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Maternal Periconceptional Exposure to Cigarette Smoking and Congenital Limb Deficiencies

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Abstract

Background—Congenital limb deficiencies (LD)s are characterised by the failure or disruption in formation of limbs or digits. Epidemiological research on maternal exposure to cigarette smoke and LDs is inconclusive.

Methods—Data from the National Birth Defects Prevention Study were used to examine LDs and maternal exposure to active or passive cigarette smoke. Mothers of LD case (n = 906) and unaffected control (n = 8352) pregnancies from October 1997 through December 2007 reported on exposure type and quantity. Logistic regression was used to estimate adjusted odds ratio (OR) and 95% confidence interval [95% CI]; interactions with folic acid (FA) intake were tested.

Results—For any LD, ORs were elevated for active (1.24 [95% CI 1.01, 1.53]), passive (home) (1.28 [95% CI 1.03, 1.59]), and 'active and passive' (1.34 [95% CI 1.05, 1.70]) exposures. The ORs for longitudinal LDs were elevated for passive (home) (1.62 [95% CI 1.14, 2.31]) and 'active and passive' (1.62 [95% CI 1.09, 2.41]) exposures. The OR for pre-axial LDs were elevated for any (1.39 [95% CI 1.01, 1.90]), active (1.53 [95% CI 1.03, 2.29]), passive (home) (1.82 [95% CI 1.23, 2.69]), and 'active and passive' (1.87 [95% CI 1.20, 2.92]) exposures. For lower limbs, ORs were elevated for passive (home) (1.44 [95% CI 1.01, 2.04]) and smoking 15 or more cigarettes/day (2.25 [95% CI 1.27, 3.97]). Interactions showed that ORs for any passive smoke exposure were 0.43 and 0.59 higher in the absence of FA intake for any and terminal transverse LDs.

Conclusions—Maternal active smoking and exposure to passive cigarette smoke emerged as a potential teratogen that affects limb and digit formation. FA was not found to mitigate the impact.

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Keywords

case–control studies; limb deficiencies; congenital; maternal exposure; musculoskeletal abnormalities; pregnancy; smoking; folic acid

Limb deficiencies (LD)s are characterised by the failure in formation or disruption of a portion of the entire upper or lower limb or digits during foetal development; the prevalence of LDs is estimated to be 5–8 per 10 000 live births.[1] Limb development in the human embryo begins as early as 4 weeks after conception; upper limb buds first appear on the 26th day and lower limb buds on the 28th day. The limb buds, which consist of mesenchymal core and ectodermal cells, differentiate into muscle, cartilage, and bone. At around the sixth week, the hand and foot plates form and separate from the limb buds; thus, the first trimester marks an important period during which various factors can lead to malformations in limb development.

The majority of LDs appear as isolated defects with 12–33% occurring with other major congenital malformations.[1] Terminal transverse LDs are the most common subtype followed by split hand/foot, longitudinal, intercalary, and mixed. Vascular disruption resulting in foetal hypoxia has been implicated in the pathogenesis of LDs.[2–5] There are several known contributors to the development of foetal hypoxia, including maternal exposure to cigarette smoke during pregnancy from either active smoking[6] or passive cigarette smoke.[7] Studies examining the relation between maternal exposure to cigarette smoke and LDs have been inconsistent with elevated odds ratios (ORs) reported for some,[8–11] but not all LD subtypes.[8,10,12–14] Fewer studies have assessed passive smoke exposure, and, of those identified, the findings have also been equivocal.[12–14] Comparison across studies is difficult, however, due to variation in the inclusion of covariables, LD subtypes examined, and type of cigarette smoke exposure (e.g. active or passive) measured.

Because a substantial number of pregnant women continue to be directly[15] and indirectly exposed to cigarette smoke,[16] data from the National Birth Defects Prevention Study (NBDPS), a large population-based case–control study, were used to examine the relation between maternal exposure to cigarette smoke and LDs. Associations between different types of maternal exposure to cigarette smoke and LD subtypes were examined while controlling for important covariables (e.g. alcohol consumption, intake of vasoactive medications and vitamin A). In addition, there is evidence suggesting that folic acid (FA) supplementation may mitigate the potential teratogenic effects of exposure to cigarette smoke on adverse foetal outcomes.[17,18] Therefore, the interaction between FA supplement intake and exposure to cigarette smoke was tested.

