HHS Public Access

Author manuscript

Birth Defects Res A Clin Mol Teratol. Author manuscript; available in PMC 2015 November 01.

Published in final edited form as:

Birth Defects Res A Clin Mol Teratol. 2014 November ; 100(11): 863–876. doi:10.1002/bdra.23292.

Maternal Periconceptional Alcohol Consumption and Congenital Limb Deficiencies

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Abstract

Background—Women of childbearing age report high rates of alcohol consumption, which may result in alcohol exposure during early pregnancy. Epidemiological research on congenital limb deficiencies (LDs) and periconceptional exposure to alcohol is inconclusive.

Methods—Data from the National Birth Defects Prevention Study (NBDPS) were examined for associations between LDs and patterns of maternal periconceptional (1 month before conception through the first trimester) alcohol consumption among LD case (n = 906) and unaffected control (n = 8352) pregnancies with expected delivery dates from 10/1997 through 12/2007. Adjusted odds ratios (aORs) and 95% confidence intervals were estimated from unconditional logistic regression analysis for all LDs combined, specific LD subtypes (preaxial/terminal transverse), and LD anatomic groups (upper/lower limbs); interactions with folic acid (FA) supplementation were tested.

Results—When compared with nondrinkers, inverse associations were found between all LDs combined, preaxial, and upper LDs and any reported periconceptional alcohol consumption (aORs ranged from 0.56–0.83), drinking without binging (aORs: 0.53–0.75), and binge drinking (≥4 drinks/occasion) (aORs: 0.64–0.94); however, none of the binge drinking aORs were statistically significant. Stratification by alcohol type showed inverse associations between all LDs combined, preaxial, transverse, and upper and lower LDs for drinking without binging of wine only (aORs: 0.39–0.67) and between all LDs combined and upper LDs for drinking without binging of combinations of alcohol (aORs: 0.63–0.87). FA did not modify observed associations.

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Conclusion—Maternal periconceptional alcohol consumption did not emerge as a teratogen for selected LDs in the NBDPS. Future studies should evaluate additional rare LDs among more highly exposed populations.

Keywords

limb deficiencies; congenital; maternal exposure; pregnancy; alcohol drinking; folic acid

Introduction

Limb deficiencies (LDs) are characterized by failure of the entire upper or lower limb, or a portion thereof, to form during embryonic development. Most LDs appear as isolated defects with 12% to 33% occurring with other major structural birth defects (Kallen et al., 1984; Froster-Iskenius and Baird, 1989; Ephraim et al., 2003; Makhoul et al., 2003). The overall birth prevalence for LDs is estimated to be 5 to 8 per 10,000 live births (Lin et al., 1993; Castilla et al., 1995; Makhoul et al., 2003). Limb development in humans 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 (Barham and Clarke, 2008). Approximately 6 weeks after conception, the hand and foot plates form, marking the first trimester as an important period of susceptibility for defects in limb development (Barham and Clarke, 2008).

Studies on the pathogenesis of LDs have identified several classes of factors that alter limb development, including maternal medication use during pregnancy (e.g., thalidomide, vasoactive medications), health conditions (e.g., insulin-dependent diabetes mellitus), and procedures received during pregnancy (e.g., chorionic villus sampling) (Froster and Baird, 1993; Holmes, 2002). Maternal exposures to addictive substances thought to have vascular-disrupting properties (e.g., cocaine, tobacco) have been shown to be associated with specific LD subtypes (Aro, 1983; Froster and Baird, 1993; Holmes, 2002). The findings from studies of maternal exposure to alcohol and LDs, although suggested by early case reports of fetal alcohol syndrome (FAS) (Spiegel et al., 1979; Herrmann et al., 1980; van Rensburg, 1981; Pauli and Feldman, 1986; Lin et al., 1991), have been less consistent. The equivocal results may be due, in part, to variability in defining maternal alcohol consumption (e.g., any consumption [Aro et al., 1984; Froster and Baird, 1992; Shaw et al., 2002] versus specific intake patterns [Martinez-Frias et al., 2004]), inclusion of LDs as part of a broader defect group (e.g., musculoskeletal defects [McDonald et al., 1992; Baumann et al., 2006]), or study differences in classifying LDs (Gold et al., 2011).

Examination of the association between maternal alcohol consumption and limb formation is further complicated by the genetic control of limb patterning. Specifically, multiple gene families (e.g., Sonic Hedgehog, Fibroblast growth factor, WNT, Homeobox) are involved in limb patterning across three axes (i.e., proximal–distal, anterior–posterior, and dorsal–ventral) (Barham and Clarke, 2008). Due to this developmental complexity, the pathogenesis of LDs is most likely multifactorial. In fact, experimental animal studies suggest several potential pathways by which alcohol exposure during pregnancy could affect limb development, including interference with folate metabolism (Hillman and Steinberg, 1982), vascular disruption (Froster and Baird, 1992), elevated homocysteine (Limpach et al.,

2000; van Mil et al., 2010), interference with retinoic acid synthesis (Limpach et al., 2000), and disrupted cholesterol metabolism (Lanoue et al., 1997; Gofflot et al., 2003; Li et al., 2007). Recovery studies in which diets of alcohol-exposed animals are supplemented with key nutrients (e.g., folic acid, retinoic acid, cholesterol) provide further evidence for understanding these pathways (Gofflot et al., 2003; Johnson et al., 2007; Li et al., 2007; Idrus and Thomas, 2011).

Continued epidemiological study of the associations between maternal alcohol consumption and LDs is warranted due to the paucity of human studies, limitations of existing studies, and continued high rates (51.5% any use, 15% binge drinking) of alcohol consumption among non-pregnant women 18 to 44 years of age (Centers for Disease Control and Prevention, 2012). The high rate of alcohol consumption among women of childbearing age increases the risk of exposure during critical stages of limb development, especially among unintended pregnancies (Finer and Zolna, 2014). The complexity of limb development requires a large-scale study with clinically derived LD subtypes and sufficient information about maternal alcohol consumption to allow a complete characterization of alcohol consumption patterns. To this end, data from the National Birth Defects Prevention Study (NBDPS), a large, population-based case-control study, were used to describe maternal reports of alcohol consumption and to examine associations between consumption and specific LD subtypes.

