Parental Risk Factors for Oral Clefts among Central Africans, Southeast Asians, and Central Americans

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Background: Several lifestyle and environmental exposures have been suspected as risk factors for oral clefts, although few have been convincingly demonstrated. Studies across global diverse populations could offer additional insight given varying types and levels of exposures. Methods: We performed an international case–control study in the Democratic Republic of the Congo (133 cases, 301 controls), Vietnam (75 cases, 158 controls), the Philippines (102 cases, 152 controls), and Honduras (120 cases, 143 controls). Mothers were recruited from hospitals and their exposures were collected from interviewer-administered questionnaires. We used logistic regression modeling to estimate logistic odds ratios (OR) and 95% confidence intervals (CI). Results: Family history of clefts was strongly associated with increased risk (maternal: OR = 4.7, 95% CI, 1.6–12.8; parental: OR = 2.4, 95% CI, 1.2–4.8) and paternal education (OR = 1.9; 95% CI, 1.4–2.5; and OR = 1.8; 95% CI, 1.1–2.9, respectively) and paternal tobacco smoking (OR = 1.5; 95% CI, 1.1–1.9) were associated with an increased risk. No other significant associations between maternal and paternal factors were found; some environmental factors including rural residency, indoor cooking with wood, chemicals and water source appeared to be associated with an increased risk in adjusted models. Conclusion: Our study represents one of the first international studies investigating risk factors for clefts among multiethnic underserved populations. Our findings suggest a multifactorial etiology including both maternal and paternal factors.

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Key words: cleft lip/palate; cleft palate; parental risk factors; maternal health; multiethnic populations

Introduction

Oral clefting is among the most common birth defects, affecting 1 to 2 in every 1000 newborns worldwide (Mossey et al., 2009). Disruptions of normal embryonic craniofacial development by environmental exposures, in particular during the first trimester, are suspected contributors to oral

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clefting. Consistent evidence demonstrates an increased risk of clefting with maternal smoking as well as secondhand smoke (Honein et al., 2007; Li et al., 2010; Little et al., 2004; Services U.S. Department of Health, 2014; Shi et al., 2008). Inconsistent evidence for an association has been observed for alcohol use, which may likely depend on the quantity and nutritional modifiers including B-vitamin intake (Shaw and Lammer, 1999; Lorente et al., 2000; Bille et al., 2007; Romitti et al., 2007b). The influence of inadequate levels of selected nutrients including folate/folic acid (B₉) (Badovinac et al., 2007; Wilcox et al., 2007) and zinc (Tamura et al., 2005; Munger et al., 2009) from dietary sources and supplements during pregnancy remains unresolved. Other possible maternal risk factors include medication use, infections and specific health conditions (Mossey et al., 2009). In particular, diabetes (Spilson et al., 2001; Hrubec et al., 2009; Stott-Miller et al., 2010), hypertension (Hurst et al., 1995), and seizures (Gadoth et al., 1987; Friis, 1989) have been implicated as potential risk factors. Although many studies of environmental factors have been based on small sample sizes, it is unlikely that one single exposure will explain most of the risk associated with clefts, but rather that diverse exposures occurring at critical times in development will be independent contributing risk factors and together result in clefting.

There are limited data on specific paternal factors and environmental exposures that may influence the risk of oral clefts. In terms of paternal exposures, the most critical factor hypothesized to affect spermatogenesis and induce alterations that may affect DNA integrity is tobacco smoking (Zhang et al., 1992; Du et al., 2006; Jianyan et al., 2010; Cresci, 2011). The external environment also represents an important source of exposures that may directly affect the mother’s health status. Indeed, selected occupational and environmental exposures including air pollutants (Marshall et al., 2010), pesticides (Romitti et al., 2007a), and contaminated water sources (Cech et al., 2008) have all been hypothesized risk factors for oral clefts.

In this study, we present data from a unique international hospital-based case-control study of four populations in the Democratic Republic of the Congo (DRC), Vietnam, the Philippines, and Honduras collected at local hospitals and during surgical missions by a nonprofit organization, Operation Smile. In comparison to the United States, these regions of the world face great challenges with their public health infrastructure and lower economic development; significantly higher reported rates of maternal mortality and lower GDP per capita are reported in these countries than in the United States. The lack of detailed maternal health indicators and birth registries poses a unique challenge to obtain data on child-maternal outcomes in these countries. It is known that rates of clefts are highest in Asians and lowest in Africans (Mossey et al., 2009), but the risk factor profiles are unknown in such regions. Here, we try to address this gap in knowledge by examining selected maternal and paternal exposures as risk factors for clefts in four underserved populations in Central Africa, Southeast Asia, and Central America.

Materials and Methods

STUDY POPULATION

Methods have been previously published (Figueiredo et al., 2014). In brief, we use a case-control study design and collected data on children with cleft lip with or without cleft palate CL(P) and cleft palate (CP) (cases) and children without any clefts (controls) and on their parents in the DRC, Vietnam, the Philippines, and Honduras. Male and female children with a diagnosis of an isolated oral cleft age 3 and under were eligible for inclusion (singleton-births only). We focused on isolated nonsyndromic oral clefts. Our protocol included an active prospective effort to screen out and exclude cases and controls presenting with other major or minor anomalies or suspected genetic syndromes. For cases, a pediatrician or clinical geneticist screened the child for additional birth defects or signs of an underlying syndrome. Any subject with a minor congenital variant or common anomaly was excluded, although there would remain the possibility of a subclinical minor cardiac or other asymptomatic visceral or structural anomaly.

All eligible case-children had to be accompanied by their biological mother (aged ≥ 18 years) and/or biological father (aged ≥ 18 years) and seeking treatment from Operation Smile Inc. during scheduled missions from 2009 to 2014. Operation Smile, an international nonprofit organization that specializes in treatment of patients with cleft lip and/or cleft palate, has provided millions of patient evaluations and hundreds of thousands of free surgeries for children and young adults born with craniofacial deformities across the globe (Campbell et al., 2011). Operation Smile uses a variety of methods to recruit patients which include marketing materials in the city and working with local health care workers and transportation services, radio stations, and team members on the ground by word of mouth. Consequently, the catchment area of the case population is difficult to entirely assess but represent an underserved population nearby the hospital of service.

Eligible controls were children age 3 and under without an orofacial cleft or other congenital malformation (including limb, craniofacial, or skeletal abnormalities) at maternity wards or clinics of participating hospitals in the same approximate catchment region as the hospitals where children were treated by Operation Smile. All hospitals were either a public government-funded institutions or clinics that serviced a lower socio-economic population as comparable as possible to the case population. Children who were twins or triplets, and whose whose mothers were under age 18, pregnant at the time of data collection, or had a subsequent pregnancy were excluded to reduce
the possibility of misreporting exposures unrelated to the pregnancy of interest.

In the DRC, cases and controls were recruited from the following hospitals in Kinshasa: Clinique Ngaliema, General Hospital of Kinshasa, Roi Baudoin Health Center, Kingasani Maternity Hospital, Lisanga Maternity and Health Center; Maman Mosalisi Maternity and Health Center; and Bondeko Maternity and Health Center at three time points: June 2011, June 2012, and June 2013. The Vietnamese cases were recruited from the Vietnam Cuba Friendship Hospital and the Hanoi Maternity Hospital in Hanoi from September to December 2012. In the Philippines, cases were recruited in Bacolod City from the HOPE Foundation Cleft Center and controls from the Corazon Montelíbano Memorial Regional Hospital on November 2012 and Tere-sita L. Jalandoni Provincial Hospital on June 2014. In Honduras, cases were recruited in Tegucigalpa from Operation Smile Honduras Clinic, in Comayagua from Santa Teresa Regional Hospital, in Choluteca from Hospital del Sur; in Santa Rosa Copan from Western Regional Hospital, and controls from San Felipe Hospital in Tegucigalpa between February and August 2014. Participation rates were reasonably high for cases and controls: DRC (68% and 83%), the Philippines (69% and 77%), Vietnam (71% and 82%), and Honduras (57% and 61%).

