respectively.

First, although we registered all CHD cases from participating clinical centers for >10 years, we had small numbers of cases for selected CHD phenotypes, and so we were unable to complete a matched sensitivity analysis, and these results may not be representative. We will continue to register these rare phenotypes to enhance the statistical power of a future investigation.

Interpretation of the Findings Compared With Other Studies

We found significantly lower odds for CHDs among the offspring of mothers with first-trimester FAS compared with those without, irrespective of multivitamin.

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7
Qu et al CHDs Benefited From First-Trimester Maternal FAS use. We found a similar and even stronger protective association of FAS and CHDs in the sensitivity analysis. Our results were consistent with a hospital-based, case-control study with 358 CHD cases (without detailed phenotype information) and 422 controls from China. The investigators found a 53% lower risk of total CHDs, higher than our 32% difference, among women with FAS for at least 1 month after conception.

Figure. Association of first-trimester maternal folic acid supplementation with congenital heart disease categories by severity and plurality, Guangdong Registry of Congenital Heart Disease, 2004–2016, China. A, By main categories according to severity and plurality of CHD lesions. B, By categories of combining the severity and plurality of CHD lesions. Adjusting for year (before vs after 2013), maternal demographics (age, education, migrants, and manual worker), maternal disease (fever, flu, and threatened abortion), maternal medication use (Chinese medication use), reproductive history (previous pregnancy with stillbirth and spontaneous/elective abortion history), maternal lifestyle factors and environmental exposures (smoking, living in newly renovated room, and residential proximity to a main road [<50 m]), paternal factors (flu, smoking, chemical agent contact); household income, gravidity, maternal antimiscarriage medication use, and paternal manual worker were excluded from the model because of their significant collinearity with maternal education, maternal age, threatened abortion, and maternal manual worker, respectively. aOR indicates adjusted odds ratio; and CHD, congenital heart disease.
with or without multivitamin use. Our results were also partly consistent with those from a cohort study of 94 CHD births and 9993 controls in China.\(^{37}\) The authors reported a 50% lower probability of multiple CHDs among women using FAS during pregnancy, similar to our results (−63%). However, our results differed from 2 European investigations, which found no association between maternal postconceptional FAS and CHDs.\(^{16,18}\) The first was a nationwide cohort study from Norway, which identified 6200 CHD cases among 517,784 singleton births without chromosomal anomalies from 1999 to 2009.\(^{16}\) The second was a birth cohort study from Denmark and Norway, which identified 2247 CHD cases among 197,123 births from 2000 to 2009.\(^{18}\) Several possibilities might account for the discrepancies. First, the typical Chinese diet has a lower intake of folate-rich meat than the typical Western diet.\(^{34}\) Thus, FAS could be more beneficial for the prevention of CHDs in areas with lower baseline or dietary folate levels.\(^{18}\) Prior to China’s 2010 free FAS program, very few Chinese mothers used FAS, and maternal folate levels were extremely low.\(^{38}\) Second, differences in the study populations might have been important. For example, methylenetetrahydrofolate reductase gene polymorphisms play an important role in the FA metabolic pathway and vary by race and ethnicity.\(^{39}\) The frequency of the methylenetetrahydrofolate reductase 677T allele, with which more FA is needed to prevent birth defects, is higher among Europeans and North Americans than Africans and Asians.\(^{39}\) The frequency of the methylenetetrahydrofolate reductase 677T allele, with which more FA is needed to prevent birth defects, is higher among Europeans and North Americans than Africans and Asians.\(^{39}\)

Table 4. Associations Between First-Trimester Maternal Folic Acid Supplementation (With or Without Multivitamin Use) and Congenital Heart Disease, by Etiologic Categories and Detailed Phenotypes, Guangdong Registry of Congenital Heart Disease, 2004–2016, China

| CHD Phenotypes            | n     | FAS=1, n (%) | aOR (95% CI)\(^{±}\) | P Value |
|---------------------------|-------|--------------|-----------------------|---------|
| Total CHDs                | 8379  | 928 (11.1)   | 0.69 (0.62–0.76)      | <0.001\(^{1†}\) |
| Conotruncal defects       | 868   | 25 (2.9)     | 0.15 (0.10–0.23)      | <0.001\(^{1†}\) |
| TGA                       | 343   | 7 (2.0)      | 0.11 (0.05–0.24)      | <0.001\(^{1†}\) |
| ToF                       | 309   | 12 (3.9)     | 0.21 (0.11–0.37)      | <0.001\(^{1†}\) |
| DORV                      | 178   | 3 (1.7)      | 0.09 (0.03–0.29)      | <0.001\(^{1†}\) |
| Truncus arteriosus        | 38    | 3 (7.9)      | 0.43 (0.12–1.47)      | 0.177    |
| AVSD                      | 184   | 5 (2.7)      | 0.14 (0.06–0.36)      | <0.001\(^{1†}\) |
| APVR                      | 77    | 5 (6.5)      | 0.39 (0.15–0.97)      | 0.043    |
| LVOTO                     | 213   | 13 (6.1)     | 0.33 (0.18–0.58)      | <0.001\(^{1†}\) |
| CoA/IAA                   | 111   | 5 (4.5)      | 0.23 (0.09–0.58)      | 0.002\(^{1†}\) |
| HLHS                      | 61    | 6 (9.8)      | 0.62 (0.25–1.50)      | 0.285    |
| vAS                       | 41    | 2 (4.9)      | 0.28 (0.07–1.16)      | 0.080    |
| RVOTO                     | 536   | 31 (5.8)     | 0.33 (0.23–0.49)      | <0.001\(^{1†}\) |
| HRHS                      | 74    | 0            | 0.07 (0.01–0.47)      | 0.007\(^{1†}\) |
| Ebstein anomaly           | 43    | 1 (2.3)      | 0.12 (0.02–0.91)      | 0.040    |
| PA                        | 68    | 0            | 0.11 (0.02–0.82)      | 0.031\(^{1†}\) |
| vPS                       | 351   | 30 (8.5)     | 0.50 (0.34–0.75)      | <0.001\(^{1†}\) |
| SV                        | 144   | 1 (0.7)      | 0.03 (0.004–0.21)     | <0.001\(^{1†}\) |
| Septal defects            | 4437  | 57 (13.0)    | 0.84 (0.75–0.95)      | 0.005\(^{1†}\) |
| VSD                       | 2863  | 366 (12.8)   | 0.85 (0.74–0.98)      | 0.022\(^{1†}\) |
| ASD                       | 1574  | 211 (13.4)   | 0.84 (0.71–0.99)      | 0.044    |
| Other specified CHDs      | 1385  | 142 (10.3)   | 0.68 (0.56–0.82)      | <0.001\(^{1†}\) |
| Unspecified CHDs          | 535   | 129 (24.1)   | 1.11 (0.80–1.53)      | 0.533    |

