# Maternal Periconceptional Exposure to Cigarette Smoking and Alcohol Consumption and Congenital Diaphragmatic Hernia

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**BACKGROUND:** Congenital diaphragmatic hernia (CDH) is a major birth defect that occurs when abdominal organs herniate through a diaphragmatic opening into the thoracic cavity and is associated with high mortality (>50%). The etiology of CDH is not well understood. **METHODS:** Using data from the National Birth Defects Prevention Study, we examined associations between CDH and maternal periconceptional exposure (1 month before through the third month of pregnancy) to cigarette smoking and alcohol. Interview reports of exposures were provided by mothers of CDH ($n$ = 503) and unaffected control ($n$ = 6703) infants delivered from October 1997 through December 2005. Any exposure (yes/no), as well as quantity (average number of cigarettes or drinks), type (active/passive smoking; beer, wine, distilled spirits), and duration (e.g., number of months exposed) were examined. Adjusted odds ratios (aORs) and 95% confidence intervals (CIs) were calculated for all CDH cases combined, selected subtypes (Bochdalek, Morgagni, not otherwise specified), and phenotypes (infants with/without additional major birth defects). **RESULTS:** The aOR for any smoking was nonsignificantly elevated for all CDH cases combined. Odds of any smoking was significant for isolated Bochdalek CDH (aOR, 1.9; 95% CI, 1.2–3.0). The aORs associated with all measures of alcohol consumption were near unity for each CDH category examined. Stratification of smoking exposure by alcohol consumption and stratification of alcohol consumption by smoking exposure did not appreciably change the aORs. **CONCLUSIONS:** These findings identified periconceptional smoking exposure as a potential risk factor for CDH. Future studies need to confirm our findings and explore possible pathways accounting for the teratogenic effect of smoking. *Birth Defects Research (Part A) 88:1040–1049, 2010.* © 2010 Wiley-Liss, Inc.

**Key words:** alcohol drinking; case and control studies; congenital diaphragmatic hernia; pregnancy; smoking

## INTRODUCTION

Congenital diaphragmatic hernia (CDH) is a severe birth defect with a prevalence of 2 to 5 per 10,000 births (Torfs et al., 1992; Stege et al., 2003; Tonks et al., 2004). CDH occurs when the organs of the abdominal cavity herniate into the thoracic cavity secondary to an underdeveloped diaphragmatic anlage or abnormal diaphragmatic muscular formation or migration. CDH is frequently associated with lung hypoplasia and/or pulmonary hypertension and high (>50%) mortality (Skari et al., 2000; Mah et al., 2009).

There are several well-described types of CDH broadly classified into posterolateral (or Bochdalek), nonposterolateral (anterior [Morgagni and pars sternalis]), and central hernias. The most common of these is the Bochdalek

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hernia, which accounts for approximately 95% of CDH (Torfs et al., 1992). Overall, a coexisting major malformation occurs in 37 to 47% of infants diagnosed with CDH (Cannon et al., 1996; Skari et al., 2000; Stege et al., 2003; Colvin et al., 2005), with the most common being cardiac, limb, orofacial, and body wall defects (Robert et al., 1997; Ackerman and Pober, 2007). Postnatal mortality for CDH is high and influenced by prenatal diagnosis, presence of additional malformations, and CDH laterality (Skari et al., 2000; Stege et al., 2003). Despite advances in treatment (Somaschini et al., 1999; Weber et al., 1998), recent studies of CDH mortality have shown little improvement in survival rates (Dott et al., 2003; Stege et al., 2003; Colvin et al., 2005; Brownlee et al., 2009; Mah et al., 2009).

Descriptive data reported for CDH have shown inconsistent associations with infant sex (David and Illingworth, 1976; Boychuk et al., 1983; Torfs et al., 1992; Robert et al., 1997; Dott et al., 2003; Yang et al., 2006) and maternal age (Torfs et al., 1992; Robert et al., 1997; Dott et al., 2003; Yang et al., 2006; Materna-Kiryluk et al., 2009). Higher prevalence of CDH has been found among non-Hispanic whites (Yang et al., 1994; Robert et al., 1997; Yang et al., 2006), multiple births (Robert et al., 1997; Mastroiacovo et al., 1999; Yang et al., 2006), and obese mothers (body mass index $\geq$30) (Waller et al., 2007). Significant associations between maternal nutritional intake during the year prior to conception and risk of CDH have also been reported with lower intake of retinol among mothers of CDH infants who did not take vitamin supplements and lower intake of vitamin A among mothers who did take vitamin supplements (Yang et al., 2008).

Studies of animals (Ackerman and Greer, 2007; Ackerman and Pober, 2007) and humans (Ackerman et al., 2005; Holder et al., 2007) have identified candidate genes involved in CDH. Briefly, *FOG2, GATA4,* and *COUP-TFII* have been implicated in lung and diaphragm development in mouse models. Some of the genes involved in lung and diaphragm development (e.g., *COUP-TFII, GATA4,* and *FOG2*), along with others (e.g., *RXR, RAR, STRA6,* and *LRP2*), have also been identified as affecting regulation of the retinoic signaling pathway. Independent evidence implicating the retinoic acid pathway comes from teratogenic animal models of CDH, notably vitamin A deficiency (Wilson et al., 1953; Warkany, 1954), and several chemicals that interfere with vitamin A metabolism (Ackerman and Pober, 2007). Specifically, exposures to several chemicals have been examined and shown to induce CDH in mouse models after either a single dose (e.g., nitrofen) or in a repeated administration, dose-dependent manner (e.g., 4-biphenyl carboxylic acid, bisdiamine[N,N′-octamethylenebis (dichloroacetamide)], SB-210661) (Iritani, 1984; Kluth et al., 1990; Greer et al., 2003; Mey et al., 2003). The suggested and unifying mechanism by which the above chemicals cause CDH is homeostatic disturbance of the retinoid system (Mey et al., 2003; Kling and Schnitzer, 2007). Further support for the involvement of the retinoid system has been demonstrated by a decrease in CDH prevalence following antenatal treatment with vitamin A (retinol) in nitrofen-treated rats (Thebaud et al., 1999; Thebaud et al., 2001; Greer et al., 2003; Babiuk et al., 2004).

