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Increased risk of orofacial clefts associated with maternal obesity: case–control study and Monte Carlo-based bias analysis

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Summary

Our objective was to evaluate whether infants born to obese or diabetic women are at higher risk of non-syndromic orofacial clefting. We conducted a population-based case–control study using Washington State birth certificate and hospitalisation data for the years 1987–2005. Cases were infants born with orofacial clefts (n = 2153) and controls infants without orofacial clefts (n = 18 070). The primary exposures were maternal obesity (body mass index ≥30) and diabetes (either pre-existing or gestational). We estimated adjusted odds ratios (ORs) to compare, for mothers of cases and controls, the proportions of obese vs. normal-weight women and diabetic vs. non-diabetic women. We additionally performed Monte Carlo-based simulation analysis to explore possible influences of biases.

Obese women had a small increased risk of isolated orofacial clefts in their offspring compared with normal-body mass index women [adjusted OR 1.26; 95% confidence interval 1.03, 1.55]. Results were similar regardless of type of cleft. Bias analyses suggest that estimates may represent underlying ORs of stronger magnitude. Results for diabetic women were highly imprecise and inconsistent. We and others have observed weak associations of similar magnitude between maternal obesity and risk of nonsyndromic orofacial clefts. These results could be due to bias or residual confounding. However, it is also possible that these results represent a stronger underlying association. More precise exposure measurement could help distinguish between these two possibilities.

Keywords

cleft lip; cleft palate; maternal obesity

Introduction

Orofacial clefts are among the most common birth defects, with an estimated prevalence of 10.5 infants per 10 000 livebirths for cleft lip with or without cleft palate and 6.4 per 10 000 for cleft palate alone.1 Children with orofacial clefts require complex and expensive surgical

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and medical treatments. While much research has been carried out on potential genetic and environmental causes, for the vast majority of cases the aetiology remains uncertain.

Maternal obesity has been found to be associated with a number of congenital malformations, including neural tube defects, spina bifida, cardiovascular anomalies and orofacial clefts.[2–4] An association between risk of orofacial clefts and maternal obesity may be due to adverse sequelae of glucose intolerance or insulin resistance, disorders that are strongly associated with obesity.[5–7] Women with pre-existing diabetes appear to be at higher risk of having an infant with orofacial clefts.[8–12] Given the increased risk of diabetes[6] and glucose intolerance[7] associated with obesity, excess adiposity may involve metabolic abnormalities similar to diabetes, and thus the biological mechanisms that result in increased rates of congenital malformations in diabetic women might be similar for obese women.

These potential mechanisms include (a) hypoxic stress to the fetus due to maternal hyperglycaemia or hyperinsulinaemia,[13–16] or (b) increased formation of advanced glycation endproducts, resulting in DNA damage.[17–19] Alternatively, the biological mechanisms through which obesity might increase the risk for infant orofacial clefts may be directly or indirectly due to excess adiposity. Excess adiposity results in disturbed secretion of adipokines and pro-inflammatory cytokines, infiltration of immune cells into adipose tissue and a chronic state of low-grade inflammation.[20,21] A further possibility is an indirect influence of excess adiposity due to bioaccumulation and release of pollutants, such as dioxins, which have been shown to cause cleft palate in mice.[22] Dioxins and other persistent organic pollutants are stored in adipose tissue, resulting in a higher body burden of these toxins in obese individuals. Pregnancy causes enhanced release of stored chemicals, thus creating the potential for pollutants, such as dioxins, to affect the developing fetus.

Few studies have been conducted explicitly to explore the link between orofacial clefts and obesity, and many of these studies have been limited by small numbers of orofacial cleft cases.[3,11,23–26] To evaluate the hypothesis that obese or diabetic (either pre-existing or gestational) women are at elevated risk of giving birth to an infant with orofacial clefting, we conducted a large population-based case–control study. We also conducted a Monte Carlo-based bias analysis to explore some possible influences on association estimates of misclassification or unmeasured confounding. Such analyses address sources of error in addition to random error, the only error accounted for by traditional confidence intervals (CIs). These analyses may be particularly useful in quantifying the limitations of results based on secondary analysis of existing data, such as from publicly available data resources.