Methods

Sample selection, case classification, and recruitment

The NBDPS is an ongoing, multisite, population-based, case–control study designed to investigate genetic and environmental risk factors for 37 major birth defect groups. Initial NBDPS sites 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 at 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. Sites contributed live births diagnosed with LDs and a limited number of sites ascertained foetal deaths (AR, CA, CDC, IA, MA, TX, and NY since 2000) and elective terminations (AR, CA, CDC, IA, TX, and NY since 2000). Each site obtained institutional review board approval for the NBDPS.

Control pregnancies were unaffected live births with an estimated date of delivery (EDD) during the same time frames as case pregnancies and randomly selected from either hospital delivery logs (AR and CDC 1997–2000; CA, NY, TX 1997–2007) or birth certificate files (AR 2000-07; CDC 2001-07; IA, MA, NC, NJ, and UT 1997–2007). Excluded were cases with defects of known or strongly suspected genetic aetiology (e.g. single gene disorders and chromosome abnormalities), cases and controls not in the custody of or not residing with their birth mothers, or cases and controls whose birth mother did not speak English or Spanish.

Case classification

Case classification was determined by local clinical geneticists at each NBDPS site using clinical information abstracted from medical records and compiled in a centralised clinical database. Clinical information on each pregnancy used to determine case classification included: method of diagnosis (i.e. a diagnosis of a cardiac defect required results from an echocardiography, catheterisation, surgery, or autopsy); laboratory results, including genetics and other specialty evaluations when available; and relevant exposures or presence of family history. Clinical geneticists reviewed available clinical information for each case pregnancy and assigned standard case definitions to determine case status. Additional information about case classification can be found in Rasmussen *et al.*[19] An NBDPS-specific modification of the CDC six-digit coding system was assigned to each case meeting definitional criteria. The development of the NBDPS codes and their relation to the International Statistical Classification of Diseases and Related Health Problems (ICD-9), the clinical modification of the ICD-9, and British Paediatric Association coding schemes can be found in Rasmussen and Moore.[20] The NBDPS codes were developed because of a lack of specificity of existing codes for certain LD subtypes (e.g. split hand or foot codes).

Case classification by site clinical geneticists was reviewed by a NBDPS clinical geneticist (R.S.O.) to ensure consistency in coding and to further classify eligible cases as isolated (no additional major and unrelated defects), multiple (one or more additional major and unrelated defects), or complex sequence (i.e. limb-body wall complex and amniotic bands).

Cases were classified into the following subtypes: longitudinal (pre-axial, post-axial, and split hand/ foot), terminal transverse (amelia excluded), amelia, intercalary, and not elsewhere classified. LDs were also classified in terms of laterality and sidedness of the deficiency (unilateral-left, unilateral-right, bilateral, and unknown), and whether an upper or lower limb was affected. To reduce pathogenetic heterogeneity, cases with amniotic band syndrome (n = 162) or any other complex sequence (n = 1) were excluded.

Recruitment

Structured, computer-assisted telephone interviews were conducted with birth mothers of cases and controls; interviews were conducted from 6 weeks to 2 years following the EDD. Median length between EDD and interview date was 9.0 months for case mothers and 7.6 months for control mothers. A standard protocol was followed for recruitment of case and control mothers.[21] Specifically, mothers were mailed a packet that included an introductory letter describing the study, $20 compensation, and interview-related materials (i.e. a sheet listing response categories and a pregnancy calendar). The packet was mailed no earlier than 6 weeks following the EDD. The recruitment protocol consisted of an 8-week follow-up period after the initial mailing of the introductory letter. Three additional attempts to reach the mother were made at 2-week intervals and consisted of a series of follow-up telephone calls or reminder letters if contact was not made by phone. If no contact was made during the 8-week recruitment period, then the mother was counted as a non-participant.

Maternal interview

The interview included, but was not limited to, detailed questions about health problems, single and multiple vitamin intake, medication use, alcohol consumption, and maternal exposure to cigarette smoke from 3 months before conception through the end of the pregnancy. For each, the mother was asked for dates of occurrence and, where applicable, the frequency with which the exposure occurred. From these questions, we derived maternal periconceptional exposures to the following covariables: vitamin A from either a single vitamin or multivitamin; vasoactive medications, which included antihypertensives (0.1%), bronchodilators (4.1%), decongestants (10.3%), migraine medications (0.6%), and non-steroidal anti-inflammatory drugs (32.4%); and alcohol consumption.