Despite evidence in humans of lower vitamin A concentrations among mothers and infants directly or indirectly exposed to cigarette smoke (Hozyasz and Chelchowska, 2004; Chelchowska et al., 2006; Yilmaz et al., 2009) and inhibition of retinol metabolism by alcohol (Deltour et al., 1996; Wang, 1999; Crabb et al., 2001; Molotkov and Duester, 2002; Felix et al., 2008), exposures to periconceptional cigarette smoking and alcohol consumption in relation to CDH risk have received little attention. Of the existing studies, inconsistent findings have been reported for smoking exposure with significant effects found for paternal smoking and elevated findings for secondary smoke exposure (Zhang et al., 1992), but nonsignificant effects reported for maternal smoking (Felix et al., 2008). We are aware of only a single study reporting periconceptional alcohol consumption and CDH risk (Felix et al., 2008).

Despite the paucity of research, the finding of lower retinol levels among infants diagnosed with CDH (Major et al., 1998) and lower nutritional intake of vitamin A or retinol among mothers of CDH infants (Yang et al., 2008) provides justification for continued study of risk factors that may influence malformations affected by regulation of the retinoid signaling pathway. Thus, the goal of this study was to examine environmental exposures (i.e., teratogens) that may interfere with retinoid homeostasis. Specifically, data from the National Birth Defects Prevention Study were used to examine associations between detailed maternal reports of periconceptional (i.e., the month before pregnancy through the end of the first trimester) cigarette smoking and alcohol exposures and CDH. Cigarette smoking exposure includes active smoking by the mother and exposure to second-hand smoke at home or work.

## MATERIALS AND METHODS

### National Birth Defects Prevention Study (NBDPS)

The NBDPS is an ongoing, multicenter, population-based, case–control study designed to investigate genetic and environmental risk factors for more than 30 major birth defects (Yoon et al., 2001; Rasmussen et al., 2003). Initial NBDPS centers included birth defect surveillance systems in seven states (Arkansas [AR], California [CA], Iowa [IA], Massachusetts [MA], New Jersey [NJ], New York [NY], and Texas [TX]), as well as the Centers for Disease Control and Prevention (CDC) in Georgia. In 2002, surveillance systems in two additional states (North Carolina [NC] and Utah [UT]) were included in the NBDPS. Each center obtained institutional review board approval for the NBDPS. A brief description of study methods is presented below.

### Subject Selection

Centers contributed live births diagnosed with CDH and a limited number of centers ascertained fetal deaths (AR, CA, CDC, IA, MA, NY, TX since 2000) and elective terminations (AR, CA, CDC, IA, NY, TX since 2000). All case infants included in the current analyses had an estimated date of delivery (EDD) on or after October 1, 1997 (CA, CDC, IA, MA, NY, TX), January 1, 1998 (AR, NJ), or January 1, 2003 (NC, UT) and on or before December 31, 2002 (NJ) or December 31, 2005 (AR, CA, CDC, IA, MA, NC, NY, TX, UT). Control infants were unaffected live births with an EDD during the same time frames and randomly selected from either hospital delivery logs (AR 1997–2000, CA, CDC 1997–2000, NY, TX) or birth certificate files (AR 2000–2005, CDC 2001–2005, IA, MA, NC, NJ, UT). Also excluded were case infants with defects of

known genetic etiology (i.e., single gene disorders, chromosome abnormalities), case and control infants not in the custody of or not residing with their birth mothers, or case and control infants whose birth mother did not speak English or Spanish.

## Case Classification

Clinical geneticists at each center reviewed clinical information to determine case eligibility using standard case definitions for the study (Rasmussen et al., 2003). Information abstracted from medical records was reviewed by one of two NBDPS clinical geneticists (B.R.P., S.A.R.) to further classify eligible case infants as isolated (no additional major, unrelated defects), multiple (one or more major, unrelated defects), or complex sequence (Pentalogy of Cantrell and limb-body wall complex). Case infants were classified by type (Bochdalek, Morgagni, or not otherwise specified [NOS] when sufficient diagnostic information was not available), by laterality (unilateral, bilateral, unknown), and by sidedness of the hernia (left, right, unknown). To reduce pathogenetic heterogeneity, case infants with complex sequences ($n = 8$), such as Pentalogy of Cantrell and limb-body wall complex, were excluded from the analyses because their etiology may be distinct from that of more common types of CDH.

## Exposure Assessment

Structured, computer-assisted telephone interviews were conducted with birth mothers of case and control infants (Yoon et al., 2001). Interviews were conducted between 6 weeks and 2 years following the EDD; interviewers asked detailed questions about maternal cigarette smoking and alcohol consumption from 3 months before conception through the delivery date. Median length between EDD and interview date was 9.0 months for case mothers and 7.6 months for control mothers. Information about cigarette smoke exposure and alcohol consumption was collected monthly for the 3 months prior to pregnancy (labeled B3, B2, and B1) and the first 3 months of pregnancy (labeled M1, M2, and M3) and by trimester for months 4–6 and 7–9 of pregnancy (labeled T2 and T3, respectively).