This study was approved by the Institutional Review Board of the University of Southern California (FWA #: 00005906), University of Santo Tomas IRB in Manila, Philippines (FWA #: A00009240), and the National Council of the Order of Physicians in Kinshasa, DRC. In Vietnam and Honduras, collaborating hospital directors reviewed the study and made revisions regarding ethical, cultural, clinical, and vocabulary appropriateness and provided an authorization for human subject research, which were reviewed by the Institutional Review Board of the University of Southern California.

DATA COLLECTION AND VARIABLE DEFINITIONS
All mothers were interviewed by a local research assistant at the participating hospitals in each of the four countries. Local doctors and volunteer recruiters were trained to interview participants in a standardized manner. Maternal questions focused on lifetime health status and exposures with an emphasis on those during the pregnancy of interest. The following information was collected: demographic characteristics (i.e., place of residence, maternal and paternal age, marital status, educational level, income, employment status, ethnicity, and race); family history of oral clefts (i.e., maternal and paternal); pregnancy characteristics (i.e., parity, birth order, health status of the mother); and exposures before (3 months) and during pregnancy (i.e., alcohol consumption, drugs, smoking, medication use, health status/onset of disease, cooking method, water source, exposure to chemicals, and irradiation). Questions were asked with respect to the first trimester for a selected number of exposures including multivitamin use, alcohol, tobacco use, and chemical exposures. Smoking was defined as regular use of tobacco products including cigarettes, cigars, and pipes. Alcohol use was defined as regular consumption of wine, beer, or liquor.

Mothers were asked to report whether a medical professional had informed them of certain medical conditions including: diabetes, hypertension, or seizures either before or at any time during their pregnancy. Medication use was defined as any medication for diabetes, colds, pain, fever, urinary tract infections, obstetric disorder, antibiotics, antidepressants, corticosteroids, and other medications. Supplement use included both multivitamins and/or folic acid-specific supplement during the first trimester. Women who reported use of any type of agricultural or industrial chemical compound for any length of time during pregnancy were considered as exposed.

Lastly, mothers were requested to report on selected paternal factors including family history of oral clefting and other health conditions, selected exposures (i.e., smoking), and lifestyle (i.e., educational level, income, employment status).

STATISTICAL ANALYSIS
We present results below including all cases with any type of oral cleft (cleft lip, cleft lip with cleft palate, and cleft palate) exclusion of cleft palate cases did not appreciably change the estimates of risk and are presented in Supplementary Tables S1 to S3, which are available online. Chi-square and Student’s t tests were used to compare demographics/lifestyle factors and risk of oral clefts. Fisher’s exact test was used for the analysis of categorical variables with small numbers. Odds ratios (ORs) and 95% confidence intervals (CI) were calculated by logistic regression. Crude and adjusted odds ratios are reported. Adjusted models included the following confounders: child’s sex, mother and father’s employment status, mother’s and father’s education level, mother’s and father’s age at time of delivery, location at birth (rural vs. urban), and country (only in combined country analysis). Additional adjustment in multivariable models for other prenatal factors (i.e., hypertension, number of pregnancy, seizures, diabetes), maternal lifestyle (i.e., family history, alcohol, smoking), paternal factors (i.e., smoking), and environmental (i.e., household tobacco, chemical use) did not substantially change the adjusted estimates of risk. For missing continuous variables (i.e., parental age and number of pregnancies), the mean values for the case group and control groups by country were imputed. For missing categorical variables, we created a missing category. In general, less than 5% of key variables were missing. Both combined and country-specific estimates of risk are presented in the tables; no appreciably significant differences were observed by country. All p-values less than 0.05 were
considered statistically significant. All statistical tests were performed using SAS 9.4.

Results
There were a total of 430 mothers who had a child with an oral cleft (60.0% male and 40.0% female, Table 1); the majority of cases were children with cleft lip and palate (55.0%), and the remaining had an isolated cleft lip (32.6%) or isolated cleft palate (12.4%). Males were more likely to have cleft lip with or without cleft palate compared with females ($p = 0.03$). No significant differences were observed by country with respect to sex or type of cleft. Four (1.0%) of the 430 mothers of children with a cleft also were born with an oral cleft. Among controls, one mother of the 754 total (0.1%) was born with a cleft. Maternal family history of clefts was highly significantly associated with risk (adjusted OR = 4.7; 95% CI, 3.0–7.2) as well as having other biological children with a cleft (adjusted OR = 5.3; 95% CI, 1.4–19.9).

Mothers of affected children were more likely to be unemployed than control mothers (47.1% vs. 51.3%; Table 1); mothers with a primary school education or less and mothers with a secondary school education had an increased risk compared with mothers with a university education (adjusted OR = 2.4; 95% CI, 1.6–3.8 and adjusted OR = 1.6; 95% CI, 1.2–2.2, respectively, Table 2). Rural residence was more common among case-mothers compared with control-mothers (42.4% vs. 23.6%, adjusted OR = 2.1; 95% CI, 1.6–2.8; Table 2).

Maternal age was associated with a borderline increased risk (5-year adjusted OR = 1.2; 95% CI, 1.0–1.3; Table 2). Gravity was also positively associated with an increased risk, but did not reach statistical significance after adjustment for maternal and paternal age and other confounders (adjusted OR = 1.1; 95% CI, 1.0–1.1). We observed no association between incomplete pregnancies and risk of a child with an oral cleft. Moreover, children with a cleft were less likely to be first-born children compared with controls (32.2% vs. 40.2%), but there was no significant association with birth order.

The highest rates of prenatal care during the first trimester were reported among Vietnamese mothers (93%) and the lowest among the Congolese (29%); in the Philippines 63% of mothers received prenatal care and 86% in Honduras. Prepregnancy rates of reported supplement use appeared infrequent overall (<6%) with higher use of multivitamins in the Philippines (10–12%) and of folic acid in Honduras (9–11%). Overall, we found no association with 1st trimester supplement use and oral clefts (adjusted OR = 1.0; 95% CI, 0.7–1.3, Table 2). A variety of other types of supplements (vitamin B₆ and iron; argile in DRC; and medicinal herbs in Vietnam) were reported, but we did not have a sufficient sample size to estimate their association with risk of oral cleft.

We examined the association between a diagnosis of selected health conditions including diabetes, hypertension, and seizures. Overall, there was an increased risk associated with a medical history of hypertension before pregnancy and seizures during pregnancy. Although medication use was higher in the case-mothers than the control-mothers it did not reach statistical significance; the most common reasons for medication use were for the treatment of fevers, cold and infections requiring antibiotics.

Smoking was uncommon among mothers; no association was observed for maternal smoking either before or during pregnancy and risk of having a child with an oral cleft. Alcohol use was more frequent among Congolese mothers, and infrequent among Vietnamese, Filipino, and Honduran mothers; we observed no significant association with an increased risk of oral clefts. Household smoking, a proxy measure of secondhand smoke exposure, was nonsignificantly associated with an increased risk of having a child with an oral cleft (adjusted OR = 1.3; 95% CI, 1.0–1.7).

Exposures to chemicals from industrial or agricultural sources, both before and during the 1st trimester of pregnancy were reported more frequently in Vietnam compared with the other countries, and overall, was significantly associated with an increased risk with having a child with an oral cleft. There were some observed differences in the source of water between case and control mothers; reported drinking of water from a well was significant with risk of an oral cleft (adjusted OR = 2.0; 95% CI, 1.5–2.9). Lower risks were observed for mothers reporting drinking public or filtered water (OR = 0.6 and 0.7, respectively).