aOR indicates adjusted odds ratio; ASD, atrial septal defect; APVR, anomalous pulmonary venous return; AVSD, atrioventricular septal defect; CHD, congenital heart disease; CoA, coarctation of aorta; DORV, double-outlet right ventricle; FAS, folic acid supplementation; HLHS, hypoplastic left heart syndrome; HRHS, hypoplastic right heart syndrome; IAA, interrupted aortic arch; LVOTO, left ventricular outflow tract obstruction; LVOTS, left ventricular outflow tract stenosis; PA, pulmonary atresia; RVOTO, right ventricular outflow tract obstruction; RVOTS, right ventricular outflow tract stenosis; SV, single ventricle; TGA, d-transposition of the great arteries; ToF, tetralogy of Fallot; vAS, valvular aortic stenosis; vPS, valvular pulmonary stenosis; and VSD, ventricular septal defect.

*Adjusted for year (before vs after 2013), maternal demographics (age, education, migrants, and manual worker), maternal disease (fever, flu, and threatened abortion), maternal medication use (Chinese medication use), reproductive history (previous pregnancy with stillbirth, and spontaneous/elective abortion history), maternal lifestyle factors and environmental exposures (smoking, living in newly renovated room, and residential proximity to a main road [≤50 m]), and paternal factors (flu, smoking, and chemical agent contact); Household income, gravidity, maternal antimiscarriage medication use, and paternal manual worker were excluded from the model due to their significant collinearity with maternal education, maternal age, threatened abortion, and paternal manual worker, respectively.

†False discovery rate Q<0.05.

‡Compare with 949 FAS in 6918 controls (13.7%).
Table 5. Predicted Congenital Heart Disease Among Newborns With Universal First-Trimester Maternal Folic Acid Supplementation in China, by Phenotypes, and Severe and Plurality Categories, Using Data From the Guangdong Registry of Congenital Heart Disease, 2004–2016, China

| CHD                  | Reduction by FAS, % | Prevalence, % | Annual Birth Number of CHDs | Reduction Number by FAS | Remaining Number of CHDs |
|----------------------|---------------------|---------------|----------------------------|-------------------------|--------------------------|
| All CHDs             | 31                  | 11.1          | 191,253                    | 59,288                  | 131,965                  |
| SV                   | 97                  | 0.14          | 2412                       | 2,234                   | 22                         |
| HRHS                 | 93                  | 0.1           | 17,23                      | 1,602                   | 121                        |
| DORV                 | 91                  | 0.28          | 4,824                      | 4,390                   | 434                        |
| TGA                  | 89                  | 0.43          | 7,409                      | 6,594                   | 815                        |
| PA                   | 89                  | 0.09          | 15,511                     | 13,800                  | 171                        |
| AVSD                 | 86                  | 0.28          | 4,824                      | 4,140                   | 675                        |
| ToF                  | 79                  | 0.32          | 5,514                      | 4,356                   | 1,158                      |
| CoA/IAA              | 77                  | 0.13          | 2,240                      | 1,725                   | 515                        |
| APVR                 | 61                  | 0.1           | 1,723                      | 1,051                   | 672                        |
| vPS                  | 50                  | 0.69          | 11,889                     | 5,945                   | 5,944                      |
| VSD                  | 16                  | 3.71          | 63,923                     | 10,228                  | 53,695                     |
| ASD                  | 16                  | 2.89          | 49,795                     | 7,967                   | 41,828                     |
| Critical CHDs        | 46                  | 3.52          | 60,650                     | 27,899                  | 32,751                     |
| Minor CHDs           | 16                  | 7.57          | 130,431                    | 20,869                  | 109,562                    |
| Multiple CHDs        | 63                  | 3.95          | 68,059                     | 42,877                  | 25,182                     |
| Single CHDs          | 13                  | 7.14          | 123,022                    | 15,993                  | 107,029                    |
| Multiple critical CHDs| 84                  | 2.63          | 45,315                     | 38,065                  | 7,250                      |
| Single critical CHDs | 18                  | 0.89          | 15,335                     | 2,760                   | 12,575                     |
| Multiple minor CHDs  | 36                  | 1.32          | 22,744                     | 8,188                   | 14,556                     |

ASD indicates atrial septal defect; APVR, anomalous pulmonary venous return; AVSD, atrioventricular septal defect; CHD, congenital heart disease; CoA, coarctation of aorta; DORV, double-outlet right ventricle; GRCHD, Guangdong Registry of Congenital Heart Disease; HRHS, hypoplastic right heart syndrome; IAA, interrupted aortic arch; PA, pulmonary atresia; SV, single ventricle; TGA, d-transposition of the great arteries; ToF, tetralogy of Fallot; vPS, valvular pulmonary stenosis; and VSD, ventricular septal defect.