**Smoking exposure.** Maternal exposure to cigarette smoking was classified as active and/or passive (exposure to cigarette smoke in the household or workplace). If mothers reported active smoking, information about the number of cigarettes smoked per day (frequency categories: <1, 1, 2–4, 5–14 [one-half pack], 15–24 [one pack], 25–34 [one and one-half packs], 35–44 [two packs], and >45) and the month(s) of exposure were collected. If mothers reported passive exposure to smoking, information about the location of exposure (household or workplace) and the month(s) during which exposure occurred was collected.

Mothers were classified as exposed to cigarette smoking if they reported active or passive smoking during the periconceptional period (defined as 1 month before conception [B1] through the first 3 months following conception [M1, M2, and M3]). Reported exposure to cigarette smoking was classified as any exposure (active and/or passive vs. none) and according to type of exposure (active only, passive only, or active+passive). These three exposure types were constructed from affirmative responses to the individual interview items, described above, for active smoking, passive smoking, or both. Among mothers who reported active smoking, exposure was further classified by number of cigarettes smoked per day (1–14 per day vs. ≥15 per day). To evaluate variability in the number of reported cigarettes smoked across months, odds ratios (ORs) were calculated using both minimum and maximum monthly values. To evaluate the effect of variation in number of periconceptional months exposed, mothers were classified by reported patterns of exposure as B1 only, B1–M1, B1–M2, B1–M3, and other (e.g., M1 only). Finally, mothers were classified by duration of exposure to passive and/or active smoke (number of periconceptional months exposed, 0–4) considering each month to be of equal exposure value; thus, duration was assigned a value of 1 whether a mother reported exposure during B1, M1, M2, or M3 only. Comparison of values for duration and pattern of active exposure showed a high concordance with 86% of mothers reporting 1 periconceptional month of exposure corresponding to reported smoking in B1 only and 98% of mothers who reported 2 or 3 periconceptional months of exposure corresponding to reported smoking in B1–M1 and B1–M2, respectively. Given potentially harmful effects of passive smoke exposure (Hozyasz and Chelchowska, 2004; Yilmaz et al., 2009), the highest reported duration for passive and/or active smoke exposure was used. For example, if the mother reported 4 months of passive smoke exposure and 3 months of active smoking, the highest reported duration would be 4 months. If only passive or active smoking exposure was reported, then the duration corresponded to the reported duration for the reported type of exposure.

**Alcohol exposure.** If mothers reported alcohol consumption, information about the month(s) in which they drank, average number of drinking days per month (frequency), average number of drinks per drinking day (quantity), and maximum number of drinks on one occasion per drinking month (variability) was collected. Alcohol exposure was classified as any drinking (yes/no), and by quantity, frequency, and variability of consumption as described elsewhere (Romitti et al., 2007). Because values for average number of drinks per day were recorded as continuous variables, overall average and maximum average values were calculated for each mother as described (Romitti et al., 2007). Similar to smoking exposure, both duration and pattern of periconceptional alcohol consumption were calculated and compared. Duration largely reflected reported patterns of exposure with reports of 1, 2, and 3 periconceptional months of exposure corresponding to patterns of B1 only, B1–M1, and B1–M2 for 70%, 92%, and 87% of mothers, respectively. In addition, binge drinking was evaluated using both sex-neutral (≥5 drinks per day) (Naimi et al., 2003) and sex-specific (≥4 drinks per day) (Wechsler et al., 1995) criteria.

**Combined cigarette smoking and alcohol exposure.** Periconceptional exposure to both cigarette smoking and alcohol was determined by constructing cross-tabulations of any exposure (yes/no) and reported type (active only/passive only/active+passive; binge drinking/drinking but no binging) or reported frequency (e.g., number of cigarettes smoked per day; number of drinks consumed per month).

### Statistical Analysis

Analyses were conducted using SAS, version 9.2 (SAS, Cary, North Carolina). Descriptive analyses used the chi-square test to compare case and control infants on sex, gestational age, and family history of CDH; maternal age at estimated date of delivery (EDD), race/ethnicity, education, gravidity, pre-pregnancy body mass index, and folic acid intake from vitamins; and NBDPS center. Crude ORs and 95% confidence intervals (CIs) were calculated to assess associations between CDH and maternal exposure to smoking and alcohol consumption. Covariates were included when a significant bivariate association was found, or if the variable showed a significant association with CDH in previous studies. Two subanalyses were conducted. First, case and control mother reports of cigarette smoking exposure and alcohol consumption were compared by 6-month intervals between EDD and date of interview to examine time-dependent recall bias. Second, changes in exposure due to learning of pregnancy were examined by comparing reported maximum amounts of monthly exposure to cigarette smoking and alcohol (M1, M2, M3) before and after the prenatal month when the mother reported finding out she was pregnant. Differences in scores were then computed for exposure before and after pregnancy recognition and compared between case and control mothers. Adjusted ORs (aORs) and 95% CIs were computed with infant sex and gestational age; family history (first- or second-degree relative with CDH); maternal age at EDD, race/ethnicity, and education; and NBDPS center as covariates. In addition, models for smoking exposure were adjusted for alcohol consumption, whereas those for alcohol consumption were adjusted for smoking exposure. Overall analysis included all CDH types (NOS, Bochdalek, Morgagni, Isolated, Multiple). Analysis of specific CDH phenotypes was restricted to those with at least five case infants.

## RESULTS

Participation in the maternal interview was 72% among case mothers and 67% among control mothers. A total of 503 mothers of case infants and 6703 mothers of control infants were interviewed. Overall, most case infants were classified as having an isolated defect and an undetermined hernia type (i.e., NOS); over 90% of these were unilateral and predominantly left-sided (Table 1). Compared to control infants, case infants were significantly more likely to be male and preterm and were also more likely to have any family history (e.g., first-degree or other relative) of CDH. Case mothers were less likely to be non-Hispanic black and more likely to be overweight. No other maternal factors differed between case and control infants.

