Journal of the American Heart Association

ORIGINAL RESEARCH

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

Yanji Qu, MD, PhD; Shao Lin, PhD; Jian Zhuang, MD, PhD; Michael S. Bloom, PhD; Maggie Smith, PhD; Zhiqiang Nie, MD, PhD; Jinzhuang Mai, MD; Yanqiu Ou, MD, PhD; Yong Wu, MD; Xiangmin Gao, MD; Hongzhuan Tan, PhD; Xiaoqing Liu, MD

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]

Correspondence to: Hongzhuan Tan, PhD, Xiangya School of Public Health, Central South University, #238 Shangmayuanling Road, Kaifu District, Changsha, Hunan 410008, China. E-mail: tanhz@csu.edu.cn and Xiaoqing Liu, MD, Guangdong Cardiovascular Institute, Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, #96 Dongchuan Road, Guangzhou 510100, China. E-mail: drliuxiaoqing@gdph.org.cn

Supplementary Materials for this article are available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.119.015652

For Sources of Funding and Disclosures, see page 12.

JAHA is available at: www.ahajournals.org/journal/jaha

CLINICAL PERSPECTIVE

What Is New?

- First-trimester maternal folic acid supplementation (FAS), even when initiated after conception, is associated with a lower risk of congenital heart diseases (CHDs) among offspring, and first-trimester multivitamin use alone does not protect against CHDs.
- Most CHD phenotypes benefit from first-trimester maternal FAS; the most severe categories, such as multiple critical CHDs and critical phenotypes like single ventricle, benefit most from FAS.
- Approximately 60000 live births with CHDs could be saved annually by universal implementation of first trimester FAS in China.

What Are the Clinical Implications?

- Women planning to get pregnant should be advised to initiate FAS as early as possible to ensure coverage of critical fetal heart development periods and to decrease the risk of CHDs.
- Mothers who missed preconceptional FAS should also supplement folic acid rather than use only multivitamins during the first trimester of pregnancy to decrease the risk of CHDs.

Nonstandard Abbreviations and Acronyms

aOR	adjusted odds ratio
CHD	congenital heart disease
FA	folic acid
FAS	folic acid supplementation
GRCHD	Guangdong Registry of Congenital Heart Disease study
NTDs	neural tube defects

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, preconceptionally 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, atrioventricular 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, atrioventricular 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. All pregnant women received basic ultrasound at 11 to 13 gestational weeks (ie, first trimester) and 15 to 20 gestational weeks (ie, second trimester). Fetuses with suspected CHDs were referred to our center for a confirmatory echocardiogram and genetic tests as needed. All newborns were also assessed for cardiac defects before discharge (usually within 72 hours). When necessary, computed tomography, cardiac catheterization, surgery, or autopsy were performed to assist the diagnosis. Each confirmed CHD diagnosis was approved by 2 senior specialists at our center. Disagreements were resolved by a third senior pediatric cardiologist.

Face-to-face interviews were conducted by obstetricians with the mothers of CHD cases and controls. A standardized, structured questionnaire was adopted to obtain information about (1) maternal sociodemographic factors (age, education, household income, occupation, ethnicity, residence, and migrant worker); (2) reproductive history (gravidity, previous pregnancy with birth defects or stillbirth, and spontaneous and elective abortion history); (3) maternal diseases, medication use, lifestyle factors, and environmental exposures during the first trimester; and (4) paternal sociodemographic factors, lifestyle factors, and environmental exposures during periconceptional periods (from 3 months before conception until the end of the first trimester of pregnancy) as previously described in detail.[22]

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 $P \leq 0.10$ 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.[27,28] 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 medication 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, antimiscarriage 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.[29] 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[30] and the number of live births in 2017.[31] We estimated the annual total number of CHD births as total birth number in 2017× prevalence of CHDs in live births; the annual number of CHD cases prevented by FAS 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

Table 1. Maternal Sociodemographic Characteristics by Congenital Heart Disease Case-Control Status, Guangdong Registry of Congenital Heart Disease, 2004–2016, China (n=15 297)

Maternal Sociodemographics	CHD Cases, n (%)	Controls, n (%)	*P* Value
Total	8379	6918	…
Age, y			
>35	774 (9.2)	488 (7.1)	<0.001
15–29	5860 (69.9)	5074 (73.3)	
30–35	1745 (20.8)	1356 (19.6)	
Education attainment			
<12 y	691 (8.4)	346 (5.1)	<0.001
≥12 y	7534 (91.6)	6471 (94.9)	
Ethnicity			
Minorities	123 (1.5)	63 (0.9)	0.002
Han	8256 (98.5)	6855 (99.1)	
Residence			
Rural	3338 (39.8)	2682 (38.8)	0.178
City	5041 (60.2)	4236 (61.2)	
Migrants*			
Yes	2260 (27)	1612 (23.3)	<0.001
No	6119 (73)	5306 (76.7)	
Household income, CNY/month/person			
<3000	4481 (54.3)	3426 (50.2)	<0.001
≥3000	3772 (45.7)	3402 (49.8)	
Manual worker†,‡			
Yes	1130 (13.5)	573 (8.3)	<0.001
No	7249 (86.5)	6345 (91.7)	

*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 first trimester).

was not associated with CHDs (aOR, 1.42; 95% CI, 0.73–2.77). We found a similar effect estimate for simultaneous use of both FAS and multivitamin to only FAS (aOR, 0.68; 95% CI, 0.57–0.81). There was no significant interaction between FAS and multivitamin use (P=0.292).

