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Keywords:	serum folate, red blood cell folate, vitamin, congenital heart diseases, periconceptional health care

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ABSTRACT (264/300)

Purpose: The Shanghai PreConception Cohort (SPCC) was set up to investigate associations between parental periconceptional key nutritional factors with the development of birth defects, with congenital heart diseases (CHD) being the primary aim. Based on nested case-control analysis, we aim to explore the optimal nutrition levels which is in relation to the offspring defects.

Participants Recruitment sought to enroll parenting-plan women and men in Shanghai city of China during 2016-18; the participants were followed up throughout the entire pregnancy till the delivery. By the end of the study enrollment in March, 2018, 24,446 women and 30% of their husbands (n=6,573) had been enrolled. It considers genetic, biological, social and other environmental exposures in relation to a similarly diverse range of health, social and CHD outcomes.

Findings to date The established cohort holds clinical, biological, nutritional, genetic and other environmental exposures of mother–infant–father triads representing a valuable resource for studying the pathogenesis of CHD. Until now, research has suggest that a large proportion of women planning pregnancy were below the optimal RBC folate concentration for preventing NTDs and the prevalence of FA supplement use was low in pregnancy planners(15.8% in men and 42.6% in women).

Future plans Ongoing cohort will describe the status of key nutrients (eg.folic acid and vitamins) biological levels and dietary intake among peri-conception population (women and husbands) in Shanghai. This study will find out the association between the biological levels of key nutrients during periconceptional period and the incidence of congenital heart defects (CHD) in newborn through a nested case-control study design. The findings will help to propose recommended nutrients levels.

Key Messages: serum folate, red blood cell folate, vitamin, congenital heart diseases, periconceptional health care

Strengths and limitations of this study

- The SPCC cohort is the first prospective birth cohort with CHD as primary outcome and recruitment starting from preconception.
- Blood samples were collected and stored which allows for direct measurement of individual exposure levels before the development of CHD and make causal inference.
- This cohort also allows for investigating associations between periconceptional maternal and paternal nutrition exposures with other birth defects, early onset-diseases, and neurodevelopment outcomes.
- The established cohort holds clinical, biological, nutritional, genetic and other environmental exposures of mother–infant–father triads representing a valuable resource for studying the pathogenesis of congenital heart diseases (CHD).
- A major strength of this study is its multicenter prospective design with preconceptional parental biological nutrients levels, and linking to the municipal medical care data sources which enable chance for longer term follow-up of participants for future studies.

Introduction

Congenital heart disease (CHD) is a common congenital malformation, which seriously affects quality of children's life [1]. CHD is a leading cause of infant death in high-income countries, and affect eight of 1 000 live births [2]. According to the report from National Health and Family Planning Commission of the People's Republic of China, CHD accounts for about a quarter of the birth defects of newborns in China, ranking the first among birth defects[3]. In a prospective, nation-wide large-scale study in more than 120 000 newborns in China in 2013, the prevalence of CHD was identified 8.94 ‰ in live births; the rate of severe CHD was 2.9 ‰[4].

The cause of CHD is multifactorial. With the development of genetic engineering technology, the genetic factors have been better understood in the past decade[5]. Multiple environmental risk factors have been reported in epidemiological studies, the maternal social variables such as occupation, educational background, health status, unhealthy life style, maternal medical history and emotional status, family history of disease, consanguineous marriages and so on [6-10]. In addition, maternal key nutrients related to the risk of offspring's CHD as a modifiable environmental factor during periconception [11, 12]. Periconceptional intake of folic acid supplement has been shown to reduce the risk for CHD [13, 14] and women worldwide have been recommended to take folic acid supplements before conception and in

the beginning of pregnancy. Awareness of the relationship between folic acid deficiency and CHD is actually a by-product result from the well-known Hungarian RCT study of folic acid supplementation to prevent neural tube defects. The study found that prenatal supplementation with a vitamin complex containing 0.8 mg of folic acid daily reduced the incidence of congenital neural tube defects. At the same time, the incidence of various heart defects has also been reduced by nearly half[15]. Longitudinal data from more than one million births in Canada over a total of 22 years from 1990 to 2011 also show that the food fortification with folic acid reduced 20-30% risk of CHD [16]. The current folic acid supplementation recommends that all women of childbearing potential be supplemented with at least 0.4 mg of folic acid daily prior to conception and during pregnancy, which is designed for preventing neural tube dysplasia[17]. However, excessive folic acid intake may increase the risk of cancer[18], vitamin B12 deficiency[19] and autism spectrum disorder[20]. The optimal dose of folic acid for preventing CHD warrants further investigation. In addition, most of previous studies only focused on the supplement of folic acid or the serum folate level during or after pregnancy, which may not be the optimal time period and way to reflect the exposure level to risk of CHD.

To investigate the association between parental periconceptional key nutritional factors such as folate with the development of CHD and to

explore the cutoff biomarker levels, we conducted Shanghai PreConception Cohort (SPCC) and a nested case-control analysis.

Who is in the cohort?

The SPCC cohort recruited parenting-plan women and men who were permanent residents and took part in the preconception clinical visit at 28 maternity institutions in 10 districts (Minhang District, Huangpu District, Xuhui District, Changning District, Jing'an District, Putuo District, Yangpu District, Pudong District，Songjiang District, Qingpu District) in Shanghai from March 2016 to December 2018. The preconception clinical visit is the preconception examination policy in the city of Shanghai providing a unique opportunity and clinical resources to support SPCC, the basis for the proposed study. Since 2010, married couples in Shanghai have been encouraged to attend a free preconception health visit at a medical clinic. In addition, these maternity institutions have strong local support and integrated maternal health-care networking and provide service to 15 000–20 000 annual deliveries in Shanghai (Figure 1). Eligible participant live in Shanghai who are preparing pregnancy in one year and intend to receive antenatal care and deliver at Shanghai. Informed, written consent is obtained from all study participants. In addition, we recruited early pregnant women at their first antenatal examination whose gestational week <14 week.

The SPCC cohort includes a longitudinal birth cohort, based on which, a nested case-control study will be conducted. The study has been registered with Clinical Trials Registry (NCT02737644). In the cohort, we recruited participants in the preconception clinical visit. Among the enrolled participants, once a woman was confirmed for pregnancy, she would have been followed throughout the entire pregnancy. CHD outcome was abstracted from Shanghai CHD screen platform that is a routine screen and diagnosis policy covering all Shanghai newborns. The nested case-control subjects included couples and women at periconcpetional stage whose baby with confirmative CHD diagnosis (case group) and four controls matched by delivery hospital and maternal age (control group) randomly selected from the rest of the cohort. Participants were identified by their unique national identification number during follow up. Serum key nutrients levels in cases and controls was examined for association analysis (Figure 2). The sample size for the case-control analysis was planned as 180 cases and 600 controls to detect a maternal folate deficiency with prevalence of 50% in controls with odds ratio of 1.6 in association with the offspring CHD at power of 80% at alpha of 0.05. Based on CHD incidence above 8.94 per 1 000 live birth[4], 20 000 pregnancies will be needed. For a continues nutrient variable with standard deviation 2.0 , 50 matched-pairs are required to achieve 90% power to detect an odds ratio of 1.3 calculated using conditional logistic regression

with a 0.05 significance level[21, 22].

The SPCC began recruitment in March 2016. By the end of the study enrollment in March, 2018, 24 446 women and 30% of their husbands (n=6 573) had been enrolled in the SPCC. At the preconception clinical visit, a questionnaire survey/interview and a health exam were conducted on couples of men (n=6 573) and women (n=9 243). The preconception questionnaires were also assigned to additional 15 203 pregnant women before 14 gestational weeks, to recall information about dietary supplements and health history during 3-6 month before pregnancy. Table 1 describes the basic demographic characteristics of the preparing pregnant participants and pregnant women, respectively. In preparing pregnant participants, the average age of female and male was 29.9±3.9 and 31.4±4.5 years respectively and there was a relatively larger proportion of both with high educational attainment. One-third and two-third of male had a habit of smoking and drinking. One-third of female had a habit of drinking. In pregnant women, the average age was 29.9±4.0 years, half of them was the first pregnancy, and most of all did not have drinking and smoking habit.

Follow-up procedure

At enrollment, the participants completed the questionnaire of key nutrients supplementation and blood samples collection. When participants were

pregnant, the same investigation conducted during early pregnancy (first antenatal visit at 16-20 gestational week). Pregnancies were followed up along with routine maternal health-care procedures. Besides, blood samples were also collected at the second (24-28 gestational week) and third trimester (32-34 gestational week). The follow-up of CHD outcome and birth data was obtained by Shanghai CHD screen platform.

The routine clinical data of participants and offspring birth data was extracted from three clinical data sources (Figure 2). First, The preconception clinical visit data was from the preconception care electronic data system supported by national and local government, including height, weight, age, infections, sexually transmitted disease, and family history, etc. Second, the pregnant routine data were obtained in maternal clinic antenatal medical record system, managed by Shanghai Center for Women and Children's Health, including height, gestational weight, last menstrual period, childbearing history, delivery outcomes, infections, hematocrit, coagulation function, liver and kidney function and so on. Third, the maternal and neonatal data at delivery came from Shanghai neonatal CHD screen platform including birth weight, CHD diagnosis, birth defects, Apgar score, etc. The maternal identification card number was applied as index variable through the three data sources. The detail of clinical variable codebook please see Appendix 1.

Comprehensive strategies were used to retain participants in the study. For mothers, we provided a variety of engagement activities including green channel in antenatal care, nutrition consulting. Site investigators at early pregnant clinics in collaborative hospitals were provided a smartphone APP to identify cohort subjects timely and manage the data and blood sample collection procedures. We provided green channel echocardiography of CHD for all site hospital to enhance the compliancy of the participants. In addition, an automated text message system is adopted to remind participants of schedule and appointment of follow-up.

Study measures

Personal characteristics questionnaires

Questionnaire 1 was administered at baseline and early pregnancy to collect information on folic acid supplement, vitamin supplement, the brand and content of nutrient supplement, maternal education, socio-demographic status, occupation, smoking status, alcohol consumption, BMI, medication, health status. In addition to the content of Questionnaire 1, Questionnaire 2 added drug information, reproductive history and health status. (Questionnaire 1 for baseline, Questionnaire 2 for the visit in early pregnancy, please see Appendix 2a & Appendix 2b).

Collection of blood samples

We kept the rest of fasting serum and EDTA anticoagulation blood samples of peripheral venous blood from routine laboratory examination. The fasting serum were divided into three light-proof EP tubes within six hours, as well as EDTA anticoagulation blood samples. After the completion of the blood sample distribution, the serum and the whole blood were stored at the site laboratory in -20 degree freezers temporarily and were transported by three trained investigators to the central biobank for storage in -80 degree freezers within two weeks. The protocol for blood sample tagging, isolation, temperary storage, transportation and permanent storage please see Appendix 3.

Blood sample examinations

EDTA anticoagulation blood was collected to measure RBC folate and serum to folate, homocysteine, vitamin D, vitamin B12 and iron protein assays. All six biomarkers are performed on an electrochemiluminescence assay (ARCHITECT i2000SR Analyzer, Abbott Laboratories, USA). A standard solution with known concentration (produced by Abbott Laboratories) was used to quality control every day before the examination. If the level of quality control were out of the range of concentration, the examination would be suspended and adjusted. External quality control was

carried out with the control lab data program from Abbott Laboratories (Abbott Laboratories, Shanghai, China). RBC folate concentrations were adjusted for hematocrit. If the RBC folate concentration is below 126.0 ng/ml or above 651.1 ng/ml, we need to adjust the serum folate level. The hematocrit data were extracted from the hospital laboratory information system. Those examinations were performed in central laboratory of Children's Hospital of Fudan University.

The serum concentration of vitamin A and vitamin E were quantitatively detected by liquid chromatography-tandem mass spectrometry in central laboratory of Children's Hospital of Fudan University. The testing instrument was triple quadrupole mass spectrometer LC/MS/MS System (NO: API 3200MDTM, ABSciex Pte.Ltd.). A standard solution of vitamin A-d6 and vitamin E-d6 were applied as internal standards.

We also planned to measure other blood chemicals using the stored serum including cholesterol, high-density lipoprotein, low-density lipoprotein, triglyceride, fasting glucose and heavy metals. Cholesterol, high-density lipoprotein, low-density lipoprotein, triglyceride, and fasting glucose were performed on Beckman coulter AU chemistry analyzers (Beckman, USA) in central laboratory of Children's Hospital of Fudan University. Serum heavy metals including Mg, Fe, Zn, Se, Mn, As, Cu and Ca, were analyzed by inductively coupled plasma mass spectrometry (ICP-

MS) (Inductively Coupled Plasma Optical iCAP6300, Themo®, USA) in standard mode [23]. The metals examination was conduct in Instrumental Analysis Center of Shanghai Jiaotong University.

CHD Outcome

The diagnosis of CHD was the primary outcome of study at this stage and obtained from Shanghai neonatal CHD screen network platform, which was initiated as routine screen for newborn in Shanghai since Jun 1st 2016. The standard protocol of CHD screening of the platform was previously described in detail [24]. All newborn babies received the screen by using double-index method (i.e. cardiac murmur auscultation and pulse oximetry) during 6-72 hours after delivery, and those screen-positive babies would receive a subsequent echocardiography for further comformative diagnosis.

SPCC also focus on the other birth defects as secondary outcomes including Down's syndrome, neural tube defects, hydrocephalus, digestive tract malformations, urinary malformations, and behavioral cognitive developmental disorder. After delivery, the infants will attend routine child care procedures organized by Shanghai child health care system which is administered by Shanghai Center for Women and Children's Health. All birth defects records which diagnosed in afterlife, as well as routine neuro-development examinations and longitudinal anthropometric data will be abstracted from the system by professional clinical team from Children's

hospital of Fudan University. The offspring of participant will be followed up to school age. After that, health data will be abstract from Shanghai Student Health Surveillance information system.

FINDINGS TO DATE

Findings from the SPCC cohort currently are limited in the available sample recruited in 2016[25]. A total of 4 122 people participated in the study, including 2 268 (55.0%) women and 1 854 men, with average age of 30.5±4.3 years, 94.5% of which were highly educated. Among preconception subjects, the proportion of using folic acid supplement was 15.8% (292/1 854) in men and 42.6% (970/2 268) in women. The proportion in women is far below the National Health and Family Planning Commission requirements (70%) of China. In women of childbearing age in Europe and North America, the proportion of folic acid supplements used also does not exceed 50% [26-28]. In the face of lower usage ratios, Canada, the United States and the United Kingdom have successively carried out folic acid fortification in grains. However, there is no such fortification in china. Our findings suggest that education of folic acid supplement knowledge is deeply needed.

As the study is still ongoing, findings of blood nutrients based on limited processed blood sample are described below. The blood nutritional

levels of the preconceptional and pregnancy was based on 553 available participants. The concentrations of RBC folate median level is 247.0 ng/ml (IQR: 184.8-340.5 ng/ml) in preconception and 416.6 ng/ml (IQR: 305.4-542.2 ng/ml) in pregnancy. Twenty percent of preconceptional subjects and 44.9% pregnant subjects had folate level over 400 ng/ml, which was suggested as optimal level for preventing neural tube development defects [29, 30]. Our findings suggest that effort is urgently needed to improve the effectiveness of folic acid supplementary for this prepare-for pregnancy population, especially before pregnancy.

STRENGTEHS AND WEAKNESS

This study has two important strengths. First, the SPCC cohort is the first prospective birth cohort with CHD as primary outcome and recruitment starting from preconception. Blood samples were collected and stored which allows for direct measurement of individual exposure levels before the development of CHD and make causal inference. Up to date, none published studies measured maternal blood levels of folate levels before conception and link it to disease outcomes. Second, this cohort also allows for investigating associations between periconceptional maternal and paternal nutrition exposures with other birth defects, early onset-diseases, and neurodevelopment outcomes. Third, a major strength of this study is its large

multicenter prospective design with opportunities for follow-up through linking to the municipal medical care data sources as well as the recalling of participants for future studies.

Two challenges of this cohort study should be considered. First, there are 200000 pregnant women giving birth every year in Shanghai, and 20000 of them will take part in the free preconceptional care in Shanghai, where our participants came from. These people may have better health awareness and may introduce selection bias. Second, in this study, biological samples (cord blood, placenta) of the newborns are not collected.

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Contributors: YZ and DW prepared the original draft of the manuscript. Substantial contributions to the conception or design of the work were made by GH and WY. YZ, DW, YY, JY, ML, MJ, YD and XC led study implementation at participating sites. DW and YZ were responsible for the day-to-day project management at each site. XM and WS were responsible for the biobank of the cohort. All authors provided critical review of the manuscript for important intellectual content and approved the final version.

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Conflict of interest: The authors have no conflicts of interest

Ethics approval: This study was approved by Ethics Committee of Children's hospital of Fudan University, Shanghai, China.

Provenance and peer review Not commissioned; externally peer reviewed.

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Table 1. Socio-demographics of participants including 6,573 couples of parents and 15,203 pregnant women that were enrolled in the Shanghai PreConception Cohort (SPCC).

Characteristics	Couples of parents completed preconception questionnaires				Additional pregnant women completed both preconception and first-trimester questionnaires	
	Male (n=6573)		Female (n=9243)		Pregnant women （n=15203）	
Age (years), Mean±SD	6573	31.4±4.5	9243	29.9±3.9	15203	29.9±4.0
Ethnics	6536		9188		15176	
Han Nationality		6403 (98%)		8966 (97.6%)		15245 (97.8%)
Other		133 (2%)		222 (2.4%)		342 (2.2%)
Educational level	6530		9147		15143	
<College		514 (7.8%)		795 (8.7%)		2052 (13.6%)
>College		6016 (92.2%)		8352 (91.3%)		13091 (86.4%)
Annual household income	6530		9147		-	
<0.1 million		1424 (21.8%)		2214 (24.2%)		
>=0.1 million		5106(78.2%)		6933 (75.8%)		
Occupation	6530		9147		14789	
Entrepreneur		157 (2.4%)		128 (1.4%)		171 (1.1%)
Farmer		26 (0.4%)		46 (0.5%)		79 (0.5%)
Self-employed		216 (3.3%)		274 (3%)		862 (5.6%)
Manager		1410 (21.6%)		1765 (19.3%)		2650 (17.2%)
Technician		2462 (37.7%)		1903 (20.8%)		3239 (20.6%)
Company clerk		2259 (34.6%)		5031 (55%)		8451 (54.7%)
Attending pregnant examination	-		-		14996	
Yes						3374 (22.5%)
No						11622 (77.5%)

Frequency of pregnant	-		-		15162	
1						7569 (49.9%)
2						4604 (49.9%)
>=3						2989 (19.7%)
Abnormal delivery history	-		-		15159	
Yes						4838 (31.2%)
No						10385 (68.7%)
Smoking history	6552		9212		15159	
Yes		2073 (31.6%)		218 (2.4%)		153 (1%)
No		4479 (68.4%)		8994 (97.6%)		15006 (99%)
Alcohol Drinking history	6448		9075		15164	
Yes		3962 (61.5%)		2865 (31.6%)		1556 (10.3%)
No		2486 (38.5%)		6210 (68.4%)		13608 (89.7%)
Location of home	6573		9243		-	
Developed districts		3034 (46.2%)		5452 (59.0%)		
Developing districts		3539 (53.8%)		3791 (41.0%)		

Table 2. Maternal biomarkers evaluated in the SPCC study

Biomarkers	Sample type	Mother			
		Baseline	16-20 weeks	24-28 weeks	32-36 weeks
Folate, ng/ mL	Serum	○	○		
RBC Folate, ng/ mL	Whole blood	○	○		
Homocysteine, μmol/L	Serum	○	○		
Vitamin D, ng/mL	Serum	○	○		
Vitamin B12, pg/mL	Serum	○	○		
Vitamin A, μg/mL	Serum	○	○		
Vitamin E, μg/mL	Serum	○	○		
Iron protein, ng/mL	Serum	○	○		
Metals (Mg, Fe, Zn, Se, Mn, As, Cu, Ca, ect) ,mg/L	Serum	○	○		
DNA	Whole blood	○	○	○	○
CHOL, HDL, LDL, TG, fasting glucose	Serum	○	○		

Note: RBC folate means red blood cell folate

Figure 1 Map showing the location of Shanghai in China and the distribution of study sites in Shanghai

Figure 2 Diagram of the protocol and follow up of Shanghai PreConceptional Cohort (SPCC)

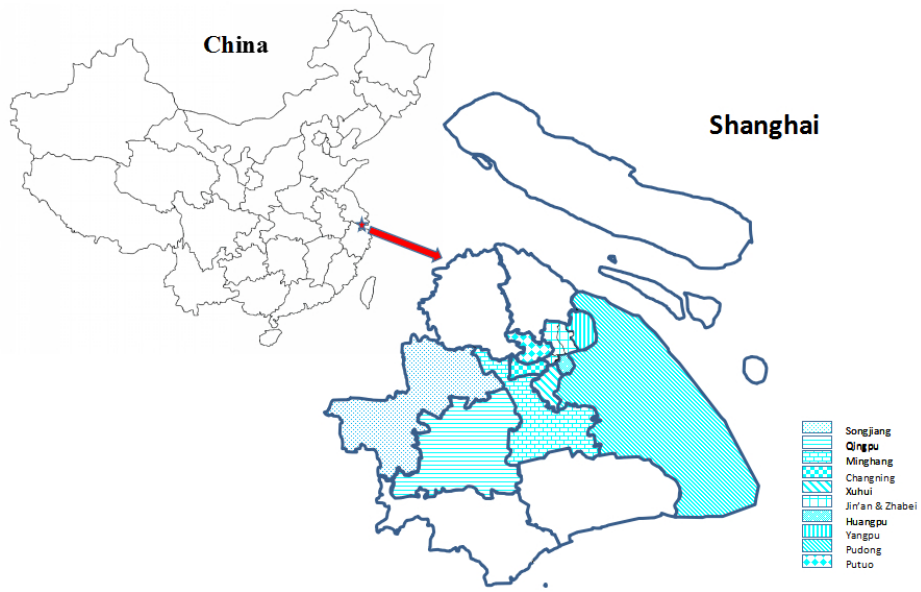


Figure 1 Map showing the location of Shanghai in China and the distribution of study sites in Shanghai

254x190mm (96 x 96 DPI)

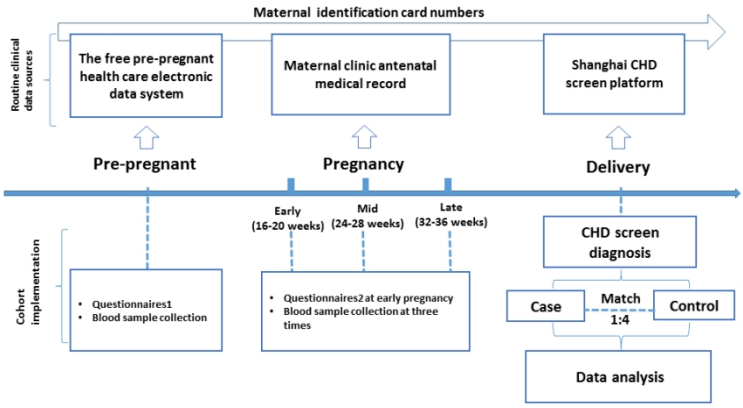


Figure 2 Diagram of the protocol and follow up of Shanghai PreConceptional Cohort (SPCC)

338x190mm (96 x 96 DPI)

Appendix 1 variable list

Table 1 Intervention trail variable list

NO	variable	
	Pre-pregnancy	
1	hospital card number	T
2	Recruited number	T
3	Recruited date	D
4	Female name	T
5	Female height	N
6	Female weight	N
7	Female age	N
8	Male name	T
9	Male height	N
10	Male weight	N
11	Male age	N
12	Tel No	T
13	Community (basis for grouping)	N
14	Nutrient Interventions	B (1=intervention 0=control)
	Nutrient first test	
15	Nutrient first test date	D
16	Serum folate	N
17	Red blood cell folate	N
18	Serum ferritin	N
19	VD(Vitamin D)	N
20	HCY(homocysteine)	N
21	Vitamin B12	N
22	LDL low density lipoprotein cholesterol	N
23	HDL high density lipoprotein cholesterol	N
24	TG total cholesterol	N
25	TC triglyceride	N
26	FBG(fasting blood-glucose)	N
27	Genotyping (BHMT (rs3733890), CBS (rs2850144, rs2851391), FIGN (rs2119289), MTHFD1 (rs2236225), MTHFR (rs1801131, rs1801133, rs3737965), MRT (rs1805087, rs28372871, rs1131450), MTRR (rs1801394, rs326119), RFC1 (rs1051266), and SHMT (rs1979277))	B
28	Clinical information (see Table 3)	-

29	Insufficient serum folate	B
30	Insufficient RBC folate	B
	Nutrient repetition measurement	
29	Nutrient repetition measurement date	D
30	Serum folate	N
31	Red blood cell folate	N
32	HCY (Homocysteine)	N
	Pregnancy	
33	Ultrasound image screen in mid-gestation	T
34	Report detail (positive)	B
35	Confirm image diagnosis	T
36	Therapeutic plan	T
37	birth defect diagnosis	T
38	Clinical information (see Table 2)	-

Note: T, text variable; D, The date type; N, Continuous variable; B, binary variable.

Table 2 Pregnant variable list in routine system

NO	variable	
	basic information	
1	hospital card number	T
2	inpationt number	T
3	name	T
4	age	N
5	Pregnant times	N
6	Delivery times	N
7	last menstrual period	D
8	Gestational week at the first visit	N
9	The first visit date	D
10	Height	N
11	Weight	N
12	Systolic blood pressure at the first visit	N
13	Diastolic blood pressure at the first visit	N
14	Occupation	T
15	Education	T
	Antenatal care record	
16	Weight at eath antenatal care	N
17	Systolic blood pressure at eath antenatal care	N
18	Diastolic blood pressure at eath antenatal care	N
19	Gestational week at eath antenatal care	N
20	Antenatal care date	D
	Lab data	
21	Cytomegalovirus	N
22	Cytomegalovirus date	D
23	Rubella virus	N
24	Rubella virus date	D
25	Toxoplasmosis	N
26	Toxoplasmosis date	D
27	Syphilis screening	N
28	Syphilis screening date	D
29	Fasting blood-glucose	N
30	Fasting blood-glucose date	D
31	HCT(hematokrit)	N
32	HCT(hematokrit) date	D
33	Serum folate	N
34	Serum folate date	D
35	HCY(homocysteine)	N
36	HCY(homocysteine) date	D

37	OGTT 0 hours	N
38	OGTT 1 hours	N
39	OGTT 2 hours	N
40	OGTT date	D
41	Triglyceride	N
42	Triglyceride date	D
43	Total cholesterol	N
44	Total cholesterol date	D
45	Hemoglobin date	N
46	Hemoglobin date	D
	Delivery date	
47	Gestational week at delivery	N
48	Delivery mode	T
49	Birth weight	N
50	Birth weight(second baby)	N
51	Systolic blood pressure at delivery	N
52	Diastolic blood pressure at delivery	N
53	Apgar scoring	N
54	Delivery date	D
55	Birth defect records	T
56	Weight blood pressure at delivery	N

Note: The data will be extracted from maternal clinic antenatal medical record system.

T, text variable; D, The date type; N, Continuous variable; B, binary variable.

Table 3 Pre-pregnant variable list in routine system

	variable	
	Basic information	
1	Wife id	T
2	Husband nation	T
3	Husband age	N
4	Husband education	T
5	Husband id	T
6	Husband occupation	T
7	Wife nation	T
8	Wife age	N
9	Wife education	T
10	Wife occupation	T
11	Tel no	T
12	Mobile phone No	T
	Medical history	
13	Female anemia	B
14	Female EH	B
15	Female heart disease	B
16	Female DM	B
17	Female epilepsia	B
18	Female thyroid disease	B
19	Female CGN	B
20	Female mental disease	B
21	Female tumour	B
22	Female TB	B
23	Female HBV	B
24	Female VD	B
25	Male anemia	B
26	Male EH	B
27	Male heart disease	B
28	Male DM	B
29	Male epilepsia	B
30	Male thyroid disease	B
31	Male CGN	B
32	Male mental disease	B
33	Male tumour	B
34	Male TB	B
35	Male HBV	B
36	Male VD	B
	Vaccine	
37	Female rubella vaccine	B
38	Female hepB vaccine	B

39	Male hepB vaccine	B
	Durg	
40	Female current medicine	B
41	Female medicine name	B
42	Male current medicine	B
43	Male medicine name	B
	Childbearing history	
44	Birth history	B
45	Pregnancy times	B
46	Live birth	B
47	Dead fetus	B
48	Dead birth	B
49	Term delivery	B
50	Premature delivery	B
51	Natural abortion	B
52	Abactio	B
53	Children number	B
54	Birth defect	B
55	Defect type	B
56	Menarche age	B
57	Period menstruation	B
58	Menstrual cycle	B
59	Menstrual quantity	B
60	LMP	D
	Family history of disease	
61	Female family history thalassemia	B
62	Female family history albinism	B
63	Female family history favism	B
64	Female family history hemophilia	B
65	Female family history CHD	B
66	Female family history DS	B
67	Female family history openNTDs	B
68	Female family history DM	B
69	Female family history dysnoesia	B
70	Female family history daysaudia	B
71	Female family history viaual disorder	B
72	Female family history neuropsychiatric	B
73	Female family history other birthdefects	B
74	Female family history fetal death	B
75	Female family history intermarry	B
76	Female family history relations	B
77	Male family history thalassemia	B
78	Male family history albinism	B

79	Male family history favism	B
80	Male family history hemophilia	B
81	Male family history CHD	B
82	Male family history DS	B
83	Male family history openNTDs	B
84	Male family history DM	B
85	Male family history dysnoesia	B
86	Male family history dysaudia	B
87	Male family history viaual disorder	B
88	Male family history neuropsychiatric	B
89	Male family history other birth defects	B
90	Male family history fetal death	B
91	Male family history intermarry	B
92	Male family history relations	B
	Anthroposomatology	
93	Female height	N
94	Female weight	N
95	Female BMI	N
96	Female heart rate	N
97	Female SBP	N
98	Female SDP	N
99	Male height	N
100	Male weight	N
101	Male BMI	N
102	Male heart rate	N
103	Male SBP	N
104	Male SDP	N
	Lab data	
105	Leucorrhea check	N
106	Clue cell	N
107	Monilia infection	N
108	Trichomomas	N
109	Cleanness	N
110	Whiff test	N
111	PH	N
112	Wom blood analysis	N
113	Female hb	N
114	Female wbc	N
115	Female rbc	N
116	Wom urine test	N
117	Female ABO	N
118	Female Rh	N
119	Female GLU	N

120	Female GLU levels	N
121	Female NG	N
122	Female chlamydia	N
123	Female syphilis	N
124	Female HIV	N
125	Female ALT	N
126	Female ALT levels	N
127	Female HBs-Ag	N
128	Female HBs-Ab	N
129	Female HBe-Ag	N
130	Female HBe-Ab	N
131	Female HBc-Ab	N
132	Female HCV-Ab	N
133	Female CMV IgM	N
134	Female CMV IgG	N
135	Female RV IgM	N
136	Female RV IgG	N
137	Female TOX IgM	N
138	Female TOX IgG	N
139	Male blood analysis	N
140	Male hb	N
141	Male wbc	N
142	Male rbc	N

Note: The data will be extracted the preconception care electronic data system T, text variable; D, The date type; N, Continuous variable; B, binary variable.

姓名 **Name**：_____

身份证号 **ID no**：□□□□□□□□□□□□□□□□□□

医院代码 **Hospital No**：_____（到时打印到问卷上）

填表日期 **Date**：_____年_____月_____日

孕前膳食补充剂调查表

Pre-pregnancy nutrition supplement questionnaire

（男女共用）

A 一般情况

A1 联系电话（请您认真填写，以助于医生和您联系）Contact number： _____(手机) _____(固定电话) _____（Email）
A2 家庭住址 Address：_____区/县_____街道/小区_____门牌号码/村
A3 您的出生日期是 Birth date _____年_____月_____日
A4 民族 Nationality ☐ 1 汉族 ☐ 2 其他（请注明）_____
A5 您的最高学历 Education ☐1 初高中以下 ☐2 大专本科 ☐3 硕士研究生以上及以上
A6 您现在的主要职业 Occupation ☐1 管理人员/干部 ☐2 技术人员 ☐3.企业主 ☐ 4 工人 ☐5 农民 ☐ 6 个体户 ☐7 其它_____
A7 上一年您的家庭年收入是：Income of a year ☐1. <2 万元 ☐ 2. (2~3.9)万 ☐3.(5.9)万 ☐4.(6~9.9)万 ☐5.(10~14.9)万 ☐6.15 万及以上 ☐9.不详
A8 填表日期 Date _____年_____月_____日

B 营养补充剂使用情况

营养补充剂种类 Nutritional supplement types	叶酸 Folic acid	复合维生素 multi-vitamins	单一维生素 Single vitamin
您在近三个月是否服用 Have you taken it in the last three months?	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否
您服用的什么品牌的营养补充剂？（如果没有对应选项请在其他处写明） Brand name	☐ 1、创盈金斯利安多维片 ☐ 2、福施福胶囊营养素 ☐ 3、汤臣倍健 ☐ 4、安利纽崔莱铁 ☐ 5、其他_____	☐ 1、爱乐维 ☐ 2、汤臣倍健孕妇专用 ☐ 3、惠氏玛特纳 ☐ 4、21 金维他 ☐ 5、其他_____	☐ V_C ☐ V_E ☐ V_{B1} ☐ V_{B2} ☐ 其他_____
您这三个月的服用频率？（如果食用频率小于每天/周一次，请填写每周/月食用几次，并勾出周/月） How often did you take in	____次/☐天 ☐周 ☐月	____次/☐天☐周☐月	____次/☐天 ☐周 ☐月

营养补充剂种类 Nutritional supplement types	铁 Fe	钙 Ca	锌 Zn
您在近三个月是否服用 Have you taken it in the last three months?	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否
您服用的什么品牌的营养补充剂？（如果没有，选项请在其他处写明） Brand name	☐ 1、汤臣倍健叶酸亚铁片 ☐ 2、安利纽崔莱铁片 ☐ 3、金康倍叶酸铁片 ☐ 4、其他_____	☐ 1、惠氏金钙尔奇 ☐ 2、君宝康孕妇钙片 ☐ 3、十月妈咪维生素 AD 钙锌咀嚼片 ☐ 4、安利钙镁片 ☐ 5、其他_____	☐ 1、美康利健 MK 硒金牡蛎锌片 ☐ 2、汤臣倍健 锌咀嚼片 ☐ 3、宫诺肽片 ☐ 4、其他_____
您这三个月的服用频率？（如果食用频率小于每天/周一次，请填写每周/月食用几次，并勾出周/月） How often did you take in	____次/☐天 ☐周 ☐月	____次/☐天☐周☐月	____次/☐天 ☐周 ☐月

B 营养补充剂使用情况

C 吸烟情况		
C1 您有吸烟史吗？ Have you smoked cigarettes ever before?	☐ 1 是	☐ 0 否***(跳至 F6)***
C2 在您最近的 3 个月内，您是否吸烟？ Did you smoke cigarettes in 3 month	☐1 是	☐ 0 否
C3 如果是，您平均每天吸多少支烟？ On average, how many cigarettes did you smoke each day in the month after your last menstrual period?	_____ 支	
C4 如果您曾经戒过烟，您戒了多少次？ How many times did you stop smoking?	_____	
C5 在您生活的大多数时间里，您是否暴露于他人烟草烟雾中？ On most days during your pregnancy, were you exposed to someone else's cigarette smoke?	☐ 1 是	☐ 0 否***（跳至 G1）***
C6 您在哪里暴露与烟草烟雾中 Where were you exposed to the smoke?		