Mothers of 177 (35.3%) case infants and 2214 (33.1%) control infants reported periconceptional cigarette smoking exposure (Table 2). Proportions of case and control mothers reporting specific types of smoking exposure (active only, passive only, active+passive) and the duration of active smoking were similar. Of those who reported periconceptional exposure, passive smoking exposure was most common followed by active+passive and active only; approximately one-half of case and control mothers who actively smoked reported smoking all 4 periconceptional months. Proportions of mothers reporting any periconceptional alcohol consumption was also similar between case and control mothers (34.6% and 36.7%, respectively). In contrast to the duration values reported for smoking, mothers who reported periconceptional alcohol consumption most commonly reported exposure for only 1 month with progressively fewer mothers reporting longer exposure. Little difference was found for type of alcohol consumed between case and control mothers. Stratification of periconceptional cigarette smoking exposure and alcohol consumption by 6-month intervals for time between the EDD and date of interview showed little difference (data not shown). In addition, no differences between case and control mothers were found for change in reported maximum number of cigarettes smoked or maximum number of drinks consumed over the periconceptional period before and after recognition of pregnancy (data not shown).

For all CDH combined, odds of any periconceptional cigarette smoking were elevated, but not significantly (Table 3). Odds of active-only smoking were slightly higher than those for passive-only or active+passive smoking. Among active smokers, the reported number of cigarettes smoked per day did not tend to increase risk. Similarly, number of months exposed to cigarette smoking did not tend to elevate risk for all cases combined. Stratification of case infants by isolated and multiple phenotypes produced similar patterns of ORs between all isolated CDH combined and isolated NOS CDH; however, ORs were most elevated among isolated Bochdalek CDH. In contrast to all CDH combined, aORs were elevated for associations between isolated Bochdalek CDH and all types of smoking exposure. Patterns of the ORs for all multiple CDH and multiple NOS CDH were similar; there were too few cases of Bochdalek CDH multiple to reliably test associations. For periconceptional alcohol exposure, the aORs were near unity for all phenotypes and CDH subtypes (Table 4). Odds for reported average drinks per month, binge drinking, and type of alcohol were also near unity for all comparisons. Small numbers limited reliable estimation of the influence of duration of alcohol exposure on CDH. Stratification of smoking exposure by alcohol exposure (yes/no) did not appreciably change the OR estimates (data not shown). Odds of any cigarette smoke exposure remained elevated for isolated Bochdalek CDH regardless of alcohol exposure. Also, increased odds for type of smoking exposure and frequency of active smoking exposure persisted among isolated Bochdalek CDH. Odds ratios for alcohol exposure did not appreciably differ when stratified by any smoking exposure (data not shown).

## DISCUSSION

This study analyzed data from the NBDPS to examine the associations between maternal cigarette smoking and alcohol exposure and CDH. Several associations emerged between exposure to cigarette smoking and CDH. Odds of any smoking were elevated, but not significantly, among all CDH combined and significantly elevated among isolated Bochdalek CDH. Odds of mothers who reported any type of smoking exposure (active, passive, active+passive) were higher for associations with isolated Bochdalek CDH.

Table 1
Selected Characteristics of Case and Control Infants and Birth Mothers, National Birth Defects Prevention Study, 1997–2005

| Characteristic | CDH ($N$ = 503) | | Controls ($N$ = 6703) | | Significance test | |
|---|---|---|---|---|---|---|
| | $n$ | %[a] | $n$ | %[a] | $X^2$ | $p$ |
| Infant | | | | | | |
| Defect status[b] | | | | | | |
| Isolated | 406 | 80.7 | — | — | | |
| NOS[c] | 304 | 74.9 | — | — | | |
| Unilateral[e] | 292 | 96.1 | — | — | | |
| Left[e] | 231 | 79.1 | — | — | | |
| Bochdalek[c] | 90 | 22.2 | — | — | | |
| Unilateral[d] | 85 | 94.4 | — | — | | |
| Left[e] | 76 | 89.4 | — | — | | |
| Morgagni[c] | 10 | 2.5 | — | — | | |
| Unilateral[e] | 6 | 60.0 | — | — | | |
| Left[e] | 3 | 50.0 | — | — | | |
| Multiple[b] | 97 | 19.3 | — | — | | |
| NOS[c] | 76 | 78.4 | — | — | | |
| Unilateral[d] | 71 | 93.4 | — | — | | |
| Left[d] | 52 | 73.2 | — | — | | |
| Bochdalek[c] | 14 | 14.4 | — | — | | |
| Unilateral[d] | 13 | 92.9 | — | — | | |
| Left[d] | 11 | 84.6 | — | — | | |
| Morgagni[c] | 6 | 6.2 | — | — | | |
| Unilateral[d] | 5 | 83.3 | — | — | | |
| Left[d] | 2 | 40.0 | — | — | | |
| Sex | | | | | 13.08 | <0.001 |
| Female | 206 | 41.0 | 3309 | 49.4 | | |
| Male | 296 | 59.0 | 3389 | 50.6 | | |
| Gestational age | | | | | 117.24 | <0.001 |
| Term (37–45 weeks) | 378 | 75.2 | 6067 | 90.5 | | |
| Preterm (<37 weeks) | 125 | 24.9 | 635 | 9.5 | | |
| Family history of CDH | | | | | 130.54 | <0.001 |
| First-degree relative | 2 | 0.4 | 2 | 0.03 | | |
| Other relative | 12 | 2.4 | 3 | 0.05 | | |
| None | 489 | 97.2 | 6698 | 99.9 | | |
| Mother | | | | | | |
| Age at delivery (years) | | | | | 2.20 | 0.699 |
| <21 | 63 | 12.5 | 984 | 14.7 | | |
| 21–25 | 122 | 24.3 | 1605 | 23.9 | | |
| 26–30 | 138 | 27.4 | 1820 | 27.2 | | |
| 31–35 | 121 | 24.1 | 1589 | 23.7 | | |
| >35 | 59 | 11.7 | 705 | 10.5 | | |
| Race and ethnicity | | | | | 5.71 | 0.127 |
| Non-Hispanic white | 311 | 62.2 | 4011 | 60.1 | | |
| Non-Hispanic black | 40 | 8.0 | 764 | 11.5 | | |
| Hispanic | 115 | 23.0 | 1491 | 22.3 | | |
| Other | 34 | 6.8 | 409 | 6.1 | | |
| Education (years) | | | | | 1.07 | 0.785 |
| <12 | 80 | 16.3 | 1128 | 16.9 | | |
| 12 | 122 | 24.5 | 1651 | 24.7 | | |
| 13–15 | 131 | 26.1 | 1805 | 27.0 | | |
| ≥16 | 169 | 33.1 | 2110 | 31.5 | | |
| Gravidity | | | | | 3.75 | 0.153 |
| 1 | 164 | 32.6 | 1955 | 29.2 | | |
| 2 | 131 | 26.0 | 1975 | 29.5 | | |
| >2 | 208 | 41.4 | 2771 | 41.4 | | |
| Pre-pregnancy body mass index | | | | | 4.68 | 0.197 |
| Underweight (<18.5) | 22 | 4.6 | 356 | 5.5 | | |
| Normal weight (18.5–24.9) | 274 | 56.7 | 3593 | 55.8 | | |
| Overweight (25.0–29.9) | 95 | 19.7 | 1448 | 22.5 | | |
| Obese (>30) | 92 | 19.0 | 1043 | 16.2 | | |
| Folic acid intake | | | | | 0.56 | 0.454 |
| Yes | 434 | 86.3 | 5701 | 85.1 | | |
| No | 69 | 13.7 | 1002 | 14.9 | | |