Materials and Methods

SAMPLE SELECTION AND RECRUITMENT

The NBDPS was a multi-site, population-based, case-control study designed to investigate genetic and environmental risk factors for 37 major birth defects. Included in the current analyses were cases with one or more eligible birth defects and unaffected live born controls with estimated dates of delivery (EDD) from October 1, 1997 through December 31, 2007. Initial NBDPS sites were birth defect surveillance programs in seven states (Arkansas [AR], California [CA], Iowa [IA], Massachusetts [MA], New Jersey [NJ], New York [NY], and Texas [TX]), and the Centers for Disease Control and Prevention (CDC) in Georgia. In 2003, surveillance systems in two additional states (North Carolina [NC] and Utah [UT]) were included in the NBDPS, and data collection ceased in NJ. All participating sites ascertained live births diagnosed with LDs, and all but NJ ascertained fetal deaths (AR, CA, CDC, IA, MA, NC, NY 2000–2007, TX, and UT) or elective terminations (AR, CA, CDC, IA, NC, NY 2000–2007, TX, and UT). Controls were identified from the same catchment areas as cases and randomly selected from either hospital delivery logs (AR 1997–2000, CA, CDC 1997–2001, NY, and TX) or birth certificate files (AR 2000–2007, CDC 2001–2007, IA, MA, NC, NJ, and UT). Excluded were cases with defects of known or strongly suspected genetic etiology (i.e., single gene disorders, chromosome abnormalities), as well as cases and controls not in the custody of or not residing with their birth mothers, or whose birth mother did not speak English or Spanish. Each site obtained institutional review board approval for the NBDPS.

CASE CLASSIFICATION

Clinical information abstracted from medical records was reviewed by a clinical geneticist at each NBDPS site, and standard definitions were used to determine case classification. Clinical information abstracted included method of diagnosis (e.g., available x-ray confirmation of absent, partially absent or "missing" bony elements of the extremities); laboratory results, including genetics and other specialty evaluations when available; relevant exposures; and family history of LDs. A NBDPS-specific modification of the CDC six-digit diagnostic coding system was assigned to each case meeting definitional criteria. The development of the NBDPS diagnostic codes and their relation to the International Statistical Classification of Diseases and Related Health Problems (ICD-9), the clinical modification of the ICD-9 (ICD9-CM), and British Paediatric Association (BPA) coding schemes can be found elsewhere (Rasmussen and Moore, 2001). The NBDPS diagnostic codes were developed due to a lack of specificity of existing codes for certain LD subtypes (e.g., split hand or foot codes). Additional information about case classification is detailed else-where (Rasmussen et al., 2003).

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 LD cases as isolated (no additional major, unrelated defects), multiple (one or more additional major, unrelated defects), or complex sequence (e.g., limb-body wall complex, amniotic bands). LD cases were classified into the following subtypes: longitudinal (preaxial, postaxial, and split hand/foot), terminal transverse (amelia excluded), amelia, intercalary, and not elsewhere classified. LD cases were also classified in terms of laterality and sidedness of the deficiency (unilateral-left, unilateral-right, bilateral, 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.

DATA COLLECTION

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. The median time between EDD and interview date was 9.0 months for case mothers and 7.6 months for control mothers. Following the mailing of an introductory packet of materials, a structured protocol was followed for recruitment of case and control mothers (Yoon et al., 2001). This protocol consisted of a series of follow-up telephone calls, or reminder letters if contact was not made by telephone, to obtain informed consent for the NBDPS interview. Overall, 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 who completed the interview were included in this analysis.

The interview included, but was not limited to, detailed questions about health problems, single and multiple vitamin intake, medication use, alcohol consumption, caffeine intake, and maternal exposure to cigarette smoke from 3 months before conception through the end of the pregnancy. For each exposure, the mother was asked for dates of occurrence and, where applicable, the frequency with which the exposure occurred. From these questions, maternal periconceptional exposure was determined for the following covariables: folic acid

and vitamin A intake from either a single vitamin or multivitamin; total caffeine exposure (mg); vasoactive medications, which included antihypertensives, bronchodilators, decongestants, migraine medications, and nonsteroidal anti-inflammatory drugs; and any exposure to active or passive cigarette smoke.

EXPOSURE ASSESSMENT

Retrospective reports for alcohol consumption were collected for each of the 3 months before conception (labeled B3, B2, and B1), each of the first 3 months of pregnancy (labeled M1, M2, and M3) and by trimester for months 4 to 6 and 7 to 9 of pregnancy (labeled T2 and T3, respectively). Periconceptional exposure was defined as 1 month prior to conception (B1) through the first 3 months of pregnancy (M1–M3). For each month alcohol was reportedly consumed, the mother was asked how many days, on average, she drank alcohol and on those days, on average, how many drinks she consumed per day. The mother was also asked about the greatest number of drinks that she consumed on one occasion during the month(s) she drank and what types of alcohol she usually consumed (beer, wine, mixed drink or shot liquor, or other type of alcohol). Responses were coded into: any (yes or no) alcohol consumption during the periconceptional period; the number of months any alcohol was consumed during the periconceptional period (0–4 months); the pattern of periconceptional consumption (no drinking, B1 only, B1 and any month of the first trimester [M1–M3], only during M1–M3); the average number of drinks consumed during the periconceptional period (none, 1–4 drinks/month, 5–15 drinks/month, 16–30 drinks/month, >30 drinks/month); binge drinking during the periconceptional period (no drinking, drinking without binging [<4 drinks/occasion], binge drinking [≥4 drinks/occasion]); and the type of alcohol consumed during the periconceptional period (no drinking, beer only, wine only, distilled spirits only, a combination of one or more types).

STATISTICAL ANALYSIS

All analyses were conducted using the Statistical Analysis System (SAS) version 9.2 statistical software (SAS Institute, Cary, NC). Descriptive analyses used the Chi-square test to compare cases and controls on the following covariables: case and control sex (male, female), birth weight (<2500, ≥2500 grams), gestational age (<37, 37–45 weeks), and family history of LD (yes, no); maternal age at EDD (<20, 20–34, ≥35 years), race/ethnicity (non-Hispanic white, non-Hispanic black, Other, Hispanic), education (<12, 12, 13–15, ≥16 years), parity (never pregnant, primipara, multipara), prepregnancy diabetes (yes, no), and prepregnancy body mass index (<18.5, 18.5–24.9, 25.0–29.9, ≥30); plurality (multiple, singleton) and planned pregnancy (yes, no); maternal chorionic villus sampling (yes, no), periconceptional exposure to contraceptive pill use (yes, no), folic acid supplementation (yes, no), vitamin A supplementation (any use; no use), vasoactive medication use (yes, no), any cigarette smoking exposure (yes, no) and milligrams of caffeine consumed (0–9 mg, 10–99 mg, 100–199 mg, 200–299 mg, ≥ 300 mg); season of conception (summer, fall, winter, spring); and NBDPS site (AR, CA, GA, IA, NC, NJ, NY, TX, UT, CDC). Excluded from analyses were mothers of cases and controls with an EDD of 2008, incomplete interviews, mothers with missing alcohol consumption for any month during preconception through the EDD (B3-T3), or mothers who reported more than 150 drinks per month (n = 10 cases and n = 77 controls).

