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Twinning and major birth defects, National Birth Defects Prevention Study, 1997–2007

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Abstract

Background—Twinning has been associated with many types of birth defects, although previous studies have had inconsistent findings. Many studies lack information about potential confounders, particularly use of fertility treatment. Our objective was to assess the association between twinning and birth defects in the National Birth Defects Prevention Study (NBDPS).

Methods—We used data from the NBDPS, a population-based, case–control study of major birth defects in the USA, to evaluate associations between twinning and birth defects. The study population included mothers of twin and singleton controls (live-born infants without major birth defects), and cases (fetuses or infants with a major birth defect) born October 1997–December 2007. Adjusted ORs and 95% CIs were estimated using multivariable logistic regression stratified by use of fertility treatment. Twin sex-pairing data and a simulation approach were used to estimate the zygosity of twins.

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Disclaimer 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.

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Results—In the unassisted conception stratum, we observed significant positive associations between twinning and 29 of 45 defect groups. The largest effect estimates were observed for multiple ventricular septal defects and cloacal exstrophy. Among mothers reporting any use of fertility treatments, we observed a significant association with twinning for 5 of 25 defect groups, with the largest effect estimates for hypoplastic left heart syndrome and omphalocele. OR estimates in the estimated monozygotic stratum were generally further from the null than in the dizygotic stratum.

Conclusions—Compared with singletons, a wide range of birth defects are significantly more common among twins. Birth defect risk in twins may be differential by use of fertility treatment.

INTRODUCTION

From 1980 to 2011, the rate of twin births in the USA has increased by 75% from ~18.9 to 33.2 twins per 1000 births.[1] Much of this increase has been attributed to increasing use of fertility treatments, which is associated with higher rates of multiple gestations.[1] In comparison with singletons, twins are at increased risk for a number of adverse outcomes including preterm birth, low birth weight and infant mortality.[2] Monozygotic (MZ) twins are at an increased risk of birth defects compared to both singletons and dizygotic (DZ) twins.[3–6] Significant associations between twinning and birth defects have been observed for a wide range of birth defects in large, registry-based studies.[6–12] However, many of these studies lacked information about important potential confounders or effect modifiers, particularly use of fertility treatment. Associations between fertility treatment use and some major birth defects have been previously observed for singletons but not for twins or higher order multiples; the underlying mechanism for this difference is not well understood.[1314]

We hypothesised that the risk of birth defects in twins compared with singletons varies depending on the specific type of birth defect and use of fertility treatments. Using data from the National Birth Defects Prevention Study (NBDPS), our objective was to assess the association between twinning and selected major birth defects stratified by use of fertility treatment and estimated zygosity.

METHODS

The NBDPS is a population-based, case–control study of major birth defects in the USA. NBDPS is a collaborative effort of the Centers for Birth Defects Research and Prevention in Arkansas, California, Georgia, Iowa, Massachusetts, North Carolina, New Jersey, New York, Texas and Utah. Institutional Review Boards at each site approved the study. Methods for the NBDPS have been described in detail previously.[1516]

Cases of selected birth defects were ascertained through population-based surveillance systems at each site, and included live-born infants (all centres), fetal deaths >20 weeks (all sites except for New Jersey), and elective terminations of pregnancy (all sites except for Massachusetts and New Jersey). Clinical record abstractions for all cases were reviewed at each site by a clinical geneticist. Infants with a known chromosomal abnormality or single-gene condition were excluded. Controls were live-born infants with no major birth defects born in the same catchment areas as cases, and were selected at random from birth hospital

logs or vital records. In the event that both twins had eligible defects, only the first-born twin was included as a study subject. We included data from pregnancies with births on 1 October 1997 through pregnancies with expected dates of delivery on or before 31 December 2007.

After they provided informed consent, mothers of case and control infants were interviewed in English or Spanish, and between 6 weeks and 2 years after the expected date of delivery, on topics including pregnancy history, demographic information and exposures that occurred during pregnancy. Regarding plurality, mothers were asked: 'In this pregnancy, how many babies were you carrying?'. If necessary for clarification, a second question was asked: 'Did you have a single baby, twins, or more babies?'. Secondary sources of plurality information were birth certificates and/or maternal medical records. In the event of discrepancies between the plurality reported in the maternal interview or birth certificate, we elected to defer to the maternal interview response, because this could be due to clerical errors or to death of a cotwin in utero. A sensitivity analysis restricted to mothers without plurality discrepancies was conducted to assess the potential impact of this source of misclassification. Mothers of triplets and higher order multiples or with missing data were excluded from the analysis.

SAS V.9.3 (Cary, North Carolina, USA) was used to conduct all analyses. We used logistic regression to assess the association between twinning and each NBDPS-eligible birth defect for which there were at least five twin cases. The following variables, selected a priori, were included in the multivariable logistic regression models: maternal age at delivery (years, continuous); race/ethnicity (non-Hispanic white, other); smoking from 1 month prior to conception through the third month of pregnancy (any, none); parity (no previous live births, at least one previous live birth); obesity (body mass index <30 kg/m^2, ≥ 30 kg/m^2); educational attainment (up to high school graduate or equivalent, more than high school); use of a folic acid-containing multivitamin from 1 month prior to conception through the first month of pregnancy (any, none); and study site. Mothers with missing data for any of the covariates were excluded from the analysis. Since the aetiology of isolated defects may differ from the aetiology of multiple defects, we also conducted subanalyses in which we excluded cases with more than one unrelated major defect.[15,17]

Preliminary models were examined for statistical interaction between twinning and use of fertility treatment. As this interaction was statistically significant ($p<0.05$) for most of the models, we stratified the results by any use of fertility treatments. Any fertility treatment was defined as use of fertility-enhancing medications (eg, clomiphene citrate) and/or maternal procedures (eg, in vitro fertilisation).