East Asians.40 Thus, the adequate FA dose to prevent CHDs may differ in different population groups.

However, we did not find an independent association between multivitamin use alone and CHDs. Our results are in accord with the aforementioned Norwegian study of postconception supplementation that reported similar results.16,41 In contrast, the Hungarian randomized clinical trial and following cohort control trial found a 43% decrease in total CHDs among women using a multivitamin supplement containing 0.8 mg FA, which is more than twice the recommended dose of FA to prevent NTDs, together with 18 other nutrients.2,13,42 This higher FA dose in the presence of other micronutrients involved in methylation and biosynthesis (eg B2, B6, and B12) might also benefit heart morphogenesis. Furthermore, preconception use might also impact the multivitamin’s effect on CHD.36 However, our results suggested that first-trimester multivitamin use did not protect against CHDs.

We detected a stronger protective association for maternal FAS with critical (ie, 46% difference) and multiple (ie, 63% difference) CHDs than on minor (ie, 16% difference) and single (ie, 13% difference) CHDs. Specifically, we found the strongest association for FAS with multiple critical CHDs (84% difference), while no association for FAS with single minor CHDs was observed. These results were consistent with previous epidemiologic studies that reported a protective effects for maternal FAS on critical CHDs, multiple CHDs, minor CHDs, and single CHDs, and they reported a stronger effect on multiple CHDs37 and single CHDs than ours.36 In contrast, the aforementioned European cohort study reported no significant association between FAS and critical CHDs compared with no FAS.16 Unfortunately, there is scarce evidence to characterize the effects of FAS on CHD categories combining severity and plurality of lesions. Our results were consistent with Li et al. (2013), who found a 73% lower prevalence of multiple critical CHDs associated with FAS for at least 1 month after conception (odds ratio, 0.27; 95% CI, 0.12–0.62).36

We found consistent protective associations for FAS with all CHD etiology categories, except
unspecified CHDs and most phenotypes. In addition, the most severe CHD category, multiple critical CHDs, and the most severe phenotype, SV, appeared to benefit most from FAS. These severe CHDs correlate with substantial mortality, morbidity, and financial costs worldwide.\(^9\) Consistent with our results, significant reductions in conotruncal defects, coarctation of aorta in left ventricular outflow tract obstruction, and ventricular septal defect had been associated with FAS in previous studies.\(^{8,17,43–46}\) Similarly, a 17-year retrospective study from Hungary reported significantly fewer children with ventricular septal defect, tetralogy of Fallot, d-transposition of the great arteries, and atrioventricular septal defect among mothers taking FA (calculated daily average, 5.6 mg) during pregnancy compared with children whose mothers did not take FA.\(^{47}\) Our large sample size enabled detailed interrogation of the associations between FAS and CHD phenotypes and categories, especially the most rare and severe ones. Our study is the first to report the protective effects of FAS on SV, anomalous pulmonary venous return, hypoplastic right heart syndrome, pulmonary atresia, and valvular pulmonary stenosis.

Our results suggested that universal first-trimester FAS in China would reduce the annual number of CHD cases by 31%. This translates to almost 60,000 CHDs prevented across China each year. For multiple critical CHDs, the most severe CHD category, 84% of cases could be prevented by FAS, preventing more than 38,000 cases in China annually. Our results also suggested that 97% of SV cases, the most severe CHD phenotype, in China could be prevented by universal adoption of first-trimester FAS. The substantial reduction in CHD cases would translate to enormous cost savings. The potential benefit of FAS has never been estimated before. Comparison of our estimation to the others in this aspect are impossible. We based our estimate on the assumption that our study results are representative of FAS prevalence and FAS-isolated CHD (CHD without gene anomaly) associations across China. More accurate predictions of the impacts of universal FAS implementation accounting for differences in socioeconomic status, environmental and lifestyle factors, and FAS prevalence across different regions is necessary to help confirm our results.

Possible Explanations and Implications

While the biologic mechanism of the protective effect of FAS on CHDs is unclear, FA could protect against CHD by influencing the critical cardiac neural crest cell migration that contributes to embryonic heart development.\(^{48,49}\) The protective associations might also be driven by elevation of maternal FA concentration during the critical periods for fetal heart development around 3 to 8 weeks of pregnancy.\(^{50}\) Red blood cell folate concentrations increase rapidly after FAS initiation,\(^{51}\) and CHD risks decrease with greater RBC folate concentration.\(^{52}\) We recommended that women planning to get pregnant initiate FAS as early as possible to ensure coverage of critical fetal heart development periods and to decrease the risk of CHDs. For mothers who missed preconceptional FAS, we recommend that they supplement FA and not use only multivitamins during the first trimester of pregnancy to decrease the risk of CHDs.

