

Maternal bronchodilator use and the risk of orofacial clefts

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BACKGROUND: Few epidemiological studies have explored the relationship between orofacial clefts and bronchodilators. We assessed whether mothers who used bronchodilators during early pregnancy were at an increased risk of delivering infants with orofacial clefts.

METHODS: We used National Birth Defects Prevention Study case–control data from mothers of 2711 infants with orofacial clefts and 6482 mothers of live born infants without birth defects, delivered during 1997 through 2005. Information on medication use from 3 months before pregnancy through delivery was collected using a standardized interview. Logistic regression was used to estimate adjusted odds ratios (aOR) and 95% confidence intervals (CIs) for maternal bronchodilator use during the periconceptional period (1 month before pregnancy through the third month of pregnancy) while controlling for other covariates.

RESULTS: We observed an association between maternal bronchodilator use during the periconceptional period and cleft lip only (CLO) (aOR = 1.77, 95% CI: 1.08–2.88). The risk of cleft palate only (CPO) (aOR = 1.53, 95% CI: 0.99–2.37) was elevated but was not statistically significant. No association was observed for maternal bronchodilator use and the risk of cleft lip with cleft palate (aOR = 0.78, 95% CI: 0.46–1.31). The most commonly used bronchodilator was albuterol (88.7%). Maternal albuterol use was associated with CLO (aOR = 1.79, 95% CI: 1.07–2.99) and CPO (aOR = 1.65, 95% CI: 1.06–2.58).

CONCLUSIONS: We observed a statistically significant association between maternal bronchodilator use during the periconceptional period and the risk of CLO after controlling for other risk factors. It is unclear whether the increased odds ratios observed in this study are due to the bronchodilators, the severity of asthma, or both, or to chance alone. Further studies to disentangle the role of asthma or asthma medications would help clarify these findings.

Key words: asthma / birth defects / bronchodilator agents / orofacial clefts

Introduction

Asthma is one of the most common chronic conditions that can complicate pregnancy. In recent national surveys (2000–2004) in the USA, ~8.4–8.8% of pregnant women reported asthma and 4.1% reported an asthma attack in the previous year (Kwon *et al.*, 2006). From the same national surveys, pregnant women who were younger, unmarried, of lower income, Puerto Rican or who lived in the northeast USA experienced more asthma attacks or asthma-related emergency visits during the previous year (Kwon *et al.*, 2006). The National Asthma Education and Prevention Program recommends the

continued use of appropriate asthma medications during pregnancy (NAEEP, 2005). Studies have suggested that well-controlled asthma is less likely to result in adverse reproductive outcomes than poorly controlled asthma (Murphy *et al.*, 2005). Several epidemiologic studies have reported increased risks of adverse pregnancy outcomes among women with asthma, including pre-eclampsia, intrauterine growth restriction, preterm birth, perinatal death and congenital malformations (Demissie *et al.*, 1998; Kallén *et al.*, 2000, 2007; Tamasi *et al.*, 2006).

Nevertheless, 39% of women with asthma reported discontinuing their asthma medication, reducing medication dosage or both during

pregnancy in a survey of 501 asthmatic women of child-bearing age (Chambers, 2003).

Asthma is treated with anti-inflammatory agents to prevent acute exacerbations and with bronchodilators to control symptoms (NAEEP, 2005). Anti-inflammatory agents include inhaled and oral corticosteroids, mast cell stabilizers and leukotriene modifiers. Bronchodilators improve ventilation to the lungs by relaxing contractions of the smooth muscle of the bronchioles. Bronchodilators include β_2 -adrenegic agonists (beta-agonists), anticholinergic agents and theophylline; of these, beta-agonists are most commonly used to treat asthma. Bronchodilators are categorized as either quick-relief or rescue medications (short-acting beta-agonists and anticholinergics) or long-term control medications (long-acting beta-agonists and theophylline). Teratogenic effects, including cleft palate, were found when bronchodilators (albuterol, theophylline) were administered to pregnant mice or rabbits (Szabo et al., 1975; Shibata et al., 2000). In epidemiological studies, the use of maternal oral corticosteroids (anti-inflammatory medications) during early pregnancy has been found to be associated with an increased risk of cleft lip, cleft palate or both (Rodriguez-Panilla and Martinez-Frias, 1998; Park-Willie et al., 2000; Carmichael et al., 2007); however, no known studies have evaluated associations between bronchodilators and specific birth defects.