Crude odds ratios (cORs), adjusted odd ratios (aORs), and corresponding 95% confidence intervals (CIs) were calculated to estimate associations between maternal periconceptional alcohol consumption and LDs. For adjusted analyses, 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 alcohol consumption changed by 10% or more after adding the covariable. Adjusted analyses are only presented for LD subtypes containing at least 100 cases (e.g., all LDs combined, preaxial and terminal transverse subtypes, and upper and lower affected limbs). Additionally, the significance of multiplicative and additive interactions (i.e., the relative excess risk due to interaction [RERI]) between any periconceptional alcohol consumption and folic acid supplementation was also tested. Significance of multiplicative interaction estimates was determined using p-values, and significance of RERI was determined using bootstrap 95% CI (Knol et al., 2007). (The RERI and boot-strap 95% CI were calculated using a computer program created by Sandra Richardson, RN, MS [personal communication, New York State Department of Health, 2011]).

Results

Overall, the most common LD subtype was terminal transverse; intercalary and amelia LDs were least frequent (Table 1). Approximately half (46.2%) of all cases were affected on the left-side, followed by right-sided and bilateral presentation. Most (69.6%) of the affected limbs were arms only. The presence of other major congenital defects differed by LD subtype; preaxial-longitudinal and amelia subtypes were more likely than other LD subtypes to have multiple defects. All other subtypes were mostly comprised of isolated defects.

Cases were more likely than controls to be male, low birth weight, preterm, and have a family history of LDs (Table 2). Case mothers were more frequently Hispanic, had a diagnosis of prepregnancy type I or type II diabetes, or had fewer years of education. Case pregnancies were more often the mother’s first, a multiple pregnancy, or unplanned. Maternal periconceptional exposures to no vitamin A supplementation, vasoactive medication, and caffeine consumption were more common among case mothers compared with controls. 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, prepregnancy body mass index, chorionic villus sampling, maternal periconceptional exposure to contraceptive pills, folic acid supplementation, or any cigarette smoking exposure. Overall, case mothers reported periconceptional alcohol consumption less frequently, reported lower amounts of alcohol consumed per month or occasion, and reported different types of alcohol consumed than control mothers. To evaluate possible response bias, the number of months between EDD and time of interview were compared for patterns of alcohol consumption for case and control mothers. No differences were found ($p > 0.05$), suggesting no response bias due to time between the infant’s birth and the interview (data not shown).

Adjusted odds ratios showed case and control mothers differed on several indicators of periconceptional alcohol consumption (Table 3). Similar findings were observed for any and preaxial LDs, and for upper affected limbs across patterns of alcohol consumption. For each

of these LDs, statistically significant inverse associations were found for any periconceptional alcohol consumption, lower average amounts of alcohol consumed (1–4 drinks/month), and drinking without binging (<4 drinks/occasion). Inverse associations were also found for preaxial LDs and higher amounts (5–15 drinks/month) of alcohol consumed, with control mothers reporting greater alcohol consumption than case mothers (Table 3). Finally, inverse associations were found for terminal transverse LDs and affected lower limbs; however, only the aORs for drinking without binging and any alcohol consumption, respectively, were statistically significant.

Although inverse associations were found between LDs and all types of alcohol consumed, the patterns of statistical significance differed by LD subtype and anatomic group (Table 3). Consumption of wine only was associated with all LDs combined, preaxial LDs, and lower affected limbs. An association was also found for consumption of distilled spirits only and preaxial LDs; however, the cell size was small, and the estimate may be unreliable. Finally, consumption of other combinations of alcohol was associated with all LDs combined and upper affected limbs. Terminal transverse and upper limbs did not show significant aORs, although other combination was marginally associated with upper limbs.

In analyses stratified by type of alcohol consumed, inverse associations were found for most patterns of alcohol consumption and most LDs among mothers who reported drinking beer only (Table 4); however, increased aORs, although nonsignificant, were found between 1–4 drinks/month of beer only, binge drinking of wine only, and binge drinking of distilled spirits only and terminal transverse LDs, and between binge drinking of wine only and upper affected limbs. Among mothers who reported drinking wine only, inverse associations were found for most patterns of consumption and LDs, with significant aORs for any consumption of wine only and an average of 1–4 drinks/month (Table 4). An inverse association was found for drinking without binging of wine only and all LDs combined; increased, albeit nonsignificant, associations were found for binge drinking of wine and terminal transverse LDs and upper affected limbs. For consumption of distilled spirits only, increased, but nonsignificant, aORs were found for associations between heavier drinking (e.g., 5–15 drinks/month) and terminal transverse LDs and upper or lower affected limbs. Of the remaining inverse associations, only the aOR between any consumption of distilled spirits only and preaxial LDs was significant, although the sample size was small making the estimate unreliable. Finally, inverse associations were found for consumption of other combinations (i.e. beer + wine, wine-+ distilled spirits, beer + distilled spirits, or beer + wine + distilled spirits) and all LDs combined or upper affected limbs but only when mothers reported drinking 1–4 drinks/month or drinking with binging. Stratification of alcohol consumption by folic acid supplementation failed to show significant additive or multiplicative interactions (data not shown).

Discussion

The current study findings did not show teratogenic associations between maternal retrospective reports of peri-conceptional alcohol consumption and LDs. In contrast, inverse associations were found, with reported consumption of alcohol during the periconceptional period less likely among case mothers compared with control mothers for selected LD

subtypes and anatomic groups. Furthermore, when stratified by type of alcohol consumed, the statistically significant inverse associations found for any of the LDs studied were limited to reports of drinking fewer than 4 drinks on any occasion and consumption of wine only or combinations of alcohol types.

Based on case reports of FAS, alcohol was expected to act as a teratogen on limb development due to several plausible biological mechanisms including interference with folate metabolism (e.g., inadequate dietary intake, increased folate clearance, and malabsorption) (El Banna et al., 1983; McMartin, 1984; McMartin et al., 1985; Eisenga et al., 1989; Halsted et al., 2002; Manari et al., 2003; Mason and Choi, 2005; Chiuve et al., 2005; Hamid and Kaur, 2007; Romanoff et al., 2007; Hamid et al., 2009), inhibition of methionine synthase activity resulting in hyperhomocysteinemia (Halsted et al., 2002; Mason and Choi, 2005), and disruption of retinoic acid homeostasis (Limpach et al., 2000) or cholesterol biosynthesis (Lanoue et al., 1997; Gofflot et al., 2003; Li et al., 2007). These mechanisms are predicated on chronic and excessive alcohol consumption, such as that observed in FAS (Hillman and Steinberg, 1982; Chiuve et al., 2005; Napoli, 2011). Of those mothers who reported periconceptional alcohol consumption, approximately half consumed, on average, 1–4 drinks per month; thus, the alcohol consumption levels reported by mothers in this study may have fallen below the teratogenic threshold. Recent studies have found little effect of light drinking on adverse birth outcomes (Henderson et al., 2007; O'Leary et al., 2010; Patra et al., 2011; Pfinder et al., 2013), and a previous study of cranio-synostosis using NBDPS data showed a similar inverse relationship with light alcohol consumption (Richardson et al., 2011). The patterns of light drinking found in the current data are consistent with these recent findings; however, the true threshold of when alcohol becomes a teratogen is unknown (Henderson et al., 2007). Therefore, public health policy recommendations for abstinence during pregnancy should not be ignored.