Exposure assessments

Periconceptional exposure to cigarette smoke—Retrospective reports for cigarette smoke exposure were collected for each of the 3 months prior to pregnancy (labelled B3, B2, and B1), each of the first 3 months of pregnancy (labelled M1, M2, and M3), and by trimester for months 4–6 and 7–9 of pregnancy (labelled T2 and T3). Maternal exposure to cigarette smoke was classified as active (mother herself smoked) or passive (exposure to smoke at home or in the workplace). If a mother reported active smoking, information about the average 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. A mother was classified as exposed to passive cigarette smoke in the home if she answered yes to the question ‘Did anyone in your household smoke cigarettes in your home between 3 months before you became pregnant to the end of your pregnancy?’ and exposed to passive cigarette smoke in the workplace or school if she said yes to ‘Did anyone smoke cigarettes near you at a work-place or school you may have attended during that year?’. If a mother reported passive exposure to smoke, information about the periconceptional month(s) during which exposure at home or work/school occurred was collected.

A mother was classified as exposed to cigarette smoke if she reported active or passive smoke exposure during any month of 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 smoke was also classified by type of exposure (active only, passive only, or 'active and passive'). 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). Duration of exposure was estimated separately for exposure to any active or any passive cigarette smoke (number of periconceptional months exposed, 0 through 4), with each month considered 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.

Periconceptional FA supplementation—Periconceptional FA supplementation was determined from interview questions asking about the use of pre-natal vitamins, multivitamins, and intake of specific vitamins or minerals from 3 months before conception through the end of the pregnancy. Follow-up questions about timing and frequency of intake were asked for each reported prenatal vitamin, multivitamin, or single vitamin/mineral. These follow-up probes included questions about start and stop dates of use and frequency of intake (e.g. once per day). Mothers were classified as using a FA supplement during the 4-month periconceptional period (B1–M3) if a supplement was taken at least 90 out of the possible 120 days. Ninety days was assigned as the minimum cut-off for use because many women do not initiate FA supplement intake until after knowledge of pregnancy.

Statistical analysis

Analyses were conducted using SAS, version 9.2 (SAS Institute, Cary, NC, USA). Descriptive analyses used the chi-square test to compare cases and controls on the following covariables: case and control sex (male and female), low birthweight (<2500 and ≥2500 g), preterm delivery (<37 and 37–45 weeks), and family history of LD (yes and no); maternal age (<20, 20–34 and ≥35 years), race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, and other), education (<12, 12, 13–15, and ≥16 years), pre-pregnancy body mass index (<18.5, 18.5–24.9, 25.0–29.9 and ≥30), and parity (no prior births, primipara, and multipara); plurality (singleton and multiple) and pregnancy intention (yes and no); maternal periconceptional exposure to contraceptive pills (yes and no), FA or multivitamin (yes and no), and vitamin A (yes and no), vasoactive medications (0, 1, and 2 or more), and alcohol (yes and no); season of conception (summer, fall, winter, and spring); and NBDPS site.

Crude OR (cOR), adjusted OR (aOR), and associated 95% confidence intervals (CI) were calculated to assess associations between LDs and maternal exposure to cigarette smoke. The significance of multiplicative and additive interactions [e.g. the relative excess risk interval (RERI)] between selected maternal cigarette smoke exposure variables and FA supplementation were also tested. Bootstrapping methods were used to estimate the interaction terms and corresponding significance tests; *P*-values were used to determine significance of the multiplicative interaction estimate, and 95% CI were used for the RERI estimates.[22] [The RERI and 95% CI were calculated by bootstrapping methods using a SAS macro developed by Marilyn Browne and Sandra Richardson (2011, pers. comm., New York State Department of Health)].

Possible confounding was examined by introducing each covariable into a model containing the exposure variable of interest. The respective covariable was included in the multivariable model if any aOR for cigarette smoke exposure changed by 10% or more after adding the covariable. Adjusted analyses are only presented for LD subtype groups containing at least 100 cases (i.e. any LD, any longitudinal LD, pre-axial LD, and terminal transverse). Analyses for LD subtypes with fewer than 100 cases are available in the supplementary tables.

Results

Participation in the maternal interview was 69% among case mothers and 65% among control mothers. A total of 906 case mothers and 8352 control mothers completed interviews. Overall, the most common LD subtype was terminal transverse; intercalary and amelia LDs were least frequent (Table 1). Approximately one half of all cases were affected on the left side, followed by right-sided and bilateral presentation. Most of the affected limbs were the arms. The presence of other major congenital defects differed by LD subtype; any longitudinal, pre-axial, and amelia subtypes were more likely than other LD subtypes to have multiple defects; all other subtypes were mostly isolated defects.