Millions of dollars in treatment costs would be saved by universal FAS. The annual CHD treatment costs reached $1.8 billion in 2002 in China, 60 times greater than direct economic losses attributable to NTDs, and 6 times the treatment cost of Down syndrome.\(^{53}\) More than $550 million in treatment costs could be saved annually by universal FAS implementation in China. Our estimates are conservative given that we did not count in the loss of stillbirths and abortions as a result of CHDs.

There is still space to increase maternal FAS in China. The prevalence of first-trimester maternal FAS was ≈12% in our study population, much lower than the 55.6% to 97.8% reported for Western countries.\(^{53}\) Based on the low uptake of FAS, the Chinese government launched the National Free Preconception Health Examination Project in 2010, providing no-cost FA to pregnant women residing in rural areas with lower socioeconomic status, and afterwards to all pregnant women living in both rural and urban areas. An increasing proportion of pregnant women used FA after the policy nationwide.\(^{54}\) Consequently, the prevalence of FAS has increased dramatically, from 0.2% before 2013 to 33.3% after 2013 in our study, although still lower than reported for Western countries. Still, we found a lower prevalence of FAS among the lower-education groups in our study. Similarly, greater preconceptional FAS uptake leading to greater blood folate levels, was previously reported among wealthier and more educated mothers.\(^{33,55,56}\) Despite the success of the National Free Preconception Health Examination Project, targeted policies to enhance FAS would still likely benefit women of lower socioeconomic status in China, even after conception.

We implemented several strategies to evaluate or control for the potential confounding and modifying effects of the National Free Preconception Health Examination Project policy change on the associations between maternal FAS and CHDs. First, we included a variable indicating pre- and post-2013 in the model to adjust for confounding. We next assessed the associations between FAS and CHDs in a stratified analysis, before and after 2013. We only found a significant protective effect of FAS on CHDs.
after 2013 (aOR, 0.71; 95% CI, 0.63–0.80). The limited number of mothers with FAS (n=24 [0.2%]) before 2013 versus n=1853 [33.3%] after 2013 may have contributed to the null result before 2013. Finally, we compared the numbers of CHD cases before 2013 (2004–2012) to those with maternal FAS after 2013 using overdispersed Poisson generalized linear models, accounting for the proportion of mothers >35 years of age, having <12 years of education attainment, and living in rural areas. We found that the policy was associated with a significantly lower number of CHDs (risk ratio, 0.19; 95% CI, 0.10–0.33). This translates to a range of 284 to 947 fewer CHD cases (assuming compliance rates of 30%–100%) associated with the policy annually (Table S4).

CONCLUSIONS AND FUTURE RESEARCH
We found a protective association for first-trimester maternal FAS but not multivitamin use only on CHDs. Almost all CHD categories and phenotypes benefited from FAS, and the most severe ones—multiple critical CHDs and SV—benefited the most. Women of child-bearing age should supplement with FA as early as possible, ensuring coverage of the critical window for fetal heart development to prevent CHDs. Additional studies, especially clinical trials, will be necessary to evaluate the appropriate FAS dose to prevent CHDs and to determine the role of preconceptional FA-containing multivitamin use on CHDs.

ARTICLE INFORMATION
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Supplementary Materials
Figures S1–S2
Tables S1–S4

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SUPPLEMENTAL MATERIAL
Figure S1. Causal diagrams of maternal folic acid supplementation (FAS) and congenital heart disease (CHD) in offspring, Guangdong Registry of Congenital Heart Disease, 2004-2016, China.
Figure S2. Flow chart of study participants, Guangdong Registry of Congenital Heart Disease, 2004-2016, China.

N = 18017

10660 cases + 7353 controls

Excluded 95 cases with chromosomal abnormalities or genetic mutation or chromosomal microarray analysis abnormalities

17922 cases

Excluded 642 non-singleton cases

17280 cases

Excluded 681 preterm cases only with PDA

16599 cases

Excluded 408 cases with non-cardiac malformation (associated CHD)

16191 cases

Excluded 625 cases and 265 controls aged ≥ 1 year

8379 cases + 6918 controls

Total study N = 15297
Table S1. Parental sociodemographic and lifestyle factors and environmental exposures according to congenital heart disease status, Guangdong Registry of Congenital Heart Disease, 2004-2016, China (n=15,297).