There are limitations to this study. Small numbers for specific LD subtypes (e.g., postaxial, split hand or foot, intercalary and amelia) precluded reliable analysis of associations with maternal periconceptional alcohol consumption. Preliminary examination of the additional rare LD subtypes showed elevated crude ORs for these deficiencies (i.e., intercalary and amelia) (data not shown). Odds of consuming more than 30 drinks per month or multiple episodes of binge drinking (≥4 drinks/occasion) were nearly 2 times higher among mothers of pregnancies affected by these subtypes; however, the estimates were unstable due to small numbers (<5 exposed). These findings reinforce the current public health policy of promoting avoidance of any alcohol consumption during pregnancy due to unknown thresholds for safe drinking. 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 due to social desirability. Mothers of pregnancies affected by an LD may have been more likely to underreport periconceptional alcohol consumption due to the well-known implications of heavy drinking during pregnancy on pregnancy outcomes. The social desirability may have been less influential on responses by control mothers due to the absence of health problems in their child. Although response bias is a possible contributor to the unexpected inverse associations observed for alcohol consumption and LDs, the percentage of mothers of case and control pregnancies who reported any alcohol consumption from 3 months before pregnancy through the end of pregnancy was similar to

national averages reported for drinking in the past 30 days by nonpregnant women of childbearing age (data not shown) (Centers for Disease Control and Prevention, 2012). Similarly, frequency of reported binge drinking (≥4 drinks/occasion) by mothers in the current study was similar to the rate reported by women from the national study mentioned above. An additional limitation of this analysis was that the occurrences of some important covariables reported in previous studies of LDs (e.g., chorionic villus sampling, migraines) were too rare to analyze. Finally, the analyses were stratified by LD subtype or anatomic group and indicators of maternal reports of alcohol consumption, which resulted in few independent statistical tests. Given the controversy surrounding adjusting multiple comparisons in epidemiological research (Rothman, 1990; Greenland, 2008), p-values were not adjusted. Significant associations identified could be due to chance, and independent replication is warranted.

Strengths of this analysis included analysis of multiple LD subtypes and anatomic groups, detailed assessment of periconceptional alcohol consumption, and control of relevant covariables. The use of a well-characterized LD classification system allowed greater specification of underlying structural involvement in each phenotype. With regard to alcohol consumption, information was collected about frequency and duration of exposure, as well as types of alcohol consumed. This information allowed creation of patterns of alcohol consumption similar to those reported in animal studies and provided a more comprehensive understanding of the degree of exposure among pregnancies affected by LDs. Another strength was the extensive set of covariables evaluated, which allowed examination of periconceptional alcohol consumption after controlling for other potential causative factors involved in LDs (e.g., vasoconstriction).

In the NBDPS data, maternal reported consumption of alcohol during the periconceptional period did not emerge as a teratogen for the development of limbs or related structures. In fact, case mothers were less likely to report alcohol consumption compared with control mothers. These inverse associations must be interpreted with caution, given that preliminary analyses of rarer LD subtypes supported the teratogenic effects of alcohol on embryonic development. Additional studies are needed to replicate the findings using larger samples, which would allow inclusion of additional rare LDs and a broader spectrum of problem drinking.

Acknowledgments

Coding of drug information in NBDPS used the Slone Drug Dictionary, under license from the Slone Epidemiology Center at Boston University, Boston, MA. We would also like to acknowledge Drs. Charlotte Hobbs, Gary Shaw, Marlene Anderka, Charlotte Druschel, Andrew Olshan, Robert Meyer, Mark Canfield, Peter Langlois, and Marcia Feldkamp. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention. There are no stated conflicts of interest.

This work was supported by cooperative agreements from the Centers for Disease Control and Prevention to the Iowa Center for Birth Defects Research and Prevention participating in the National Birth Defects Prevention Study (U01/DD000492) and the Birth Defects Study To Evaluate Pregnancy exposureS (U01/DD001035).

References

Aro T. Maternal diseases, alcohol consumption and smoking during pregnancy associated with reduction limb defects. Early Hum Dev. 1983; 9:49–57. [PubMed: 6667650]

Aro T, Haapakoski J, Heinonen OP. A multivariate analysis of the risk indicators of reduction limb defects. Int J Epidemiol. 1984; 13:459–464. [PubMed: 6519885]

Barham G, Clarke NM. Genetic regulation of embryological limb development with relation to congenital limb deformity in humans. J Child Orthop. 2008; 2:1–9. [PubMed: 19308596]

Baumann P, Schild C, Hume RF, Sokol RJ. Alcohol abuse–a persistent preventable risk for congenital anomalies. Int J Gynaecol Obstet. 2006; 95:66–72. [PubMed: 16926014]

Castilla EE, Cavalcanti DP, Dutra MG, et al. Limb reduction defects in South America. Br J Obstet Gynaecol. 1995; 102:393–400. [PubMed: 7612534]

Centers for Disease Control and Prevention. Alcohol use and binge drinking among women of childbearing age — United States, 2006–2010. MMWR Morb Mortal Wkly Rep. 2012; 61:534–538. [PubMed: 22810267]

Chiuve SE, Giovannucci EL, Hankinson SE, et al. Alcohol intake and methylenetetrahydrofolate reductase polymorphism modify the relation of folate intake to plasma homocysteine. Am J Clin Nutr. 2005; 82:155–162. [PubMed: 16002814]

Eisenga BH, Collins TD, McMartin KE. Differential effects of acute ethanol on urinary excretion of folate derivatives in the rat. J Pharmacol Exp Ther. 1989; 248:916–922. [PubMed: 2703978]

El Banna N, Picciano MF, Simon J. Effects of chronic alcohol consumption and iron deficiency on maternal folate status and reproductive outcome in mice. J Nutr. 1983; 113:2059–2070. [PubMed: 6684677]

Ephraim PL, Dillingham TR, Sector M, et al. Epidemiology of limb loss and congenital limb deficiency: a review of the literature. Arch Phys Med Rehabil. 2003; 84:747–761. [PubMed: 12736892]

Finer LB, Zolna MR. Shifts in intended and unintended pregnancies in the United States, 2001–2008. Am J Public Health. 2014; 104(Suppl 1):S43–S48. [PubMed: 24354819]

