

# The Prenatal High Risk Factors in Non-syndromic Cleft Lip or/and Palate Fetuses: A Nested Case-control Study Based on Maternal Health Care Records of a Population

**Bing Li**

Guangdong Women and Children Hospital

**Xiao-hua Xiao**

Boai Hospital of Zhongshan Affiliated to Southern Medical University

**Ang Chen**

Boai Hospital of Zhongshan Affiliated to Southern Medical University

**Ying Li**

Guangdong Women and Children Hospital

**Hui-fang Lin**

Guangdong Women and Children Hospital

**Hua-zhao Lin**

Guangdong Women and Children Hospital

**Feng-hua Liu** (✉ [522276188@qq.com](mailto:522276188@qq.com))

Guangdong Women and Children Hospital

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## Research Article

**Keywords:** cleft lip or/and palate, prenatal risk factors, maternal health care records, nested case-control study

**Posted Date:** March 16th, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-288084/v1>

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# **The prenatal high risk factors in non-syndromic cleft lip or/and palate fetuses: a nested case-control study based on maternal health care records of a population**

Bing Li,<sup>1\*</sup> Xiao-hua Xiao,<sup>2\*</sup> Ang Chen,<sup>3</sup> Ying Li,<sup>1</sup> Hui-fang Lin,<sup>1</sup> Hua-zhao Lin,<sup>1</sup> Feng-hua Liu<sup>4</sup>

<sup>1</sup>Department of Health Education, Guangdong Women and Children Hospital

<sup>2</sup>Department of Dean, Boai Hospital of Zhongshan Affiliated to Southern Medical University

<sup>3</sup>Department of Science and Education, Boai Hospital of Zhongshan Affiliated to Southern Medical University

<sup>4</sup>Department of Reproductive Health and Infertility, Guangdong Women and Children Hospital

\*Bing Li and Xiao-hua Xiao contributed equally to this work, and were co-first author.

## **Correspondence:**

Professor Feng-hua Liu, Department of Reproductive Health and Infertility, Guangdong Women and Children Hospital, No. 521, XingNan Road, Guangzhou 511442, Guangdong, China; liushine2006@163.com.

Bing Li: 522276188@qq.com

Xiao-hua Xiao: drxxh@qq.com

Ang Chen: 361782947@qq.com

Ying Li: 13302283975@163.com

Hui-fang Lin: 414580339@qq.com

Hua-zhao Lin: 76991033@qq.com

Feng-hua Liu: liushine2006@163.com

Funding: Funded by grants from the Ministry of Science and Technology of the People's Republic of China (project ID 2018YFC1002600, 2018YFC1002604, and 2018YFC1002601).

Abbreviations:

NSCL/P: non-syndromic cleft lip or/and palate

CL/P: Cleft lip or/and palate

BMI: Body Mass Index

HBsAg: hepatitis B surface antigen

G6PD: glucose-6-phosphate dehydrogenase deficiency

MLRM: multiple logistic regression model

VIF: variance expansion factor

CIs: confidence intervals

## ABSTRACT

**Background:** To determine the associations of different prenatal factors with the development of non-syndromic cleft lip or/and palate (NSCL/P), as there are still no consensus to the risk factors of NSCL/P.

**Methods:** Nested case-control study based on the gestational period health care record data of all singleton NSCL/P (n=197) and fetuses without birth defects (n=192706) born in-hospital of Zhongshan between 2016 and 2019.

**Results:** Vagina infected (OR 3.43, 95%CI 1.34-7.19), assisted conception (OR 11.57, 95% CI 0.65-54.29), folic acid intake <3 moths during pre- and early pregnancy (OR 1.57, 95% CI 1.10-2.30), incomplete placenta or rough surface placenta (OR 4.65, 95% CI 3.23-6.57), abnormal amniotic fluid (OR 2.74, 95% CI 2.04, 3.67), low birth weight of newborns (OR 5.44, 95% CI 3.41-8.54), preterm delivery (OR 3.29, 95% CI 2.07-5.24), and post-term delivery (OR 5.94, 95% CI 0.97-18.93) were associated with a higher risk of NSCL/P.

