Journal of Exposure Science & Environmental Epidemiology www.nature.com/jes

ARTICLE

Maternal exposure to ambient ozone and fetal congenital heart defects: a national multicenter study in China

Yaqi Wang[1,4], Yanping Ruan[2,4], Xiaoyu Wan[1], Huan Wang[1], Jianhui Guo[1], Jing Wei[3], Sheng Ma[1], Yihua He[2]✉, Zhiyong Zou[1]✉ and Jing Li[1]✉

BACKGROUND: Ambient O_3 has demonstrated an aggravated increasing trend in the context of global warming. The available evidence of maternal exposure to ambient O_3 on fetal congenital heart defects (CHD) is still limited, especially in high polluted areas.

OBJECTIVE: To examine associations of maternal exposure to ambient O_3 during early pregnancy with fetal CHDs.

METHODS: We conducted a national multicenter study in 1313 hospitals from 26 provinces in China and collected a total of 27,817 participants at high risk of CHD from 2013 to 2021. Exposure to ambient O_3 during the embryonic period, preconception, the first trimester and periconception was assessed by extracting daily concentrations from a validated grid dataset at each subject's residential district. CHDs were diagnosed based on fetal echocardiography.

RESULTS: Each 10 μg/m^3 increase of exposure to ambient O_3 during the embryonic period was approximately linearly associated with a 12.7% (odds ratio [OR]: 1.127, 95% confidence interval [CI]: 1.098, 1.155) increase in odds of pooled CHD ($p < 0.001$). The associations remain robust after adjusting for ambient $PM_{2.5}$ and NO_2 exposure. The odds of different types of CHD in association with ambient O_3 exposure varied greatly. We observed significant association of ambient O_3 exposure with ventricular septal defect (VSD), tetralogy of Fallot (TOF); pulmonary stenosis (PS), pulmonary atresia (PA), transposition of great arteries (TGA) and persistent left superior vena cava (PLSVC), with TOF demonstrating the strongest estimates (OR: 1.194, 95% CI:1.107, 1.288). The estimates for preconception, the first trimester and periconception demonstrate consistent findings with the main analyses, indicating stronger associations of ambient O_3 exposure during the periconception period.

IMPACT:

- Our study provides evidence that higher ambient O_3 during early pregnancy was significantly associated with increased odds of fetal CHD.
- Our findings suggest that pregnant women, clinical practitioners, and policy makers need to pay more attention to the exposure to higher ambient O_3 during early pregnancy to reduce the risk of developing CHD and to improve outcomes across the life span.

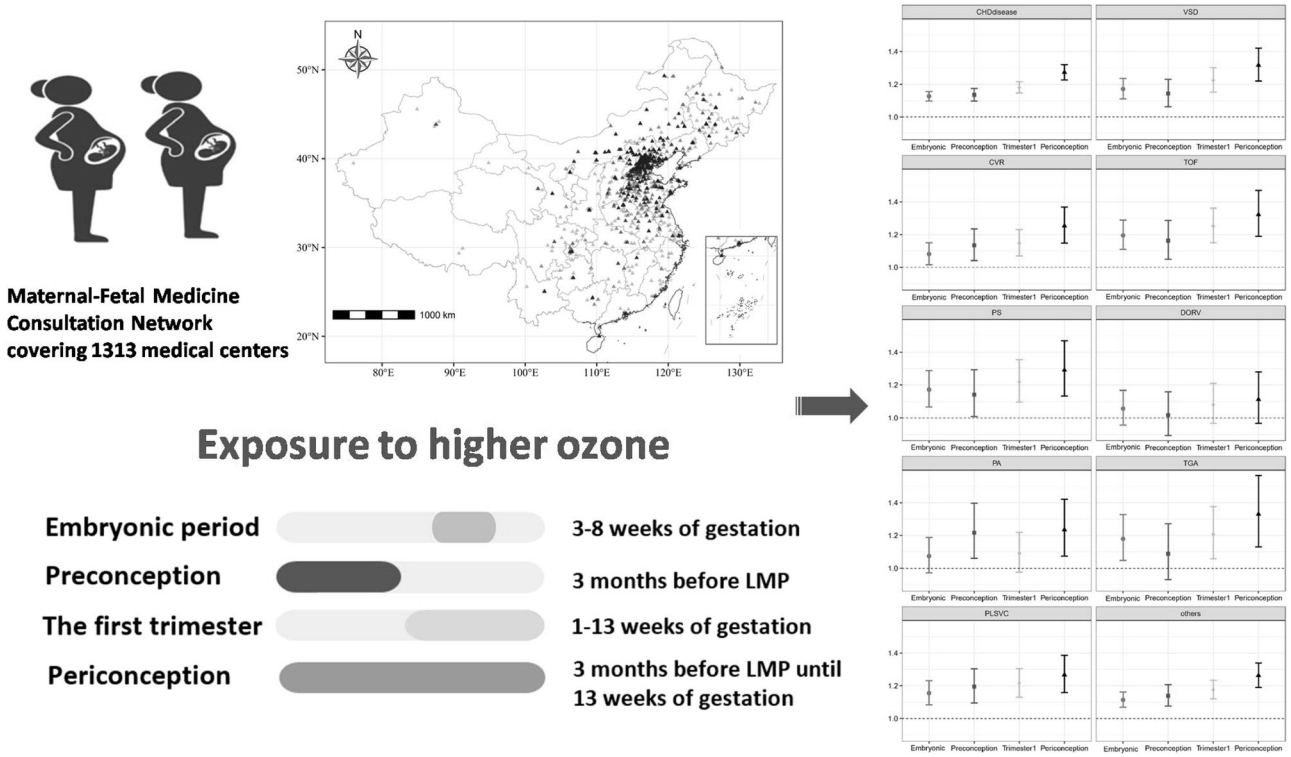
KEYWORDS: Congenital heart defects; Air pollution; Ozone; Embryonic period; Periconception period.

Journal of Exposure Science & Environmental Epidemiology (2025) 35:511–519; https://doi.org/10.1038/s41370-024-00716-4

[1]Institute of Child and Adolescent Health, School of Public Health, Peking University, National Health Commission Key Laboratory of Reproductive Health, Beijing 100191, China. [2]Department of Echocardiography, Maternal-Fetal Medicine Research Consultation Center, Beijing Anzhen Hospital, Capital Medical University, Beijing 100029, China. [3]Department of Atmospheric and Oceanic Science, Earth System Science Interdisciplinary Center, University of Maryland, College Park, MD 20740, USA. [4]These authors contributed equally: Yaqi Wang, Yanping Ruan. ✉email: heyihuaecho@hotmail.com; harveyzou2002@bjmu.edu.cn; jing.li@hsc.pku.edu.cn

Received: 20 March 2024 Revised: 19 August 2024 Accepted: 21 August 2024
Published online: 31 August 2024

Graphical Abstract



INTRODUCTION

Congenital heart disease (CHD) is the most common type of birth defect, and remains the primary cause of infant death from birth defects [1]. According to Global Burden of Disease 2019 study, CHD affected almost 9‰ infants worldwide and 61.6% CHD cases occurred in Asia [2]. In China, the mean prevalence of CHD was approximately 8.5 per 1000 live births [2], and 25,311 died due to CHD in 2019 [3]. Previous studies indicates that genetic factors account for approximately 20% of CHDs, while the remaining 80% is unknown [4]. Under the scenario of global climate change, the impacts of ambient air pollution on CHD have attracted increasing attention.