As shown in the Figure—Panel A, we found protective associations between FAS and all CHD severity and plurality categories, including critical and minor CHDs, and multiple and single CHDs. The associations were stronger for critical CHDs (aOR, 0.54; 95% CI, 0.47–0.62) than for minor CHDs (aOR, 0.84; 95% CI, 0.75–0.95), and for multiple CHDs (aOR, 0.37; 95% CI, 0.31–0.44) than for single CHDs (aOR, 0.87; 95% CI, 0.78–0.97). The Figure—Panel B showed joint severity and plurality CHD categories. We found the strongest protective association for FAS with multiple critical CHDs (aOR, 0.16; 95% CI, 0.12–0.22), followed by multiple minor CHDs (aOR, 0.64; 95% CI, 0.52–0.79), and single critical CHDs (aOR, 0.82; 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

Table 2. Associations Between First-Trimester Maternal Folic Acid Supplementation and Maternal Sociodemographic Characteristics, Guangdong Registry of Congenital Heart Disease, 2004–2016, China (n=15 297)

Characteristics	FAS=1, n (%)	FAS=0, n (%)	cORs (95% CI)	aORs (95% CI)*
Total	1877 (12.3)	13 420 (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)	12 206 (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)	13 259 (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)	11 885 (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, 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.

†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 first trimester).

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 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 (1.00–3.66)	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 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.

†Multiplicative interaction between FAS and multivitamin use: aOR, 0.69; 95% CI, 0.34 to 1.38; P=0.292.

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 to a 0.4-mg dose of FA to prevent NTDs.[32] Third, we did not have access to the information of individual start time of maternal FAS. There might be misclassification between first-trimester maternal FAS with periconceptional FAS (starting FAS from 3 months before conception). However, this probability was extremely low because unplanned pregnancies were common, together with a very low proportion (<15%) of preconceptional FAS among the mothers supplementing FA in the first trimester of pregnancy in China.[33] Although the exact starting time of maternal FAS is not available in our study, we asked the participating mothers if they supplemented FA continuously in the first trimester, which covered the critical window for fetal heart development. In addition, the starting time of maternal FAS should not be substantially different between the cases and controls because most FA supplements were prescribed by obstetricians according to a standard procedure at the initial antenatal visit early in the first trimester of pregnancy. Furthermore, we made every effort to reduce the reporting bias between the cases and controls using the following strategies: (1) We used the standardized and the same approach in collecting the information of FAS and other exposures in both the cases and controls; (2) brand name of FA product and weekly frequency of FA use were inquired if first-trimester FAS was admitted; (3) pregnancy calendar was used to aid mothers to recall FAS and other exposures; and (4) cases and controls were frequency matched on enrollment year and inquired in the same year in each participating center to minimize recall difference. Still, we plan to incorporate FAS start time in our future work. Fourth, in terms of confounders, we excluded CHD cases with gene-related cases, noncardiac anomalies, and nonsingleton births to decrease the possibility of confounding from inherited conditions. We further adjusted for most known noninherited risk factors of CHD. However, we did not collect information about dietary intake of FA-containing foods, which may lead to remaining confounding. However, the typical Chinese diet has a lower intake of folate-rich meat than the typical Western diet,[34] and FA fortification foods are not available in China. In addition, FA intake is strongly influenced by dietary patterns related to sociodemographic characteristics.[35] We adjusted for maternal and paternal sociodemographic factors, which were likely to adjust in great part for dietary FA. Fifth, 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