Previous studies have suggested that the magnitude of the association between twinning and birth defects varies by zygosity, with stronger associations observed for MZ twins than for DZ twins.[4,12] However, the NBDPS does not routinely collect information on cotwins, including zygosity or sex. Although not able to directly obtain zygosity information, we were able to obtain information on the sex of many of the cotwins through linkage with birth certificates. Using information relating to mother's name, study infant's name and the study infant's date of birth to link records with the birth certificate of the cotwin, we were able to identify the sex of the cotwin for 176 of 227 control twin pairs (77.5%; 112 like-sex pairs

and 64 unlike-sex pairs) and 875 of 1250 case twin pairs (70.0%; 603 like-sex pairs and 272 unlike-sex pairs). Unlike-sex twin pairs can be assumed to be DZ, but the zygosity of like-sex pairs cannot be identified without further information. Although twin sex-pairing is an imperfect classification of zygosity, we estimated crude ORs (cORs) and 95% CIs for the association between twinning and specific birth defects for the like-sex and unlike-sex pairs.[46]

Since the interpretation of like-sex twin associations is limited, we also used a simulation modelling approach to classify the zygosity of the like-sex twins, which has been previously described in detail.[18] The modelling parameters were defined using an approach described by Hardin *et al*.[19] Using the proportion of male twins in our sample, we estimated the proportion of DZ twins among control twins, and the proportion of MZ twins among the like-sex twins. We then used a Monte Carlo sampling approach, constructing 1000 data sets in which like-sex twins were randomly designated as MZ or DZ based on our estimates of the distribution of MZ twins in our sample. In each dataset, we estimated ORs for each birth defect of interest using logistic regression, and obtained summary ORs and 95% uncertainty intervals for each association. We were not able to obtain cotwin data for participants from the Utah study site; therefore, the twin (17 controls and 104 cases) and singleton (599 controls and 1702 cases) infants from this study site were excluded from these subanalyses.

RESULTS

Among the 8470 mothers of controls, ~2.7% (n=227) of mothers reported a twin pregnancy; 5.9% of case mothers (n=1250/21 079) reported a twin pregnancy. The interview participation rate was 68.4% for case mothers and 65.7% for control mothers. There were 51 infants (17 controls and 34 cases) whose mother did not answer the plurality question in the interview, but who were identified as singletons on birth certificates and were classified as singletons for this analysis. Approximately 18.1% of controls identified as twins in the maternal interview were documented as singletons in the clinical records (n=41/227); for cases, this proportion was 14.6% (n=182/1250). All infants reported as singletons during the interview were also identified as singletons on birth certificates. One control mother was excluded due to missing information on plurality in both information sources. After excluding mothers with missing covariate information, the final sample included 7872 mothers of controls (218 twins; 7654 singletons), and 20 809 mothers of cases (1182 twins; 19 627 singletons). Any use of fertility treatment was much more common among twin pregnancies than singleton pregnancies for both controls (32.6% and 3.2%, respectively), and cases (29.2% and 4.2%, respectively) (table 1). For both control and case mothers, mothers of twins were more likely to be older than 29 years, non-Hispanic white, have attained more than a high school education and have taken a folic acid-containing multi-vitamin during the periconceptional period than mothers of singletons. A relatively large proportion of twin pregnancies were from the Massachusetts study site (23.4% of twin controls and 20.3% of twin cases, compared to 12.5% of singleton controls and 13.2% of singleton cases), where there is a state mandate that insurers provide coverage for fertility treatments, including in vitro fertilisation.

Of the 50 NBDPS-eligible birth defect categories with at least five twins, 34 (68%) were significantly associated with twinning in the analysis that was not stratified by use of fertility treatment; adjusted ORs were above the null for all defects, ranging from 1.1 (for gastroschisis) to 9.2 (for multiple ventricular septal defects (VSD)) (table 2). The associations with the largest magnitude were multiple VSDs, intercalary limb deficiency and cloacal exstrophy.

After stratifying by use of fertility treatments, there were fewer defect categories that met our criterion of having at least five twin pregnancies among mothers of cases who reported use of fertility treatments (24 of the 50 examined in the non-stratified analysis); we were able to examine 45 of 50 defect categories among women who did not report use of fertility treatments. Defects significantly associated with twinning among women who reported any use of fertility treatment were anotia/microtia, perimembranous VSD, secundum atrial septal defect (ASD), hypoplastic left heart syndrome and omphalocele; the association with anotia/microtia was only significant in this stratum (table 2). The majority (28 of 34) of the significant associations observed in the non-stratified analysis remained significant when the analysis was restricted to mothers reporting unassisted conception. Biliary atresia was significantly associated with twinning only among women reporting unassisted conception; we were not able to test this association among mothers reporting use of fertility treatment. Only four defect groups were significantly associated with twinning in both the use of fertility treatment and unassisted conception strata: perimembranous VSD, secundum ASD, hypoplastic left heart syndrome and omphalocele.

In the sensitivity analysis restricted to infants with isolated birth defects, results were similar, although with less power than the overall analysis; only hypoplastic left heart syndrome was significantly associated with twinning in the fertility treatment stratum (adjusted OR 2.7, 95% CI 1.1 to 6.7).