As a highly reactive and strongly oxidative ambient pollutant, ambient ozone (O_3) has demonstrated an aggravated increasing trend in the context of global warming in recent years, and has emerged as a major global public health concern [5]. Few previous studies have investigated the association between maternal exposure to ambient O_3 and the odds of CHD with inconsistent results. Two studies in Foshan city [6] and Changsha city, China utilized data from local birth defect surveillance system and identified CHD cases by physical examination after birth. They both assessed the ambient O_3 exposure using data from air quality monitoring stations, and reported that maternal exposure to ambient O_3 during the first trimester was significantly associated with increased odds of overall CHD. In contrast, other two studies in Canada [7] and Europe [8] which also used population based congenital abnormality surveillance data did not observe a significant association. Previous research mainly focused on birth CHD; however, prenatal detection of CHD plays a crucial role in ensuring timely interventions, enhancing family support, and improving the perioperative clinical condition of neonates with CHD [9]. Thus, further investigation with a specific emphasis on fetal CHD is warranted. In addition, due to the lack of complete information or insufficient sample size for certain subtypes of CHDs, it remains unknown if and how ambient O_3 exposure is associated with an increased risk of specific CHD types including ventricular septal defect (VSD), tetralogy of Fallot (TOF), valvular pulmonary stenosis (PS) and double outlet right ventricle (DORV), which can be of great importance and interest to frail individuals, clinical practitioners, and public health policymakers in reducing risk of developing specific cardiovascular diseases (CVDs). Furthermore, most previous studies were conducted in regions with lower ambient O_3 level, more investigations especially in regions with different ambient O_3 exposure ranges were warranted.

To fill the knowledge gap, we used data from Maternal-Fetal Medicine Consultation Network covering 1313 medical centers in China, and conducted a nationwide cross-sectional study to explore the impacts of exposure to ambient O_3 during early pregnancy on odds of CHD and its subtypes, and also to identify potential susceptible exposure windows.

METHODS

Study design and study population

We conducted a cross-sectional study among 28,487 pregnant women who underwent initial screening in 1313 medical centers in China. Figure 1A presents the distribution of referral hospitals. Among them, individuals identified with high risk of CHD were referred to National Maternal-Fetal Medicine Center for Fetal Heart Disease at Beijing Anzhen Hospital to obtain a definitive diagnosis of CHD when they presented with common indications for fetal echocardiogram referral according to the American Heart Association (AHA) scientific statement [10]. Pregnant women at a high risk of fetal CHD were identified based on several criteria, including structural cardiac abnormalities detected during obstetric ultrasound, suspected fetal bradycardia and irregular fetal rhythm, maternal metabolic diseases (e.g., gestational diabetes mellitus and uncontrolled phenylketonuria), fetal karyotype abnormalities, monochorionic twinning, maternal first-trimester infections (e.g., rubella virus), family history of CHD, or other indications as recommended by the American Heart Association [10]. Finally, all data was kept and managed by Maternal-Fetal Medicine Center of AnZhen hospital. By excluding 133 women without age information, 144 women lacking data on gestation week at examination and 393 women with duplicated medical records, we finally

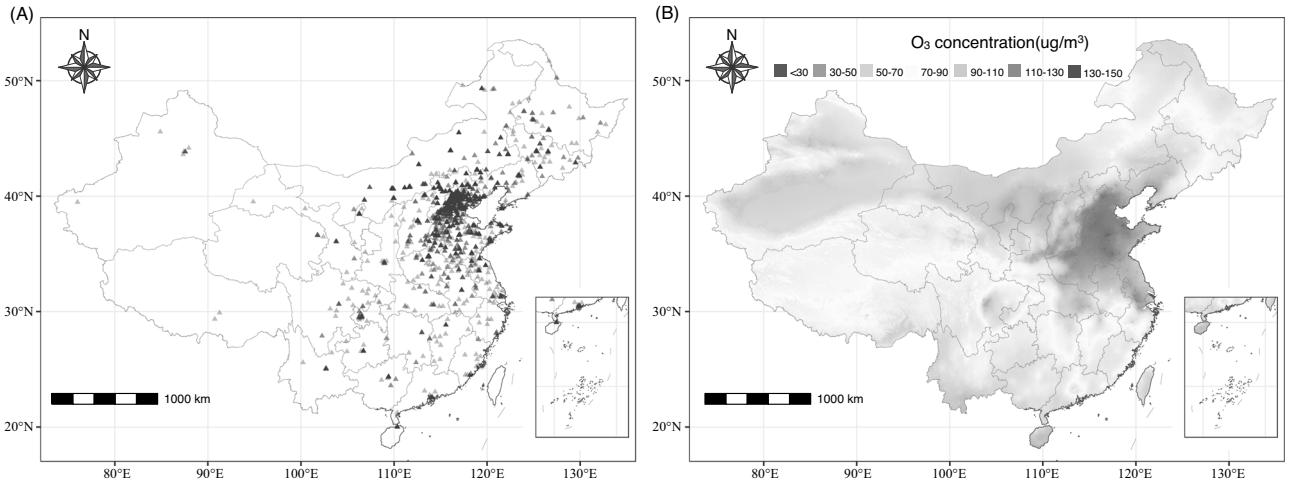


Fig. 1 Spatial distributions of 1313 Maternal-Fetal Medicine centers in China and ambient O_3 concentrations in warm season from 2013 to 2021. A The red triangle gives the location of Maternal-Fetal Medicine centers. **B** The ambient O_3 concentrations was calculated as the average of daily mean concentrations from April through September.

included 27,817 pregnant women as the study subjects. The flowchart of the participants included in this study is shown in Figure S1. This study was approved by the Institutional Review Board of Beijing Anzhen Hospital, Capital Medical University (ID: 2022060X), and written informed consent was signed by all subjects.

Exposure assessment

Ambient daily Maximum 8 h average (MDA8) O_3 concentrations with a spatial resolution of 10 km × 10 km in China between 2013 and 2021 were obtained from the Tracking Air Pollution in China (TAP) [11], which was developed and maintained by Tsinghua University in collaboration with Peking University, Nanjing University, Fudan University, Chinese Academy of Meteorological Sciences, etc. The TAP database used a data-fusion algorithm for ambient O_3 estimation that combined in situ observations, satellite remote sensing measurements, and model results from the community multiscale air quality model [11]. Five-fold cross-validation coefficient of determination (R^2) was 0.70, and the mean modeling error (measured using the root-mean-squared error [RMSE]) is 26 μg/m^3, which accounts for 29% of the mean level (Xue et al. 2020).

As embryonic period (weeks 3-8 of gestation) was a critical time for cardiac development of the fetus [12], it is widely accepted to consider this period as an exposure window in environmental epidemiological studies on CHD. Therefore, we extracted ambient daily MDA8 O_3 concentrations for each subject at her geocoded residential address during embryonic period from the TAP, and averaged them to assess an individual-level exposure to ambient O_3. The available maternal residential address was at the district level in the study. The gestational week was measured from the first day of the last menstrual period (LMP). Likewise, we also assessed the exposure to ambient O_3 during other three key periods: (1) preconception (3 months before LMP); (2) the first trimester (1–13 weeks of gestation); (3) periconception (3 months before LMP until 13 weeks into pregnancy). These periods were considered as critical time when maternal risk factors may be associated with adverse pregnancy outcome [1].