**Conclusions:** The status of placenta and fluid were discovered as risk factors with NSCL/P, which strengthens the importance role of interaction between the placental barrier and various environmental factors on the development of NSCL/P.

**Keyword:** cleft lip or/and palate, prenatal risk factors, maternal health care records, nested case-control study

## Background

Cleft lip or/and palate (CL/P) are most common serial congenital anomalies to affect the orofacial region, and the overall incidence of CL/P is approximately 1 in every 700 to 1000 births with wide variation across geographic areas, ethnic group and nature of cleft itself [1-3]. CL/P occurs between the 6<sup>th</sup> and 12<sup>th</sup> week of pregnancy during early embryologic development, and has complex etiology thought to involve genetic influences with variable interactions from environmental factors [4-6].

Non-syndromic cleft lip or/and palate (NSCL/P) accounts for 70% of CL/P cases, and it is isolated anomaly without other congenital anomalies. Several previous studies have reported more than 30 genetic risk loci that are responsible for pathogenesis of NSCL/P, but the major cause of NSCL/P has not been fully elucidated due to complex interaction between genetic and environmental risk factors [7, 8]. Meanwhile, the reported environmental factors associate with NSCL/P are variational in different studies, and there was few study focus on prenatal factors of NSCL/P. However, the prenatal physiologic and pathological changes reflect the effects of environmental factors on fetuses, further large sample sizes study on prenatal factors of NSCL/P is needed.

The aim of this study was to determine the associations of different prenatal factors with the development of NSCL/P. The gestational period health care record data of 192903 fetuses born between 2016 and 2019 in one city region were used to explore the prenatal risk factors on NSCL/P.

## Methods

### Study design and participants

This nested case-control study included 192903 singleton fetuses were born (included artificial

termination of death pregnancy) in-hospital from January 1, 2016 to December 31, 2019 in Zhongshan City of China. We extracted the maternal health care record data of total 204119 pregnant women and their 206850 fetuses from the regional information system databases of maternal and children health care of Zhongshan during this period, and all the the private identity information was excluded when we extracted the data. All the target fetuses delivered between 2016 and 2019, we excluded 5446 twins and multiple pregnancies, 23 singleton fetuses with syndromic cleft lip or/and palate (SCL/P), and 8478 singleton fetuses with other birth defects (no cleft lip or/and palate), and then there were 192903 singleton fetuses were included in the final pregnancy cohort. In the final cohort, the cases were 197 singleton fetuses with non-syndromic cleft lip or/and palate (NSCL/P), the controls were 192706 singleton fetuses without NSCL/P and without other birth defects.

We confirm that all methods were carried out in accordance with relevant guidelines and regulations of *ethical review of biomedical research involving human beings* issued by Health and Family Planning Commission of the people's Republic of China in 2016. This study used the deidentified historical health record data belong to information centre of health bureau of Zhongshan city, and informed consent was waived by the ethical review board of Guangdong Women and Children Hospital.

From the real records data, we got these variables in prenatal period as candidate risk factors, included mother's age, education level, job, per capita household income, pre-pregnancy body mass index (BMI), folic acid intake during pre- and early pregnancy, risk factors exposure in early pregnancy, illness history, assisted conception, prenatal Down's screening, liver function test, renal function test, urine occult blood, hepatitis B surface antigen (HBsAg), syphilis test, scarred uterus, gestational diabetes mellitus, hypertensive disorder, thalassemia carrier, anemia, thyroid dysfunction or abnormalities, vagina infected, glucose-6-phosphate dehydrogenase deficiency (G6PD), incomplete placenta, placental roughness, torsion of umbilical cord, umbilical cord around neck, amniotic fluid abnormal, fetal position, and gender of newborns.

### **Statistical analysis**

The data were analysed using R version 3.6.2 (2019-12-12). Firstly, all variables were tested by  $\chi^2$  tests. Secondly, all variables were used to set-up a multiple logistic regression model (MLRM), and variance expansion factor (VIF) scores were calculated to verify the multicollinearity of independent variables. The variables of illness history and HBsAg were excluded from the MLRM for their VIF scores >10, and a new MLRM was set-up. And then a final simple MLRM was set-up using the variables with the p value <0.05 in the new MLRM, the ORs and their 95% confidence intervals (CIs) of these variables were calculated. The missing values of variables were recoded to unknown for analysis. In all analyses, p values <0.05 were considered significant.