As in the primary analyses, twinning was positively associated with most birth defects in the analyses stratified by twin sex-pairing; the magnitude of the association was generally larger in the like-sex stratum. Anotia/microtia and dextro-transposition of the great arteries were positively associated with twinning in the unlike-sex stratum, but negatively associated with twinning in the like-sex stratum, although not all of these associations reached statistical significance. The OR estimates in the estimated MZ stratum were further from the null than for those in the estimated DZ stratum for all associations tested except for anotia/microtia, dextro-transposition of the great arteries, and diaphragmatic hernia (table 3). The simulation estimates for the MZ stratum were also generally larger in magnitude than in the like-sex twin stratum. The DZ simulation results and the results from the unlike-sex twin stratum did not display the same pattern of associations of larger magnitude in the simulated stratum; only five of the associations in the simulated stratum were further from the null.

DISCUSSION

We examined associations between selected major structural birth defects and twinning in a population-based case–control study, and found that, compared to singletons, twin pregnancies were associated with an increased risk for birth defects in nearly every organ

system. This is the first study of which we are aware, in which the association between twinning and birth defects could be assessed separately among mothers who did and did not use fertility treatments. Our unadjusted results were generally similar to registry-based studies that have examined the association between twinning and specific birth defects. Twins have been observed to have increased risk for a number of specific defects that have been observed in multiple studies (either in crude or adjusted analyses), including anencephaly,[7810] hydrocephalus,[810] tetralogy of Fallot,[8–10] pulmonary valve stenosis,[8–10] coarctation of the aorta,[810] cleft lip with or without cleft palate,[910] oesophageal atresia with or without tracheoesophageal fistula,[8–10] anorectal atresia[89] and hypospadias.[7–10] Several previously unreported associations were observed in our analysis for some common combinations of heart defects, such as coarctation of the aorta with VSD, pulmonary valve stenosis with ASD and ASD with VSD, and for rare defects such as Dandy Walker syndrome. A significant association with spina bifida was observed for multiple births in studies using data from Florida and Zhejiang Province, China, but we did not observe an increased risk of spina bifida in any of our analyses.[710] However, these studies included higher order multiples in their analyses. In addition, elective terminations were not included for the Massachusetts NBDPS Center, which represented about 25% of the twins, and could have contributed to the lower observed rates of spina bifida among twins in our study.

For most defect groups studied, the magnitude of the association with twinning was larger among mothers who did not report use of fertility treatments than among those who did report use of fertility treatments, although the small sample size of women reporting use of fertility treatments inhibits our ability to see associations in that stratum. Results from the zygosity simulation suggested that the association with certain birth defects may be stronger for MZ twins compared with DZ twins.

Explanations for the observed associations between twinning and birth defects are not clear, although our results suggest that there may be differences depending on use of fertility treatments, zygosity of twins and the birth defect being considered. Proposed mechanisms include a shared aetiology of birth defects and twinning, particularly for MZ twins, or consequences of twinning itself, such as crowding, insufficient nutrition supply, or, for MZ twins, vascular interchange.[32021] One mechanism that may be a factor in the aetiology of both twinning and birth defects is subfertility. Factors associated with sub-fertility, such as advanced maternal age and obesity, have also been previously associated with twinning.[2223] Owing to small sample size we were unable to further stratify the zygosity subanalyses by use of fertility treatment; therefore, some of the difference in the associations for MZ and DZ twins may be explained by differential rates of use of fertility treatment in these mothers.

Studies have also shown a higher rate of birth defects among couples with a history of infertility, even without the use of fertility treatments.[2024] For some defects, such as cloacal exstrophy, other potential pathogenic mechanisms include an early disturbance in blastogenesis, or a partial or complete duplication of the organising centre within a single embryonic disc, which increases the risk of mesodermal insufficiency, and could cause failure of cloacal membrane development.[25]

Our study has several limitations. First, our study was limited by the relatively small sample size of many defect groups after stratifying by use of fertility treatment and estimated zygosity. Information on fertility treatments and other pregnancy exposures was self-reported during a retrospective maternal interview, so we cannot rule out exposure misclassification resulting from inaccurate recall; the average age of infants at the maternal interview was 9 months for controls and 11 months for cases. Approximately 30% of case and control mothers eligible for the NBDPS did not participate in the interview. Owing to small sample size, we were also unable to stratify by type of fertility treatment. For mothers who reported use of fertility treatment, we were unable to determine the number of embryos implanted, which is a strong predictor of twinning, and may be related to risk of birth defects.[14] Another limitation is that mothers more frequently reported twin pregnancies during the interview than were recorded on the birth certificate or maternal hospital records. However, results from a sensitivity analysis restricted to mothers without plurality discrepancies did not differ from the primary analysis, supporting that this potential misclassification had a minimal effect.

We assessed the risk of birth defects in only one twin, as only one of the twins was included in the study. Therefore, the unit of observation in our study was the included twin rather than the risk of birth defects in the twin pregnancy, and may not accurately represent the risk of birth defects in the cotwin, as it was possible for the cotwin to have the same birth defect(s), different birth defect(s), or none.

We were also unable to directly determine zygosity. This limitation is important because the risk of birth defects has been observed to be greater for MZ twins than for DZ twins.[1112] Information on zygosity is particularly difficult to obtain, as the gold-standard for determining zygosity is genetic analysis.[26] Even self-report of zygosity from parents is not a reliable source of information on zygosity.[27] However, our simulation modelling approach may be a useful alternative to the more rudimentary sex-pair analysis for like-sex twins, as the uncertainty caused by the potential misclassification is taken into account. The simulation provided some evidence that using like-sex as a crude proxy for monozygosity may result in an underestimate of the risk of birth defects in MZ twins. In addition, we were able to assess birth defects associations in the estimated DZ stratum that we were not able to assess in the unlike-sex stratum as a result of small cell sizes. Although the risk of birth defects in twins has also been shown to vary by chorionicity, we were not able to assess this.[11]

Strengths of this study include use of a demographically diverse, population-based sample from 10 regions across the USA, with careful review and classification of birth defects. We were able to assess the association between twinning and specific birth defects accounting for important potential confounders and effect modifiers, in particular, use of fertility treatments.