| Characteristics                                      | Total | CHD Case, n (%) | Control, n (%) | P-value |
|------------------------------------------------------|-------|-----------------|----------------|---------|
| **Total**                                            | 15,297 | 8379            | 6918           |         |
| **Maternal socio-demographics**                       |       |                 |                |         |
| Age (years)                                          |       |                 |                |         |
| >35                                                  | 1262  | 774 (9.2)       | 488 (7.1)      | <0.001  |
| 30-35                                                | 3101  | 1745 (20.8)     | 1356 (19.6)    |         |
| 15-29                                                | 10,934| 5860 (69.9)     | 5074 (73.3)    |         |
| Educational attainment                               |       |                 |                |         |
| <12 years                                            | 1037  | 691 (8.4)       | 346 (5.1)      | <0.001  |
| ≥12 years                                            | 14,005| 7534 (91.6)     | 6471 (94.9)    |         |
| Ethnicity                                            |       |                 |                |         |
| Minorities                                          | 186   | 123 (1.5)       | 63 (0.9)       | 0.002   |
| Han                                                  | 15,111| 8256 (98.5)     | 6855 (99.1)    |         |
| Residence                                            |       |                 |                |         |
| Rural                                                | 6020  | 3338 (39.8)     | 2682 (38.8)    | 0.178   |
| Urban                                                | 9277  | 5041 (60.2)     | 4236 (61.2)    |         |
| Migrants*                                            |       |                 |                |         |
| Yes                                                  | 3872  | 2260 (27)       | 1612 (23.3)    | <0.001  |
| No                                                   | 11,425| 6119 (73)       | 5306 (76.7)    |         |
| Household income (CNY/month/person)                  |       |                 |                |         |
| <3000                                                | 7907  | 4481 (54.3)     | 3426 (50.2)    | <0.001  |
| ≥3000                                                | 7174  | 3772 (45.7)     | 3402 (49.8)    |         |
| Manual worker †‡                                      |       |                 |                |         |
| Yes                                                  | 1703  | 1130 (13.5)     | 573 (8.3)      | <0.001  |
| No                                                   | 13,594| 7249 (86.5)     | 6345 (91.7)    |         |
| **Maternal disease**: §                              |       |                 |                |         |
| Cardiac disease (including CHD)                      |       |                 |                |         |
| Yes                                                  | 28    | 21 (0.3)        | 7 (0.1)        | 0.038   |
| No                                                   | 15,269| 8358 (99.7)     | 6911 (99.9)    |         |
| Fever (>38.5 °C)                                     |       |                 |                |         |
| Yes                                                  | 371   | 306 (3.7)       | 65 (0.9)       | <0.001  |
| No                                                   | 14,926| 8073 (96.3)     | 6853 (99.1)    |         |
| Cold/ flu                                            |       |                 |                |         |
| Yes                                                  | 777   | 603 (7.2)       | 174 (2.5)      | <0.001  |
| No                                                   | 14,520| 7776 (92.8)     | 6744 (97.5)    |         |
| Other viral infection (hepatitis/ syphilis/ rubella/ HIV/ herpes) |       |                 |                |         |
| Yes                                                  | 225   | 141 (1.7)       | 84 (1.2)       | 0.017   |
| No                                                   | 15,072| 8238 (98.3)     | 6834 (98.8)    |         |
| Diabetes ‡                                           |       |                 |                |         |
| Yes                                                  | 379   | 221 (2.6)       | 158 (2.3)      | 0.162   |
| Condition                        | Yes          | No           | p-value |
|---------------------------------|--------------|--------------|---------|
| Hypertension                    | 148          | 15,149       | <0.001  |
| Threatened abortion             | 727          | 14,567       | <0.001  |
| Thyroid disorder                | 54           | 15,243       | 0.508   |
| Thalassemia                     | 115          | 15,182       | 0.259   |
| Maternal medicine use           |              |              |         |
| Chinese (patent) medicine       | 208          | 15,089       | <0.001  |
| Antibiotic                      | 141          | 15,156       | <0.001  |
| Anti-miscarriage medication     | 334          | 14,963       | <0.001  |
| Maternal lifestyle factors and environmental exposures |              |              |         |
| Prepregnancy BMI (kg/m^2)       |              |              |         |
| Over weight (≥24)               | 455          | 14,099       | 0.536   |
| Low weight (<18.5)              | 743          | 7716         | 0.061   |
| Normal (18.5-24.9)              |              |              |         |
| Alcohol intake                  |              |              |         |
| Yes                             | 87           | 15,210       | <0.001  |
| No                              |              |              |         |
| Smoking                         |              |              |         |
| Yes                             | 141          | 15,156       | <0.001  |
| No                              |              |              |         |
| Passive smoking                 |              |              |         |
| Yes                             | 1892         | 14,405       | 0.09    |
| No                              |              |              |         |
| Chemical agent contact          |              |              |         |
| Yes                             | 210          | 15,087       | 0.211   |
| No                              |              |              |         |
| Ionizing radiation exposure     |              |              |         |
| Yes                             | 83           | 15,214       | 0.061   |
| No                              |              |              |         |
| Living in newly renovated room  |              |              |         |
| Yes                             |              |              |         |
| No                              |              |              |         |
|                                      | Yes            | No             | p-value |
|--------------------------------------|----------------|----------------|---------|
| Yes                                   | 392            | 14,905         | <0.001  |
| Residential proximity to a main road <50 m ‡ | 317 (3.8)    | 8062 (96.2)    |         |
| Yes                                   | 75 (1.1)       | 6843 (98.9)    |         |
| No                                    | 1721           | 13,576         | <0.001  |
| Pets exposure                         | 1076 (12.8)    | 7303 (87.2)    |         |
| Yes                                   | 645 (9.3)      | 6273 (90.7)    |         |
| No                                    | 243 (2.9)      | 8136 (97.1)    | <0.001  |
| Pets exposure                         | 98 (1.4)       | 6820 (98.6)    |         |
| Residential proximity to a main road <50 m ‡ | 317 (3.8)    | 8062 (96.2)    |         |
| Yes                                   | 75 (1.1)       | 6843 (98.9)    |         |
| No                                    | 1721           | 13,576         | <0.001  |
| Pets exposure                         | 1076 (12.8)    | 7303 (87.2)    |         |
| Yes                                   | 645 (9.3)      | 6273 (90.7)    |         |
| No                                    | 243 (2.9)      | 8136 (97.1)    | <0.001  |
| Pets exposure                         | 98 (1.4)       | 6820 (98.6)    |         |
| Reproductive history                  |               |                |         |
| Gravidity                             |               |                |         |
| ≥3                                   | 3089           | 1810 (21.8)    |         |
| 2                                    | 4710           | 2682 (32.2)    | <0.001  |
| 1                                    | 7392           | 3829 (46.0)    |         |
| Previous pregnancy with birth defect  |               |                |         |
| Yes                                   | 106            | 95 (1.1)       | <0.001  |
| No                                    | 15,191         | 8284 (98.9)    |         |
| Previous pregnancy with stillbirths   |               |                |         |
| Yes                                   | 112            | 87 (1.0)       | <0.001  |
| No                                    | 15,185         | 8292 (99.0)    |         |
| Spontaneous/ elective abortion history |               |                |         |
| Yes                                   | 1037           | 644 (7.7)      | <0.001  |
| No                                    | 14,260         | 7735 (92.3)    |         |
| Paternal factors ‡                    |               |                |         |
| Manual worker †                       |               |                |         |
| Yes                                   | 3057           | 1895 (22.6)    | <0.001  |
| No                                    | 12,240         | 6484 (77.4)    |         |
| Cold/ Flu                             |               |                |         |
| Yes                                   | 215            | 147 (1.8)      | <0.001  |
| No                                    | 15,082         | 8232 (98.2)    |         |
| Alcohol intake ‡                      |               |                |         |
| Yes                                   | 885            | 611 (7.3)      | <0.001  |
| No                                    | 14,412         | 7768 (92.7)    |         |
| Smoking ‡                             |               |                |         |
| Yes                                   | 2592           | 1664 (19.9)    | <0.001  |
| No                                    | 12,705         | 6715 (80.1)    |         |
| Chemical agent contact #              |               |                |         |
| Yes                                   | 114            | 91 (1.1)       | <0.001  |
| No                                    | 15,183         | 8288 (98.9)    |         |