Cases were more likely than controls to be male, low birthweight, preterm, and have a family history of LDs (Table 2). Case mothers were more likely to be Hispanic and have fewer years of education. Case pregnancies were more likely to be the mother's first birth, a multiple pregnancy, and unintended. Maternal periconceptional exposures to vitamin A, vasoactive medication, and alcohol were more common among case mothers compared with control mothers. Case pregnancies were more often conceived in the winter, and variation across site was also found. No differences between case and control mothers were found for maternal age at EDD and pre-pregnancy body mass index or maternal periconceptional exposure to contraceptive pills and FA or multivitamins.

Case and control mothers reported similar exposures to any periconceptional cigarette smoke and active smoking. Case mothers were more likely to report exposure to passive cigarette smoke, with or without active smoking (Table 3). Proportions of case and control mothers reporting specific types of smoke exposure ('active only', 'passive only', and 'active and passive'), duration of 'active and passive' smoke, and the number of average cigarettes smoked per day by mothers who reported active smoking were similar. Among those exposed to cigarette smoke, passive smoke only was the most common mode of exposure (43.1%) followed by 'active and passive' (35.7%) and 'active only' (21.2%). Of mothers reporting active smoking, over one half (53.8%) smoked all four months of the periconceptional period. Most mothers (85.1%) that reported passive exposure (home or work) were exposed all 4 months. To evaluate possible response bias, case and control mothers were compared on changes in reported frequency of cigarette smoke exposure after pregnancy recognition and by 6-month intervals between EDD and date of interview. No response bias was found (data not shown).

aORs showed that case and control mothers differed on several indicators of exposure to cigarette smoke (see Table 4). The aORs for any exposure to cigarette smoke and active

smoke were significantly elevated for any LD and pre-axial longitudinal LDs. Odds of passive exposure in the home were elevated for any LD, any longitudinal LD, and pre-axial longitudinal LDs. Odds of exposure to 'active and passive' cigarette smoke were elevated for any LD, any longitudinal LD, and the pre-axial longitudinal LD subtype. Terminal transverse LDs were not significantly associated with any indicator of periconceptional exposure to cigarette smoke. Odds of passive exposure to cigarette smoke in the home and actively smoking greater than 15 cigarettes a day were higher for cases with affected lower limbs (see Table 5). Adjusted analyses for duration of exposure to active smoking and passive smoke in the home could only be reliably estimated for any LD for which no associations were found (data not shown).

The mitigation of the association of cigarette smoke cigarette exposure on limb development by FA supplementation was examined. To maximise power, the cigarette smoke exposure variables were limited to: any cigarette smoke; active smoking only; any passive (home or work) cigarette smoke only; and 'active and passive' (home or work) cigarette smoke. No exposure to 'active and passive' cigarette smoke was the reference group for each exposure. Tests of the multiplicative interactions were not significant for any of the LD subtypes evaluated (data not shown). The RERIs calculated for additive interactions between exposure to passive (home or work) smoke only and FA supplementation were significant for any LD and the terminal transverse subtype. The risk ratio (RR) for passive (home or work) cigarette smoke exposure and any LD was 0.74 [95% CI 0.45, 1.16] among mothers who supplemented with FA, and the RR for exposure among mothers who did not supplement was 1.18 [95% CI 0.93, 1.49]. The RERI between mothers who did and did not use FA supplements was significant (RERI = 1.18–0.74 = 0.43 [95% CI 0.04, 0.78]), indicating higher RR among mothers who were exposed to any passive (home or work) cigarette smoke and did not take FA supplements compared with exposed mothers who did take supplements. Similarly, the RR for terminal transverse was 0.61 [95% CI 0.33, 1.14] among mothers who used FA supplements and 1.22 [95% CI 0.90, 1.65] among mothers who did not take supplements; the statistically significant RERI was 0.59 [95% CI 0.10, 0.99]. The non-significant RERIs for the pre-axial subtype and upper or lower affected limbs suggests FA supplementation does not mitigate the associations of cigarette smoke exposure with these LDs.

Comments

Maternal reports of exposure to cigarette smoke were compared between mothers of cases diagnosed with LDs and mothers of non-malformed controls. Odds of exposure to active cigarette smoke, passive (home) smoke, and combined exposure to multiple types of cigarette smoke were higher among mothers of cases diagnosed with any LD, any longitudinal LD, and the pre-axial longitudinal LD subtype. Analysis of upper and lower affected limbs (regardless of subtype) showed elevated ORs for exposure to passive cigarette smoke and actively smoking 15 or more cigarettes a day for lower limbs only. Analyses for effect modification of exposure to cigarette smoke by FA supplement intake showed higher cORs among mothers who were exposed to cigarette smoke but did not take FA supplements. Statistical tests of the interactions, however, only showed significant

additive interactions for any LD and the terminal transverse subtype with higher cOR for exposure to cigarette smoke in the absence of FA supplementation.