☐ 1 仅在家中 ☐2 仅在工作单位 ☐3 在家和在工作单位均暴露
D 酒精
D1 您最近三个月的饮酒情况？During the 3 months before or during your pregnancy, did you ever drink any alcoholic beverages? ☐0.从未饮酒； ☐1.尝试饮酒（曾饮至少半瓶或一听啤酒，一小盅白酒等）； ☐2.现在饮酒（过去 30 天，至少有一天喝过一杯酒）； ☐3.重度饮酒（过去 30 天，至少有一天在 2 小时内喝过五杯酒）； ☐4.醉酒（过去 12 个月内，因喝酒太多而感到头晕/头疼/嗜睡等醉酒症状）。

核查人员签名：_____

Name of pregnancy： _____

ID no： _____

孕期危险因素暴露调查表

Pregnancy risk factor exposure questionnaire

A 一般情况 General information

A1 您的出生日期是 Birth date _____年_____月_____日
A2 民族 Nationality ☐ 1 汉族 Han ☐2 其他 other_____
A3 您的最高学历 Education ☐1 初高中以下 Mid ☐2 大专本科 High ☐3 硕士研究生以上及以上 College
A4 您现在的主要职业 Occupation ☐1 管理人员/干部 ☐2 技术人员 ☐3.企业主 ☐ 4 工人 ☐5 农民 ☐ 6 个体户 ☐7 其它_____
A6 家庭住址：Address _____区/县_____街道/小区_____门牌号码/村
A7 联系电话（请您认真填写，以助于医生和您联系）：Contact number _____(手机) _____(固定电话) _____(Email) _____(微信号)

B 本次妊娠情况

B1 您孕前体重通常为？ Current weight _____（公斤 Kg）			
B2 您身高是？ Height _____(厘米 cm)			
B3 您的腰围是？Waist _____（厘米 cm）			
B4 您此次怀孕的末次月经时间？ _____年_____月_____日 What was the first day of the menstrual period that came right before this pregnancy (LMP)?			
B5 孕期是否发生过重大负性生活事件而使您的精神受到刺激？ ☐ 1 是 ☐ 0 否 Have you ever experienced the negative events which irritate you and generate some negative emotion?			
B6 生这个孩子是您第几次怀孕？ _____次 How many times have you been pregnant?			
B7 是否有不良生育史？ Did you have the adverse reproductive history？ ☐ 1 有 ***（继续回答 B6.1）*** ☐ 0 无 ***（跳至 B7）***			
B6.1	流产史 Abortion	☐ 1 有	☐ 0 无
B6.2	早产史 Preterm	☐ 1 有	☐ 0 无
B6.3	死产史 Stillbith	☐ 1 有	☐ 0 无
B8 您是否有糖尿病和高血压疾病？Do you have hypertension or diabetes ☐ 1 是 ***(继续回答 B8)*** ☐ 0 否 ***（跳至 C1）*** ☐ 999 不知道 ***（跳至 C1）***			
B9 您的直系亲属中是否患有糖尿病、高血压疾病？ s there the family history of hypertension or diabetes in children's immediate family members ☐ 1 是 ***(继续回答 B8)*** ☐ 0 否 ***（跳至 C1）*** ☐ 999 不知道 ***（跳至 C1）***			
B10 若有，请选择出该亲属与您的关系（**可多选**）**If so, please choose the relationship with the child**			

☐1. 父亲 ☐2. 母亲 ☐3. 爷爷 ☐4. 奶奶 ☐5. 兄弟 ☐6. 姐妹

C 叶酸使用

C1 在您末次月经前三个月内，您是否服用过叶酸？ ☐1 是 ☐0 否 Did you take folic acid in the month before your last period?	
C2 在您末次月经之后至今，您是否服用过叶酸？ ☐1 是 ☐ 0 否 Did you take any folic acid after your last period anddduring pregnancy ?	

	叶酸 1	叶酸 2	叶酸 3
C3 药物名称（商品名） Brand name			
C4 使用时间 When did you take it?	☐1 孕前 before pregnancy ☐2 孕后 pregnancy ☐3 孕前孕后都有 always	☐1 孕前 before pregnancy ☐2 孕后 pregnancy ☐3 孕前孕后都有 always	☐1 孕前 before pregnancy ☐2 孕后 pregnancy ☐3 孕前孕后都有 always
C5 是否在怀孕期间一直使用？ Did you take it during the rest of your pregnancy?	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否
C6 是否停止使用过？ Did you stop taking it?	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否
C7 使用频率？ How often did you take it?	____次/☐天 ☐周 ☐月	____次/☐天 ☐周 ☐月	____次/☐天 ☐周 ☐月
C8 每次的使用量 What is the usage per time?	一次_____片	一次_____片	一次_____片

D 维生素使用

D1 在您末次月经的前三个月内，您是否服用过维生素？ ☐1 是 ☐0 否 Did you take any vitamins in the three months before your last period ？			
D2 在您末次月经之后至今，您是否服用过维生素？ ☐1 是 ☐0 否 ***（跳至 E1）*** Did you take any vitamins after your last period and during pregnancy?			
	维生素 1	维生素 2	维生素 3
D3 维生素名称 Vitamin name			
D4 维生素商品名称 Brand name			

D5 是否是医生给药？ Did your doctor give it to you?	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否
D6 是否包括叶酸？ Does it contain folic acid?	☐1 是 ☐0 否 ☐999 不知道	☐1 是 ☐0 否 ☐999 不知道	☐1 是 ☐0 否 ☐999 不知道
D7 使用时间 When did you take it?	☐1 孕前 before pregnancy ☐2 孕后 pregnancy ☐3 孕前孕后都有 always	☐1 孕前 before pregnancy ☐2 孕后 pregnancy ☐3 孕前孕后都有 always	☐1 孕前 before pregnancy ☐2 孕后 pregnancy ☐3 孕前孕后都有 always
D8 是否停止使用过？ Did you stop taking it?	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否
D9 使用频率？ How often did you take it?	____次/☐天 ☐周 ☐月	____次/☐天 ☐周 ☐月	____次/☐天 ☐周 ☐月
D10 每次的使用量 What is the usage per time?	一次_____片	一次_____片	一次_____片

E 其他营养补充剂使用情况 Other nutritional supplement use

营养补充剂种类 Nutritional supplement types	铁 Fe	钙 Ca	锌 Zn
您在近三个月是否服用 Did you take it around the time you became pregnant?	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否
您服用的什么品牌的营养补充剂？（如果没有，选项请在其他处写明）Brand name	☐ 1、汤臣倍健叶酸亚铁片 ☐ 2、安利纽崔莱铁片 ☐ 3、金康倍叶酸铁片 ☐ 4、其他_____	☐ 1、惠氏金钙尔奇 ☐ 2、君宝康孕妇钙片 ☐ 3、十月妈咪维生素 AD 钙锌咀嚼片 ☐ 4、安利钙镁片 ☐ 5、其他_____	☐ 1、美康利健 MK 硒金牡蛎锌片 ☐ 2、汤臣倍健 锌咀嚼片 ☐ 3、宫诺肽片 ☐ 4、其他_____
您这三个月的服用频率？（如果食用频率小于每天/周一次，请填写每周/月食用几次，并勾出周/月）How often did you take it?	____次/☐天 ☐周 ☐月	____次/☐天☐周☐月	____次/☐天 ☐周 ☐月

F 草本药物使用

F1 在您末次月经前三个月内，您是否使用过任何一种草本药物/传统医学药物？Did you take any herbal supplements/traditional Chinese medicine in the three months before your last period ☐1 是 ☐0 否			
F2 在您末次月经之后，您是否使用过任何一种草本药物/传统医学药物 Did you take any herbal supplements/traditional Chinese medicine after your last period and during pregnancy? ？ ☐1 是 ☐0 否 ***（跳至 F1）***			
	草本药物 1 Herbal 1	草本药物 2 Herbal 2	草本药物 3Herbal 3
F3 草本药物类型 Name of herb	☐1 中成药 ☐2 中医草药	☐1 中成药 ☐2 中医草药	☐1 中成药 ☐2 中医草药
F4 药物名称（如果选择中医草药，请填写中药功效） The use of traditional Chinese medicine			
F5 用药持续多少天 How long did you take in?	_____天	_____天	_____天

G 吸烟情况

G1 您在怀孕前或者怀孕期间吸烟吗？ Did you smoke cigarettes before or during your pregnancy with the baby?	☐1 是	☐0 否***(跳至 F9)***
G2 在您末次月经的当月，您是否吸烟？ Did you smoke during the month before your last menstrual period ?	☐1 是	☐0 否
G3 在您末次月经的一个月后（末次月经结束直至一个月后），您是否吸烟？ ***(跳至 5)*** Did you smoke during the month after your last menstrual period, that is between LMP and LMP+1 month	☐1 是	☐0 否
G4 如果是，您平均每天吸多少支烟？ On average, how many cigarettes did you smoke each day in the month after your last menstrual period ？ 支		
G5 在您怀孕期间，您是否吸烟？ Did you smoke during your pregnancy?	☐1 是	☐0 否***(跳至 F7)***
G6 在您怀孕期间，您平均每天吸多少支烟？ On average how many cigarettes did you smoke each	_____ 支	

G7 在您末次月经期间至今，您是否戒过吸烟？ ☐1 是 ☐0 否*（跳至 F9）* Did you stop smoking at any time between your last menstrual period and the end of your pregnancy?
G8 您戒烟有多少次？ How many times did you stop? _____ 次
G9 在您怀孕的大多数时间里，您是否暴露于他人烟草烟雾中？ On most days during your pregnancy, were you exposed to someone else's cigarette smoke? ☐1 是 ☐0 否*（跳至 G1）*
G10 您在哪里暴露与烟草烟雾中？ Where were you exposed to the smoke? ☐1 仅在家中 ☐2 仅在工作单位 ☐3 在家和在工作单位均暴露

H 酒精

H1 在您怀孕前三个月至今，您是否饮用过任何含有酒精的饮料？ ☐1 是 ☐0 否 During the 3 months before or during your pregnancy, did you ever drink any alcoholic beverages?
H2 在这三个月内，您通常每次饮几杯酒？ _____ 杯 On those days that you drank, how many drinks did you have?

I 环境暴露情况

I1 怀孕前三个月到现走，您是否染烫发? ☐1 是 ☐0 否 Did you dye perm in the first three months of pregnancy?
I2 怀孕前三个月到现在，后您工作的地点或家里是否装修过? ☐ 1 是 ☐0 否 Did you exposed to formaldehyde in the first three months of pregnancy?
I3 怀孕前后您是否接触过下列物质？ Have you been exposed to the following substances before and after pregnancy? (Toxic chemicals) ☐1 除草剂 ☐2 杀虫剂 ☐3 灭鼠剂 ☐4 有机溶剂 ☐5 消毒剂 ☐6 金属制剂 ☐7 有害气体 ☐8 有害固体

J 药物使用情况

以下问题是有关于您在末次月经之前三个月内至今期间的用药情况

	治疗高血压药物 Medication for hypertension	胰岛素 Insulin for diabetes	口服降血糖药物 Oral hypoglycemic for diabetes	抗癫痫药物 Medications for epilepsy	每天都要服用的药物 Medications at least once a day
1 您是否使用过？ Did you take?	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否
2 您使用的药物名称？ What	_____	_____	_____	_____	_____

did you take?					

如果下一个问题令您感到不安且不愿意回答，请在“拒答”上打✓，我们将对您所有回答进行严格保密!

	安定\有助于您放松药物 Valium\drugs to help you relax	使您感觉良好\精力旺盛 Make you feel good\have more energy	美沙酮\氧可酮\其他止痛药 Methadone oxymoron\other pain killers	可卡因 Cocaine or crack cocaine	海洛因 Heroin	大麻 Marijuana
1 您是否使用过？ Did you take?	☐1 是 ☐0 否 ☐2 拒答 ☐9 不清楚	☐1 是 ☐0 否 ☐2 拒答 ☐9 不清楚	☐1 是 ☐0 否 ☐2 拒答 ☐9 不清楚	☐1 是 ☐0 否 ☐2 拒答 ☐99 不清楚	☐1 是 ☐0 否 ☐2 拒答 ☐9 不清楚	☐1 是 ☐0 否 ☐2 拒答 ☐9 不清楚
2 您使用的药物名称？ What did you take?	_____	_____	_____	_____	_____	_____

I 您在怀孕期间是否患过以下疾病？

I.3 发热性疾病及呼吸道感染 ☐1 是 ☐0 否

Febrile illness and respiratory infections

I.3.1 您发烧时的最高温度是多少？ _____℃

What was the highest temperature of your fever during your illness?

I.3.2 您发烧有几天？ _____天

How long did you have a fever?

I.6 其他_____

调查结束，谢谢您的配合！

调查员姓名 _____

调查日期 ___ ___/ ___ ___/ ___ ___

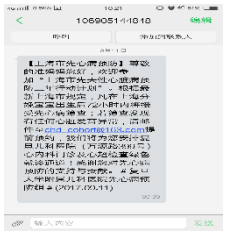
年 月 日

Management of blood samples

Recognition and Collection

Subjects with marks need to collect the blood sample

Cohord card &
Recognized label



Rercuiting
message

Recognized & Collected
blood sample

ID no checking

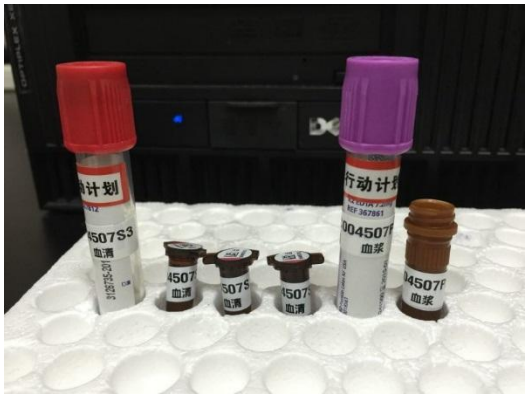
Blood sample dividing

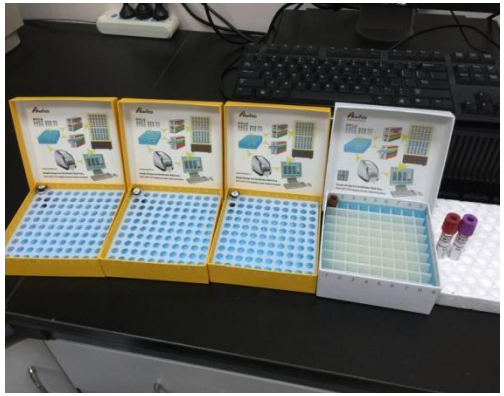
- Repacked within 6 hours after blood collection
- Serum is collected in three parts, no less than 200ul each
- 1ml of whole blood and divided it into the frozen storage tube.
- Taging the antifreezing label to the tube(red for serum & purple for whole blood)

Antifreezing label
Purple(two identical Numbers are a group)
Red (four identical Numbers are a group)

Blood sample transportation

- Keep away from light
- **Blood samples are** saved at -20 degrees in eath site
- Samples will be collected from each site every two weeks
- Guarantee -10 degrees during transportation
- **Blood samples are s**aved at -80 degrees in central biobank





BMJ Open

Cohort profile: Shanghai PreConception Cohort (SPCC) for association of periconceptional parental key nutrition factors with health outcomes of children: I -congenital heart disease

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Keywords:	red blood cell folate, vitamin, congenital heart diseases, periconceptional health care

Cohort profile: Shanghai PreConception Cohort (SPCC) for association of periconceptional parental key nutrition factors with health outcomes of children: I -congenital heart disease

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ABSTRACT (293)

Purpose: The Shanghai PreConception Cohort (SPCC) was established initially to investigate associations of parental periconceptional nutritional factors with congenital heart disease (CHD), and has extended to children growth, development and pediatric diseases.

Participants Prepare-for-pregnant couples who presented at Shanghai pre-conception examination clinics and early-pregnant women before 14 gestational weeks were enrolled to comprise a periconceptional baseline study population. General characteristics, routine clinical data, consumption of diet supplements, such as folic acid and multivitamins, were collected. Blood samples were collected at pre-conception, early, middle and late gestation respectively by standard procedures. Multiple nutrition factors in blood sample of participants that were selected by case-control design will be examined, including folates, homocysteine, vitamin A, vitamin D, vitamin E and metals. Genomic DNA was extracted.

Findings to date The baseline population included 8045 preconceptional couples, 3054 single women, and 15 615 early-pregnant women, respectively. Birth data from 12 402 births were collected and follow-up of the cohort for more outcomes is ongoing. Currently, 151 CHDs were identified after birth. Pilot analysis in a small subgroup showed that only about 15% of 656 pre-conception women and 49% of early-pregnant women had red blood cell folate concentration meeting the international recommendation for preventing neural tube defects.

Future plans

Once a sufficient number of CHD cases is achieved, we will investigate quantitative association of preconceptional red blood cell folate levels with CHD using nested case-control design. The SPCC cohort will be followed up for 18 years to investigate extensive outcomes of growth, development, obesity, and common and rare diseases during childhood and adolescence according to our plan. Blood nutrition factors will be examined in participants selected for specific aims. The SPCC cohort will also allow for prospective cohort studies on extensive research questions.

Trial registration number: NCT 02737644.

Key Messages: red blood cell folate, vitamin, congenital heart diseases, periconceptional health care.

Strengths and limitations of this study

- The SPCC cohort is the first prospective birth cohort with CHD as primary outcome with recruitment starting from preconception stage. Temporal sequence of exposures and outcomes can be achieved for causal inference of birth defects and other diseases that occur during early stage of gestation.
- Preconception blood samples were appropriately collected and stored which allow examination of individual blood levels for nutrition factors and other exposures.
- Clinical data and blood samples from both father and mother from before conception were collected, which will allow for testing the effect of both maternal and paternal genetic and nutrition factors on fetal and children diseases.
- Although response rate was high (over 95%), pre-conception participants were recruited from the population who voluntarily presented at Shanghai city pre-conception physical examination sites. They may have a stronger willingness for a healthy pregnancy, which may induce selection bias.
- Biological samples (cord blood, placenta) of the newborns were not collected.

Introduction

Congenital heart disease (CHD) is a common congenital malformation, which seriously affects quality of children's life [1]. CHD is a leading cause of infant death in high-income countries affecting eight of 1000 live births [2]. According to the report from National Health and Family Planning Commission of the People's Republic of China, CHD accounts for about a quarter of the birth defects of newborns in China, ranking the first among birth defects[3]. In a prospective, nation-wide large-scale study in more than 120 000 newborns in China in 2013, the prevalence of CHD in live births was identified 8.94 ‰; the rate of severe CHD was 2.9 ‰[4].

The cause of CHD is multifactorial. With the development of genetic engineering technology, the genetic factors have been better understood in the past decade[5]. Multiple environmental risk factors have been reported in epidemiological studies, the maternal social variables such as occupation, educational background, health status, unhealthy life style, maternal medical history and emotional status, family history of disease, consanguineous marriages and so on [6-10]. In addition, maternal key nutrients related to the risk of offspring's CHD as a modifiable environmental factor during periconception [11, 12]. The periconceptional intake of folic acid supplement has been shown to reduce the risk of CHD [13, 14] and women worldwide have been recommended to take folic acid supplements before

conception and in the beginning of pregnancy. Awareness of the relationship between folic acid deficiency and CHD is actually a by-product finding from the well-known Hungarian RCT study of folic acid supplementation to prevent neural tube defects. The study found that prenatal supplementation with a vitamin complex containing 0.8 mg of folic acid daily reduced the incidence of congenital neural tube defects. At the same time, the incidence of various heart defects have also been reduced by nearly half[15]. Longitudinal data from more than one million births in Canada over a total of 22 years from 1990 to 2011 also show that food fortification with folic acid reduced risk of CHD by 20-30% [16]. The current folic acid supplementation recommends that all women of childbearing potential be supplemented with at least 0.4 mg folic acid daily prior to conception and during pregnancy, which is designed for preventing neural tube dysplasia[17]. However, excessive folic acid intake may increase the risk of cancer[18], vitamin B12 deficiency[19], and autism spectrum disorder[20]. The optimal dose of folic acid for preventing CHD warrants further investigation. In addition, most previous studies only focused on the supplement of folic acid or the serum folate level during or after pregnancy, which may not be the optimal time period and way to reflect the exposure level to risk of CHD.

To investigate the association between parental periconceptional key

nutritional factors such as folate with the development of CHD and to explore the cutoff biomarker levels, we conducted Shanghai PreConception Cohort (SPCC) and a nested case-control analysis.

The SPPC cohort was initiated primarily to study CHD. However, based on the strengths of its baseline data collection, it has received attention and support, with improved additional extensive outcomes for children that will be followed up longer term.

Who is in the cohort?

The SPCC cohort recruited parent-planning women and men who were permanent residents and who voluntarily presented at preconception clinical clinics at 28 maternity institutions in 10 districts of Shanghai (Minhang District, Huangpu District, Xuhui District, Changning District, Jing'an District, Putuo District, Yangpu District, Pudong District， Songjiang District, Qingpu District) from March 2016 to December 2018. The preconception examination policy in the city of Shanghai provides a unique opportunity and clinical resources to support recruitment of SPCC. Since 2010, married couples in Shanghai have been encouraged to attend a free preconception health examination. In addition, these maternity institutions receive strong local administrative support and integrated maternal health care networking, providing service to 150 000–200 000 annual deliveries in Shanghai. Couples living in Shanghai who present at preconception clinics,

who are preparing for pregnancy within one year, who plan to receive antenatal care and deliver in Shanghai were eligible for the study. Written informed consent was obtained from all study participants before any data collection. In addition, we recruited early-pregnant women at their first antenatal examination who were at gestational week <14 weeks. These two groups of participants comprised a periconceptional baseline study population.

The first primary outcome of the SPCC cohort is CHD. The hypothesis is that maternal pre-conceptional serum or red blood cell (RBC) folate concentration is quantitatively associated with offspring CHD. The study design and protocol has been registered with Clinical Trials Registry (NCT 02737644).

As shown in Figure 1, the baseline population will be followed up to delivery, and their babies will be followed up until 18 years old (Figure 1).

Follow-up procedure

At enrollment, the participants completed the questionnaire of key nutrient supplementation and blood sample collection. When participants were pregnant, the same investigations (questionnaire/blood sample collection) were conducted during early pregnancy (first antenatal visit at 16-20 gestational weeks). Pregnancies were followed up along with routine maternal health care procedures. Blood samples were also collected at the

second (24-28 gestational weeks) and third trimester (32-34 gestational week). The follow-up of CHD outcome and birth data was obtained through Shanghai CHD screen platform (Figure 1).

As shown in Figure 1, outcomes at birth, during infant to childhood (preschool phase), and between 7 to 18 years (school ages) will be collected or extracted from multiple public platforms and data sources. Firstly, preconception clinical visit data from preconception care electronic data systems supported by national and local government, including height, weight, age, infections, sexually transmitted disease, and family history were collected. Secondly, the routine pregnant data were obtained in maternal clinic antenatal medical record systems, managed by Shanghai Center for Women and Children's Health, including height, gestational weight, last menstrual period, childbearing history, delivery outcomes, infections, hematocrit, coagulation function, liver and kidney function, and so on. Thirdly, the maternal and neonatal data at delivery came from Shanghai neonatal CHD screen platform including birth weight, CHD diagnosis, birth defects, and Apgar score, etc. In addition, we will work with the Shanghai Student Health and Fitness Surveillance Center to obtain outcome data. The personal national identification card number of participants are applied as index variables through the multiple data sources. The detailed variable list and codebook of data collection is presented in Appendix 1.

During the first phase of the cohort, from preconception to delivery, comprehensive strategies were used to retain participants in the study. For mothers, we provided a variety of engagement activities including green channel (fast track) to their antenatal care to provide convenience and save their time in hospitals. We also provided a contact number on the participant card to answer their calls or queries about the study procedures. Site investigators at early pregnant clinics in collaborative hospitals were provided a smartphone APP to help identify recruited cohort participants timely and manage data and blood sample collection procedures. We also provided green channel echocardiography for diagnosing CHD for all site hospitals to enhance the compliancy of the participants. In addition, an automated text message system is adopted to remind participants of schedules and appointment of follow-up.

Study measures

Personal characteristics questionnaires

As shown in Figure 1, Questionnaire 1 was administered during recruitment at pre-conception examination sites and Questionnaire 2 was administered at early pregnancy sites to collect information on consumption of folic acid supplement, vitamin supplement, the brand and content of nutrient supplement. Information of demographics, maternal education, socio-demographic status, occupation, smoking status, alcohol consumption, BMI,

medication, and health status were also included. In addition to the content of Questionnaire 1, Questionnaire 2 added drug information, reproductive history, and health status. Questionnaire 1 for baseline and Questionnaire 2 for the first antenatal visit at early pregnancy are presented in Appendix 2a and Appendix 2b.

Collection of blood samples

In this study, the rest blood samples for routine clinical examination were collected. The blood sample for routine clinical examination was usually 5 ml and extracted in the morning. Routine clinical examination followed which was performed at room temperature. The rest blood samples (fasting serum and EDTA anticoagulation) of peripheral venous blood from routine laboratory examination were kept. These blood samples were temporarily stored in a 4℃ refrigerator for dispensing within 6 hours and transferred to a -20 4℃. After completion of blood sample distribution the serum and the whole blood were stored at the site laboratory and then transported by three trained investigators to the central biobank for storage in -80 ℃ freezers within two weeks. During the collection and transfer process samples were labeled and recorded in the sample system. In order to detect chemicals (folate) that are sensitive to light sampling tubes were made of a light-proof material and the process of collecting blood samples were completely

protected from light.

Examination of key nutrition factors in blood samples

The examinations will be conducted in participants selected by nested case-control designs based on specific aims.

(1) *RBC folate, serum folate, serum homocysteine, vitamin D, vitamin B12 and serum ferritin*

EDTA anticoagulation blood was collected to measure RBC folate, serum folate, serum homocysteine, vitamin D, vitamin B12 and serum ferritin assays. All six biomarkers were analyzed on an electrochemiluminescence assay (ARCHITECT i2000SR Analyzer, Abbott Laboratories, USA). A standard solution with known concentration (produced by Abbott Laboratories) was used daily to quality control before the measurement. If the quality control level was out of concentration range, the measurement would be suspended and adjusted. External quality control was carried out with the control lab data program from Abbott Laboratories (Abbott Laboratories, Shanghai, China). RBC folate concentrations were adjusted for hematocrit. If the RBC folate concentration is below 126.0 ng/ml or above 651.1 ng/ml, adjustment was needed based on serum folate level. The hematocrit data were extracted from the hospital laboratory information system. Those examinations were performed in central laboratory of Children's Hospital of Fudan University.

(2) Vitamin A and vitamin E

The serum concentration of vitamin A and vitamin E were quantitatively detected by liquid chromatography-tandem mass spectrometry in central laboratory of Children's Hospital of Fudan University. The testing instrument was triple quadrupole mass spectrometer LC/MS/MS System (NO: API 3200MDTM, ABSciex Pte.Ltd.). A standard solution of vitamin A-d6 and vitamin E-d6 were applied as internal standards.

(3) *Glycemic and lipid profiles*

Fasting serum cholesterol, high-density lipoprotein, low-density lipoprotein, triglyceride, and fasting glucose were performed on Beckman coulter AU chemistry analyzers (Beckman, USA) in central laboratory of Children's Hospital of Fudan University.

(4) Metals

Serum levels including Mg, Fe, Zn, Se, Mn, As, Cu and Ca, were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) (Inductively Coupled Plasma Optical iCAP6300, Themo®, USA) in standard mode [21]. The metals examination was conducted in Instrumental Analysis Center of Shanghai Jiaotong University which is a national key laboratory.

(5) Genomic DNA extraction

Genomic DNA were extracted using a magnetic bead-based kit (TGuide M16 Automatic Nucleic Acid Extractor (OSE-M16), TIANGEN BIOTECH

(BEIJING) CO. LTD, China) from 2 ml of EDTA anticoagulated whole blood sample after blood routine test and stored for future study. An average 150 ng DNA are available. Similar to blood chemicals, future genetic variants genotyping will be performed in subjects that selected participants according to nested case-control design for specific aims. Currently, there are no candidate genes or variants that are listed. DNA was extracted from the EDTA anticoagulation blood samples.

Outcomes -CHD in neonates

The diagnosis of CHD was the primary outcome of the study at this stage and obtained from Shanghai neonatal CHD screen network platform, which was initiated as routine screen for newborns in Shanghai since Jun 1st 2016. The standard protocol of CHD screening of the platform was previously described in detail [22]. All newborn babies received the screen by using double-index method (i.e. cardiac murmur auscultation and pulse oximetry) during 6-72 hours after delivery, and those screen-positive babies would receive a subsequent echocardiography for further confirmative diagnosis.

SPCC will also collect other birth defects as secondary outcomes, including Down's syndrome, neural tube defects, hydrocephalus, digestive tract malformations, urinary malformations, and behavioral cognitive developmental disorder. After delivery, the infants attended routine child care procedures organized by Shanghai child health care system which is

administered by Shanghai Center for Women and Children's Health. All birth defect records which were diagnosed in after birth, as well as routine neuro-development examinations and longitudinal anthropometric data were abstracted from the system by a professional clinical team from Children's Hospital of Fudan University (for details of the types of birth defect please see Appendix 3)

Statistical methods

To investigate the association of maternal pre-conception nutrition levels with offspring CHD risk, a nested case-control study will be conducted. The control will be matched by age and site.

The sample size for the nested case-control analysis was planned as 180 cases and 720 matched controls to detect a maternal folate deficiency with prevalence of 50% in controls with odds ratio of 1.6 in association to achieve a power of 80% at alpha of 0.05. Based on CHD incidence above 8.94 per 1 000 live births [4], 20 000 pregnancies will be needed. For a continuous nutrient variable with standard deviation 2.0, 50 matched-pairs (1:4) are required to achieve 90% power to detect an odds ratio of 1.3 calculated using conditional logistic regression with a 0.05 significance level [23, 24]. Once a sufficient number of CHD cases is achieved, the quantitative association

of pre-conceptional RBC folate levels with CHD using nested case-control design will be investigated.

Conditional multivariate logistic regression will be used for association analysis with offspring affected status of CHD being the dependent variable, nutrition factors levels as exposure and adjusted for all potential paternal and maternal covariates. Odds ratios (OR) and 95% confidence intervals (95%CI) will be reported. To explore a potential cutoff point of the nutrition levels that significantly increases the risk of CHD, a dummy variable will be set up by categorizing the maternal pre-conception nutrition levels based on the distribution of the control group. The does-response relationship will be also be analyzed. Sensitive analysis will include non-conditional logistic regression analysis, or generalized estimation equations (GEE) model, or generalized linear models when necessary.

FINDINGS TO DATE

The SPCC started recruitment in March 2016. As shown in Figure 2, by December 2018, we consecutively recruited 19 144/19 563 (97.9%) participants at preconception settings, including 8045 couples and 3054 single women, and an additional 15 615/16 201 (96.4%) pregnant women at maternity hospitals with gestational age <16. Table 1 describes the basic demographic characteristics of the preparing-for-pregnant participants and pregnant women, respectively. The average age of the preconception

population was 29.9 (SD 3.9) years for females and 31.4 (SD 4.5) years for male, one-third of males and 2.4% of females were smokers, and two-thirds of males, and one-third of females had a habit of drinking alcohol. In pregnant women, the average age was 29.9 (SD 4.0) years, with half of them having a first pregnancy. Compared with the preconception females, they were similar in age but different in education levels and occupation, the prevalence of smoking and alcohol drinking were much lower (The descriptive data of Table 1 was partly included in another manuscript which is under review of Public Health Nutrition with Manuscript number of PHN-RES-2019-0914).

By the end of November 2018, the last participants recruited at early pregnancy were due for delivery, however, by now we have achieved birth records of 12 402 newborns. The follow-up of outcomes of the rest of the participants is ongoing (shown in Figure 2). A total of 151 cases of CHD were identified through the CHD screening platform, 131 cases of which were from the early pregnancy sample, the remaining 20 cases were from the preconception sample. The prevalence of CHD is 10.5‰ (131/12 402) based on the present available data.

We conducted a small pilot study in April 2017 to explore blood levels of nutrition factors, including serum folate, RBC folate, vitamin A, vitamin E, and vitamin D. The blood samples from 627 females were selected

consecutively from the preconception sample according to who was identified pregnant. In addition, 597 women who were consecutively recruited from the antenatal care clinics were selected. As shown in Table 2, the concentrations of RBC folate median level is 247.0 ng/ml (IQR: 184.8-340.5 ng/ml) in preconception women and 417.4 ng/ml (IQR: 308.6-544.2 ng/ml) in early-pregnant women. Twenty percent of preconceptional participants and 44.9% of pregnant participants had a folate level over 400 ng/ml, which was suggested as optimal level for preventing neural tube development defects [25, 26]. These results suggest that effort is urgently needed to improve the intake of folic acid supplementation in the prepare-for-pregnancy population, especially before pregnancy.

Based on SPCC, the possible scope of research questions, available types and number of biosamples and biomarkers that can be examined is shown in Table 3.

FUTURE PLANS

We have a complete plan to follow-up offspring to the age of 18 years old. The cohort will be financially supported by different grants. The current manuscript focuses on the first phase, the establishment of the baseline and our first main outcome, the CHD. The data collection plan for infants and children (from birth to 6 years old, pre-school stage), as well as school age (from 6 to 18 years old), are included (please see variable list of data

collection plan: Appendix 4). Multiple outcomes for pediatrics, including growth, physical and neuro-developments, obesity, and common and rare diseases will be investigated. Nested case-control design will be used for rare outcomes.

STRENGTHS AND LIMITATIONS

Compared with existing birth cohorts, there are three important strengths in our cohort. Firstly, the SPCC cohort is the first prospective birth cohort with CHD as primary outcome and recruitment starting from preconception. Blood samples were collected and stored which allows for direct measurement of individual exposure levels before the development of CHD and make causal inference. Temporal sequence of exposures and outcomes can be achieved for causal inference of birth defects and other diseases that occur during early stage of gestation. Up to date, no published studies have measured maternal blood folate levels before conception and link it to disease outcomes. Secondly, this cohort also allows for the investigation of associations between periconceptional maternal and paternal nutrition exposures with other birth defects, early onset-diseases, and neuro-development outcomes. Preconception blood samples were appropriately collected and stored which allows for the examination of individual blood levels of nutrition factors and other exposures. Thirdly, both paternal and

maternal clinical data and blood samples before conception were collected, which will allow for testing effect of both maternal and paternal genetic and nutrition factors to fetal and children diseases.

Two limitations of this cohort study should be considered. Firstly, there are approximately 200 000 pregnant women giving birth every year in Shanghai, and approximately 20 000 of them will take part in the free preconceptional care in Shanghai, where participants were recruited consecutively. Although response rate was high (over 95%), pre-conception participants were recruited from a population voluntarily present in Shanghai city with pre-conception physical examination sites, who may have a stronger willingness for a healthy pregnancy. This may induce selection bias. Secondly, in this study, biological samples (cord blood, placenta) of the newborns are not collected. We plan to give new informed consent to the family who are willing to participate in future studies, to collect biological samples not mentioned before. In addition, electrochemiluminescence assay was used to examine serum and RBC folate concentrations, which is different from microbiologic assay that is used widely. This will not bias the association analysis but comparison with international populations needs caution.

Collaboration

Investigators with an interest in hypotheses related to SPCC (and that meet the requirements of current approvals) are welcome to contact Dr. Guoying Huang or Weili Yan. A 'Research Collaboration application' should be send to the corresponding author by Email. The application should include a brief description of the project.

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SPCC group

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Contributors: Substantial contributions to the conception or design of the work were made by GH and WY. YZ and DW prepared the original draft of the manuscript. YZ, DW, YY, JY, ML, MJ, YD and XC led study implementation at participating sites. DW and YZ were responsible for the day-to-day project management at each site. XM and WS were responsible for the biobank of the cohort. All authors provided critical review of the manuscript for important intellectual content and approved the final version.

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Table 1. Socio-demographics of participants including 6,573 couples (parents) and 15,203 pregnant women that were enrolled in the Shanghai PreConception Cohort (SPCC).

Characteristics	Couples (parents) who completed preconception questionnaires				Additional pregnant women who completed both preconception and first-trimester questionnaires		P^*
	Male (n=6573)		Female (n=9243)		Pregnant women （n=15203）		
Age (years), Mean±SD	6573	31.4±4.5	9243	29.9±3.9	15203	29.9±4.0	0.995
Ethnicity	6536		9188		15176		0.258
Han Nationality		6403 (98%)		8966 (97.6%)		15245 (97.8%)	
Other		133 (2%)		222 (2.4%)		342 (2.2%)	
Educational level	6530		9147		15143		<0.001
<College		514 (7.8%)		795 (8.7%)		2052 (13.6%)	
>=College		6016 (92.2%)		8352 (91.3%)		13091 (86.4%)	
Annual household income	6530		9147		NA		
< ￥10 000		1424 (21.8%)		2214 (24.2%)			
>=￥10 000		5106(78.2%)		6933 (75.8%)			
Occupation	6530		9147		14789		<0.001
Entrepreneur		157 (2.4%)		128 (1.4%)		171 (1.1%)	
Farmer		26 (0.4%)		46 (0.5%)		79 (0.5%)	
Self-employed		216 (3.3%)		274 (3%)		862 (5.6%)	
Manager		1410 (21.6%)		1765 (19.3%)		2650 (17.2%)	
Technician		2462 (37.7%)		1903 (20.8%)		3239 (20.6%)	

Company clerk		2259 (34.6%)		5031 (55%)		8451 (54.7%)	
Attending preconception pregnant examination	NA		NA		14996		–
Yes						3374 (22.5%)	
No						11622 (77.5%)	
Times of pregnancy	NA		NA		15162		
1						7569 (49.9%)	
2						4604 (30.4%)	
>=3						2989 (19.7%)	
Miscarriage or stillbirth	NA		NA		15159		
Yes						4838 (31.2%)	
No						10385 (68.7%)	
Smoking history	6552		9212		15159		<0.001
Yes		2073 (31.6%)		218 (2.4%)		153 (1.0%)	
No		4479 (68.4%)		8994 (97.6%)		15006 (99%)	
Alcohol Drinking history	6448		9075		15164		<0.001
Yes		3962 (61.5%)		2865 (31.6%)		1556 (10.3%)	
No		2486 (38.5%)		6210 (68.4%)		13608 (89.7%)	
Location of home	6573		9243		NA		
Developed districts		3034 (46.2%)		5452 (59.0%)			
Developing districts		3539 (53.8%)		3791 (41.0%)			

* Comparisons between pre-conception females and pregnant women.