Methods

Data sources and linkage

We used two existing linked data sources from Washington State: the state birth certificate registry data and birth hospitalisation discharge records. The birth certificate data contain demographic information and information on maternal and infant conditions and complications. We used unique identifiers for each infant to link these data to the Comprehensive Hospital Abstract Reporting System (CHARS), which includes hospital discharge records of all non-federal hospitals in Washington State. From CHARS, we obtained procedure and diagnosis codes for the infant and mother during the birth hospitalisation. We used this linked dataset, which is population-based, to ascertain both cases and controls. This study was ruled exempt from Institutional Review Board review by the Human Subjects Division, University of Washington.

Study population

Cases—Eligible cases were all liveborn singletons with orofacial clefts born in the years 1987–2005 in Washington State. We identified cases with orofacial clefts from the checkbox for 'cleft lip/palate' on the birth certificate (n = 356); diagnostic code from the hospital discharge data (n = 902); or both (n = 1087). The primary outcome was cleft lip and/or palate, excluding cases with known chromosomal anomalies. The following three ICD-9 diagnostic codes identified case subjects from hospital discharge data: 749.0 (cleft palate), 749.1 (cleft lip) and 749.2 (cleft lip and palate). We included only the first birth for women with more than one birth in the database. We excluded cases with known chromosomal anomalies, identified from either the checkbox on the birth certificate for 'other chromosomal anomalies' or 'Down's syndrome' or from hospital discharge data ICD-9 codes 758.0–758.9.

We focused the primary analyses on the 'isolated cleft' case group, those with no additional congenital malformations. For these analyses, we excluded all cases with major malformations identified from checkboxes on the birth certificate or with ICD-9 diagnostic codes indicating any other congenital anomalies (codes 740–748 and 750–759), except infants whose only other malformations were minor according to the New York State Department of Health Congenital Malformations Registry Summary Report.27

Cleft lip with or without cleft palate may have a different aetiology and different risk factors compared with cleft palate alone.28 Therefore, we subdivided the case group into two categories: (1) cleft lip with (ICD-9 code 749.2) or without (749.1) cleft palate (CL ± P), and (2) cleft palate only (CPO; 749.0). Heterogeneity of categories in the birth certificate data across birth years prevented complete categorisation of the entire dataset, resulting in the exclusion of 181 isolated cases for these analyses. To ensure mutual exclusivity of categories, we reclassified cases as CL ± P if a diagnosis of cleft lip (CLO) or cleft lip and palate (CL + P) was recorded in addition to a diagnosis of CPO. We categorised 1050 cases with isolated CL ± P, and 491 cases with isolated CPO.

Controls—Controls were a random sample of infants born during the period 1987–2005 without a diagnosis of an orofacial cleft and were frequency matched to the cases by birth year. We selected eight controls for each case, yielding an initial total of 18 116 controls. We included only the first birth for multiparous women. As with cases, we excluded controls identified with chromosomal anomalies. For analyses involving the isolated cleft cases, we also excluded control infants with any major congenital malformation according to the same criteria as for cases.27

Exposures

The primary exposure of interest was obesity, defined as body mass index (BMI) ≥ 30. We calculated BMI as weight (in kilograms) divided by the square of height (in metres), and classified women according to the categories recommended by the World Health Organization (obese ≥30; overweight ≥25 and <30; normal ≥18.5 and <25; and underweight <18.5).29 For births occurring after 1991, we determined pre-pregnancy weight from a text box on the birth certificate. Because maternal weight was not recorded prior to 1992, births prior to that year were not eligible for this analysis. For the period 1992–2002, height was not available in the birth certificate data and was obtained by linking to Washington State driver's license records. Using a deterministic linkage procedure, 78.4% of mothers were matched on birth records to driver's license records.30 From 2003 onwards, both height and weight were available on the birth certificate. Pre-pregnancy weight on the birth certificate is sourced primarily from medical records prior to or at the first prenatal visit. For women

with no medical records on file with the delivery doctor, pre-pregnancy weight is based on self-report.