The absence of an association between maternal exposure to cigarette smoke and terminal transverse LDs is consistent with findings presented by Werler *et al*.,[10] which analysed NBDPS data collected between 1998 and 2004, but contrary to two previous independent studies that showed higher odds of maternal exposure to cigarette smoke among cases born with terminal transverse LDs.[8,9] The findings in this study are also inconsistent with Källen[8] and Czeizel *et al*.[9] because of the demonstrated associations between maternal reports of exposure to cigarette smoke and the pre-axial LD not found in the previous studies. The discrepancy between studies could be due in part to the collapsing of LD subtypes into broader categories. For example, Källen[8] examined associations between maternal active smoking and combined LD subtypes into broader classes (e.g. any longitudinal). The overall diagnosis of longitudinal LD was not associated with exposure to active cigarette smoke; only ORs for the pre-axial longitudinal LD subtype were elevated. Another possible reason for discrepancies could be that neither Källen[8] nor Czeizel *et al*.[9] assessed maternal exposure to passive smoke, which was predictive in this study. The finding of higher odds of passive exposure among mothers of LD cases is consistent with one other study that included assessment of paternal cigarette smoking.[12] Finally, the inconsistencies with other findings could be due, in part, to changes in the classification scheme of LDs that have occurred over the years, making direct comparisons of findings difficult.

There are multiple biological mechanisms that could account for the observed associations between exposure to cigarette smoke and LDs. One is that exposure to cigarette smoke may contribute to LDs because of the increased likelihood of chronic foetal hypoxia.[5] Chronic hypoxia is hypothesised to trigger a ‘lower limb reflex’,[23] which is a compensatory mechanism by which circulatory flow is directed away from the vascular beds of the lower limb, skeletal muscles, skin, and mesentery, and towards the foetal heart and brain.[24] This vasoconstriction may result in cell death or haemodynamic changes that can impact delivery of vital nutrients. The ‘lower limb reflex’ hypothesis is consistent with the findings of elevated odds of maternal exposure to cigarette smoke among infants having lower LDs and is supported by studies suggesting an association between other lower limb structural defects and maternal exposure to cigarette smoke (e.g. clubfoot/talipes equinovarus).[25]

Another plausible mechanism is the effect of homocysteine on retinol conversion. Animal models have shown a dose-dependent association between the induction of congenital defects and hyperhomocysteinemia.[26] Homocysteine has been shown to be elevated among smokers,[27] including pregnant women[28,29] and non-smokers exposed to passive cigarette smoke.[30] Hyperhomocysteinemia interferes with the conversion of retinol to retinoic acid and signalling by retinoic acid receptors.[26] Retinoic acid is important to skeletal development because it controls developmental genes and acts as a morphogen.[31] Morphogens are essential to informing cells of where they are located (i.e. limbs) and their purpose (i.e. digits). Retinoic acid is also key to the regulation (e.g. turning on) of the sonic hedgehog gene,[32] which polarises the zone of polarising activity and activates *Hoxd* gene activity in a sequential transcriptional pattern. Interference with the actions of the zone of polarising activity and *Hoxd* can subsequently affect limb patterning and development.

Limitations of the study included small numbers for specific LD subtypes (i.e. post-axial, split hand or foot, and amelia). Even though the exposure assessment improves upon methods of previous studies (e.g. birth certificates), the use of retrospective maternal reports may introduce response bias by underestimating exposures due to active maternal smoking. However, potential response bias was evaluated by examining differences between case and control mothers on changes in rates of exposure to cigarette smoke over the periconceptional period, after recognition of pregnancy, or as a function of time between EDD and date of interview. Although none of the comparisons were statistically significant, evaluations of biological markers not available in the NBDPS are needed to provide a more definitive evaluation of response bias. Another limitation is the measurement of frequency or average number of cigarettes. The number of cigarettes smoked per day was collected in categories, which prohibited detailed examination of dose–response associations between LDs and number of cigarettes actively smoked. In addition, the assessment of exposure to passive cigarette smoke was less exhaustive than that for active cigarette smoking. Questions were limited to location exposed (home or work) and did not gather detailed information about frequency and intensity of exposure; furthermore, changes in work policies on smoking in the workplace could not be systematically evaluated. Finally, this study attempted to include important covariables reported in previous studies of LDs; however, the occurrence of some (i.e. chorionic villus sampling and migraines) were too rare to analyse.