Orofacial clefts are birth defects in which the tissues of the palate and/or lip fail to fuse properly during fetal development. In this study, we divided orofacial clefts into three groups: cleft lip with cleft palate (CLP), cleft lip only (CLO) and cleft palate only (CPO). As most studies do not separate CLP from CLO, it is unclear to what extent the epidemiologic features and origin of these defects differ. A recent study (Harville et al., 2007) suggests that epidemiologic assessments of orofacial clefts should, when possible, include separate analyses of CLP and CLO. In the USA, prevalence estimates of cleft lip with or without cleft palate (CL/P) and CPO are 10.48 and 6.39 per 10 000 live births, respectively (CDC, 2006). The etiology of these birth defects is not well understood; however, many studies suggest that both genetic and environmental factors are involved. A specific gene, interferon regulatory factor 6, was identified in 2004 (Zucchero et al., 2004), and polymorphisms in this gene result in a 3-fold increased risk of these defects. Several studies involving the exploration and analysis of candidate genes such as *TK32B*, *EVC*, *PTCHI*, *SUMO1*, *TGFA* and *TNSI* have provided further evidence of their role in the causation of non-syndromic orofacial clefts (Beaty et al., 2009; Carter et al., 2009; Sull et al., 2010). In previous studies, orofacial clefts have been associated with older maternal and/or paternal age (Honein et al., 2007; Green et al., 2010), exposure to organic solvents (Gordon and Shy, 1981; Chevrier et al., 2006), oral corticosteroid use (Carmichael et al., 2007), alcohol consumption (Romitti et al., 2007), cigarette smoking (Bille et al., 2007; Honein et al., 2007; Shi et al., 2007), less use of multivitamins (Itikala et al., 2001), race/ethnicity (Khoury et al., 1983; Croen et al., 1998; Forrester and Merz, 2004), epilepsy and antiepileptic drugs (Dravet et al., 1992; Abrishamchian et al., 1994), and family history of orofacial clefts (Leite and Koifman, 2009).

We are not aware of previous studies that explore the link between maternal bronchodilator use and orofacial clefts. The objective of the study is to assess whether mothers who used bronchodilators during early pregnancy were at an increased risk of delivering infants with orofacial clefts.

Materials and Methods

Design and study population

This analysis used data from the ongoing National Birth Defects Prevention Study (NBDPS), a multi-site, population-based, case-control study designed to evaluate the genetic and environmental risk factors associated with more than 30 categories of birth defects in the USA. Detailed methodology used in the NBDPS is described elsewhere (Yoon et al., 2001). Infants with single-gene disorders or chromosome abnormalities are excluded from the study. Adopted children, those in foster care, or those whose mothers did not speak English or Spanish are also excluded from the study. Clinical information abstracted from medical records was reviewed by clinical geneticists at each center, and strict criteria were used to determine case eligibility. In addition, a single clinical geneticist (S.A.R.) classified infants with orofacial clefts as isolated (an orofacial cleft, but no other major birth defects) or as having multiple defects (one or more major, unrelated birth defects in addition to orofacial cleft) (Rasmussen et al., 2003).

The study population in this analysis included infants with orofacial clefts identified from birth defects surveillance systems in 10 states [Arkansas, California, Georgia (Centers for Disease Control and Prevention), Iowa, Massachusetts, New Jersey, New York, North Carolina, Texas and Utah]. Case infants included live births, fetal deaths of 20 or more weeks of gestation or greater than 550 g (nine sites), and elective terminations (eight sites). Control infants were live born infants without birth defects randomly selected from birth certificates or birth hospital records in the same geographic areas as the case infants. A total of 2773 case infants and 6652 control infants had estimated dates of delivery (EDD) from 1 October 1997 through 31 December 2005. Infants ($n = 6$) whose mothers used the beta-agonist terbutaline (which is used also to prevent preterm labor) and infants ($n = 73$) whose mothers did not complete the medications questions were excluded from the analysis. Also excluded were infants ($n = 153$) whose mothers used an anti-inflammatory medication commonly used to treat asthma but did not report use of a bronchodilator. All activities involved in this study were approved by the institutional review boards of each of the participating study centers and the Centers for Disease Control and Prevention.