We classified diabetes as 'pre-existing' if indicated on either data source (ICD-9 code 250); 'gestational' if indicated on either data source (ICD-9 code 648.0) with no recorded indication of pre-existing diabetes; and 'unknown' if diabetes was indicated on the birth certificate with no additional diagnosis information in the CHARS data.

Confounders

We used directed acyclic graphs31 to identify potential confounders, including in the directed acyclic graphs all covariates identified from the literature as potentially associated with maternal obesity or risk of orofacial clefts. Based on this analysis, we enumerated the following confounders from the birth certificates: (1) maternal education (years), (2) maternal age (years), (3) maternal smoking during pregnancy (binary indicator), and (4) maternal race. Maternal race is used to classify infant's race in Washington State and is based on a combination of self-report and information obtained from medical records. Most demographic variables had a high level of completeness (for example, 97.3% for race and 99.9% for age), but education was available only for 67.3% of women. We collapsed the 15 race categories into seven (White, Black, American Indian/Alaska Native, Asian, Native Hawaiian/Pacific Islander, Latino, other). We additionally adjusted for year of birth because of the frequency matching. These covariates were included in all regression models.

Statistical analysis

We estimated the distributions of maternal characteristics (age, marital status, race, income, education, smoking status and diabetic status during pregnancy) by infants' case status.

In the primary analyses, we evaluated the odds ratio (OR) estimating the association between risk of orofacial clefting with exposure to maternal obesity vs. normal-weight (excluding underweight or overweight but not obese) by fitting adjusted logistic regression models. We estimated 95% CI for all association estimates. Women with pre-existing diabetes were excluded from this analysis and subsequent analyses regarding maternal weight or BMI. We performed this analysis for all cases, as well as fitting models separately for CL ± P and CPO. We carried out the analyses for cases and controls where other major malformations were present and where clefting was an isolated condition.

In secondary analyses we used a different BMI exposure threshold, comparing risk for all overweight mothers (including obese mothers) to risk for normal-weight mothers. We also explored possible dose–response relationships by estimating the ORs associated with a 10-point increment in BMI value and with a 50-point increment in weight in two separate logistic regression models. The latter analysis made use of a larger dataset as a substantial number of women were missing height information.

Finally, we fitted adjusted logistic regression models to compare the proportion of cases and unaffected controls whose mothers had pre-existing or gestational diabetes.

Secondary analyses—Based upon the published literature,32–35 we identified the following potential modifiers of the relationship between obesity and infant clefting: infant sex and prenatal care during the first 2 months of pregnancy (identified from the birth certificate). We fitted models with a multiplicative interaction term for each potential effect modifier with obesity and performed a likelihood ratio test.

To address the fact that data sources for weight and height differed across the study period, we repeated analyses separately for the years 1992–2002 and 2003–05. For some key

covariates, a large proportion of participants were missing data, primarily because of the lack of certain fields in the birth certificate for earlier years. We thus imputed missing values conditional upon covariates by using a 'MICE' procedure (multiple imputation by chained equations), developed for use in STATA as 'ICE'.36 This program uses a sequence of regression equations to impute missing data conditional on other predictors, and cycles through the equations until all variables have complete data. The variables that we imputed were: (a) the exposure variable (BMI), (b) adjustment variables (education, age, smoking status), and (c) relevant predictors of these variables. We identified predictors by using results from previous research, Akaike's information criterion37,38 values, and stepwise linear regression.

We conducted all statistical analyses described thus far by using STATA statistical software (version 10.0, Stata Corp.).