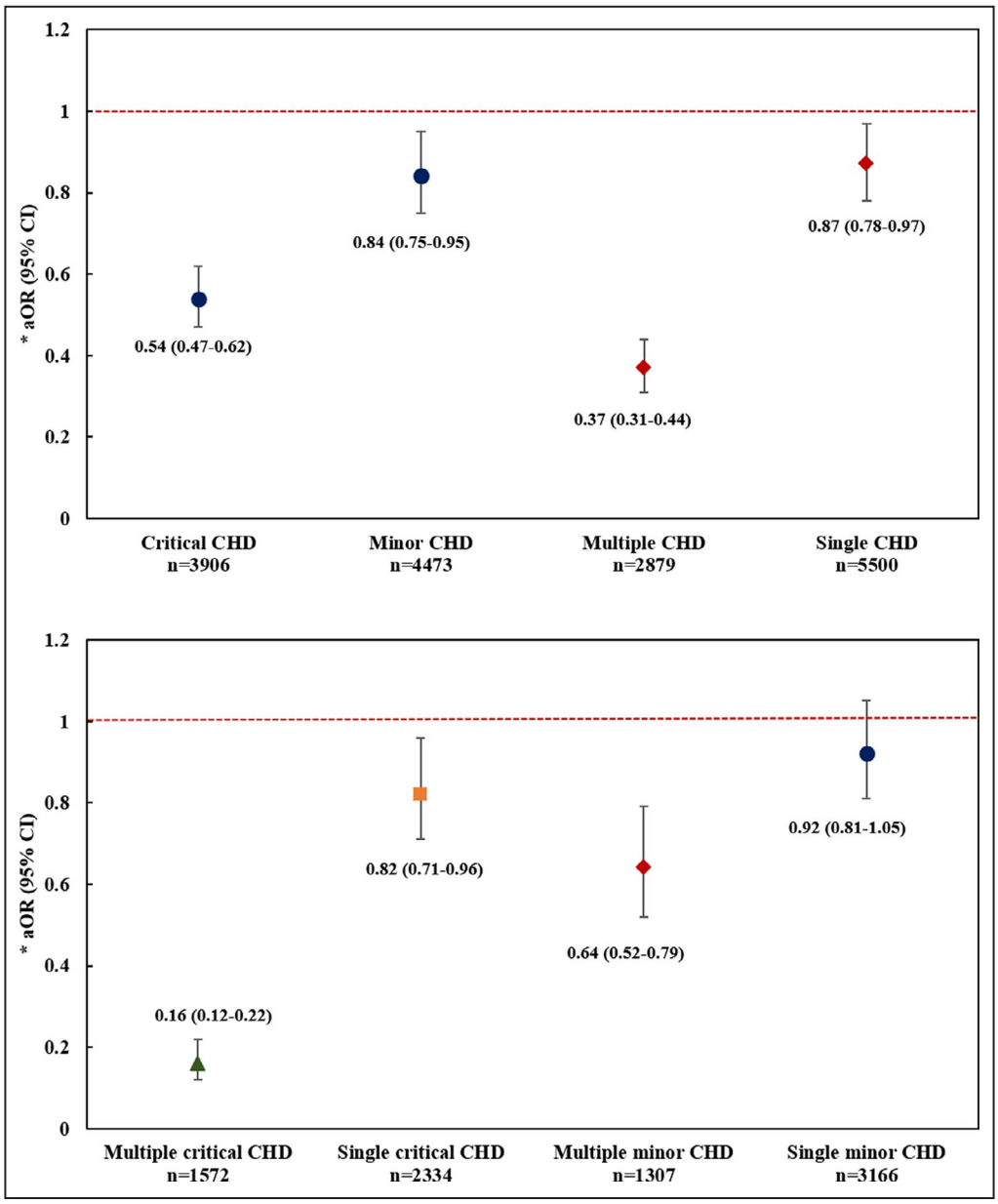

Figure. Association of first-trimester maternal folic acid supplementation with congenital heart disease categories by severity and plurality, Guangdong Registry of Congenital Heart Disease, 2004–2016, China.

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.

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.[36] 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,

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‡
Conotruncal defects	868	25 (2.9)	0.15 (0.10–0.23)	<0.001‡
TGA	343	7 (2.0)	0.11 (0.05–0.24)	<0.001‡
ToF	309	12 (3.9)	0.21 (0.11–0.37)	<0.001‡
DORV	178	3 (1.7)	0.09 (0.03–0.29)	<0.001‡
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‡
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‡
CoA/IAA	111	5 (4.5)	0.23 (0.09–0.58)	0.002‡
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‡
HRHS	74	0	0.07 (0.01–0.47)	0.007‡
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‡
vPS	351	30 (8.5)	0.50 (0.34–0.75)	0.001‡
SV	144	1 (0.7)	0.03 (0.004–0.21)	<0.001‡
Septal defects	4437	577 (13.0)	0.84 (0.75–0.95)	0.005‡
VSD	2863	366 (12.8)	0.85 (0.74–0.98)	0.022‡
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‡
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 maternal manual worker, respectively.

†Compare with 949 FAS in 6918 controls (13.7%).

‡False discovery rate Q<0.05.

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

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, %	GRCHD 2004–2016, China			
		Based on 17.23 Million Live Births in China in 2017			
		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	2340	72
HRHS	93	0.1	1723	1602	121
DORV	91	0.28	4824	4390	434
TGA	89	0.43	7409	6594	815
PA	89	0.09	1551	1380	171
AVSD	86	0.28	4824	4149	675
ToF	79	0.32	5514	4356	1158
CoA/IAA	77	0.13	2240	1725	515
APVR	61	0.1	1723	1051	672
vPS	50	0.69	11 889	5945	5944
VSD	16	3.71	63 923	10 228	53 695
ASD	16	2.89	49 795	7967	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	7250
Single critical CHDs	18	0.89	15 335	2760	12 575
Multiple minor CHDs	36	1.32	22 744	8188	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 B_2, B_6, and B_{12}) 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 CHDs[37] 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.[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.[10] 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 periconceptional 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 childbearing 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

Received December 21, 2019; accepted May 29, 2020.

Affiliations

From the Department of Epidemiology and Health Statistics, Xiangya School of Public Health, Central South University, Changsha, Hunan, (Y.Q., H.T.); Guangdong Cardiovascular Institute, WHO Collaborating Center for Research and Training in Cardiovascular Diseases, Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, Guangzhou, Guangdong, China (Y.Q., J.Z., Z.N., J.M., Y.O., Y.W., X.G., X.L.); Department of Environmental Health Sciences (S.L., M.S.B., M.S.), and Department of Epidemiology and Biostatistics, University at Albany, State University of New York, Rensselaer, NY (S.L., M.S.B.).

Acknowledgments

The authors thank all participants and doctors from the 40 clinical centers in 21 cities of Guangdong Province, China. All authors reviewed and approved the final version for submission.

Sources of Funding

This study was funded by the National Key Research and Development Program (2018YFC1002600), the Natural Science Foundation of Guangdong Province (2018A030313785), the Science and Technology Planning Project of Guangdong Province (2014A050503048, 2017A070701013, 2017B090904034, 2017030314109, and 2019B020230003), Guangdong Peak Project (DFJH201802), Guangdong Provincial Key Laboratory of South China Structural Heart Disease (2012A061400008), National Natural Science Foundation of China (81903287), and Natural Science Foundation of Guangdong Province (2018A030313329).