## **Results**

### **General characters**

In all 192903 fetuses, there were 197 (0.10 %) non-syndromic cleft lip or/and palate (NSCL/P), among them, there were 74 (37.56%) cleft lips without palates, 43 (21.83%) cleft palates without lips, and 80 (40.61%) cleft lips with palates. The prenatal general characteristics of all fetuses and their mothers are given in table 1. By  $\chi^2$  tests, it showed education of mothers, pre-pregnancy BMI grades of mothers, and folic acid intake during pre- and early pregnancy might associated with

NSCP.

### **Prenatal risk factors**

The prenatal risk factors of all fetuses and their mothers are given in table 2. By  $\chi^2$  tests, it showed risk factors exposure in early pregnancy, vagina infected, incomplete placenta, rough placenta, abnormal amniotic fluid, illness history of mothers, and prenatal Down's screening might associated with NSCP.

The results of the final simple logistic regression model were given in table 3. Risk factors exposure in early pregnancy, scarred uterus, vagina infected, assisted conception, incomplete placenta, placental roughness, abnormal amniotic fluid, not head presentation, folic acid intake <3 bottles during pre- and early pregnancy, mother's education is primary school or no schooling, and high risk of prenatal Down's screening were confirmed as the prenatal high risk factors on NSC/P.

### **Discussion**

Orofacial clefts particularly cleft lip or/and cleft palate (CL/P) being a major public health problem, affecting thousands of children worldwide each year, with a frequency of 1 in 500 affecting more than 2.6 million people in China [7, 9]. Many researches have focused on NSCL/P, especially with a target to distinguish the underlying genetic risk loci behind pathogenicity of CL/P, but as they all together accounts for only 20%–25% of NSCL/P heritability.<sup>7</sup> Most cases of CL/P are diagnosed in utero by ultrasound after 20 weeks' gestation, but some are undetected and only discovered at delivery and a small percentage of clefts are diagnosed during childhood [10, 11]. So it is important to confirm the prenatal environmental and biological risk factors for auxiliary early diagnosis and etiology on NSCL/P.

The previous researches are still no consensus exists as to the cause of NSCL/P, researchers generally agree that genetic, nutritional, and environmental factors contribute to their formation.<sup>12</sup> Various reported environmental factors include smoking, alcohol use, some medications, folate deficiency, maternal obesity, maternal diseases such as diabetes and stress during pregnancy, chemical exposure, smoke from cooking indoors exposure [1, 12-22]. But we found the risk factors exposure in early pregnancy had no association with NSCL/P when we took into account extensive prenatal factors, these factors include fever and other sicknesses, take medicines, radiation exposure, smoke, or drink wine, etc., however, each of these factors were seldom in pregnant couples for the popular practice of health care before pregnancy among the mothers of this study, so we took into account these risk factors together. It was different from some previous reports, this study showed maternal BMI, higher level of maternal education, family history, high or low parental age at time of childbirth, and maternal diseases such as diabetes were not associated with NSCL/P [1, 23, 24].

This study reflects only more than 3 months of daily intake of 0.4 mg folic acid before and during early pregnancy may help to reduce the risk of NSCL/P. However, there was a high heterogeneity between the previous studies on the role of folate in the etiology of CL/P, such as population characteristics, variation in timing of exposure and supplement types, and publication bias. A review has reported that high-dose folic acid probably has a role in prevention of recurrence of isolated CL/P in high-risk individuals, but not cleft palate [25]. Another review has reported that mandatory folic acid fortification of wheat and/or maize flour may have beneficial

effects on non-syndromic CL/P [26]. A recent meta-analysis also has shown that the risk of non-syndromic orofacial clefts was reduced among pregnant women with folic acid-containing supplements during the etiologically relevant period [27]. In Zhongshan of China, every pregnant woman is recommended daily intake of 0.4 mg folic acid in 3 months before pregnancy and in first 3 months of pregnancy. This study also showed that less than 3 months of daily intake of 0.4 mg folic acid during pre- and early pregnancy was associated with the development of NSCL/P.