Exposure assessment

Asthma medications are generally classified as bronchodilator or anti-inflammatory. The Boston University Slone Epidemiology Center Drug Dictionary, a computerized database of prescription and non-prescription drugs that links drug products to their respective active ingredients, was used to classify asthma medications.

In this study, we defined 'exposed' as any mother who reported use of a bronchodilator at least once during the period 1 month before conception through the third month of her pregnancy. This four-month period was designated as the periconceptual period in the analysis. It includes the time of lip and palate formation. Included as bronchodilators were all beta-agonists, anticholinergics and methyxanthines (theophylline) commonly used to treat asthma. In addition, we looked at the effect of using both bronchodilator and anti-inflammatory asthma medication during the periconceptual period, which may be an indicator of asthma severity. Anti-inflammatory medications included were those commonly used to treat asthma. Anti-inflammatory asthma medications reported by study mothers included fluticasone, prednisone, beclomethasone, triamcinolone, cromolyn sodium, budesonide, montelukast, zafirlukast, methylprednisolone, flunisolide and prednisolone.

Mothers who reported using bronchodilators 'as needed' rather than reporting the timing of exposure were subjected to close examination to minimize misclassification. Those who used bronchodilators 'as

needed', but the period of use could not be determined were removed from the main analysis. We attempted to determine the route of administration of the bronchodilator (oral or inhaled) based on the mother's report or known formulation of the medication. However, because the route of administration was not available for many participants, the variable was not considered in the final analysis. In addition, we compared odds ratios for maternal bronchodilator use during the periconceptional period to those for use only after the third month of pregnancy (4th–9th months of pregnancy).

Statistical analysis and potential confounders

We examined the crude association between orofacial clefts and maternal and infant characteristics, which included maternal age at delivery, BMI, parity, race/ethnicity, education, alcohol consumption, smoking habits, folic acid-containing vitamin use, cocaine/crack use, marijuana use, fever during the first trimester, as well as infant sex. Associations between orofacial clefts and the use of vasoactive medicines, which included aspirin, ibuprofen, acetaminophen, pseudoephedrine, phenylpropanolamine and methylenedioxymethamphetamine, were also examined. In addition, we looked at associations between maternal and infant characteristics and bronchodilator use.

Bronchodilator use only and the combined use of bronchodilators and anti-inflammatory medications (fluticasone, prednisone, beclomethasone, triamcinolone, cromolyn sodium, budesonide, montelukast, zafirlukast, methylprednisolone, flunisolide and prednisolone) were evaluated in separate models for each type of orofacial cleft, including both isolated and multiple defects, as well as for isolated defects only. Exposure to albuterol use was examined separately for each type of orofacial cleft. Mothers who used anti-inflammatory medications were excluded from the analysis of bronchodilator use and orofacial clefts. The adjusted odd ratios (aOR) and 95% confidence intervals (CIs) were estimated using unconditional logistic regression. The following covariates were included in all models because of evidence in the literature that they may be associated with orofacial clefts or use of bronchodilators: maternal age at delivery, race/ethnicity, level of education, alcohol consumption, smoking habits, marijuana use, folic acid-containing vitamin use, vasoactive medication use and infant sex.