Outcome identification

CHDs were diagnosed based on fetal echocardiography using a Voluson E8-RAB4-8 machine equipped with a 2- to 8 MHz transducer (GE Healthcare, Little Chalfont, United Kingdom). Two specially trained echocardiologists independently reviewed each of the echocardiograms of CHD cases. Fetal echocardiographic images were acquired in accordance with the guidelines and standards established by the AHA [10] and the International Society of Ultrasound in Obstetrics and Gynecology (ISUOG) [13]. All cases were classified using international classification of disease 10 codes, and an a priori decision was made to analyze the following common subgroups of anomalies: ventricular septal defect (VSD; Q21.0), vascular ring of aorta (VR, Q25.45), persistent left superior vena cava (PLSVC, Q26.1), tetralogy of Fallot (TOF, Q21.3), valvular pulmonary stenosis (PS, Q22.1), double outlet right ventricle (DORV, Q20.1), pulmonary atresia (PA, Q22.0) and d-transposition of the great arteries (TGA, Q20.3). The accuracy of fetal echocardiography in the diagnosis of CHD has been validated by comparing with autopsy findings. The diagnostic coincidence rate for major cardiac abnormalities was 98.8% [14].

Covariate measures

Data on demographics, lifestyle factors, medical history were collected on a brief, study staff-administered questionnaire. Demographic and lifestyle information for subjects and spouses included age, occupation, smoking status, alcohol consumption, radioactive substances exposure, keeping pets and renovation during 3 months before conception. For clarity, renovation refers to activities aimed at enhancing the aesthetic and functional aspects of indoor spaces, such as adding new paintings and furniture, repainting walls, building cabinets, and installing flooring. Medical history included history of diseases (diabetes mellitus, gestational diabetes mellitus, upper respiratory infection during 3 months before conception, anemia during the first trimester, phenylketonuria, metabolic disorders, thyroid disease and connective tissue diseases), history of drug use during the first trimester, history of fetus CHD, adverse results of prenatal genetic test and psychological stress during pregnancy. The presence of any aforementioned medical history, as well as the presence of pets or renovation in the household during the three months preceding conception, was defined as having risk factors. Clinical information, including gestational weeks, fetus number and conception mode was abstracted from electronic medical records.

Grid data on daily 24 h mean $PM_{2.5}$ (spatial resolution: 1 km × 1 km) during 2013–2021 and NO_2 (spatial resolution: 10 km × 10 km) during 2013–2020 were obtained from the ChinaHighAirPollutants (CHAP) dataset (available at: https://weijing-rs.github.io/product.html), which has been validated in previous studies (Wei et al. 2020; Wei et al. 2023). Daily meteorological data on temperature and relative humidity at 9 × 9 km spatial resolution were obtained from the fifth generation of European Re-Analysis (ERA5)-Land reanalysis data set [15]. We included average temperature and relative humidity during the embryonic period as natural cubic splines with three degrees of freedom. These splines were constructed to capture any potential nonlinear relationships between temperature, relative humidity, and the odds of CHD as previous studies have shown evidence for J-shape or U-shape relationships [16].

Statistical Analysis

We employed logistic regression models to investigate the association of maternal exposure to ambient O_3 during the embryonic period with CHD. All models were adjusted for maternal age (continuous), maternal occupation status (categorical: Employed, Unemployed, Unknown), paternal smoking status (categorical: Smoking or ever smoked, Never), paternal alcohol consumption (categorical: Drinking or ever drank, Never), gestational weeks (categorical: 15–28 weeks, 29–40 weeks), conception mode (categorical: Natural, in vitro fertilization [IVF]), fetus number (categorical: Singleton, Multiple), presence of risk factors (categorical: Yes, No), season of conception (categorical: Warm, Cool), average

Table 1. Descriptive statistics for the study population during 2013–2021.

Characteristics	Total ($N = 27,817$)	CHD ($N = 4842$)	No CHD ($N = 22,975$)	*P*-value
Maternal factors				
Maternal age years, mean (SD)	31.0 (4.3)	30.5 (4.5)	31.1 (4.2)	< 0.001
Maternal occupation status, No. (%)				< 0.001
Employed	21,994 (79.1)	3435 (70.9)	18,559 (80.8)	
Unemployed	4912 (17.7)	1287 (26.6)	3625 (15.8)	
Unknown	911 (3.3)	120 (2.5)	791 (3.4)	
Paternal factors				
Paternal age years, mean (SD)	32.5 (5.1)	31.8 (5.1)	32.7 (5.0)	< 0.001
Smoking status, No. (%)				<0.001
Smoking or ever smoked	2466 (8.9)	532 (11.0)	1934 (8.4)	
Never	25,351 (91.1)	4310 (89.0)	21,041 (91.6)	
Alcohol consumption, No. (%)				< 0.001
Drinking or ever drank	1137 (4.1)	260 (5.4)	877 (3.8)	
Never	26,680 (95.9)	4582 (94.6)	22,098 (96.2)	
Clinical factors				
Gestational weeks, No. (%)[a]				< 0.001
15 ≤ GW ≤ 28	23,646 (85.0)	3722 (76.9)	19,924 (86.7)	
29 ≤ GW ≤ 40	4171 (15.0)	1120 (23.1)	3051 (13.3)	
Conception mode, No. (%)				0.794
Natural	26,758 (96.2)	4654 (96.1)	22,104 (96.2)	
IVF	1059 (3.8)	188 (3.9)	871 (3.8)	
Fetus number, No. (%)				0.002
Singleton	27,246 (97.9)	4714 (97.4)	22,532 (98.1)	
Multiple	571 (2.1)	128 (2.6)	443 (1.9)	
Presence of risk factors, No. (%)[b]				< 0.001
Yes	19,230 (69.1)	3684 (76.1)	15,546 (67.7)	
No	8587 (30.9)	1158 (23.9)	7429 (32.3)	
Season of conception, No. (%)[c]				0.516
Cool	14,207 (51.1)	2494 (51.5)	11,713 (51.0)	
Warm	13,610 (48.9)	2348 (48.5)	11,262 (49.0)	

CHD Congenital heart defects, *SD* Standard deviation, *GW* Gestational week, *IVF* in vitro fertilization.
[a]Gestational week represented the age of the fetus at the time when a pregnant women underwent a fetal echocardiography.
[b]Risk factors included the following information: commodities (diabetes mellitus, gestational diabetes mellitus, upper respiratory infections within 3 months before conception, anemia during the first trimester, phenylketonuria metabolic disorders, thyroid disease and connective tissue diseases), history of CHD, adverse results of prenatal genetic test results, radioactive substances exposure, mental stress and household environment (keeping pets decoration) during the early pregnancy. The presence of any aforementioned history was defined as having risk factors.
[c]Warm season was defined as April to September; Cool season was defined as October to December and January to March.

temperature (natural cubic spline function; degree freedom [*df*] = 3) and relative humidity (natural cubic spline function; *df* = 3) during embryonic period. We estimated odds ratios (ORs) and their 95% confidence intervals (95% CIs) for CHD associated with each 10 μg/m^3 increase of the exposure to ambient O_3 by including the exposure as a continuous variable in the model. In categorical analysis, we divided the exposure into quartiles (Quartile 1-Quartile 4) based on its distribution among all observations and estimated ORs of Quartile 2-Quartile 4 in comparison with Quartile 1. Linear trends across quartiles were tested by including the median of each quartile range in the model as a continuous variable. In addition, A natural cubic spline function (*df* = 3) of the exposure were applied to plot exposure–response curves, and used likelihood ratio tests to examine possible nonlinearities of these associations.