In Table 3, mothers reported on paternal factors. Overall, the mean age of case-fathers was similar to control-fathers (32.5 ± 7.7 vs. 32.5 ± 7.5). Five of 430 case-fathers were born with a cleft compared with one control-father. Significantly more case-fathers had a family history of an oral cleft in their families compared with control-fathers (18.4% vs. 2.0%, adjusted OR = 10.5; 95% CI, 5.9–18.8). Fathers of cases tended to have similar employment rates to those of control children (91.8% vs. 91.8%), but lower levels of completion of a secondary school or university education (77.8% vs. 88.1%, combined). Smoking was far more common among fathers than mothers in our overall study population and we observed a statistically significant increased risk of having a child with an orofacial cleft among fathers who had ever smoked compared with those that did not (adjusted OR = 1.5; 95% CI, 1.1–1.9).

Discussion
Understanding the broader implications of risk for oral clefts in diverse ethnic/racial populations in resource-limited settings is likely to provide additional insight into the underlying etiology. We observed strong evidence for a
| Characteristics of Children, Mothers, and Fathers Included in This Study | Vietnam | DRC | Philippines | Honduras | All |
|---|---|---|---|---|---|
| N | 75 | 158 | 133 | 301 | 102 | 152 | 120 | 143 | 430 | 754 |
| **Child** | | | | | | | | | | |
| Age (months) | 12.6 ± 8.6 | 0.2 ± 2.9 | 10.7 ± 8.9 | 0.9 ± 3.2 | 20.9 ± 11.8 | 0.02 ± 0.1 | 16.3 ± 13.0 | 0.3 ± 2.3 | 15.0 ± 11.5 | 0.5 ± 2.6 |
| Sex (N, %) | Male | 40 (54.1) | 95 (60.5) | 77 (58.3) | 156 (52.0) | 65 (63.7) | 80 (52.6) | 75 (62.5) | 72 (50.4) | 257 (60.0) | 403 (53.6) |
| Female | 34 (46.0) | 62 (39.5) | 55 (41.7) | 144 (48.0) | 37 (36.3) | 72 (47.4) | 45 (37.5) | 71 (49.7) | 171 (40.0) | 349 (46.4) |
| Type of cleft (N, %) | CL | 18 (24.3) | 53 (39.9) | 32 (31.4) | 36 (30.5) | 139 (32.6) | 17 (11.3) | 27 (22.5) | 11 (7.7) | 83 (19.3) | 36 (4.8) |
| CP | 7 (9.5) | 13 (9.8) | 15 (14.7) | 18 (15.3) | 53 (12.4) | 18 (11.3) | 18 (15.3) | 53 (12.4) | 36 (5.1) | 139 (32.6) | 18 (11.3) |
| Unknown type of cleft | 1 | 2 | 2 | 1 | 3 |
| **Mother** | | | | | | | | | | |
| Age at birth | 26.6 ± 5.2 | 28.6 ± 4.8 | 28.6 ± 6.6 | 27.9 ± 5.7 | 29.1 ± 7.4 | 27.0 ± 6.7 | 26.6 ± 6.9 | 24.3 ± 5.3 | 27.8 ± 6.8 | 27.2 ± 5.9 |
| Family history of clefts | 0.7 (9.7) | 3 (1.9) | 8 (6.1) | 5 (1.7) | 41 (40.2) | 17 (11.3) | 27 (22.5) | 11 (7.7) | 83 (19.3) | 36 (4.8) |
| Other child with cleft | 1 (4.0) | 2 (3.1) | 0 (0) | 1 (0.5) | 6 (8.0) | 0 (0) | 4 (5.1) | 0 (0) | 11 (3.9) | 3 (0.7) |
| Education level | University | 15 (20.8) | 83 (59.7) | 18 (13.9) | 54 (18.0) | 25 (25.3) | 58 (38.4) | 18 (15.0) | 31 (21.7) | 76 (17.7) | 226 (30.8) |
| Secondary | 57 (79.2) | 50 (36.0) | 80 (61.5) | 221 (73.7) | 90 (59.6) | 76 (50.3) | 39 (32.5) | 63 (44.1) | 235 (54.7) | 410 (55.9) |
| None/Primary | 0 (0) | 6 (4.3) | 32 (24.6) | 25 (8.3) | 15 (15.2) | 17 (11.3) | 63 (52.5) | 49 (34.3) | 110 (25.6) | 97 (13.2) |
| Employment status | Unemployed | 10 (13.5) | 6 (3.8) | 78 (59.5) | 151 (50.7) | 80 (80.0) | 122 (81.3) | 57 (47.5) | 86 (60.1) | 225 (52.9) | 365 (48.7) |
| Employed | 64 (86.5) | 152 (96.2) | 53 (40.5) | 147 (49.3) | 20 (20.0) | 28 (18.7) | 63 (52.5) | 57 (39.9) | 200 (47.1) | 384 (51.3) |
| Location at birth | City | 15 (26.3) | 110 (72.9) | 99 (83.9) | 201 (62.2) | 50 (53.2) | 93 (66.0) | 53 (49.1) | 87 (65.4) | 217 (57.6) | 491 (76.4) |
| Rural | 42 (73.7) | 41 (27.2) | 19 (16.1) | 17 (7.8) | 44 (46.8) | 48 (34.0) | 55 (50.9) | 46 (34.6) | 160 (42.4) | 152 (23.6) |
| # of pregnancies | 1.8 ± 0.9 | 2.0 ± 1.6 | 3.6 ± 2.1 | 3.1 ± 2.0 | 2.6 ± 2.0 | 2.3 ± 1.7 | 2.8 ± 2.0 | 2.1 ± 1.4 | 2.8 ± 2.0 | 2.5 ± 1.8 |
| Supplement use | No | 71 (98.6) | 155 (98.7) | 104 (98.1) | 238 (98.8) | 87 (88.8) | 134 (89.3) | 103 (86.6) | 127 (88.8) | 365 (92.4) | 654 (94.7) |
| Pre-pregnancy | 1 (1.4) | 2 (1.3) | 2 (1.9) | 3 (1.2) | 11 (11.2) | 16 (10.7) | 16 (13.5) | 16 (11.2) | 30 (7.6) | 37 (5.4) |
|                  | Vietnam | Cases | Controls | DRC | Cases | Controls | Philippines | Cases | Controls | Honduras | Cases | Controls | All | Cases | Controls |
|------------------|---------|-------|----------|-----|-------|----------|-------------|-------|----------|----------|-------|----------|-----|-------|----------|
| N                |         | 75    | 158      | 133 | 301   |           | 102         | 152   |          | 120     | 143   |          | 430 | 754   |           |
| No               |         | 69(84.0) | 153 (99.4) | 119 (94.4) | 286 (99.0) | 95 (94.9) | 147 (100) | 94 (87.9) | 137 (95.0) | 392 (98.0) | 717 (98.4) |
| Pre-pregnancy    |         | 62 (98.4) | 151 (99.3) | 116 (95.1) | 281 (98.3) | 96 (96.9) | 147 (100) | 105 (98.1) | 137 (96.8) | 392 (98.0) | 722 (99.0) |
| Pregnancy       |         | 66 (93.0) | 151 (97.4) | 101 (80.8) | 220 (77.5) | 91 (92.9) | 146 (97.3) | 119 (100) | 142 (100) | 377 (91.3) | 659 (90.2) |
| Medication use   |         | 66 (94.3) | 147 (94.2) | 97 (74.1) | 201 (77.5) | 91 (92.9) | 146 (97.3) | 119 (100) | 142 (100) | 377 (91.3) | 659 (90.2) |
| Alcohol use      |         | 66 (94.3) | 147 (94.2) | 97 (74.1) | 201 (77.5) | 91 (92.9) | 146 (97.3) | 119 (100) | 142 (100) | 377 (91.3) | 659 (90.2) |
| Seizures         |         | 66 (94.3) | 147 (94.2) | 97 (74.1) | 201 (77.5) | 91 (92.9) | 146 (97.3) | 119 (100) | 142 (100) | 377 (91.3) | 659 (90.2) |
| Hypertension     |         | 66 (94.3) | 147 (94.2) | 97 (74.1) | 201 (77.5) | 91 (92.9) | 146 (97.3) | 119 (100) | 142 (100) | 377 (91.3) | 659 (90.2) |
| No               |         | 66 (94.3) | 147 (94.2) | 97 (74.1) | 201 (77.5) | 91 (92.9) | 146 (97.3) | 119 (100) | 142 (100) | 377 (91.3) | 659 (90.2) |
| Pregnancy       |         | 66 (94.3) | 147 (94.2) | 97 (74.1) | 201 (77.5) | 91 (92.9) | 146 (97.3) | 119 (100) | 142 (100) | 377 (91.3) | 659 (90.2) |
| Pre-pregnancy    |         | 66 (94.3) | 147 (94.2) | 97 (74.1) | 201 (77.5) | 91 (92.9) | 146 (97.3) | 119 (100) | 142 (100) | 377 (91.3) | 659 (90.2) |
| Pregnancy       |         | 66 (94.3) | 147 (94.2) | 97 (74.1) | 201 (77.5) | 91 (92.9) | 146 (97.3) | 119 (100) | 142 (100) | 377 (91.3) | 659 (90.2) |