Disclosures

Dr Bloom served as a paid consultant to the Guangdong Cardiovascular Institute. The remaining authors have no disclosures to report.

Supplementary Materials

Figures S1–S2
Tables S1–S4

REFERENCES

1. Gomes S, Lopes C, Pinto E. Folate and folic acid in the periconceptional period: recommendations from official health organizations in thirty-six countries worldwide and WHO. *Public Health Nutr*. 2016;19:176–189.
2. Czeizel AE, Dobó M, Vargha P. Hungarian cohort-controlled trial of periconceptional multivitamin supplementation shows a reduction in certain congenital abnormalities. *Birth Defects Res A Clin Mol Teratol*. 2004;11:853–861.
3. Hoffman JI, Kaplan S. The incidence of congenital heart disease. *J Am Coll Cardiol*. 2002;39:1890–1900.
4. Hoffman J. The global burden of congenital heart disease. *Cardiovasc J Afr*. 2013;24:141–145.
5. Centers for Disease Control. Critical congenital heart defects in the United States. Available at: https://www.cdc.gov/ncbddd/heartdefects/features/cchd-keyfindings.html. Accessed September 17, 2019.
6. ADAMS FH. Congenital heart disease; comments regarding incidence and natural history. *Calif Med*. 1959;3:213–216.
7. Triedman JK, Newburger JW. Trends in congenital heart disease: the next decade. *Circulation*. 2016;133:2716–2733.
8. Arth AC, Tinker SC, Simeone RM, Ailes EC, Cragan JD, Grosse SD. Inpatient hospitalization costs associated with birth defects among persons of all ages—United States, 2013. *MMWR Morb Mortal Wkly Rep*. 2017;66:41–46.
9. Simeone RM, Oster ME, Cassell CH, Armour BS, Gray DT, Honein MA. Pediatric inpatient hospital resource use for congenital heart defects. *Birth Defects Res A Clin Mol Teratol*. 2014;100:934–943.
10. China Disabled Persons Federation. [Action plan to improve the quality of birth and reduce birth defects and disabilities in China (2002–2010)]. *Chin J Reprod Health*. 2002;3:98–101.
11. McClung N, Glidewell J, Farr SL. Financial burdens and mental health needs in families of children with congenital heart disease. *Congenit Heart Dis*. 2018;13:554–562.
12. Obeid R, Holzgreve W, Pietrzik K. Folate supplementation for prevention of congenital heart defects and low birth weight: an update. *Cardiovasc Diagn Ther*. 2019;9:S424–S433.
13. Czeizel AE. Reduction of urinary tract and cardiovascular defects by periconceptional multivitamin supplementation. *Am J Med Genet*. 1996;62:179–183.
14. Feng Y, Wang S, Chen R, Tong X, Wu Z, Mo X. Maternal folic acid supplementation and the risk of congenital heart defects in offspring: a meta-analysis of epidemiological observational studies. *Sci Rep*. 2015;5:8506.
15. Xu A, Cao X, Lu Y, Li H, Zhu Q, Chen X, Jiang H, Li X. A meta-analysis of the relationship between maternal folic acid supplementation and the risk of congenital heart defects. *Int Heart J*. 2016;57:725–728.
16. Leirgul E, Gildestad T, Nilsen RM, Fomina T, Brodwall K, Greve G, Vollset SE, Holmstrøm H, Tell GS, Øyen N. Periconceptional folic acid supplementation and infant risk of congenital heart defects in Norway 1999–2009. *Paediatr Perinat Epidemiol*. 2015;29:391–400.
17. Bedard T, Lowry RB, Sibbald B, Harder JR, Trevenen C, Horobec V, Dyck JD. Folic acid fortification and the birth prevalence of congenital heart defect cases in Alberta, Canada. *Birth Defects Res A Clin Mol Teratol*. 2013;97:564–570.
18. Øyen N, Olsen SF, Basit S, Leirgul E, Strøm M, Carstensen L, Granström C, Tell GS, Magnus P, Vollset SE, et al. Association between maternal folic acid supplementation and congenital heart defects in offspring in birth cohorts from Denmark and Norway. *J Am Heart Assoc*. 2019;8:e011615. DOI: 10.1161/JAHA.118.011615.
19. Czeizel AE. The primary prevention of birth defects: multivitamins or folic acid? *Int J Med Sci*. 2004;1:50–61.
20. Department of Health. *Folic Acid and the Prevention of Disease. Report of the Committee on Medical Aspects of Food and Nutrition*