Strengths of the study included analysis of multiple LD subtypes, detailed assessment of exposure to active cigarette smoke, and detailed examination of important covariables. Analysis of individual LD subtypes showed associations between specific LD subtypes and periconceptional maternal exposure to cigarette smoke; although the results should be replicated using larger samples for subtypes. With regard to cigarette smoke, information was collected about frequency and duration of exposure, as well as passive exposure at home and work. This information provides a more complete understanding of the degree of exposure among pregnancies affected by LDs. Another strength was the extensive set of covariables evaluated, which allowed examination of cigarette smoke exposure after controlling for other potential factors involved in LDs (e.g. vasoactive medication use).

Based on the findings, maternal exposure to cigarette smoke during the periconceptional period is a potential teratogen that affects limb and digit formation, and FA supplement intake was not found to mitigate the impact. Importantly, this study suggests that passive exposure to cigarette smoke might affect limb development regardless of maternal active smoking status. These findings add to a growing literature on passive smoke exposure and adverse foetal outcomes. Because of multiple, albeit correlated, comparisons that increase the likelihood of significant findings due to chance, additional research is needed to replicate these findings using larger sample sizes to explore specific pathogenic mechanisms and examine potential interventions to reduce toxicity of exposure.

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Table 1

Description of limb deficiencies, National Birth Defects Prevention Study (1997–2007)[a]

	Any LD		Isolated		Multiple	
Characteristic	**No.**	**%[b]**	**No.**	**%[c]**	**No.**	**%[c]**
Any LD[d]	906		656	72.4	250	27.6
Any longitudinal[d]	283	31.2	140	49.5	143	50.5
Pre-axial longitudinal	210	22.8	79	37.6	131	62.4
Post-axial longitudinal	71	7.7	54	76.1	17	23.9
Split hand or foot longitudinal	66	8.2	49	74.2	17	25.8
Amelia	18	2.1	7	38.9	11	61.1
Terminal transverse	505	55.2	431	85.4	74	14.7
Intercalary	49	5.6	36	73.5	13	26.5
Laterality[d]						
Left	420	46.4	334	79.5	86	20.5
Right	282	31.1	205	72.7	77	27.3
Bilateral[e]	187	20.6	105	56.2	82	43.9
Unknown/unilateral, side unknown	17	1.9	12	70.6	5	29.4
Affected limbs						
Arms	632	69.8	457	72.3	175	27.7
Legs	216	23.8	160	74.1	56	25.9
Both	58	6.4	39	67.2	19	32.8

No., number of cases.

[a] NBDPS codes: longitudinal [longitudinal, not otherwise specified (NBDPS codes: 755250-755254, 755350-755354), pre-axial (NBDPS codes: 755260-755269; 755360-755369), post-axial (NBDPS codes: 755270-755274; 755370-755374), and split hand/ foot (NBDPS codes: 755255-755259; 755355-755359)], terminal transverse (NBDPS codes: 755200-755209, 755240-755249; 755300-755309, 755340-755249), amelia (NBDPS codes: 755205-755209; 755305-755309), intercalary (NBDPS codes: 755210-755239; 755310-755339), and not elsewhere classified (NEC) (NBDPS codes: 755280-755289; 755380-755389).

[b] Percent of total number of cases with any LD (n = 906).

[c] Percent within LD type for isolated/multiple frequencies of the LD.

[d] Number and percent may be greater than the total number of any LD because of multiple subtype diagnoses.

[e] Bilateral may include more than one LD subtype.

Table 2

Selected characteristics of case and control pregnancies and birth mothers, National Birth Defects Prevention Study (1997–2007)

	Controls		Any LD	
Characteristic	**No.**	**%**	**No.**	**%**
Totals	8352		906	
Case and control characteristics				
Sex***				
Female	4102	49.2	386	43.0
Male	4242	50.8	512	57.0
Low birthweight ***				
<2500 g	466	5.6	233	25.9
≥2500 g	7851	94.4	667	74.1
Preterm delivery***				
Term (37–45 weeks)	7564	90.6	666	73.9
Preterm (<37 weeks)	787	9.4	235	26.1
Family history of LD***				
Yes	11	0.1	7	0.8
No	8341	99.9	899	99.2
Maternal characteristics				
Age at EDD (years)				
<20	856	10.3	100	11.0
20–34	6322	75.7	696	76.8
≥35	1174	14.1	110	12.1
Race/ethnicity**				
Non-Hispanic white	4942	59.4	507	56.0
Non-Hispanic black	927	11.1	89	9.8
Hispanic	1908	22.9	254	28.1
Other	545	6.6	55	6.1
Education (years)*				
<12	1429	17.1	162	17.9
12	2017	24.2	245	27.1
13–15	2260	27.1	256	28.3
16 or more	2937	31.6	242	26.7
Pre-pregnancy body mass index				
Underweight (<18.5)	433	5.4	49	5.7
Normal weight (18.5–24.9)	4405	55.0	447	52.0
Overweight (25.0–29.9)	1830	22.8	204	23.7
Obese (≥30)	1343	16.8	160	18.6
Parity*				
Never pregnant	2435	29.2	302	33.3