**TABLE 1. Continued**
| Vietnam | DRC | Philippines | Honduras | All |
|---------|-----|-------------|----------|-----|
| N       |     |             |          |     |
| Cases   | 75  | 158         | 133      | 301 |
| Controls| 158 | 133         | 301      |    |
| Cases   | 102 | 152         | 120      | 143 |
| Controls| 152 | 120         | 143      |    |
| Cases   | 430 | 754         |          |    |
| Controls| 754 |            |          |    |

**Tobacco use**

| No | 75 (100) | 158 (100) | 133 (100) | 301 (99.7) | 102 (95.9) | 152 (92.0) | 120 (98.3) | 143 (99.3) | 415 (98.6) | 737 (98.2) |
|-----|----------|-----------|-----------|------------|------------|------------|------------|------------|------------|-----------|
| Pre-pregnancy | 0 (0) | 0 (0) | 0 (0) | 1 (0.3) | 4 (4.1) | 12 (8.0) | 2 (1.7) | 1 (0.7) | 6 (1.4) | 14 (1.9) |
| No | 70 (100) | 156 (100) | 132 (99.3) | 300 (99.7) | 92 (96.8) | 146 (98.0) | 113 (98.3) | 143 (100) | 407 (98.6) | 745 (99.5) |
| 1st trimester | 0 (0) | 0 (0) | 1 (0.8) | 1 (0.3) | 3 (3.2) | 3 (2.0) | 2 (1.7) | 0 (0) | 6 (1.5) | 4 (0.5) |

**Household tobacco**

| No | 20 (28.17) | 70 (44.3) | 102 (79.1) | 247 (84.0) | 32 (3.2) | 40 (2.67) | 28 (2.35) | 27 (1.89) | 173 (21.4) | 272 (36.5) |
|-----|------------|-----------|------------|------------|----------|------------|------------|------------|-------------|---------|
| Yes | 51 (71.8) | 88 (55.7) | 27 (20.9) | 47 (16.0) | 67 (67.7) | 110 (73.3) | 130 (76.6) | 143 (100) | 110 (80.6) | 272 (36.5) |

**Indoor cooking with wood**

| No | 41 (59.4) | 126 (81.3) | 75 (58.1) | 220 (74.8) | 9 (9.3) | 19 (12.7) | 51 (42.9) | 87 (61.3) | 176 (42.5) | 452 (60.1) |
|-----|------------|-----------|------------|------------|--------|-----------|----------|------------|-------------|---------|
| Yes | 21 (49.1) | 29 (18.7) | 54 (41.9) | 74 (25.2) | 88 (90.7) | 131 (87.3) | 58 (47.1) | 55 (38.7) | 238 (57.5) | 289 (39.0) |

**Industrial chemical use**

| No | 59 (90.8) | 151 (96.8) | 117 (90.0) | 290 (96.8) | 89 (93.7) | 144 (96.6) | 117 (98.3) | 141 (99.3) | 382 (93.4) | 726 (98.0) |
|-----|------------|-----------|------------|------------|----------|------------|----------|------------|-------------|---------|
| 1st trimester | 6 (9.2) | 5 (3.2) | 13 (10.0) | 4 (1.4) | 6 (6.3) | 5 (3.4) | 2 (1.7) | 1 (0.7) | 27 (6.6) | 12 (2.0) |

**Agricultural chemical use**

| No | 58 (87.9) | 154 (98.7) | 120 (99.2) | 282 (100) | 88 (95.7) | 140 (96.6) | 115 (96.6) | 141 (99.3) | 381 (95.7) | 717 (98.9) |
|-----|------------|-----------|------------|------------|----------|------------|----------|------------|-------------|---------|
| 1st trimester | 8 (12.1) | 2 (1.3) | 1 (0.8) | 0 (0) | 4 (4.4) | 5 (3.5) | 4 (3.4) | 1 (0.7) | 17 (4.3) | 8 (1.1) |

**Water source**

| No | 39 (52.0) | 159 (88.0) | 120 (90.2) | 288 (96.0) | 59 (51.5) | 117 (78.0) | 86 (72.3) | 113 (79.6) | 304 (71.9) | 657 (87.6) |
|-----|------------|-----------|------------|------------|----------|------------|----------|------------|-------------|---------|
| Well water | 36 (48.0) | 19 (12.0) | 13 (9.8) | 12 (4.0) | 37 (35.5) | 33 (22.0) | 33 (27.7) | 29 (20.4) | 119 (28.1) | 93 (12.4) |
| No | 63 (84.0) | 104 (65.8) | 30 (22.6) | 36 (12.0) | 83 (85.5) | 114 (76.0) | 91 (76.5) | 108 (76.1) | 267 (63.1) | 362 (48.3) |
| Public water | 12 (16.0) | 54 (34.2) | 103 (73.7) | 264 (88.0) | 13 (15.5) | 36 (24.0) | 28 (23.5) | 34 (23.9) | 156 (36.9) | 388 (51.7) |
| No | 63 (84.0) | 97 (61.4) | 130 (97.7) | 285 (95.0) | 72 (75.0) | 102 (68.0) | 109 (91.6) | 133 (93.7) | 374 (88.4) | 617 (82.3) |
| Filtered water | 12 (16.0) | 61 (38.6) | 3 (2.3) | 15 (5.0) | 24 (25.0) | 48 (32.0) | 10 (8.4) | 9 (6.3) | 49 (11.6) | 133 (17.7) |