Twinning was associated with an increased risk of many major structural birth defects in the NBDPS. Although there appears to be a differential risk of birth defects by use of fertility treatment, future studies with larger sample sizes are needed to confirm these findings.

These results may better assist clinicians in counselling women on the risk of birth defects associated with a twin pregnancy.

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References

1. Martin JA, Hamilton BE, Ventura SJ, et al. Births: final data for 2011. Natl Vital Stat Rep. 2013; 62:1–90.
2. Chauhan SP, Scardo JA, Hayes E, et al. Twins: prevalence, problems, and preterm births. Am J Obstet Gynecol. 2010; 203:305–15. [PubMed: 20728073]
3. Schinzel AA, Smith DW, Miller JR. Monozygotic twinning and structural defects. J Pediatr. 1979; 95:921–30. [PubMed: 501497]
4. Hardin J, Carmichael SL, Selvin S, et al. Increased prevalence of cardiovascular defects among 56,709 California twin pairs. Am J Med Genet Part A. 2009; 149A:877–86. [PubMed: 19353581]
5. Cragan JD, Martin ML, Waters GD, et al. Increased risk of small intestinal atresia among twins in the United States. Arch Pediatr Adolesc Med. 1994; 148:733–9. [PubMed: 8019630]
6. Rider RA, Stevenson DA, Rinsky JE, et al. Association of twinning and maternal age with major structural birth defects in Utah, 1999 to 2008. Birth Defects Res A Clin Mol Teratol. 2013; 97:554–63. [PubMed: 23913417]
7. Zhang XH, Qiu LQ, Huang JP. Risk of birth defects increased in multiple births. Birth Defects Res A Clin Mol Teratol. 2011; 91:34–8. [PubMed: 20890935]
8. Li SJ, Ford N, Meister K, et al. Increased risk of birth defects among children from multiple births. Birth Defects Res A Clin Mol Teratol. 2003; 67:879–85. [PubMed: 14745942]
9. Mastroiacovo P, Castilla EE, Arpino C, et al. Congenital malformations in twins: an international study. Am J Med Genet. 1999; 83:117–24. [PubMed: 10190482]
10. Tang Y, Ma CX, Cui W, et al. The risk of birth defects in multiple births: a population-based study. Matern Child Health J. 2006; 10:75–81. [PubMed: 16240077]
11. Glinianaia SV, Rankin J, Wright C. Congenital anomalies in twins: a register-based study. Hum Reprod. 2008; 23:1306–11. [PubMed: 18387962]
12. Layde PM, Erickson JD, Falek A, et al. Congenital malformation in twins. Am J Hum Genet. 1980; 32:69–78. [PubMed: 7189094]
13. Reefhuis J, Honein MA, Schieve LA, et al. Assisted reproductive technology and major structural birth defects in the United States. Hum Reprod. 2009; 24:360–6. [PubMed: 19010807]
14. Hansen M, Kurinczuk JJ, de Klerk N, et al. Assisted reproductive technology and major birth defects in Western Australia. Obstet Gynecol. 2012; 120:852–63. [PubMed: 22996103]
15. Rasmussen SA, Olney RS, Holmes LB, et al. Guidelines for case classification for The National Birth Defects Prevention Study. Birth Defects Res A Clin Mol Teratol. 2003; 67:193–201. [PubMed: 12797461]
16. Reefhuis J, Gilboa SM, Anderka M, et al. National Birth Defects Prevention Study. The National Birth Defects Prevention Study: A review of the methods. Birth Defects Res A Clin Mol Teratol. 2015; 103:656–69. [PubMed: 26033852]
17. Khoury MJ, Erickson JD, James LM. Etiologic heterogeneity of neural tube defects: clues from epidemiology. Am J Epidemiol. 1982; 115:538–48. [PubMed: 7041633]

18. Dawson AL, Tinker SC, Jamieson DJ, et al. the National Birth Defects Prevention Study. Epidemiology of twinning in the National Birth Defects Prevention Study, 1997 to 2007. Birth Defects Res A Clin Mol Teratol. 2015; 103:85–99. [PubMed: 25359509]
19. Hardin J, Selvin S, Carmichael SL, et al. The estimated probability of dizygotic twins: a comparison of two methods. Twin Res Hum Genet. 2009; 12:79–85. [PubMed: 19210182]
20. Zhu JL, Basso O, Obel C, et al. Infertility, infertility treatment, and congenital malformations: Danish national birth cohort. BMJ. 2006; 333:679. [PubMed: 16893903]
21. Li Z, Gindler J, Wang H, et al. Folic acid supplements during early pregnancy and likelihood of multiple births: a population-based cohort study. Lancet. 2003; 361:380–4. [PubMed: 12573374]
22. Beemsterboer SN, Homburg R, Gorter NA, et al. The paradox of declining fertility but increasing twinning rates with advancing maternal age. Hum Reprod. 2006; 21:1531–2. [PubMed: 16497698]
23. Reddy UM, Branum AM, Klebanoff MA. Relationship of maternal body mass index and height to twinning. Obstet Gynecol. 2005; 105:593–7. [PubMed: 15738030]
24. Davies MJ, Moore VM, Willson KJ, et al. Reproductive technologies and the risk of birth defects. N Engl J Med. 2012; 366:1803–13. [PubMed: 22559061]
25. Siebert JR, Rutledge JC, Kapur RP. Association of cloacal anomalies, caudal duplication, and twinning. Pediatr Dev Pathol. 2005; 8:339–54. [PubMed: 16010492]
26. Chen W, Chang H-W, Wu M-Z, et al. Diagnosis of zygosity by questionnaire and polymarker polymerase chain reaction in young twins. Behav Genet. 1999; 29:115–23. [PubMed: 10405460]
27. Goldsmith HH. A zygosity questionnaire for young twins: a research note. Behav Genet. 1991; 21:257–69. [PubMed: 1863259]