This study revealed that vagina infected fungi, bacteria, trichomonad, or gonorrhoea as a total may increase the risk of NSCL/P. A previous report has shown maternal self-reported genital tract infections were associated with CL/P, but the strength of that conclusion was limited [28]. Meanwhile, in our study, the number of vagina infected (only 6 of 197 NSCL/P) was small and the occurrence of infected timing couldn't be determined in early pregnancy, so further study is still needed to confirm the association between vagina infected and NSCL/P.

We found assisted conception was associated with NSCL/P. Patricia Fauque et al. have reported a moderately increased risk of defects (i.e. CL/P, etc. the 15 relevant subgroups of malformations) subsisted after IVF in a large study [29]. A meta-Analysis has reported that the IVF/ICSI singleton pregnancies were significantly associated with high birth prevalence of congenital malformations (included CL/P), but it remains uncertain whether detected differences represent true or methodological differences [30]. However, this study only had a small number of assisted conception pregnancies (100 of 192706 no NSCL/P vs. 1 of 197 NSCL/P), so the strength was limited.

We found several new prenatal factors increased the risk of the development of NSCL/P, they were incomplete placenta or rough surface placenta, abnormal amniotic fluid, and preterm or post-term delivery of newborns. Similar to the report, we also found low birth weight of newborns was associated with NSCL/P [24]. According to the results of this study, placental barrier may play a key role in the effect of environmental factor on NSCL/P, and a well status of placenta decreases the adverse effects of prenatal risk factors exposure. Meanwhile, maybe there are common risk factors between NSCL/P and preterm and low birth weight.

There are, however, several limitations. Firstly, we couldn't include all the previous reported risk factors of NSCL/P in this study, because we used the existing health care record databases to analyze. Secondly, although there was a large number in this retrospect cohort, but the number of NSCL/P was small (197 NSCL/P vs. 192706 no NSCL/P). Several risk factors showed in this study are needed further study to confirm.

## **Conclusion**

Vagina infected, assisted conception, and insufficiency folic acid intake before and during early pregnancy, low birth weight of newborns were associated with a higher risk of the development of NSCL/P, which similar to previous studies. Our study adds several new prenatal risk factors of NSCL/P, includes incomplete placenta or rough surface placenta, abnormal amniotic fluid, preterm delivery or post-term delivery. This study strengthens the importance role of interaction between the placental barrier and various environmental factors on the development of NSCL/P.

## **Abbreviations**

NSCL/P: non-syndromic cleft lip or/and palate; CL/P: Cleft lip or/and palate; BMI: Body Mass

Index; HBsAg: hepatitis B surface antigen; G6PD: glucose-6-phosphate dehydrogenase deficiency  
MLRM: multiple logistic regression model; VIF: variance expansion factor; CIs: confidence intervals

### **Acknowledgements**

We would like to thank the management team of health bureau of Zhongshan City for extracting the deidentified data from regional maternal and children health care information system.

### **Authors's contributors**

BL, XH X, and FH L conceptualised and designed the study, reviewed and revised the manuscript. BL conducted the statistical analysis, drafted the initial manuscript. AC contributed to acquire and sort the data. YL, HF L, and HZ L contributed to sort the data. All authors read and approved the final manuscript.

### **Funding**

Funded by grants from the Ministry of Science and Technology of the People's Republic of China (project ID 2018YFC1002600, 2018YFC1002604, and 2018YFC1002601).

### **Availability of data and materials**

Deidentified data will be available on reasonable request to Bing Li or Feng-hua Liu.

### **Ethics approval and consent to participate**

This study was approved by the ethical review board of Guangdong Women and Children Hospital, approval number: 201801023 (decision 2018-07-18). The authors declare that the experiments comply with the relevant guidelines and regulations of *ethical review of biomedical research involving human beings* issued by Health and Family Planning Commission of the people's Republic of China in 2016. We used the deidentified historical health record data belong to information centre of health bureau of Zhongshan city, and informed consent was waived by the ethical review board of Guangdong Women and Children Hospital.

### **Consent for publication**

Not applicable.

### **Competing interests**

The authors declare that they have no competing interests.

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