Policy. London: H.M. The Stationery Office. Available at: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/743522/Folic_Acid_and_the_Prevention_of_Disease__2000_.pdf. Accessed November 29, 2019.
21. Ou Y, Mai J, Zhuang J, Liu X, Wu Y, Gao X, Nie Z, Qu Y, Chen J, Kielb C, et al. Risk factors of different congenital heart defects in Guangdong, China. *Pediatr Res*. 2016;79:549–558.
22. Liu X, Nie Z, Chen J, Guo X, Ou Y, Chen G, Mai J, Gong W, Wu Y, Gao X, et al. Does maternal environmental tobacco smoke interact with social-demographics and environmental factors on congenital heart defects? *Environ Pollut*. 2018;234:214–222.
23. Botto LD, Lin AE, Riehle-Colarusso T, Malik S, Correa A. Seeking causes: classifying and evaluating congenital heart defects in etiologic studies. *Birth Defects Res A Clin Mol Teratol*. 2007;79:714–727.
24. Oyen N, Poulsen G, Boyd HA, Wohlfahrt J, Jensen PK, Melbye M. National time trends in congenital heart defects, Denmark, 1977–2005. *Am Heart J*. 2009;157:467–473.e1.
25. Liberman RF, Getz KD, Lin AE, Higgins CA, Sekhavat S, Markenson GR, Anderka M. Delayed diagnosis of critical congenital heart defects: trends and associated factors. *Pediatrics*. 2014;134:e373–e381.
26. Slaughter JL, Cua CL, Notestine JL, Rivera BK, Marzec L, Hade EM, Maitre NL, Klebanoff MA, Ilgenfritz M, Le VT, et al. Early prediction of spontaneous patent ductus arteriosus (PDA) closure and PDA-associated outcomes: a prospective cohort investigation. *BMC Pediatr*. 2019;19:333.
27. Greenland S, Pearl J, Robins JM. Causal diagrams for epidemiologic research. *Epidemiology*. 1999;10:37–48.
28. Textor J, van der Zander B, Gilthorpe MS, Liskiewicz M, Ellison GT. Robust causal inference using directed acyclic graphs: the R package "dagitty." *Int J Epidemiol*. 2016;45:1887–1894.
29. Storey JD. A direct approach to false discovery rates. *J R Stat Soc Series B Stat Method*. 2002;479–498.
30. Qu Y, Liu X, Zhuang J, Chen G, Mai J, Guo X, Ou Y, Chen J, Gong W, Gao X, et al. Incidence of congenital heart disease: the 9-year experience of the Guangdong registry of congenital heart disease, China. *PLoS One*. 2016;11:e0159257.
31. Statistics from the National Bureau of China. Live births number in 2017. Available at: http://www.stats.gov.cn/tjsj/zxfb/201802/t20180228_1585631.html. Accessed September 17, 2019.
32. Berry RJ, Li Z, Erickson JD, Li S, Moore CA, Wang H, Mulinare J, Zhao P, Wong LY, Gindler J, et al. Prevention of neural-tube defects with folic acid in China. China-U.S. Collaborative Project for Neural Tube Defect Prevention. *N Engl J Med*. 1999;341:1485–1490.
33. Yan J, Zheng YZ, Cao LJ, Liu YY, Li W, Huang GW. Periconceptional folic acid supplementation in Chinese women: a cross-sectional study. *Biomed Environ Sci*. 2017;30:737–748.
34. Sebastiani G, Herranz BA, Borras-Novell C, Alsina CM, Aldecoa-Bilbao V, Andreu-Fernandez V, Pascual Tutusaus M, Ferrero Martínez S, Gómez Roig MD, García-Algar O. The effects of vegetarian and vegan diet during pregnancy on the health of mothers and offspring. *Nutrients*. 2019;11:E557.
35. Yang J, Dang S, Cheng Y, Qiu H, Mi B, Jiang Y, Qu P, Zeng L, Wang Q, Li Q, et al. Dietary intakes and dietary patterns among pregnant women in Northwest China. *Public Health Nutr*. 2017;20:282–293.
36. Li X, Li S, Mu D, Liu Z, Li Y, Lin Y, Chen X, You F, Li N, Deng K, et al. The association between periconceptional folic acid supplementation and congenital heart defects: a case–control study in China. *Prev Med*. 2013;56:385–389.
37. Mao B, Qiu J, Zhao N, Shao Y, Dai W, He X, Cui H, Lin X, Lv L, Tang Z, et al. Maternal folic acid supplementation and dietary folate intake and congenital heart defects. *PLoS One*. 2017;12:e0187996.
38. Ren A, Zhang L, Li Z, Hao L, Tian Y, Li Z. Awareness and use of folic acid, and blood folate concentrations among pregnant women in northern China-an area with a high prevalence of neural tube defects. *Reprod Toxicol*. 2006;3:431–436.
39. Yang B, Fan S, Zhi X, Xia R, Wang Y, Zheng Q, Sun G. Geographical and ethnic distribution of MTHFR gene polymorphisms and their associations with diseases among Chinese population. *Clin Genet*. 2017;3:243–258.
40. ALFRED. The ALlele FREquency Database. Available at: https://alfred.med.yale.edu/alfred/SiteTable1A_working.asp?siteuid=SI001032G. Accessed October 27, 2019.
41. Werler MM, Hayes C, Louik C, Shapiro S, Mitchell AA. Multivitamin supplementation and risk of birth defects. *Am J Epidemiol*. 1999;7:675–682.
42. Czeizel AE. Prevention of congenital abnormalities by periconceptional multivitamin supplementation. *BMJ*. 1993;306:1645–1648.
43. Czeizel AE, Vereczkey A, Szabó I. Folic acid in pregnant women associated with reduced prevalence of severe congenital heart defects in their children: a national population-based case–control study. *Eur J Obstet Gynecol Reprod Biol*. 2015;193:34–39.
44. Liu S, Joseph KS, Luo W, León JA, Lisonkova S, Van den Hof M, Evans J, Lim K, Little JR, Sauve RS, et al. Effect of folic acid food fortification in Canada on congenital heart disease subtypes. *Circulation*. 2016;134:647–655.
45. Botto LD, Mulinare J, Erickson JD. Occurrence of congenital heart defects in relation to maternal multivitamin use. *Am J Epidemiol*. 2000;9:878–884.
46. van Beynum IM, Kapusta L, Bakker MK, den Heijer M, Blom HJ, de Walle HE. Protective effect of periconceptional folic acid supplements on the risk of congenital heart defects: a registry-based case-control study in the northern Netherlands. *Eur Heart J*. 2010;4:464–471.
47. Czeizel AE, Vereczkey A, Szabo I. Folic acid in pregnant women associated with reduced prevalence of severe congenital heart defects in their children: a national population-based case-control study. *Eur J Obstet Gynecol Reprod Biol*. 2015;193:34–39.
48. Case AP, Ramadhani TA, Canfield MA, Beverly L, Wood R. Folic acid supplementation among diabetic, overweight, or obese women of childbearing age. *J Obstet Gynecol Neonatal Nurs*. 2007;4:335–341.
49. Butterworth CE Jr, Hatch KD, Macaluso M, Cole P, Sauberlich HE, Soong SJ, Borst M, Baker VV. Folate deficiency and cervical dysplasia. *JAMA*. 1992;4:528–533.
50. Betts JG. *Anatomy & Physiology*. 2013:787–846. Available at: http://cnx.org/content/col11496/1.6/. Accessed November 20, 2019.
51. Hao L, Yang QH, Li Z, Bailey LB, Zhu JH, Hu DJ, Zhang BL, Erickson JD, Zhang L, Gindler J, et al. Folate status and homocysteine response to folic acid doses and withdrawal among young Chinese women in a large-scale randomized double-blind trial. *Am J Clin Nutr*. 2008;2:448–457.
52. Jiang YL, Sun NH, Xiang Y, Li SL, Qi QW, Liu JT, Bian XM, Yang JQ. [Study on the correlation of serum folate and red blood cell folate level with birth defects and unexplained recurrent pregnancy loss]. *Zhonghua Fu Chan Ke Za Zhi*. 2007;42:448–452.
53. Oliver EM, Grimshaw KE, Schoemaker AA, Keil T, McBride D, Sprikkelman AB, Ragnarsdottir HS, Trendelenburg V, Emmanouil E, Reche M, et al. Dietary habits and supplement use in relation to national pregnancy recommendations: data from the EuroPrevall birth cohort. *Matern Child Health J*. 2014;18:2408–2425.
54. Liu M, Chen J, Liu J, Zhang S, Wang Q, Shen H, Zhang Y. Socioeconomic inequality in periconceptional folic acid supplementation in China: a census of 0.9 million women in their first trimester of pregnancy. *BMC Pregnancy Childbirth*. 2017;17:422.
55. Medveczky E, Puhó E. Parental employment status and neural-tube defects and folic acid/multivitamin supplementation in Hungary. *Eur J Obstet Gynecol Reprod Biol*. 2004;115:178–184.
56. Brough L, Rees GA, Crawford MA, Dorman EK. Social and ethnic difference in folic acid use preconception and during early pregnancy in the UK: effect on maternal folate status. *J Hum Nutr Diet*. 2009;2:100–107.