To address some potential influences on OR estimates of exposure misclassification and the presence of any unmeasured confounders, we conducted Monte Carlo-based bias analyses. We used the Excel program, 'SensTool' developed by Fox *et al*.39 Self-reported weight and height among the obese has an estimated 74% sensitivity and 99% specificity.40 We examined this level of misclassification as well as more extreme conditions. For all of the probabilistic bias parameters, we used uniform or trapezoidal distributions and varied the distributions to values that seemed plausible and to implausibly extreme values.

Using 500 repetitions for each analysis, we ran multiple simulations assuming non-differential exposure misclassification with sensitivity ranging from 0.60 to 0.99 and specificity ranging from 0.75 to 0.99. We assumed the prevalence of an unmeasured confounder to range from 0.25 to 0.40 for exposed subjects and 0.10 to 0.30 for unexposed subjects. We varied the association of this unmeasured confounder with orofacial clefting from 1.50 to 3.00. We experimented with misclassification and unmeasured confounder effects independently and together.

Results

Infants with orofacial clefts were more likely to be male (58%) than female. Case mothers were less commonly of Black race (2.4%) or Latino ethnicity (8.0%) compared with controls (4.1% and 9.6% respectively), but more likely to be American Indians or Alaska Natives (3.5%) compared with controls (2.4%). A higher proportion of case mothers smoked during pregnancy (20.6% of cases vs. 16.1% of controls). Cases and controls did not differ materially with respect to maternal age, marital status, median family income or education (Table 1).

Body mass index data were missing for 39.6% of mothers in the period 1992–2002 because of lack of height data from driver's licenses, and for 16.3% of women for birth years 2003–05. Women with and without BMI information did not differ substantially in terms of age or marital status (not shown). Lack of BMI data was more common for women with less than 12 years of education, Latino women and women who smoked during pregnancy. The proportion missing BMI information and the association of this lack with covariates were virtually the same in cases and controls.

There were a total of 2153 cases, 1722 of whom had isolated clefts. The group with isolated clefts consisted of 1050 cases with CL ± P (61.0%), 491 cases with CPO (28.5%) and 181 unspecified cases (10.5%) (Table 2).

Case–control comparisons for maternal BMI and weight

Obese women had a small increased risk of isolated orofacial clefts in their offspring compared with women of normal BMI [adjusted OR 1.26; 95% CI 1.03, 1.55]. The associations were similar for CL ± P and CPO (Table 3). The pattern was also similar for the total group that additionally included non-isolated cases.

Compared with normal-weight women, overweight women had an elevated adjusted odds of isolated CL ± P in their offspring [adjusted OR 1.30; 95% CI 1.07, 1.58]. However, we observed no increased risk of isolated CPO [adjusted OR 0.92; 95% CI 0.69, 1.22]. The pattern was similar whether non-isolated cases were included or excluded.

Excluding underweight women, the adjusted odds of isolated CL ± P in the infant was 23% higher for each 10-point increase in BMI [95% CI 1.05, 1.45] and 18% higher for each 50-point increment in pre-pregnancy weight, adjusted for height [95% CI 1.04, 1.35]. We observed a similar pattern when using all orofacial cleft cases as the outcome, but with a slight attenuation of all ORs (results not shown).

Case–control comparisons for maternal diabetes

There were too few cases with maternal pre-existing diabetes to obtain valid adjusted estimates of the ORs. In unadjusted analyses, we observed a 93% greater risk of any type of clefts for mothers with pre-existing diabetes compared with mothers with no diagnosis of diabetes [95% CI 1.28, 2.92], with a 2.19 times higher risk of CL ± P [95% CI 1.33, 3.60]. A similar pattern was observed for isolated orofacial clefts (and isolated CL ± P), but with an attenuation of estimates (Table 4).

Diagnosis with gestational diabetes was not associated with risk of isolated orofacial clefts [adjusted OR 0.88; 95% CI 0.63, 1.23] or clefts in the total sample [adjusted OR 0.90; 95% CI 0.67, 1.21].