Table 1
Selected Characteristics of Case and Control Infants and Birth Mothers, National Birth Defects Prevention Study, 1997–2005 (continued)

| | CDH ($N$ = 503) | | Controls ($N$ = 6703) | | Significance test | |
|---|---|---|---|---|---|---|
| Characteristic | $n$ | %[a] | $n$ | %[a] | $X^2$ | $p$ |
| Study center | | | | | 11.19 | 0.263 |
| Arkansas | 59 | 11.7 | 838 | 12.5 | | |
| California | 68 | 13.5 | 849 | 12.7 | | |
| Iowa | 53 | 10.5 | 751 | 11.2 | | |
| Massachusetts | 67 | 13.3 | 855 | 12.8 | | |
| New Jersey | 40 | 8.0 | 573 | 8.6 | | |
| New York | 36 | 7.2 | 591 | 8.8 | | |
| Texas | 56 | 11.1 | 766 | 11.4 | | |
| CDC/Atlanta | 71 | 14.1 | 724 | 10.8 | | |
| North Carolina | 20 | 4.0 | 393 | 5.9 | | |
| Utah | 33 | 6.6 | 363 | 5.4 | | |

CDH, congenital diaphragmatic hernia; NOS, not otherwise specified. Numbers vary because of incomplete or missing data.
[a]Due to rounding, percentages may not total 100.
[b]Percentages of total cases.
[c]Percentages within Isolated or Multiple.
[d]Percentages within NOS, Bochdalek, or Morgagni.
[e]Percentages within Unilateral.

Table 2
Reported Patterns of Periconceptional Exposure for Cigarette Smoking and Alcohol for Case and Control Mothers, National Birth Defects Prevention Study, 1997–2005

| | CDH | | Controls | | Significance test | |
|---|---|---|---|---|---|---|
| Exposure | $n$ | %[a] | $n$ | %[a] | $X^2$ | $p$ |
| Cigarette smoking | | | | | | |
| Total | 503 | | 6703 | | | |
| Any periconceptional exposure[b] | | | | | 1.01 | 0.326 |
| No | 324 | 64.7 | 4468 | 66.9 | | |
| Yes | 177 | 35.3 | 2214 | 33.1 | | |
| Type of exposure[b] | | | | | 3.45 | 0.327 |
| Active+passive smoking | 54 | 10.8 | 768 | 11.5 | | |
| Active smoking only | 48 | 9.6 | 500 | 7.5 | | |
| Passive smoking only | 75 | 15.0 | 946 | 14.1 | | |
| Duration of active smoking[c] | | | | | 4.21 | 0.378 |
| 1 month | 13 | 2.6 | 174 | 2.6 | | |
| 2 months | 26 | 5.2 | 271 | 4.0 | | |
| 3 months | 17 | 3.4 | 154 | 2.3 | | |
| 4 months | 46 | 9.1 | 673 | 10.0 | | |
| Alcohol | | | | | | |
| Total | 503 | | 6703 | | | |
| Any periconceptional exposure[d] | | | | | 0.91 | 0.359 |
| No | 325 | 65.4 | 4205 | 63.3 | | |
| Yes | 172 | 34.6 | 2442 | 36.7 | | |
| Type(s) of alcohol[e] | | | | | 3.25 | 0.517 |
| Beer only | 40 | 8.1 | 505 | 7.6 | | |
| Wine only | 44 | 8.9 | 680 | 10.2 | | |
| Distilled spirits only | 24 | 4.8 | 429 | 6.5 | | |
| Beer + wine | 24 | 4.8 | 320 | 4.8 | | |
| Beer + distilled spirits | 15 | 3.0 | 201 | 3.0 | | |
| Wine + distilled spirits | 14 | 2.8 | 183 | 2.8 | | |
| Beer + wine + distilled spirits | 9 | 1.8 | 121 | 1.8 | | |
| Duration of alcohol consumption[d] | | | | | 6.27 | 0.180 |
| 1 month | 81 | 16.3 | 1300 | 19.6 | | |
| 2 months | 61 | 12.3 | 744 | 11.2 | | |
| 3 months | 18 | 3.6 | 175 | 2.6 | | |
| 4 months | 12 | 2.4 | 223 | 3.4 | | |