We performed stratified analysis by maternal age (≤ 35, > 35 years), maternal occupation status, paternal smoking status, presence of risk factors, conception mode, fetus number and conception season. Likelihood ratio tests were used to examine their potential effect modifications. Several sensitivity analyses were used to test the robustness of our results. First, we explored the respective impact of ambient O_3 exposure on CHD during preconception, the first trimester and periconception by including three exposures in separate logistic regression models. Second, we adjusted for exposure to fine particulate matter ($PM_{2.5}$) and nitrogen dioxide (NO_2) in the model. We also conducted a sensitivity analysis by further adjusting the model for maternal smoking status and alcohol consumption. Third, we used different *df* (2 and 4) for the natural cubic splines in the model to compare them with the exposure-response curves in the main analysis using 3 *df*. Finally, the COVID-19 pandemic in 2020–2021 may have affected both exposure to ambient O_3 (e.g., due to changes in outdoor activities) and CHD. Since we lack data on the COVID-19 status of our subjects during pregnancy, we could not include this variable as a covariate. Therefore, we conducted a sensitivity analysis on subjects enrolled before the pandemic (2013-2019). R version 4.2.2 was used for data analysis and visualization [17]. A two-sided $p < 0.05$ was considered statistically significant.

RESULTS

Study population characteristics

From 2013 to 2021, we enrolled 27,817 subjects with high-risk factors of CHD based on data from Maternal-Fetal Medicine Consultation Network covering 1313 medical centers in China

Table 2. Estimated ORs and 95% CIs[a] of CHD combined and its certain types associated with exposure to ambient O_3 during embryonic period in China 2013–2021.

Disease	*N* (%)	Per 10 ug/m^3 increase of exposure	Quartile of exposure to ambient O_3[b] ug/m^3				*P*-trend[c]
			18.9–56.4	56.4–2.1	92.1–95.6	95.6–134.1	
Overall CHD	4842 (17.4%)	1.127 (1.098, 1.155)	1.00 (Ref)	1.318 (1.186, 1.465)	2.175 (1.807, 2.617)	1.973 (1.555, 2.503)	< 0.001
VSD	886 (3.2%)	1.170 (1.109, 1.235)	1.00 (Ref)	1.400 (1.123, 1.745)	2.528 (1.694, 3.771)	2.132 (1.272, 3.571)	0.002
VR	568 (2.1%)	1.063 (0.955, 1.136)	1.00 (Ref)	1.060 (0.805, 1.396)	1.636 (0.995, 2.690)	1.540 (0.821, 2.892)	0.192
TOF	426 (1.5%)	1.194 (1.107, 1.288)	1.00 (Ref)	1.250 (0.920, 1.698)	3.005 (1.711, 5.278)	2.705 (1.311, 5.583)	0.025
PS	284 (1.0%)	1.172 (1.067, 1.287)	1.00 (Ref)	1.259 (0.850, 1.864)	2.322 (1.154, 4.671)	3.945 (1.629, 9.553)	0.002
DORV	231 (0.8%)	1.056 (0.954, 1.170)	1.00 (Ref)	1.412 (0.916, 2.177)	2.112 (0.996, 4.476)	1.179 (0.446, 3.119)	0.688
PA	194 (0.7%)	1.117 (1.000, 1.247)	1.00 (Ref)	1.156 (0.743, 1.800)	2.401 (1.066, 5.411)	1.709 (0.585, 4.990)	0.635
TGA	163 (0.6%)	1.167 (1.035, 1.316)	1.00 (Ref)	1.604 (0.982, 2.622)	2.756 (1.136, 6.684)	4.185 (1.304, 13.43)	0.005
PLSVC[d]	681 (2.4%)	1.108 (1.042, 1.177)	1.00 (Ref)	1.228 (0.955, 1.58)	1.983 (1.262, 3.116)	1.793 (1.013, 3.176)	0.009
Others	1409 (5.1%)	1.118 (1.071, 1.167)	1.00 (Ref)	1.392 (1.164, 1.664)	2.380 (1.732, 3.268)	2.119 (1.411, 3.181)	0.003

CHD Congenital heart defects, *VSD* Ventricular septal defect, *VR* Vascular ring of aorta, *TOF* Tetralogy of Fallot, *PS* Pulmonary stenosis, *DORV* Double outlet right ventricle; PA, pulmonary atresia, *TGA*, transposition of great arteries, *PLSVC* persistent left superior vena cava, *OR* Odds ratio, *CI* confidence interval.

[a]Odds ratios (ORs) and 95% CIs were estimated using logistic regression models. All models were adjusted for maternal age, maternal occupation status, paternal smoking status, paternal alcohol consumption, gestational week, conception mode, fetus number, presence of risk factors, season of conception, average temperature and relative humidity during embryonic period (3–8 weeks of gestation).

[b]Assessed by averaging daily mean O_3 concentrations during embryonic period (3–8 weeks of gestation).

[c]*P* for linear trend was tested by including the median of each quartile range as a continuous variable in the model.

[d]The most common type of PLSVC in the study was PLSVC draining into the right atrium via the coronary sinus. In cases with this type of PLSVC, the convergence of somatic venous blood to the right atrium remains uninterrupted and there is no apparent impact on the overall hemodynamics of the heart, indicating a lack of evident pathophysiologic significance.

during 2013–2021. Among all the subjects, 4842 (17.4%) were diagnosed with CHD. For certain types of CHD, 886 (3.2%), 568 (2.1%), 426 (1.5%), 284 (1.0%), 231 (0.8%), 194 (0.7%), 163 (0.6%) and 681 (2.4%) subjects were diagnosed with VSD, VR, TOF, PS, DORV, PA, TGA and PLSVC. Median age of the subjects was 31 years, and 22,056 (79.3%) of them were under 35 years. In terms of clinical factors, 1059 (3.8%) subjects were conceived by IVF, 571 (2.1%) experienced multiple pregnancy and 69.1% had at least one risk factors for CHD (Table 1). Additionally, we observed significant differences between the CHD and non-CHD populations, including maternal occupation status and clinical factors. Specifically, there was a higher proportion of unemployed mothers in the CHD group compared to the non-CHD group. The CHD group also exhibited a higher proportion of pregnancies with shorter gestational weeks at the time of examination, a greater prevalence of twin pregnancies, and a higher proportion with pregnancy risk factors (all *P*-value < 0.05).

Air pollution distributions

Based on the TAP data in 2013–2021, daily mean ambient O_3 concentrations in China ranged from 1 μg/m^3 to 377.2 μg/m^3, with an overall average of 84.5 μg/m^3 and an average of 95.8 μg/m^3, 71.6 μg/m^3, in warm season and cool season, respectively. The spatial distribution of seasonal ambient O_3 concentrations in China is presented in Fig. 1. For all subjects, the average ambient O_3 exposure during the embryonic period across all study subjects was 95.6 μg/m^3, ranging from 18.9 to 134.1 μg/m^3 (Table S1). The average ambient O_3 exposure during preconception period, the first trimester and periconception period was 91.9 μg/m^3 (SD: 39.1 μg/m^3), 95.9 μg/m^3 (SD: 39.9 μg/m^3) and 93.8 (SD: 29.7 μg/m^3), respectively (Table 1).