Secondary analyses

After fitting an interaction term with infant sex, maternal obesity was associated with a 51% adjusted increased risk of isolated CL ± P in male infants [95% CI 1.11, 2.04] and a 7% decreased risk in female infants [95% CI 0.58, 1.48; *P* for interaction = 0.08]. The corresponding ORs for CPO were 1.32 for male infants [95% CI 0.78, 2.22] and 1.13 for female infants [95% CI 0.71, 1.79; *P* for interaction = 0.65]. We did not observe any meaningful effect modification by prenatal care during the first 2 months of pregnancy, regardless of clefting case group (results not shown).

Results differed only very slightly when the data source varied across the study period. After imputing missing data for all subjects, results were slightly attenuated for the relationship between maternal obesity and orofacial clefting [adjusted OR 1.19; 95% CI 1.01, 1.40].

Monte Carlo-based bias analyses

Results of the bias analysis for non-differential misclassification under various assumptions produced OR estimates ranging from 1.39 to 2.94 for isolated CL ± P (Table 5). Modelling the potential influence on estimates of an unmeasured confounder resulted in OR estimates ranging from 1.17 to 1.57 when the prevalence of the confounder was assumed to be similar for exposed and unexposed individuals. In order for the bias analyses to yield an OR of one, there would have had to be no misclassification and more extreme conditions than the most extreme we studied: prevalence of an unmeasured confounder ranging between 0.25 to 0.40 for exposed and 0.10 to 0.30 for unexposed, with a confounder outcome OR association ranging from 1.50 to 3.00.

Discussion

In the Washington State population we studied, obese mothers had a mildly increased risk of orofacial clefts in their offspring compared with women of normal BMI. By using birth certificate and hospital discharge information, the lack of precise information on confounders may have limited our ability to adjust completely, even for measured confounders. Thus the very modest observed elevation in risk could be due to residual confounding. However, it is also possible that imprecision of the primary exposure could have biased the results. Our measurement of maternal BMI was based primarily on self-report, and as people tend to underestimate weight and overestimate height on average,41 such non-differential misclassification is most likely to bias results towards the null.

Nonetheless, a notable feature of the results we obtained is the close agreement with other studies that were of similar magnitude. Our results are similar to those obtained in individual studies,4،11،23،25،26 in which adjusted ORs ranged from 1.3 to 2.2, and to those obtained through a meta-analysis of the relationship of maternal overweight and obesity with orofacial clefting risk [OR for CL + P: 1.20; 95% CI 1.03, 1.40].2 Although there have also been results reported that are closer to the null,3،42،43 heterogeneity of categorisation of type of clefting and small sample sizes may explain the observed lack of association.

Despite the relatively weak association, its close agreement with other similar studies motivated us to perform Monte Carlo-based bias analyses. This type of analysis combines estimated ORs based on the observed data and estimates based on the assumed prior distributions for bias sources. This probabilistic method allows for a range of possible sensitivity and specificity values to be evaluated. The resulting simulation intervals portray the uncertainty from the combination of both random and systematic errors.39،44 The bias analyses cannot provide any stronger evidence against the null hypothesis than the original data.45 Results of the bias analyses are dependent also on the parameters selected as well as other assumptions (such as that errors are independent).39 However, this type of analysis provides a quantitative model of how, given these assumptions, specific sources of bias may have affected the results obtained. This may be particularly important when one relies on existing data sources, as we did, as it allows for a quantitative assessment of the inadequacies that often exist when performing secondary analysis of existing data, such as missing variables or incomplete data. The results of such bias analyses suggest that, as a result of misclassification of BMI, it is possible that the estimated ORs reflect underlying true ORs of much stronger magnitude than those observed. In the bias analyses, the magnitude of the simulated OR was particularly influenced by low specificity. Although specificity of the exposure is likely to be closer to 99% than 75%,41 we included this range because inaccuracies in birth certificate data can be fairly common for medical data.46 The bias analyses also suggested that if the presence of an unmeasured confounder was responsible for producing non-null results, this unknown risk factor would have had to be very common, to have been unmeasured in all studies and to have had a very strong association with orofacial clefts. Conditional on the assumed bias models, these analyses suggest that although we and others have observed only weak associations, they may represent a true positive association between maternal obesity and orofacial clefting risk in offspring.