**Father**

| Age at birth | 29.7 ± 6.0 | 32.8 ± 5.6 | 35.6 ± 6.3 | 35.9 ± 6.4 | 32.8 ± 8.2 | 29.9 ± 8.5 | 30.4 ± 8.6 | 27.8 ± 6.9 | 32.5 ± 7.7 | 32.5 ± 7.5 |
|-------------|------------|-----------|------------|------------|------------|------------|------------|------------|-------------|---------|
| Family history of cleft | 6 (8.3) | 1 (0.7) | 8 (6.4) | 1 (0.3) | 43 (45.7) | 9 (6.2) | 18 (15.8) | 4 (2.9) | 77 (18.4) | 15 (2.0) |
| Education level | | | | | | | | | | |
|                        | Vietnam Cases | Vietnam Controls | DRC Cases | DRC Controls | Philippines Case | Philippines Controls | Honduras Cases | Honduras Controls | All Cases | All Controls |
|------------------------|---------------|------------------|-----------|--------------|------------------|----------------------|-----------------|------------------|-----------|--------------|
| N                      | 75            | 158              | 133       | 301          | 102              | 152                  | 120             | 143              | 430       | 754          |
| University             | 12 (17.4)     | 81 (60.0)        | 51 (41.8) | 164 (57.8)   | 33 (34.0)        | 56 (37.1)            | 9 (8.0)         | 23 (17.0)        | 105 (26.3)| 324 (46.0)   |
| Secondary              | 56 (81.2)     | 51 (37.8)        | 63 (51.6) | 113 (39.8)   | 48 (49.5)        | 66 (43.7)            | 39 (34.8)       | 67 (49.6)        | 206 (51.5)| 297 (42.1)   |
| None/Primary           | 1 (1.4)       | 3 (2.2)          | 8 (6.6)   | 7 (2.5)      | 16 (16.5)        | 29 (19.2)            | 64 (57.1)       | 45 (33.3)        | 89 (22.3)| 84 (11.9)    |
| Employment status      |               |                  |           |              |                  |                      |                 |                  |           |              |
| Unemployed             | 1 (1.4)       | 0 (0)            | 25 (19.5) | 32 (10.9)    | 4 (4.2)          | 24 (15.9)            | 4 (3.5)         | 5 (3.7)          | 34 (8.2)| 61 (8.2)     |
| Employed               | 73 (98.7)     | 158 (100)        | 103 (80.5)| 262 (89.1)   | 92 (95.8)        | 127 (84.1)           | 111 (96.5)      | 132 (96.4)       | 379 (91.8)| 679 (91.8)   |
| Tobacco use            |               |                  |           |              |                  |                      |                 |                  |           |              |
| No                     | 27 (37.0)     | 74 (46.9)        | 55 (71.4) | 174 (85.7)   | 33 (33.0)        | 55 (36.9)            | 75 (66.4)       | 100 (72.5)       | 190 (52.3)| 403 (62.2)   |
| Yes                    | 46 (63.0)     | 84 (53.2)        | 22 (28.6) | 29 (14.3)    | 67 (67.0)        | 94 (63.1)            | 38 (33.6)       | 38 (37.5)        | 173 (47.7)| 245 (37.8)   |

aData missing from 2011 DRC collection.

bIncludes parent and parent’s relatives born with an oral cleft.

cIncludes multivitamins and folic acid supplements.