Froster-Iskenius UG, Baird PA. Limb reduction defects in over one million consecutive livebirths. Teratology,. 1989; 39:127–135. [PubMed: 2784595]

Froster UG, Baird PA. Congenital defects of the limbs and alcohol exposure in pregnancy: data from a population based study. Am J Med Genet. 1992; 44:782–785. [PubMed: 1481846]

Froster UG, Baird PA. Maternal factors, medications, and drug exposure in congenital limb reduction defects. Environ Health Perspect. 1993; 101(Suppl 3):269–274. [PubMed: 8143629]

Gofflot F, Hars C, Illien F, et al. Molecular mechanisms underlying limb anomalies associated with cholesterol deficiency during gestation: implications of Hedgehog signaling. Hum Mol Genet. 2003; 12:1187–1198. [PubMed: 12719383]

Gold NB, Westgate MN, Holmes LB. Anatomic and etiological classification of congenital limb deficiencies. Am J Med Genet A. 2011; 155A:1225–1235. [PubMed: 21557466]

Greenland S. Multiple comparisons and association selection in general epidemiology. Int J Epidemiol. 2008; 37:430–434. [PubMed: 18453632]

Halsted CH, Villanueva JA, Devlin AM, Chandler CJ. Metabolic interactions of alcohol and folate. J Nutr. 2002; 132(Suppl):2367S–2372S. [PubMed: 12163694]

Hamid A, Kaur J. Long-term alcohol ingestion alters the folate-binding kinetics in intestinal brush border membrane in experimental alcoholism. Alcohol. 2007; 41:441–446. [PubMed: 17936512]

Hamid A, Wani NA, Kaur J. New perspectives on folate transport in relation to alcoholism-induced folate malabsorption–association with epigenome stability and cancer development. FEBS J. 2009; 276:2175–2191. [PubMed: 19292860]

Henderson J, Gray R, Brocklehurst P. Systematic review of effects of low-moderate prenatal alcohol exposure on pregnancy outcome. BJOG. 2007; 114:243–252. [PubMed: 17233797]

Herrmann J, Pallister PD, Opitz JM. Tetraectrodactyly and other skeletal manifestations in the fetal alcohol syndrome. Eur J Pediatr. 1980; 133:221–226. [PubMed: 7389734]

Hillman RS, Steinberg SE. The effects of alcohol on folate metabolism. Annu Rev Med. 1982; 33:345–354. [PubMed: 6805415]

Holmes LB. Teratogen-induced limb defects. Am J Med Genet. 2002; 112:297–303. [PubMed: 12357474]

Idrus NM, Thomas JD. Fetal alcohol spectrum disorders: experimental treatments and strategies for intervention. Alcohol Res Health. 2011; 34:76–85. [PubMed: 23580044]

Johnson CS, Zucker RM, Hunter ES III, Sulik KK. Perturbation of retinoic acid (RA)-mediated limb development suggests a role for diminished RA signaling in the teratogenesis of ethanol. Birth Defects Res A Clin Mol Teratol. 2007; 79:631–641. [PubMed: 17676605]

Kallen B, Rahmani TM, Winberg J. Infants with congenital limb reduction registered in the Swedish Register of Congenital Malformations. Teratology. 1984; 29:73–85. [PubMed: 6701808]

Knol MJ, van der Tweel I, Grobbee DE, et al. Estimating interaction on an additive scale between continuous determinants in a logistic regression model. Int J Epidemiol. 2007; 36:1111–1118. [PubMed: 17726040]

Lanoue L, Dehart DB, Hinsdale ME, et al. Limb, genital, CNS, and facial malformations result from gene/environment-induced cholesterol deficiency: further evidence for a link to sonic hedgehog. Am J Med Genet. 1997; 73:24–31. [PubMed: 9375918]

Li YX, Yang HT, Zdanowicz M, et al. Fetal alcohol exposure impairs Hedgehog cholesterol modification and signaling. Lab Invest. 2007; 87:231–240. [PubMed: 17237799]

Limpach A, Dalton M, Miles R, Gadson P. Homocysteine inhibits retinoic acid synthesis: a mechanism for homocysteine-induced congenital defects. Exp Cell Res. 2000; 260:166–174. [PubMed: 11010821]

Lin S, Marshall EG, Davidson GK, et al. Evaluation of congenital limb reduction defects in upstate New York. Teratology,. 1993; 47:127–135. [PubMed: 8446926]

Lin YS, Chang FM, Liu CH. Fetal alcohol syndrome: report of a case. J Formos Med Assoc. 1991; 90:411–414. [PubMed: 1680974]

Makhoul IR, Goldstein I, Smolkin T, et al. Congenital limb deficiencies in newborn infants: prevalence, characteristics and prenatal diagnosis. Prenat Diagn. 2003; 23:198–200. [PubMed: 12627419]

Manari AP, Preedy VR, Peters TJ. Nutritional intake of hazardous drinkers and dependent alcoholics in the UK. Addict Biol. 2003; 8:201–210. [PubMed: 12850779]

Martinez-Frias ML, Bermejo E, Rodriguez-Pinilla E, Frias JL. Risk for congenital anomalies associated with different sporadic and daily doses of alcohol consumption during pregnancy: a case-control study. Birth Defects Res A Clin Mol Teratol. 2004; 70:194–200. [PubMed: 15108246]

Mason JB, Choi SW. Effects of alcohol on folate metabolism: implications for carcinogenesis. Alcohol. 2005; 35:235–241. [PubMed: 16054985]

McDonald AD, Armstrong BG, Sloan M. Cigarette, alcohol, and coffee consumption and congenital defects. Am J Public Health. 1992; 82:91–93. [PubMed: 1536342]

McMartin KE. Increased urinary folate excretion and decreased plasma folate levels in the rat after acute ethanol treatment. Alcohol Clin Exp Res. 1984; 8:172–178. [PubMed: 6375428]

McMartin KE, Shiao CQ, Collins TD, Redetzki HM. Acute ethanol ingestion by humans and subacute treatment of rats increase urinary folate excretion. Alcohol. 1985; 2:473–477. [PubMed: 4026968]

Napoli JL. Effects of ethanol on physiological retinoic acid levels. IUBMB Life. 2011; 63:701–706. [PubMed: 21766417]

O'Leary CM, Nassar N, Kurinczuk JJ, et al. Prenatal alcohol exposure and risk of birth defects. Pediatrics. 2010; 126:e843–850. [PubMed: 20876169]

Patra J, Bakker R, Irving H, et al. Dose-response relationship between alcohol consumption before and during pregnancy and the risks of low birthweight, preterm birth and small for gestational age (SGA)-a systematic review and meta-analyses. BJOG. 2011; 118:1411–1421. [PubMed: 21729235]