BMI, body mass index; CHD, congenital heart disease.

* Migrants: people living and working outside their origin;
† Manual worker: working in handicraft industry, working by hand, or operating machine in manufactory;
‡ Exposure window: during periconceptional period (3 months before pregnancy to the end of the 1st trimester);
§ Exposure window: in the 1st trimester of pregnancy (within 3 months after pregnancy);
¥ Maternal diabetes: included pregestational and gestational, type I and type II diabetes;
† Threatened abortion: symptom of vaginal bleeding occurs in the first 20 weeks of pregnancy with or without abdominal cramps, indicating a possible miscarriage;
‡ Anti-miscarriage medication: medication use to prevent miscarriage;
§ Alcohol intake: a reported alcohol intake of on average at least 50 ml/d without specifying wine;
$ Smoking: on average consume at least one cigarette per day;
& Passive smoking: maternal self-reported exposure to environmental tobacco smoke at home, workplace, or both;
# Chemical agent contact: occupational or long-term use of any of the harmful chemicals comprising any organic solvent or farm chemicals (including Herbicides, Pesticides, and Rodenticides);
△ Living in newly renovated room: pregnant women moving into a new house within 6 months after decoration.
Table S2. Association of first trimester maternal folic acid supplementation and parental factors, Guangdong Registry of Congenital Heart Disease, 2004-2016, China (n=15,297).