Results

A total of 2711 case infants, which includes 397 with multiple defects, were identified after excluding 2 case infants whose mothers used terbutaline (which is used also to prevent preterm labor), 14 case infants whose mothers did not complete the medication questions and 46 case infants whose mothers reported an anti-inflammatory medication commonly used to treat asthma but did not report use of a bronchodilator. Of the case infants included in the analysis, 614 had CLO, 938 had CPO and 1159 had CLP. A total of 6482 control infants were included in the final analysis after excluding 59 whose mothers did not complete the medication questions and 4 whose mothers used terbutaline and 107 infants whose mothers reported an anti-inflammatory medication commonly used to treat asthma but did not report use of a bronchodilator. Participation rates for the study were 74% among mothers of infants with orofacial clefts and 67% among control mothers. The median time between EDD and interview was 9 months for case mothers and 7 months for control mothers.

Table I shows the use of different bronchodilators among case and control mothers. Of the mothers who used bronchodilators

($n = 247$), 81 case and 138 control mothers used albuterol during the periconceptional period. Salmeterol was the second most frequently used asthma medication among case ($n = 6$) and control ($n = 21$) mothers.

The associations between maternal and infant characteristics and orofacial clefts are shown in Table II. Compared with controls, crude analysis revealed that younger maternal age (≤ 29 years old), low maternal BMI (< 18.5), low level of maternal education (≤ 12 years), maternal Hispanic race/ethnicity, male infant sex, maternal smoking and a family history were significantly associated with CLP; maternal use of folic acid-containing vitamins showed a protective effect with CLP, and maternal Hispanic race/ethnicity showed a protective effect with CLO and CPO. There was a protective effect between non-Hispanic black race/ethnicity and CLO, CPO and CLP. Maternal BMI, smoking, vasoactive medication use, male infant sex and a family history were associated with CLO. Finally, maternal smoking, female infant sex and a family history were associated with CPO. There was no significant difference between mothers of case infants with CLO, CPO and CLP, and mothers of control infants with regard to, parity, use of cocaine/crack, use of marijuana, consumption of alcohol during the periconceptional period or maternal fever in the first trimester of pregnancy.

The aORs of the associations between each type of orofacial cleft and bronchodilator use during the periconceptional period are presented in Table III. The results suggest an association between maternal bronchodilator use only and CLO (aOR = 1.77, 95% CI: 1.08–2.88). The association was slightly higher when only isolated case infants were examined (aOR = 1.88, 95% CI: 1.15–3.07). The risk of CPO (aOR = 1.53, 95% CI: 0.99–2.37) was elevated but was not statistically significant. No significant association was found between maternal bronchodilator use and CLP (aOR = 0.78, 95% CI: 0.46–1.31) nor when we grouped CLO and CLP (aOR = 1.11, 95% CI: 0.76–1.62; data not shown) after controlling for maternal age at delivery, race/ethnicity, level of education, alcohol

Table I Maternal bronchodilator use during the periconceptional period among infants with orofacial clefts and non-malformed control infants, NBDPS 1997–2005.

Bronchodilator use ($n = 247$) ^a	<i>n</i>	% ^b	Cases, $n = 89$ (%)	Controls, $n = 158$ (%)
Albuterol	219	88.7	81 (3.05) ^c	138 (2.17)
Salmeterol	27	10.9	6 (0.23)	21 (0.34)
Pirbuterol	6	2.4	3 (0.12)	3 (0.05)
Ipratropium bromide	5	2.0	1 (0.04)	4 (0.06)
Theophylline	5	2.0	2 (0.08)	3 (0.05)
Epinephrine (Primatene)	2	0.8	1 (0.04)	1 (0.02)
Metaproterenol	1	0.4	0 (0.00)	1 (0.02)
Levalbuterol	0	0	0	0

^a*n* includes bronchodilator users regardless of use of an anti-inflammatory.

^bPercentage who used a specific bronchodilator out of all bronchodilator users.

Total is not equal to 100% due to multiple medication use.

^c $P < 0.05$ in χ^2 test between cases and controls.

Table II Maternal and infant characteristics among infants with orofacial clefts and non-malformed control infants, NBDPS Study, 1997–2005.