What is already known on this subject

The rate of twin births in the USA has increased by 75% from 1980 to 2011. In comparison with singletons, twins have an increased risk of birth defects. Although use of fertility treatments has been considered the primary reason for the observed increase in twinning, few studies have examined the impact of use of fertility treatment on twins' risk of birth defects.

What this study adds

Our results suggest that the risk of birth defects in twins, as compared to singletons, may be greater among mothers who did not report use of fertility treatments than among those who did report use of fertility treatments. These results may better assist clinicians in counselling women on the risk of birth defects associated with a twin pregnancy.

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Table 1

Characteristics of mothers of twin and singleton infants, National Birth Defects Prevention Study, 1997–2007

	Controls			Cases		
Maternal characteristics	**Twins (n=218) n (%)**	**Singletons (n=7654) n (%)**	**p Value***	**Twins (n=1182) n (%)**	**Singletons (n=19 627) n (%)**	**p Value***
Any fertility treatment used			<0.01			<0.01
No	147 (67.4)	7408 (96.8)		837 (70.8)	18 798 (95.8)	
Yes	71 (32.6)	246 (3.2)		345 (29.2)	829 (4.2)	
Assisted reproductive technology	39 (17.9)	39 (0.5)	<0.01	178 (15.1)	166 (0.9)	<0.01
Clomiphene citrate	19 (8.7)	130 (1.7)	<0.01	118 (10.0)	433 (2.2)	<0.01
Age at delivery (years)			<0.01			<0.01
<25	38 (17.4)	2554 (33.4)		276 (23.4)	6778 (34.5)	
25–29	49 (22.5)	2105 (27.5)		288 (24.4)	5209 (26.5)	
30–34	77 (35.3)	1922 (25.1)		355 (30.0)	4763 (24.3)	
>34	54 (24.8)	1073 (14.0)		263 (22.3)	2877 (14.7)	
Race/ethnicity			<0.01			<0.01
Non-Hispanic white	153 (70.2)	4683 (61.2)		786 (66.5)	11 944 (60.9)	
Non-Hispanic black	31 (14.2)	882 (11.5)		141 (11.9)	2025 (10.3)	
Hispanic	23 (10.6)	1583 (20.7)		168 (14.2)	4320 (22.0)	
Other	11 (5.0)	506 (6.6)		87 (7.3)	1338 (6.8)	
Smoking†			0.88			<0.01
Yes	41 (18.8)	1471 (19.2)		194 (16.4)	4300 (21.9)	
No	177 (81.2)	6183 (80.8)		988 (83.6)	15 327 (78.1)	
Prepregnancy body mass index (kg/m^2)			0.80			0.62
<30	180 (82.6)	6370 (83.2)		945 (80.0)	15 808 (80.5)	
≥30	38 (17.4)	1284 (16.8)		237 (20.1)	3819 (19.5)	
Parity			0.66			0.17
No previous live births	91 (41.7)	3082 (40.3)		533 (45.1)	8450 (43.1)	
One or more previous live births	127 (58.3)	4572 (59.7)		649 (54.9)	11 177 (57.0)	
Education			<0.01			<0.01
High school graduate or less	57 (26.2)	3047 (39.8)		363 (30.7)	8485 (43.2)	

Maternal characteristics	Controls			Cases		
	Twins (n=218) n (%)	Singletons (n=7654) n (%)	p Value*	Twins (n=1182) n (%)	Singletons (n=19 627) n (%)	p Value*
More than high school graduate	161 (73.9)	4607 (60.2)		819 (69.3)	11 142 (56.8)	
Folic acid-containing multivitamin use‡			<0.01			<0.01
Yes	136 (62.4)	3979 (52.0)		744 (62.9)	9896 (50.4)	
No	82 (37.6)	3675 (48.0)		438 (37.1)	9731 (49.6)	
Study site			<0.01			<0.01
Arkansas	19 (8.7)	988 (12.9)		132 (11.2)	2681 (13.7)	
California	6 (2.8)	910 (11.9)		114 (9.6)	2548 (13.0)	
Georgia	23 (10.6)	801 (10.5)		141 (11.9)	2197 (11.2)	
Iowa	35 (16.1)	867 (11.3)		93 (7.9)	1911 (9.7)	
Massachusetts	51 (23.4)	959 (12.5)		240 (20.3)	2581 (13.2)	
New Jersey	26 (11.9)	479 (6.3)		96 (8.1)	1366 (7.0)	
New York	11 (5.1)	682 (5.1)		103 (8.7)	1334 (6.8)	
North Carolina	14 (6.4)	518 (6.8)		58 (4.9)	1057 (5.4)	
Texas	19 (8.7)	868 (11.3)		108 (9.1)	2315 (11.8)	
Utah	14 (6.4)	582 (7.6)		97 (8.2)	1057 (8.3)	

*Mantel-Haenszel χ^2 statistics were used to compare group differences for each characteristic of interest.