| Characteristics                                | Total   | FAS, n (%) | no FAS, n (%) | P-value |
|------------------------------------------------|---------|------------|---------------|---------|
| **Total**                                      | 15,297  | 1877 (12.3)| 13,420 (87.7) |         |
| **Multivitamin use**                           |         |            |               |         |
| Yes                                            | 655     | 611 (93.3) | 44 (6.7)      | <0.001  |
| No                                             | 14,642  | 1266 (8.6) | 13,376 (91.4) |         |
| **Maternal socio-demographics**                 |         |            |               |         |
| Age (years)                                    |         |            |               |         |
| >35                                            | 1262    | 178 (14.1) | 1084 (85.9)   | <0.001  |
| 30-35                                          | 3101    | 437 (14.1) | 2664 (85.9)   | <0.001  |
| 15-29                                          | 10,934  | 1262 (11.5)| 9672 (88.5)   |         |
| Education attainment                           |         |            |               |         |
| ≥12 years                                      | 14,005  | 1799 (12.8)| 12,206 (87.2) | <0.001  |
| <12 years                                      | 1037    | 52 (5.0)   | 985 (95.0)    |         |
| Ethnicity                                      |         |            |               |         |
| Minorities                                     | 186     | 25 (13.4)  | 161 (86.6)    | 0.624   |
| Han                                            | 15,111  | 1852 (12.3)| 13,259 (87.7) |         |
| Residence                                      |         |            |               |         |
| Rural                                          | 6020    | 651 (10.8) | 5369 (89.2)   | <0.001  |
| City                                           | 9277    | 1226 (13.2)| 8051 (86.8)   |         |
| Migrants †                                     |         |            |               |         |
| Yes                                            | 3872    | 389 (10.0) | 3483 (90.0)   | <0.001  |
| No                                             | 11,425  | 1488 (13.0)| 9937 (87.0)   |         |
| Household income (CNY/month/person)            |         |            |               |         |
| ≥3000                                          | 7174    | 1235 (17.2)| 5939 (82.8)   | <0.001  |
| <3000                                          | 7907    | 590 (7.5)  | 7317 (92.5)   |         |
| Manual worker ‡‡                                |         |            |               |         |
| Yes                                            | 1703    | 168 (9.9)  | 1535 (90.1)   | 0.001   |
| No                                             | 13,594  | 1709 (12.6)| 11,885 (87.4) |         |
| **Maternal disease ‡**                         |         |            |               |         |
| Cardiac disease (including CHD)                |         |            |               |         |
| Yes                                            | 28      | 3 (10.7)   | 25 (89.3)     | 0.802   |
| No                                             | 15,269  | 1874 (12.3)| 13,395 (87.7) |         |
| Fever (>38.5 °C)                               |         |            |               |         |
| Yes                                            | 371     | 60 (16.2)  | 311 (83.8)    | 0.020   |
| No                                             | 14,926  | 1817 (12.2)| 13,109 (87.8) |         |
| Other virus infection (Hepatitis/ Syphilis/ Rubella/ HIV/ Herpes) | | | | |
| Yes                                            | 225     | 31 (13.8)  | 194 (86.2)    | 0.488   |
| No                                             | 15,072  | 1846 (12.2)| 13,226 (87.8) |         |
| Diabetes †                                     |         |            |               |         |
| Yes                                            | 379     | 104 (27.4)| 275 (72.6)    | <0.001  |
| No                                             | 14,918  | 1773 (11.9)| 13,145 (88.1) |         |
| Condition                          | Yes | No       | p-value |
|-----------------------------------|-----|----------|---------|
| Hypertension                      |     |          |         |
| Yes                               | 148 | 18 (12.2)| 130 (87.8)| 0.968 |
| No                                | 15,149 | 1859 (12.3)| 13,290 (87.7) |
| Threatened abortion               |     |          |         |
| Yes                               | 727 | 152 (20.9)| 575 (79.1)| <0.001 |
| No                                | 14,567 | 1725 (11.8)| 12,842 (88.2) |
| Thalassemia                       |     |          |         |
| Yes                               | 115 | 32 (27.8)| 83 (72.2)| <0.001 |
| No                                | 15,182 | 1845 (12.2)| 13,337 (87.8) |
| Maternal medicine use             |     |          |         |
| Chinese (patent) medicinal        |     |          |         |
| Yes                               | 208 | 45 (21.6)| 163 (78.4)| <0.001 |
| No                                | 15,089 | 1832 (12.1)| 13,257 (87.9) |
| Antibiotic                        |     |          |         |
| Yes                               | 141 | 16 (11.3)| 125 (88.7)| 0.737 |
| No                                | 15,156 | 1861 (12.3)| 13,295 (87.7) |
| Anti-miscarriage medication       |     |          |         |
| Yes                               | 334 | 201 (60.2)| 133 (39.8)| <0.001 |
| No                                | 14,963 | 1676 (11.2)| 13,287 (88.8) |
| Maternal lifestyle factors and environmental exposures |     |          |         |
| Prepregnancy BMI (kg/m^2)         |     |          |         |
| Over weight (≥24)                 | 455 | 148 (32.5)| 307 (67.5)|         |
| Low weight (<18.5)                | 743 | 375 (50.5)| 368 (49.5)| <0.001 |
| Normal (18.5-24.9)                | 14,099 | 1354 (9.6)| 12,745 (90.4) |
| Alcohol intake                    |     |          |         |
| Yes                               | 87  | 10 (11.5)| 77 (88.5)| 0.825 |
| No                                | 15,210 | 1867 (12.3)| 13,343 (87.7) |
| Smoking                           |     |          |         |
| Yes                               | 141 | 9 (6.4)| 132 (93.6)| 0.032 |
| No                                | 15,156 | 1868 (12.3)| 13,288 (87.7) |
| Passive smoking                   |     |          |         |
| Yes                               | 1892 | 603 (32.1)| 1289 (68.1)| <0.001 |
| No                                | 13,405 | 1274 (9.5)| 12,131 (90.5) |
| Chemical agent contact            |     |          |         |
| Yes                               | 210 | 43 (20.5)| 167 (79.5)| <0.001 |
| No                                | 15,087 | 1834 (12.2)| 13,253 (87.8) |
| Ionizing radiation exposure       |     |          |         |
| Yes                               | 83  | 11 (13.3)| 72 (86.7)| 0.784 |
| No                                | 15,214 | 1866 (12.3)| 13,348 (87.7) |
| Living in newly renovated room    |     |          |         |
| Yes                               | 392 | 30 (7.7)| 362 (92.3)| 0.005 |
| No                                | 14,905 | 1847 (12.4)| 13,058 (87.6) |
| Residential proximity to a main road <50 m |     |          |         |
| Yes                               | 1721 | 158 (9.2)| 1563 (90.8)| <0.001 |
| Pets exposure | No | Yes | No |
|---------------|----|-----|----|
|               | 13,576 | 1719 (12.7) | 11,857 (87.3) |
|               | 14,956 | 1876 (12.5) | 13,080 (87.5) |
|               | <0.001 |