	Controls (n = 6482) n (%)	Cleft lip only Cases (n = 614) n (%)	Cleft palate only Cases (n = 938) n (%)	Cleft lip with cleft palate Cases (n = 1159) n (%)
Maternal age at delivery				
≤24 years old	2188 (33.8)	215 (35.0)	295 (31.5)	470 (40.6) ^a
25–29 years old	1697 (26.2)	167 (27.2)	252 (26.9)	307 (26.5) ^a
>29 years old (Ref)	2597 (40.1)	232 (37.8)	391 (41.7)	382 (33.0)
Maternal BMI				
< 18.5 kg/m ²	350 (5.6)	47 (8.0) ^a	54 (6.0)	83 (7.5) ^a
≥ 18.5 kg/m ²	5868 (94.4)	540 (92.0)	853 (94.1)	1020 (92.5)
Parity				
0 births	2608 (40.3)	259 (42.2)	370 (39.5)	471 (40.7)
≥ 1 birth	3868 (59.7)	355 (57.8)	566 (60.5)	686 (59.3)
Maternal race/ethnicity				
White non-Hispanic (Ref)	3809 (59.0)	411 (67.1)	607 (64.9)	667 (57.8)
Black non-Hispanic	756 (11.7)	37 (6.0) ^a	69 (7.4) ^a	70 (6.1) ^a
Hispanic	1472 (22.8)	125 (20.4) ^a	188 (20.1) ^a	341 (29.5) ^a
Others	417 (6.5)	40 (6.5)	71 (7.6)	77 (6.7)
Maternal level of education				
≤ 12 years	2722 (42.3)	255 (41.6)	415 (44.6)	621 (53.9) ^a
> 12 years	3714 (57.7)	358 (58.4)	515 (55.4)	532 (46.1)
Maternal alcohol consumption ^b				
Yes	2399 (37.3)	251 (41.0)	372 (40.1)	421 (36.6)
No	4026 (62.7)	362 (59.1)	556 (59.9)	729 (63.4)
Infant sex				
Male	3276 (50.6)	397 (64.8) ^a	424 (45.3) ^a	770 (66.7) ^a
Female	3201 (49.4)	216 (35.2)	513 (54.8)	385 (33.3)
Maternal smoking ^b				
Yes	1246 (19.3)	150 (24.4) ^a	219 (23.5) ^a	298 (25.8) ^a
No	5204 (80.7)	464 (75.6)	712 (76.5)	856 (74.2)
Folic acid containing vitamins				
Yes	3257 (50.3)	309 (50.3)	467 (49.8)	515 (44.4) ^a
No	3225 (49.8)	305 (49.7)	471 (50.2)	644 (55.6)
Cocaine or crack use ^b				
Yes	46 (0.7)	6 (1.0)	10 (1.1)	7 (0.6)
No	6404 (99.3)	608 (99.0)	921 (98.9)	1146 (99.4)
Any vasoactive medications ^{b,c}				
Yes	1619 (25.0)	186 (30.3) ^a	262 (27.9)	291 (25.1)
No	4863 (75.0)	428 (69.7)	676 (72.1)	868 (74.9)
Marijuana use ^b				
Yes	312 (4.8)	40 (6.5)	50 (5.4)	64 (5.6)
No	6137 (95.2)	574 (93.5)	882 (94.6)	1089 (94.5)
Fever in first trimester				
Yes	690 (10.6)	79 (12.9)	114 (12.2)	129 (11.1)
No	5792 (89.4)	535 (87.1)	824 (87.9)	1030 (88.9)
Family history of orofacial cleft				
Yes	1 (0.0)	2 (0.3) ^a	5 (0.5) ^a	11 (1.0) ^a

Continued

Table III Periconceptional maternal use of bronchodilators only, both bronchodilators and anti-inflammatory medications and the risk of orofacial clefts, NBDPS, 1997–2005.