CDH, congenital diaphragmatic hernia. Numbers vary because of incomplete or missing data.
[a]Percentage of total.
[b]Missing or incomplete data for cigarette smoking distributed as follows: case mothers = 2; control mothers = 21.
[c]Missing or incomplete data for cigarette smoking distributed as follows: case mothers = 0; control mothers = 3.
[d]Missing or incomplete data for alcohol consumption distributed as follows: case mothers = 6; control mothers = 56.
[e]Missing or incomplete data for alcohol consumption distributed as follows: case mothers = 8; control mothers = 60.

Table 3
Adjusted Odds Ratio Estimates for Infant Phenotype Associated with Maternal Reports of Cigarette Smoking, National Birth Defects Prevention Study, 1997–2005

| | | CDH Phenotypes[a] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Controls ($N$ = 6703) | All ($N$ = 503)[b] | | Isolated ($N$ = 406)[b] | | Multiple ($N$ = 97)[b] | | Isolated NOS ($N$ = 304) | | Isolated Bochdalek ($N$ = 90) | | Multiple NOS ($N$ = 76) | |
| Exposure | $N$ | $N$ | OR (95% CI) | $N$ | OR (95% CI) | $N$ | OR (95% CI) | $N$ | OR (95% CI) | $N$ | OR (95% CI) | $N$ | OR (95% CI) |
| Any smoking[c] | | | | | | | | | | | | | |
| No smoking exposure | 4468 | 324 | Reference | 266 | Reference | 58 | Reference | 212 | Reference | 46 | Reference | 48 | Reference |
| Active and/or passive smoking | 2214 | 177 | 1.2 (0.9–1.4) | 139 | 1.1 (0.9–1.4) | 38 | 1.3 (0.8–2.0) | 91 | 0.9 (0.7–1.2) | 44 | 1.9 (1.2–3.0) | 27 | 1.1 (0.7–1.9) |
| Type of smoking[c] | | | | | | | | | | | | | |
| Active only | 500 | 48 | 1.4 (1.0–1.9) | 36 | 1.2 (0.9–1.8) | 12 | 1.7 (0.9–3.4) | 24 | 1.1 (0.7–1.7) | 11 | 1.9 (0.9–4.0) | 11 | 1.9 (1.0–3.9) |
| Passive only | 946 | 75 | 1.2 (0.9–1.5) | 57 | 1.1 (0.8–1.5) | 18 | 1.4 (0.8–2.5) | 38 | 0.9 (0.6–1.3) | 17 | 1.7 (1.0–3.2) | 12 | 1.1 (0.6–2.2) |
| Active+passive | 768 | 54 | 0.9 (0.7–1.3) | 46 | 1.0 (0.7–1.4) | 8 | 0.8 (0.3–1.7) | 29 | 0.8 (0.5–1.2) | 16 | 2.0 (1.0–3.8) | 4 | nc |
| Cigarettes/day[d] | | | | | | | | | | | | | |
| 1∼14/day | 888 | 80 | 1.2 (0.9–1.6) | 65 | 1.2 (0.9–1.6) | 15 | 1.1 (0.6–2.0) | 44 | 1.1 (0.8–1.5) | 19 | 1.7 (1.0–3.0) | 12 | 1.1 (0.6–2.2) |
| ≥15/day | 380 | 21 | 0.7 (0.5–1.2) | 16 | 0.7 (0.4–1.2) | 5 | 1.0 (0.4–2.5) | 9 | 0.5 (0.2–1.0) | 7 | 1.5 (0.6–3.5) | 3 | nc |
| Duration[c,e] | | | | | | | | | | | | | |
| 1 month | 181 | 19 | 1.4 (0.9–2.4) | 11 | 1.0 (0.5–1.9) | 8 | 3.0 (1.4–6.7) | 7 | 0.7 (0.3–1.7) | 4 | nc | 7 | 3.3 (1.4–7.6) |
| 2 months | 229 | 19 | 1.2 (0.7–2.0) | 17 | 1.4 (0.8–2.3) | 2 | nc | 14 | 1.4 (0.8–2.5) | 2 | nc | 2 | nc |
| 3 months | 134 | 15 | 1.8 (1.0–3.1) | 10 | 1.5 (0.7–2.9) | 5 | 2.8 (1.1–7.3) | 6 | 1.1 (0.5–2.6) | 4 | nc | 5 | 3.4 (1.3–9.1) |
| 4 months | 1670 | 124 | 1.0 (0.8–1.3) | 101 | 1.0 (0.8–1.4) | 23 | 1.0 (0.6–1.7) | 64 | 0.8 (0.6–1.1) | 34 | 1.9 (1.2–3.2) | 13 | 0.7 (0.3–1.3) |

CDH, congenital diaphragmatic hernia; CI, confidence interval; nc, not calculated; NOS, not otherwise specified; OR, odds ratio. Numbers vary because of incomplete or missing data.

[a]Adjusted for infant sex, gestational age, first- or second-degree family history, maternal age, race and ethnicity, periconceptional alcohol consumption (yes/no), and study center.

[b]Case phenotypes included NOS, Bochdalek, and Morgagni; all laterality (unknown, unilateral, bilateral); and all sidedness (left, right, unknown).