In unadjusted analyses, we observed an increased risk of clefts for mothers with pre-existing diabetes. Although the analysis was limited by the small number of exposed cases, this finding is supported by previous research.8–12 However, we did not observe an association for gestational diabetes, contrary to results from several previous studies.8،24 The lack of a positive finding of an association between maternal gestational diabetes and orofacial clefts might appear to suggest that abnormal glucose metabolism is not involved in the aetiology

of clefting. However, gestational diabetes develops towards the latter part of gestation, while orofacial clefts develop within the first 2 months of pregnancy. Thus, the discrepant findings regarding established vs. gestational diabetes may not detract from the overall hypothesis regarding impaired glucose metabolism. In addition, women with abnormalities of glucose metabolism due to insulin resistance may still have a normal result on a formal oral glucose tolerance test, and such underdiagnosis could have attenuated an association if one exists.47

In secondary analyses, we observed an increased risk of CL ± P associated with maternal obesity in male infants, but no increased risk in female infants. CL + P is more common in males, while CPO is more common in females.34 Although this finding is not conclusive, it is not entirely implausible. Orofacial clefts have been postulated to be caused by maternal hormone imbalance.35 In addition, inflammatory cytokines produced by adipose tissue appear to increase the expression of oestrogen-producing enzymes.48,49 Based on these relationships, one could speculate that abnormalities in the maternal hormonal profile caused by obesity, such as excess oestrogen, might be detrimental only to a male fetus.

This study has several strengths. It is population based and relatively robust against selection bias. As with other registry-based studies, analyses included data for a very large number of subjects ascertained over a long period of time. In some studies, conclusions about the relationship between maternal obesity and orofacial clefting have been limited by small numbers of cases.11,23,26,43 The birth certificate data and hospital discharge records are rich data sources with respect to information on potential confounders. In addition, accessing information from both data sources is likely to have increased the completeness of case ascertainment and ascertainment of medical conditions. For example, the sensitivity of ascertaining gestational diabetes from the birth certificate has been estimated to be 64% from birth certificates alone and 93% from both data sources combined.46

As orofacial clefts are usually easily visually identifiable, they are likely to be recorded at birth more reliably than many other birth defects. However, the possibility does remain that we missed some cases (particularly infants with CPO) that were identified after the birth hospitalisation.

A potentially important limitation of this study was the considerable amount of missing data for maternal BMI and pre-pregnancy weight, primarily because maternal weight was not recorded prior to 1992, and partly because of missing height data for the period 1992–2002. Women with missing BMI data were also more likely to have less than 12 years of education than women without missing data. As all analyses were adjusted for educational level, this limitation is not expected to have biased our results substantially. In addition, we conducted secondary analyses with imputation of missing data, in part to evaluate whether the association between missing BMI and other covariates could have biased the results. We observed a slight attenuation of results for the primary analyses, but no substantial difference. While these results are reassuring, the possibility for bias remains if missing BMI was also related to unmeasured covariates that are also strongly associated with risk of orofacial clefting.

Conclusions

In this population-based case–control study using birth certificate and hospital discharge data, obese and overweight women had a mildly elevated risk of orofacial clefts in the offspring. While it is possible that the relatively weak association is due to chance, the magnitude agrees closely with other similar studies. Moreover, a detailed simulation-based bias analysis suggests that the results are unlikely to be attributable solely to an unmeasured confounder. The bias analysis also provides plausible scenarios under which the true ORs

would be of greater magnitude than those we observed. A more precise measurement of BMI or, to the extent that the association is mediated by insulin resistance, the examination of insulin-resistant obese women, could yield stronger results. Despite the limitations of this study, our findings are consistent with the hypothesis that maternal obesity is a modifiable risk factor for orofacial clefts in the offspring.