†Any reported use 1 month prior to pregnancy through 3 months after conception.

‡Any reported use 1 month prior to pregnancy through the first month of pregnancy.

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Table 2

Adjusted ORs and 95% CIs for associations between twinning and birth defects, stratified by use of fertility treatment, National Birth Defects Prevention Study, 1997–2007

	Total			Any use of fertility treatment			No fertility treatment used		
	Plurality			Plurality			Plurality		
Type of birth defect[†]	Twin (n)	Singleton (n)	Adjusted OR[‡] (95% CI)[*]	Twin (n)	Singleton (n)	Adjusted OR[‡] (95% CI)	Twin (n)	Singleton (n)	Adjusted OR[‡] (95% CI)
Controls	218	7654	Ref.	71	246	Ref.	147	7408	Ref.
Central nervous system									
Anencephaly	21	381	**2.2 (1.4 to 3.4)**	4	14	NC	17	367	**2.5 (1.5 to 4.2)**
Spina bifida	27	820	1.2 (0.8 to 1.8)	8	29	1.0 (0.5 to 2.4)	19	791	1.2 (0.7 to 2.0)
Encephalocele	5	147	1.3 (0.5 to 3.2)	0	8	NC	5	139	1.9 (0.8 to 4.7)
Holoprosencephaly	7	106	**2.7 (1.2 to 5.8)**	2	7	NC	5	99	2.5 (1.0 **to** 6.3)
Hydrocephalus	26	329	**2.9 (1.9 to 4.4)**	0	12	NC	26	317	**4.2 (2.8 to 6.6)**
Dandy Walker malformation	8	108	**2.8 (1.3 to 5.8)**	2	4	NC	6	104	**3.0 (1.3 to 7.0)**
Eye									
Cataracts and other lens defects	10	230	1.6 (0.8 to 3.0)	3	8	1.5 (0.4 to 5.9)	7	223	1.7 (0.8 to 3.8)
Glaucoma	5	119	1.6 (0.6 to 3.9)	2	2	NC	3	117	NC
Anotia/microtia	20	418	**1.8 (1.1 to 3.0)**	10	16	**2.4 (1.0 to 5.6)**	10	402	1.2 (0.6 to 2.3)
Cardiovascular									
Heterotaxia	6	291	1.3 (0.6 to 2.9)	0	8	NC	6	283	0.9 (0.4 to 2.2)
Dextro-transposition of great arteries	21	534	1.4 (0.9 to 2.1)	7	19	1.3 (0.5 to 3.1)	14	515	1.4 (0.8 to 2.4)
Tetralogy of Fallot	53	772	**2.3 (1.7 to 3.1)**	11	44	0.9 (0.4 to 1.7)	42	728	**2.9 (2.0 to 4.1)**
Atrioventricular septal defects	9	238	1.3 (0.6 to 2.5)	1	12	NC	8	226	1.8 (0.9 to 3.6)
Total anomalous pulmonary venous return	8	196	1.5 (0.8 to 3.2)	2	5	NC	6	191	1.6 (0.7 to 3.6)
Perimembranous ventricular septal defect	108	1320	**2.8 (2.2 to 3.6)**	33	58	**2.0 (1.2 to 3.3)**	75	1262	**3.0 (2.3 to 4.0)**
Conoventricular septal defect	11	107	**3.2 (1.7 to 6.2)**	3	3	NC	8	104	**3.8 (1.8 to 8.1)**
Multiple ventricular septal defects	13	52	**9.2 (4.9 to 17.4)**	5	2	NC	8	50	**8.5 (3.9 to 18.3)**
Secundum atrial septal defect	142	1557	**3.4 (2.7 to 4.2)**	37	59	**2.4 (1.4 to 3.9)**	105	1498	**3.6 (2.8 to 4.7)**
Atrial septal defect, not otherwise specified	9	238	**2.4 (1.6 to 3.5)**	10	19	1.9 (0.9 to 4.4)	23	465	**2.3 (1.5 to 3.7)**