	Controls		Any LD	
Characteristic	**No.**	**%**	**No.**	**%**
Primipara	2455	29.4	261	28.8
Multipara	3461	41.4	343	37.9
Maternal pregnancy characteristics				
Plurality***				
Multiple	251	3.0	57	6.3
Singleton	8096	97.0	849	93.7
Pregnancy Intention**				
Yes	5051	60.7	506	56.2
No	3277	39.4	395	43.8
Maternal periconceptional behaviours				
Contraceptive pill use				
Yes	640	7.7	74	8.2
No	7712	82.3	832	91.8
Folic acid/multivitamin intake				
Yes	3040	36.4	323	35.6
No	5312	63.6	583	64.4
Vitamin A intake*				
Yes	3931	47.2	389	43.2
No	4390	52.8	511	56.8
Vasoactive medication intake***				
0	5388	65.9	550	61.9
1	2279	27.9	250	28.2
2 or more	510	6.2	88	9.9
Alcohol use**				
No	5239	63.3	610	68.1
Yes	3041	36.7	286	31.9
Season of conception*				
Fall	2163	25.9	232	25.6
Winter	2079	24.9	254	28.0
Spring	2041	24.4	231	25.5
Summer	2069	24.8	189	20.9
Study site***				
Arkansas	1055	12.6	92	10.2
California	1017	12.2	143	15.8
Iowa	928	11.1	89	9.8
Massachusetts	1027	12.3	110	12.1
New Jersey	573	6.9	84	9.3
New York	722	8.6	63	7.0
Texas	969	11.6	109	12.0
Center for Disease Control and Prevention	880	10.5	91	10.0

	Controls		Any LD	
Characteristic	**No.**	**%**	**No.**	**%**
North Carolina	570	6.8	37	4.1
Utah	611	7.3	88	9.7

* $P < 0.05$;

** $P < 0.01$;

*** $P < 0.001$.

Numbers vary because of incomplete or missing data. Because of rounding, percentages may not total 100.

Table 3

Maternal periconceptional exposure to cigarette smoke and limb deficiencies, National Birth Defects Prevention Study (1997–2007)

	Controls (*n* = 8352)		Any LD (*n* = 906)	
Cigarette smoking exposure	**No.**	**%**	**No.**	**%**
Any active or passive				
No active or passive	5664	68.0	589	65.3
Active or passive	2666	32.0	313	34.7
Any active				
No active or passive	5664	78.7	589	75.7
Active[a]	1537	21.3	189	24.3
Any passive (home)				
No active or passive	5664	82.2	589	79.1
Passive (home)[b]	1231	17.9	156	20.9
Any passive (work)				
No active or passive	5664	83.2	589	81.6
Passive (work)[c]	1148	16.9	133	18.4
Type of smoke				
No active or passive	5664	68.1	589	65.3
Active only	626	7.5	73	8.1
Passive only (home or work)	1126	13.5	124	13.8
Active and passive	906	10.9	116	12.9
Cigarettes/day[a]				
No active or passive	5664	78.8	589	75.8
1–14/day	1270	17.7	154	19.8
≥15/day	253	3.5	34	4.4
Duration of active smoking				
No active or passive	5664	78.7	589	75.7
1 month	217	3.0	26	3.3
2 months	324	4.5	42	5.4
3 months	177	2.5	20	2.6
4 months	819	11.4	101	13.0
Duration of passive (home) smoke				
No active or passive	5664	73.6	589	71.1
1 month	139	1.8	10	1.2
2 months	116	1.5	14	1.7
3 months	95	1.2	10	1.2
4 months	1685	21.9	206	24.9

No., number of cases or controls.

Numbers vary because of incomplete or missing data. Due to rounding, percentages may not total 100.

[a]May include mothers who report exposure to passive (home or work) cigarette smoke.

[b] May include mothers who report exposure to active or passive (work) cigarette smoke.

[c] May include mothers who report exposure to active or passive (home) cigarette smoke.

