Sex Differences in Birth Defects: A Study of Opposite-Sex Twins

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BACKGROUND: Sex differences in structural birth defects are often confounded by environmental risk factors. Opposite-sex twins provide a unique model for detecting sex differences in birth defects while maximally controlling environmental risk factors in a natural setting. **METHODS:** Population data from the Florida Birth Defects Registry were analyzed. A total of 4768 pairs of twins who were discordant for sex and born between 1996 and 2001 were analyzed. The McNemar test was used to compare the differences between a male twin and his twin sister for the risk of developing specific defects and organ-system defects. **RESULTS:** Of 4768 twin pairs, 225 males (4.72%) and 175 females (3.67%) had birth defects. Among opposite-sex twin pairs, males had a 29% higher risk for birth defects than their twin sisters. Compared to their twin sisters, males had a 5.4 times higher risk for pyloric stenosis and a 2.4 times higher risk for obstructive genitourinary defect, but only one-tenth the risk for congenital hip dislocation. **CONCLUSIONS:** Sex differences in birth defects exist between opposite-sex twins. *Birth Defects Research (Part A) 73:876–880, 2005.* © 2005 Wiley-Liss, Inc.

Key words: birth defect; sex difference; twin

INTRODUCTION

Sex differences in a variety of specific birth defects have been observed for over 40 years (Gittelsohn and Milham, 1964; Fernando et al., 1978; Lubinsky, 1997). Recently, Lary and Paulozzi (2001) reported sex differences in the prevalence of birth defects in a large population study. It is known that environmental risk factors, such as pesticides, can affect fetal development and may lead to birth defects in infants (Garry et al., 2002). Also, environmental risk factors may change the sex ratio of infants, which may be an indicator of intrauterine exposure to external toxins (Davis et al., 1998; del Rio Gomez et al., 2002; Fukuda et al., 2002). Therefore, sex differences in birth defects can be confounded by environmental risk factors. A study that rigorously controls environmental factors is needed to determine the role of sex in the etiology of birth defects. In a study that assessed the differential risks to males and females for congenital malformations, Shaw et al. (2003) adjusted environmental risk factors by controlling some of the maternal sociodemographic factors and sibling conditions. In the present study we utilized a statewide population of opposite-sex twins to determine whether there are sex differences in birth defects between twin siblings.

MATERIALS AND METHODS

The data for this study were obtained from the Florida Birth Defects Registry (FBDR), which is a statewide, population-based, passive surveillance system. This registry records birth defects that occur within the first year of life in children whose mothers are residents of Florida at the time of delivery. The registry consists of 4 statewide source data sets: the Florida Agency of Health Care and Administration (AHCA) hospital discharge diagnosis data files; Florida Birth Vital Statistics (BVS); Children's Medical Services (CMS) Regionalized Perinatal Intensive Care Centers Data Reporting System (RPICC); and the CMS Early Intervention Program (EIP) Data Reporting System. Diagnoses are recorded using the International Classification of Diseases 9th edition (ICD-9) codes. These data sets were merged to eliminate duplication and develop a single, comprehensive inventory of birth defects in the state. Data-

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quality studies have shown that the combination of the 4 data sets provides 93% overall sensitivity and 95% overall specificity for ascertaining all birth defects in the Centers for Disease Control and Prevention (CDC) reporting list (Carter and Grove, 1999).

The children's sex was recorded on the birth certificate and verified by other medical records. In the analysis of total birth defects, a child with ≥1 defect was counted only once. On the other hand, in the analysis at the organ-system level, each type of birth defect was counted separately when a child had >1 type of defect.

Sex differences in opposite-sex twins were analyzed for 40 of the 45 birth defects reported to the CDC. We excluded diagnoses specific to 1 sex, such as hypospadias. Sex-specific risks for defects at the organ-system level were also examined.

The male and female twins were a matched pair from a common mother; therefore, their birth-defects outcomes may be correlated. The McNemar test, a test for comparing occurrence rates in dependent samples (Rao, 1998), was used to determine the difference between a male twin and his twin sister in the risk of developing specific defects and defects grouped at the organ-system level. The 95% confidence intervals (CIs) of the corresponding relative risks (RRs) were also calculated.