Type of birth defect[†]	Total			Any use of fertility treatment			No fertility treatment used		
	Plurality			Plurality			Plurality		
	Twin (n)	Singleton (n)	Adjusted OR[‡] (95% CI)[*]	Twin (n)	Singleton (n)	Adjusted OR[‡] (95% CI)	Twin (n)	Singleton (n)	Adjusted OR[‡] (95% CI)
Tricuspid atresia	10	109	**3.2 (1.7 to 6.3)**	4	1	NC	6	108	**2.7 (1.2 to 6.3)**
Pulmonary valve stenosis	84	937	**3.0 (2.3 to 3.9)**	16	36	1.6 (0.8 to 3.0)	68	901	**3.6 (2.7 to 4.9)**
Pulmonary atresia	16	163	**3.5 (2.1 to 6.0)**	3	4	NC	13	159	**4.1 (2.3 to 7.5)**
Coarctation of the aorta	54	716	**2.5 (1.8 to 3.4)**	15	37	1.4 (0.7 to 2.7)	39	679	**2.8 (2.0 to 4.1)**
Aortic stenosis	21	309	**2.2 (1.4 to 3.5)**	5	17	1.0 (0.4 to 2.8)	16	292	**2.8 (1.6 to 4.7)**
Hypoplastic left heart syndrome	25	413	**2.1 (1.4 to 3.3)**	10	13	**2.8 (1.2 to 6.7)**	15	400	**1.9 (1.1 to 3.2)**
Single ventricle	9	239	1.3 (0.7 to 2.6)	0	3	NC	9	236	1.9 (0.9 to 3.7)
Coarctation of aorta with ventricular septal defect	15	195	**2.7 (1.5 to 4.6)**	3	11	NC	12	184	**3.3 (1.8 to 6.0)**
Pulmonary valve stenosis with atrial septal defect	15	142	**3.8 (2.2 to 6.6)**	3	2	NC	12	140	**4.0 (2.2 to 7.5)**
Pulmonary valve stenosis with ventricular septal defect	6	107	2.0 (0.9 to 4.7)	2	2	NC	4	105	NC
Atrial septal defect with ventricular septal defect	39	540	**2.4 (1.7 to 3.4)**	8	24	1.1 (0.5 to 2.7)	31	516	**3.0 (2.0 to 4.5)**
Orofacial									
Cleft lip±cleft palate	101	2020	**1.8 (1.4 to 2.4)**	30	80	1.4 (0.8 to 2.2)	71	1940	**1.9 (1.4 to 2.5)**
Cleft palate	44	1087	1.4 (1.0 to 1.9)	14	55	0.9 (0.5 to 1.6)	30	1032	1.4 (1.0 to 2.1)
Choanal atresia	8	101	**2.3 (1.1 to 4.9)**	4	7	NC	4	94	NC
Gastrointestinal									
Duodenal atresia	5	140	1.2 (0.5 to 3.1)	3	8	NC	2	132	NC
Oesophageal atresia with/without tracheoesophageal fistula	53	444	**3.6 (2.6 to 5.0)**	20	40	1.6 (0.9 to 2.9)	33	404	**4.2 (2.8 to 6.2)**
Biliary atresia	7	124	2.0 (0.9 to 4.4)	0	7	NC	7	117	**3.0 (1.4 to 6.6)**
Small intestinal atresia	22	301	**2.7 (1.7 to 4.3)**	3	8	NC	19	293	**3.2 (2.0 to 5.3)**
Anorectal atresia	49	681	**2.6 (1.9 to 3.5)**	15	28	1.9 (1.0 to 3.8)	34	653	**2.6 (1.8 to 3.9)**
Genitourinary									
Renal agenesis/hypoplasia	5	112	1.7 (0.7 to 4.2)	0	2	NC	5	110	2.2 (0.9 **to** 5.6)
Hypospadias[§]	113	1480	**2.2 (1.7 to 2.9)**	53	100	1.6 (1.0 to 2.7)	60	1380	**2.3 (1.6 to 3.2)**
Musculoskeletal									

Type of birth defect[†]	Total			Any use of fertility treatment			No fertility treatment used		
	Plurality			Plurality			Plurality		
	Twin (n)	Singleton (n)	Adjusted OR[‡] (95% CI)[*]	Twin (n)	Singleton (n)	Adjusted OR[‡] (95% CI)	Twin (n)	Singleton (n)	Adjusted OR[‡] (95% CI)
Cloacal exstrophy	7	54	**4.9 (2.2 to 10.9)**	1	8	NC	6	46	**6.5 (2.7 to 15.5)**
Intercalary limb deficiency	6	42	**5.2 (2.1 to 12.4)**	3	3	NC	3	39	NC
Longitudinal limb deficiency	21	304	**2.6 (1.6 to 4.1)**	8	14	2.1 (0.8 to 5.1)	13	290	**2.4 (1.3 to 4.3)**
Preaxial limb deficiency	14	186	**2.9 (1.7 to 5.1)**	7	10	2.7 (1.0 to 7.3)	7	176	2.1 (1.0 to 4.7)
Transverse limb deficiency	26	465	**2.0 (1.3 to 3.0)**	7	21	1.2 (0.5 to 2.9)	19	444	**2.2 (1.3 to 3.6)**
Craniosynostosis	50	962	**1.7 (1.2 to 2.3)**	24	51	1.6 (0.9 to 2.8)	26	911	1.4 (0.9 to 2.1)
Diaphragmatic hernia	25	566	1.5 (1.0 to 2.3)	10	19	1.8 (0.8 to 4.0)	15	547	1.4 (0.8 to 2.4)
Omphalocele	26	290	**3.0 (1.9 to 4.6)**	10	12	**2.8 (1.2 to 6.9)**	16	278	**3.0 (1.7 to 5.0)**
Gastroschisis	19	898	1.1 (0.7 to 1.9)	0	5	NC	19	893	1.3 (0.8 to 2.2)
Amniotic band sequence	23	221	**3.7 (2.3 to 5.7)**	4	7	NC	19	214	**5.3 (3.2 to 8.8)**

[*] Bold values indicate p<0.05.

[†] Includes isolated and multiple defects.

[‡] Adjusted for maternal age at delivery (continuous), race, parity, obesity, education, smoking, use of a folic acid-containing multivitamins and study site.

[§] Controls limited to male infants.

NC, not calculated because ≤4 twin pregnancies.