CI, confidence interval; DRC, Democratic Republic of the Congo; OR, odds ratio.
|                          | Vietnam |          | DRC |          | Philippines |          | Honduras |          | All |          |
|--------------------------|---------|----------|-----|----------|-------------|----------|----------|----------|-----|----------|
|                          | Crude OR (95% CI) | Adjusted OR (95% CI) | Crude OR (95% CI) | Adjusted OR (95% CI) | Crude OR (95% CI) | Adjusted OR (95% CI) | Crude OR (95% CI) | Adjusted OR (95% CI) | Crude OR (95% CI) | Adjusted OR (95% CI) |
| Age at birth             | 0.6 (0.5-0.9)† | 1.0 (0.6-1.6) | 1.1 (0.9-1.3) | 1.2 (1.0-1.6) | 1.2 (1.0-1.5) † | 1.0 (0.7-1.4) | 1.4 (1.1-1.7) † | 1.1 (0.9-1.5) | 1.1 (1.0-1.2) | 1.2 (1.0-1.3) † |
| Family history of clefts | 5.6 (1.4-22.2)† | 2.1 (0.5-9.2) | 3.8 (1.2-11.9) † | 5.0 (1.5-16.8) † | 5.3 (2.8-10.1) † | 5.2 (2.6-10.4) † | 3.5 (1.6-7.4) † | 4.4 (1.9-9.8) † | 4.8 (3.2-7.3) † | 4.7 (3.0-7.2) † |
| Other children with clefts | 1.3 (0.1-15.2) | 1.2 (0.1-15.6) | – | – | – | – | – | – | 5.7 (1.6-20.6)† | 5.3 (1.4-19.9)† |
| Education level          |         |          |     |         |         |          |         |          |     |          |
| University               | 1       | 1        | 1   | 1        | 1        | 1        | 1        | 1        | 1   | 1        |
| Secondary                | 6.3 (3.2-12.3)† | 3.4 (1.5-7.3)† | 1.1 (0.6-2.0) | 0.9 (0.5-1.7) | 1.8 (1.0-3.2)† | 1.5 (0.8-2.9) | 1.1 (0.5-2.2) | 1.2 (0.6-2.6) | 1.7 (1.3-2.3)† | 1.6 (1.2-2.2)† |
| None/Primary             | –       | –        | 3.8 (1.8-8.1)† | 2.7 (1.2-6.2)† | 2.0 (0.9-4.7) | 1.8 (0.7-5.1) | 2.2 (1.1-4.4)† | 1.3 (0.6-2.9) | 3.4 (2.3-4.9)† | 2.4 (1.6-3.8)† |
| Employment Status        |         |          |     |         |         |          |         |          |     |          |
| Unemployed               | 1       | 1        | 1   | 1        | 1        | 1        | 1        | 1        | 1   | 1        |
| Employed                 | 0.3 (0.1-0.7)† | 0.2 (0.05-0.6)† | 0.7 (0.5-1.1) | 0.7 (0.4-1.0) | 1.1 (0.6-2.1) | 0.8 (0.4-1.7) | 1.7 (1.0-2.7)† | 1.8 (1.0-3.0) | 0.8 (0.7-1.1) | 0.9 (0.7-1.2) |
| Location                 |         |          |     |         |         |          |         |          |     |          |
| City                     | 1       | 1        | 1   | 1        | 1        | 1        | 1        | 1        | 1   | 1        |
| Rural                    | 7.5 (3.8-15.0)† | 8.4 (3.8-18.3)† | 2.3 (1.1-4.6)† | 1.9 (0.9-4.0) | 1.7 (1.0-2.9) | 1.7 (1.0-3.2) | 2.0 (1.2-3.3)† | 1.3 (0.7-2.3) | 2.4 (1.8-3.1)† | 2.1 (1.6-2.8)† |
| # of pregnancies         | 0.9 (0.7-1.1) | 0.9 (0.7-1.3) | 1.1 (1.0-1.2)† | 1.1 (1.0-1.3) | 1.1 (0.9-1.3) | 1.0 (0.9-1.2) | 1.3 (1.1-1.5)† | 1.1 (0.9-1.4) | 1.1 (1.0-1.2) | 1.1 (1.0-1.1) |
| Supplement use           |         |          |     |         |         |          |         |          |     |          |
| No                       | 1       | 1        | 1   | 1        | 1        | 1        | 1        | 1        | 1   | 1        |
| Pre-pregnancy            | 1.1 (0.1-12.2) | 0.8 (0.01-108.7) | 1.5 (0.3-9.3) | 2.0 (0.2-16.2) | 1.1 (0.5-2.4) | 1.1 (0.4-2.6) | 1.2 (0.6-2.6) | 1.3 (0.6-3.0) | 1.5 (0.9-2.4) | 1.3 (0.8-2.2) |
| 1st trimester            | 0.6 (0.3-1.1) | 0.9 (0.4-2.2) | 1.0 (0.4-2.4) | 1.0 (0.4-2.5) | 1.4 (0.8-2.5) | 1.7 (0.9-3.2) | 0.7 (0.5-1.2) | 0.7 (0.4-1.3) | 1.2 (0.9-1.6) | 1.0 (0.7-1.3) |
| Diabetes                 |         |          |     |         |         |          |         |          |     |          |
| No                       | 1       | 1        | 1   | 1        | 1        | 1        | 1        | 1        | 1   | 1        |
| Pre-pregnancy            | –       | –        | 2.3 (0.3-16.6) | 1.2 (0.2-10.1) | –       | –       | 0.3 (0.1-1.3) | 0.3 (0.05-1.4) | 1.2 (0.5-3.0) | 1.2 (0.5-3.0) |
| No                       | 1       | 1        | 1   | 1        | 1        | 1        | 1        | 1        | 1   | 1        |
| Pregnancy                | –       | –        | 2.4 (0.5-11.9) | 1.5 (0.2-9.6) | –       | –       | 0.3 (0.1-1.2) | 0.2 (0.04-1.3) | 1.3 (0.5-3.0) | 1.2 (0.5-2.9) |
| Hypertension             |         |          |     |         |         |          |         |          |     |          |
| No                       | 1       | 1        | 1   | 1        | 1        | 1        | 1        | 1        | 1   | 1        |
| Pre-pregnancy            | –       | –        | 5.6 (1.4-22.1) | 3.4 (0.8-14.4) | 1.5 (0.4-5.4) | 1.6 (0.4-6.0) | 2.6 (1.0-6.8)† | 2.6 (0.9-7.5) | 3.2 (1.7-6.1)† | 2.6 (1.3-5.1)† |
| No                       | 1       | 1        | 1   | 1        | 1        | 1        | 1        | 1        | 1   | 1        |
|                          | Vietnam Crude OR (95% CI) | Vietnam Adjusted OR (95% CI) | DRC Crude OR (95% CI) | DRC Adjusted OR (95% CI) | Philippines Crude OR (95% CI) | Philippines Adjusted OR (95% CI) | Honduras Crude OR (95% CI) | Honduras Adjusted OR (95% CI) | All Crude OR (95% CI) | All Adjusted OR (95% CI) |
|--------------------------|---------------------------|-----------------------------|-----------------------|--------------------------|-----------------------------|--------------------------------|---------------------------|--------------------------|-----------------|--------------------------|
| Pregnancy                |                           |                             |                       |                          |                             |                                |                           |                          |                 |                          |
|                         | 2.4 (0.2-39.6)            | 4.5 (0.02-979.3)            | 1.2 (0.6-2.5)         | 1.1 (0.5-2.3)            | 0.9 (0.4-2.1)               | 0.8 (0.3-2.0)                  | 2.1 (0.9-4.8)            | 2.3 (0.9-5.9)         | 1.4 (0.9-2.2) | 1.3 (0.9-2.1)            |
| Seizures                 |                           |                             |                       |                          |                             |                                |                           |                          |                 |                          |
| No                      | 1                          | 1                           | 1                     | 1                        | 1                           |                                 | 1                         | 1                        | 1                | 1                        |
| Pre-pregnancy            | –                          | –                           | 1.4 (0.3-5.9)         | 0.8 (0.2-3.8)            | –                           |                                 | 1.3 (0.2-9.4)            | 0.5 (0.1-4.4)         | 2.1 (0.8-5.8) | 2.0 (0.7-5.8)            |
| Pregnancy               | –                          | –                           | 2.9 (0.9-9.7)         | 2.2 (0.6-8.3)            | 4.6 (0.5-45.2)              | 11.5 (0.8-168.9)              | 1.8 (0.3-11.1)         | 0.9 (0.1-6.6)         | 2.8 (1.1-6.9) | 2.9 (1.1-7.4)            |
| Medication use           |                           |                             |                       |                          |                             |                                |                           |                          |                 |                          |
| No                      | 1                          | 1                           | 1                     | 1                        | 1                           |                                 | 1                         | 1                        | 1                | 1                        |
| Pre-pregnancy            | 2.9 (0.7-11.0)            | 4.1 (0.8-21.3)              | 0.8 (0.5-1.4)         | 0.8 (0.5-1.5)            | 2.8 (0.8-9.9)               | 2.8 (0.8-10.1)                | –                         | –                        | 0.9 (0.6-1.3) | 1.2 (0.8-1.9)            |
| Alcohol use              |                           |                             |                       |                          |                             |                                |                           |                          |                 |                          |
| No                      | 1                          | 1                           | 1                     | 1                        | 1                           |                                 | 1                         | 1                        | 1                | 1                        |
| Pre-pregnancy            | 1.0 (0.3-3.3)             | 0.7 (0.2-2.9)               | 0.7 (0.4-1.1)         | 0.8 (0.5-1.2)            | 0.6 (0.3-1.3)               | 0.7 (0.3-1.6)                 | 1.5 (0.4-5.9)            | 2.7 (0.6-11.7)         | 0.7 (0.5-0.9) | 0.8 (0.6-1.2)            |
| Tobacco use              |                           |                             |                       |                          |                             |                                |                           |                          |                 |                          |
| No                      | 1                          | 1                           | 1                     | 1                        | 1                           |                                 | 1                         | 1                        | 1                | 1                        |
| Pre-pregnancy            | –                          | –                           | –                     | –                        | 0.5 (0.2-1.6)               | 0.9 (0.2-3.2)                 | 2.5 (0.2-27.6)           | 2.5 (0.2-31.3)         | 0.8 (0.3-20) | 0.8 (0.3-21)            |
| Household tobacco        |                           |                             |                       |                          |                             |                                |                           |                          |                 |                          |
| Yes                     | 2.0 (1.1-3.7)             | 1.8 (0.9-3.7)               | 1.4 (0.8-2.4)         | 1.2 (0.7-2.1)            | 0.8 (0.4-1.3)               | 0.9 (0.5-1.7)                 | 1.3 (0.7-2.4)            | 2.1 (1.0-4.2)         | 1.2 (1.0-16) | 1.3 (1.0-1.7)            |
| Indoor cooking with wood |                           |                             |                       |                          |                             |                                |                           |                          |                 |                          |
| No                      | 1                          | 1                           | 1                     | 1                        | 1                           |                                 | 1                         | 1                        | 1                | 1                        |
| Yes                     | 3.0 (1.6-5.6)             | 1.5 (0.7-3.2)               | 2.1 (1.4-3.3)         | 1.7 (1.0-2.7)            | 1.4 (0.6-3.3)               | 1.3 (0.5-3.2)                 | 2.1 (1.3-3.5)            | 1.7 (0.9-3.2)         | 2.1 (1.7-2.7) | 1.6 (1.2-2.1)            |
| Industrial chemicals     |                           |                             |                       |                          |                             |                                |                           |                          |                 |                          |
| No                      | 1                          | 1                           | 1                     | 1                        | 1                           |                                 | 1                         | 1                        | 1                | 1                        |
| Yes                     |                            |                             |                       |                          |                             |                                |                           |                          |                 |                          |
|                | Vietnam |         | DRC |         | Philippines |         | Honduras |         | All |         |
|----------------|---------|---------|-----|---------|-------------|---------|----------|---------|-----|---------|
|                | Crude OR (95% CI) | Adjusted OR (95% CI) | Crude OR (95% CI) | Adjusted OR (95% CI) | Crude OR (95% CI) | Adjusted OR (95% CI) | Crude OR (95% CI) | Adjusted OR (95% CI) | Crude OR (95% CI) | Adjusted OR (95% CI) |
| **1st trimester** | 3.1 (0.9–10.4) | 2.0 (0.5–8.7) | 8.1 (2.6–25.2) | 7.3 (2.3–23.9) | 1.9 (0.6–6.5) | 2.0 (0.5–7.2) | 2.4 (0.2–26.9) | 2.3 (0.2–27.9) | 3.4 (1.8–6.5) | 3.9 (2.0–7.6) |
| **Agricultural chemicals** |         |         |     |         |             |         |           |         |     |         |
| No | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 1st trimester | 10.6 (2.2–51.5) | † | 12.2 (2.1–71.1) | † | – | – | 1.3 (0.3–4.9) | 0.7 (0.2–3.0) | 4.9 (0.5–44.5) | 2.8 (0.3–29.3) | 4.0 (1.7–9.3) | 1.1 (1.1–6.7) |
| **Water source** |         |         |     |         |             |         |           |         |     |         |
| No | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Public water | 0.4 (0.2–0.7) | † | 0.4 (0.2–1.0) | † | 0.5 (0.3–1.0) | † | 0.5 (0.3–1.0) | † | 0.6 (0.3–1.2) | 1.0 (0.6–1.7) | 0.8 (0.4–1.6) | 0.5 (0.4–0.7) | † | 0.6 (0.5–0.8) | † |
| No | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Filtered water | 0.3 (0.2–0.6) | † | 0.2 (0.1–0.4) | † | 0.4 (0.1–1.5) | 0.9 (0.2–3.6) | 0.7 (0.4–1.3) | 0.9 (0.4–1.6) | 1.4 (0.5–3.5) | 1.6 (0.6–4.4) | 0.6 (0.4–0.9) | † | 0.7 (0.5–0.9) | † |