Table 4

Adjusted odds ratio estimates for maternal reports of periconceptional exposure to cigarette smoke and limb deficiencies, National Birth Defects Prevention Study (1997–2007)

	Controls	Any LD		Any longitudinal		Pre-axial longitudinal		Terminal transverse	
Cigarette smoking exposure	**No.[a]**	**No.[a]**	**aOR [95% CI]**	**No.[a]**	**aOR [95% CI]**	**No.[a]**	**aOR [95% CI]**	**No.[a]**	**aOR [95% CI]**
No active or passive	5210	529	1.00 [Referent]	159	1.00 [Referent]	112	1.00 [Referent]	299	1.00 [Referent]
Any active or passive	2494	289	1.14 [0.97, 1.35]	96	1.24 [0.93, 1.64]	79	1.39 [1.01, 1.90]	157	1.13 [0.91, 1.40]
Any active[b]	1439	172	1.24 [1.01, 1.53]	58	1.39 [0.98, 1.98]	46	1.53 [1.03, 2.29]	91	1.18 [0.90, 1.56]
Any passive (home)[c]	1148	146	1.28 [1.03, 1.59]	56	1.62 [1.14, 2.31]	48	1.82 [1.23, 2.69]	67	1.08 [0.80, 1.45]
Any passive (work)[d]	1081	125	1.14 [0.91, 1.41]	42	1.25 [0.87, 1.81]	36	1.42 [0.94, 2.13]	70	1.14 [0.86, 1.52]
Cigarettes/day[b]									
1–14/day	1197	139	1.21 [0.97, 1.51]	46	1.32 [0.91, 1.92]	37	1.48 [0.97, 2.25]	73	1.14 [0.85, 1.52]
≥15/day	230	33	1.48 [1.00, 2.20]	12	1.86 [0.98, 3.53]	9	1.95 [0.93, 4.09]	18	1.38 [0.82, 2.31]
Type of smoke									
Active only	582	63	1.11 [0.83, 1.47]	19	1.13 [0.68, 1.87]	13	1.08 [0.59, 1.98]	39	1.19 [0.83, 1.70]
Passive only	1053	117	1.06 [0.85, 1.32]	38	1.08 [0.75, 1.57]	33	1.27 [0.84, 1.91]	66	1.19 [0.83, 1.70]
Active and passive	853	109	1.34 [1.05, 1.70]	39	1.62 [1.09, 2.41]	33	1.87 [1.20, 2.92]	52	1.16 [0.83, 1.62]

Covariates entered into multivariate logistic model: study site; sex; maternal race/ethnicity and education; season of conception; pregnancy intention; periconceptional vasoactive medication use and folic acid intake; and any periconceptional alcohol consumption.

[a] Number (No.) of case and control infants with no missing data.

[b] May include mothers who report exposure to passive (home or work) cigarette smoke.

[c] May include mothers who report exposure to active or passive (work) cigarette smoke.

[d] May include mothers who report exposure to active or passive (home) cigarette smoke.

Table 5

Adjusted odds ratio estimates for maternal reports of periconceptional exposure to cigarette smoke and upper/lower affected limbs, National Birth Defects Prevention Study (1997–2007)

	Controls	Upper		Lower	
Cigarette smoking exposure	**No.[a]**	**No.[a]**	**aOR [95% CI]**	**No.[a]**	**aOR [95% CI]**
No active or passive	5210	408	1.00 [Referent]	153	1.00 [Referent]
Any active and passive	2494	210	1.06 [0.88, 1.28]	99	1.26 [0.95, 1.66]
Any active[b]	1439	128	1.16 [0.92, 1.46]	60	1.40 [0.99, 1.98]
Any passive (home)[c]	1148	104	1.14 [0.89, 1.44]	53	1.44 [1.01, 2.04]
Any passive (work)[d]	1081	95	1.08 [0.85, 1.38]	37	1.10 [0.75, 1.62]
Cigarettes/day[b]					
1–14/day	1197	105	1.15 [0.90, 1.47]	44	1.25 [0.86, 1.82]
≥15/day	230	23	1.26 [0.79, 1.99]	16	2.25 [1.27, 3.97]
Type of smoke					
Active only	582	45	1.02 [0.73, 1.41]	24	1.40 [0.88, 2.21]
Passive only	1053	82	0.96 [0.75, 1.23]	39	1.13 [0.78, 1.63]
Active and passive	853	83	1.27 [0.97, 1.67]	36	1.38 [0.92, 2.07]

Numbers vary because of incomplete or missing data. $n = 58$ have both upper and lower affected limb(s) and are included in the respective counts.

[a] Number (No.) of case and control infants with no missing data.

[b] May include mothers who report exposure to passive (home or work) cigarette smoke.

[c] May include mothers who report exposure active or passive (work) cigarette smoke.

[d] May include mothers who report exposure active or passive (home) cigarette smoke.