	n = 6482 controls (%)	Cleft lip only		Cleft palate only		Cleft lip with cleft palate	
		n = 614 cases (%)	aOR ^a , 95% CI	n = 938 cases (%)	aOR ^a , 95% CI	n = 1159 cases (%)	aOR ^a , 95% CI
Bronchodilator use (no anti-inflammatory use)							
BI–P3 ^b	114 (1.79)	20 (3.35)	1.77 (1.08–2.88)	26 (2.84)	1.53 (0.99–2.37)	17 (1.50)	0.78 (0.46–1.31)
P4–P9 ^c	58 (0.91)	7 (1.17)	1.26 (0.57–2.80)	4 (0.44)	0.49 (0.18–1.36)	6 (0.53)	0.59 (0.25–1.38)
Unexposed	6207 (97.30)	570 (95.48)	Ref.	887 (96.73)	Ref.	1114 (97.98)	Ref.
Bronchodilator and anti-inflammatory use							
BI–P3 ^b	39 (0.62)	4 (0.70)	1.06 (0.38–3.01)	5 (0.56)	0.85 (0.33–2.18)	9 (0.80)	1.26 (0.60–2.65)
P4–P9 ^c	11 (0.18)	0	—	3 (0.34)	1.87 (0.51–6.78)	1 (0.09)	—
Unexposed	6207 (99.20)	570 (99.30)	Ref.	887 (99.11)	Ref.	1114 (99.11)	Ref.
Albuterol use (no other bronchodilator use)							
BI–P3 ^b	101 (1.59)	18 (3.03)	1.79 (1.07–2.99)	25 (2.73)	1.65 (1.06–2.58)	15 (1.32)	0.76 (0.44–1.33)
P4–P9 ^c	55 (0.86)	7 (1.18)	1.34 (0.60–2.98)	4 (0.44)	0.52 (0.19–1.44)	6 (0.53)	0.64 (0.27–1.49)
Unexposed	6207 (97.55)	570 (95.80)	Ref.	887 (96.83)	Ref.	1114 (98.15)	Ref.

aOR, adjusted odds ratio; CI, confidence interval. Bold values indicate statistically significant aORs.

^aAdjusted for maternal age at delivery, race/ethnicity, level of education, alcohol consumption and smoking habits, vitamin/folic acid use, marijuana use, vasoactive medications use and infant sex.

^bOne month before pregnancy through third month.

^cFourth through ninth month of pregnancy.

consumption, smoking habits, marijuana use, folic acid-containing vitamin use, maternal use of any vasoactive medication as well as infant sex. Additionally, no significant associations were found between maternal use of both bronchodilators and anti-inflammatory medications during the periconceptional period and CLO (aOR = 1.06, 95% CI: 0.38–3.01), CPO (aOR = 0.85, 95% CI: 0.33–2.18) or CLP (aOR = 1.26, 95% CI: 0.60–2.65). Table III also shows the risks associated with maternal albuterol use. A statistically significant association was found between maternal albuterol use and both CLO (aOR = 1.79, 95% CI: 1.07–2.99) and CPO (aOR = 1.65, 95% CI: 1.06–2.58). For isolated cases only, the odds ratio for maternal albuterol use and CLO was slightly higher (aOR = 1.90, 95% CI: 1.14–3.18) but for CPO the odds ratio was lower and not significant (aOR = 1.48, 95% CI: 0.89–2.47). The odds ratios among mothers who used bronchodilators during the 4th–9th months of pregnancy only were not significant. When mothers with family history of orofacial clefts ($n = 19$) were removed from the analysis, the odds ratios were unchanged.

Discussion

Mothers of infants with CLO were statistically significantly more likely to use bronchodilators during the periconceptional period than mothers of control infants. In addition, a statistically significant association was observed between maternal albuterol use and both CLO and CPO. While the risk of CPO for infants whose mothers used any bronchodilators was elevated but not statistically significant, the risk of CPO was statistically significant for infants whose mothers used albuterol only. No statistical association was found with maternal bronchodilator use when we grouped CLO and CLP. Among mothers who reported bronchodilator use, most reported using albuterol. The odds ratios were slightly higher when we examined infants classified as having isolated CLO. No associations were observed between the use of both bronchodilators and anti-inflammatory medications during the periconceptional period and the any type of orofacial cleft.