| Reproductive history | Gravidity | ≥3 | 2 | 1 |
|----------------------|-----------|----|---|---|
|                      |           | 3089 | 421 (13.6) | 2668 (86.4) |
|                      |           | 4710 | 601 (12.8) | 4109 (87.2) |
|                      |           | 7392 | 836 (11.3) | 6556 (88.7) |
| Previous pregnancy with birth defect | Yes | 106 | 15 (14.2) | 91 (85.8) |
|                      | No        | 15,191 | 1862 (12.3) | 13,329 (87.7) |
| Previous pregnancy with stillbirths | Yes  | 112 | 24 (21.4) | 88 (78.6) |
|                                  | No      | 15,185 | 1853 (12.2) | 13,332 (87.8) |
| Spontaneous/elective abortion history | Yes | 1037 | 226 (21.8) | 811 (78.2) |
|                                  | No      | 14,260 | 1651 (11.6) | 12,609 (88.4) |
| Paternal factors | Manual worker | Yes | 3057 | 416 (13.6) | 2641 (86.4) |
|                    |          | No  | 12,240 | 1461 (11.9) | 10,779 (88.1) |
| Cold/Flu | Yes | 215 | 107 (49.8) | 108 (50.2) |
|           | No  | 15,082 | 1770 (11.7) | 13,312 (88.3) |
| Alcohol intake | Yes | 885 | 101 (11.4) | 784 (88.6) |
|                   | No  | 14,412 | 1776 (12.3) | 12,636 (87.7) |
| Smoking | Yes | 2592 | 622 (24) | 1970 (76) |
|         | No  | 12,705 | 1255 (9.9) | 11,450 (90.1) |
| Chemical agent contact | Yes | 114 | 27 (23.7) | 87 (76.3) |
|                   | No  | 15,183 | 1850 (12.2) | 13,333 (87.8) |

FAS, folic acid supplementation; BMI, body mass index; CHD, congenital heart disease;

* Migrants: people living and working outside their origin;
† Manual worker: working in handicraft industry, working by hand, or operating machine in manufactory;
‡ Exposure window: during periconceptional period (3 months before pregnancy to the end of the 1st trimester);
§ Exposure window: in the 1st trimester of pregnancy (within 3 months after pregnancy);
¶ Maternal diabetes: included pregestational and gestational, type I and type II diabetes;
£ Threatened abortion: symptom of vaginal bleeding occurs in the first 20 weeks of pregnancy with or without abdominal cramps, indicating a possible miscarriage;
∮ Anti-miscarriage medication: medication use to prevent miscarriage;
‡ Alcohol intake: a reported alcohol intake of on average at least 50 ml/d without specifying wine;
Smoking: on average consume at least one cigarette per day;
Passive smoking: maternal self-reported exposure to environmental tobacco smoke at home, workplace, or both;
Chemical agent contact: occupational or long-term use of any of the harmful chemicals comprising any organic solvent or farm chemicals (including Herbicides, Pesticides, and Rodenticides);
Living in newly renovated room: pregnant women moving into a new house within 6 months after decoration.
Table S3. Sensitivity analysis of the associations between first trimester maternal folic acid supplementation and congenital heart disease, by etiologic categories, in 4726 matched cases and controls, Guangdong Registry of Congenital Heart Disease, 2004-2016, China.

| CHD etiology categories | Paired n * | aOR (95%CI) † | P-value |
|--------------------------|------------|---------------|---------|
| Total CHDs               | 4726       | 0.57 (0.44-0.75) | <0.001 |
| Conotruncal defects      | 338        | The coefficients did not converge | NA      |
| AVSD                     | 103        | The coefficients did not converge | NA      |
| APVR                     | 29         | The coefficients did not converge | NA      |
| LVOTO                    | 89         | The coefficients did not converge | NA      |
| RVOTO                    | 260        | The coefficients did not converge | NA      |
| SV                       | 52         | The coefficients did not converge | NA      |
| Septal defects           | 2733       | 0.58 (0.42-0.81) | 0.001  |
| Other specified CHDs     | 1002       | 0.18 (0.07-0.50) | 0.001  |
| Unspecified CHDs         | 120        | The coefficients did not converge | NA      |

aOR: adjusted odds ratio; APVR, anomalous pulmonary venous return; AVSD, atrioventricular septal defect; CHD, congenital heart disease; CI, confidence interval; LVOTO, left ventricle outflow tract obstruction; RVOTO, right ventricle outflow tract obstruction; SV, single ventricle.

* Condition on enrollment hospitals, date of conception (±3 months) and baby sex.
† Adjusted for year (pre vs. post 2013), maternal demographics (age, education, migrants, and manual worker), maternal disease (fever, flu, and threatened abortion), maternal medicine use (Chinese medicine use), reproductive history (previous pregnancy with stillbirth, and spontaneous/elective abortion history), maternal lifestyle factors and environmental exposures (smoking, living in newly renovated room, and residential proximity to a main road (<50m)), and paternal factors (flu, smoking, and chemical agent contact); household income, gravidity, maternal anti-miscarriage medicine use, and paternal manual worker were excluded from the model due to significant collinearity with maternal education, maternal age, threatened abortion, and maternal manual worker, respectively.
Table S4. Annual number of congenital heart disease avoided by the folic acid policy under different compliance rate among the pregnant women, Guangdong Registry of Congenital Heart Disease, 2004-2016, China.

| Compliance rate | N of CHDs avoided by the policy | 95% CIs  |
|-----------------|--------------------------------|---------|
| 100%            | 947                            | 783-1052|
| 90%             | 852                            | 705-947 |
| 80%             | 758                            | 626-842 |
| 70%             | 663                            | 548-736 |
| 60%             | 568                            | 470-631 |
| 50%             | 474                            | 392-526 |
| 40%             | 379                            | 313-421 |
| 30%             | 284                            | 235-316 |

CHDs, congenital heart diseases; CI, confidence intervals; N, number; Estimation was based on 1169 CHD cases in 2012.