Pauli RM, Feldman PF. Major limb malformations following intrauterine exposure to ethanol: two additional cases and literature review. Teratology. 1986; 33:273–280. [PubMed: 3526621]

Pfinder M, Kunst AE, Feldmann R, et al. Preterm birth and small for gestational age in relation to alcohol consumption during pregnancy: stronger associations among vulnerable women? Results from two large Western-European studies. BMC Pregnancy Childbirth. 2013; 13:49. [PubMed: 23433310]

Rasmussen SA, Moore CA. Effective coding in birth defects surveillance. Teratology. 2001; 64(Suppl 1):S3–S7. [PubMed: 11745837]

Rasmussen SA, Olney RS, Holmes LB, et al. Guidelines for case classification for the National Birth Defects Prevention Study. Birth Defects Res Part A Clin Mol Teratol. 2003; 67:193–201. [PubMed: 12797461]

Richardson S, Browne ML, Rasmussen SA, et al. Associations between periconceptional alcohol consumption and cranio-synostosis, omphalocele, and gastroschisis. Birth Defects Res Part A Clin Mol Teratol. 2011; 91:623–630. [PubMed: 21630421]

Romanoff RL, Ross DM, McMartin KE. Acute ethanol exposure inhibits renal folate transport, but repeated exposure upregulates folate transport proteins in rats and human cells. J Nutr. 2007; 137:1260–1265. [PubMed: 17449590]

Rothman KJ. No adjustments are needed for multiple comparisons. Epidemiology. 1990; 1:43–46. [PubMed: 2081237]

Shaw GM, Nelson V, Carmichael SL, et al. Maternal periconceptional vitamins: interactions with selected factors and congenital anomalies? Epidemiology. 2002; 13:625–630. [PubMed: 12410002]

Spiegel PG, Pekman WM, Rich BH, et al. The orthopedic aspects of the fetal alcohol syndrome. Clin Orthop Relat Res. 1979:58–63. Mar-Apr. [PubMed: 455851]

van Mil NH, Oosterbaan AM, Steegers-Theunissen RP. Teratogenicity and underlying mechanisms of homocysteine in animal models: a review. Reprod Toxicol. 2010; 30:520–531. [PubMed: 20656016]

van Rensburg LJ. Major skeletal defects in the fetal alcohol syndrome. A case report. S Afr Med J. 1981; 59:687–688. [PubMed: 7194516]

Yoon PW, Rasmussen SA, Lynberg MC, et al. The National Birth Defects Prevention Study. Public Health Rep. 2001; 116(Suppl 1):32–40. [PubMed: 11889273]

TABLE 1

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

	All LDs Combined		Isolated LDs		Multiple LDs	
Characteristic	***n***	**%**[b]	***n***	**%**[c]	***n***	**%**[c]
All LDs combined[d]	896		649	72	247	28
Preaxial-longitudinal	208	23	78	38	130	63
Postaxial-longitudinal	71	8	54	76	17	24
Split hand or foot-longitudinal	65	7	49	75	16	25
Amelia	18	2	7	39	11	61
Terminal transverse	500	58	426	85	74	15
Intercalary	48	5	36	75	12	25
Laterality[d]						
Left	414	46	329	79	85	20
Right	281	31	205	73	76	27
Bilateral[e]	184	21	103	56	81	44
Unknown/unilateral, side unknown	17	2	12	71	5	29
LD anatomic groups						
Upper	624	70	451	72	173	28
Lower	215	24	159	74	56	26
Both	57	6	39	68	18	32

LD, limb deficiency.

[a] Excluded if incomplete interview, missing alcohol consumption for any month between 3 month before conception through the end of pregnancy, reported average consumption over 150 drinks per month, or estimated year of birth was 2008. Due to rounding, percentages may not total 100.

[b] Percent of all LDs combined (*n*=896).

[c] Percent within LD characteristic for isolated or multiple defect frequencies.

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

[e] Bilateral may include more than one subtype.

TABLE 2

Selected Characteristics of Limb Deficiency Cases, Controls, and Birth Mothers, National Birth Defects Prevention Study (1997–2007)[a]

	All LDs Combined		Controls	
Characteristic	***n***	**%**	***n***	**%**
Totals	896		8275	
Case and control characteristics				
Sex[d]				
Female	381	43	4073	49
Male	507	57	4194	51
Birth weight (grams)[d]				
<2,500	228	26	456	6
≥2,500	662	74	7785	94
Gestational age (weeks)[d]				
Term (37–45)	662	74	7500	91
Preterm (<37)	229	26	774	9
Family history of LD[d]				
Yes	7	0.8	11	0.1
No	889	99	8264	99
Maternal characteristics				
Age at delivery (years)				
<20	98	11	849	10
20–34	691	77	6264	76
≥35	107	12	1162	14
Race and ethnicity[c]				
non-Hispanic white	503	56	4900	59
non-Hispanic black	88	10	921	11
Hispanic	255	28	1915	23
Other	50	6	537	6
Education (years)[b]				
<12	162	18	1417	17
12	241	27	1992	24
13–15	253	28	2237	27
≥16	239	27	2621	32
Parity[b]				
Never pregnant	296	33	2413	29
Primlpara	259	29	2436	29
Multipara	341	38	3425	41
Pre-pregnancy type I or II diabetes[d]				

	All LDs Combined		Controls	
Characteristic	***n***	**%**	***n***	**%**
Yes	27	3	50	1
No	869	97	8213	99
Pre-pregnancy body mass Index (kg/m^2)				
Underweight (<18.5)	49	6	428	5
Normal weight (18.5 - 24.9)	440	52	4370	55
Overweight (25.0 – 29.9)	204	24	1808	23
Obese (≥30)	157	18	1330	17
Maternal pregnancy characteristics				
Plurality[d]				
Multiple	57	6	246	3
Singleton	839	94	8029	97
Planned pregnancy[c]				
Yes	501	56	5014	61
No	395	44	3261	39
Maternal periconceptional pregnancy behaviors[e]				
Chorionic villus sampling				
Yes	34	4	228	3
No	850	96	7936	97
Contraceptive pill use				
Yes	74	8	634	8
No	822	92	7641	92
Folic acid supplementation				
Yes	579	65	5260	64
No	317	35	3015	36
Vitamin A supplementation[b]				
Any use	385	43	3904	47
No use	505	57	4340	53
Vasoactive medications[b]				
Yes	335	38	2754	34
No	544	62	5350	66
Any cigarette smoking exposure				
Yes	308	34	2629	32
No	586	66	5631	68
Total caffeine consumption (mg)[b]				
0–10	157	17	1844	22
10–99	315	35	2650	32
100–199	219	24	1874	23
200–299	112	12	1027	12
≥300	93	10	880	11