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.

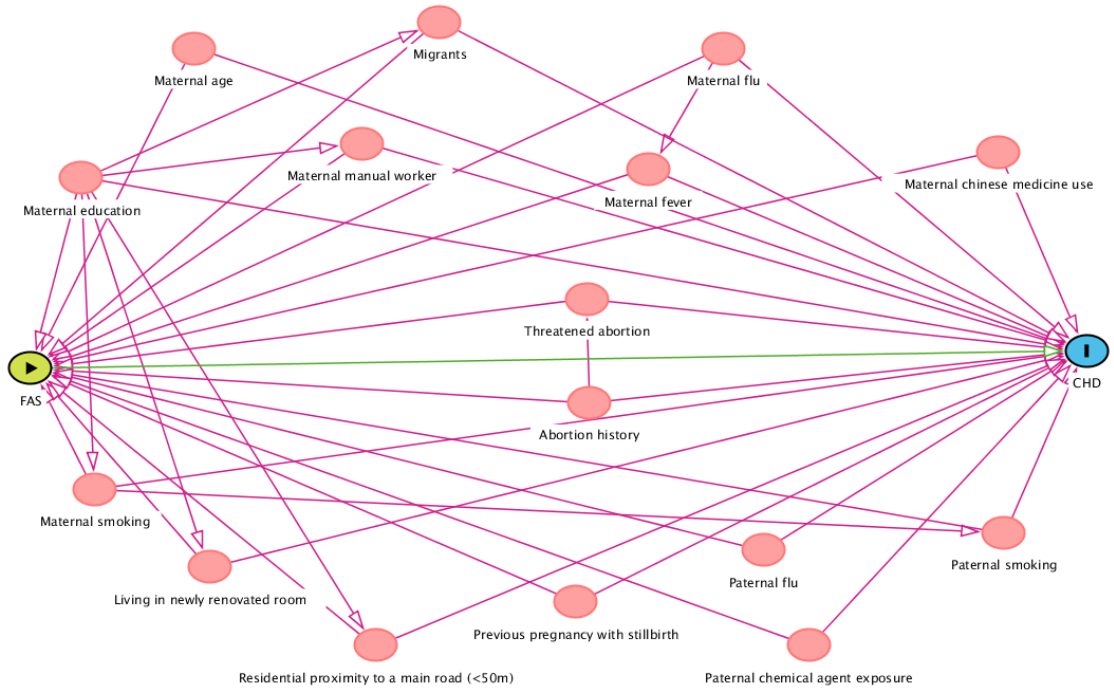

Figure S2. Flow chart of study participants, Guangdong Registry of Congenital Heart Disease, 2004-2016, China.