[c]Missing or incomplete: all CDH case mothers = 2; control mothers = 21.

[d]Missing or incomplete: all CDH case mothers = 1; control mothers = 7.

[e]Exposure to smoking duration calculated as maximum exposure from active and/or passive smoking.

Table 4
Adjusted Odds Ratio Estimates for Infant Phenotype Associated with Maternal Reports of Alcohol Consumption, National Birth Defects Prevention Study, 1997–2005

| | | CDH phenotypes[a] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Controls ($N$ = 6703) | All ($N$ = 503)[b] | | Isolated ($N$ = 406)[b] | | Multiple ($N$ = 97)[b] | | Isolated NOS ($N$ = 304) | | Isolated Bochdalek ($N$ = 90) | | Multiple NOS ($N$ = 76) | |
| Exposure | $N$ | $N$ | OR (95% CI) | $N$ | OR (95% CI) | $N$ | OR (95% CI) | $N$ | OR (95% CI) | $N$ | OR (95% CI) | $N$ | OR (95% CI) |
| Periconceptional alcohol consumption[c] | | | | | | | | | | | | | |
| No alcohol consumption | 4205 | 325 | Reference | 261 | Reference | 64 | Reference | 198 | Reference | 57 | Reference | 51 | Reference |
| Any alcohol consumption | 2442 | 172 | 1.1 (0.9–1.4) | 140 | 1.1 (0.9–1.4) | 32 | 1.1 (0.7–1.7) | 104 | 1.1 (0.9–1.5) | 31 | 1.1 (0.7–1.7) | 24 | 1.1 (0.7–1.9) |
| Drinks/month[d] | | | | | | | | | | | | | |
| 1–15 | 1901 | 131 | 0.9 (0.7–1.1) | 107 | 0.9 (0.7–1.1) | 24 | 0.9 (0.6–1.5) | 78 | 0.8 (0.6–1.1) | 26 | 1.0 (0.6–1.7) | 18 | 0.9 (0.5–1.5) |
| 16–30 | 341 | 30 | 1.1 (0.7–1.7) | 25 | 1.1 (0.7–1.7) | 5 | 1.1 (0.4–2.8 | 19 | 1.1 (0.7–1.9) | 5 | 1.1 (0.4–2.8) | 4 | nc |
| >30 | 182 | 10 | 0.7 (0.4–1.4) | 8 | 0.7 (0.3–1.5) | 2 | nc | 7 | 0.9 (0.4–1.9) | 0 | nc | 1 | nc |
| Binge drinking[e] | | | | | | | | | | | | | |
| Drinking but no binge episode | 1893 | 134 | 0.9 (0.6–1.3) | 111 | 0.9 (0.6–1.4) | 23 | 1.0 (0.4–2.1) | 82 | 1.0 (0.6–1.5) | 25 | 0.8 (0.3–1.8) | 17 | 1.0 (0.4–2.3) |
| 1 or more binge episode | 524 | 37 | 0.9 (0.7–1.1) | 29 | 0.9 (0.7–1.1) | 8 | 0.9 (0.6–1.5) | 22 | 0.9 (0.6–1.1) | 6 | 1.0 (0.6–1.7) | 6 | 0.9 (0.5–1.5) |
| Alcohol type[f] | | | | | | | | | | | | | |
| Beer | 505 | 40 | 1.0 (0.7–1.5) | 32 | 1.0 (0.7–1.5) | 8 | 1.0 (0.5–2.2) | 19 | 0.8 (0.5–1.3) | 12 | 1.6 (0.8–3.1) | 6 | 1.0 (0.4–2.4) |
| Wine | 680 | 44 | 0.8 (0.6–1.1) | 39 | 0.8 (0.6–1.2) | 5 | 0.6 (0.2–1.5) | 34 | 1.0 (0.6–1.4) | 5 | 0.6 (0.2–1.6) | 5 | 0.7 (0.3–1.9) |
| Distilled spirit | 429 | 24 | 0.7 (0.5–1.2) | 20 | 0.8 (0.5–1.2) | 4 | nc | 10 | 0.5 (0.3–1.0) | 9 | 1.5 (0.7–3.2) | 2 | nc |
| 2 or more | 825 | 62 | 1.0 (0.7–1.3) | 48 | 0.9 (0.7–1.3) | 14 | 1.3 (0.7–2.3) | 41 | 1.0 (0.7–1.5) | 4 | nc | 10 | 1.1 (0.6–2.3) |
| Duration[c] | | | | | | | | | | | | | |
| 1 month | 1300 | 81 | 0.8 (0.6–1.1) | 68 | 0.8 (0.6–1.1) | 13 | 0.7 (0.4–1.3) | 48 | 0.8 (0.5–1.1) | 16 | 0.9 (0.5–1.6) | 8 | 0.6 (0.3–1.2) |
| 2 months | 744 | 61 | 1.1 (0.8–1.4) | 49 | 1.0 (0.7–1.4) | 12 | 1.1 (0.6–2.2) | 39 | 1.1 (0.7–1.5) | 10 | 1.0 (0.5–2.0) | 10 | 1.2 (0.6–2.6) |
| 3 months | 175 | 18 | 1.4 (0.8–2.3) | 14 | 1.3 (0.7–2.3) | 4 | nc | 10 | 1.3 (0.6–2.4) | 4 | nc | 4 | nc |
| 4 months | 223 | 12 | 0.7 (0.4–1.3) | 9 | 0.6 (0.3–1.2) | 3 | nc | 7 | 0.6 (0.3–1.4) | 1 | nc | 2 | nc |

CDH, congenital diaphragmatic hernia; CI, confidence interval; nc, not calculated; NOS, not otherwise specified; OR, odds ratio. Numbers vary because of incomplete or missing data.
[a]Adjusted for infant sex, gestational age, maternal age, race and ethnicity, periconceptional cigarette smoking (yes/no), first- or second-degree family history, and study center.
[b]Case phenotypes included NOS, Bochdalek, and Morgagni; all laterality (unknown, unilateral, bilateral); and all sidedness (left, right, unknown).
[c]Missing or incomplete: all CDH case mothers = 6; control mothers = 56.
[d]Missing or incomplete: all CDH case mothers = 7; control mothers = 11.
[e]Missing or incomplete: all CDH case mothers = 7; control mothers = 81.
[f]Missing or incomplete: all CDH case mothers = 8; control mothers = 60.