Characteristic	All LDs Combined		Controls	
	n	%	*n*	%
Season of conception[b]				
Fall	224	25	2058	25
Winter	253	28	2062	25
Spring	226	25	2020	24
Summer	193	21	2135	26
Study site[d]				
Arkansas	92	10	1042	13
California	143	16	1013	12
lowa	88	10	904	11
Massachusetts	109	12	1025	12
New Jersey	83	9	565	7
New York	62	7	715	9
Texas	106	12	961	12
CDC	89	10	876	11
North Carolina	37	4	568	7
Utah	87	10	606	7
Maternal periconceptional alcolnol consumption[e]				
Any consumption[c]				
No	610	68	5239	63
Yes	286	32	3036	37
Average amount consumed (drinks/month)				
None	610	68	5239	63
1–4	124	14	1390	17
5–15	90	10	958	12
16–30	44	5	427	5
>30	22	2	227	3
Any binge episodesc				
No drinking	610	68	5239	63
Drinking without binging (<4 drinks/ occasion)	174	19	2019	24
Binge drinking (≥4 drinks/occasion)	107	12	990	12
Type of alcohoi consumed[b]				
No drinking	610	68	5239	63
Beer only	68	8	618	7
Wine only	68	8	850	10
Distilled spirits only	54	6	523	6
Other combination	96	11	1037	12

LD, limb deficiency.

[a] Excluded if incomplete interview, missing alcohol consumption for any month between 3 months before conception through the end of pregnancy, reported average consumption over 150 drinks per month, or estimated year of delivery was 2008. Numbers within characteristic categories vary because of incomplete or missing data. Due to rounding, percentages may not total 100.

[b] $p<0.05$.

[c] $p<0.01$.

[d] $p<0.001$.

[e] Periconceptionai = 1 month before through 3 months after conception.

TABLE 3

Adjusted Odds Ratio Estimates for Maternal Reports of Periconceptional Alcohol Consumption and Limb Deficiencies, National Birth Defects Prevention Study (1997–2007)[a]

Periconceptional Alcohol Consumption[b]	Controls	All LDs Combined		LD Subtypes				LD Groups			
				Preaxial		Terminal Transverse		Upper Limbs		Lower Limbs	
	n	*n*	aOR (95% CI)	*n*	aOR (95% CI)	*n*	aOR (95% CI)	*n*	aOR (95% CI)	*n*	aOR (95% CI)
Any consumption											
No	5239	610	Reference	154	Reference	332	Reference	468	Reference	184	Reference
Yes	3036	286	**0.76 (0.64–0.89)**	54	**0.56 (0.40–0.79)**	168	0.83 (0.67–1.02)	213	**0.75 (0.62–0.90)**	88	**0.73 (0.55–0.96)**
Average amount consumed (drinks/month)											
1–4	1390	124	**0.74 (0.60–0.92)**	24	**0.57 (0.36–0.90)**	74	0.81 (0.62–1.07)	91	**0.71 (0.55–0.91)**	40	0.79 (0.55–1.13)
5–15	958	90	0.78(0.61–1.00)	16	**0.55 (0.32–0.94)**	55	0.88 (0.64–1.20)	69	0.81 (0.62–1.07)	26	0.69 (0.44–1.07)
16–30	427	44	0.76 (0.53–1.07)	8	0.52 (0.24–1.13)	23	0.75 (0.47–1.20)	34	0.78 (0.53–1.15)	12	0.60 (0.31–1.16)
>30	227	22	0.70 (0.43–1.12)	6	0.75 (0.32–1.74)	11	0.72 (0.38–1.34)	15	0.66 (0.38–1.16)	8	0.74 (0.34–1.62)
Any binge episodes											
Drinking without binging (<4 drinks/occasion)	2019	174	**0.71 (0.58–0.85)**	22	**0.53 (0.35–0.80)**	100	**0.75 (0.59–0.96)**	128	**0.67 (0.54–0.83)**	31	0.75 (0.54–1.04)
Binge drinking (≥4 drinks/occasion)	990	107	0.84 (0.66–1.06)	32	0.64 (0.39–1.03)	63	0.94 (0.70–1.26)	82	0.89 (0.69–1.16)	55	0.68 (0.44–1.04)
Type of alcohol consumed											
Beer only	618	68	0.85 (0.65–1.14)	13	0.63 (0.35–1.16)	42	0.97 (0.68–1.39)	47	0.80 (0.58–1.12)	25	0.96 (0.61–1.52)
Wine only	850	68	**0.65 (0.49–0.86)**	10	**0.41 (0.20–0.82)**	40	0.70 (0.49–1.01)	58	0.74 (0.54–1.01)	14	**0.38 (0.20–0.70)**
Distilled spirits only	523	54	0.82 (0.61–1.11)	7	**0.40 (0.19–0.87)**	32	0.92 (0.63–1.36)	35	0.71 (0.49–1.02)	21	0.99 (0.62–1.61)
Other combination	1037	96	**0.75 (0.59–0.95)**	24	0.73 (0.45–1.16)	54	0.80 (0.59–1.10)	73	0.76 (0.57–0.99)	28	0.70 (0.46–1.07)

aOR, adjusted odds ratio; CI, confidence interval; LD, limb deficiency.

[a] Excluded if incomplete interview, missing alcohol consumption for any month from 3 months before conception through the end of the pregnancy, reported average consumption over 150 drinks per month,-or estimated year of delivery was 2008. Covariables: infant sex; maternal pre-pregnancy body mass index and education; chorionic villus sampling; periconceptional vasoactive medication use; and any cigarette smoke exposure. Numbers within characteristic categories vary because of incomplete or missing data. Due to rounding, percentages may not total 100.

[b] Periconceptional = 1 month before conception through 3 months after conception.