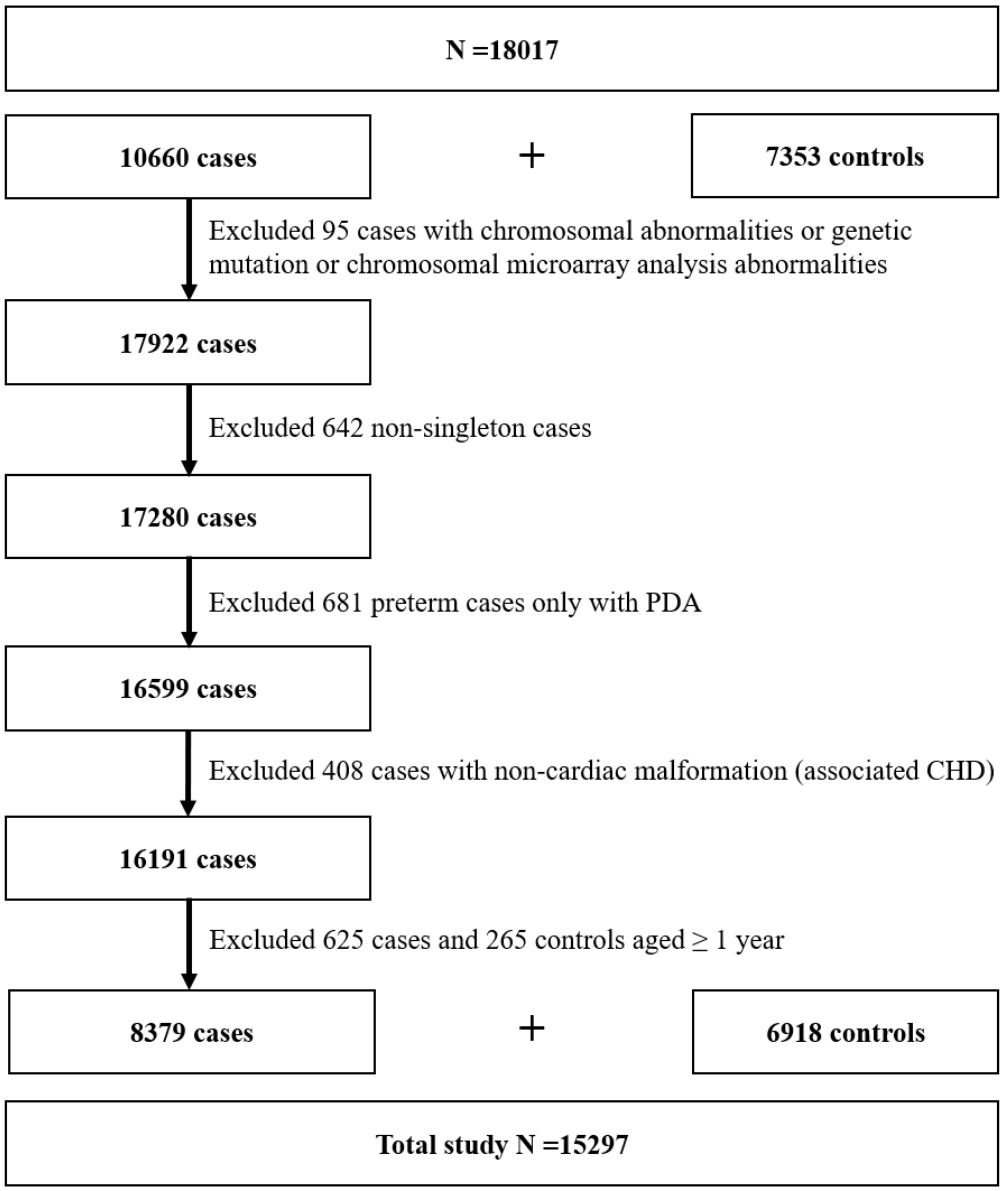

Table S1. Parental sociodemographic and lifestyle factors and environmental exposures according to congenital heart disease status, Guangdong Registry of Congenital Heart Disease, 2004-2016, China (n=15,297).