Previous studies that assessed the teratogenic effects of medication on orofacial clefts have focused on the use of corticosteroids (oral, inhaled and topical) and have produced mixed results. Carmichael et al. (2007) examined corticosteroid use in the NBDPS and found a moderately increased risk of CL/P (aOR = 1.7, 95% CI: 1.1–2.6) and no association for CPO (aOR = 0.5, 95% CI: 0.2–1.3). In contrast, in a population-based, case–control study conducted by Czeizel et al. (1997), no statistically significant associations were observed between corticosteroid treatment during the second and third months of pregnancy and a number of different congenital anomalies, including CL/P. We are not aware of other epidemiological studies that investigate the teratogenic effects of bronchodilators as a group or albuterol individually on orofacial clefts.

Human placental transfer of albuterol and other bronchodilators has been demonstrated *in vitro* (Nandakumaran et al., 1981; Sodha and Schneider, 1983; Omarini et al., 1993). The US Food and Drug Administration has classified albuterol and salmeterol as pregnancy category C medications (Product information, 2000). Pregnancy category C is given to medications for which animal studies have shown harm or no animal studies are available and no adequate and well-controlled studies in humans are available. The second bronchodilator most commonly reported by pregnant women is salmeterol

(10.9%), a long-acting, inhaled beta-agonist widely recommended during pregnancy. Among mothers of infants with CLO, none reported using salmeterol during the periconceptional period. Based on a study by Wilton et al. (1998), among 47 infants whose mothers used salmeterol during the first trimester of pregnancy, the only birth defect found was one case of Aarskog syndrome, a single-gene condition unlikely to be associated with salmeterol exposure.

The present analysis has several strengths. The study is population-based and has a large sample size, allowing us to look at individual types of bronchodilators. In addition, the NBDPS benefits from a systematic case review by clinical geneticists at each center to ensure case eligibility and use of a detailed interview instrument to collect data on many potentially confounding variables. Finally, studies have shown that CLO, CPO and CLP may not be associated with the same risk factors at the same level or share the same etiology (Carmichael et al., 2007; Harville et al., 2007; Romitti et al., 2007). Our in-depth case classification allowed us to analyze these defects separately and to also examine isolated orofacial clefts.

This study has certain limitations worth noting. Medication use was ascertained only by maternal self-report, and no information is available on the dosage. Recall bias cannot be ruled out since mothers of affected children may attempt to find a causal explanation such as medication use during pregnancy, compared with mothers of infants without birth defects (Werler et al., 1989; Rockenbauer et al., 2001). To minimize this potential bias, the interview included questions about specific types of medicines, as well as the timing of these exposures by using a pregnancy calendar. Although this analysis has controlled for some confounders, the results may be influenced by factors not accounted for. Asthma status/severity, for which data were not available in this study, may have also played a role. Recent studies have suggested that maternal asthma (Blais et al., 2010) or the combination of both asthma (controlled versus uncontrolled and severe versus mild) and asthma medication use, particularly bronchodilators (Lin et al., 2009), may play a role in the risk of specific congenital malformations.

In conclusion, our results reveal a statistically significant association between maternal bronchodilator use during the periconceptional period and the risk of CLO after controlling for other risk factors. However, it is unclear whether the association observed in this study is due to the bronchodilators, the severity of asthma or both or to chance alone. There was no association between the use of both bronchodilators and anti-inflammatory medications and orofacial clefts. The use of both types of asthma medication may indicate more severe asthma; however, it may also indicate better therapeutic control of asthma, which in turn might explain the lack of association observed in our study. Further studies to disentangle the role of asthma or asthma medications or both are needed to help clarify these findings.

Authors' roles

J.P.M. played a role in study design, data analysis and interpretation, as well as manuscript preparation. S.L. was involved in conception and study design, manuscript preparation and critical review of manuscript. M.B. contributed to conception and study design, manuscript preparation and critical review of manuscript. K.C. took part in acquisition of data, exposure assessment and critical review of manuscript. A.C.

played a role in Study design, critical review of manuscript. E.B. was involved in study design, critical review of manuscript. S.R. contributed to study design and clinical expertise, as well as critical review of manuscript. P.R. took part in conception and study design, as well as critical review of manuscript. C.D. was involved in conception and study design, clinical expertise and critical review of manuscript. All authors participated in the reporting stage and have seen and approved the final version to be published.

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