Table 2. Distributions of biomarkers in women before and at early gestation (pilot study)

Biomarker	PreConception		Early pregnancy	
	n	level	n	level
Serum folate，ng/ mL	620	9.7(6.5, 13.8)	577	14.5(11.2, 16.4)
RBC folate，ng/ mL	570	247.0(184.8, 340.5)	587	417.4(308.6, 544.2)
Homocysteine，μmol/L	624	6.5(5.2, 8.6)	599	4.2(3.5, 5.2)
Vitamin B12，pg/mL	625	495.2(394.2, 639.0)	600	388.5(289.4, 511.4)
Vitamin D，ng/ mL	607	16.3±6.0	578	15.5±6.1

Table 3. Biosample collected and biomarkers that can be examined in the SPCC cohort

Biosample Available in participants	Available Sample type and volume	Time		
		Preconception+early pregnancy (Baseline)	24-28 weeks	32-36 weeks
Mother		(n=25487)	(n=8668)	(n=7522)
	Serum, 200 ul*3	Yes	Yes	Yes
	Whole blood	Yes	Yes	Yes
	Genomic DNA, 150 ng	Yes	Yes	Yes
Father		(n= 7151)	-	-
	Serum, 200 ul*3	Yes	NA	NA
	Whole blood	Yes	NA	NA
	Genomic DNA, 150 ng	Yes	NA	NA
Child	NA			

Scope of research questions:

1. Quantitative association of pre-conceptional key nutrition levels (such as serum and RBC folates and related biomarkers, such as homocysteine and vitamin B12) with CHD, currently, and other folate sensitive birth defects.
2. Quantitative association of periconceptional maternal and paternal key nutrition levels (preconception levels, and dynamic levels during gestation) with important maternal and neonatal gestational complications, neuro-development of infants, childhood obesity, and clinical pediatric diseases.
3. Periconcpetional maternal and paternal folate concentration with Autism spectrum disorder, allergy and asthma in children.

Biomarkers that will be examined in different types of biosamples:

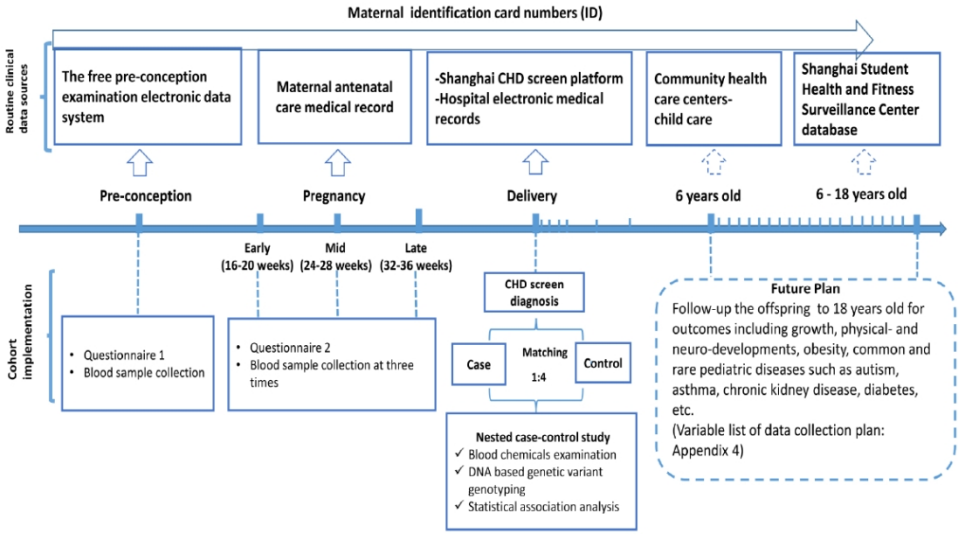
1. Biomarkers based on serum sample:
a) Folate and related markers: serum folate, homocysteine
b) Other Vitamins: vitamin A, vitamin B12, vitamin E, and vitamin D
c) Marco and micro metals: Mg, Fe, Zn, Se, Mn, As, Cu, Ca, etc., mg/L
d) Serum ferritin
e) Fasting glycemic and lipid profiles: fasting glucose, total cholesterol, triglyceride, high-density lipoprotein, low-density lipoprotein
2. Whole blood sample: RBC folate
3. Genomic DNA sample: candidate genetic variants or genome-wide variants are all possible to examined

Figure 1 Diagram of the protocol and follow-up of Shanghai PreConceptional Cohort (SPCC)

Frame of SPCC cohort protocol. The baseline population of SPCC cohort were females and males at periconception stage: couples who are prepare-for-pregnancy, and pregnant women at early gestation stage. The cohort includes three phases, from periconception to birth (peri-natal phase), from newborns to 6 years old (birth to pre-school age), and from 7-18 years (school age). The current manuscript focuses on the first phase, with congenital heart disease as the primary outcome, and will cover other folate sensitive birth defects.

Figure 2 Flow chart diagram

By December 2018, we consecutively recruited 19 144/19 563 (97.9%) participants at preconception settings, including 8045 couples and 3054 single women at pre-pregnancy sites, and an additional 15 615/16 201 (96.4%) pregnant women at maternity hospitals with gestational age <16 were recruited. By the end of November 2018, the last participants recruited at early pregnancy were due for delivery, by now we have achieved birth records of 12402 newborns. The follow-up of outcomes of the rest of participants is ongoing (shown in Figure 2). A total of 151 cases of CHD were identified through the CHD screening platform, 131 cases of which were from the early pregnancy sample, the remaining 20 cases were from preconception sample. The prevalence of CHD is 10.5 ‰ (131/12 402) based on the present available data.



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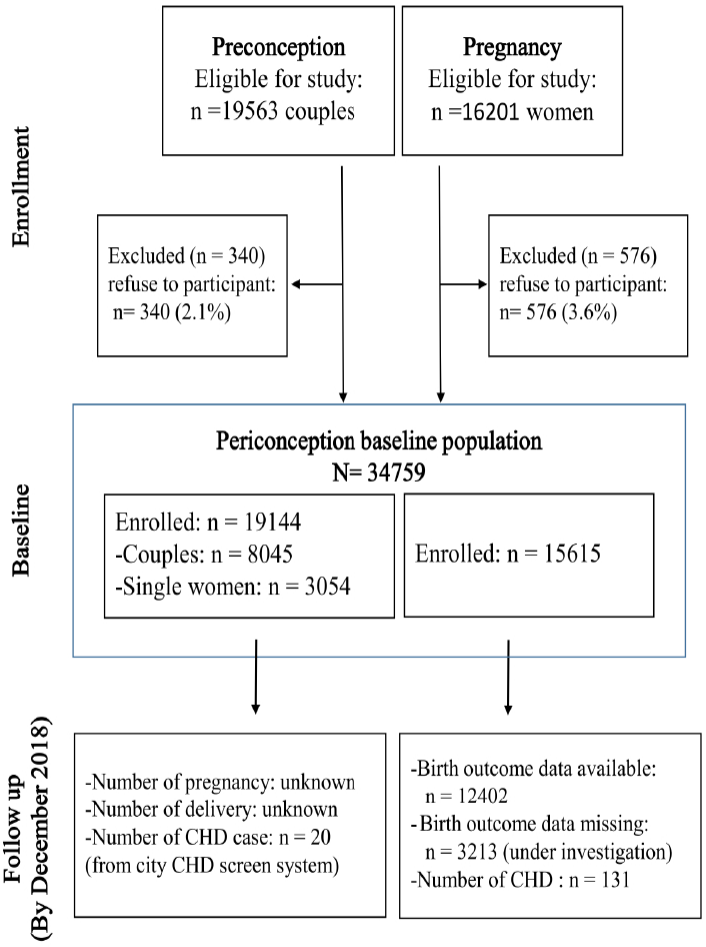

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Appendix 1 Variable list

Pre-pregnant variable list

No	Variables	Data type
	General information	
1	Wife id	Text
2	Husband nation	Text
3	Husband age	N
4	Husband education	Text
5	Husband id	Text
6	Husband occupation	Text
7	Wife nation	Text
8	Wife age	N
9	Wife education	Text
10	Wife occupation	Text
11	Tel No	Text
12	Mobile phone No	Text
	Medical history	
13	Female anemia	Category
14	Female EH	Category
15	Female heart disease	Category
16	Female DM	Category
17	Female epilepsia	Category
18	Female thyroid disease	Category
19	Female CGN	Category
20	Female mental disease	Category
21	Female tumour	Category
22	Female TB	Category
23	Female HBV	Category
24	Female VD	Category
25	Male anemia	Category
26	Male EH	Category
27	Male heart disease	Category
28	Male DM	Category
29	Male epilepsia	Category
30	Male thyroid disease	Category
31	Male CGN	Category
32	Male mental disease	Category
33	Male tumour	Category
34	Male TB	Category
35	Male HBV	Category
36	Male VD	Category

	Vaccine	
37	Female rubella vaccine	Category
38	Female hepB vaccine	Category
39	Male hepB vaccine	Category
	Drug use	
40	Female current medicine	Category
41	Female medicine name	Category
42	Male current medicine	Category
43	Male medicine name	Category
	Childbearing history	
44	Birth history	Category
45	Pregnancy times	Category
46	Live birth	Category
47	Dead fetus	Category
48	Dead birth	Category
49	Term delivery	Category
50	Premature delivery	Category
51	Natural abortion	Category
52	Abactio	Category
53	Children number	Category
54	Birth defect	Category
55	Defect type	Category
56	Menarche age	Category
57	Period menstruation	Category
58	Menstrual cycle	Category
59	Menstrual quantity	Category
60	LMP	Date
	Family history of disease	
61	Female family history thalassemia	Category
62	Female family history albinism	Category
63	Female family history favism	Category
64	Female family history hemophilia	Category
65	Female family history CHD	Category
66	Female family history DS	Category
67	Female family history openNTDs	Category
68	Female family history DM	Category
69	Female family history dysnoesia	Category
70	Female family history daysaudia	Category
71	Female family history viaual disorder	Category
72	Female family history neuropsychiatric	Category
73	Female family history other birth defects	Category
74	Female family history fetal death	Category
75	Female family history intermarry	Category

76	Female family history relations	Category
77	Male family history thalassemia	Category
78	Male family history albinism	Category
79	Male family history favism	Category
80	Male family history hemophilia	Category
81	Male family history CHD	Category
82	Male family history DS	Category
83	Male family history openNTDs	Category
84	Male family history DM	Category
85	Male family history dysnoesia	Category
86	Male family history dysaudia	Category
87	Male family history viaual disorder	Category
88	Male family history neuropsychiatric	Category
89	Male family history other birth defects	Category
90	Male family history fetal death	Category
91	Male family history intermarry	Category
92	Male family history relations	Category
	Anthroposomatology	
93	Female height	Numeric
94	Female weight	Numeric
95	Female BMI	Numeric
96	Female heart rate	Numeric
97	Female SBP	Numeric
98	Female SDP	Numeric
99	Male height	Numeric
100	Male weight	Numeric
101	Male BMI	Numeric
102	Male heart rate	Numeric
103	Male SBP	Numeric
104	Male SDP	Numeric
	Lab data	
105	Leucorrhea check	Numeric
106	Clue cell	Numeric
107	Monilia infection	Numeric
108	Trichomomas	Numeric
109	Cleanness	Numeric
110	Whiff test	Numeric
111	PH	Numeric
112	Wom blood analysis	Numeric
113	Female hb	Numeric
114	Female wbc	Numeric
115	Female rbc	Numeric
116	Wom urine test	Numeric

117	Female ABO	Numeric
118	Female Rh	Numeric
119	Female GLU	Numeric
120	Female GLU levels	Numeric
121	Female NG	Numeric
122	Female chlamydia	Numeric
123	Female syphilis	Numeric
124	Female HIV	Numeric
125	Female ALT	Numeric
126	Female ALT levels	Numeric
127	Female HBs-Ag	Numeric
128	Female HBs-Ab	Numeric
129	Female HBe-Ag	Numeric
130	Female HBe-Ab	Numeric
131	Female HBc-Ab	Numeric
132	Female HCV-Ab	Numeric
133	Female CMV IgM	Numeric
134	Female CMV IgG	Numeric
135	Female RV IgM	Numeric
136	Female RV IgG	Numeric
137	Female TOX IgM	Numeric
138	Female TOX IgG	Numeric
139	Male blood analysis	Numeric
140	Male hb	Numeric
141	Male wbc	Numeric
142	Male rbc	Numeric

During-pregnancy variable list

	Basic information	
1	Hospital card number	Text
2	Inpatient number	Text
3	Name	Text
4	Age	Numeric
5	Pregnant times	Numeric
6	Delivery times	Numeric
7	Last menstrual period	Date
8	Gestational week at the first visit	Numeric
9	The first visit date	Date
10	Height	Numeric
11	Weight	Numeric
12	Systolic blood pressure at the first visit	Numeric
13	Diastolic blood pressure at the first visit	Numeric

14	Occupation	Text
15	Education	Text
	Antenatal care record	
16	Weight at each antenatal care	Numeric
17	Systolic blood pressure at each antenatal care	Numeric
18	Diastolic blood pressure at each antenatal care	Numeric
19	Gestational week at each antenatal care	Numeric
20	Antenatal care date	Date
	Lab data	
21	Cytomegalovirus	Numeric
22	Cytomegalovirus examination date	Date
23	Rubella virus	Numeric
24	Rubella virus examination date	Date
25	Toxoplasmosis	Numeric
26	Toxoplasmosis examination date	Date
27	Syphilis screening	Numeric
28	Syphilis screening examination date	Date
29	Fasting blood-glucose	Numeric
30	Fasting blood-glucose examination date	Date
31	HCT(hematokrit)	Numeric
32	HCT(hematokrit) examination date	Date
33	Serum folate	Numeric
34	Serum folate examination date	Date
35	HCY(homocysteine)	Numeric
36	HCY(homocysteine) examination date	Date
37	OGTT 0 hours	Numeric
38	OGTT 1 hours	Numeric
39	OGTT 2 hours	Numeric
40	OGTT examination date	Date
41	Triglyceride	Numeric
42	Triglyceride examination date	Date
43	Total cholesterol	Numeric
44	Total cholesterol examination date	Date
45	Hemoglobin	Numeric
46	Hemoglobin examination date	Date
	Delivery date	
47	Gestational week at delivery	Numeric
48	Delivery mode	Text
49	Birth weight	Numeric
50	Birth weight(second baby)	Numeric
51	Systolic blood pressure at delivery	Numeric
52	Diastolic blood pressure at delivery	Numeric
53	Apgar scoring	Numeric

54	Delivery date	Date
55	Birth defect records	Text
56	Weight blood pressure at delivery	Numeric
Offspring variable list		
	0 - 6 years (6 weeks, 6 months, 12 months, 36 months, 60 months)	
1	Name	Text
2	ID number	Text
3	Weight	Numeric
4	Height/ Length	Numeric
5	Vision detection	Numeric
6	DDST: Denver developmental screening test	Numeric
	6 - 18 years (each year)	
7	Weight	Numeric
8	Height	Numeric
9	Diastolic blood pressure	Numeric
10	Systolic blood pressure	Numeric
11	Vision detection	Numeric
12	Vital capacity	Numeric
13	Physical fitness measurement (running, jumping, solid balls, etc)	Text
The clinical diagnosis during 0 – 18 years will be extracted from the routine medical system each year.		

姓名 Name：_____

身份证号 ID no：□□□□□□□□□□□□□□□□□□

医院代码 Hospital No：_____（到时打印到问卷上）

填表日期 Date：_____年_____月_____日

孕前膳食补充剂调查表

Pre-pregnancy nutrition supplement questionnaire

（男女共用）

A 一般情况

A1 联系电话（请您认真填写，以助于医生和您联系）Contact number： _____(手机) _____(固定电话) _____（Email）
A2 家庭住址 Address：_____区/县_____街道/小区_____门牌号码/村
A3 您的出生日期是 Birth date _____年_____月_____日
A4 民族 Nationality ☐1 汉族 ☐2 其他（请注明）_____
A5 您的最高学历 Education ☐1 初高中以下 ☐2 大专本科 ☐3 硕士研究生以上及以上
A6 您现在的主要职业 Occupation ☐1 管理人员/干部 ☐2 技术人员 ☐3.企业主 ☐4 工人 ☐5 农民 ☐6 个体户 ☐7 其它_____
A7 上一年您的家庭年收入是：Income of a year ☐1. <2 万元 ☐ 2. (2~3.9)万 ☐3.(5.9)万 ☐4.(6~9.9)万 ☐5.(10~14.9)万 ☐6.15 万及以上 ☐9.不详
A8 填表日期 Date _____年_____月_____日

B 营养补充剂使用情况

营养补充剂种类 Nutritional supplement types	**叶酸 Folic acid**	**复合维生素 multi-vitamins**	**单一维生素 Single vitamin**
您在近三个月是否服用 Have you taken it in the last three months?	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否
您服用的什么品牌的营养补充剂？（如果没有对应选项请在其他处写明） Brand name	☐ 1、创盈金斯利安多维片 ☐ 2、福施福胶囊营养素 ☐ 3、汤臣倍健 ☐ 4、安利纽崔莱铁 ☐ 5、其他_____	☐ 1、爱乐维 ☐ 2、汤臣倍健孕妇专用 ☐ 3、惠氏玛特纳 ☐ 4、21 金维他 ☐ 5、其他_____	☐ V_C ☐ V_E ☐ V_{B1} ☐ V_{B2} ☐ 其他_____
您这三个月的服用频率？（如果食用频率小于每天/周一次，请填写每周/月食用几次，并勾出周/月） How often did you take in	____次/☐天 ☐周 ☐月	____次/☐天☐周☐月	____次/☐天 ☐周 ☐月

营养补充剂种类 Nutritional supplement types	铁 Fe	钙 Ca	锌 Zn
您在近三个月是否服用 Have you taken it in the last three months?	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否
您服用的什么品牌的营养补充剂？（如果没有，选项请在其他处写明） Brand name	☐ 1、汤臣倍健叶酸亚铁片 ☐ 2、安利纽崔莱铁片 ☐ 3、金康倍叶酸铁片 ☐ 4、其他_____	☐ 1、惠氏金钙尔奇 ☐ 2、君宝康孕妇钙片 ☐ 3、十月妈咪维生素 AD 钙锌咀嚼片 ☐ 4、安利钙镁片 ☐ 5、其他_____	☐ 1、美康利健 MK 硒金牡蛎锌片 ☐ 2、汤臣倍健 锌咀嚼片 ☐ 3、宫诺肽片 ☐ 4、其他_____

您这三个月的服用频率？（如果食用频率小于每天/周一次，请填写每周/月食用几次，并勾出周/月）How often did you take in	____次/☐天 ☐周 ☐月	____次/☐天☐周☐月	____次/☐天 ☐周 ☐月

B 营养补充剂使用情况

C 吸烟情况
C1 您有吸烟史吗？ ☐ 1 是 ☐ 0 否***(跳至 F6)*** Have you smoked cigarettes ever before?
C2 在您最近的 3 个月内，您是否吸烟？ ☐1 是 ☐ 0 否 Did you smoke cigarettes in 3 month
C3 如果是，您平均每天吸多少支烟？ _____ 支 On average, how many cigarettes did you smoke each day in the month after your last menstrual period?
C4 如果您曾经戒过烟，您戒了多少次？ _____ How many times did you stop smoking?
C5 在您生活的大多数时间里，您是否暴露于他人烟草烟雾中？ On most days during your pregnancy, were you exposed to someone else's cigarette smoke? ☐ 1 是 ☐ 0 否 ***（跳至 G1）***
C6 您在哪里暴露与烟草烟雾中 Where were you exposed to the smoke? ☐ 1 仅在家中 ☐2 仅在工作单位 ☐3 在家和在工作单位均暴露
D 酒精
D1 您最近三个月的饮酒情况？During the 3 months before or during your pregnancy, did you ever drink any alcoholic beverages? ☐0.从未饮酒； ☐1.尝试饮酒（曾饮至少半瓶或一听啤酒，一小盅白酒等）； ☐2.现在饮酒（过去 30 天，至少有一天喝过一杯酒）； ☐3.重度饮酒（过去 30 天，至少有一天在 2 小时内喝过五杯酒）； ☐4.醉酒（过去 12 个月内，因喝酒太多而感到头晕/头疼/嗜睡等醉酒症状）。

核查人员签名：_____

Name of pregnancy： _____

ID no： _____

孕期危险因素暴露调查表

Pregnancy risk factor exposure questionnaire

A 一般情况 General information

A1 您的出生日期是 Birth date _____年_____月_____日
A2 民族 Nationality ☐ 1 汉族 Han ☐2 其他 other_____
A3 您的最高学历 Education ☐1 初高中以下 Mid ☐2 大专本科 High ☐3 硕士研究生以上及以上 College
A4 您现在的主要职业 Occupation ☐1 管理人员/干部 ☐2 技术人员 ☐3.企业主 ☐ 4 工人 ☐5 农民 ☐ 6 个体户 ☐7 其它_____
A6 家庭住址：Address _____区/县_____街道/小区_____门牌号码/村
A7 联系电话（请您认真填写，以助于医生和您联系）：Contact number _____(手机) _____(固定电话) _____(Email) _____(微信号)

B 本次妊娠情况

B1 您孕前体重通常为？ Current weight _____（公斤 Kg）			
B2 您身高是？ Height _____(厘米 cm)			
B3 您的腰围是？Waist _____（厘米 cm）			
B4 您此次怀孕的末次月经时间？ _____年_____月_____日 What was the first day of the menstrual period that came right before this pregnancy (LMP)?			
B5 孕期是否发生过重大负性生活事件而使您的精神受到刺激？ ☐ 1 是 ☐ 0 否 Have you ever experienced the negative events which irritate you and generate some negative emotion?			
B6 生这个孩子是您第几次怀孕？ _____次 How many times have you been pregnant?			
B7 是否有不良生育史？ Did you have the adverse reproductive history？ ☐ 1 有***（继续回答 B6.1）*** ☐ 0 无***（跳至 B7）***			
B6.1	流产史 Abortion	☐ 1 有	☐ 0 无
B6.2	早产史 Preterm	☐ 1 有	☐ 0 无
B6.3	死产史 Stillbith	☐ 1 有	☐ 0 无
B8 您是否有糖尿病和高血压疾病？Do you have hypertension or diabetes ☐ 1 是 ***(继续回答 B8)*** ☐ 0 否 ***（跳至 C1）*** ☐ 999 不知道 ***（跳至 C1）***			
B9 您的直系亲属中是否患有糖尿病、高血压疾病？ s there the family history of hypertension or diabetes in children's immediate family members ☐ 1 是 ***(继续回答 B8)*** ☐ 0 否 ***（跳至 C1）*** ☐ 999 不知道 ***（跳至 C1）***			
B10 若有，请选择出该亲属与您的关系**（可多选）If so, please choose the relationship with the child**			

☐1. 父亲 ☐2. 母亲 ☐3. 爷爷 ☐4. 奶奶 ☐5. 兄弟 ☐6. 姐妹

C 叶酸使用

C1 在您末次月经前三个月内，您是否服用过叶酸？ ☐1 是 ☐0 否 Did you take folic acid in the month before your last period?			
C2 在您末次月经之后至今，您是否服用过叶酸？ ☐1 是 ☐ 0 否 Did you take any folic acid after your last period anddduring pregnancy ?			
	叶酸 1	叶酸 2	叶酸 3
C3 药物名称（商品名） Brand name			
C4 使用时间 When did you take it?	☐1 孕前 before pregnancy ☐2 孕后 pregnancy ☐3 孕前孕后都有 always	☐1 孕前 before pregnancy ☐2 孕后 pregnancy ☐3 孕前孕后都有 always	☐1 孕前 before pregnancy ☐2 孕后 pregnancy ☐3 孕前孕后都有 always
C5 是否在怀孕期间一直使用？ Did you take it during the rest of your pregnancy?	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否
C6 是否停止使用过？ Did you stop taking it?	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否
C7 使用频率？ How often did you take it?	____次/☐天 ☐周 ☐月	____次/☐天 ☐周 ☐月	____次/☐天 ☐周 ☐月
C8 每次的使用量 What is the usage per time?	一次_____片	一次_____片	一次_____片

D 维生素使用

D1 在您末次月经的前三个月内，您是否服用过维生素？ ☐1 是 ☐0 否 Did you take any vitamins in the three months before your last period ？			
D2 在您末次月经之后至今，您是否服用过维生素？ ☐1 是 ☐0 否 ***（跳至 E1）*** Did you take any vitamins after your last period and during pregnancy?			
	维生素 1	维生素 2	维生素 3
D3 维生素名称 Vitamin name			
D4 维生素商品名称 Brand name			

D5 是否是医生给药？ Did your doctor give it to you?	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否
D6 是否包括叶酸？ Does it contain folic acid?	☐1 是 ☐0 否 ☐999 不知道	☐1 是 ☐0 否 ☐999 不知道	☐1 是 ☐0 否 ☐999 不知道
D7 使用时间 When did you take it?	☐1 孕前 before pregnancy ☐2 孕后 pregnancy ☐3 孕前孕后都有 always	☐1 孕前 before pregnancy ☐2 孕后 pregnancy ☐3 孕前孕后都有 always	☐1 孕前 before pregnancy ☐2 孕后 pregnancy ☐3 孕前孕后都有 always
D8 是否停止使用过？ Did you stop taking it?	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否
D9 使用频率？ How often did you take it?	____次/☐天 ☐周 ☐月	____次/☐天 ☐周 ☐月	____次/☐天 ☐周 ☐月
D10 每次的使用量 What is the usage per time?	一次_____片	一次_____片	一次_____片

E 其他营养补充剂使用情况 Other nutritional supplement use

营养补充剂种类 Nutritional supplement types	铁 Fe	钙 Ca	锌 Zn
您在近三个月是否服用 Did you take it around the time you became pregnant?	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否
您服用的什么品牌的营养补充剂？（如果没有，选项请在其他处写明）Brand name	☐ 1、汤臣倍健叶酸亚铁片 ☐ 2、安利纽崔莱铁片 ☐ 3、金康倍叶酸铁片 ☐ 4、其他_____	☐ 1、惠氏金钙尔奇 ☐ 2、君宝康孕妇钙片 ☐ 3、十月妈咪维生素 AD 钙锌咀嚼片 ☐ 4、安利钙镁片 ☐ 5、其他_____	☐ 1、美康利健 MK 硒金牡蛎锌片 ☐ 2、汤臣倍健 锌咀嚼片 ☐ 3、宫诺肽片 ☐ 4、其他_____
您这三个月的服用频率？（如果食用频率小于每天/周一次，请填写每周/月食用几次，并勾出周/月）How often did you take it?	____次/☐天 ☐周 ☐月	____次/☐天☐周☐月	____次/☐天 ☐周 ☐月

F 草本药物使用

F1 在您末次月经前三个月内，您是否使用过任何一种草本药物/传统医学药物？Did you take any herbal supplements/traditional Chinese medicine in the three months before your last period ☐1 是 ☐0 否			
F2 在您末次月经之后，您是否使用过任何一种草本药物/传统医学药物 Did you take any herbal supplements/traditional Chinese medicine after your last period and during pregnancy? ？ ☐1 是 ☐0 否 ***（跳至F1）***			
	草本药物 1 Herbal 1	草本药物 2 Herbal 2	草本药物 3Herbal 3
F3 草本药物类型 Name of herb	☐1 中成药 ☐2 中医草药	☐1 中成药 ☐2 中医草药	☐1 中成药 ☐2 中医草药
F4 药物名称（如果选择中医草药，请填写中药功效） The use of traditional Chinese medicine			
F5 用药持续多少天 How long did you take in?	_____天	_____天	_____天

G 吸烟情况

G1 您在怀孕前或者怀孕期间吸烟吗？ ☐1 是 ☐0 否***(跳至F9)*** Did you smoke cigarettes before or during your pregnancy with the baby?
G2 在您末次月经的当月，您是否吸烟？ ☐1 是 ☐0 否 Did you smoke during the month before your last menstrual period ?
G3 在您末次月经的一个月后（末次月经结束直至一个月后），您是否吸烟？ ***(跳至5)*** Did you smoke during the month after your last menstrual period, that is between LMP and LMP+1 month ☐1 是 ☐0 否
G4 如果是，您平均每天吸多少支烟？ On average, how many cigarettes did you smoke each day in the month after your last menstrual period ？ 支
G5 在您怀孕期间，您是否吸烟？ ☐1 是 ☐0 否***(跳至F7)*** Did you smoke during your pregnancy?
G6 在您怀孕期间，您平均每天吸多少支烟？ _____ 支 On average how many cigarettes did you smoke each

G7 在您末次月经期间至今，您是否戒过吸烟？ ☐1 是 ☐0 否 ***（跳至 F9）*** Did you stop smoking at any time between your last menstrual period and the end of your pregnancy?
G8 您戒烟有多少次？ How many times did you stop? _____ 次
G9 在您怀孕的大多数时间里，您是否暴露于他人烟草烟雾中？ On most days during your pregnancy, were you exposed to someone else's cigarette smoke? ☐1 是 ☐0 否 ***（跳至 G1）***
G10 您在哪里暴露与烟草烟雾中？ Where were you exposed to the smoke? ☐1 仅在家中 ☐2 仅在工作单位 ☐3 在家和在工作单位均暴露

H 酒精

H1 在您怀孕前三个月至今，您是否饮用过任何含有酒精的饮料？ ☐1 是 ☐0 否 During the 3 months before or during your pregnancy, did you ever drink any alcoholic beverages?
H2 在这三个月内，您通常每次饮几杯酒？ _____ 杯 On those days that you drank, how many drinks did you have?

I 环境暴露情况

I1 怀孕前三个月到现走，您是否染烫发? ☐1 是 ☐0 否 Did you dye perm in the first three months of pregnancy?
I2 怀孕前三个月到现在，后您工作的地点或家里是否装修过? ☐ 1 是 ☐0 否 Did you exposed to formaldehyde in the first three months of pregnancy?
I3 怀孕前后您是否接触过下列物质？ Have you been exposed to the following substances before and after pregnancy? (Toxic chemicals) ☐1 除草剂 ☐2 杀虫剂 ☐3 灭鼠剂 ☐4 有机溶剂 ☐5 消毒剂 ☐6 金属制剂 ☐7 有害气体 ☐8 有害固体

J 药物使用情况

以下问题是有关于您在末次月经之前三个月内至今期间的用药情况

	治疗高血压药物 Medication for hypertension	胰岛素 Insulin for diabetes	口服降血糖药物 Oral hypoglycemic for diabetes	抗癫痫药物 Medications for epilepsy	每天都要服用的药物 Medications at least once a day
1 您是否使用过？ Did you take?	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否

2 您使用的药物名称？ What did you take?	_____	_____	_____	_____	_____

如果下一个问题令您感到不安且不愿意回答，请在"拒答"上打√，我们将对您所有回答进行严格保密!

	安定\有助于您放松药物 Valium\drugs to help you relax	使您感觉良好\精力旺盛 Make you feel good\have more energy	美沙酮\氧可酮\其他止痛药 Methadone oxymoron\other pain killers	可卡因 Cocaine or crack cocaine	海洛因 Heroin	大麻 Marijuana
1 您是否使用过？ Did you take?	☐1 是 ☐0 否 ☐2 拒答 ☐9 不清楚	☐1 是 ☐0 否 ☐2 拒答 ☐9 不清楚	☐1 是 ☐0 否 ☐2 拒答 ☐9 不清楚	☐1 是 ☐0 否 ☐2 拒答 ☐99 不清楚	☐1 是 ☐0 否 ☐2 拒答 ☐9 不清楚	☐1 是 ☐0 否 ☐2 拒答 ☐9 不清楚
2 您使用的药物名称？ What did you take?	_____	_____	_____	_____	_____	_____

I 您在怀孕期间是否患过以下疾病？

I.3 发热性疾病及呼吸道感染 ☐1 是 ☐0 否

Febrile illness and respiratory infections

I.3.1 您发烧时的最高温度是多少？ _____℃

What was the highest temperature of your fever during your illness?

I.3.2 您发烧有几天？ _____天

How long did you have a fever?

I.6 其他_____

调查结束，谢谢您的配合！

调查员姓名 _____

调查日期 ___ ___/ ___ ___/ ___ ___

年 月 日

Types of fetus defects and birth defects

Diagnosis
Anencephalus
Spina bifida
Encephalocele
Congenital Hydrocephalus
Cleft Palate
Cleft Lip
Cleft Lip with Cleft Palate
Microtia (including Anotia)
Deformity of external ear(s) (except Microtia and Anotia)
Esophageal atresia or stenosis
Anorectal atresia (including Congenital Anorectal Malformations)
Hypospadia
Ectopocystis
Pes Equinovarus
Polydactylism
Syndactylia
Limb shortening
Congenital Diaphragmatic Hernia
Pcromphalus
Celoschisis
Conjoined Twins
Trisomy 21 syndrome
Congenital heart disease
Others

Note: Down's syndrome, neural tube defects, congenital heart defects, hydrocephalus, digestive tract malformations and urinary malformations are most common defects in China. Defects were detected by

prenatal Down's syndrome screening, NT examination and Ultrasound image examination during the second trimester; and the number and type of birth defects after childbirth are diagnosed by professional clinical team. Down syndrome (DS), caused by the trisomy, translocation, or partial trisomy of chromosome 21, is the most common genetic cause of intellectual disability. DS is diagnosed by neonatologist. Neural tube defects including spina bifida and hypospadias, were diagnosed by ultrasound examination and pediatric neurosurgeon. Congenital heart disease was diagnosis by Doppler ultrasound and CHD screening (pulse oximetry plus cardiac murmurs). Hydrocephalus, digestive tract malformations, urinary malformations and other defects also were also diagnosed either by ultrasound or some other specific diagnosis methods.

Future Plan of Shanghai PreConception Cohort, Shanghai, China (SPCC)

The Shanghai PreConception Cohort (SPCC) is a long-term cohort and meets the "Cohort profile" requirement. We have a complete plan to follow-up offspring to the age of 18 years old. The cohort will be financially supported by different grants. The current manuscript focuses on the first phase, the establishment of the baseline and our first main outcome, the congenital heart disease (CHD).

The second phase of this cohort study is from birth to 6 years old. Follow-up work will rely on Shanghai Community health care centers-child care. The third phase is from 6 to the age of 18 (school age). We will cooperate with Shanghai Student Health and Fitness Surveillance Center for the follow-up of the school age children.

Multiple outcomes for pediatrics, including growth, physical and neuro-developments, obesity, common and rare diseases will be investigated. Nested case-control design will be used for rare outcomes.

The data collection plan for infants and children

	At birth	6 weeks	6 months	12 months	36 months	60 months	6 – 18 years*
General information (gender, ID number, Home Address, etc)	√	√					
Delivery outcome (Congenital malformation, preterm birth, miscarriage, stillbirth)	√						
Body measurement(weight, height, waistline, hipline)	√	√	√	√	√	√	√
Diet investigation (questionnaire)	√	√	√	√	√	√	√
Neurobehavioral developmental assessment (DDST[$])		√	√	√	√	√	
Anthropometrics data (Shanghai Community health care centers-child care and Shanghai Student Health and Fitness Surveillance Center) weight, height, waist circumference	√	√	√	√	√	√	√
Physical fitness measurement (running, jumping, solid balls, etc)							√
Blood pressure measurement, annually							√
Hemachrome (anemia)							√
Renal functions, at grade 9 and 12							√
Cardiovascular-related chronic diseases (Hypertension, Obesity, Diabetes/Pre-diabetes and Dyslipidemia)							√
Venous blood[#]							√

Note: * Shanghai Student Health and Fitness Surveillance Center routinely conducts all-round physical examinations for students in the school as a health care policy for Shanghai students. It is usually in September-November of each year.

$ DDST: Denver developmental screening test.

Venous blood will be collected at 12, 15 and 18 years of age.