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Table 1

Characteristics of mothers delivering singleton infants with orofacial clefts and controls, Washington State 1987–2005[a]

Characteristic	Cases (*n* = 2153) *n* (%)	Controls (*n* = 18 070) *n* (%)
Maternal age (years)		
<20	272 (13)	1953 (11)
20–34	1637 (76)	13 983 (77)
≥35	244 (11)	2120 (12)
Maternal marital status		
Married	1507 (70)	12 996 (72)
Unmarried	640 (30)	5020 (28)
Maternal race		
White	1674 (80)	13 617 (77)
Black/African American	51 (2)	722 (4)
American Indian/Alaska Native	73 (4)	419 (2)
Asian	121 (6)	1073 (6)
Hawaiian/Pacific Islander	10 (1)	72 (0)
Hispanic/Latino	167 (8)	1682 (10)
Other	2 (0)	6 (0)
Median family income ($)[b]		
<30 000	538 (27)	4441 (27)
30 000–55 000	1202 (61)	9966 (60)
> 55 000	244 (12)	2301 (14)
Maternal education (years)		
<12	274 (19)	2239 (18)
12	450 (31)	3739 (31)
>12	715 (50)	6197 (51)
Maternal smoking during pregnancy		
Yes	425 (21)	2775 (16)
No	1640 (79)	14 459 (84)
Maternal diabetes		
Pre-existing	28 (1)	122 (1)
Gestational	74 (3)	683 (4)
Unknown type	1 (0)	8 (0)
No diabetes	2050 (95)	17 257 (96)

[a] Excludes infants with chromosomal anomalies. Includes infants with other major congenital malformations. Numbers may not add to the totals because of missing data. Percentages have been rounded and may not total 100.

[b] Calculated as an estimate of the median income level of maternal residence census track using 2000 US Census data.

Table 2

Distribution of body mass index (BMI) among women who delivered singleton infants with orofacial clefts and controls, stratified by type of clefting

BMI (kg/m^2)	CL ± P *n* (%)	CPO *n* (%)	Unspecified *n* (%)	Controls *n* (%)
Isolated: excluding other major malformations (*n* = 1722)				
≥30	101 (10)	51 (10)	11 (6)	1405 (8)
25–29.9	131 (13)	40 (8)	12 (7)	1895 (11)
18.5–24.9	261 (25)	142 (29)	36 (20)	4692 (27)
<18.5	27 (3)	15 (3)	6 (3)	381 (2)
Missing BMI	530 (51)	243 (50)	116 (64)	9202 (52)
Total (= 100%)	1050	491	181	17 575
Isolated and non-isolated: not excluding other major malformations (*n* = 2153)				
≥30	115 (10)	61 (9)	15 (6)	1452 (8)
25–29.9	149 (12)	57 (8)	18 (7)	1945 (11)
18.5–24.9	304 (25)	191 (28)	45 (18)	4829 (27)
<18.5	30 (3)	22 (3)	6 (2)	392 (2)
Missing BMI	618 (51)	353 (52)	169 (67)	9452 (52)
Total (= 100%)	1216	684	253	18 070

Percentages have been rounded and may not total 100.

CL ± P, cleft lip with or without cleft palate; CPO, cleft palate only.

Table 3

Adjusted ORs for the association between orofacial clefts in the infant and maternal obesity [body mass index (BMI) ≥30 kg/m^2][a] or overweight (BMI 25.0–29.9 kg/m^2)[a] stratified by type of malformation[b]

	Isolated[c]		All clefts (isolated and non-isolated)	
Cleft type	**Cases exposed (*n*)**	**OR [95% CI]**	**Cases exposed (*n*)**	**OR [95% CI]**
Obese women (BMI ≥ 30)				
All clefts	149	1.26 [1.03, 1.55]	176	1.19 [0.98, 1.43]
CL ± P	93	1.29 [1.00, 1.67]	106	1.25 [0.99, 1.59]
CPO	46	1.21 [0.85, 1.72]	56	1.04 [0.76, 1.42]
Overweight women (BMI 25.0–29.9)				
All clefts	321	1.15 [0.98, 1.34]	384	1.11 [0.96, 1.28]
CL ± P	216	1.30 [1.07, 1.58]	246	1.27 [1.06, 1.53]
CPO	85	0.92 [0.69, 1.22]	108	0.84 [0.66, 1.08]

[a] Compared with women of normal weight (BMI ≥18.5 to <25). BMI is in kg/m^2.