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Table 3

Association between twinning and selected birth defects stratified by estimated zygosity, National Birth Defects Prevention Study, 1997–2007

Type of birth defect	Singleton (n)	Unlike-sex twin pairs (n)	Unlike-sex twin crude OR (95% CI)*	Estimated dizygotic twin crude OR (95% uncertainty interval)	Like-sex twin pairs (n)	Like-sex twin crude OR (95% CI)	Estimated monozygotic twin crude OR (95% uncertainty interval)
Controls	7072†	62	Ref.	Ref.	109	Ref.	Ref.
Central nervous system							
Anencephaly	352	4	NC	1.5 (1.0 to 2.1)	12	**2.2 (1.2 to 4.0)**	**2.9 (1.3 to 4.6)**
Spina bifida	747	4	NC	0.8 (0.5 to 1.1)	15	**1.3 (0.7 to 2.2)**	**1.7 (0.9 to 2.6)**
Hydrocephalus	307	2	NC	**1.8 (1.1 to 2.7)**	17	**3.5 (2.1 to 6.0)**	**4.6 (2.3 to 7.1)**
Cataracts and other lens defects	202	4	NC	**1.9 (1.2 to 2.6)**	5	1.7 (0.7 to 4.3)	2.2 (0.6 to 4.2)
Anotia/microtia	383	9	**2.5 (1.3 to 5.0)**	**1.7 (1.3 to 2.1)**	5	0.8 (0.3 to 1.9)	1.1 (0.3 to 2.0)
Cardiovascular							
Dextro-transposition of great arteries	502	8	1.8 (0.9 to 3.8)	1.2 (0.9 to 1.5)	6	0.8 (0.3 to 1.8)	1.0 (0.3 to 1.8)
Tetralogy of Fallot	737	9	1.4 (0.7 to 2.8)	**1.8 (1.4 to 2.3)**	32	**2.8 (1.9 to 4.2)**	**3.6 (2.3 to 5.1)**
Perimembranous ventricular septal defects	1320	27	**2.5 (1.6 to 3.9)**	**2.5 (2.1 to 2.9)**	58	**3.0 (2.2 to 4.2)**	**3.9 (2.8 to 5.4)**
Secundum atrial septal defect	1408	22	**1.8 (1.1 to 2.9)**	**2.2 (1.8 to 2.5)**	75	**3.4 (2.5 to 4.6)**	**4.2 (3.2 to 5.6)**
Pulmonary valve stenosis	822	13	1.7 (0.9 to 3.1)	**2.2 (1.8 to 2.7)**	45	**3.5 (2.4 to 4.9)**	**4.5 (3.1 to 6.2)**
Coarctation of the aorta	621	16	**2.9 (1.7 to 5.1)**	**2.6 (2.1 to 3.1)**	28	**2.9 (1.9 to 4.4)**	**3.8 (2.5 to 5.6)**
Aortic stenosis	254	6	**2.7 (1.2 to 6.3)**	**2.2 (1.6 to 2.8)**	8	**2.0 (1.0 to 4.2)**	2.6 (0.8 to 4.4)
Hypoplastic left heart syndrome	362	7	2.2 (1.0 to 4.8)	**1.7 (1.3 to 2.2)**	8	1.4 (0.7 to 2.9)	1.9 (0.7 to 3.1)
Orofacial							
Cleft lip±cleft palate	2020	28	**1.7 (1.1 to 2.6)**	**1.3 (1.1 to 1.6)**	36	1.2 (0.8 to 1.8)	1.6 (1.0 to 2.3)
Cleft palate	1087	11	1.2 (0.7 to 2.4)	1.2 (0.9 to 1.5)	23	1.5 (0.9 to 2.3)	**1.9 (1.2 to 2.7)**
Gastrointestinal							
Oesophageal atresia with/without tracheoesophageal fistula	444	7	1.9 (0.9 to 4.3)	**2.7 (2.0 to 3.5)**	27	**4.2 (2.8 to 6.5)**	**5.5 (3.4 to 8.0)**
Small intestinal atresia	301	5	2.0 (0.8 to 5.0)	**1.8 (1.2 to 2.4)**	9	2.1 (1.0 to 4.1)	2.7 (1.0 to 4.5)
Anorectal atresia	681	8	1.4 (0.7 to 2.9)	**1.7 (1.3 to 2.2)**	26	**2.6 (1.7 to 4.0)**	**3.0 (1.9 to 4.2)**
Hypospadias≠	1480	27	**2.2 (1.3 to 3.6)**	**2.1 (1.7 to 2.5)**	55	**2.6 (1.8 to 3.5)**	**3.3 (2.2 to 4.7)**
Musculoskeletal							

Type of birth defect	Singleton (n)	Unlike-sex twin pairs (n)	Unlike-sex twin crude OR (95% CI)*	Estimated dizygotic twin crude OR (95% uncertainty interval)	Like-sex twin pairs (n)	Like-sex twin crude OR (95% CI)	Estimated monozygotic twin crude OR (95% uncertainty interval)
Longitudinal limb deficiency	304	6	**2.5 (1.1 to 5.8)**	**2.3 (1.6 to 3.0)**	11	**2.6 (1.4 to 4.9)**	**3.4 (1.6 to 5.5)**
Transverse limb deficiency	465	6	1.6 (0.7 to 3.8)	**1.6 (1.1 to 2.0)**	12	1.8 (1.0 to 3.4)	**2.4 (1.2 to 3.8)**
Craniosynostosis	962	14	**2.0 (1.1 to 3.6)**	**1.4 (1.2 to 1.7)**	14	1.1 (0.7 to 2.0)	1.5 (0.7 to 2.2)
Diaphragmatic hernia	566	9	2.0 (1.0 to 4.0)	**1.4 (1.1 to 1.7)**	8	1.0 (0.5 to 2.1)	1.3 (0.5 to 2.2)
Omphalocele	290	2	NC	2.2 (0.9 to 4.2)	12	**2.9 (1.6 to 5.3)**	**3.4 (1.8 to 5.7)**
Gastroschisis	898	5	0.7 (0.3 to 1.8)	0.7 (0.5 to 1.1)	11	0.9 (0.5 to 1.7)	1.4 (0.9 to 2.0)

*Bold values indicate p<0.05.

†Cases and controls from the Utah study site were not included in these analyses.

‡Controls limited to male infants.

NC, not calculated because ≤4 twin pregnancies.