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Cohort profile: Shanghai PreConception Cohort (SPCC) for association of periconceptional parental key nutrition factors with health outcomes of children: I -congenital heart disease

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Cohort profile: Shanghai PreConception Cohort (SPCC) for association of periconceptional parental key nutrition factors with health outcomes of children: I -congenital heart disease

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ABSTRACT (293)

Purpose: The Shanghai PreConception Cohort (SPCC) was established initially to investigate associations of parental peri-conception nutritional factors with congenital heart disease (CHD), and has extended to children growth, development and pediatric diseases.

Participants Prepare-for-pregnant couples who presented at Shanghai preconception examination clinics and early-pregnant women before 14 gestational weeks were enrolled to comprise a peri-conception baseline study population. General characteristics, routine clinical data, consumption of diet supplements, such as folic acid and multivitamins, were collected. Blood samples were collected at preconception, early, middle and late gestation respectively by standard procedures. Multiple nutrition factors in blood sample of participants that were selected by case-control design will be examined, including folates, homocysteine, vitamin A, vitamin D, vitamin E and metals. Genomic DNA was extracted.

Findings to date The baseline population included 8045 preconception couples, 3054 single women, and 15 615 early-pregnant women, respectively. Birth data from 12 402 births were collected and follow-up of the cohort for more outcomes is ongoing. Currently, 151 CHDs were identified after birth. Pilot analysis in a small subgroup showed that only about 15% of 656 preconceptional women and 49% of early-pregnant women had red blood cell folate concentration meeting the international recommendation for preventing neural tube defects.

Future plans

Once a sufficient number of CHD cases is achieved, we will investigate quantitative association of preconceptional red blood cell folate levels with CHD using nested case-control design. The SPCC cohort will be followed up for 18 years to investigate extensive outcomes of growth, development, obesity, and common and rare diseases during childhood and adolescence according to our plan. Blood nutrition factors will be examined in participants selected for specific aims. The SPCC cohort will also allow for prospective cohort studies on extensive research questions.

Trial registration number: NCT 02737644.

Key Messages: red blood cell folate, vitamin, congenital heart diseases,

peri-conception health care

Strengths and limitations of this study

- The SPCC cohort is the first prospective birth cohort with CHD as primary outcome with recruitment starting from preconception stage. Temporal sequence of exposures and outcomes can be achieved for causal inference of birth defects and other diseases that occur during early stage of gestation.
- Preconception blood samples were appropriately collected and stored which allow examination of individual blood levels for nutrition factors and other exposures.
- Clinical data and blood samples from both father and mother from before conception were collected, which will allow for testing the effect of both maternal and paternal genetic and nutrition factors on fetal and children diseases.
- Although response rate was high (over 95%), pre-conception participants were recruited from the population who voluntarily presented at Shanghai city preconception physical examination sites. They may have a stronger willingness for a healthy pregnancy, which may induce selection bias.
- Biological samples (cord blood, placenta) of the newborns were not collected.

Introduction

Congenital heart disease (CHD) is a common congenital malformation, which seriously affects quality of children's life [1]. CHD is a leading cause of infant death in high-income countries affecting eight of 1000 live births [2]. According to the report from National Health and Family Planning Commission of the People's Republic of China, CHD accounts for about a quarter of the birth defects of newborns in China, ranking the first among birth defects [3]. In a prospective, nation-wide large-scale study in more than 120 000 newborns in China in 2013, the prevalence of CHD in live births was identified 8.94 ‰; the rate of severe CHD was 2.9 ‰ [4].

The cause of CHD is multifactorial. With the development of genetic engineering technology, the genetic factors have been better understood in the past decade [5]. Multiple environmental risk factors have been reported in epidemiological studies, the maternal social variables such as occupation, educational background, health status, unhealthy life style, maternal medical history and emotional status, family history of disease, consanguineous marriages and so on [6-10]. In addition, maternal key nutrients related to the risk of offspring's CHD as a modifiable environmental factor during peri-conception [11, 12]. The peri-conception intake of folic acid supplement has been shown to reduce the risk of CHD

[13, 14] and women worldwide have been recommended to take folic acid supplements before conception and in the beginning of pregnancy. Awareness of the relationship between folic acid deficiency and CHD is actually a by-product finding from the well-known Hungarian RCT study of folic acid supplementation to prevent neural tube defects. The study found that prenatal supplementation with a vitamin complex containing 0.8 mg of folic acid daily reduced the incidence of congenital neural tube defects. At the same time, the incidence of various heart defects have also been reduced by nearly half [15]. Longitudinal data from more than one million births in Canada over a total of 22 years from 1990 to 2011 also show that food fortification with folic acid reduced risk of CHD by 20-30% [16]. The current folic acid supplementation recommends that all women of childbearing potential be supplemented with at least 0.4 mg folic acid daily prior to conception and during pregnancy, which is designed for preventing neural tube defects [17]. However, excessive folic acid intake may increase the risk of cancer [18], vitamin B12 deficiency [19], and autism spectrum disorder [20]. The optimal dose of folic acid for preventing CHD warrants further investigation. In addition, most previous studies only focused on the supplement of folic acid or the serum folate level during or after pregnancy, which may not be the optimal period and way to reflect the exposure level to risk of CHD.

To investigate the association between parental peri-conception key nutritional factors such as folate with the development of CHD and to explore the cutoff biomarker levels, we conducted Shanghai PreConception Cohort (SPCC) and a nested case-control analysis.

The SPCC cohort was initiated primarily to study CHD. However, based on the strengths of its baseline data collection, it has received attention and support, with improved additional extensive outcomes for children that will be followed up longer term.

Who is in the cohort?

The SPCC cohort recruited parent-planning women and men who were permanent residents and who voluntarily presented at preconception clinical clinics at 28 maternity institutions in 10 districts of Shanghai (Minhang District, Huangpu District, Xuhui District, Changning District, Jing'an District, Putuo District, Yangpu District, Pudong District，Songjiang District, Qingpu District) from March 2016 to December 2018. The preconception examination policy in the city of Shanghai provides a unique opportunity and clinical resources to support recruitment of SPCC. Since 2010, married couples in Shanghai have been encouraged to attend a free preconception health examination. In addition, these maternity institutions receive strong local administrative support and integrated maternal health care networking, providing service to 150 000–200 000

annual deliveries in Shanghai. Couples who were present at preconception clinics, living in Shanghai, preparing for pregnancy within one year, and planning to receive antenatal care and to delivering in Shanghai, were eligible for the study. Written informed consents were obtained from all participants before any data collection. In addition, we recruited early-pregnant women at their first antenatal examination who were at gestational week <14 weeks. These two groups of participants comprised a peri-conception baseline study population.

The first primary outcome of the SPCC cohort is CHD. The hypothesis is that maternal preconception serum or red blood cell (RBC) folate concentration is quantitatively associated with offspring CHD. The study design and protocol has been registered with Clinical Trials Registry (NCT 02737644).

As shown in Figure 1, the baseline population will be followed up to delivery, and their babies will be followed up until 18 years old (Figure 1).

Follow-up procedure

At enrollment, the participants completed the questionnaire of key nutrient supplementation and blood sample collection. When participants got pregnant, the same investigations (questionnaire/blood sample collection) were conducted during early pregnancy (first antenatal visit at 16-20 gestational weeks). Pregnancies were followed up along with routine

maternal health care procedures. Blood samples were also collected at the second (24-28 gestational weeks) and third trimester (32-34 gestational week). The follow-up of CHD outcome and birth data was obtained through Shanghai Neonatal CHD Screen Platform (Figure 1).

As shown in Figure 1, outcomes at birth, during infant to childhood (preschool phase), and between 7 to 18 years (school ages) will be collected or extracted from multiple public platforms and data sources. Firstly, preconception clinical visit data from Preconception Care Electronic Data System supported by national and local government, including height, weight, age, infections, sexually transmitted disease, and family history were collected. Secondly, the routine pregnant data were obtained in Maternal Clinic Antenatal Medical Record System, managed by Shanghai Center for Women and Children's Health, including height, gestational weight, last menstrual period, childbearing history, delivery outcomes, infections, hematocrit, coagulation function, liver and kidney function, and so on. Thirdly, the maternal and neonatal data at delivery came from Shanghai Neonatal CHD Screen Platform including birth weight, CHD diagnosis, birth defects, and Apgar score, etc. In addition, we will work with the Shanghai Student Health and Fitness Surveillance Center to obtain outcome data. The personal national identification card number of participants are applied as index variables through the multiple

data sources. The detailed variable list and codebook of data collection is presented in Appendix 1.

During the first phase of the cohort, from preconception to delivery, comprehensive strategies were used to retain participants in the study. For mothers, we provided a variety of engagement activities including green channel (fast track) to their antenatal care to provide convenience and save their time in hospitals. We also provided a contact number on the participant card to answer their calls or queries about the study procedures. Site investigators at early pregnant clinics in collaborative hospitals were provided a smartphone APP to help identify recruited cohort participants timely and manage data and blood sample collection procedures. We also provided green channel echocardiography for diagnosing CHD for all site hospitals to enhance the compliancy of the participants. In addition, an automated text message system is adopted to remind participants of schedules and appointment of follow-up.

Study measures

Personal characteristics questionnaires

As shown in Figure 1, Questionnaire 1 was administered during recruitment at preconception examination sites and Questionnaire 2 was administered at early pregnancy sites to collect information on consumption of folic acid supplement, vitamin supplement, the brand and

content of nutrient supplement. Information of demographics, maternal education, socio-demographic status, occupation, smoking status, alcohol consumption, BMI, medication, and health status were also included. In addition to the content of Questionnaire 1, Questionnaire 2 added drug information, reproductive history, and health status. Questionnaire 1 for baseline and Questionnaire 2 for the first antenatal visit at early pregnancy are presented in Appendix 2a and Appendix 2b.

Collection of blood samples

In this study, the rest blood samples for routine clinical blood test were collected. The blood sample for routine clinical examination was usually 5 ml and extracted in the morning. Routine clinical blood test was performed at room temperature. The rest blood samples (fasting serum and EDTA anticoagulation) of peripheral venous blood from routine laboratory clinical blood test were kept. These blood samples were temporarily stored in a 4℃ refrigerator for dispensing within 6 hours and transferred to a -20 4℃. After completion of blood sample distribution the serum and the whole blood were stored at the site laboratory, and then transported by three trained investigators to the central laboratory for storage in -80 ℃ freezers within two weeks. Sampling tubes were made of a light-proof material and the process of collecting blood samples were completely protected from

light.

Examination of key nutrition factors in blood samples

The examinations will be conducted in participants selected by nested case-control designs based on specific aims.

(1) *RBC folate, serum folate, serum homocysteine, vitamin D, vitamin B12 and serum ferritin*

EDTA anticoagulation blood was collected to measure RBC folate, serum folate, serum homocysteine, vitamin D, vitamin B12 and serum ferritin assays. All six biomarkers were analyzed on an electrochemiluminescence assay (ARCHITECT i2000SR Analyzer, Abbott Laboratories, USA). A standard solution with known concentration (produced by Abbott Laboratories) was used daily to quality control before the measurement. If the quality control level was out of concentration range, the measurement would be suspended and adjusted. External quality control was carried out with the control lab data program from Abbott Laboratories (Abbott Laboratories, Shanghai, China). RBC folate concentrations were adjusted for hematocrit. If the RBC folate concentration is below 126.0 ng/ml or above 651.1 ng/ml, adjustment was needed based on serum folate level. The hematocrit data were extracted from the hospital laboratory information system. Those examinations were performed in central laboratory of Children's Hospital of Fudan University.

(2) Vitamin A and vitamin E

The serum concentration of vitamin A and vitamin E were quantitatively detected by liquid chromatography-tandem mass spectrometry in central laboratory of Children's Hospital of Fudan University. The testing instrument was triple quadrupole mass spectrometer LC/MS/MS System (NO: API 3200MDTM, ABSciex Pte.Ltd.). A standard solution of vitamin A-d6 and vitamin E-d6 were applied as internal standards.

(3) *Glycemic and lipid profiles*

Fasting serum cholesterol, high-density lipoprotein, low-density lipoprotein, triglyceride, and fasting glucose were performed on Beckman coulter AU chemistry analyzers (Beckman, USA) in central laboratory of Children's Hospital of Fudan University.

(4) Metals

Serum levels including Mg, Fe, Zn, Se, Mn, As, Cu and Ca, were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) (Inductively Coupled Plasma Optical iCAP6300, Themo®, USA) in standard mode [21]. The metals examination was conducted in Instrumental Analysis Center of Shanghai Jiaotong University which is a national key laboratory.

(5) Genomic DNA extraction

Genomic DNA of all participants were extracted using a magnetic bead-based kit (TGuide M16 Automatic Nucleic Acid Extractor

(OSE-M16), TIANGEN BIOTECH (BEIJING) CO. LTD, China) from 2 ml of EDTA anticoagulated whole blood sample after routine blood test. Genomic DNA samples were stored for future study. An average 150 ng DNA were available. Similar to blood chemicals, future genetic variants genotyping will be performed in selected participants according to nested case-control design for specific aims. Currently, there are no candidate genes or variants that are listed.

Outcomes -CHD in neonates

The diagnosis of CHD was the primary outcome of the study at this stage and obtained from Shanghai Neonatal CHD Screen Platform, which was initiated as routine screen for newborns in Shanghai since Jun 1st 2016. The standard protocol of CHD screening of the platform was previously described in detail [22]. All newborn babies received the screen by using double-index method (i.e. cardiac murmur auscultation and pulse oximetry) during 6-72 hours after delivery, and those screen-positive babies would receive a subsequent echocardiography for further confirmative diagnosis.

SPCC will also collect other birth defects as secondary outcomes, including Down's syndrome, neural tube defects, hydrocephalus, digestive tract malformations, urinary malformations, and behavioral cognitive developmental disorder. After delivery, the infants attended routine childcare procedures organized by Shanghai Child Health Care System,

which is administered by Shanghai Center for Women and Children's Health. All birth defects records, which were diagnosed after birth, as well as routine neuro-development examinations and longitudinal anthropometric data, were abstracted from the system by a professional clinical team from Children's Hospital of Fudan University (for details of the types of birth defect please see Appendix 3)

Statistical methods

To investigate the association of maternal preconception nutrition levels with offspring CHD risk, a nested case-control study will be conducted. The control will be matched by age and site.

The sample size for the nested case-control analysis was planned as 180 CHD cases and 720 matched controls to detect a maternal folate deficiency with prevalence of 50% in controls with odds ratio of 1.6 in association to achieve a power of 80% at alpha of 0.05. Based on CHD incidence above 8.94 per 1 000 live births [4], 20 000 pregnancies will be needed. For a continuous nutrient variable with standard deviation 2.0, 50 matched-pairs (1:4) are required to achieve 90% power to detect an odds ratio of 1.3 calculated using conditional logistic regression with a 0.05 significance level [23, 24]. Once a sufficient number of CHD cases is achieved, the quantitative association of preconception RBC folate levels with CHD using nested case-control design will be investigated.

Conditional multivariate logistic regression will be used for association analysis with offspring affected status of CHD being the dependent variable, nutrition factors levels as exposure and adjusted for all potential paternal and maternal covariates. Odds ratios (OR) and 95% confidence intervals (95%CI) will be reported. To explore a potential cutoff point of the nutrition levels that significantly increases the risk of CHD, a dummy variable will be set up by categorizing the maternal preconception nutrition levels based on the distribution of the control group. The does-response relationship will be also be analyzed. Sensitive analysis will include non-conditional logistic regression analysis, or generalized estimation equations (GEE) model, or generalized linear models when necessary.

FINDINGS TO DATE

The SPCC started recruitment in March 2016. As shown in Figure 2, by December 2018, we consecutively recruited 19 144/19 563 (97.9%) participants at preconception settings, including 8045 couples and 3054 single women, and an additional 15 615/16 201 (96.4%) pregnant women at maternity hospitals with gestational age <14 weeks. Table 1 describes the basic demographic characteristics of the preparing-for-pregnant participants and pregnant women, respectively. The average age of the preconception population was 29.9 (SD 3.9) years for females and 31.4 (SD 4.5) years for male, one-third of males and 2.4% of females were

smokers, and two-thirds of males, and one-third of females had a habit of drinking alcohol. In pregnant women, the average age was 29.9 (SD 4.0) years, with half of them having a first pregnancy. Compared with the preconception females, they were similar in age but different in education levels and occupation, the prevalence of smoking and alcohol drinking were much lower. (The descriptive data of Table 1 was partly included in another manuscript, which is under review of Maternal & Child Nutrition with Manuscript number of MCN-08-19-OA-4056).

By the end of December 2018, the last participants recruited at early pregnancy were due for delivery, however, we have achieved birth records of 12 402 newborns. The follow-up of outcomes of the rest of the participants is ongoing (shown in Figure 2). A total of 151 cases of CHD were identified through the CHD screening platform, 131 cases from the early pregnancy sample, and the remaining 20 cases were from the preconception sample. The prevalence of CHD in live births is 10.5 ‰ (131/12 402) based on the present available data.

We conducted a small pilot study in April 2017 to explore blood levels of nutrition factors, including serum folate, RBC folate, vitamin A, vitamin E, and vitamin D. The blood samples from 627 females were selected consecutively from the preconception sample according to who was identified pregnant. In addition, 597 women who were consecutively

recruited from the antenatal care clinics were selected. As shown in Table 2, the concentrations of RBC folate median level is 247.0 ng/ml (IQR: 184.8-340.5 ng/ml) in preconception women and 417.4 ng/ml (IQR: 308.6-544.2 ng/ml) in early-pregnant women. Twenty percent of preconceptional participants and 44.9% of pregnant participants had a folate level over 400 ng/ml, which was suggested as optimal level for preventing neural tube development defects [25, 26]. These results suggest that effort is urgently needed to improve the intake of folic acid supplementation in the prepare-for-pregnancy population, especially before pregnancy.

Based on SPCC, the possible scope of research questions, available types and number of bio-samples and biomarkers that can be examined is shown in Table 3.

FUTURE PLANS

We have a complete plan to follow-up offspring to the age of 18 years old. The cohort will be financially supported by different grants. The current manuscript focuses on the first phase, the establishment of the baseline and our first main outcome, the CHD. The data collection plan for infants and children (from birth to 6 years old, pre-school stage), as well as school age (from 6 to 18 years old), are included. During the stage of 0 to 6 years old, the data of neurodevelopment will be collected from routine childcare

clinical visit at birth, 6 weeks, 6 months, 12 months, 36 months, and 60 months through the cooperating medical institutions. Physical measurements data and dietary intake information also can be collected at this stage. During the stage of 6 to 18 years old, we plan to follow up their growth (height, weight, blood pressure, et al.) mainly relying on the annual physical examination of Shanghai Student Health and Fitness Surveillance Center System. Multiple outcomes for children, including growth and development, cardiovascular diseases, neurodevelopment, metabolic diseases, obesity, and hypertension will be investigated. Please see Appendix 4 for details.

STRENGTHS AND LIMITATIONS

Compared with existing birth cohorts, there are three important strengths in our cohort. Firstly, the SPCC cohort is the first prospective birth cohort with CHD as primary outcome and recruitment starting from preconception. Blood samples were collected and stored which allows for direct measurement of individual exposure levels before the development of CHD and make causal inference. Temporal sequence of exposures and outcomes can be achieved for causal inference of birth defects and other diseases that occur during early stage of gestation. Up to date, no published studies have measured maternal blood folate levels before conception and link it to disease outcomes. Secondly, this cohort also allows for the investigation of

associations between peri-conception maternal and paternal nutrition exposures with other birth defects, early onset-diseases, and neuro-development outcomes. Preconception blood samples were appropriately collected and stored which allows for the examination of individual blood levels of nutrition factors and other exposures. Thirdly, both paternal and maternal clinical data and blood samples before conception were collected, which will allow for testing effect of both maternal and paternal genetic and nutrition factors to fetal and children diseases.

Two limitations of this cohort study should be considered. Firstly, there are approximately 200 000 pregnant women giving birth every year in Shanghai, and approximately 20 000 of them will take part in the free preconception care in Shanghai, where participants were recruited consecutively. Although response rate was high (over 95%), preconception participants were recruited from a population voluntarily present in Shanghai city with preconception physical examination sites, who may have a stronger willingness for a healthy pregnancy. This may induce selection bias. Secondly, in this study, biological samples (cord blood, placenta) of the newborns are not collected. We plan to give new informed consent to the family who are willing to participate in future studies, to collect biological samples not mentioned before. In addition,

electrochemiluminescence assay was used to examine serum and RBC folate concentrations, which is different from microbiologic assay that is used widely. This will not bias the association analysis but comparison with international populations needs caution.

Collaboration

Investigators with an interest in hypotheses related to SPCC (and that meet the requirements of current approvals) are welcome to contact Dr. Guoying Huang or Weili Yan. A 'Research Collaboration application' should be send to the corresponding author by Email. The application should include a brief description of the project.

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Contributors: Substantial contributions to the conception or design of the work were made by GH and WY. YZ and DW prepared the original draft of the manuscript. YZ, DW, YY, JY, ML, MJ, YD and XC led study implementation at participating sites. DW and YZ were responsible for the day-to-day project management at each site. XM and WS were responsible for the biobank of the cohort. All authors provided critical review of the manuscript for important intellectual content and approved the final version.

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Table 1. Socio-demographics of participants including 6,573 couples (parents) and 15,203 pregnant women that were enrolled in the Shanghai PreConception Cohort (SPCC).

Characteristics	Couples (parents) who completed preconception questionnaires				Additional pregnant women who completed both preconception and first-trimester questionnaires		P^*
	Male (n=6573)		Female (n=9243)		Pregnant women （n=15203）		
Age (years), Mean±SD	6573	31.4±4.5	9243	29.9±3.9	15203	29.9±4.0	0.995
Ethnicity	6536		9188		15176		0.258
Han Nationality		6403 (98%)		8966 (97.6%)		15245 (97.8%)	
Other		133 (2%)		222 (2.4%)		342 (2.2%)	
Educational level	6530		9147		15143		<0.001
<College		514 (7.8%)		795 (8.7%)		2052 (13.6%)	
>=College		6016 (92.2%)		8352 (91.3%)		13091 (86.4%)	
Annual household income	6530		9147		NA		
< ￥10 000		1424 (21.8%)		2214 (24.2%)			
>=￥10 000		5106(78.2%)		6933 (75.8%)			
Occupation	6530		9147		14789		<0.001
Entrepreneur		157 (2.4%)		128 (1.4%)		171 (1.1%)	
Farmer		26 (0.4%)		46 (0.5%)		79 (0.5%)	
Self-employed		216 (3.3%)		274 (3%)		862 (5.6%)	
Manager		1410 (21.6%)		1765 (19.3%)		2650 (17.2%)	
Technician		2462 (37.7%)		1903 (20.8%)		3239 (20.6%)	

Company clerk		2259 (34.6%)		5031 (55%)		8451 (54.7%)	
Attending preconception pregnant examination	NA		NA		14996		–
Yes						3374 (22.5%)	
No						11622 (77.5%)	
Times of pregnancy	NA		NA		15162		
1						7569 (49.9%)	
2						4604 (30.4%)	
>=3						2989 (19.7%)	
Miscarriage or stillbirth	NA		NA		15159		
Yes						4838 (31.2%)	
No						10385 (68.7%)	
Smoking	6552		9212		15159		<0.001
Yes		2073 (31.6%)		218 (2.4%)		153 (1.0%)	
No		4479 (68.4%)		8994 (97.6%)		15006 (99%)	
Alcohol drinking	6448		9075		15164		<0.001
Yes		3962 (61.5%)		2865 (31.6%)		1556 (10.3%)	
No		2486 (38.5%)		6210 (68.4%)		13608 (89.7%)	
Location of home	6573		9243		NA		
Developed districts		3034 (46.2%)		5452 (59.0%)			
Developing districts		3539 (53.8%)		3791 (41.0%)			

* Comparisons between preconception females and pregnant women. *t* tests were used to compare numerical variable (age). Chi-square tests were used to compare categorical variables (ethnicity, educational level occupation, smoking and alcohol drinking).

Table 2. Distributions of biomarkers in women before and at early gestation (pilot study)

Biomarker	Preconception		Early pregnancy	
	n	level	n	level
Serum folate, Median (IQR), ng/ mL	620	9.7 (6.5, 13.8)	577	14.5 (11.2, 16.4)
RBC folate, Median (IQR), ng/ mL	570	247.0 (184.8, 340.5)	587	417.4 (308.6, 544.2)
Homocysteine, Median (IQR), µmol/L	624	6.5 (5.2, 8.6)	599	4.2 (3.5, 5.2)
Vitamin B12, Median (IQR), pg/mL	625	495.2 (394.2, 639.0)	600	388.5 (289.4, 511.4)
Vitamin D, Mean ±SD, ng/ mL	607	16.3 ±6.0	578	15.5 ±6.1

Table 3. Bio-samples collected and biomarkers that can be examined in the SPCC cohort

Bio-samples Available in participants	Available sample type and volume	Time		
		Preconception+early pregnancy (Baseline)	24-28 weeks	32-36 weeks
Mother		(n=25487)	(n=8668)	(n=7522)
	Serum, 200 ul*3	Yes	Yes	Yes
	Whole blood	Yes	Yes	Yes
	Genomic DNA, 150 ng	Yes	Yes	Yes
Father		(n= 7151)	-	-
	Serum, 200 ul*3	Yes	NA	NA
	Whole blood	Yes	NA	NA
	Genomic DNA, 150 ng	Yes	NA	NA
Child	NA			

Scope of research questions:

1. Quantitative association of preconceptional key nutrition levels (such as serum and RBC folates and related biomarkers, such as homocysteine and vitamin B12) with CHD, currently, and other folate sensitive birth defects.
2. Quantitative association of peri-conceptional maternal and paternal key nutrition levels (preconception levels, and dynamic levels during gestation) with important maternal and neonatal gestational complications, neuro-development of infants, childhood obesity, and clinical pediatric diseases.
3. Peri-concpetional maternal and paternal folate concentration with Autism spectrum disorder, allergy and asthma in children.

Biomarkers that will be examined in different types of bio-samples:

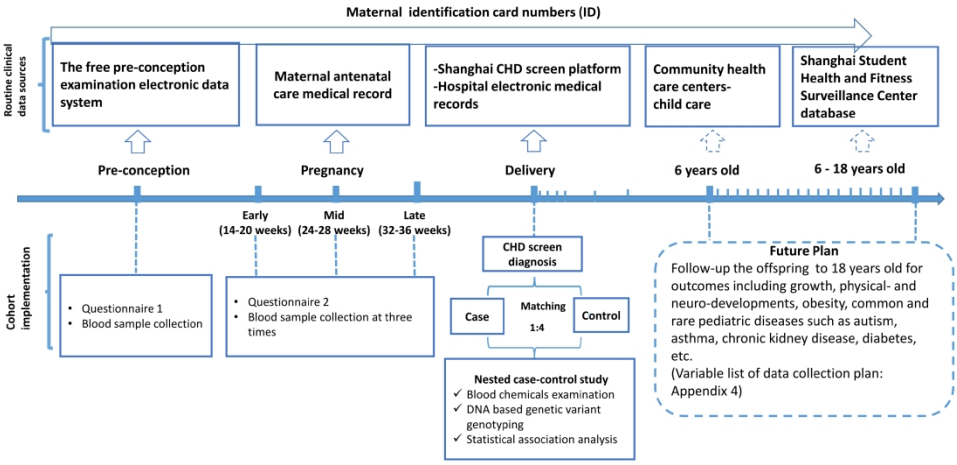
1. Biomarkers based on serum sample:
a) Folate and related markers: serum folate, homocysteine
b) Other Vitamins: vitamin A, vitamin B12, vitamin E, and vitamin D
c) Marco and micro metals: Mg, Fe, Zn, Se, Mn, As, Cu, Ca, etc., mg/L
d) Serum ferritin
e) Fasting glycemic and lipid profiles: fasting glucose, total cholesterol, triglyceride, high-density lipoprotein, low-density lipoprotein
2. Whole blood sample: RBC folate
3. Genomic DNA sample: candidate genetic variants or genome-wide variants are all possible to examined

Figure 1 Diagram of the protocol and follow-up of Shanghai PreConceptional Cohort (SPCC)

The baseline population of SPCC cohort was females and males at peri-conception stage (couples who are prepare-for-pregnancy) and pregnant women at early gestation stage. The cohort includes three phases, from peri-conception to birth (Phase I, peri-natal phase), from birth to 6 years old (Phase II, infant and pre-school age), and from 7 to 18 years (Phase III, school age). The current manuscript focuses on the first phase, with congenital heart disease as the primary outcome, and will cover other folate sensitive birth defects.

Figure 2 Flow chart diagram

By December 2018, we consecutively recruited 19 144/19 563 (97.9%) participants at preconception settings, including 8045 couples and 3054 single women at pre-pregnancy sites, and an additional 15 615/16 201 (96.4%) pregnant women at maternity hospitals with gestational age <14 weeks were recruited. By the end of December 2018, the last participants recruited at early pregnancy were due for delivery, we have achieved birth records of 12 402 newborns in Maternal Clinic Antenatal Medical Record System. The follow-up of outcomes of the rest of participants is ongoing (shown in Figure 2). A total of 151 cases of CHD were identified through Shanghai Neonatal CHD Screen Platform, 131 cases were from the early pregnancy sample, the remaining 20 cases were from the preconception sample. The prevalence of CHD in liver births is 10.5 ‰ (131/12 402) based on the present available data. In the preconception sample, from March 2016 to May 2018 (the latest data extraction in Maternal Clinic Antenatal Medical Record System), the number of pregnancies and delivers were 1538 and 975 respectively.



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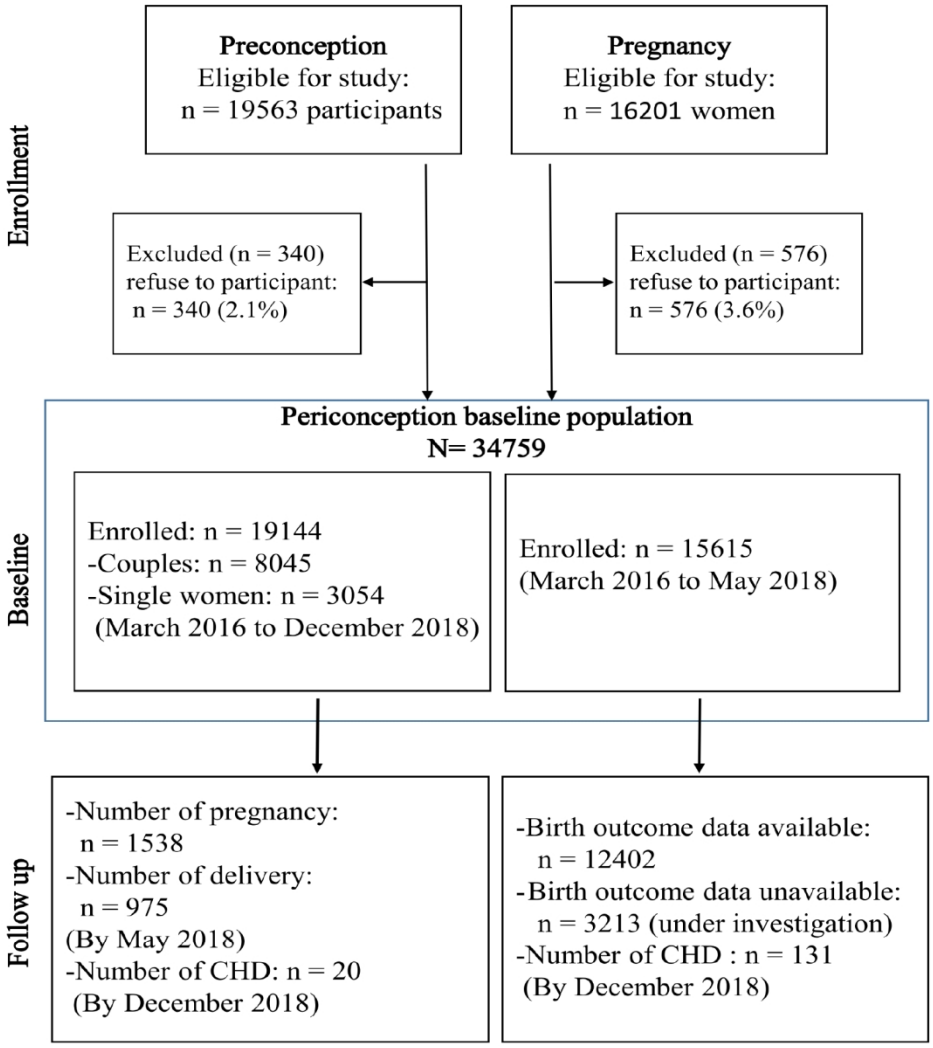

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Appendix 1 Variable list

Pre-pregnant variable list

No	Variables	Data type
	General information	
1	Wife id	Text
2	Husband nation	Text
3	Husband age	N
4	Husband education	Text
5	Husband id	Text
6	Husband occupation	Text
7	Wife nation	Text
8	Wife age	N
9	Wife education	Text
10	Wife occupation	Text
11	Tel No	Text
12	Mobile phone No	Text
	Medical history	
13	Female anemia	Category
14	Female EH	Category
15	Female heart disease	Category
16	Female DM	Category
17	Female epilepsia	Category
18	Female thyroid disease	Category
19	Female CGN	Category
20	Female mental disease	Category
21	Female tumour	Category
22	Female TB	Category
23	Female HBV	Category
24	Female VD	Category
25	Male anemia	Category
26	Male EH	Category
27	Male heart disease	Category
28	Male DM	Category
29	Male epilepsia	Category
30	Male thyroid disease	Category
31	Male CGN	Category
32	Male mental disease	Category
33	Male tumour	Category
34	Male TB	Category
35	Male HBV	Category
36	Male VD	Category

	Vaccine	
37	Female rubella vaccine	Category
38	Female hepB vaccine	Category
39	Male hepB vaccine	Category
	Drug use	
40	Female current medicine	Category
41	Female medicine name	Category
42	Male current medicine	Category
43	Male medicine name	Category
	Childbearing history	
44	Birth history	Category
45	Pregnancy times	Category
46	Live birth	Category
47	Dead fetus	Category
48	Dead birth	Category
49	Term delivery	Category
50	Premature delivery	Category
51	Natural abortion	Category
52	Abactio	Category
53	Children number	Category
54	Birth defect	Category
55	Defect type	Category
56	Menarche age	Category
57	Period menstruation	Category
58	Menstrual cycle	Category
59	Menstrual quantity	Category
60	LMP	Date
	Family history of disease	
61	Female family history thalassemia	Category
62	Female family history albinism	Category
63	Female family history favism	Category
64	Female family history hemophilia	Category
65	Female family history CHD	Category
66	Female family history DS	Category
67	Female family history openNTDs	Category
68	Female family history DM	Category
69	Female family history dysnoesia	Category
70	Female family history daysaudia	Category
71	Female family history viaual disorder	Category
72	Female family history neuropsychiatric	Category
73	Female family history other birth defects	Category
74	Female family history fetal death	Category
75	Female family history intermarry	Category

76	Female family history relations	Category
77	Male family history thalassemia	Category
78	Male family history albinism	Category
79	Male family history favism	Category
80	Male family history hemophilia	Category
81	Male family history CHD	Category
82	Male family history DS	Category
83	Male family history openNTDs	Category
84	Male family history DM	Category
85	Male family history dysnoesia	Category
86	Male family history dysaudia	Category
87	Male family history viaual disorder	Category
88	Male family history neuropsychiatric	Category
89	Male family history other birth defects	Category
90	Male family history fetal death	Category
91	Male family history intermarry	Category
92	Male family history relations	Category
	Anthroposomatology	
93	Female height	Numeric
94	Female weight	Numeric
95	Female BMI	Numeric
96	Female heart rate	Numeric
97	Female SBP	Numeric
98	Female SDP	Numeric
99	Male height	Numeric
100	Male weight	Numeric
101	Male BMI	Numeric
102	Male heart rate	Numeric
103	Male SBP	Numeric
104	Male SDP	Numeric
	Lab data	
105	Leucorrhea check	Numeric
106	Clue cell	Numeric
107	Monilia infection	Numeric
108	Trichomomas	Numeric
109	Cleanness	Numeric
110	Whiff test	Numeric
111	PH	Numeric
112	Wom blood analysis	Numeric
113	Female hb	Numeric
114	Female wbc	Numeric
115	Female rbc	Numeric
116	Wom urine test	Numeric

117	Female ABO	Numeric
118	Female Rh	Numeric
119	Female GLU	Numeric
120	Female GLU levels	Numeric
121	Female NG	Numeric
122	Female chlamydia	Numeric
123	Female syphilis	Numeric
124	Female HIV	Numeric
125	Female ALT	Numeric
126	Female ALT levels	Numeric
127	Female HBs-Ag	Numeric
128	Female HBs-Ab	Numeric
129	Female HBe-Ag	Numeric
130	Female HBe-Ab	Numeric
131	Female HBc-Ab	Numeric
132	Female HCV-Ab	Numeric
133	Female CMV IgM	Numeric
134	Female CMV IgG	Numeric
135	Female RV IgM	Numeric
136	Female RV IgG	Numeric
137	Female TOX IgM	Numeric
138	Female TOX IgG	Numeric
139	Male blood analysis	Numeric
140	Male hb	Numeric
141	Male wbc	Numeric
142	Male rbc	Numeric

During-pregnancy variable list

	Basic information	
1	Hospital card number	Text
2	Inpatient number	Text
3	Name	Text
4	Age	Numeric
5	Pregnant times	Numeric
6	Delivery times	Numeric
7	Last menstrual period	Date
8	Gestational week at the first visit	Numeric
9	The first visit date	Date
10	Height	Numeric
11	Weight	Numeric
12	Systolic blood pressure at the first visit	Numeric
13	Diastolic blood pressure at the first visit	Numeric

14	Occupation	Text
15	Education	Text
	Antenatal care record	
16	Weight at each antenatal care	Numeric
17	Systolic blood pressure at each antenatal care	Numeric
18	Diastolic blood pressure at each antenatal care	Numeric
19	Gestational week at each antenatal care	Numeric
20	Antenatal care date	Date
	Lab data	
21	Cytomegalovirus	Numeric
22	Cytomegalovirus examination date	Date
23	Rubella virus	Numeric
24	Rubella virus examination date	Date
25	Toxoplasmosis	Numeric
26	Toxoplasmosis examination date	Date
27	Syphilis screening	Numeric
28	Syphilis screening examination date	Date
29	Fasting blood-glucose	Numeric
30	Fasting blood-glucose examination date	Date
31	HCT(hematokrit)	Numeric
32	HCT(hematokrit) examination date	Date
33	Serum folate	Numeric
34	Serum folate examination date	Date
35	HCY(homocysteine)	Numeric
36	HCY(homocysteine) examination date	Date
37	OGTT 0 hours	Numeric
38	OGTT 1 hours	Numeric
39	OGTT 2 hours	Numeric
40	OGTT examination date	Date
41	Triglyceride	Numeric
42	Triglyceride examination date	Date
43	Total cholesterol	Numeric
44	Total cholesterol examination date	Date
45	Hemoglobin	Numeric
46	Hemoglobin examination date	Date
	Delivery date	
47	Gestational week at delivery	Numeric
48	Delivery mode	Text
49	Birth weight	Numeric
50	Birth weight(second baby)	Numeric
51	Systolic blood pressure at delivery	Numeric
52	Diastolic blood pressure at delivery	Numeric
53	Apgar scoring	Numeric

54	Delivery date	Date
55	Birth defect records	Text
56	Weight blood pressure at delivery	Numeric
Offspring variable list		
	0 - 6 years (6 weeks, 6 months, 12 months, 36 months, 60 months)	
1	Name	Text
2	ID number	Text
3	Weight	Numeric
4	Height/ Length	Numeric
5	Vision detection	Numeric
6	DDST: Denver developmental screening test	Numeric
	6 - 18 years (each year)	
7	Weight	Numeric
8	Height	Numeric
9	Diastolic blood pressure	Numeric
10	Systolic blood pressure	Numeric
11	Vision detection	Numeric
12	Vital capacity	Numeric
13	Physical fitness measurement (running, jumping, solid balls, etc)	Text
The clinical diagnosis during 0 – 18 years will be extracted from the routine medical system each year.		