RESULTS

Out of 1,175,538 live births that were reported in Florida between 1996 and 2001, 4768 pairs of twins were available for analysis. Among the twin pairs, 225 males and 175 females were found to have birth defects, representing a birth-defects rate of 471.9 per 10,000 for males and 367.0 per 10,000 for females. In 31 pairs both the brother and sister had birth defects. Table 1 presents the frequency and RR of birth defects by sex in Florida for all births between 1996 and 2001. The results are reported for 40 specific birth defects, as well as grouped at the organ system level.

Overall, among opposite-sex twins, males had a significantly higher rate of birth defects than their twin sisters ($P < .01$). The male twin was found to have a 29% higher risk of birth defects than his twin sister (RR = 1.29). That is, among twins consisting of 1 male and 1 female, the rate of birth defects in males is 29% higher than that in females.

At the organ-system level among opposite-sex twins, the male twin had a 2.9 times higher risk of gastrointestinal defects ($P < .01$) and a 2.7 times higher risk of urogenital defects ($P < .01$) compared with his twin sister.

Regarding specific birth defects, significant differences were found between opposite-sex twin siblings in gastrointestinal, urogenital, and musculoskeletal defects. Compared with their twin sisters, male infants had a 5.4 times higher risk of pyloric stenosis and a 2.4 times higher risk of obstructive genitourinary defect, but one-tenth the risk of congenital hip dislocation (RR = 0.13). The McNemar test detected that male twins also had a higher risk of diaphragmatic hernia ($P < .05$); however, the RR of this defect could not be calculated because no cases were observed in females. There was no significant difference between male and female twin siblings in the opposite-sex twin population for most heart defects and all oral cleft defects. Although females had higher risks than their twin brothers in several organ system categories (RR < 1), none of the differences were statistically significant. No difference in defects of the ear and eye was observed, probably because of the small sample size (Table 1).

DISCUSSION

In opposite-sex twins, the male twin had about a 29% higher risk of having a birth defect than his twin sister. This elevated risk of a birth defect in males is consistent with other observations in the general population. However, these twins were found to have a higher rate of birth defects than the general population: 472 per 10,000 vs. 225 per 10,000 in males ($P < .01$) and 367 per 10,000 vs. 175 per 10,000 in females ($P < .01$), excluding genital organs and sex chromosome defects (Shaw et al., 2003). This elevated risk for opposite-sex dizygotic twins may reflect the physical and/or nutritional intrauterine restraint for twins compared to singletons (Schinzel et al., 1979; Li et al., 2003).

In terms of etiology, sex differences can be roughly classified as being caused before and after differentiation of the male gonads during fetal development. We will first discuss birth defects that are associated with factors that arise after gonadal differentiation, as most of the positive findings in this study are in that category.

Male gonadal differentiation starts at the eighth week, when the fetal level of testosterone is observed to be much higher in male fetuses (Reyes et al., 1974). The subsequent hormonal and physiologic differences in male and female fetuses may explain some of the sex differences in birth defects.

The male twins were found to have a higher risk of pyloric stenosis than their twin sisters in this study. Maccoby et al. (1979) observed that among male infants, the first-borns had higher concentrations of testosterone. This finding is correlated with the fact that first-borns had a higher rate of pyloric stenosis. James (2004) suggested that higher intrauterine androgen levels contributed by the mother and child may be a cause of pyloric stenosis. Some studies (Ibanez et al., 1999, 2000; Szathmari, 2001; Francois and de Zegher, 1997; Szathmari et al., 2001) observed hyperandrogenism that occurred postnatally in babies born small for gestational age. Among these, girls with hyperandrogenism may sometimes develop pyloric stenosis under the influence of high androgen levels (James, 2004). In our study, male twins in the opposite-sex twin population were found to have higher rates of pyloric stenosis compared to the general population (56 per 10,000 vs. 26 per 10,000 ($P < .01$)), whereas females twins did not (10 per 10,000 vs. 6 per 10,000 ($P = .09$)) (Shaw et al., 2003). This increased risk of pyloric stenosis in male twins may be due to the high intrauterine testosterone level, because the growth of twins is restrained relative to that of singletons. The different reaction to the higher because of testosterone may reflect different sensitivities to this hormone by male and female twin siblings, affecting the smooth muscle around the pylorus. However, further evidence of a difference in fetal androgen levels between twins and singletons is needed.