[b] ORs are adjusted for birth year (continuous), maternal age (continuous), race (categorical), years of education (continuous) and smoking during pregnancy (binary).

[c] Isolated = excluding other major malformations.

CL ± P, cleft lip with/without cleft palate; CPO, cleft palate only.

Table 4

Unadjusted ORs for the association between orofacial clefts in the infant and maternal pre-existing diabetes stratified by type of malformation

	Isolated[a]		All clefts (isolated and non-isolated)	
Cleft type	**Cases exposed (*n*)**	**OR [95% CI]**	**Cases exposed (*n*)**	**OR [95% CI]**
All clefts	17	1.48 [0.89, 2.47]	28	1.93 [1.28, 2.92]
CL ± P	13	1.85 [1.04, 3.30]	18	2.19 [1.33, 3.60]
CPO	3	0.92 [0.29, 2.89]	6	1.31 [0.57, 2.98]

[a] Isolated = excluding other major malformations.

CL ± P, cleft lip with/without cleft palate; CPO, cleft palate only.

Table 5

Bias analysis: estimated impact of exposure misclassification and presence of unmeasured confounder on OR[a]

Sensitivity (range)	Specificity (range)	P_O[b]	P_N[c]	OR_{CO}[d]	Simulation OR [95%CI]
Conditions for simulating influence of non-differential misclassification					
0.74 (0.72, 0.76)	0.99 (0.99, 0.99)				1.38 [1.07, 1.79]
0.70 (0.65, 0.75)	0.90 (0.85, 0.95)				1.63 [1.17, 2.24]
0.70 (0.60, 0.80)	0.90 (0.80, 0.99)				1.63 [1.16, 2.54]
0.90 (0.80, 0.99)	0.90 (0.80, 0.99)				1.57 [1.10, 2.56]
0.80 (0.75, 0.85)	0.80 (0.75, 0.85)				2.94 [1.67, 25.18]
Conditions for simulating influence of an unmeasured confounder					
1.00 (1.00, 1.00)	1.00 (1.00, 1.00)	0.25, 0.35	0.20, 0.30	1.50, 2.00	1.30 [0.97, 1.71]
1.00 (1.00, 1.00)	1.00 (1.00, 1.00)	0.25, 0.35	0.20, 0.30	2.00, 3.00	1.25 [0.95, 1.71]
1.00 (1.00, 1.00)	1.00 (1.00, 1.00)	0.30, 0.40	0.10, 0.20	1.50, 2.00	1.17 [0.83, 1.62]
Conditions for simulating simultaneous influence of an unmeasured confounder and non-differential misclassification					
0.70 (0.65, 0.75)	0.90 (0.85, 0.95)	0.25, 0.35	0.20, 0.30	1.50, 2.00	1.57 [1.11, 2.20]
0.70 (0.65, 0.75)	0.90 (0.85, 0.95)	0.30, 0.40	0.10, 0.20	2.00, 3.00	1.40 [0.99, 2.04]

[a] Crude OR is calculated for isolated cleft lip with/without cleft palate: [OR = 1.33; 95% CI 1.05, 1.69]. Results obtained indicate simulation OR based on bias parameters entered into spreadsheet.

[b] P_O = prevalence of confounder among obese mothers (minimum to maximum).

[c] P_N = prevalence of confounder among normal-weight mothers (minimum to maximum).

[d] OR_{CO} = odds ratio of confounder and orofacial clefts (minimum to maximum).