Adjusted by child's sex, mother and father's employment status (employed/unemployed), mother’s and father’s education (completed primary school or less/ completed secondary school or more), mother’s and father’s age at time of delivery, location at birth (rural/urban), and country (for OR on combined country data).

*5-year OR given.

*p is significant at the 0.05 level.

†Includes parent and parent’s relatives born with an oral cleft.

‡Includes multivitamins and folic acid supplements.

CI, confidence interval; DRC, Democratic Republic of the Congo; OR, odds ratio.
|                          | Vietnam          |                | DRC            |                | Philippines     |                | Honduras       |                | All            |                |
|--------------------------|------------------|----------------|----------------|----------------|------------------|----------------|----------------|----------------|----------------|----------------|
|                          | Crude OR (95% CI)| Adjusted OR (95% CI) | Crude OR (95% CI) | Adjusted OR (95% CI) | Crude OR (95% CI) | Adjusted OR (95% CI) | Crude OR (95% CI) | Adjusted OR (95% CI) | Crude OR (95% CI) | Adjusted OR (95% CI) |
| **Age at birth**<sup>a</sup> | 0.6 (0.4–0.8)<sup>b</sup> | 0.7 (0.5–1.2) | 1.0 (0.8–1.1) | 0.8 (0.7–1.1) | 1.2 (1.1–1.4)<sup>b</sup> | 1.2 (0.9–1.5) | 1.2 (1.1–1.5)<sup>b</sup> | 1.1 (0.9–1.4) | 1.0 (0.9–1.1) | 0.9 (0.8–1.0) |
| **Family history** of cleft<sup>c</sup> | 14.1 (1.7–119.3)<sup>b</sup> | 12.3 (1.0–154.7) | 19.3 (2.4–155.6)<sup>b</sup> | 21.7 (2.6–184.2)<sup>b</sup> | 12.6 (5.8–27.5)<sup>b</sup> | 12.8 (5.6–29.0)<sup>b</sup> | 6.2 (2.1–19.1)<sup>b</sup> | 5.7 (1.7–18.9)<sup>b</sup> | 10.9 (6.2–19.3)<sup>b</sup> | 10.5 (5.9–18.8)<sup>b</sup> |
| **Education level**       |                  |                |                |                |                  |                |                |                |                |                |
| University                | 1                | 1              | 1              | 1              | 1                | 1              | 1              | 1              | 1              | 1              |
| Secondary                 | 7.4 (3.6–15.1)<sup>b</sup> | 4.4 (1.9–10.1)<sup>b</sup> | 1.8 (1.2–2.8)<sup>b</sup> | 1.5 (0.9–2.4) | 1.2 (0.7–2.2) | 1.0 (0.5–1.8) | 1.5 (0.6–3.5) | 2.0 (0.8–5.2) | 2.1 (1.6–2.8)<sup>b</sup> | 1.9 (1.4–2.5)<sup>b</sup> |
| None/Primary              | 2.2 (0.2–23.4) | ---            | 3.7 (1.3–10.6)<sup>b</sup> | 1.9 (0.5–6.6) | 0.9 (0.4–2.0) | 0.6 (0.2–1.3) | 3.3 (1.5–8.6)<sup>b</sup> | 4.2 (1.5–11.5)<sup>b</sup> | 3.3 (2.3–4.7)<sup>b</sup> | 1.8 (1.1–2.9)<sup>b</sup> |
| **Employment Status**     |                  |                |                |                |                  |                |                |                |                |                |
| Unemployed                | 1                | 1              | 1              | 1              | 1                | 1              | 1              | 1              | 1              | 1              |
| Employed                  | ---              | ---            | 0.5 (0.3–0.9)<sup>b</sup> | 0.8 (0.4–1.4) | 4.3 (1.5–12.9)<sup>b</sup> | 5.2 (1.5–18.6) | 1.1 (0.3–4.0) | 0.7 (0.2–2.6) | 1.0 (0.6–1.6) | 0.9 (0.6–1.5) |
| **Tobacco use**<sup>d</sup> |                  |                |                |                |                  |                |                |                |                |                |
| No                       | 1                | 1              | 1              | 1              | 1                | 1              | 1              | 1              | 1              | 1              |
| Yes                      | 1.5 (0.9–2.7) | 1.3 (0.6–2.6) | 2.4 (1.3–4.5)<sup>b</sup> | 2.1 (1.0–4.2) | 1.2 (0.7–2.0) | 1.5 (0.8–2.7) | 1.3 (0.8–2.3) | 1.5 (0.8–2.6) | 1.5 (1.2–1.9)<sup>b</sup> | 1.5 (1.1–1.9)<sup>b</sup> |

Adjusted by child’s sex, mother and father’s employment status (employed/unemployed), mother and father’s education (completed primary school or less/ completed secondary school or more), mother’s and father’s age at time of delivery, location at birth (rural/urban), and country (for OR on combined country data).

<sup>a</sup>5-year OR given.

<sup>b</sup>p is significant at the 0.05 level.

<sup>c</sup>Includes parent and parent’s relatives born with an oral cleft.

<sup>d</sup>Data missing from 2011 DRC collection.

CI, confidence interval; DRC, Democratic Republic of the Congo; OR, odds ratio.
family history of oral clefts; the estimates of risk appeared stronger for one or more affected relatives from the paternal side compared with the maternal side. Higher education among mothers was a significant protective factor; whereas advanced maternal age, other children born with an oral cleft, pregestational hypertension, and gestational seizures were associated with an increased risk. Tobacco use was infrequent among mothers; however, paternal smoking was significantly associated with an increased risk of having a child with an oral cleft. Several environmental factors including rural residence, cooking with wood, public water source, and exposure to chemical and agricultural products were significant risk factors, but need to be replicated in other studies.