TABLE 4

Adjusted Odds Ratio Estimates for Maternal Reports of Periconceptional Alcohol Consumption and Limb Deficiencies Stratified by Alcohol Type, National Birth Defects Prevention (1997–2007)[a]

Periconceptional Alcohol Consumption[b]	Controls	All LDs Combined		LD Subtypes: Preaxlal		LD Subtypes: Terminal Transverse		LD Groups: Upper Limbs		LD Groups: Lower Limbs	
	n	*n*	aOR (95% CI)	*n*	aOR (95% CI)	*n*	aOR (95% CI)	*n*	aOR (95% CI)	*n*	aOR (95% CI)
Beer only											
Any consumption											
No	5239	610	Reference	154	Reference	332	Reference	468	Reference	184	Reference
Yes	618	68	0.87 (0.65–1.15)	13	0.66 (0.36–1.21)	42	0.97 (0.68–1.39)	47	0.82 (0.59–1.14)	25	0.96 (0.60–1.52)
Average amount consumed (drinks/month)											
1–4	265	30	0.90 (0.60–1.37)	4	nc	22	1.22 (0.75–1.98)	23	0.92 (0.58–1.47)	9	0.88 (0.43–1.81)
5–15	195	21	0.92 (0.57–1.48)	5	0.91 (0.37–2.27)	12	0.92 (0.49–1.72)	14	0.86 (0.49–1.50)	8	0.97 (0.45–2.11)
16–30	95	9	0.74 (0.37–1.49)	2	nc	4	nc	7	0.77 (0.35–1.69)	3	nc
>30	55	5	0.75 (0.30-1.89)	2	nc	2	nc	2	nc	3	nc
Any binge episodes											
Drinking without binging (≥4 drinks/occasion)	349	36	0.82 (0.56–1.20)	8	0.42 (0.15-1.15)	24	1.02 (0.64-1.61)	23	0.67 (0.41-1.07)	13	0.99 (0.54-1.80)
Binge drinking (≥4 drinks/occasion)	261	30	0.93 (0.62–1.39)	5	0.96 (0.46–2.02)	16	0.88 (0.51–1.52)	24	1.04 (0.67–1.61)	10	0.86 (0.43–1.72)
Wine only											
Any consumption											
No	5239	610	Reference	154	Reference	332	Reference	468	Reference	184	Reference
Yes	850	68	**0.65 (0.49–0.87)**	10	**0.43 (0.21–0.86)**	40	0.70 (0.48–1.01)	58	0.75 (0.55–1.02)	14	**0.37 (0.20–0.70)**
Average amount consumed (drinks/month)											
1–4	506	40	**0.66 (0.46–0.94)**	8	0.55 (0.25–1.20)	22	0.67 (0.42–1.07)	34	0.73 (0.49 1.08)	9	**0.46 (0.22–0.96)**
5–15	223	18	0.67 (0.40–1.12)	1	nc	13	0.85 (0.46–1.56)	15	0.80 (0.46–1.38)	3	nc
16–30	97	9	0.66 (0.30–1.43)	1	nc	4	nc	8	0.76 (0.33–1.76)	2	nc
>30	19	0	nc	0	nc	0	nc	0	nc	0	nc
Any binge episodes											
Drinking witlnout binging (<4 drinks/occasion)	750	55	**0.60 (0.44–0.82)**	10	**0.49 (0.24–0.99)**	30	**0.60 (0.39–0.90)**	47	0.67 (0.47–0.94)	12	**0.39 (0.20–0.75)**
Binge drinking (≥4 drinks/occasion)	99	12	0.96 (0.51–1.81)	0	nc	9	1.25 (0.60–2.63)	10	1.18 (0.61–2.30)	2	nc

Periconceptional Alcohol Consumption[b]	Controls	All LDs Combined		LD Subtypes				LD Groups			
				Preaxial		Terminal Transverse		Upper Limbs		Lower Limbs	
	n	*n*	aOR (95% CI)	*n*	aOR (95% CI)	*n*	aOR (95% CI)	*n*	aOR (95% CI)	*n*	aOR (95% CI)
Distilled spirits only											
Any consumption											
No	5239	610	Reference	154	Reference	332	Reference	468	Reference	184	Reference
Yes	523	54	0.83 (0.61–1.13)	7	0.43 (0.20–0.93)	32	0.93 (0.63–1.37)	35	0.72 (0.50–1.05)	21	0.99 (0.61–1.61)
Average amount consumed (drinks/month)											
1–4	297	31	0.87 (0.60–1.29)	6	0.67 (0.29–1.54)	16	0.83 (0.50–1.41)	19	0.71 (0.44–1.14)	12	1.08 (0.59–1.98)
5–15	147	15	0.87 (0.50–1.49)	0	nc	10	1.10 (0.57–2.14)	13	1.00 (0.56–1.79)	4	nc
16–30	43	5	0.54 (0.17–1.77)	0	nc	3	nc	1	nc	4	nc
>30	23	3	nc	1	nc	3	nc	2	nc	1	nc
Any binge episodes											
Drinking without binging (≥4 drinks/occasion)	335	36	0.88 (0.61–1.26)	6	0.60 (0.26–1.37)	18	0.83 (0.51–1.37)	24	0.76 (0.49–1.18)	13	1.04 (0.58–1.85)
Binge drinking (≥4 drinks/occasion)	179	18	0.79 (0.47–1.31)	1	nc	14	1.15 (0.64–2.07)	11	0.68 (0.36–1.28)	8	0.96 (0.44–2.10)
Combination											
Any consumption											
No	5239	610	Reference	154	Reference	332	Reference	468	Reference	184	Reference
Yes	1037	96	**0.76 (0.59–0.96)**	24	0.74 (0.46–1.19)	54	0.81 (0.59–1.11)	73	0.76 (0.57–1.00)	28	0.74 (0.48–1.13)
Average amount consumed (drinks/month)											
1–4	317	23	**0.62 (0.40–0.97)**	6	0.70 (0.30–1.61)	14	0.68 (0.38–1.21)	15	**0.52 (0.30–0.91)**	10	0.92 (0.48–1.78)
5–15	392	36	0.76 (0.52–1.09)	10	0.83 (0.41–1.66)	20	0.78 (0.48–1.27)	27	0.74 (0.48–1.12)	11	0.80 (0.43–1.51)
16–30	192	21	0.89 (0.55–1.43)	5	0.70 (0.25–1.94)	12	0.99 (0.54–1.81)	18	1.01 (0.60–1.68)	3	nc
>30	128	14	0.78 (0.42–1.43)	3	nc	6	0.72 (0.31–1.66)	11	0.87 (0.45–1.69)	4	nc
Any binge episodes											
Drinking without binging (<4 drinks/occasion)	579	47	0.68 (0.49–0.95)	11	0.66 (0.34–1.27)	28	0.74 (0.48–1.12)	34	0.63 (0.43–0.93)	17	0.87 (0.52–1.46)
Binge drinking (≥4 drinks/occasion)	449	47	0.82 (0.59–1.15)	13	0.84 (0.46–1.56)	24	0.84 (0.54–1.30)	37	0.87 (0.60–1.26)	11	0.60 (0.31–1.16)

aOR, adjusted odds ratio; CI, confidence intervals; LD, limb deficiency; nc, not calculated.

[a] Excluded if incomplete interview, missing alcohol consumption for any month from 3 months before conception through the end of the pregnancy, reported average consumption over 150 drinks per month, or estimated year of delivery was 2008. Covariables: infant sex; maternal pre-pregnancy body mass index and education; chorionic villus sampling; periconceptional vasoactive medication use; and any cigarette smoke exposure.

[b] Periconceptional = 1 month before conception through 3 months after conception.