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 ℃)				
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

No	14,918	8158 (97.4)	6760 (97.7)	
Hypertension				
Yes	148	115 (1.4)	33 (0.5)	<0.001
No	15,149	8264 (98.6)	6885 (99.5)	
Threatened abortion [¶]				
Yes	727	546 (6.5)	181 (2.6)	<0.001
No	14,567	7831 (93.5)	6736 (97.4)	
Thyroid disorder				
Yes	54	32 (0.4)	22 (0.3)	0.508
No	15,243	8347 (99.6)	6896 (99.7)	
Thalassemia				
Yes	115	69 (0.8)	46 (0.7)	0.259
No	15,182	8310 (99.2)	6872 (99.3)	
Maternal medicine use [§]				
Chinese (patent) medicine				
Yes	208	168 (2.0)	40 (0.6)	<0.001
No	15,089	8211 (98.0)	6878 (99.4)	
Antibiotic				
Yes	141	118 (1.4)	23 (0.3)	<0.001
No	15,156	8261 (98.6)	6895 (99.7)	
Anti-miscarriage medication [£]				
Yes	334	228 (2.7)	106 (1.5)	<0.001
No	14,963	8151 (97.3)	6812 (98.5)	
Maternal lifestyle factors and environmental exposures [§]				
Prepregnancy BMI (kg/m^2)				
Over weight (≥24)	455	265 (3.2)	190 (2.7)	
Low weight (<18.5)	743	398 (4.7)	345 (5.0)	0.536
Normal (18.5-24.9)	14,099	7716 (92.1)	6383 (92.3)	
Alcohol intake [ɸ]				
Yes	87	65 (0.8)	22 (0.3)	<0.001
No	15,210	8314 (99.2)	6896 (99.7)	
Smoking [$]				
Yes	141	119 (1.4)	22 (0.3)	<0.001
No	15,156	8260 (98.6)	6896 (99.7)	
Passive smoking [&]				
Yes	1892	1002 (12.0)	890 (12.9)	0.09
No	14,405	7377 (88.0)	6028 (87.1)	
Chemical agent contact [#]				
Yes	210	124 (1.5)	86 (1.2)	0.211
No	15,087	8255 (98.5)	6832 (98.8)	
Ionizing radiation exposure				
Yes	83	54 (0.6)	29 (0.4)	0.061
No	15,214	8325 (99.4)	6889 (99.6)	
Living in newly renovated room [‡, △]				

Yes	392	317 (3.8)	75 (1.1)	<0.001
No	14,905	8062 (96.2)	6843 (98.9)	
Residential proximity to a main road <50 m ‡				
Yes	1721	1076 (12.8)	645 (9.3)	<0.001
No	13,576	7303 (87.2)	6273 (90.7)	
Pets exposure				
Yes	341	243 (2.9)	98 (1.4)	<0.001
No	14,956	8136 (97.1)	6820 (98.6)	
Reproductive history				
Gravidity				
≥3	3089	1810 (21.8)	1279 (18.6)	<0.001
2	4710	2682 (32.2)	2028 (29.5)	
1	7392	3829 (46.0)	3563 (51.9)	
Previous pregnancy with birth defect				
Yes	106	95 (1.1)	11 (0.2)	<0.001
No	15,191	8284 (98.9)	6907 (99.8)	
Previous pregnancy with stillbirths				
Yes	112	87 (1.0)	25 (0.4)	<0.001
No	15,185	8292 (99.0)	6893 (99.6)	
Spontaneous/ elective abortion history				
Yes	1037	644 (7.7)	393 (5.7)	<0.001
No	14,260	7735 (92.3)	6525 (94.3)	
Paternal factors ‡				
Manual worker †				
Yes	3057	1895 (22.6)	1162 (16.8)	<0.001
No	12,240	6484 (77.4)	5756 (83.2)	
Cold/ Flu				
Yes	215	147 (1.8)	68 (1.0)	<0.001
No	15,082	8232 (98.2)	6850 (99.0)	
Alcohol intake ¥				
Yes	885	611 (7.3)	274 (4.0)	<0.001
No	14,412	7768 (92.7)	6644 (96.0)	
Smoking $				
Yes	2592	1664 (19.9)	928 (13.4)	<0.001
No	12,705	6715 (80.1)	5990 (86.6)	
Chemical agent contact #				
Yes	114	91 (1.1)	23 (0.3)	<0.001
No	15,183	8288 (98.9)	6895 (99.7)	

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)	
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 ℃)				
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)	

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

No	13,576	1719 (12.7)	11,857 (87.3)	
Pets exposure				
Yes	341	1 (0.3)	340 (99.7)	<0.001
No	14,956	1876 (12.5)	13,080 (87.5)	
Reproductive history				
Gravidity				
≥3	3089	421 (13.6)	2668 (86.4)	
2	4710	601 (12.8)	4109 (87.2)	0.002
1	7392	836 (11.3)	6556 (88.7)	
Previous pregnancy with birth defect				
Yes	106	15 (14.2)	91 (85.8)	0.554
No	15,191	1862 (12.3)	13,329 (87.7)	
Previous pregnancy with stillbirths				
Yes	112	24 (21.4)	88 (78.6)	0.003
No	15,185	1853 (12.2)	13,332 (87.8)	
Spontaneous/ elective abortion history				
Yes	1037	226 (21.8)	811 (78.2)	<0.001
No	14,260	1651 (11.6)	12,609 (88.4)	
Paternal factors ‡				
Manual worker †				
Yes	3057	416 (13.6)	2641 (86.4)	0.012
No	12,240	1461 (11.9)	10,779 (88.1)	
Cold/ Flu				
Yes	215	107 (49.8)	108 (50.2)	<0.001
No	15,082	1770 (11.7)	13,312 (88.3)	
Alcohol intake ʃ				
Yes	885	101 (11.4)	784 (88.6)	0.423
No	14,412	1776 (12.3)	12,636 (87.7)	
Smoking $				
Yes	2592	622 (24)	1970 (76)	<0.001
No	12,705	1255 (9.9)	11,450 (90.1)	
Chemical agent contact #				
Yes	114	27 (23.7)	87 (76.3)	<0.001
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;

ʃ 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.