Association of ambient O_3 With CHD

We found that an increase of 10 μg/m^3 in ambient O_3 exposure during the embryonic period was significantly associated with a 12.7% increase in odds of pooled CHD (OR, 1.127; 95% CI, 1.098–1.155). Likelihood ratio tests detected slight but significant departures from linearity for the association between ambient O_3 exposure and pooled CHD (*P* for nonlinear trend < 0.001). As demonstrated by both categorical analyses and the exposure-response curves (Table 2; Fig. 2), the estimates for pooled CHD with significant nonlinear associations increased monotonically with increasing exposures at relatively lower levels but slightly attenuated at higher exposure levels.

In terms of specific types of CHD, exposure to ambient O_3 during the embryonic period demonstrated a significant association with an increased odds of VSD, TOF, PS, PA, TGA and PLSVC. The ORs were 1.170 (1.109, 1.235) for VSD, 1.194 (1.107, 1.288) for TOF, 1.172 (1.067, 1.287) for PS, 1.117 (1.000, 1.247) for PA, 1.167 (1.035, 1.316) for TGA and 1.108 (1.042, 1.177) for PLSVC, respectively (Table 2). No significant association was identified with VR and DORV. We found no evidence of departure from linearity for VSD, TOF, PS and TGA (all *P* for nonlinear trend > 0.05). The estimates monotonically increased with increasing exposures for all conditions, except for TGA where the curve slightly attenuated at higher exposure levels (Fig. 2).

Stratified and sensitivity analyses

In the stratified analyses, we did not observe any significant effect modification by maternal age, maternal occupation status, paternal smoking status, presence of risk factor, conception mode and fetus number (all *P* for effect modification >0.05; Fig. 3), whereas we find effect modification by the season of conception, with the association between O_3 and CHD combined being stronger in the warm season compared to the cool season (*P* for effect modification < 0.001; Table S2). Figure 4 and Table S3-S5 showed the estimated ORs of CHD associated with ambient O_3 exposure during preconception, the first trimester and periconception, respectively. Overall, the

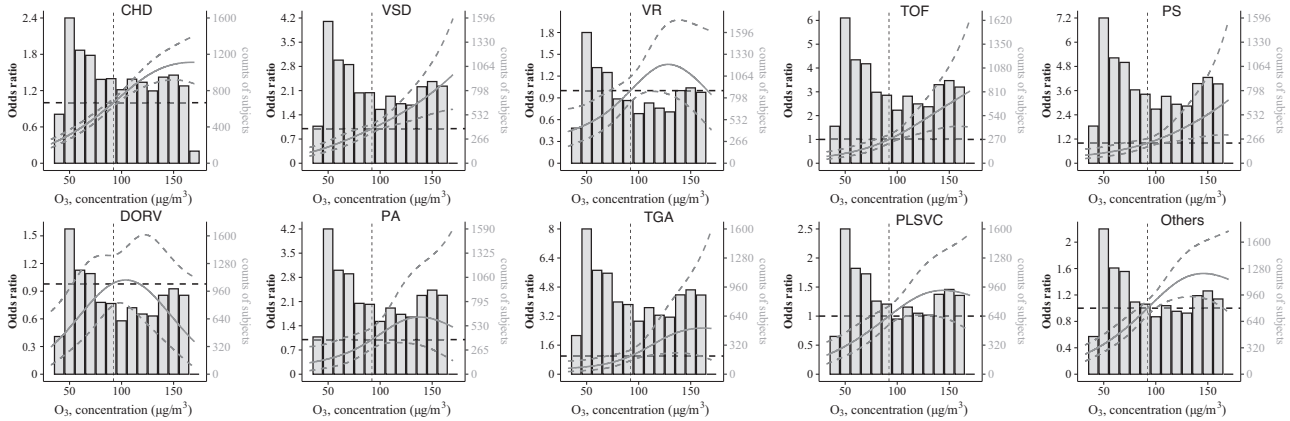


Fig. 2 Exposure-response curves of the association of exposure to ambient O_3 during the embryonic period with CHD combined and certain types of CHD. The yellow box along each x-axis presented the 2.5th to 97.5th percentiles of ambient O_3 concentrations during the embryonic period. ORs were estimated by comparing with the median value (92.1 μg/m³) of O_3 during the embryonic period. The red solid lines with two blue dashed lines represent odds and its 95% CI of CHD combined and certain subtypes respectively. CHD, Congenital heart defects; VSD, ventricular septal defect; VR, vascular ring of aorta; TOF, tetralogy of Fallot; PS, pulmonary stenosis; DORV, double outlet right ventricle, PA pulmonary atresia, TGA Transposition of great arteries, PLSVC persistent left superior vena cava, OR odds ratio, CI confidence interval.

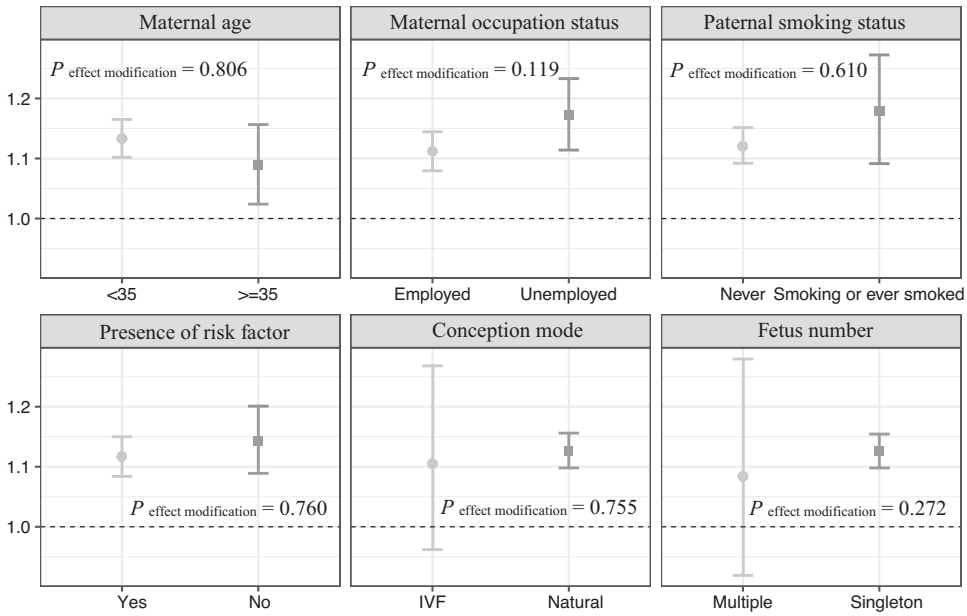


Fig. 3 Association of maternal ambient O_3 exposure during the embryonic period with CHD combined stratified by maternal or clinical characteristics (per increase of 10 μg/m³ in O_3). Stratified analyses were performed by maternal age (≤35, >35 years), maternal occupation status, paternal smoking status, presence of risk factors, conception mode and fetus number. *P* values from likelihood ratio tests assess whether the association between ambient O_3 exposure and CHD combined were modified by stratified factors. Risk factors included the following information: commodities (diabetes mellitus, gestational diabetes mellitus, upper respiratory infection within 3 months before conception, anemia during the first trimester, phenylketonuria metabolic disorders, connective tissue diseases and thyroid disease), drug use during the first trimester, history of CHD, adverse results of prenatal genetic test results, radioactive substances exposure, mental stress and household environment (keeping pets or renovation) during the early pregnancy. The presence of any aforementioned history was defined as having risk factors. CHD, congenital heart defect; OR, odds ratio; O_3, ozone.

estimates demonstrate consistent findings with the main analyses, indicating stronger associations of ambient O_3 exposure during periconception with pooled CHD, VSD, VR, TOF, PS, PA, TGA and PLSVC compared with during preconception and first trimester (Fig. 4). Additionally, adjustment for exposure to $PM_{2.5}$ and NO_2 yielded similar associations with slightly higher estimates for pooled CHD, VSD, VR, PS and TGA (Table S6; Table S7). After adjusting for maternal smoking and alcohol consumption, the model results remained stable and consistent with the main analysis (Table S8). The analysis with an exclusion of subjects who were enrolled during the COVID-19 pandemic also gave similar results (Table S9). Using 2 *df* for the natural cubic spline in the model yielded smoother curves as expected (Figure S3), and the use of 4 *df* yielded very similar results (Figure S4).