Several studies suggest that a family history of oral clefts increases risk of both cleft lip with or without palate and cleft palate (Fraser, 1989; Dixon et al., 2011). Previous studies have reported an 8.25 increased odds (95% CI, 3.12–23.52) associated with any family history (Salihu et al., 2014) and 6.86-fold increased risk (95% CI, 3.39–13.87) for an affected paternal relative and 4.61-fold increased risk (95% CI, 2.34–9.06) for a maternal relative (Acuna-Gonzalez et al., 2011). The recurrence risk to siblings is greater than that predicted by familial aggregation of environmental risk factors (Wyszynski et al., 1998). Concordance rates for cleft lip, cleft lip and palate, and cleft palate alone are higher in monozygotic twin pairs than in dizygotic pairs (Little and Bryan, 1986). Several genetic variants have been identified through linkage studies (Letra et al., 2012; Bureau et al., 2014; Ludwig et al., 2014) and genome-wide association studies (Birnbaum et al., 2009; Grant et al., 2009; Beaty et al., 2010; Mangold et al., 2010; Ludwig et al., 2012; Figueiredo et al., 2014). Of the number of suspected environmental causes, smoking is the exposure most consistently associated with oral clefts and is estimated to account for up to 20% of all cases (Mossey et al., 2009; Services U.S. Department of Health, 2014). Cigarette smoke is a complex mixture of toxic and teratogenic chemicals and has been reported to exert an adverse effect on the development of several vulnerable fetal structures (Anblagan et al., 2013; Ekblad et al., 2015). Both maternal smoking before and during pregnancy has been shown to be associated with an increased risk of having a child with a cleft (Little et al., 2004; Honein et al., 2007; Shi et al., 2008; Li et al., 2010). In our data, we had low overall reported smoking among mothers consistent with smoking patterns in African, Asian, and Central American countries, and consequently were not able to determine this association. However, a greater proportion of our study population reported smoking among fathers, and children of fathers who smoked had an increased risk of having a child with a cleft. Previous studies have also observed paternal smoking to be associated with an increased risk (Savitz et al., 1991; Zhang et al., 1992b; Krapels et al., 2006; Mirilas et al., 2011). Although the mechanism is still unclear, passive smoking during pregnancy is a risk factor for clefts (Li et al., 2011); additionally, paternal tobacco smoking has been linked to DNA alterations in sperm (Savitz et al., 1991; Axelsson et al., 2013) that may directly interfere with craniofacial development.

Overall, our data generally suggest that good maternal health and prenatal care lower the risk of clefting. Maternal alcohol use, which is a known folate-antagonist and a well-known cause of fetal alcohol syndrome, has been inconsistently associated with risk of oral clefts (Shaw and Lammer, 1999; Lorente et al., 2000; Bille et al., 2007; Romitti et al., 2007b) and our analysis showed no association. We also did not find that early or continuing prenatal visits was associated with lower risk in any of the countries, although most of the women enrolled in this study reported some form of prenatal care. We observed that a diagnosis of hypertension before pregnancy was associated with an increased risk in agreement with other research (Hurst et al., 1995). Hypertensive disorders and antihypertensive medications use in early pregnancy have both been suggested to affect fetal development through teratogenic mechanisms (van Gelder et al., 2010). Other conditions, including diabetes (Spilson et al., 2001; Hrubec et al., 2009; Stott-Miller et al., 2010), seizures (Gadoth et al., 1987; Friis, 1989), and colds (Lin et al., 2014) have also been linked to oral clefts. Whether such conditions directly or indirectly affect palatogenesis by use of medications is possible (Hviid and Molgaard-Nielsen, 2011) but unclear to date.

Several maternal reproductive factors have been inconsistently associated with risk of clefts including previous incomplete pregnancies, previous spontaneous abortions, short inter-pregnancy interval, and birth order (Shiota, 1989; Felix-Schollaart et al., 1992). We did not confirm these associations; however, we did observe an increased risk with birth order (similar to gravidity) that could be explained by adjusting for maternal age. A recent meta-analysis reported that clefting is associated with increasing birth order peaking at an odds ratio of 3.67 (95% CI, 3.36–3.99) in children birth order of 4 or more (Vieira and Orioli, 2002); however, as the authors pointed out by an editorial (Zeiger and Beaty, 2002), maternal age, smoking, nutrition, and other potential confounders could not be accounted. Indeed, women with more children are generally older and increasing number of pregnancies especially with short intervals may be associated with nutritional deficiency (Luo et al., 2013; Csermely et al., 2014) or accumulation of DNA alterations due to lifestyle/environmental exposures (i.e., smoking). Furthermore, older maternal age is often correlated with older paternal age. A recent meta-analysis found that fathers 40 years of age or older and mothers aged 35 years of older were more likely to have an affected child than parents aged between 20 and 29 years (Herkrath et al., 2012). Bille et al. found high paternal age was associated with clefts;
however, maternal age did not show the same relationship (Billy et al., 2005). Further research is needed to untangle the independent effects of maternal and paternal reproductive factors on risk of clefts.

Several socio-economic and environmental factors were found in our study to be significant factors associated with risk of having a child with a cleft. Our research indicated that low parental education level appeared to be a risk factor for having a child with a cleft in agreement with some studies (Hemminki et al., 1980; Laumon et al., 1996), but not all (Carmichael et al., 2003). The underlying reason is unknown but could be associated with prenatal care, nutrition and other lifestyle behaviors, although our analysis did not observe strong correlations between these factors. Of interest is our finding that maternal exposures to chemicals from industrial or agricultural sources were more common among mothers of cases compared with mothers of controls. Rural residence including working on farms and well water consumption was higher among case-mothers compared with control-mothers. Previous studies have also suggested that rural residence (Messer et al., 2010) and a variety of teratogens present in the environment (Chevrier et al., 2006; Mirilas et al., 2011) and water source (Chevrier et al., 2008) as potential risk factors for clefts. Considering the patient recruitment strategy of Operation Smile and our methodology of collecting controls there is a potential for biased estimates and our reported associations need to be replicated in population-based studies with more detailed environmental and neighborhood measurements. However, conducting population-based studies in underserved populations may prove difficult given the lack of registries and consequently hospital-based studies may be the most practical approach despite their inherent limitations.

Our analysis has limitations and notable strengths. First, we may be subject to selection and recall bias. We recruited controls among women giving birth at maternity wards, while cases were recruited from hospitals but not at the time of surgery and were older than controls. We did not observe appreciable differences when limiting cases and controls to the same year of birth; although potential selection bias cannot entirely be ruled out. Nevertheless, no research in these underserved populations exists to assess this potential issue and future studies are needed to confirm our results. Overall, recruitment of controls coincided in time with case reporting; however, the control mothers were, on average, more educated and more likely to live in urban areas compared with the mothers of cases. To provide additional protection against this type of bias, we reanalyzed the data using conditional logistic regression after 1:1 case–control matching based on confounding characteristics and did not observe any significant differences in the estimates of risk, further strengthening our internal validity. Lower education and rural residence at birth have also been associated with clefts in other studies (Messer et al., 2010; Lin et al., 2014; Materna-Kiryukh et al., 2014). Second, even though we found no appreciable differences in our estimates of risk for any cleft type versus cleft lip with or without cleft palate, our limited sample size did not permit us to examine risk estimates for isolated cleft palate. Lastly, although we requested mothers to report on selected paternal exposures, there may still be misclassification (albeit nondifferential) and future studies need to consider engaging fathers as study participants to examine in greater detail paternal-specific exposures. There are several advantages in this international study of diverse underserved populations including the large variability in exposures, which is in contrast to the majority of studies on non-Hispanic Whites in high-income folic acid-fortified countries, where mothers maintain a fairly consistent and monitored health status during pregnancy to promote fetal health and development. Our study represents one of the first initiatives to examine multiethnic underserved populations and evaluate the combined effect of maternal and paternal factors for oral clefts.

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