姓名 Name：_____

身份证号 ID no：□□□□□□□□□□□□□□□□□□

医院代码 Hospital No：_____（到时打印到问卷上）

填表日期 Date：_____年_____月_____日

孕前膳食补充剂调查表

Pre-pregnancy nutrition supplement questionnaire

（男女共用）

A 一般情况

A1 联系电话（请您认真填写，以助于医生和您联系）Contact number： _____(手机) _____(固定电话) _____（Email）
A2 家庭住址 Address：_____区/县_____街道/小区_____门牌号码/村
A3 您的出生日期是 Birth date _____年_____月_____日
A4 民族 Nationality ☐1 汉族 ☐2 其他（请注明）_____
A5 您的最高学历 Education ☐1 初高中以下 ☐2 大专本科 ☐3 硕士研究生以上及以上
A6 您现在的主要职业 Occupation ☐1 管理人员/干部 ☐2 技术人员 ☐3.企业主 ☐4 工人 ☐5 农民 ☐6 个体户 ☐7 其它_____
A7 上一年您的家庭年收入是：Income of a year ☐1. <2 万元 ☐ 2. (2~3.9)万 ☐3.(5.9)万 ☐4.(6~9.9)万 ☐5.(10~14.9)万 ☐6.15 万及以上 ☐9.不详
A8 填表日期 Date _____年_____月_____日

B 营养补充剂使用情况

营养补充剂种类 Nutritional supplement types	**叶酸 Folic acid**	**复合维生素 multi-vitamins**	**单一维生素 Single vitamin**
您在近三个月是否服用 Have you taken it in the last three months?	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否
您服用的什么品牌的营养补充剂？（如果没有对应选项请在其他处写明） Brand name	☐ 1、创盈金斯利安多维片 ☐ 2、福施福胶囊营养素 ☐ 3、汤臣倍健 ☐ 4、安利纽崔莱铁 ☐ 5、其他_____	☐ 1、爱乐维 ☐ 2、汤臣倍健孕妇专用 ☐ 3、惠氏玛特纳 ☐ 4、21 金维他 ☐ 5、其他_____	☐ V_C ☐ V_E ☐ V_{B1} ☐ V_{B2} ☐ 其他_____
您这三个月的服用频率？（如果食用频率小于每天/周一次，请填写每周/月食用几次，并勾出周/月） How often did you take in	____次/☐天 ☐周 ☐月	____次/☐天☐周☐月	____次/☐天 ☐周 ☐月

营养补充剂种类 Nutritional supplement types	铁 Fe	钙 Ca	锌 Zn
您在近三个月是否服用 Have you taken it in the last three months?	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否
您服用的什么品牌的营养补充剂？（如果没有，选项请在其他处写明） Brand name	☐ 1、汤臣倍健叶酸亚铁片 ☐ 2、安利纽崔莱铁片 ☐ 3、金康倍叶酸铁片 ☐ 4、其他_____	☐ 1、惠氏金钙尔奇 ☐ 2、君宝康孕妇钙片 ☐ 3、十月妈咪维生素 AD 钙锌咀嚼片 ☐ 4、安利钙镁片 ☐ 5、其他_____	☐ 1、美康利健 MK 硒金牡蛎锌片 ☐ 2、汤臣倍健 锌咀嚼片 ☐ 3、宫诺肽片 ☐ 4、其他_____

您这三个月的服用频率？（如果食用频率小于每天/周一次，请填写每周/月食用几次，并勾出周/月）How often did you take in	____次/☐天 ☐周 ☐月	____次/☐天☐周☐月	____次/☐天 ☐周 ☐月

B 营养补充剂使用情况

C 吸烟情况
C1 您有吸烟史吗？ ☐ 1 是 ☐ 0 否***(跳至 F6)*** Have you smoked cigarettes ever before?
C2 在您最近的 3 个月内，您是否吸烟？ ☐1 是 ☐ 0 否 Did you smoke cigarettes in 3 month
C3 如果是，您平均每天吸多少支烟？ _____ 支 On average, how many cigarettes did you smoke each day in the month after your last menstrual period?
C4 如果您曾经戒过烟，您戒了多少次？ _____ How many times did you stop smoking?
C5 在您生活的大多数时间里，您是否暴露于他人烟草烟雾中？ On most days during your pregnancy, were you exposed to someone else's cigarette smoke? ☐ 1 是 ☐ 0 否 ***（跳至 G1）***
C6 您在哪里暴露与烟草烟雾中 Where were you exposed to the smoke? ☐ 1 仅在家中 ☐2 仅在工作单位 ☐3 在家和在工作单位均暴露
D 酒精
D1 您最近三个月的饮酒情况？During the 3 months before or during your pregnancy, did you ever drink any alcoholic beverages? ☐0.从未饮酒； ☐1.尝试饮酒（曾饮至少半瓶或一听啤酒，一小盅白酒等）； ☐2.现在饮酒（过去 30 天，至少有一天喝过一杯酒）； ☐3.重度饮酒（过去 30 天，至少有一天在 2 小时内喝过五杯酒）； ☐4.醉酒（过去 12 个月内，因喝酒太多而感到头晕/头疼/嗜睡等醉酒症状）。

核查人员签名：_____

Name of pregnancy： _____

ID no： _____

孕期危险因素暴露调查表

Pregnancy risk factor exposure questionnaire

A 一般情况 General information

A1 您的出生日期是 Birth date _____年_____月_____日
A2 民族 Nationality ☐ 1 汉族 Han ☐2 其他 other_____
A3 您的最高学历 Education ☐1 初高中以下 Mid ☐2 大专本科 High ☐3 硕士研究生以上及以上 College
A4 您现在的主要职业 Occupation ☐1 管理人员/干部 ☐2 技术人员 ☐3.企业主 ☐ 4 工人 ☐5 农民 ☐ 6 个体户 ☐7 其它_____
A6 家庭住址：Address _____区/县_____街道/小区_____门牌号码/村
A7 联系电话（请您认真填写，以助于医生和您联系）：Contact number _____(手机) _____(固定电话) _____(Email) _____(微信号)

B 本次妊娠情况

B1 您孕前体重通常为？ Current weight _____（公斤 Kg）			
B2 您身高是？ Height _____(厘米 cm)			
B3 您的腰围是？Waist _____（厘米 cm）			
B4 您此次怀孕的末次月经时间？ _____年_____月_____日 What was the first day of the menstrual period that came right before this pregnancy (LMP)?			
B5 孕期是否发生过重大负性生活事件而使您的精神受到刺激？ ☐ 1 是 ☐ 0 否 Have you ever experienced the negative events which irritate you and generate some negative emotion?			
B6 生这个孩子是您第几次怀孕？ _____次 How many times have you been pregnant?			
B7 是否有不良生育史？ Did you have the adverse reproductive history？ ☐ 1 有***（继续回答 B6.1）*** ☐ 0 无***（跳至 B7）***			
B6.1	流产史 Abortion	☐ 1 有	☐ 0 无
B6.2	早产史 Preterm	☐ 1 有	☐ 0 无
B6.3	死产史 Stillbith	☐ 1 有	☐ 0 无
B8 您是否有糖尿病和高血压疾病？Do you have hypertension or diabetes ☐ 1 是 ***(继续回答 B8)*** ☐ 0 否 ***（跳至 C1）*** ☐ 999 不知道 ***（跳至 C1）***			
B9 您的直系亲属中是否患有糖尿病、高血压疾病？ s there the family history of hypertension or diabetes in children's immediate family members ☐ 1 是 ***(继续回答 B8)*** ☐ 0 否 ***（跳至 C1）*** ☐ 999 不知道 ***（跳至 C1）***			
B10 若有，请选择出该亲属与您的关系**（可多选）If so, please choose the relationship with the child**			

☐1. 父亲 ☐2. 母亲 ☐3. 爷爷 ☐4. 奶奶 ☐5. 兄弟 ☐6. 姐妹

C 叶酸使用

C1 在您末次月经前三个月内，您是否服用过叶酸？ ☐1 是 ☐0 否 Did you take folic acid in the month before your last period?			
C2 在您末次月经之后至今，您是否服用过叶酸？ ☐1 是 ☐ 0 否 Did you take any folic acid after your last period anddduring pregnancy ?			
	叶酸 1	叶酸 2	叶酸 3
C3 药物名称（商品名） Brand name			
C4 使用时间 When did you take it?	☐1 孕前 before pregnancy ☐2 孕后 pregnancy ☐3 孕前孕后都有 always	☐1 孕前 before pregnancy ☐2 孕后 pregnancy ☐3 孕前孕后都有 always	☐1 孕前 before pregnancy ☐2 孕后 pregnancy ☐3 孕前孕后都有 always
C5 是否在怀孕期间一直使用？ Did you take it during the rest of your pregnancy?	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否
C6 是否停止使用过？ Did you stop taking it?	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否
C7 使用频率？ How often did you take it?	____次/☐天 ☐周 ☐月	____次/☐天 ☐周 ☐月	____次/☐天 ☐周 ☐月
C8 每次的使用量 What is the usage per time?	一次_____片	一次_____片	一次_____片

D 维生素使用

D1 在您末次月经的前三个月内，您是否服用过维生素？ ☐1 是 ☐0 否 Did you take any vitamins in the three months before your last period ？			
D2 在您末次月经之后至今，您是否服用过维生素？ ☐1 是 ☐0 否 ***（跳至 E1）*** Did you take any vitamins after your last period and during pregnancy?			
	维生素 1	维生素 2	维生素 3
D3 维生素名称 Vitamin name			
D4 维生素商品名称 Brand name			

D5 是否是医生给药？ Did your doctor give it to you?	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否
D6 是否包括叶酸？ Does it contain folic acid?	☐1 是 ☐0 否 ☐999 不知道	☐1 是 ☐0 否 ☐999 不知道	☐1 是 ☐0 否 ☐999 不知道
D7 使用时间 When did you take it?	☐1 孕前 before pregnancy ☐2 孕后 pregnancy ☐3 孕前孕后都有 always	☐1 孕前 before pregnancy ☐2 孕后 pregnancy ☐3 孕前孕后都有 always	☐1 孕前 before pregnancy ☐2 孕后 pregnancy ☐3 孕前孕后都有 always
D8 是否停止使用过？ Did you stop taking it?	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否
D9 使用频率？ How often did you take it?	____次/☐天 ☐周 ☐月	____次/☐天 ☐周 ☐月	____次/☐天 ☐周 ☐月
D10 每次的使用量 What is the usage per time?	一次_____片	一次_____片	一次_____片

E 其他营养补充剂使用情况 Other nutritional supplement use

营养补充剂种类 Nutritional supplement types	铁 Fe	钙 Ca	锌 Zn
您在近三个月是否服用 Did you take it around the time you became pregnant?	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否
您服用的什么品牌的营养补充剂？（如果没有，选项请在其他处写明）Brand name	☐ 1、汤臣倍健叶酸亚铁片 ☐ 2、安利纽崔莱铁片 ☐ 3、金康倍叶酸铁片 ☐ 4、其他_____	☐ 1、惠氏金钙尔奇 ☐ 2、君宝康孕妇钙片 ☐ 3、十月妈咪维生素 AD 钙锌咀嚼片 ☐ 4、安利钙镁片 ☐ 5、其他_____	☐ 1、美康利健 MK 硒金牡蛎锌片 ☐ 2、汤臣倍健 锌咀嚼片 ☐ 3、宫诺肽片 ☐ 4、其他_____
您这三个月的服用频率？（如果食用频率小于每天/周一次，请填写每周/月食用几次，并勾出周/月）How often did you take it?	____次/☐天 ☐周 ☐月	____次/☐天☐周☐月	____次/☐天 ☐周 ☐月

F 草本药物使用

F1 在您末次月经前三个月内，您是否使用过任何一种草本药物/传统医学药物？Did you take any herbal supplements/traditional Chinese medicine in the three months before your last period ☐1 是 ☐0 否			
F2 在您末次月经之后，您是否使用过任何一种草本药物/传统医学药物 Did you take any herbal supplements/traditional Chinese medicine after your last period and during pregnancy? ？ ☐1 是 ☐0 否 ***（跳至F1）***			
	草本药物 1 Herbal 1	草本药物 2 Herbal 2	草本药物 3Herbal 3
F3 草本药物类型 Name of herb	☐1 中成药 ☐2 中医草药	☐1 中成药 ☐2 中医草药	☐1 中成药 ☐2 中医草药
F4 药物名称（如果选择中医草药，请填写中药功效） The use of traditional Chinese medicine			
F5 用药持续多少天 How long did you take in?	_____天	_____天	_____天

G 吸烟情况

G1 您在怀孕前或者怀孕期间吸烟吗？ ☐1 是 ☐0 否***(跳至F9)*** Did you smoke cigarettes before or during your pregnancy with the baby?
G2 在您末次月经的当月，您是否吸烟？ ☐1 是 ☐0 否 Did you smoke during the month before your last menstrual period ?
G3 在您末次月经的一个月后（末次月经结束直至一个月后），您是否吸烟？ ***(跳至5)*** Did you smoke during the month after your last menstrual period, that is between LMP and LMP+1 month ☐1 是 ☐0 否
G4 如果是，您平均每天吸多少支烟？ On average, how many cigarettes did you smoke each day in the month after your last menstrual period ？ 支
G5 在您怀孕期间，您是否吸烟？ ☐1 是 ☐0 否***(跳至F7)*** Did you smoke during your pregnancy?
G6 在您怀孕期间，您平均每天吸多少支烟？ _____ 支 On average how many cigarettes did you smoke each

G7 在您末次月经期间至今，您是否戒过吸烟？ ☐1 是 ☐0 否 ***（跳至 F9）*** Did you stop smoking at any time between your last menstrual period and the end of your pregnancy?
G8 您戒烟有多少次？ How many times did you stop? _____ 次
G9 在您怀孕的大多数时间里，您是否暴露于他人烟草烟雾中？ On most days during your pregnancy, were you exposed to someone else's cigarette smoke? ☐1 是 ☐0 否 ***（跳至 G1）***
G10 您在哪里暴露与烟草烟雾中？ Where were you exposed to the smoke? ☐1 仅在家中 ☐2 仅在工作单位 ☐3 在家和在工作单位均暴露

H 酒精

H1 在您怀孕前三个月至今，您是否饮用过任何含有酒精的饮料？ ☐1 是 ☐0 否 During the 3 months before or during your pregnancy, did you ever drink any alcoholic beverages?
H2 在这三个月内，您通常每次饮几杯酒？ _____ 杯 On those days that you drank, how many drinks did you have?

I 环境暴露情况

I1 怀孕前三个月到现走，您是否染烫发? ☐1 是 ☐0 否 Did you dye perm in the first three months of pregnancy?
I2 怀孕前三个月到现在，后您工作的地点或家里是否装修过? ☐ 1 是 ☐0 否 Did you exposed to formaldehyde in the first three months of pregnancy?
I3 怀孕前后您是否接触过下列物质？ Have you been exposed to the following substances before and after pregnancy? (Toxic chemicals) ☐1 除草剂 ☐2 杀虫剂 ☐3 灭鼠剂 ☐4 有机溶剂 ☐5 消毒剂 ☐6 金属制剂 ☐7 有害气体 ☐8 有害固体

J 药物使用情况

以下问题是有关于您在末次月经之前三个月内至今期间的用药情况

	治疗高血压药物 Medication for hypertension	胰岛素 Insulin for diabetes	口服降血糖药物 Oral hypoglycemic for diabetes	抗癫痫药物 Medications for epilepsy	每天都要服用的药物 Medications at least once a day
1 您是否使用过？ Did you take?	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否

2 您使用的药物名称？ What did you take?	_____	_____	_____	_____	_____

如果下一个问题令您感到不安且不愿意回答，请在"拒答"上打√，我们将对您所有回答进行严格保密!

	安定\有助于您放松药物 Valium\drugs to help you relax	使您感觉良好\精力旺盛 Make you feel good\have more energy	美沙酮\氧可酮\其他止痛药 Methadone oxymoron\other pain killers	可卡因 Cocaine or crack cocaine	海洛因 Heroin	大麻 Marijuana
1 您是否使用过？ Did you take?	☐1 是 ☐0 否 ☐2 拒答 ☐9 不清楚	☐1 是 ☐0 否 ☐2 拒答 ☐9 不清楚	☐1 是 ☐0 否 ☐2 拒答 ☐9 不清楚	☐1 是 ☐0 否 ☐2 拒答 ☐99 不清楚	☐1 是 ☐0 否 ☐2 拒答 ☐9 不清楚	☐1 是 ☐0 否 ☐2 拒答 ☐9 不清楚
2 您使用的药物名称？ What did you take?	_____	_____	_____	_____	_____	_____

I 您在怀孕期间是否患过以下疾病？

I.3 发热性疾病及呼吸道感染 ☐1 是 ☐0 否

Febrile illness and respiratory infections

I.3.1 您发烧时的最高温度是多少？ _____℃

What was the highest temperature of your fever during your illness?

I.3.2 您发烧有几天？ _____天

How long did you have a fever?

I.6 其他_____

调查结束，谢谢您的配合！

调查员姓名 _____

调查日期 ___ ___/ ___ ___/ ___ ___

年 月 日

Types of fetus defects and birth defects

Diagnosis
Anencephalus
Spina bifida
Encephalocele
Congenital Hydrocephalus
Cleft Palate
Cleft Lip
Cleft Lip with Cleft Palate
Microtia (including Anotia)
Deformity of external ear(s) (except Microtia and Anotia)
Esophageal atresia or stenosis
Anorectal atresia (including Congenital Anorectal Malformations)
Hypospadia
Ectopocystis
Pes Equinovarus
Polydactylism
Syndactylia
Limb shortening
Congenital Diaphragmatic Hernia
Pcromphalus
Celoschisis
Conjoined Twins
Trisomy 21 syndrome
Congenital heart disease
Others

Note: Down's syndrome, neural tube defects, congenital heart defects, hydrocephalus, digestive tract malformations and urinary malformations are most common defects in China. Defects were detected by

prenatal Down's syndrome screening, NT examination and Ultrasound image examination during the second trimester; and the number and type of birth defects after childbirth are diagnosed by professional clinical team. Down syndrome (DS), caused by the trisomy, translocation, or partial trisomy of chromosome 21, is the most common genetic cause of intellectual disability. DS is diagnosed by neonatologist. Neural tube defects including spina bifida and hypospadias, were diagnosed by ultrasound examination and pediatric neurosurgeon. Congenital heart disease was diagnosis by Doppler ultrasound and CHD screening (pulse oximetry plus cardiac murmurs). Hydrocephalus, digestive tract malformations, urinary malformations and other defects also were also diagnosed either by ultrasound or some other specific diagnosis methods.

Future Plan of Shanghai PreConception Cohort, Shanghai, China (SPCC)

The Shanghai PreConception Cohort (SPCC) is a long-term cohort and meets the "Cohort profile" requirement. We have a complete plan to follow-up offspring to the age of 18 years old. The cohort will be financially supported by different grants. The current manuscript focuses on the first phase, the establishment of the baseline and our first main outcome, the congenital heart disease (CHD).

The second phase of this cohort study is from birth to 6 years old. Follow-up work will rely on Shanghai Community health care centers-child care. The third phase is from 6 to the age of 18 (school age). We will cooperate with Shanghai Student Health and Fitness Surveillance Center for the follow-up of the school age children.

Multiple outcomes for pediatrics, including growth, physical and neuro-developments, obesity, common and rare diseases will be investigated. Nested case-control design will be used for rare outcomes.

The data collection plan for infants and children

	At birth	6 weeks	6 months	12 months	36 months	60 months	6 – 18 years*
General information (gender, ID number, Home Address, etc)	√	√					
Delivery outcome (Congenital malformation, preterm birth, miscarriage, stillbirth)	√						
Body measurement(weight, height, waistline, hipline)	√	√	√	√	√	√	√
Diet investigation (questionnaire)	√	√	√	√	√	√	√
Neurobehavioral developmental assessment (DDST[$])		√	√	√	√	√	
Anthropometrics data (Shanghai Community health care centers-child care and Shanghai Student Health and Fitness Surveillance Center) weight, height, waist circumference	√	√	√	√	√	√	√
Physical fitness measurement (running, jumping, solid balls, etc)							√
Blood pressure measurement, annually							√
Hemachrome (anemia)							√
Renal functions, at grade 9 and 12							√
Cardiovascular-related chronic diseases (Hypertension, Obesity, Diabetes/Pre-diabetes and Dyslipidemia)							√
Venous blood[#]							√

Note: * Shanghai Student Health and Fitness Surveillance Center routinely conducts all-round physical examinations for students in the school as a health care policy for Shanghai students. It is usually in September-November of each year.

$ DDST: Denver developmental screening test.

Venous blood will be collected at 12, 15 and 18 years of age.

BMJ Open

Cohort profile: Shanghai PreConception Cohort (SPCC) for association of periconceptional parental key nutrition factors with health outcomes of children: I -congenital heart disease

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Keywords:	red blood cell folate, vitamin, congenital heart diseases, periconceptional health care

Cohort profile: Shanghai PreConception Cohort (SPCC) for association of periconceptional parental key nutrition factors with health outcomes of children: I -congenital heart disease

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ABSTRACT (290)

Purpose: The Shanghai PreConception Cohort (SPCC) was established initially to investigate associations of parental periconception nutritional factors with congenital heart disease (CHD), and has extended to children growth, development and pediatric diseases.

Participants Prepare-for-pregnant couples who presented at Shanghai preconception examination clinics and early-pregnant women before 14 gestational weeks were enrolled to comprise a periconception baseline study population. General characteristics, routine clinical data, consumption of diet supplements, such as folic acid and multivitamins, were collected. Blood samples were collected at preconception, early, middle and late gestation respectively by standard procedures. Multiple nutrition factors in blood sample of participants that were selected by case-control design will be examined, including folates, homocysteine, vitamin A, vitamin D, vitamin E and metals. Genomic DNA was extracted.

Findings to date The baseline population included 8045 preconception couples, 3054 single women, and 15 615 early-pregnant women, respectively. Birth data from 12 402 births were collected and follow-up of the cohort for more outcomes is ongoing. Currently, 151 CHDs were identified after birth. Pilot analysis in a small subgroup showed that only about 15% of 656 preconceptional women and 49% of early-pregnant women had red blood cell folate concentration meeting the international recommendation for preventing neural tube defects.

Future plans Once a sufficient number of CHD cases is achieved, we will investigate quantitative association of preconceptional red blood cell folate levels with CHD using nested case-control design. The SPCC cohort will be followed up for 18 years to investigate extensive outcomes of growth, development, obesity, and common and rare diseases during childhood and adolescence according to our plan. Blood nutrition factors will be examined in participants selected for specific aims. The SPCC cohort will also allow for prospective cohort studies on extensive research questions.

Registration: NCT 02737644.

Key Messages: red blood cell folate, vitamin, congenital heart diseases,

periconception health care

Strengths and limitations of this study

- The SPCC cohort is the first prospective birth cohort with CHD as primary outcome with recruitment starting from preconception stage. Temporal sequence of exposures and outcomes can be achieved for causal inference of birth defects and other diseases that occur during early stage of gestation.
- Preconception blood samples were appropriately collected and stored which allow examination of individual blood levels for nutrition factors and other exposures.
- Clinical data and blood samples from both father and mother from before conception were collected, which will allow for testing the effect of both maternal and paternal genetic and nutrition factors on fetal and children diseases.
- Although response rate was high (over 95%), preconception participants were recruited from the population who voluntarily presented at Shanghai city preconception physical examination sites. They may have a stronger willingness for a healthy pregnancy, which may induce selection bias.
- Biological samples (cord blood, placenta) of the newborns were not collected.

INTRODUCTION

Congenital heart disease (CHD) is a common congenital malformation, which seriously affects quality of children's life [1]. CHD is a leading cause of infant death in high-income countries affecting eight of 1000 live births [2]. According to the report from National Health and Family Planning Commission of the People's Republic of China, CHD accounts for about a quarter of the birth defects of newborns in China, ranking the first among birth defects [3]. In a prospective, nation-wide large-scale study in more than 120 000 newborns in China in 2013, the prevalence of CHD in live births was identified 8.94 ‰; the rate of severe CHD was 2.9 ‰ [4].

The cause of CHD is multifactorial. With the development of genetic engineering technology, the genetic factors have been better understood in the past decade [5]. Multiple environmental risk factors have been reported in epidemiological studies, the maternal social variables such as occupation, educational background, health status, unhealthy life style, maternal medical history and emotional status, family history of disease, consanguineous marriages and so on [6-10]. In addition, maternal key nutrients related to the risk of offspring's CHD as a modifiable environmental factor during periconception [11, 12]. The periconception intake of folic acid supplement has been shown to reduce the risk of CHD

[13, 14] and women worldwide have been recommended to take folic acid supplements before conception and in the beginning of pregnancy. Awareness of the relationship between folic acid deficiency and CHD is actually a by-product finding from the well-known Hungarian RCT study of folic acid supplementation to prevent neural tube defects. The study found that prenatal supplementation with a vitamin complex containing 0.8 mg of folic acid daily reduced the incidence of congenital neural tube defects. At the same time, the incidence of various heart defects have also been reduced by nearly half [15]. Longitudinal data from more than one million births in Canada over a total of 22 years from 1990 to 2011 also show that food fortification with folic acid reduced risk of CHD by 20-30% [16]. The current folic acid supplementation recommends that all women of childbearing potential be supplemented with at least 0.4 mg folic acid daily prior to conception and during pregnancy, which is designed for preventing neural tube defects [17]. However, excessive folic acid intake may increase the risk of cancer [18], vitamin B12 deficiency [19], and autism spectrum disorder [20]. The optimal dose of folic acid for preventing CHD warrants further investigation. In addition, most previous studies only focused on the supplement of folic acid or the serum folate level during or after pregnancy, which may not be the optimal period and way to reflect the exposure level to risk of CHD.

To investigate the association between parental periconception key nutritional factors such as folate with the development of CHD and to explore the biomarker cutoff levels, we conducted Shanghai PreConception Cohort (SPCC) and a nested case-control analysis.

The SPCC cohort was initiated to study CHD as the primary outcome. Based on the strengths of its baseline data collection, it has received attention and support, with improved additional extensive outcomes for children that will be followed up longer term.

Cohort description

Setting and participants

The SPCC cohort recruited parent-planning women and men who were permanent residents and who voluntarily presented at preconception clinical clinics at 28 maternity institutions in 10 districts of Shanghai (Minhang District, Huangpu District, Xuhui District, Changning District, Jing'an District, Putuo District, Yangpu District, Pudong District, Songjiang District, Qingpu District) from March 2016 to December 2018. The preconception examination policy in the city of Shanghai provides a unique opportunity and clinical resources to support recruitment of SPCC. Since 2010, married couples in Shanghai have been encouraged to attend a free preconception health examination. In addition, these maternity

institutions receive strong local administrative support and integrated maternal health care networking, providing service to 150 000–200 000 annual deliveries in Shanghai. Couples who were present at preconception clinics, living in Shanghai, preparing for pregnancy within one year, and planning to receive antenatal care and to delivering in Shanghai, were eligible for the study. Written informed consents were obtained from all participants before any data collection. In addition, we recruited early-pregnant women at their first antenatal examination who were at gestational week <14 weeks. These two groups of participants comprised a periconceptional baseline study population.

Baseline data collection and antenatal visits

Baseline data collection was designed according to the hypothesis that maternal preconception serum or red blood cell (RBC) folate concentration is quantitatively associated with offspring CHD. As shown in Figure 1, the baseline population will be followed up to delivery, and their babies will be followed up until 18 years old. At enrollment, the participants completed a questionnaire (Questionnaire 1) of key nutrients supplementation and blood sample collection. When participants got pregnant, as well as the participants recruited at early gestation, investigations included administration of Questionnaire 2 and blood sample collection at the first antenatal visit <14 gestational weeks. Pregnancies were followed up till

birth along with routine maternal health care procedures. Blood samples were also collected at the second (24-28 gestational weeks) and third trimester (32-34 gestational week). The study design and protocol has been registered with Clinical Trials Registry (NCT 02737644).

Outcomes -CHD in neonates

The diagnosis of CHD was the primary outcome of the study at this stage and obtained from Shanghai Neonatal CHD Screen Platform, which was initiated as routine screen for newborns in Shanghai since Jun 1st 2016. The standard protocol of CHD screening of the platform was previously described in detail [21]. All newborn babies received the screen by using double-index method (i.e. cardiac murmur auscultation and pulse oximetry) during 6-72 hours after delivery, and those screen-positive babies would receive a subsequent echocardiography for further confirmative diagnosis.

SPCC will also collect other birth defects as secondary outcomes, including Down's syndrome, neural tube defects, hydrocephalus, digestive tract malformations, urinary malformations, and behavioral cognitive developmental disorder. After delivery, the infants attended routine childcare procedures organized by Shanghai Child Health Care System, which is administered by Shanghai Center for Women and Children's Health. All birth defects records, which were diagnosed after birth, as well as routine neuro-development examinations and longitudinal

anthropometric data, were abstracted from the system by a professional clinical team from Children's Hospital of Fudan University (for details of the types of birth defects please see Appendix 1)

Questionnaire 1 and 2 (Demographics, disease history and diet supplements)

As shown in Figure 1, Questionnaire 1 was administered during recruitment at preconception examination sites for collecting information of demographics, maternal education, socio-economic status, occupation, smoking status, alcohol consumption, BMI, medication, and health status were also included. Questionnaire 2 was administered at early pregnancy sites to collect information on consumption of folic acid supplement, vitamin supplement, the brand and content of nutrient supplement. Besides the content of Questionnaire 1, Questionnaire 2 added drug information, reproductive history, and health status. The structure of Questionnaire 1 and Questionnaire 2 are presented in Appendix 2a and Appendix 2b.

Specimens

In this study, the rest blood samples for routine clinical blood test were collected. The blood sample for routine clinical examination was usually 5 ml and extracted in the morning. Routine clinical blood test was performed at room temperature. The rest blood samples (fasting serum and EDTA anticoagulation) of peripheral venous blood from routine laboratory clinical

blood test were kept. These blood samples were temporarily stored in a 4℃ refrigerator for dispensing within 6 hours and transferred to a -20 4 ℃. After completion of blood sample distribution the serum and the whole blood were stored at the site laboratory, and then transported by three trained investigators to the central laboratory for storage in -80 ℃ freezers within two weeks. Sampling tubes were made of a light-proof material and the process of collecting blood samples were completely protected from light.

Laboratory examinations of key nutrition factors

The examinations will be conducted in participants selected by nested case-control designs based on specific aims.

(1) RBC folate, serum folate, serum homocysteine, vitamin D, vitamin B12 and serum ferritin

EDTA anticoagulation blood was collected to examine RBC folate, serum folate, serum homocysteine, vitamin D, vitamin B12 and serum ferritin assays. All six biomarkers were analyzed on an electrochemiluminescence assay (ARCHITECT i2000SR Analyzer, Abbott Laboratories, USA). A standard solution with known concentration (produced by Abbott Laboratories) was used daily to quality control before the measurement. If the quality control level was out of concentration range, the measurement

would be suspended and adjusted. External quality control was carried out with the control lab data program from Abbott Laboratories (Abbott Laboratories, Shanghai, China). RBC folate concentrations were adjusted for hematocrit. If the RBC folate concentration is below 126.0 ng/ml or above 651.1 ng/ml, adjustment was needed based on serum folate level. The hematocrit data were extracted from the hospital laboratory information system. Those examinations were performed in central laboratory of Children's Hospital of Fudan University.

(2) Vitamin A and vitamin E

The serum concentration of vitamin A and vitamin E were quantitatively detected by liquid chromatography-tandem mass spectrometry in central laboratory of Children's Hospital of Fudan University. The testing instrument was triple quadrupole mass spectrometer LC/MS/MS System (NO: API 3200MDTM, ABSciex Pte.Ltd.). A standard solution of vitamin A-d6 and vitamin E-d6 were applied as internal standards.

(3) *Glycemic and lipid profiles*

Fasting serum cholesterol, high-density lipoprotein, low-density lipoprotein, triglyceride, and fasting glucose were performed on Beckman coulter AU chemistry analyzers (Beckman, USA) in central laboratory of Children's Hospital of Fudan University.

(4) Metals

Serum levels including Mg, Fe, Zn, Se, Mn, As, Cu and Ca, were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) (Inductively Coupled Plasma Optical iCAP6300, Themo®, USA) in standard mode [22]. The metals examination was conducted in Instrumental Analysis Center of Shanghai Jiaotong University which is a national key laboratory.

(5) Genomic DNA extraction

Genomic DNA of all participants were extracted using a magnetic bead-based kit (TGuide M16 Automatic Nucleic Acid Extractor (OSE-M16), TIANGEN BIOTECH (BEIJING) CO. LTD, China) from 2 ml of EDTA anticoagulated whole blood sample after routine blood test. Genomic DNA samples were stored for future study. An average 150 ng DNA were available. Similar to blood chemicals, future genetic variants genotyping will be performed in selected participants according to nested case-control design for specific aims. Currently, there are no candidate genes or variants that are listed.

Procedures for routine data extraction and cohort retention

As shown in Figure 1, outcomes at birth, during infant to childhood (preschool phase), and between 7 to 18 years (school ages) will be collected or extracted from multiple public platforms and data sources. Firstly, preconception clinical visit data (height, weight, age, infections, sexually transmitted disease, and family history) was collected from

Preconception Care Electronic Data System, supported by national and local government. Secondly, the routine pregnant data was obtained in Maternal Clinic Antenatal Medical Record System, managed by Shanghai Center for Women and Children's Health, including height, gestational weight, last menstrual period, childbearing history, delivery outcomes, infections, hematocrit, coagulation function, liver and kidney function, and so on. Thirdly, the maternal and neonatal data at delivery came from Shanghai Neonatal CHD Screen Platform including birth weight, CHD diagnosis, birth defects, and Apgar score, etc. In addition, we will work with the Shanghai Student Health and Fitness Surveillance Center to obtain outcome data. The personal national identification card numbers of participants are applied as index variables through the multiple data sources. The detailed variables list and codebook of data collection are presented in Appendix 3.