DISCUSSION

In this large nationwide cross-sectional study, we found an approximately linear exposure-response association of exposure

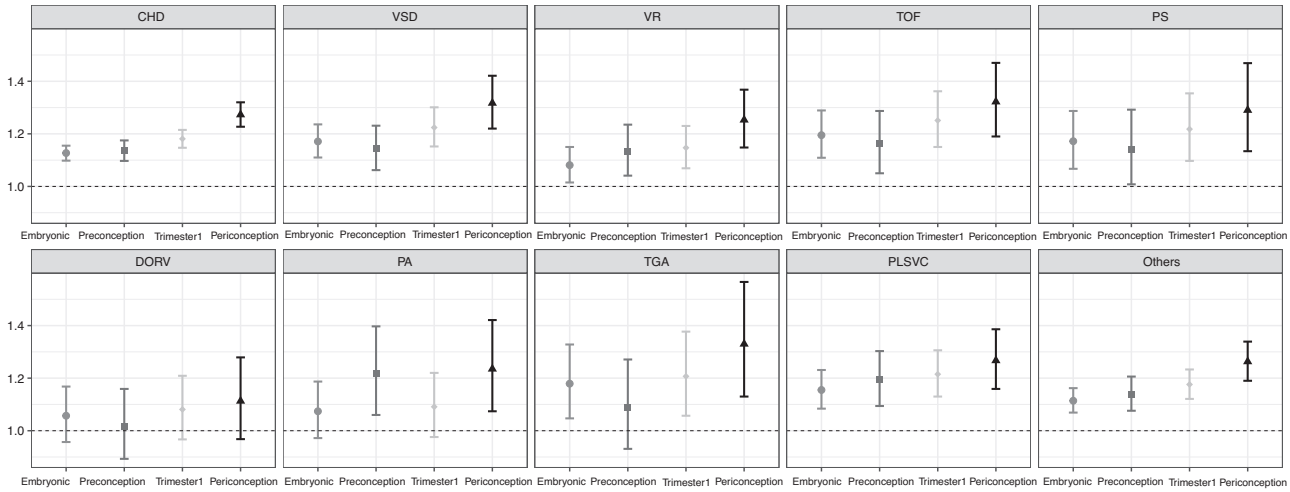


Fig. 4 Estimated ORs and 95% CIs of CHD combined and certain types of CHD associated with each 10 μg/m^3 increase of exposure to ambient O_3 during embryonic period, preconception period, the first trimester and periconception period. The horizontal black dashed lines represent a reference level of 1. CHD, Congenital heart defects; VSD, ventricular septal defect; VR, vascular ring of aorta; TOF, tetralogy of Fallot; PS, pulmonary stenosis; DORV, double outlet right ventricle; PA pulmonary atresia, TGA transposition of great arteries, PLSVC persistent left superior vena cava, OR odds ratio, CI confidence interval.

to ambient O_3 during embryonic period ranging from 18.9 ug/m^3 to 134.1 ug/m^3 with increase odds of overall CHD and certain types of CHD including VSD, TOF, PS, PA, TGA and PLSVC. These associations did not vary across maternal age, maternal occupation status, paternal smoking status, presence of risk factors, conception mode and fetus number. In addition, sensitivity analyses demonstrated that similar associations were also observed during the first trimester and the periconception period. Our findings suggest that policies targeting air quality improvement and measures to decrease ambient O_3 exposure have potential benefits for reducing the odds of CHD in fetuses.

Our study identified significant differences between the CHD and No CHD groups. The CHD group had a higher proportion of unemployed mothers, indicating lower socioeconomic status (SES), which is linked to limited healthcare access, lower health literacy, poor prenatal care, and higher psychosocial stress [18], all of which may contribute to CHD. Additionally, the CHD group had more pregnancies with shorter gestational weeks at examination, a higher prevalence of twin pregnancies, and more pregnancy risk factors like diabetes and hypertension. CHD may be detected earlier due to targeted screening or abnormal ultrasounds, resulting in earlier gestational age at diagnosis. Twin pregnancies pose higher risks due to increased physiological demands and shared placental circulation, leading to complications such as CHD [19]. Moreover, risk factors like diabetes, hypertension, and infections can negatively impact fetal development and increase the risk of CHD.

Several studies have investigated the association of maternal exposure to ambient O_3 with the odds of pooled CHD and the results were inconsistent. Some studies in China, Canada and Europe did not identify a significant association with CHD [7, 8, 20, 21]. In contrast, the other studies observed that exposure to ambient O_3 during the embryonic period and the first trimester was significantly associated with increased odds of CHD [6, 22–24], with an increase of less than 10% in odds for each 10 μg/m^3 increase in ambient O_3 during the first trimester. These effect estimates are lower than those found in our study. However, the comparison may need to be interpreted with caution due to heterogeneity in the study population. Previous studies were executed utilizing regional birth registry systems, and CHD cases were ascertained among all neonates of the residents. In contrast, the subjects in this study were patients with high-risk factors of CHD seeking a definitive diagnosis in hospital settings. CHD cases were identified in both neonates and in pregnancies terminated after prenatal diagnosis of fetal anomalies and late miscarriages. Thus, the proportion of CHD cases in the study was 17.4%, which markedly exceeded the prevalence reported in prior studies. Secondly, ambient O_3 exposure levels were various in those studies. The mean ambient O_3 exposure in our study was 95.1 μg/m^3, which exceeded the mean ambient O_3 concentrations documented in most prior studies (mean range: 26.5 μg/m^3 to 72.4 μg/m^3); In addition, highly heterogeneous in CHD definitions, air pollutants assessments and the adjustment set also account for the discrepancy.

With regard to CHD types, VSD was one of the most common CHD subtypes at birth. We observed that exposure to ambient O_3 during embryonic period was significantly associated with increased odds of VSD, which was also reported by two Chinese studies [1, 25]. TOF is the most common form of cyanotic congenital heart disease. Two studies observed maternal exposure to ambient O_3 during the first trimester was associated with increased odds of TOF, which was consistent with our findings [23, 26]. Quite a few studies have investigated PS [7, 27] and TG [7, 20, 25] with maternal exposure to ambient O_3 and most studies did not find significant association, which was inconsistent with our results. Insufficient sample size of these types of CHD in previous studies may restricted the statistical power to estimate the association and may not provide robust results. For other types of CHD, we were the first to assess the association of maternal ambient O_3 exposure with VR, PA, PLSVC and DORV, and detect significant association for PLSVC and PA but found no association for VR and DORV.