Although few studies have examined the associations between cigarette smoking or alcohol consumption and CDH, our findings are consistent with a previous study that showed evidence of exposure to cigarette smoking increasing risk for CDH (Zhang et al., 1992). In contrast, our findings were not consistent with Felix et al. (2008), who reported an association between any alcohol use during the periconceptional period and CDH. The lack of a positive association between alcohol and CDH in this study may be due in part to the reported drinking patterns of the mothers. Of the women who drank, most (~82%) reported drinking only in the month before pregnancy (B1) or in the months either side of conception (B1 and M1). If we adhere to a 'critical exposure period' model, then the pattern of alcohol consumption may not have overlapped or may not have occurred during diaphragm development (4–10 weeks). Conversely, reported maternal exposure to cigarette smoke most often extended throughout the entire periconceptional period, which includes gestational weeks 4–10. This proposed argument of dose- and time-dependent effects during the 'critical exposure period' is consistent with several teratogenic mouse models where repeated administration over an extended period of time is required to induce CDH, though the nitrofen model remains a notable exception. The limited overlap in exposure periods for smoking and alcohol might also account for the findings from the stratified analyses in which alcohol consumption did not appear to modify any associations between cigarette smoking exposure and CDH.

The mechanism by which fetal exposure to cigarette smoking might contribute to CDH is unknown. However, the observed lower levels of vitamin A measured in cord blood of CDH infants exposed to cigarette smoking (Yilmaz et al., 2009) could reflect a perturbation in the retinoic acid pathway, which has been identified as a primary mechanism in mouse models (Greer et al., 2003; Mey et al., 2003; Ackerman and Greer, 2007). Also consistent, and biologically plausible, is the effect of homocysteine on retinol conversion among smokers. Homocysteine has been shown to be elevated among smokers (O'Callaghan et al., 2002; Marszall and Czarnowski, 2007), including pregnant women (Ozerol et al., 2004), and high levels of homocysteine have been found to interfere with the conversion of retinol to retinoic acid (Limpach et al., 2000; Refsum, 2001). Thus, elevated homocysteine may mimic the effect of known RALDH2 antagonists (e.g., nitrofen, BPCA, bisdiamine, SB-210661) on vitamin A levels by preventing retinol conversion.

Although the exposure assessment used in this study provides advantages over previous studies, the use of retrospective reports for cigarette smoking and alcohol consumption could have resulted in reporting biases. Specifically, exposures for case mothers may have been underreported because of the social stigma associated with these behaviors during pregnancy or differential recall between case and control mothers may have occurred. However, a previous study (Verkerk et al., 1994) found that prospective and retrospective reports of cigarette and alcohol use during pregnancy produced similar exposure levels, suggesting little bias if present. Furthermore, no differences were found between case and control mothers for change in cigarette smoking exposure and alcohol consumption over the periconceptional period, after recognition of the pregnancy, and as a function of time between estimated date of delivery and interview.

Additional limitations of this study include the assessment of passive smoking exposure, binge drinking episodes, and alcohol concentration in a drink. Questions inquiring about the source of passive smoking requested detailed information about location of exposure (workplace or household), but not frequency or duration. Therefore, the smoking type variable, as constructed, assumes varying degrees of passive smoking exposure with greater exposure among mothers that reported active+passive smoke than mothers that reported active smoking only due to multiple sources of passive smoke exposure in the active+passive group. Our approximation of the frequency of binge drinking episodes may have been underestimated due to the manner in which the information was collected. Mothers were asked to report average number of days and average number of drinks per day they drank as well as the largest number of drinks on one occasion during each specified time frame of pregnancy. Frequency of binge drinking episodes was derived from the reported averages, which could underestimate the number of actual episodes, especially among women with infrequent and/or low monthly averages (e.g., <5 drinks on average). Furthermore, queries about drink volume were not defined in terms of standard drinks but rather as a 'glass' of alcohol resulting in a possible inaccurate estimates of actual amount consumed.

Finally, clinical certainty of subtype (e.g., Bochdalek, Morgagni) was absent for a large proportion of case infants who were thus classified as 'not otherwise specified' (NOS). It is possible that if sufficient clinical information had been available, some of these NOS infants would have been classified Bochdalek given that this is the most common subtype of congenital diaphragmatic hernia. As a result, interpretation of the findings of unity for associations between cigarette smoking exposure and NOS CDH are difficult.

Among the strengths of this study are the use of a large, population-based sample and the availability of more detailed information on alcohol consumption and cigarette smoking than has been previously reported. With regard to cigarette smoking and alcohol consumption, rather than only assessing any exposure, this study was able to ascertain information about frequency and quantity during critical periods of development. The data also allowed examination of mode of administration for smoking exposure (e.g., active and/or passive) and type of alcohol consumed (e.g., beer, wine, distilled spirits). Finally, the large sample size allowed for examination of differences in risk by phenotype and subtype. Based on these data, periconceptional exposure to cigarette smoking may increase risk of CDH, though further investigation is warranted. Additional studies should also examine the hypothesized pathways accounting for the risk effect of smoking presented in this paper.

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