During the first phase of the cohort, from preconception to delivery, comprehensive strategies were used to retain participants in the study. For mothers, we provided a variety of engagement activities including green channel (fast track) to their antenatal care to provide convenience and save their time in hospitals. We also provided a contact number on the participant card to answer their calls or queries about the study procedures. Site investigators at early pregnant clinics in collaborative hospitals were

provided a smartphone APP to help identify recruited cohort participants timely and manage data and blood sample collection procedures. We also provided green channel echocardiography for diagnosing CHD for all site hospitals to enhance the compliancy of the participants. In addition, an automated text message system was adopted to remind participants of schedules and appointment of follow-up.

Characteristics of study participants

The SPCC started recruitment in March 2016. As shown in Figure 2, by December 2018, we consecutively recruited 19 144/19 563 (97.9%) participants at preconception settings, including 8045 couples and 3054 single women, and an additional 15 615/16 201 (96.4%) pregnant women at maternity hospitals with gestational age <14 weeks. Table 1 describes the basic demographic characteristics of the preparing-for-pregnant participants and pregnant women, respectively. The average age of the preconception population was 29.9 (SD 3.9) years for females and 31.4 (SD 4.5) years for male, one-third of males and 2.4% of females were smokers, and two-thirds of males, and one-third of females had a habit of drinking alcohol. In pregnant women, the average age was 29.9 (SD 4.0) years, with half of them having a first pregnancy. Compared with the preconception females, they were similar in age but different in education levels and occupation, the prevalence of smoking and alcohol drinking

were much lower. (Table 1 was partly included in another manuscript, which is under review of Maternal & Child Nutrition with Manuscript number of MCN-08-19-OA-4056).

Statistical methods

To investigate the association of maternal preconception nutrition levels with offspring CHD risk, a nested case-control study will be conducted. The control will be matched by age and site.

The sample size for the nested case-control analysis was planned as 180 CHD cases and 720 matched controls to detect a maternal folate deficiency with prevalence of 50% in controls with odds ratio of 1.6 in association to achieve a power of 80% at alpha of 0.05. Based on CHD incidence above 8.94 per 1 000 live births [4], 20 000 pregnancies will be needed. For a continuous nutrient variable with standard deviation 2.0, 50 matched-pairs (1:4) are required to achieve 90% power to detect an odds ratio of 1.3 calculated using conditional logistic regression with a 0.05 significance level [23, 24]. Once a sufficient number of CHD cases is achieved, the quantitative association of preconception RBC folate levels with CHD using nested case-control design will be investigated.

Conditional multivariate logistic regression will be used for association analysis with offspring affected status of CHD being the dependent variable, nutrition factors levels as exposure and adjusted for all potential

paternal and maternal covariates. Odds ratios (OR) and 95% confidence intervals (95%CI) will be reported. To explore a potential cutoff point of the nutrition levels that significantly increases the risk of CHD, a dummy variable will be set up by categorizing the maternal preconception nutrition levels based on the distribution of the control group. The does-response relationship will be also be analyzed. Sensitive analysis will include non-conditional logistic regression analysis, or generalized estimation equations (GEE) model, or generalized linear models when necessary.

Future plan

We have a complete plan to follow-up offspring of SPCC population to the age of 18 years old. The cohort will be financially supported by different grants. The current manuscript focuses on the first phase, the establishment of the baseline and our first main outcome, the CHD. The data collection plan for infants and children (from birth to 6 years old, pre-school stage), as well as school age (from 6 to 18 years old), are included. During the stage of 0 to 6 years old, the data of neurodevelopment will be collected from routine childcare clinical visit at birth, 6 weeks, 6 months, 12 months, 36 months, and 60 months through the cooperating medical institutions. Physical measurements data and dietary intake information also can be collected at this stage. During the stage of 6 to 18 years old, we plan to follow up their growth (height, weight, blood pressure, etc.) mainly relying

on the annual physical examination of Shanghai Student Health and Fitness Surveillance Center System. Multiple outcomes for children, including growth and development, cardiovascular diseases, neurodevelopment, metabolic diseases, obesity status, and hypertension will be investigated. Please see Appendix 4 for details.

Patient and public involvement

Patients and public were not involved in the design or conduct of this study.

Findings to date

By the end of December 2018, the last participants recruited at early pregnancy were due for delivery, however, we have achieved birth records of 12 402 newborns. The follow-up of outcomes of the rest of the participants is ongoing (shown in Figure 2). A total of 151 cases of CHD were identified through the CHD screening platform, 131 cases from the early pregnancy sample, and the remaining 20 cases were from the preconception sample. The prevalence of CHD in live births is 10.5 ‰ (131/12 402) based on the present available data.

We conducted a small pilot study in April 2017 to explore blood levels of nutrition factors, including serum folate, RBC folate, vitamin A, vitamin E, and vitamin D. The blood samples from 627 females were selected

consecutively from the preconception sample according to who was identified pregnant. In addition, 597 women who were consecutively recruited from the antenatal care clinics were selected. As shown in Table 2, the concentrations of RBC folate median level is 247.0 ng/ml (IQR: 184.8-340.5 ng/ml) in preconception women and 417.4 ng/ml (IQR: 308.6-544.2 ng/ml) in early-pregnant women. Twenty percent of preconceptional participants and 44.9% of pregnant participants had a folate level over 400 ng/ml, which was suggested as optimal level for preventing neural tube development defects [25, 26]. These results suggest that effort is urgently needed to improve the intake of folic acid supplementation in the prepare-for-pregnancy population, especially before pregnancy.

Based on SPCC, the possible scope of research questions, available types and number of bio-samples and biomarkers that can be examined is shown in Table 3.

Strengths and limitations

Compared with existing birth cohorts, there are three important strengths in our cohort. Firstly, the SPCC cohort is the first prospective birth cohort with CHD as primary outcome and recruitment starting from preconception. Blood samples were collected and stored which allows for direct

measurement of individual exposure levels before the development of CHD and make causal inference. Temporal sequence of exposures and outcomes can be achieved for causal inference of birth defects and other diseases that occur during early stage of gestation. Up to date, no published studies have measured maternal blood folate levels before conception and link it to disease outcomes. Secondly, this cohort also allows for the investigation of associations between periconception maternal and paternal nutrition exposures with other birth defects, early onset-diseases, and neuro-development outcomes. Preconception blood samples were appropriately collected and stored which allows for the examination of individual blood levels of nutrition factors and other exposures. Thirdly, both paternal and maternal clinical data and blood samples before conception were collected, which will allow for testing effect of both maternal and paternal genetic and nutrition factors to fetal and children diseases.

Two limitations of this cohort study should be considered. Firstly, there are approximately 200 000 pregnant women giving birth every year in Shanghai, and approximately 20 000 of them will take part in the free preconception care in Shanghai, where participants were recruited consecutively. Although response rate was high (over 95%), preconception participants were recruited from a population voluntarily present in

Shanghai city with preconception physical examination sites, who may have a stronger willingness for a healthy pregnancy. This may induce selection bias. Secondly, in this study, biological samples (cord blood, placenta) of the newborns are not collected. We plan to give new informed consent to the family who are willing to participate in future studies, to collect biological samples not mentioned before. In addition, electrochemiluminescence assay was used to examine serum and RBC folate concentrations, which is different from microbiologic assay that is used widely. This will not bias the association analysis but comparison with international populations needs caution.

Collaboration

Investigators with an interest in hypotheses related to SPCC (and that meet the requirements of current approvals) are welcome to contact Dr. Guoying Huang or Weili Yan. A 'Research Collaboration application' should be send to the corresponding author by Email. The application should include a brief description of the project.

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SPCC group

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Table 1. Socio-demographics of participants including 6573 couples (parents) and 15 203 pregnant women that were enrolled in the Shanghai PreConception Cohort (SPCC).

Characteristics	Couples (parents) who completed preconception questionnaires				Additional pregnant women who completed both preconception and first-trimester questionnaires		*P**
	Male (n=6573)		Female (n=9243)		Pregnant women （n=15203）		
Age (years), Mean±SD	6573	31.4±4.5	9243	29.9±3.9	15203	29.9±4.0	0.995
Ethnicity	6536		9188		15176		0.258
Han Nationality		6403 (98%)		8966 (97.6%)		15245 (97.8%)	
Other		133 (2%)		222 (2.4%)		342 (2.2%)	
Educational level	6530		9147		15143		<0.001
<College		514 (7.8%)		795 (8.7%)		2052 (13.6%)	
>=College		6016 (92.2%)		8352 (91.3%)		13091 (86.4%)	
Annual household income	6530		9147		NA		
< ￥10 000		1424 (21.8%)		2214 (24.2%)			
>=￥10 000		5106(78.2%)		6933 (75.8%)			
Occupation	6530		9147		14789		<0.001
Entrepreneur		157 (2.4%)		128 (1.4%)		171 (1.1%)	
Farmer		26 (0.4%)		46 (0.5%)		79 (0.5%)	
Self-employed		216 (3.3%)		274 (3%)		862 (5.6%)	
Manager		1410 (21.6%)		1765 (19.3%)		2650 (17.2%)	
Technician		2462 (37.7%)		1903 (20.8%)		3239 (20.6%)	

Company clerk		2259 (34.6%)		5031 (55%)		8451 (54.7%)	
Attending preconception pregnant examination	NA		NA		14996		–
Yes						3374 (22.5%)	
No						11622 (77.5%)	
Parity	NA		NA		15162		
1						7569 (49.9%)	
2						4604 (30.4%)	
>=3						2989 (19.7%)	
Miscarriage or stillbirth	NA		NA		15159		
Yes						4838 (31.2%)	
No						10385 (68.7%)	
Smoking	6552		9212		15159		<0.001
Yes		2073 (31.6%)		218 (2.4%)		153 (1.0%)	
No		4479 (68.4%)		8994 (97.6%)		15006 (99%)	
Alcohol drinking	6448		9075		15164		<0.001
Yes		3962 (61.5%)		2865 (31.6%)		1556 (10.3%)	
No		2486 (38.5%)		6210 (68.4%)		13608 (89.7%)	
Location of home	6573		9243		NA		
Developed districts		3034 (46.2%)		5452 (59.0%)			
Developing districts		3539 (53.8%)		3791 (41.0%)			

* Comparisons between preconception females and pregnant women. *t* tests were used to compare numerical variable (age). Chi-square tests were used to compare categorical variables (ethnicity, educational level occupation, smoking and alcohol drinking). Of 6573 preconceptional males, values were missing in ethnicity (n=37), educational level (n=43), annual household income (n=43), occupation

(n=43), smoking (n=21), and alcohol drinking (n=125). Of 9243 preconceptional females, values were missing in ethnicity (n=55), educational level (n=96), annual household income (n=96), occupation (n=96), smoking (n=31), and alcohol drinking (n=168). Of 15203 pregnant women, values were missing in ethnicity (n=27), educational level (n=60), occupation (n=414), smoking (n=31), attending preconception pregnant examination (n=207), parity (n=41), miscarriage or stillbirth (n=44), smoking (n=44), and alcohol drinking (n=39).

Table 2. Distributions of biomarkers in women before and at early gestation (pilot study)

Biomarker	Preconception		Early pregnancy	
	n	level	n	level
Serum folate, Median (IQR), ng/ mL	620	9.7 (6.5, 13.8)	577	14.5 (11.2, 16.4)
RBC folate, Median (IQR), ng/ mL	570	247.0 (184.8, 340.5)	587	417.4 (308.6, 544.2)
Homocysteine, Median (IQR), μmol/L	624	6.5 (5.2, 8.6)	599	4.2 (3.5, 5.2)
Vitamin B12, Median (IQR), pg/mL	625	495.2 (394.2, 639.0)	600	388.5 (289.4, 511.4)
Vitamin D, Mean ±SD, ng/ mL	607	16.3 ±6.0	578	15.5 ±6.1

Table 3. Bio-samples collected and biomarkers that can be examined in the SPCC cohort

Bio-samples Available in participants	Available sample type and volume	Time		
		Preconception + early pregnancy (Baseline)	24-28 weeks	32-36 weeks
Mother		(n=25487)	(n=8668)	(n=7522)
	Serum, 200 ul*3	Yes	Yes	Yes
	Whole blood	Yes	Yes	Yes
	Genomic DNA, 150 ng	Yes	Yes	Yes
Father		(n= 7151)	-	-
	Serum, 200 ul*3	Yes	NA	NA
	Whole blood	Yes	NA	NA
	Genomic DNA, 150 ng	Yes	NA	NA
Child	NA			

Scope of research questions:

1. Quantitative association of preconceptional key nutrition levels (such as serum and RBC folates and related biomarkers, such as homocysteine and vitamin B12) with CHD, currently, and other folate sensitive birth defects.
2. Quantitative association of periconceptional maternal and paternal key nutrition levels (preconception levels, and dynamic levels during gestation) with important maternal and neonatal gestational complications, neuro-development of infants, childhood obesity, and clinical pediatric diseases.
3. Periconcpetional maternal and paternal folate concentration with Autism spectrum disorder, allergy and asthma in children.

Biomarkers that will be examined in different types of bio-samples:

1. Biomarkers based on serum sample:

a) Folate and related markers: serum folate, homocysteine
b) Other Vitamins: vitamin A, vitamin B12, vitamin E, and vitamin D
c) Marco and micro metals: Mg, Fe, Zn, Se, Mn, As, Cu, Ca, etc., mg/L
d) Serum ferritin
e) Fasting glycemic and lipid profiles: fasting glucose, total cholesterol, triglyceride, high-density lipoprotein, low-density lipoprotein

2. Whole blood sample: RBC folate
3. Genomic DNA sample: candidate genetic variants or genome-wide variants are all possible to examined

Figure 1 Diagram of the protocol and follow-up of Shanghai PreConceptional Cohort (SPCC)

The baseline population of SPCC cohort was females and males at periconception stage (couples who are prepare-for-pregnancy) and pregnant women at early gestation stage. The cohort includes three phases, from periconception to birth (Phase I, peri-natal phase), from birth to 6 years old (Phase II, infant and pre-school age), and from 7 to 18 years (Phase III, school age). The current manuscript focuses on the first phase, with congenital heart disease as the primary outcome, and will cover other folate sensitive birth defects.

Figure 2 Flow chart diagram

By December 2018, we consecutively recruited 19 144/19 563 (97.9%) participants at preconception settings, including 8045 couples and 3054 single women at pre-pregnancy sites, and an additional 15 615/16 201 (96.4%) pregnant women at maternity hospitals with gestational age <14 weeks were recruited. By the end of December 2018, the last participants recruited at early pregnancy were due for delivery, we have achieved birth records of 12 402 newborns in Maternal Clinic Antenatal Medical Record System. The follow-up of outcomes of the rest of participants is ongoing (shown in Figure 2). A total of 151 cases of CHD were identified through Shanghai Neonatal CHD Screen Platform, 131 cases were from the early pregnancy sample, the remaining 20 cases were from the preconception sample. The prevalence of CHD in liver births is 10.5 ‰ (131/12 402) based on the present available data. In the preconception sample, from March 2016 to May 2018 (the latest data extraction in Maternal Clinic Antenatal Medical Record System), the number of pregnancies and delivers were 1538 and 975 respectively.

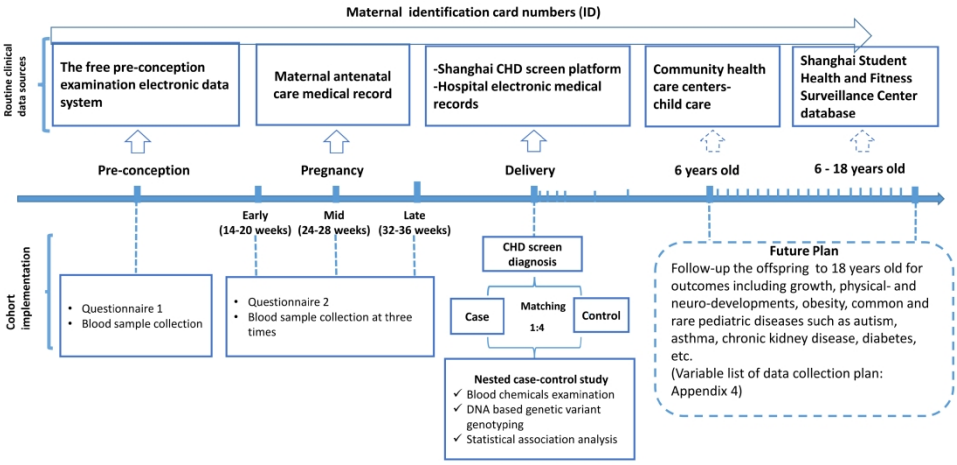

Figure 1 Diagram of the protocol and follow-up of Shanghai PreConceptional Cohort (SPCC)
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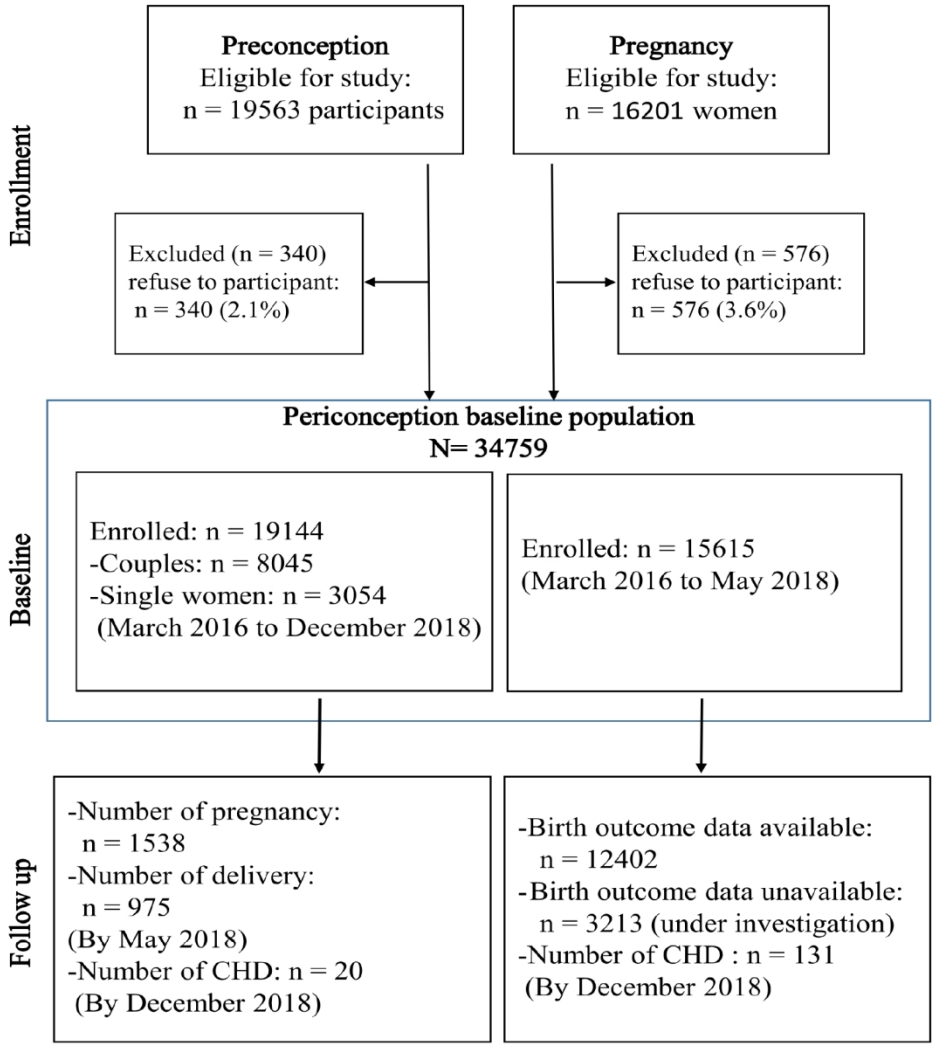

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Appendix 1 Types of fetus defects and birth defects

Types of fetus defects and birth defects

Diagnosis
Anencephalus
Spina bifida
Encephalocele
Congenital Hydrocephalus
Cleft Palate
Cleft Lip
Cleft Lip with Cleft Palate
Microtia (including Anotia)
Deformity of external ear(s) (except Microtia and Anotia)
Esophageal atresia or stenosis
Anorectal atresia (including Congenital Anorectal Malformations)
Hypospadia
Ectopocystis
Pes Equinovarus
Polydactylism
Syndactylia
Limb shortening
Congenital Diaphragmatic Hernia
Pcromphalus
Celoschisis
Conjoined Twins
Trisomy 21 syndrome
Congenital heart disease
Others

Note: Down's syndrome, neural tube defects, congenital heart defects, hydrocephalus, digestive tract

malformations and urinary malformations are most common defects in China. Defects were detected by prenatal Down's syndrome screening, NT examination and Ultrasound image examination during the second trimester; and the number and type of birth defects after childbirth are diagnosed by professional clinical team. Down syndrome (DS), caused by the trisomy, translocation, or partial trisomy of chromosome 21, is the most common genetic cause of intellectual disability. DS is diagnosed by neonatologist. Neural tube defects including spina bifida and hypospadias, were diagnosed by ultrasound examination and pediatric neurosurgeon. Congenital heart disease was diagnosis by Doppler ultrasound and CHD screening (pulse oximetry plus cardiac murmurs). Hydrocephalus, digestive tract malformations, urinary malformations and other defects also were also diagnosed either by ultrasound or some other specific diagnosis methods.

Appendix 2a pre-pregnant questionnaire

姓名 Name：_____

身份证号 ID no：□□□□□□□□□□□□□□□□□□

医院代码 Hospital No：_____（到时打印到问卷上）

填表日期 Date：_____年_____月_____日

孕前膳食补充剂调查表

Pre-pregnancy nutrition supplement questionnaire

（男女共用）

A 一般情况

A1 联系电话（请您认真填写，以助于医生和您联系）Contact number： _____(手机) _____(固定电话) _____（Email）
A2 家庭住址 Address：_____区/县_____街道/小区_____门牌号码/村
A3 您的出生日期是 Birth date _____年_____月_____日
A4 民族 Nationality ☐ 1 汉族 ☐ 2 其他（请注明）_____
A5 您的最高学历 Education ☐1 初高中以下 ☐2 大专本科 ☐3 硕士研究生以上及以上
A6 您现在的主要职业 Occupation ☐1 管理人员/干部 ☐2 技术人员 ☐3.企业主 ☐ 4 工人 ☐5 农民 ☐ 6 个体户 ☐7 其它_____
A7 上一年您的家庭年收入是：Income of a year ☐1. <2 万元 ☐ 2. (2~3.9)万 ☐3.(5.9)万 ☐4.(6~9.9)万 ☐5.(10~14.9)万 ☐6.15 万及以上 ☐9.不详
A8 填表日期 Date _____年_____月_____日

B 营养补充剂使用情况

营养补充剂种类 Nutritional supplement types	**叶酸 Folic acid**	**复合维生素 multi-vitamins**	**单一维生素 Single vitamin**
您在近三个月是否服用 Have you taken it in the last three months?	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否
您服用的什么品牌的营养补充剂？（如果没有对应选项请在其他处写明） Brand name	☐ 1、创盈金斯利安多维片 ☐ 2、福施福胶囊营养素 ☐ 3、汤臣倍健 ☐ 4、安利纽崔莱铁 ☐ 5、其他_____	☐ 1、爱乐维 ☐ 2、汤臣倍健孕妇专用 ☐ 3、惠氏玛特纳 ☐ 4、21 金维他 ☐ 5、其他_____	☐ V_C ☐ V_E ☐ V_{B1} ☐ V_{B2} ☐ 其他_____
您这三个月的服用频率？（如果食用频率小于每天/周一次，请填写每周/月食用几次，并勾出周/月） How often did	____次/☐天 ☐周 ☐月	____次/☐天☐周☐月	____次/☐天 ☐周 ☐月

营养补充剂种类 Nutritional supplement types	铁 Fe	钙 Ca	锌 Zn
您在近三个月是否服用 Have you taken it in the last	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否

you take in			

three months?			
您服用的什么品牌的营养补充剂？（如果没有，选项请在其他处写明） **Brand name**	☐ 1、汤臣倍健叶酸亚铁片 ☐ 2、安利纽崔莱铁片 ☐ 3、金康倍叶酸铁片 ☐ 4、其他_____	☐ 1、惠氏金钙尔奇 ☐ 2、君宝康孕妇钙片 ☐ 3、十月妈咪维生素 AD 钙锌咀嚼片 ☐ 4、安利钙镁片 ☐ 5、其他_____	☐ 1、美康利健 MK 硒金牡蛎锌片 ☐ 2、汤臣倍健 锌咀嚼片 ☐ 3、宫诺肽片 ☐ 4、其他_____
您这三个月的服用频率？（如果食用频率小于每天/周一次，请填写每周/月食用几次，并勾出周/月） **How often did you take in**	____次/☐天 ☐周 ☐月	____次/☐天☐周☐月	____次/☐天 ☐周 ☐月

B 营养补充剂使用情况

C 吸烟情况
C1 您有吸烟史吗？ ☐1 是 ☐0 否***(跳至 F6)*** Have you smoked cigarettes ever before?
C2 在您最近的 3 个月内，您是否吸烟？ ☐1 是 ☐0 否 Did you smoke cigarettes in 3 month
C3 如果是，您平均每天吸多少支烟？ _____ 支 On average, how many cigarettes did you smoke each day in the month after your last menstrual period？
C4 如果您曾经戒过烟，您戒了多少次？ _____ How many times did you stop smoking?
C5 在您生活的大多数时间里，您是否暴露于他人烟草烟雾中？ On most days during your pregnancy, were you exposed to someone else's cigarette smoke? ☐1 是 ☐0 否***（跳至 G1）***
C6 您在哪里暴露与烟草烟雾中 Where were you exposed to the smoke？ ☐1 仅在家中 ☐2 仅在工作单位 ☐3 在家和在工作单位均暴露
D 酒精
D1 您最近三个月的饮酒情况？During the 3 months before or during your pregnancy, did you ever drink any alcoholic beverages? ☐0.从未饮酒； ☐1.尝试饮酒（曾饮至少半瓶或一听啤酒，一小盅白酒等）； ☐2.现在饮酒（过去 30 天，至少有一天喝过一杯酒）； ☐3.重度饮酒（过去 30 天，至少有一天在 2 小时内喝过五杯酒）； ☐4.醉酒（过去 12 个月内，因喝酒太多而感到头晕/头疼/嗜睡等醉酒症状）。

核查人员签名：_____

Appendix 2b pregnant questionnaire

Name of pregnancy： _____

ID no： _____

孕期危险因素暴露调查表

Pregnancy risk factor exposure questionnaire

A 一般情况 General information

A1 您的出生日期是 Birth date _____年_____月_____日
A2 民族 Nationality ☐ 1 汉族 Han ☐2 其他 other_____
A3 您的最高学历 Education ☐1 初高中以下 Mid ☐2 大专本科 High ☐3 硕士研究生以上及以上 College
A4 您现在的主要职业 Occupation ☐1 管理人员/干部 ☐2 技术人员 ☐3.企业主 ☐ 4 工人 ☐5 农民 ☐ 6 个体户 ☐7 其它_____
A6 家庭住址：Address _____区/县_____街道/小区_____门牌号码/村
A7 联系电话（请您认真填写，以助于医生和您联系）：Contact number _____(手机) _____(固定电话) _____(Email) _____(微信号)

B 本次妊娠情况

B1 您孕前体重通常为？ Current weight _____（公斤 Kg)
B2 您身高是？ Height _____(厘米 cm)
B3 您的腰围是？Waist _____（厘米 cm）
B4 您此次怀孕的末次月经时间？ _____年_____月_____日 What was the first day of the menstrual period that came right before this pregnancy (LMP)?
B5 孕期是否发生过重大负性生活事件而使您的精神受到刺激？ ☐ 1 是 ☐ 0 否 Have you ever experienced the negative events which irritate you and generate some negative emotion?
B6 生这个孩子是您第几次怀孕？ _____次 How many times have you been pregnant?
B7 是否有不良生育史？ Did you have the adverse reproductive history? ☐ 1 有***（继续回答 B6.1）*** ☐ 0 无***（跳至 B7）***
B6.1 流产史 Abortion ☐ 1 有 ☐ 0 无
B6.2 早产史 Preterm ☐ 1 有 ☐ 0 无
B6.3 死产史 Stillbith ☐ 1 有 ☐ 0 无
B8 您是否有糖尿病和高血压疾病？Do you have hypertension or diabetes ☐ 1 是 ***(继续回答 B8)*** ☐ 0 否***（跳至 C1）*** ☐ 999 不知道***（跳至 C1）***
B9 您的直系亲属中是否患有糖尿病、高血压疾病？ s there the family history of hypertension or diabetes in children's immediate family members ☐ 1 是 ***(继续回答 B8)*** ☐ 0 否***（跳至 C1）*** ☐ 999 不知道***（跳至 C1）***
B10 若有，请选择出该亲属与您的关系**（可多选）If so, please choose the relationship with the child**
☐1. 父亲 ☐2. 母亲 ☐3. 爷爷 ☐4. 奶奶 ☐5. 兄弟 ☐6. 姐妹

C 叶酸使用

C1 在您末次月经前三个月内，您是否服用过叶酸？ ☐1 是 ☐0 否 Did you take folic acid in the month before your last period?			
C2 在您末次月经之后至今，您是否服用过叶酸？ ☐1 是 ☐ 0 否 Did you take any folic acid after your last period anddduring pregnancy ?			
	叶酸 1	叶酸 2	叶酸 3
C3 药物名称（商品名） Brand name			
C4 使用时间 When did you take it?	☐1 孕前 before pregnancy ☐2 孕后 pregnancy ☐3 孕前孕后都有 always	☐1 孕前 before pregnancy ☐2 孕后 pregnancy ☐3 孕前孕后都有 always	☐1 孕前 before pregnancy ☐2 孕后 pregnancy ☐3 孕前孕后都有 always
C5 是否在怀孕期间一直使用？ Did you take it during the rest of your pregnancy?	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否
C6 是否停止使用过？ Did you stop taking it?	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否
C7 使用频率？ How often did you take it?	____次/☐天 ☐周 ☐月	____次/☐天 ☐周 ☐月	____次/☐天 ☐周 ☐月
C8 每次的使用量 What is the usage per time?	一次_____片	一次_____片	一次_____片

D 维生素使用

D1 在您末次月经的前三个月内，您是否服用过维生素？ ☐1 是 ☐0 否 Did you take any vitamins in the three months before your last period ？			
D2 在您末次月经之后至今，您是否服用过维生素？ ☐1 是 ☐0 否 ***（跳至 E1）*** Did you take any vitamins after your last period and during pregnancy?			
	维生素 1	维生素 2	维生素 3
D3 维生素名称 Vitamin name			
D4 维生素商品名称 Brand name			
D5 是否是医生给药？ Did your doctor give it to you?	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否
D6 是否包括叶酸？ Does it contain folic acid?	☐1 是 ☐0 否 ☐999 不知道	☐1 是 ☐0 否 ☐999 不知道	☐1 是 ☐0 否 ☐999 不知道

D7 使用时间 When did you take it?	☐1 孕前 before pregnancy ☐2 孕后 pregnancy ☐3 孕前孕后都有 always	☐1 孕前 before pregnancy ☐2 孕后 pregnancy ☐3 孕前孕后都有 always	☐1 孕前 before pregnancy ☐2 孕后 pregnancy ☐3 孕前孕后都有 always
D8 是否停止使用过？ Did you stop taking it?	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否
D9 使用频率？ How often did you take it?	____次/☐天 ☐周 ☐月	____次/☐天 ☐周 ☐月	____次/☐天 ☐周 ☐月
D10 每次的使用量 What is the usage per time?	一次_____片	一次_____片	一次_____片

E 其他营养补充剂使用情况 Other nutritional supplement use

营养补充剂种类 Nutritional supplement types	铁 Fe	钙 Ca	锌 Zn
您在近三个月是否服用 Did you take it around the time you became pregnant?	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否
您服用的什么品牌的营养补充剂？（如果没有，选项请在其他处写明）Brand name	☐ 1、汤臣倍健叶酸亚铁片 ☐ 2、安利纽崔莱铁片 ☐ 3、金康倍叶酸铁片 ☐ 4、其他_____	☐ 1、惠氏金钙尔奇 ☐ 2、君宝康孕妇钙片 ☐ 3、十月妈咪维生素 AD 钙锌咀嚼片 ☐ 4、安利钙镁片 ☐ 5、其他_____	☐ 1、美康利健 MK 硒金牡蛎锌片 ☐ 2、汤臣倍健 锌咀嚼片 ☐ 3、宫诺肽片 ☐ 4、其他_____
您这三个月的服用频率？（如果食用频率小于每天/周一次，请填写每周/月食用几次，并勾出周/月）How often did you take it?	____次/☐天 ☐周 ☐月	____次/☐天☐周☐月	____次/☐天 ☐周 ☐月

F 草本药物使用

F1 在您末次月经前三个月内，您是否使用过任何一种草本药物/传统医学药物？Did you take any herbal supplements/traditional Chinese medicine in the three months before your last period ☐ 1 是 ☐ 0 否

F2 在您末次月经之后，您是否使用过任何一种草本药物/传统医学药物 Did you take any herbal supplements/traditional Chinese medicine after your last period and during pregnancy? ？ ☐1 是 ☐0 否 ***（跳至F1）***			
	草本药物 1 Herbal 1	草本药物 2 Herbal 2	草本药物 3Herbal 3
F3 草本药物类型 Name of herb	☐1 中成药 ☐2 中医草药	☐1 中成药 ☐2 中医草药	☐1 中成药 ☐2 中医草药
F4 药物名称（如果选择中医草药，请填写中药功效） The use of traditional Chinese medicine			
F5 用药持续多少天 How long did you take in?	_____天	_____天	_____天

G 吸烟情况

G1 您在怀孕前或者怀孕期间吸烟吗？ Did you smoke cigarettes before or during your pregnancy with the baby?	☐1 是	☐0 否***(跳至F9)***
G2 在您末次月经的当月，您是否吸烟？ Did you smoke during the month before your last menstrual period ?	☐1 是	☐0 否
G3 在您末次月经的一个月后（末次月经结束直至一个月后），您是否吸烟？ ***(跳至5)*** Did you smoke during the month after your last menstrual period, that is between LMP and LMP+1 month	☐1 是	☐0 否
G4 如果是，您平均每天吸多少支烟？ On average, how many cigarettes did you smoke each day in the month after your last menstrual period ？ 支		
G5 在您怀孕期间，您是否吸烟？ Did you smoke during your pregnancy?	☐1 是	☐0 否***(跳至F7)***
G6 在您怀孕期间，您平均每天吸多少支烟？ On average how many cigarettes did you smoke each	_____ 支	
G7 在您末次月经期间至今，您是否戒过吸烟？ Did you stop smoking at any time between your last menstrual period and the end of your pregnancy?	☐1 是	☐0 否 ***（跳至F9）***
G8 您戒烟有多少次？ How many times did you stop?		_____ 次
G9 在您怀孕的大多数时间里，您是否暴露于他人烟草烟雾中？ On most days during your pregnancy, were you exposed to someone else's cigarette smoke?	☐1 是	☐0 否 ***（跳至G1）***

G10 您在哪里暴露与烟草烟雾中？ Where were you exposed to the smoke? ☐1 仅在家中 ☐2 仅在工作单位 ☐3 在家和在工作单位均暴露

H 酒精

H1 在您怀孕前三个月至今，您是否饮用过任何含有酒精的饮料？ During the 3 months before or during your pregnancy, did you ever drink any alcoholic beverages?	☐1 是 ☐0 否
H2 在这三个月内，您通常每次饮几杯酒？ On those days that you drank, how many drinks did you have?	_____ 杯

I 环境暴露情况

I1 怀孕前三个月到现走，您是否染烫发? Did you dye perm in the first three months of pregnancy?	☐1 是	☐0 否
I2 怀孕前三个月到现在，后您工作的地点或家里是否装修过? Did you exposed to formaldehyde in the first three months of pregnancy?	☐ 1 是	☐0 否
I3 怀孕前后您是否接触过下列物质？ Have you been exposed to the following substances before and after pregnancy? (Toxic chemicals) ☐1 除草剂 ☐2 杀虫剂 ☐3 灭鼠剂 ☐4 有机溶剂 ☐5 消毒剂 ☐6 金属制剂 ☐7 有害气体 ☐8 有害固体		

J 药物使用情况

以下问题是有关于您在末次月经之前三个月内至今期间的用药情况

	治疗高血压药物 Medication for hypertension	胰岛素 Insulin for diabetes	口服降血糖药物 Oral hypoglycemic for diabetes	抗癫痫药物 Medications for epilepsy	每天都要服用的药物 Medications at least once a day
1 您是否使用过？ Did you take?	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否
2 您使用的药物名称？ What did you take?	_____	_____	_____	_____	_____

如果下一个问题令您感到不安且不愿意回答，请在"拒答"上打√，我们将对您所有回答进行严格保密!