In this study, we found a stronger association between ambient O_3 exposure during embryonic period and CHD combined in the warm season compared to the cool season. The finding can be explained by several factors. Intense sunlight and higher temperatures in the warm season promote photochemical reactions, leading to higher O_3 concentrations and increased exposure levels [28]. Additionally, people tend to engage in more outdoor activities during this season, which may increase the risk of exposure to high O_3 levels, especially for pregnant women. Furthermore, high temperatures and humidity can make the respiratory system more sensitive and vulnerable [29], thereby enhancing the impact of pollutants on fetal development.

Our results suggested that except the embryonic period, periconception period was also a critical exposure window to induce damage to cardiac development of the fetus.

Periconception period included the 3 months before conception when the preantral follicle in the ovary gradually matures and ovulates and the first trimester when the major organs and structures of the fetus were formed [30]. This period is a critical time when maternal risk factors may be associated with adverse pregnancy outcome [31]. Maternal exposure to ambient O_3 during periconception period can affect oocyte quality, reduce embryo viability and development, or alter placental function, leading to impaired fetal growth [32]. Moreover, we observed the smallest effect estimates during the embryonic period, followed by the preconception period, with larger effect sizes in the first trimester and the largest OR value during the periconception period. This trend may reflect the cumulative nature of exposure, as the periconception period encompasses all previous periods, resulting in the highest OR value. To be noticed, maternal ambient O_3 exposure levels are correlated across the different time periods (Figure S5), which imply that the ORs for different time periods may not be entirely independent. Our results suggest that effective actions such as controlling emissions from industrial enterprises or modifying time-activity patterns (e.g., staying indoors on high O_3 days) can help reduce ambient O_3 exposure and prevent fetal CHD, particularly for pregnant women at high risk of CHD.

Several mechanisms have been proposed to explain the relationship between ambient O_3 and CHD. Migration of crest cells, septation of the ventricles and outflow tracts, and the formation of the endocardial tube are critical stages of cardiac development in embryonic period [33]. Ambient O_3 is known to cause robust increases in a number of proinflammatory cytokines, which have been documented to alter neural cell migration and result in outflow tract defect [34]. Also, evidence has indicated that maternal exposure to ambient O_3 can induce fibrinolysis and endothelial cell dysfunction, which may affect placenta function, and thus contribute to cardiac malformation [31]. In addition, growing evidence proves that exposure to air pollutants could cause epigenetic alternations and gene expression, which are closely related to CHD [35].

The strength of our study included the reliable maternal-fetal records and considerable fetal CHD cases ($n = 4844$). Our study used medical data from the National Clinical Research Center for Cardiovascular Diseases, covering 1313 referral hospitals in China during 2013–2021, which provided large sample size of study population and employed an expert panel of clinicians to rigorously review medical chart data and to maximize the validity of case classification. Furthermore, we investigated the association of maternal ambient O_3 exposure during various exposure windows with fetal CHD, TOF, DORV, and VSD in offspring, extending the understanding of CHD prevention and ambient O_3 exposure. Moreover, the range of air pollutant exposure was wide and offered us the possibility to quantify detailed exposure-response associations, especially at high exposure levels. Finally, we incorporated a rich set of confounders to obtain more precise estimates, and a series of sensitivity analyses indicated that our results were robust.

Our study also has several limitations. First, the cross-sectional design of the study limits our ability to determine the temporal relationship between exposure and outcomes and make causal inferences. Second, we used district-specific air pollution and meteorological factors and to assign individual exposure for all women during their pregnancy. Exposure misclassification could be present due to the lack of information on the exact residential address, maternal activity patterns, and residential mobility during pregnancy. Moreover, exposure misclassification may occur because we used ambient O_3 exposure as a proxy for individual actual ozone exposure. Further, although we adjusted fora number of potential confounders in the analysis, we were unable to account for certain important factors (e.g., socioeconomic status, pre-pregnancy BMI), which may lead to residual confounding to our results. Finally, the subjects in this study were women with high-risk factor of CHD, which limited the generalizability of our results to general population. Additionally, there are challenges in applying our findings to areas with lower levels of ambient O_3 pollution.

Our findings extend the evidence investigating the possible association between ambient O_3 and the odds of CHD using a large sample from National Maternal-Fetal Medicine Consultation Network, covering a wide geographic scope in China, which allowed analyses of individual types of CHD. Given CHD as the leading cause of deaths from non-communicable diseases (NCDs) in populations under 5 years, along with the 2030 sustainable development goals aiming to decrease premature deaths associated with NCDs [2], it is vital to effectively manage risk factors that contribute to the development of CHD. Our findings suggest that pregnant women, clinical practitioners, and policy makers need to pay more attention to the exposure to higher ambient O_3 during early pregnancy to reduce the risk of developing CHD and to improve outcomes across the life span.

CONCLUSIONS

In conclusion, based on this large nationwide cross-sectional study in China, we found that higher exposure to ambient O_3 during early pregnancy was significantly associated with increased odds of CHD combined and certain types of CHD including VSD, TOF, PS, PA, PLVSC and TGA. Our findings underscore the necessity of reducing exposure to elevated ambient O_3 levels during early pregnancy to diminish the risk of CHD, particularly among individuals at high risk for developing CHD. Well-designed longitudinal studies with more detailed exposure assessment are warranted to validate our findings.

DATA AVAILABILITY

The data for O_3 are available at http://tapdata.org.cn/. The data for $PM_{2.5}$ are available at https://doi.org/10.5281/zenodo.3753614. The clinical data are not publicly available.

REFERENCES

1. Yuan X, Liang F, Zhu J, Huang K, Dai L, Li X, et al. Maternal exposure to $PM_{2.5}$ and the risk of congenital heart defects in 1.4 million births: a nationwide surveillance-based study. Circulation. 2023;147:565–74.
2. Su Z, Zou Z, Hay SI, Liu Y, Li S, Chen H, et al. Global, regional, and national time trends in mortality for congenital heart disease, 1990-2019: an age-period-cohort analysis for the Global Burden of Disease 2019 study. EClin Med. 2022;43:101249.
3. Zhao QM, Liu F, Wu L, Ma XJ, Niu C, Huang GY. Prevalence of congenital heart disease at live birth in China. J Pediatr. 2019;204:53–8.
4. Snijder CA, Vlot IJ, Burdorf A, Obermann-Borst SA, Helbing WA, Wildhagen MF, et al. Congenital heart defects and parental occupational exposure to chemicals. Hum Reprod. 2012;27:1510–7.
5. Vicedo-Cabrera AM, Sera F, Liu C, Armstrong B, Milojevic A, Guo Y, et al. Short term association between ozone and mortality: global two stage time series study in 406 locations in 20 countries. BMJ. 2020;368:m108.
6. Yang Y, Lin Q, Liang Y, Ruan Z, Acharya BK, Zhang S, et al. Maternal air pollution exposure associated with risk of congenital heart defect in pre-pregnancy overweighted women. Sci Total Environ. 2020;712:136470.
7. Lavigne E, Lima I, Hatzopoulou M, Van Ryswyk K, Decou ML, Luo W, et al. Spatial variations in ambient ultrafine particle concentrations and risk of congenital heart defects. Environ Int. 2019;130:104953.
8. Dadvand P, Rankin J, Rushton S, Pless-Mulloli T. Ambient air pollution and congenital heart disease: a register-based study. Environ Res. 2011;111:435–41.
9. Peyvandi S, De Santiago V, Chakkarapani E, Chau V, Campbell A, Poskitt KJ, et al. Association of prenatal diagnosis of critical congenital heart disease with postnatal brain development and the risk of brain injury. JAMA Pediatr. 2016;170:e154450.
10. Donofrio MT, Moon-Grady AJ, Hornberger LK, Copel JA, Sklansky MS, Abuhamad A, et al. Diagnosis and treatment of fetal cardiac disease: a scientific statement from the American Heart Association. Circulation. 2014;129:2183–242.