	安定\有助于您放松药物 Valium\drugs to help you	使您感觉良好\精力旺盛 Make you feel	美沙酮\氧可酮\其他止痛药 Methadone oxymoron\other	可卡因 Cocaine or crack cocaine	海洛因 Heroin	大麻 Marijuana

	relax	good\have more energy	pain killers			
1 您是否使用过？ Did you take?	☐1 是 ☐0 否 ☐2 拒答 ☐9 不清楚	☐1 是 ☐0 否 ☐2 拒答 ☐9 不清楚	☐1 是 ☐0 否 ☐2 拒答 ☐9 不清楚	☐1 是 ☐0 否 ☐2 拒答 ☐99 不清楚	☐1 是 ☐0 否 ☐2 拒答 ☐9 不清楚	☐1 是 ☐0 否 ☐2 拒答 ☐9 不清楚
2 您使用的药物名称？ What did you take?	_____	_____	_____	_____	_____	_____

I 您在怀孕期间是否患过以下疾病？

I.3 发热性疾病及呼吸道感染 ☐1 是 ☐0 否

Febrile illness and respiratory infections

I.3.1 您发烧时的最高温度是多少？ _____℃

What was the highest temperature of your fever during your illness?

I.3.2 您发烧有几天？ _____天

How long did you have a fever?

I.6 其他_____

调查结束，谢谢您的配合！

调查员姓名 _____

调查日期 ___ ___/ ___ ___/ ___ ___

年 月 日

Appendix 3 Variable list

Pre-pregnant variable list

No	Variables	Data type
	General information	
1	Wife id	Text
2	Husband nation	Text
3	Husband age	N
4	Husband education	Text
5	Husband id	Text
6	Husband occupation	Text
7	Wife nation	Text
8	Wife age	N
9	Wife education	Text
10	Wife occupation	Text
11	Tel No	Text
12	Mobile phone No	Text
	Medical history	
13	Female anemia	Category
14	Female EH	Category
15	Female heart disease	Category
16	Female DM	Category
17	Female epilepsia	Category
18	Female thyroid disease	Category
19	Female CGN	Category
20	Female mental disease	Category
21	Female tumour	Category
22	Female TB	Category
23	Female HBV	Category
24	Female VD	Category
25	Male anemia	Category
26	Male EH	Category
27	Male heart disease	Category
28	Male DM	Category
29	Male epilepsia	Category
30	Male thyroid disease	Category
31	Male CGN	Category
32	Male mental disease	Category
33	Male tumour	Category
34	Male TB	Category
35	Male HBV	Category
36	Male VD	Category

	Vaccine	
37	Female rubella vaccine	Category
38	Female hepB vaccine	Category
39	Male hepB vaccine	Category
	Drug use	
40	Female current medicine	Category
41	Female medicine name	Category
42	Male current medicine	Category
43	Male medicine name	Category
	Childbearing history	
44	Birth history	Category
45	Pregnancy times	Category
46	Live birth	Category
47	Dead fetus	Category
48	Dead birth	Category
49	Term delivery	Category
50	Premature delivery	Category
51	Natural abortion	Category
52	Abactio	Category
53	Children number	Category
54	Birth defect	Category
55	Defect type	Category
56	Menarche age	Category
57	Period menstruation	Category
58	Menstrual cycle	Category
59	Menstrual quantity	Category
60	LMP	Date
	Family history of disease	
61	Female family history thalassemia	Category
62	Female family history albinism	Category
63	Female family history favism	Category
64	Female family history hemophilia	Category
65	Female family history CHD	Category
66	Female family history DS	Category
67	Female family history openNTDs	Category
68	Female family history DM	Category
69	Female family history dysnoesia	Category
70	Female family history daysaudia	Category
71	Female family history viaual disorder	Category
72	Female family history neuropsychiatric	Category
73	Female family history other birth defects	Category
74	Female family history fetal death	Category
75	Female family history intermarry	Category

76	Female family history relations	Category
77	Male family history thalassemia	Category
78	Male family history albinism	Category
79	Male family history favism	Category
80	Male family history hemophilia	Category
81	Male family history CHD	Category
82	Male family history DS	Category
83	Male family history openNTDs	Category
84	Male family history DM	Category
85	Male family history dysnoesia	Category
86	Male family history dysaudia	Category
87	Male family history viaual disorder	Category
88	Male family history neuropsychiatric	Category
89	Male family history other birth defects	Category
90	Male family history fetal death	Category
91	Male family history intermarry	Category
92	Male family history relations	Category
	Anthroposomatology	
93	Female height	Numeric
94	Female weight	Numeric
95	Female BMI	Numeric
96	Female heart rate	Numeric
97	Female SBP	Numeric
98	Female SDP	Numeric
99	Male height	Numeric
100	Male weight	Numeric
101	Male BMI	Numeric
102	Male heart rate	Numeric
103	Male SBP	Numeric
104	Male SDP	Numeric
	Lab data	
105	Leucorrhea check	Numeric
106	Clue cell	Numeric
107	Monilia infection	Numeric
108	Trichomomas	Numeric
109	Cleanness	Numeric
110	Whiff test	Numeric
111	PH	Numeric
112	Wom blood analysis	Numeric
113	Female hb	Numeric
114	Female wbc	Numeric
115	Female rbc	Numeric
116	Wom urine test	Numeric

117	Female ABO	Numeric
118	Female Rh	Numeric
119	Female GLU	Numeric
120	Female GLU levels	Numeric
121	Female NG	Numeric
122	Female chlamydia	Numeric
123	Female syphilis	Numeric
124	Female HIV	Numeric
125	Female ALT	Numeric
126	Female ALT levels	Numeric
127	Female HBs-Ag	Numeric
128	Female HBs-Ab	Numeric
129	Female HBe-Ag	Numeric
130	Female HBe-Ab	Numeric
131	Female HBc-Ab	Numeric
132	Female HCV-Ab	Numeric
133	Female CMV IgM	Numeric
134	Female CMV IgG	Numeric
135	Female RV IgM	Numeric
136	Female RV IgG	Numeric
137	Female TOX IgM	Numeric
138	Female TOX IgG	Numeric
139	Male blood analysis	Numeric
140	Male hb	Numeric
141	Male wbc	Numeric
142	Male rbc	Numeric

During-pregnancy variable list

	Basic information	
1	Hospital card number	Text
2	Inpatient number	Text
3	Name	Text
4	Age	Numeric
5	Pregnant times	Numeric
6	Delivery times	Numeric
7	Last menstrual period	Date
8	Gestational week at the first visit	Numeric
9	The first visit date	Date
10	Height	Numeric
11	Weight	Numeric
12	Systolic blood pressure at the first visit	Numeric
13	Diastolic blood pressure at the first visit	Numeric

14	Occupation	Text
15	Education	Text
	Antenatal care record	
16	Weight at each antenatal care	Numeric
17	Systolic blood pressure at each antenatal care	Numeric
18	Diastolic blood pressure at each antenatal care	Numeric
19	Gestational week at each antenatal care	Numeric
20	Antenatal care date	Date
	Lab data	
21	Cytomegalovirus	Numeric
22	Cytomegalovirus examination date	Date
23	Rubella virus	Numeric
24	Rubella virus examination date	Date
25	Toxoplasmosis	Numeric
26	Toxoplasmosis examination date	Date
27	Syphilis screening	Numeric
28	Syphilis screening examination date	Date
29	Fasting blood-glucose	Numeric
30	Fasting blood-glucose examination date	Date
31	HCT(hematokrit)	Numeric
32	HCT(hematokrit) examination date	Date
33	Serum folate	Numeric
34	Serum folate examination date	Date
35	HCY(homocysteine)	Numeric
36	HCY(homocysteine) examination date	Date
37	OGTT 0 hours	Numeric
38	OGTT 1 hours	Numeric
39	OGTT 2 hours	Numeric
40	OGTT examination date	Date
41	Triglyceride	Numeric
42	Triglyceride examination date	Date
43	Total cholesterol	Numeric
44	Total cholesterol examination date	Date
45	Hemoglobin	Numeric
46	Hemoglobin examination date	Date
	Delivery date	
47	Gestational week at delivery	Numeric
48	Delivery mode	Text
49	Birth weight	Numeric
50	Birth weight(second baby)	Numeric
51	Systolic blood pressure at delivery	Numeric
52	Diastolic blood pressure at delivery	Numeric
53	Apgar scoring	Numeric

54	Delivery date	Date
55	Birth defect records	Text
56	Weight blood pressure at delivery	Numeric
Offspring variable list		
	0 - 6 years (6 weeks, 6 months, 12 months, 36 months, 60 months)	
1	Name	Text
2	ID number	Text
3	Weight	Numeric
4	Height/ Length	Numeric
5	Vision detection	Numeric
6	DDST: Denver developmental screening test	Numeric
	6 - 18 years (each year)	
7	Weight	Numeric
8	Height	Numeric
9	Diastolic blood pressure	Numeric
10	Systolic blood pressure	Numeric
11	Vision detection	Numeric
12	Vital capacity	Numeric
13	Physical fitness measurement (running, jumping, solid balls, etc)	Text
The clinical diagnosis during 0 – 18 years will be extracted from the routine medical system each year.		

Future Plan of Shanghai PreConception Cohort, Shanghai, China (SPCC)

The Shanghai PreConception Cohort (SPCC) is a long-term cohort and meets the "Cohort profile" requirement. We have a complete plan to follow-up offspring to the age of 18 years old. The cohort will be financially supported by different grants. The current manuscript focuses on the first phase, the establishment of the baseline and our first main outcome, the congenital heart disease (CHD).

The second phase of this cohort study is from birth to 6 years old. Follow-up work will rely on Shanghai Community health care centers-child care. The third phase is from 6 to the age of 18 (school age). We will cooperate with Shanghai Student Health and Fitness Surveillance Center for the follow-up of the school age children.

Multiple outcomes for pediatrics, including growth, physical and neuro-developments, obesity, common and rare diseases will be investigated. Nested case-control design will be used for rare outcomes.

The data collection plan for infants and children

	At birth	6 weeks	6 months	12 months	36 months	60 months	6 – 18 years*
General information (gender, ID number, Home Address, etc)	√	√					
Delivery outcome (Congenital malformation, preterm birth, miscarriage, stillbirth)	√						
Body measurement(weight, height, waistline, hipline)	√	√	√	√	√	√	√
Diet investigation (questionnaire)	√	√	√	√	√	√	√
Neurobehavioral developmental assessment (DDST[$])		√	√	√	√	√	
Anthropometrics data (Shanghai Community health care centers-child care and Shanghai Student Health and Fitness Surveillance Center) weight, height, waist circumference	√	√	√	√	√	√	√
Physical fitness measurement (running, jumping, solid balls, etc)							√
Blood pressure measurement, annually							√
Hemachrome (anemia)							√
Renal functions, at grade 9 and 12							√
Cardiovascular-related chronic diseases (Hypertension, Obesity, Diabetes/Pre-diabetes and Dyslipidemia)							√
Venous blood[#]							√

Note: * Shanghai Student Health and Fitness Surveillance Center routinely conducts all-round physical examinations for students in the school as a health care policy for Shanghai students. It is usually in September-November of each year.

$ DDST: Denver developmental screening test.

Venous blood will be collected at 12, 15 and 18 years of age.

BMJ Open

Cohort profile: The Shanghai PreConception Cohort (SPCC) for the association of periconceptional parental key nutritional factors with health outcomes of children with congenital heart disease

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Cohort profile: The Shanghai PreConception Cohort (SPCC) for the association of periconceptional parental key nutritional factors with health outcomes of children with congenital heart disease

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ABSTRACT (292)

Purpose: The Shanghai PreConception Cohort (SPCC) was initially established to investigate the associations of parental periconceptional nutritional factors with congenital heart disease (CHD) but has further analyzed child growth and development and pediatric diseases.

Participants Preparing-for-pregnancy couples who presented at Shanghai preconception examination clinics and early pregnant women before 14 gestational weeks were enrolled to comprise the periconceptional baseline study population. General characteristics, routine clinical data, and consumption of diet supplements, such as folic acid and multivitamins, were collected. Blood samples were obtained at preconception and early, middle, and late gestation using standard procedures. Multiple nutritional factors, including folate, homocysteine, vitamin A, vitamin D, vitamin E, and metals, in the blood samples of participants, selected using a case-control design, were examined. Genomic DNA was extracted.

Findings to date The baseline population included 8045 preconception couples, 3054 single women, and 15,615 early-pregnant women. Data from 12,402 births were collected, and follow-up of the cohort for other outcomes is ongoing. Currently, 151 cases of CHD were identified after birth. The pilot analysis in a small subgroup showed that approximately 20.0% of preconception women and 44.9% of early-pregnant women had red blood

cell folate levels that met the international recommendation for preventing neural tube defects.

Future plans

Once a sufficient number of CHD cases are achieved, we will investigate the quantitative association of preconception red blood cell folate levels with CHD using a nested case-control design. The SPCC will be followed for 18 years to investigate extensive outcomes of growth, development, obesity, and common and rare diseases during childhood and adolescence according to our plan. Blood nutritional factors will be examined in participants selected for specific aims. The SPCC will also allow for prospective cohort studies on extensive research questions.

Trial registration number: NCT 02737644

Strengths and limitations of this study

- The SPCC is the first prospective birth cohort with CHD as primary outcome with recruitment starting from the preconception stage. Temporal sequence of exposures and outcomes can be achieved for causal inference of birth defects and other diseases that develop during the early stage of gestation.
- Preconception blood samples were appropriately collected and stored, which allow for the examination of individual blood levels for nutritional factors and other exposures.
- Preconception clinical data and blood samples from both the father and mother were collected to determine the effect of both maternal and paternal genetic and nutritional factors on fetal and pediatric diseases.
- Although the response rate was high (>95%), preconception participants were recruited from the population who voluntarily presented at preconception physical examination sites in Shanghai. They may have stronger willingness for a healthy pregnancy, which may induce selection bias.
- Biological samples (cord blood, placenta) of the newborns were not collected.

INTRODUCTION

Congenital heart disease (CHD) is a common congenital malformation that seriously affects children's quality of life.[1] CHD is a leading cause of infant death in high-income countries, affecting 8 of 1000 live births.[2] According to the report from National Health and Family Planning Commission of the People's Republic of China, CHD accounts for about a quarter of the birth defects of newborns in China, ranking first among birth defects.[3] In a prospective, nationwide large-scale study in >120,000 newborns in China in 2013, the prevalence of CHD in live births was 8.94‰. The incidence of severe CHD was 2.9‰.[4]

The cause of CHD is multifactorial. With the development of genetic engineering technology, genetic factors have been better understood in the past decade.[5] Multiple environmental risk factors have been reported in epidemiological studies, maternal social variables such as occupation, educational background, health status, unhealthy lifestyle, maternal medical history and emotional status, family history of the disease, and consanguineous marriages.[6-10] Additionally, maternal key nutrients are related to the risk of offspring's CHD as a modifiable environmental factor during periconception.[11, 12] The periconceptional intake of folic acid supplement has been shown to reduce the risk of CHD,[13, 14] and women worldwide have been recommended to use folic acid supplements before

conception and at the beginning of pregnancy. Awareness of the relationship between folic acid deficiency and CHD is actually a byproduct finding from the well-known Hungarian randomized control trials (RCT) study of folic acid supplementation to prevent neural tube defects. The study found that prenatal supplementation with a vitamin complex containing 0.8 mg of folic acid daily reduced the incidence of congenital neural tube defects. Additionally, the incidence of various heart defects has also been reduced by nearly half.[15] Longitudinal data from >1,000,000 births in Canada in 22 years, from 1990 to 2011, also showed that food fortification with folic acid reduced the risk of CHD by 20–30% [14] The current guideline for folic acid supplementation recommends that all women of childbearing potential be supplemented with at least 0.4 mg folic acid daily prior to conception and during pregnancy, which is designed to prevent neural tube defects.[16] However, excessive folic acid intake may increase the risk of cancer,[17] vitamin B12 deficiency,[18] and autism spectrum disorder.[19] The optimal dose of folic acid for preventing CHD warrants further investigation. Additionally, most previous studies only focused on the folic acid supplement or serum folate level during or after pregnancy, which may not be the optimal period and method to reflect the exposure level to the risk of CHD.

To investigate the association between parental periconceptional key

nutritional factors, such as folate level, with the development of CHD and explore the cutoff biomarker levels, we conducted the Shanghai PreConception Cohort (SPCC) and a nested case-control analysis.

The SPCC was initiated primarily to evaluate CHD. However, based on the strengths of its baseline data collection, it has received attention and support, with improved additional extensive outcomes for children that will be followed for a longer term.

COHORT DESCRIPTION

Who is in the cohort?

The SPCC recruited parent-planning women and men who were permanent residents and voluntarily presented at preconception clinical clinics at 28 maternity institutions in 10 districts of Shanghai (Minhang District, Huangpu District, Xuhui District, Changning District, Jing'an District, Putuo District, Yangpu District, Pudong District, Songjiang District, and Qingpu District) from March 2016 to December 2018. The preconception examination policy in the city of Shanghai provides a unique opportunity and clinical resources to support recruitment in the SPCC. Since 2010, married couples in Shanghai have been encouraged to attend a free preconception health examination. Moreover, these maternity institutions receive strong local administrative support and integrated maternal healthcare networking, providing service to 150,000–200,000 annual deliveries in Shanghai. Couples who were present

at preconception clinics, living in Shanghai, preparing for pregnancy within 1 year, and planning to receive antenatal care and deliver in Shanghai, were eligible for the study. Written informed consent was obtained from all participants before data collection. Additionally, we recruited early-pregnant women at their first antenatal examination who were at <14 gestational weeks. These two groups of participants comprised the periconceptional baseline study population.

The first primary outcome of the SPCC is CHD. The hypothesis is that maternal preconception serum or red blood cell (RBC) folate level is quantitatively associated with CHD development in the offspring. The study design and protocol have been registered in the ClinicalTrials Registry (NCT 02737644).

As shown in Figure 1, the baseline population will be followed until delivery, and their infants will be followed until 18 years of age (Figure 1).

Follow-up procedure

Upon enrollment, the participants completed the questionnaire of key nutrient supplementation and blood sample collection. When participants became pregnant, the same investigations (questionnaire/blood sample collection) were conducted during early pregnancy (first antenatal visit at 16–20 gestational weeks). Pregnancies were followed along with routine maternal healthcare procedures. Blood samples were also collected at the

second (24–28 gestational weeks) and third trimester (32–34 gestational weeks). The follow-up data of CHD outcome and birth were obtained through the Shanghai Neonatal CHD Screening Platform (Figure 1).

As shown in Figure 1, outcomes at birth, from infancy to childhood (preschool phase) and between 7 and 18 years (school ages), will be collected or extracted from multiple public platforms and data sources. First, preconception clinical visit data from the Preconception Care Electronic Data System, supported by the national and local government, were collected, including height, weight, age, infections, sexually transmitted disease, and family history. Second, the routine pregnancy data were obtained from the Maternal Clinic Antenatal Medical Record System, managed by the Shanghai Center for Women and Children's Health, including height, gestational weight, last menstrual period, childbearing history, delivery outcomes, infections, hematocrit, coagulation function, and liver and kidney function. Lastly, the maternal and neonatal data at delivery were obtained from the Shanghai Neonatal CHD Screening Platform, including birth weight, CHD diagnosis, birth defects, and Apgar score. Additionally, we will work with the Shanghai Student Health and Fitness Surveillance Center to obtain outcome data. The personal national identification card numbers of participants are applied as index variables through multiple data sources. The detailed variable list and codebook of data collection are presented in

Appendix 1.

During the first phase of the cohort, from preconception to delivery, comprehensive strategies were used to retain participants in the study. For mothers, we provided a variety of engagement activities including green channel (fast track) to their antenatal care to provide convenience and save their time in hospitals. We also provided a contact number on the participant card to answer their calls or queries about the study procedures. Site investigators at early pregnancy clinics in collaborative hospitals were provided a smartphone application to help timely identification of recruited cohort participants and to manage data and blood sample collection procedures. We also provided green channel echocardiography for diagnosing CHD in all site hospitals to enhance the compliance of participants. Moreover, an automated text message system is adopted to remind participants of schedules and follow-up appointments.

Study measures

Personal characteristics questionnaires

As shown in Figure 1, Questionnaire 1 was administered during recruitment at preconception examination sites, and Questionnaire 2 was administered at early pregnancy sites to collect information on the consumption of folic acid and vitamin supplements and on the brand and content of nutritional supplement. Information on the demographics, maternal education,

sociodemographic status, occupation, smoking status, alcohol consumption, body mass index (BMI), medication, and health status was also included. In addition to the content of Questionnaire 1, Questionnaire 2 added drug information, reproductive history, and health status. Questionnaire 1 for baseline and Questionnaire 2 for the first antenatal visit at early pregnancy are presented in Appendix 2a and Appendix 2b.

Blood sample collection

In this study, the remaining blood samples for routine clinical blood examination were collected. The blood sample for routine clinical examination was usually 5 mL and extracted in the morning. Routine clinical blood examination was performed at room temperature (20℃–25℃). The remaining samples (fasting serum and Ethylene Diamine Tetraacetic Acid (EDTA) anticoagulation) of peripheral venous blood from routine laboratory clinical blood examination were retained. These blood samples were temporarily stored in a 4℃ refrigerator for dispensing within 6 h and transferred to a -20℃ refrigerator. After completion of blood sample distribution, the serum and whole blood were stored at the site laboratory and then transported by three trained investigators to the central laboratory for storage in -80℃ freezers for 2 weeks. Sampling tubes were made of a light-proof material, and the process of collecting blood samples was completely protected from light.

Examination of key nutritional factors in blood samples

The examinations will be conducted in participants selected by nested case-control designs based on specific aims.

(1) *RBC folate, serum folate, serum homocysteine, vitamin D, vitamin B12, and serum ferritin*

EDTA anticoagulated blood samples were collected to measure RBC folate, serum folate, serum homocysteine, vitamin D, vitamin B12, and serum ferritin. All six biomarkers were analyzed using electrochemiluminescence assays (ARCHITECT i2000SR Analyzer, Abbott Laboratories, USA). A standard solution with known level (produced by Abbott Laboratories) was used daily to control the quality before the measurement. If the quality control level was out of range, the measurement would be suspended and adjusted. External quality control was conducted with the control laboratory data program from Abbott Laboratories (Abbott Laboratories, Shanghai, China). RBC folate levels were adjusted for hematocrit. If the RBC folate level is <126.0 ng/mL or >651.1 ng/mL, adjustment was needed based on the serum folate level. The hematocrit data were extracted from the hospital laboratory information system. These examinations were performed in the central laboratory of the Children's Hospital of Fudan University.

(2) Vitamin A and E

The serum vitamin A and E levels were quantitatively determined by liquid chromatography tandem-mass spectrometry in the central laboratory of the

Children's Hospital of Fudan University. The testing instrument was triple quadrupole mass spectrometer LC/MS/MS system (API 3200MDTM, AB Sciex Pte. Ltd.). A standard solution of vitamin A-d6 and E-d6 was applied as an internal standard.

(3) *Glycemic and lipid profiles*

Fasting serum cholesterol, high-density lipoprotein, low-density lipoprotein, triglyceride, and glucose levels were measured using the Beckman Coulter AU chemistry analyzers (Beckman Coulter Inc., USA) in the central laboratory of the Children's Hospital of Fudan University.

(4) Metals

Serum levels of Mg, Fe, Zn, Se, Mn, As, Cu, and Ca were analyzed by inductively coupled plasma mass spectrometry (Inductively Coupled Plasma Optical iCAP6300, Thermo Scientific, USA) in standard mode.[20] The metal examination was conducted in the Instrumental Analysis Center of Shanghai Jiao Tong University, which is a national key laboratory.

(5) Genomic DNA extraction

Genomic DNA of all participants was extracted using a magnetic bead-based kit (TGuide M16 Automatic Nucleic Acid Extractor (OSE-M16), Tiangen Biotech (Beijing) Co., Ltd., China) from 2 mL of EDTA anticoagulated whole blood sample after routine blood examination. Genomic DNA samples were stored for future studies. An average of 150 ng DNA was

available. Similar to that of blood chemicals, future genetic variant genotyping will be performed in selected participants according to the nested case-control design for specific aims. Currently, there are no candidate genes or variants that are listed.

Outcomes: CHD in neonates

The diagnosis of CHD was the primary outcome of the study at this stage and obtained from the Shanghai Neonatal CHD Screening Platform, which was initiated as a routine screening tool for newborns in Shanghai since June 1, 2016. The standard protocol of CHD screening in the platform was previously described in detail.[21] All newborns underwent the screening using a double-index method (i.e., cardiac murmur auscultation and pulse oximetry) at 6–72 h after delivery, and screening-positive newborns would undergo subsequent echocardiography for further confirmative diagnosis.

SPCC will also collect data on other birth defects as secondary outcomes, including Down's syndrome, neural tube defects, hydrocephalus, digestive tract malformations, urinary malformations, and behavioral cognitive developmental disorder. After delivery, the infants underwent routine childcare procedures organized by the Shanghai Child Health Care System, which are administered by the Shanghai Center for Women and Children's Health. All records of birth defects, which were diagnosed after birth, as well as routine neurodevelopmental examinations and longitudinal

anthropometric data, were abstracted from the system by a professional clinical team from the Children's Hospital of Fudan University (for details of the types of birth defects, please see Appendix 3)

Statistical methods

To investigate the association of maternal preconception nutrition factor levels with offspring CHD risk, a nested case-control study will be conducted. The control will be matched by age and site.

The sample size for the nested case-control analysis was planned as 180 CHD cases and 720 matched controls to detect maternal folate deficiency with a prevalence of 50% in controls (with an odds ratio [OR] of 1.6) to achieve a power of 80% at an alpha of 0.05. Based on CHD incidence >8.94 per 1,000 live births,[4] 20,000 pregnancies will be needed. For a continuous nutrient variable with standard deviation of 2.0, 50 matched pairs (1:4) are required to achieve 90% power to detect an OR of 1.3 calculated using conditional logistic regression with a 0.05 significance level.[22, 23] Once a sufficient number of CHD cases are achieved, the quantitative association of preconception RBC folate levels with CHD development using nested case-control design will be investigated.

Conditional multivariate logistic regression will be used in the association analysis with offspring CHD status being the dependent variable and nutrition factor levels as exposure variables, after adjustment for all potential

paternal and maternal covariates. ORs and 95% confidence intervals will be reported. To explore a potential cutoff point of the nutrition factor levels that significantly increases the risk of CHD, a dummy variable will be set by categorizing the maternal preconception nutrition factor levels based on the distribution of the control group. The dose-response relationship will be also be analyzed. Sensitivity analysis will include non-conditional logistic regression analysis or generalized estimating equations model or generalized linear models, when necessary.

FINDINGS TO DATE

The SPCC started recruitment in March 2016. As shown in Figure 2, by December 2018, we consecutively recruited 19,144/19,563 (97.9%) participants at preconception settings, including 8045 couples and 3054 single women, and an additional 15,615/16,201 (96.4%) pregnant women at maternity hospitals with gestational age <14 weeks. Table 1 describes the basic demographic characteristics of the preparing-for-pregnancy participants and pregnant women. The average age of the preconception population was 29.9 (SD, 3.9) years for women and 31.4 (SD, 4.5) years for men, 31.4% of men and 2.2% of women were smokers, and 61.4% of men, and 30.9 of women had an alcohol drinking habit. In pregnant women, the average age was 29.9 (SD, 4.0) years, with half having their first pregnancy. Preconception women were similar in age but different in education levels

and occupation. The prevalence of smoking and alcohol drinking was much lower. The descriptive data of Table 1 are partly included in another manuscript.

Table 1. Sociodemographic characteristics of participants, including 8,045 couples (parents) and 15,615 pregnant women, who were enrolled in the Shanghai PreConception Cohort

Characteristics	Couples (parents) who completed preconception questionnaires				Additional pregnant women who completed both preconception and first-trimester questionnaires		*P*-value*
	Men (n=8045)		Women (n=11099)		Pregnant women （n=15615）		
Age (years), mean±SD	8045	31.4±4.5	11099	29.9±3.9	15615	29.9±4.0	0.995
Ethnicity, n (%)	8001		11032		15587		0.407
Han nationality		7843 (98.0)		10773 (97.6)		15245 (97.8)	
Others		158 (2.0)		259 (2.4)		342 (2.2)	
Educational level, n (%)	7996		10988		15553		<0.001
High school or less		613 (7.7)		947 (8.6)		2117 (13.6)	
College or above		7383 (92.3)		10041 (91.4)		13436 (86.4)	
Annual household income, n (%)	7134		8747		NA		–
<￥100,000		1555 (21.8)		2114 (24.2)			
≥￥100,000		5579 (78.2)		6636 (75.8)			
Occupation, n (%)	7937		10909		15453		<0.001
Entrepreneur		190 (2.4)		156 (1.4)		171 (1.1)	
Farmer		35 (0.4)		49 (0.5)		79 (0.5)	
Self-employed		261 (3.3)		329 (3.0)		862 (5.6)	
Manager		1712 (21.6)		2101 (19.3)		2650 (17.2)	
Technician		2998 (37.7)		2269 (20.8)		2403 (15.6)	
Company clerk		678 (8.5)		857 (7.9)		3422 (22.1)	
Others		2063(26.0)		5148 (47.2)		5866 (37.9)	

Attending preconception pregnancy examination, n (%)	NA		NA	15594	–
Yes				3374 (22.5)	
No				11654 (77.5)	
Number of pregnancies, n (%)	NA		NA	15028	–
1				7804 (49.9)	
2				4598 (30.4)	
≥3				3192 (19.7)	
Miscarriage or stillbirth, n (%)	NA		NA	15532	–
Yes				4838 (31.1)	
No				10694 (68.9)	
Smoking, n (%)	8018		11064	15571	<0.001
Yes		2517 (31.4)	248 (2.2)	161 (1.0)	
No		5501 (68.6)	10816 (97.8)	15410 (99.0)	
Alcohol drinking, n (%)	7883		10906	15566	<0.001
Yes		4840 (61.4)	3374 (30.9)	1599 (0.3)	
No		3043 (38.6)	7532 (69.1)	13976 (89.7)	
Location of home, n (%)	8045		11099	NA	–
Developed districts		4473 (55.6)	4728 (42.6)		
Developing districts		3572 (44.4)	6371 (57.4)		

*Comparisons between preconception women and pregnant women. t-tests were used to compare numerical variable (age). Chi-square tests were used to compare categorical variables (ethnicity, educational level, occupation, smoking, and alcohol drinking). Of 8045 preconception men, values were missing in ethnicity (n=44), educational level (n=49), annual household income (n=911), occupation (n=108), smoking (n=27), and alcohol drinking (n=162). Of 11099 preconception women, values were missing in ethnicity (n=67), educational level (n=111), annual household income (n=2352), occupation (n=190), smoking (n=35), and alcohol drinking (n=193). Of 15615 pregnant women, values were missing in ethnicity (n=28), educational level (n=62), occupation (n=162), attending preconception pregnant examination (n=21), parity (n=587), miscarriage or stillbirth (n=83), smoking (n=44), and alcohol drinking (n=49).

By the end of December 2018, the last participants recruited at early pregnancy were due for delivery; however, we have obtained birth records of only 12,402 newborns. The follow-up of outcomes of the remaining participants is ongoing (shown in Figure 2). A total of 151 cases of CHD were identified through the CHD screening platform: 131 cases from the early pregnancy sample and the remaining 20 cases from the preconception sample. The prevalence of CHD in live births is 10.5‰ (131/12,402) based on the present available data.

We conducted a small pilot study in April 2017 to determine blood levels of nutritional factors, including serum folate, RBC folate, vitamin A, vitamin E, and vitamin D. The blood samples from 627 women were selected consecutively from the preconception sample according to those who were identified as pregnant. Additionally, 597 women who were consecutively recruited from the antenatal care clinics were selected. As shown in Table 2, the median RBC folate levels were 247.0 ng/mL (Interquartile range (IQR), 184.8–340.5 ng/mL) in preconception women and 417.4 ng/mL (IQR, 308.6–544.2 ng/mL) in early-pregnant women. Moreover, 20.0% of preconceptional participants and 44.9% of pregnant participants had a folate level >400 ng/mL, which was suggested as optimal level for preventing neural tube development defects.[24, 25] These results suggest that effort is urgently needed to improve folic acid supplementation in the preparing-for-

pregnancy population, especially before pregnancy.

Table 2. Distributions of biomarkers in women before and at early gestation (pilot study)

Biomarker	Preconception		Early pregnancy	
	n	Level	n	Level
Serum folate, median (IQR), ng/ mL	620	9.7 (6.5, 13.8)	577	14.5 (11.2, 16.4)
RBC folate, median (IQR), ng/ mL	570	247.0 (184.8, 340.5)	587	417.4 (308.6, 544.2)
Homocysteine, median (IQR), μmol/L	624	6.5 (5.2, 8.6)	599	4.2 (3.5, 5.2)
Vitamin B12, median (IQR), pg/mL	625	495.2 (394.2, 639.0)	600	388.5 (289.4, 511.4)
Vitamin D, mean ±SD, ng/mL	607	16.3±6.0	578	15.5±6.1

Based on the SPCC, the possible scope of research questions and available types and number of biosamples and biomarkers that can be examined are shown in Table 3.

Table 3. Biosamples collected and biomarkers that can be examined in the SPCC

Biosamples available in participants	Available sample type and volume	Time		
		Preconception+early pregnancy (Baseline)	24–28 weeks	32–36 weeks
Mother		(n=25,487)	(n=8668)	(n=7522)
	Serum, 200 µL*3	Yes	Yes	Yes
	Whole blood	Yes	Yes	Yes
	Genomic DNA, 150 ng	Yes	Yes	Yes
Father		(n=7151)	-	-
	Serum, 200 µL*3	Yes	NA	NA
	Whole blood	Yes	NA	NA
	Genomic DNA, 150 ng	Yes	NA	NA
Child	NA			

Scope of research questions

1. Quantitative association of preconception key nutrition factor levels (e.g., serum and RBC folates and related biomarkers, such as homocysteine and vitamin B12) with CHD, currently, and other folate-sensitive birth defects.
2. Quantitative association of periconceptional maternal and paternal key nutrition factor levels (preconception and dynamic levels during gestation) with important maternal and neonatal gestational complications, neurodevelopment of infants, childhood obesity, and clinical pediatric diseases.
3. Periconceptional maternal and paternal folate level with autism spectrum disorder, allergy, and asthma in children.

Biomarkers that will be examined in different types of biosamples:

1. Biomarkers based on serum sample:

a) Folate and related markers: serum folate, homocysteine
b) Other vitamins: vitamin A, vitamin B12, vitamin E, and vitamin D
c) Macro- and micrometals: Mg, Fe, Zn, Se, Mn, As, Cu, Ca, etc. – mg/L
d) Serum ferritin
e) Fasting glycemic and lipid profiles: fasting glucose, total cholesterol, triglyceride, high-density lipoprotein, and low-density lipoprotein

2. Whole blood sample: RBC folate
3. Genomic DNA sample: candidate genetic variants or genome-wide variants are possibly examined

FUTURE PLANS

We have a complete plan to follow offspring until the age of 18 years. The cohort will be financially supported by different grants. The current manuscript focuses on the first phase, the establishment of the baseline and our first main outcome, CHD. The data collection plan for infants and children (from birth to 6 years [pre-school stage] and from 6 to 18 years [school age]) is included. During the stage of 0–6 years, the neurodevelopmental data will be collected from routine childcare clinical visits at birth, 6 weeks, 6 months, 12 months, 36 months, and 60 months through the cooperating medical institutions. Physical measurement data and dietary intake information can also be collected at this stage. During the stage of 6–18 years, we plan to follow their growth (height, weight, blood pressure), mainly relying on the annual physical examination results of the Shanghai Student Health and Fitness Surveillance Center System. Multiple outcomes for children, including growth and development, cardiovascular diseases, neurodevelopment, metabolic diseases, obesity, and hypertension, will be investigated. Please see Appendix 4 for details.