11. Xue T, Zheng Y, Geng G, Xiao Q, Meng X, Wang M, et al. Estimating spatiotemporal variation in ambient ozone exposure during 2013-7 using a data-fusion model. Environ Sci Technol. 2020;54:14877–88.
12. Dadvand P, Rankin J, Rushton S, Pless-Mulloli T. Association between maternal exposure to ambient air pollution and congenital heart disease: a register-based spatiotemporal analysis. Am J Epidemiol. 2011;173:171–82.
13. International Society of Ultrasound in Obstetrics and Gynecology, Carvalho JS, Allan LD, Chaoui R, Copel JA, DeVore GR, et al. ISUOG Practice Guidelines (updated): sonographic screening examination of the fetal heart. Ultrasound Obstet Gynecol. 2013;41:348–59.
14. Gao S, Han J, Yu S, Guo Y, Ruan Y, Fu Y, et al. Comparison of fetal echocardiogram with fetal cardiac autopsy findings in fetuses with congenital heart disease. J Matern Fetal Neonatal Med. 2021;34:3844–50.
15. Xin Y, Yang Y, Chen X, Yue X, Liu Y, Yin C. Evaluation of IMERG and ERA5 precipitation products over the Mongolian Plateau. Sci Rep. 2022;12:21776.
16. Guo J, Ruan Y, Wang Y, Wang H, Ma S, Wan X, et al. Maternal exposure to extreme cold events and risk of congenital heart defects: a large multicenter study in China. Environ Sci Technol. 2024;58:3737–46.
17. R Core Team, 2023. R: a language and environment for statistical computing. R Foundation for Statistical Computing. https://www.r-project.org/.
18. Schultz WM, Kelli HM, Lisko JC, Varghese T, Shen J, Sandesara P, et al. Socioeconomic status and cardiovascular outcomes: challenges and interventions. Circulation. 2018;137:2166–78.
19. Barigye O, Pasquini L, Galea P, Chambers H, Chappell L, Fisk NM. High risk of unexpected late fetal death in monochorionic twins despite intensive ultrasound surveillance: a cohort study. PLoS Med. 2005;2:e172.
20. Huang CC, Chen BY, Pan SC, Ho YL, Guo YL. Prenatal exposure to $PM_{2.5}$ and congenital heart diseases in Taiwan. Sci Tot. Environ. 2019;655:880–6.
21. Zhang H, Zhang X, Zhao X, Cheng G, Chang H, Ye X, et al. Maternal exposure to air pollution and congenital heart diseases in Henan China: a register-based case-control study. Ecotoxicol Environ Saf. 2022;229:113070.
22. Jiang W, Liu Z, Ni B, Xie W, Zhou H, Li X. Independent and interactive effects of air pollutants and ambient heat exposure on congenital heart defects. Reprod Toxicol. 2021;104:106–13.
23. Zhang B, Zhao J, Yang R, Qian Z, Liang S, Bassig BA, et al. Ozone and other air pollutants and the risk of congenital heart defects. Sci Rep. 2016;6:34852.
24. Agay-Shay K, Friger M, Linn S, Peled A, Amitai Y, Peretz C. Air pollution and congenital heart defects. Environ Res. 2013;124:28–34.
25. Hwang BF, Lee YL, Jaakkola JJ. Air pollution and the risk of cardiac defects: a population-based case-control study. Medicine (Baltimore). 2015;94:e1883.
26. Vinikoor-Imler LC, Stewart TG, Luben TJ, Davis JA, Langlois PH. An exploratory analysis of the relationship between ambient ozone and particulate matter concentrations during early pregnancy and selected birth defects in Texas. Environ Pollut. 2015;202:1–6.
27. Ritz B, Yu F, Fruin S, Chapa G, Shaw GM, Harris JA. Ambient air pollution and risk of birth defects in Southern California. Am J Epidemiol. 2002;155:17–25.
28. Lu X, Zhang L, Wang X, Gao M, Li K, Zhang Y, et al. Rapid increases in warm-season surface ozone and resulting health impact in China since 2013. Environ Sci Technol Lett. 2020;74:240–7.
29. Lepeule J, Litonjua AA, Gasparrini A, Koutrakis P, Sparrow D, Vokonas PS, et al. Lung function association with outdoor temperature and relative humidity and its interaction with air pollution in the elderly. Environ Res. 2018;165:110–7.
30. Xie X, Kong BH, Duan T. Gynecology and Obstetrics. 9th Edition, People's Health Publishing House, Beijing, 2018.
31. Luderer U, Lim J, Ortiz L, Nguyen JD, Shin JH, Allen BD, et al. Exposure to environmentally relevant concentrations of ambient fine particulate matter ($PM_{2.5}$) depletes the ovarian follicle reserve and causes sex-dependent cardiovascular changes in apolipoprotein E null mice. Part Fibre Toxicol. 2022;19:5.
32. Van Brusselen D, Kayembe-Kitenge T, Mbuyi-Musanzayi S, Lubala Kasole T, Kabamba Ngombe L, Musa Obadia P, et al. Metal mining and birth defects: a case-control study in Lubumbashi Democratic Republic of the Congo. Lancet Planet Health. 2020;4:e158–e167.
33. Gittenberger-de Groot AC, Bartelings MM, Deruiter MC, Poelmann RE. Basics of cardiac development for the understanding of congenital heart malformations. Pediatr Res. 2005;57:169–76.
34. Sun L, Liu C, Xu X, Ying Z, Maiseyeu A, Wang A, et al. Ambient fine particulate matter and ozone exposures induce inflammation in epicardial and perirenal adipose tissues in rats fed a high fructose diet. Part Fibre Toxicol. 2013;10:43.
35. Day DB, Xiang J, Mo J, Li F, Chung M, Gong J, et al. Association of ozone exposure with cardiorespiratory pathophysiologic mechanisms in healthy adults. JAMA Intern Med. 2017;177:1344–53.

AUTHOR CONTRIBUTIONS

WYQ and RYP were co-first authors, and contributed equally to this work. WYQ and RYP conducted investigations and data analysis, prepared the first draft and finalized the manuscript based on comments from all other authors. WXY, WH, GJH, WJ and MS contributed to the data analysis, review and editing of the current manuscript. LJ, ZZY and HYH were co-corresponding authors, and contributed equally to the study design protocol, clinical data collection and the overall guidance. All authors had full access to the data in the study and accepted the responsibility for submitting this study for publication.

FUNDING

This work was supported by the National Natural Science Foundation of China (82073573 to ZZ, U21A20523 to YH), the Beijing Key Laboratory of Maternal-Fetal Medicine in Fetal Heart Disease (BZ0308 to YH), and the National Key Research and Development Program of China (2022YFC3703502 to JL).

COMPETING INTERESTS

The authors declare no competing interests.

ETHICAL APPROVAL

This study was approved by the Institutional Review Board of Beijing Anzhen Hospital, Capital Medical University (ID: 2022060X), and written informed consent was signed by all subjects.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41370-024-00716-4.

Correspondence and requests for materials should be addressed to Yihua He, Zhiyong Zou or Jing Li.