STRENGTHS AND LIMITATIONS

Compared with existing birth cohorts, there are three important strengths in our cohort. First, the SPCC is the first prospective birth cohort with CHD as primary outcome and recruitment starting from preconception. Blood

samples were collected and stored, which allows direct measurement of individual exposure levels before the development of CHD and causal inference. Temporal sequence of exposures and outcomes can be achieved for causal inference of birth defects and other diseases that develop during the early stage of gestation. To date, no published studies have measured maternal blood folate levels before conception and associated it to disease outcomes. Second, this cohort also allows the investigation of associations between periconceptional maternal and paternal nutrition exposures and other birth defects, early-onset diseases, and neurodevelopmental outcomes. Preconception blood samples were appropriately collected and stored, which allows the examination of individual blood levels of nutritional factors and other exposures. Lastly, both paternal and maternal clinical data and blood samples before conception were collected, which will allow for the testing of the effects of both maternal and paternal genetic and nutritional factors on fetal and pediatric diseases.

Two limitations of this cohort study should be considered. First, there are approximately 200,000 pregnant women giving birth annually in Shanghai, and approximately 20,000 of them will participate in the free preconception care in Shanghai, where participants were recruited consecutively. Although the response rate was high (>95%), preconception participants were recruited from a population who voluntarily presented in Shanghai

preconception physical examination sites, who may have a stronger willingness for a healthy pregnancy. This may induce selection bias. Second, in this study, biological samples (cord blood, placenta) of the newborns are not collected. We plan to provide new informed consent to the family who are willing to participate in future studies to collect biological samples not previously mentioned. Furthermore, electrochemiluminescence assay was used to examine serum and RBC folate levels, which is different from the widely used microbiological assay. This will not result in bias in the association analysis, but comparison with international populations needs caution.

COLLABORATION

Investigators with an interest in hypotheses related to SPCC (who meet the requirements of current approvals) are welcome to contact Dr. Guoying Huang or Weili Yan. A “Research Collaboration application” should be sent to the corresponding author by email. The application should include a brief description of the project.

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Contributors: Substantial contributions to the conception or design of the study were made by GH and WY. YZ and DW prepared the original draft of the manuscript. YZ, DW, YY, JY, ML, MJ, YD, and XC led the study implementation at participating sites. DW and YZ were responsible for the day-to-day project management at each site. XM and WS were responsible for the biobank of the cohort. All authors provided critical review of the manuscript for important intellectual content and approved the final version.

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Patient and public involvement: No patients were directly involved in the design and conception of this study.

Conflict of interest: The authors have no conflicts of interest.

Ethics approval: This study was approved by the Ethics Committee of the Children's Hospital of Fudan University, Shanghai, China.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement: The study data are not freely available due to confidentiality reasons, but the research team welcomes potential collaboration with other researchers. For further information, contact the author GH (gyhuang@shmu.edu.cn)

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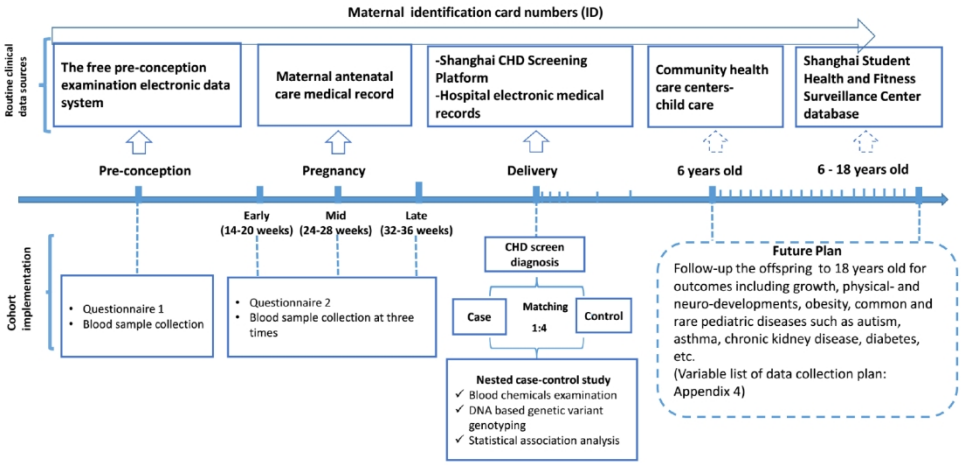
Figure 1. Protocol and follow-up of the Shanghai PreConceptional Cohort (SPCC)

The baseline population of SPCC consisted of women and men at the periconception stage (couples who are preparing for pregnancy) and pregnant women at the early gestation stage. The cohort includes three phases, from periconception to birth (Phase I, perinatal phase), from birth to 6 years (Phase II, infant and preschool age), and from 7 to 18 years (Phase III, school age). The current manuscript focuses on the first phase, with congenital heart disease as the primary outcome, and will cover other folate-sensitive birth defects.

Figure 2. Flowchart

By December 2018, we consecutively recruited 19,144/19,563 (97.9%) participants at preconception settings, including 8045 couples and 3054 single women at pre-pregnancy sites, and additional 15,615/16,201 (96.4%) pregnant women at maternity hospitals with gestational age <14 weeks were recruited. By the end of December 2018, the last participants recruited at early pregnancy were due for delivery. We have achieved birth records of 12,402 newborns in the Maternal Clinic Antenatal Medical Record System. The follow-up of outcomes of the remaining participants is ongoing. A total of 151 cases of CHD were identified through the Shanghai Neonatal CHD Screening Platform: 131 cases from the early pregnancy sample and the remaining 20 cases from the preconception sample. The prevalence of CHD in live births is 10.5‰ (131/12, 402) based on the present available data. In the preconception sample, from March 2016 to May 2018 (the latest data extraction in the Maternal Clinic Antenatal Medical Record System), the number of pregnancies and deliveries were 1538 and 975, respectively.



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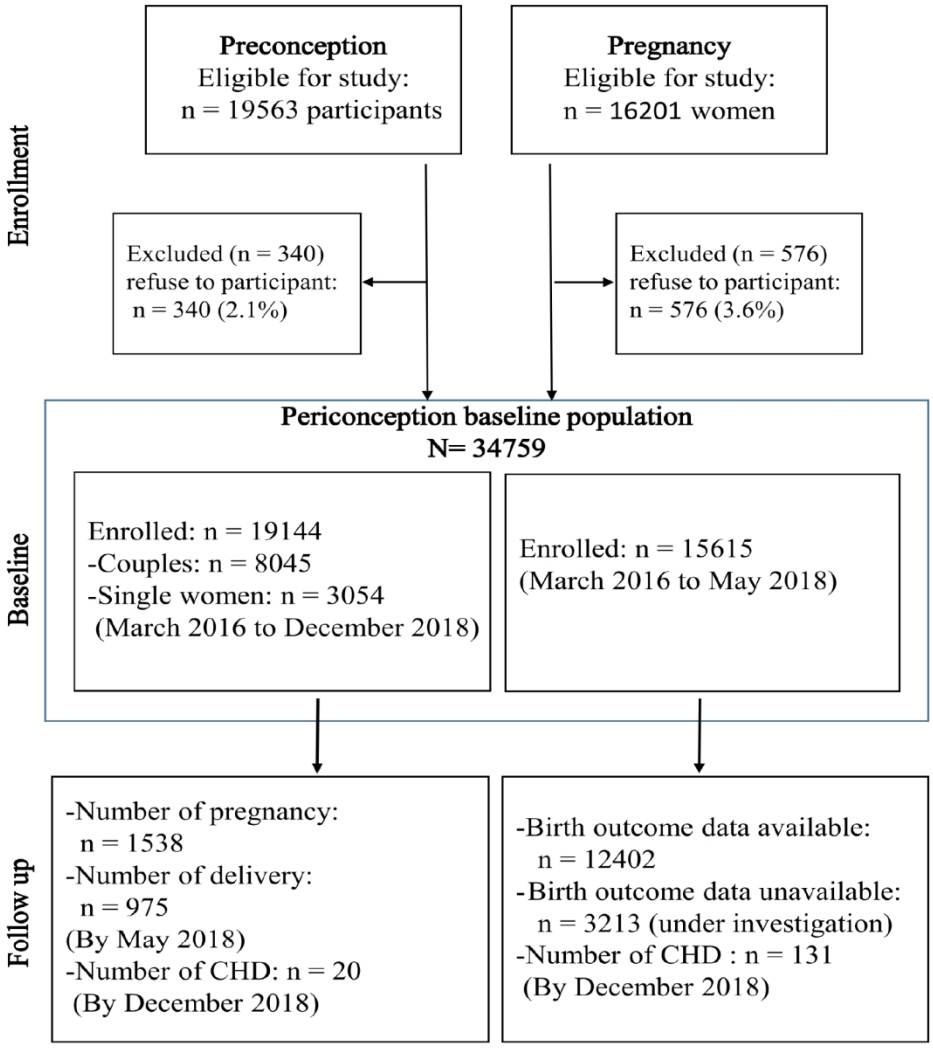

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Appendix 1 Types of fetus defects and birth defects

Types of fetus defects and birth defects

Diagnosis
Anencephalus
Spina bifida
Encephalocele
Congenital Hydrocephalus
Cleft Palate
Cleft Lip
Cleft Lip with Cleft Palate
Microtia (including Anotia)
Deformity of external ear(s) (except Microtia and Anotia)
Esophageal atresia or stenosis
Anorectal atresia (including Congenital Anorectal Malformations)
Hypospadia
Ectopocystis
Pes Equinovarus
Polydactylism
Syndactylia
Limb shortening
Congenital Diaphragmatic Hernia
Pcromphalus
Celoschisis
Conjoined Twins
Trisomy 21 syndrome
Congenital heart disease
Others

Note: Down's syndrome, neural tube defects, congenital heart defects, hydrocephalus, digestive tract

malformations and urinary malformations are most common defects in China. Defects were detected by prenatal Down's syndrome screening, NT examination and Ultrasound image examination during the second trimester; and the number and type of birth defects after childbirth are diagnosed by professional clinical team. Down syndrome (DS), caused by the trisomy, translocation, or partial trisomy of chromosome 21, is the most common genetic cause of intellectual disability. DS is diagnosed by neonatologist. Neural tube defects including spina bifida and hypospadias, were diagnosed by ultrasound examination and pediatric neurosurgeon. Congenital heart disease was diagnosis by Doppler ultrasound and CHD screening (pulse oximetry plus cardiac murmurs). Hydrocephalus, digestive tract malformations, urinary malformations and other defects also were also diagnosed either by ultrasound or some other specific diagnosis methods.

Appendix 2a pre-pregnant questionnaire

姓名 Name：_____

身份证号 ID no：□□□□□□□□□□□□□□□□□□

医院代码 Hospital No：_____（到时打印到问卷上）

填表日期 Date：_____年_____月_____日

孕前膳食补充剂调查表

Pre-pregnancy nutrition supplement questionnaire

（男女共用）

A 一般情况

A1 联系电话（请您认真填写，以助于医生和您联系）Contact number： _____(手机) _____(固定电话) _____（Email）
A2 家庭住址 Address：_____区/县_____街道/小区_____门牌号码/村
A3 您的出生日期是 Birth date _____年_____月_____日
A4 民族 Nationality ☐ 1 汉族 ☐ 2 其他（请注明）_____
A5 您的最高学历 Education ☐1 初高中以下 ☐2 大专本科 ☐3 硕士研究生以上及以上
A6 您现在的主要职业 Occupation ☐1 管理人员/干部 ☐2 技术人员 ☐3.企业主 ☐ 4 工人 ☐5 农民 ☐ 6 个体户 ☐7 其它_____
A7 上一年您的家庭年收入是：Income of a year ☐1. <2 万元 ☐ 2. (2~3.9)万 ☐3.(5.9)万 ☐4.(6~9.9)万 ☐5.(10~14.9)万 ☐6.15 万及以上 ☐9.不详
A8 填表日期 Date _____年_____月_____日

B 营养补充剂使用情况

营养补充剂种类 Nutritional supplement types	**叶酸 Folic acid**	**复合维生素 multi-vitamins**	**单一维生素 Single vitamin**
您在近三个月是否服用 Have you taken it in the last three months?	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否
您服用的什么品牌的营养补充剂？（如果没有对应选项请在其他处写明） Brand name	☐ 1、创盈金斯利安多维片 ☐ 2、福施福胶囊营养素 ☐ 3、汤臣倍健 ☐ 4、安利纽崔莱铁 ☐ 5、其他_____	☐ 1、爱乐维 ☐ 2、汤臣倍健孕妇专用 ☐ 3、惠氏玛特纳 ☐ 4、21 金维他 ☐ 5、其他_____	☐ V_C ☐ V_E ☐ V_{B1} ☐ V_{B2} ☐ 其他_____
您这三个月的服用频率？（如果食用频率小于每天/周一次，请填写每周/月食用几次，并勾出周/月） How often did	____次/☐天 ☐周 ☐月	____次/☐天☐周☐月	____次/☐天 ☐周 ☐月

营养补充剂种类 Nutritional supplement types	铁 Fe	钙 Ca	锌 Zn
您在近三个月是否服用 Have you taken it in the last	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否

you take in			

three months?			
您服用的什么品牌的营养补充剂？（如果没有，选项请在其他处写明） Brand name	☐ 1、汤臣倍健叶酸亚铁片 ☐ 2、安利纽崔莱铁片 ☐ 3、金康倍叶酸铁片 ☐ 4、其他_____	☐ 1、惠氏金钙尔奇 ☐ 2、君宝康孕妇钙片 ☐ 3、十月妈咪维生素 AD 钙锌咀嚼片 ☐ 4、安利钙镁片 ☐ 5、其他_____	☐ 1、美康利健 MK 硒金牡蛎锌片 ☐ 2、汤臣倍健 锌咀嚼片 ☐ 3、宫诺肽片 ☐ 4、其他_____
您这三个月的服用频率？（如果食用频率小于每天/周一次，请填写每周/月食用几次，并勾出周/月） How often did you take in	____次/☐天 ☐周 ☐月	____次/☐天☐周☐月	____次/☐天 ☐周 ☐月

B 营养补充剂使用情况

C 吸烟情况
C1 您有吸烟史吗？ ☐1 是 ☐0 否***(跳至 F6)*** Have you smoked cigarettes ever before?
C2 在您最近的 3 个月内，您是否吸烟？ ☐1 是 ☐0 否 Did you smoke cigarettes in 3 month
C3 如果是，您平均每天吸多少支烟？ _____ 支 On average, how many cigarettes did you smoke each day in the month after your last menstrual period？
C4 如果您曾经戒过烟，您戒了多少次？ _____ How many times did you stop smoking?
C5 在您生活的大多数时间里，您是否暴露于他人烟草烟雾中？ On most days during your pregnancy, were you exposed to someone else's cigarette smoke? ☐1 是 ☐0 否***（跳至 G1）***
C6 您在哪里暴露与烟草烟雾中 Where were you exposed to the smoke？ ☐1 仅在家中 ☐2 仅在工作单位 ☐3 在家和在工作单位均暴露
D 酒精
D1 您最近三个月的饮酒情况？During the 3 months before or during your pregnancy, did you ever drink any alcoholic beverages? ☐0.从未饮酒； ☐1.尝试饮酒（曾饮至少半瓶或一听啤酒，一小盅白酒等）； ☐2.现在饮酒（过去 30 天，至少有一天喝过一杯酒）； ☐3.重度饮酒（过去 30 天，至少有一天在 2 小时内喝过五杯酒）； ☐4.醉酒（过去 12 个月内，因喝酒太多而感到头晕/头疼/嗜睡等醉酒症状）。

核查人员签名：_____

Appendix 2b pregnant questionnaire

Name of pregnancy：_____

ID no：_____

孕期危险因素暴露调查表

Pregnancy risk factor exposure questionnaire

A 一般情况 General information

A1 您的出生日期是 Birth date _____年_____月_____日
A2 民族 Nationality ☐ 1 汉族 Han ☐2 其他 other_____
A3 您的最高学历 Education ☐1 初高中以下 Mid ☐2 大专本科 High ☐3 硕士研究生以上及以上 College
A4 您现在的主要职业 Occupation ☐1 管理人员/干部 ☐2 技术人员 ☐3.企业主 ☐ 4 工人 ☐5 农民 ☐ 6 个体户 ☐7 其它_____
A6 家庭住址：Address _____区/县_____街道/小区_____门牌号码/村
A7 联系电话（请您认真填写，以助于医生和您联系）：Contact number _____(手机) _____(固定电话) _____(Email) _____(微信号)

B 本次妊娠情况

B1 您孕前体重通常为？ Current weight _____（公斤 Kg)
B2 您身高是？ Height _____(厘米 cm)
B3 您的腰围是？Waist _____（厘米 cm）
B4 您此次怀孕的末次月经时间？ _____年_____月_____日 What was the first day of the menstrual period that came right before this pregnancy (LMP)?
B5 孕期是否发生过重大负性生活事件而使您的精神受到刺激？ ☐ 1 是 ☐ 0 否 Have you ever experienced the negative events which irritate you and generate some negative emotion?
B6 生这个孩子是您第几次怀孕？ _____次 How many times have you been pregnant?
B7 是否有不良生育史？ Did you have the adverse reproductive history? ☐ 1 有***（继续回答 B6.1）*** ☐ 0 无***（跳至 B7）***
B6.1 流产史 Abortion ☐ 1 有 ☐ 0 无
B6.2 早产史 Preterm ☐ 1 有 ☐ 0 无
B6.3 死产史 Stillbith ☐ 1 有 ☐ 0 无
B8 您是否有糖尿病和高血压疾病？Do you have hypertension or diabetes ☐ 1 是 ***(继续回答 B8)*** ☐ 0 否***（跳至 C1）*** ☐ 999 不知道***（跳至 C1）***
B9 您的直系亲属中是否患有糖尿病、高血压疾病？ s there the family history of hypertension or diabetes in children's immediate family members ☐ 1 是 ***(继续回答 B8)*** ☐ 0 否***（跳至 C1）*** ☐ 999 不知道***（跳至 C1）***
B10 若有，请选择出该亲属与您的关系**（可多选）If so, please choose the relationship with the child**
☐1. 父亲 ☐2. 母亲 ☐3. 爷爷 ☐4. 奶奶 ☐5. 兄弟 ☐6. 姐妹

C 叶酸使用

C1 在您末次月经前三个月内，您是否服用过叶酸？ ☐1 是 ☐0 否 Did you take folic acid in the month before your last period?			
C2 在您末次月经之后至今，您是否服用过叶酸？ ☐1 是 ☐ 0 否 Did you take any folic acid after your last period anddduring pregnancy ?			
	叶酸 1	叶酸 2	叶酸 3
C3 药物名称（商品名） Brand name			
C4 使用时间 When did you take it?	☐1 孕前 before pregnancy ☐2 孕后 pregnancy ☐3 孕前孕后都有 always	☐1 孕前 before pregnancy ☐2 孕后 pregnancy ☐3 孕前孕后都有 always	☐1 孕前 before pregnancy ☐2 孕后 pregnancy ☐3 孕前孕后都有 always
C5 是否在怀孕期间一直使用？ Did you take it during the rest of your pregnancy?	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否
C6 是否停止使用过？ Did you stop taking it?	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否
C7 使用频率？ How often did you take it?	____次/☐天 ☐周 ☐月	____次/☐天 ☐周 ☐月	____次/☐天 ☐周 ☐月
C8 每次的使用量 What is the usage per time?	一次_____片	一次_____片	一次_____片

D 维生素使用

D1 在您末次月经的前三个月内，您是否服用过维生素？ ☐1 是 ☐0 否 Did you take any vitamins in the three months before your last period ？			
D2 在您末次月经之后至今，您是否服用过维生素？ ☐1 是 ☐0 否 ***（跳至 E1）*** Did you take any vitamins after your last period and during pregnancy?			
	维生素 1	维生素 2	维生素 3
D3 维生素名称 Vitamin name			
D4 维生素商品名称 Brand name			
D5 是否是医生给药？ Did your doctor give it to you?	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否
D6 是否包括叶酸？ Does it contain folic acid?	☐1 是 ☐0 否 ☐999 不知道	☐1 是 ☐0 否 ☐999 不知道	☐1 是 ☐0 否 ☐999 不知道

D7 使用时间 When did you take it?	☐1 孕前 before pregnancy ☐2 孕后 pregnancy ☐3 孕前孕后都有 always	☐1 孕前 before pregnancy ☐2 孕后 pregnancy ☐3 孕前孕后都有 always	☐1 孕前 before pregnancy ☐2 孕后 pregnancy ☐3 孕前孕后都有 always
D8 是否停止使用过？ Did you stop taking it?	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否
D9 使用频率？ How often did you take it?	____次/☐天 ☐周 ☐月	____次/☐天 ☐周 ☐月	____次/☐天 ☐周 ☐月
D10 每次的使用量 What is the usage per time?	一次_____片	一次_____片	一次_____片

E 其他营养补充剂使用情况 Other nutritional supplement use

营养补充剂种类 Nutritional supplement types	铁 Fe	钙 Ca	锌 Zn
您在近三个月是否服用 Did you take it around the time you became pregnant?	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否
您服用的什么品牌的营养补充剂？（如果没有，选项请在其他处写明）Brand name	☐ 1、汤臣倍健叶酸亚铁片 ☐ 2、安利纽崔莱铁片 ☐ 3、金康倍叶酸铁片 ☐ 4、其他_____	☐ 1、惠氏金钙尔奇 ☐ 2、君宝康孕妇钙片 ☐ 3、十月妈咪维生素 AD 钙锌咀嚼片 ☐ 4、安利钙镁片 ☐ 5、其他_____	☐ 1、美康利健 MK 硒金牡蛎锌片 ☐ 2、汤臣倍健 锌咀嚼片 ☐ 3、宫诺肽片 ☐ 4、其他_____
您这三个月的服用频率？（如果食用频率小于每天/周一次，请填写每周/月食用几次，并勾出周/月）How often did you take it?	____次/☐天 ☐周 ☐月	____次/☐天☐周☐月	____次/☐天 ☐周 ☐月

F 草本药物使用

F1 在您末次月经前三个月内，您是否使用过任何一种草本药物/传统医学药物？Did you take any herbal supplements/traditional Chinese medicine in the three months before your last period ☐ 1 是 ☐ 0 否

F2 在您末次月经之后，您是否使用过任何一种草本药物/传统医学药物 Did you take any herbal supplements/traditional Chinese medicine after your last period and during pregnancy? ？ ☐1 是 ☐0 否 ***（跳至F1）***			
	草本药物 1 Herbal 1	草本药物 2 Herbal 2	草本药物 3Herbal 3
F3 草本药物类型 Name of herb	☐1 中成药 ☐2 中医草药	☐1 中成药 ☐2 中医草药	☐1 中成药 ☐2 中医草药
F4 药物名称（如果选择中医草药，请填写中药功效） The use of traditional Chinese medicine			
F5 用药持续多少天 How long did you take in?	_____天	_____天	_____天

G 吸烟情况

G1 您在怀孕前或者怀孕期间吸烟吗？ Did you smoke cigarettes before or during your pregnancy with the baby?	☐1 是	☐0 否***(跳至F9)***
G2 在您末次月经的当月，您是否吸烟？ Did you smoke during the month before your last menstrual period ?	☐1 是	☐0 否
G3 在您末次月经的一个月后（末次月经结束直至一个月后），您是否吸烟？ ***(跳至5)*** Did you smoke during the month after your last menstrual period, that is between LMP and LMP+1 month	☐1 是	☐0 否
G4 如果是，您平均每天吸多少支烟？ On average, how many cigarettes did you smoke each day in the month after your last menstrual period ？ 支		
G5 在您怀孕期间，您是否吸烟？ Did you smoke during your pregnancy?	☐1 是	☐0 否***(跳至F7)***
G6 在您怀孕期间，您平均每天吸多少支烟？ On average how many cigarettes did you smoke each	_____ 支	
G7 在您末次月经期间至今，您是否戒过吸烟？ Did you stop smoking at any time between your last menstrual period and the end of your pregnancy?	☐1 是	☐0 否 ***（跳至F9）***
G8 您戒烟有多少次？ How many times did you stop?		_____ 次
G9 在您怀孕的大多数时间里，您是否暴露于他人烟草烟雾中？ On most days during your pregnancy, were you exposed to someone else's cigarette smoke?	☐1 是	☐0 否 ***（跳至G1）***

G10 您在哪里暴露与烟草烟雾中？ Where were you exposed to the smoke? ☐1 仅在家中 ☐2 仅在工作单位 ☐3 在家和在工作单位均暴露

H 酒精

H1 在您怀孕前三个月至今，您是否饮用过任何含有酒精的饮料？ ☐1 是 ☐0 否 During the 3 months before or during your pregnancy, did you ever drink any alcoholic beverages?
H2 在这三个月内，您通常每次饮几杯酒？ _____ 杯 On those days that you drank, how many drinks did you have?

I 环境暴露情况

I1 怀孕前三个月到现走，您是否染烫发? Did you dye perm in the first three months of pregnancy?	☐1 是	☐0 否
I2 怀孕前三个月到现在，后您工作的地点或家里是否装修过? Did you exposed to formaldehyde in the first three months of pregnancy?	☐ 1 是	☐0 否
I3 怀孕前后您是否接触过下列物质？ Have you been exposed to the following substances before and after pregnancy? (Toxic chemicals) ☐1 除草剂 ☐2 杀虫剂 ☐3 灭鼠剂 ☐4 有机溶剂 ☐5 消毒剂 ☐6 金属制剂 ☐7 有害气体 ☐8 有害固体		

J 药物使用情况

以下问题是有关于您在末次月经之前三个月内至今期间的用药情况

	治疗高血压药物 Medication for hypertension	胰岛素 Insulin for diabetes	口服降血糖药物 Oral hypoglycemic for diabetes	抗癫痫药物 Medications for epilepsy	每天都要服用的药物 Medications at least once a day
1 您是否使用过？ Did you take?	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否	☐1 是 ☐0 否
2 您使用的药物名称？ What did you take?	_____	_____	_____	_____	_____

如果下一个问题令您感到不安且不愿意回答，请在"拒答"上打√，我们将对您所有回答进行严格保密!

	安定\有助于您放松药物 Valium\drugs to help you	使您感觉良好\精力旺盛 Make you feel	美沙酮\氧可酮\其他止痛药 Methadone oxymoron\other	可卡因 Cocaine or crack cocaine	海洛因 Heroin	大麻 Marijuana

	relax	good\have more energy	pain killers			
1 您是否使用过？ Did you take?	☐1 是 ☐0 否 ☐2 拒答 ☐9 不清楚	☐1 是 ☐0 否 ☐2 拒答 ☐9 不清楚	☐1 是 ☐0 否 ☐2 拒答 ☐9 不清楚	☐1 是 ☐0 否 ☐2 拒答 ☐99 不清楚	☐1 是 ☐0 否 ☐2 拒答 ☐9 不清楚	☐1 是 ☐0 否 ☐2 拒答 ☐9 不清楚
2 您使用的药物名称？ What did you take?	_____	_____	_____	_____	_____	_____

I 您在怀孕期间是否患过以下疾病？

I.3 发热性疾病及呼吸道感染 ☐1 是 ☐0 否

Febrile illness and respiratory infections

I.3.1 您发烧时的最高温度是多少？ _____℃

What was the highest temperature of your fever during your illness?

I.3.2 您发烧有几天？ _____天

How long did you have a fever?

I.6 其他_____

调查结束，谢谢您的配合！

调查员姓名 _____

调查日期 ___ ___/ ___ ___/ ___ ___

年 月 日

Appendix 3 Variable list

Pre-pregnant variable list

No	Variables	Data type
	General information	
1	Wife id	Text
2	Husband nation	Text
3	Husband age	N
4	Husband education	Text
5	Husband id	Text
6	Husband occupation	Text
7	Wife nation	Text
8	Wife age	N
9	Wife education	Text
10	Wife occupation	Text
11	Tel No	Text
12	Mobile phone No	Text
	Medical history	
13	Female anemia	Category
14	Female EH	Category
15	Female heart disease	Category
16	Female DM	Category
17	Female epilepsia	Category
18	Female thyroid disease	Category
19	Female CGN	Category
20	Female mental disease	Category
21	Female tumour	Category
22	Female TB	Category
23	Female HBV	Category
24	Female VD	Category
25	Male anemia	Category
26	Male EH	Category
27	Male heart disease	Category
28	Male DM	Category
29	Male epilepsia	Category
30	Male thyroid disease	Category
31	Male CGN	Category
32	Male mental disease	Category
33	Male tumour	Category
34	Male TB	Category
35	Male HBV	Category
36	Male VD	Category

	Vaccine	
37	Female rubella vaccine	Category
38	Female hepB vaccine	Category
39	Male hepB vaccine	Category
	Drug use	
40	Female current medicine	Category
41	Female medicine name	Category
42	Male current medicine	Category
43	Male medicine name	Category
	Childbearing history	
44	Birth history	Category
45	Pregnancy times	Category
46	Live birth	Category
47	Dead fetus	Category
48	Dead birth	Category
49	Term delivery	Category
50	Premature delivery	Category
51	Natural abortion	Category
52	Abactio	Category
53	Children number	Category
54	Birth defect	Category
55	Defect type	Category
56	Menarche age	Category
57	Period menstruation	Category
58	Menstrual cycle	Category
59	Menstrual quantity	Category
60	LMP	Date
	Family history of disease	
61	Female family history thalassemia	Category
62	Female family history albinism	Category
63	Female family history favism	Category
64	Female family history hemophilia	Category
65	Female family history CHD	Category
66	Female family history DS	Category
67	Female family history openNTDs	Category
68	Female family history DM	Category
69	Female family history dysnoesia	Category
70	Female family history daysaudia	Category
71	Female family history viaual disorder	Category
72	Female family history neuropsychiatric	Category
73	Female family history other birth defects	Category
74	Female family history fetal death	Category
75	Female family history intermarry	Category

76	Female family history relations	Category
77	Male family history thalassemia	Category
78	Male family history albinism	Category
79	Male family history favism	Category
80	Male family history hemophilia	Category
81	Male family history CHD	Category
82	Male family history DS	Category
83	Male family history openNTDs	Category
84	Male family history DM	Category
85	Male family history dysnoesia	Category
86	Male family history dysaudia	Category
87	Male family history viaual disorder	Category
88	Male family history neuropsychiatric	Category
89	Male family history other birth defects	Category
90	Male family history fetal death	Category
91	Male family history intermarry	Category
92	Male family history relations	Category
	Anthroposomatology	
93	Female height	Numeric
94	Female weight	Numeric
95	Female BMI	Numeric
96	Female heart rate	Numeric
97	Female SBP	Numeric
98	Female SDP	Numeric
99	Male height	Numeric
100	Male weight	Numeric
101	Male BMI	Numeric
102	Male heart rate	Numeric
103	Male SBP	Numeric
104	Male SDP	Numeric
	Lab data	
105	Leucorrhea check	Numeric
106	Clue cell	Numeric
107	Monilia infection	Numeric
108	Trichomomas	Numeric
109	Cleanness	Numeric
110	Whiff test	Numeric
111	PH	Numeric
112	Wom blood analysis	Numeric
113	Female hb	Numeric
114	Female wbc	Numeric
115	Female rbc	Numeric
116	Wom urine test	Numeric

117	Female ABO	Numeric
118	Female Rh	Numeric
119	Female GLU	Numeric
120	Female GLU levels	Numeric
121	Female NG	Numeric
122	Female chlamydia	Numeric
123	Female syphilis	Numeric
124	Female HIV	Numeric
125	Female ALT	Numeric
126	Female ALT levels	Numeric
127	Female HBs-Ag	Numeric
128	Female HBs-Ab	Numeric
129	Female HBe-Ag	Numeric
130	Female HBe-Ab	Numeric
131	Female HBc-Ab	Numeric
132	Female HCV-Ab	Numeric
133	Female CMV IgM	Numeric
134	Female CMV IgG	Numeric
135	Female RV IgM	Numeric
136	Female RV IgG	Numeric
137	Female TOX IgM	Numeric
138	Female TOX IgG	Numeric
139	Male blood analysis	Numeric
140	Male hb	Numeric
141	Male wbc	Numeric
142	Male rbc	Numeric

During-pregnancy variable list

	Basic information	
1	Hospital card number	Text
2	Inpatient number	Text
3	Name	Text
4	Age	Numeric
5	Pregnant times	Numeric
6	Delivery times	Numeric
7	Last menstrual period	Date
8	Gestational week at the first visit	Numeric
9	The first visit date	Date
10	Height	Numeric
11	Weight	Numeric
12	Systolic blood pressure at the first visit	Numeric
13	Diastolic blood pressure at the first visit	Numeric

14	Occupation	Text
15	Education	Text
	Antenatal care record	
16	Weight at each antenatal care	Numeric
17	Systolic blood pressure at each antenatal care	Numeric
18	Diastolic blood pressure at each antenatal care	Numeric
19	Gestational week at each antenatal care	Numeric
20	Antenatal care date	Date
	Lab data	
21	Cytomegalovirus	Numeric
22	Cytomegalovirus examination date	Date
23	Rubella virus	Numeric
24	Rubella virus examination date	Date
25	Toxoplasmosis	Numeric
26	Toxoplasmosis examination date	Date
27	Syphilis screening	Numeric
28	Syphilis screening examination date	Date
29	Fasting blood-glucose	Numeric
30	Fasting blood-glucose examination date	Date
31	HCT(hematokrit)	Numeric
32	HCT(hematokrit) examination date	Date
33	Serum folate	Numeric
34	Serum folate examination date	Date
35	HCY(homocysteine)	Numeric
36	HCY(homocysteine) examination date	Date
37	OGTT 0 hours	Numeric
38	OGTT 1 hours	Numeric
39	OGTT 2 hours	Numeric
40	OGTT examination date	Date
41	Triglyceride	Numeric
42	Triglyceride examination date	Date
43	Total cholesterol	Numeric
44	Total cholesterol examination date	Date
45	Hemoglobin	Numeric
46	Hemoglobin examination date	Date
	Delivery date	
47	Gestational week at delivery	Numeric
48	Delivery mode	Text
49	Birth weight	Numeric
50	Birth weight(second baby)	Numeric
51	Systolic blood pressure at delivery	Numeric
52	Diastolic blood pressure at delivery	Numeric
53	Apgar scoring	Numeric

54	Delivery date	Date
55	Birth defect records	Text
56	Weight blood pressure at delivery	Numeric
Offspring variable list		
	0 - 6 years (6 weeks, 6 months, 12 months, 36 months, 60 months)	
1	Name	Text
2	ID number	Text
3	Weight	Numeric
4	Height/ Length	Numeric
5	Vision detection	Numeric
6	DDST: Denver developmental screening test	Numeric
	6 - 18 years (each year)	
7	Weight	Numeric
8	Height	Numeric
9	Diastolic blood pressure	Numeric
10	Systolic blood pressure	Numeric
11	Vision detection	Numeric
12	Vital capacity	Numeric
13	Physical fitness measurement (running, jumping, solid balls, etc)	Text
The clinical diagnosis during 0 – 18 years will be extracted from the routine medical system each year.		

Future Plan of Shanghai PreConception Cohort, Shanghai, China (SPCC)

The Shanghai PreConception Cohort (SPCC) is a long-term cohort and meets the "Cohort profile" requirement. We have a complete plan to follow-up offspring to the age of 18 years old. The cohort will be financially supported by different grants. The current manuscript focuses on the first phase, the establishment of the baseline and our first main outcome, the congenital heart disease (CHD).

The second phase of this cohort study is from birth to 6 years old. Follow-up work will rely on Shanghai Community health care centers-child care. The third phase is from 6 to the age of 18 (school age). We will cooperate with Shanghai Student Health and Fitness Surveillance Center for the follow-up of the school age children.

Multiple outcomes for pediatrics, including growth, physical and neuro-developments, obesity, common and rare diseases will be investigated. Nested case-control design will be used for rare outcomes.

The data collection plan for infants and children

	At birth	6 weeks	6 months	12 months	36 months	60 months	6 – 18 years*
General information (gender, ID number, Home Address, etc)	√	√					
Delivery outcome (Congenital malformation, preterm birth, miscarriage, stillbirth)	√						
Body measurement(weight, height, waistline, hipline)	√	√	√	√	√	√	√
Diet investigation (questionnaire)	√	√	√	√	√	√	√
Neurobehavioral developmental assessment (DDST[$])		√	√	√	√	√	
Anthropometrics data (Shanghai Community health care centers-child care and Shanghai Student Health and Fitness Surveillance Center) weight, height, waist circumference	√	√	√	√	√	√	√
Physical fitness measurement (running, jumping, solid balls, etc)							√
Blood pressure measurement, annually							√
Hemachrome (anemia)							√
Renal functions, at grade 9 and 12							√
Cardiovascular-related chronic diseases (Hypertension, Obesity, Diabetes/Pre-diabetes and Dyslipidemia)							√
Venous blood[#]							√

Note: * Shanghai Student Health and Fitness Surveillance Center routinely conducts all-round physical examinations for students in the school as a health care policy for Shanghai students. It is usually in September-November of each year.

$ DDST: Denver developmental screening test.

Venous blood will be collected at 12, 15 and 18 years of age.