In opposite-sex twins, males were more likely to have obstructive genitourinary tract defects than their twin sisters. This may reflect the fact that males have a longer and more complicated lower urinary tract. Lary and Paulozzi (2001) observed substantial excess defects in the reproductive system in males compared with females, which account for about half of all birth defects in the general population. The obstructive genitourinary tract defects

Table 1
Frequency and Relative Risk (Males Compared With Females) of Birth Defects by Sex: Florida, 1996–2001

Birth defects	Male		Female		Relative risk (RR)[b]	95% Confidence interval[b]	*P* value[c]
	Frequency	Rate[a]	Frequency	Rate[a]			
Central nervous system defects							
Anencephalus	2	4.2	4	8.4	0.50	0.09	2.73
Encephalocele	0	0.0	0	0.0	na	na	na
Hydrocephalus without spina bifida	9	18.9	13	27.3	0.69	0.31	1.56
Microcephalus	0	0.0	3	6.3	na	na	na*
Spina bifida without anencephalus	4	8.4	6	12.6	0.67	0.19	2.36
Total CNS defects	15	31.5	26	54.5	0.58	0.31	1.07*
Autosomal chromosomal defects							
Down syndrome	5	10.5	10	21.0	0.50	0.17	1.46
Trisomy 13	1	2.1	0	0.0	na	na	na
Trisomy 18	0	0.0	3	6.3	na	na	na*
Total autosomal chromosomal defects	6	12.6	13	27.3	0.46	0.18	1.21
Gastrointestinal defects							
Biliary atresia	0	0.0	1	2.1	na	na	na
Esophageal atresia/tracheoesophageal fistula	0	0.0	1	2.1	na	na	na
Hirschsprung's disease (congenital megacolon)	2	4.2	1	2.1	2.00	0.18	22.06
Pyloric stenosis	27	56.6	5	10.5	**5.40**	**2.14**	**13.60*****
Rectal and large intestinal atresia/stenosis	0	0.0	2	4.2	na	na	na
Total GI defects	29	60.8	10	21.0	**2.90**	**1.44**	**5.84*****
Genital and urinary defects							
Bladder exstrophy	1	2.1	0	0.0	na	na	na
Obstructive genitourinary defect	24	50.3	10	21.0	**2.40**	**1.15**	**5.02*****
Renal agenesis/hypoplasia	2	4.2	0	0.0	na	na	na
Total GU defects	27	56.6	10	21.0	**2.70**	**1.31**	**5.58*****
Heart defects							
Aortic valve stenosis	3	6.3	0	0.0	na	na	na*
Atrial septal defect	60	125.8	69	144.7	0.87	0.63	1.21
Coarctation of aorta	1	2.1	2	4.2	0.50	0.05	5.51
Common truncus	1	2.1	2	4.2	0.50	0.05	5.51
Ebstein's anomaly	0	0.0	0	0.0	na	na	na
Endocardial cushion defect	2	4.2	1	2.1	2.00	0.18	22.06
Hypoplastic left heart syndrome	0	0.0	0	0.0	na	na	na
Pulmonary valve atresia and stenosis	11	23.1	14	29.4	0.79	0.38	1.62
Tetralogy of Fallot	5	10.5	5	10.5	1.00	0.29	3.45
Transposition of great arteries	1	2.1	3	6.3	0.33	0.03	3.20
Tricuspid valve atresia and stenosis	1	2.1	2	4.2	0.50	0.01	5.51
Ventricular septal defect	37	77.6	43	90.2	0.86	0.56	1.32
Total heart defects	109	228.6	111	232.8	0.98	0.77	1.25
Musculoskeletal defects							
Congenital hip dislocation	1	2.1	8	16.8	**0.13**	**0.02**	**0.99****
Diaphragmatic hernia	4	8.4	0	0.0	na	na	na**
Gastroschisis/omphalocele	5	10.5	2	4.2	2.50	0.63	9.99
Reduction deformity: lower limbs	0	0.0	1	2.1	na	na	na
Reduction deformity: upper limbs	0	0.0	3	6.3	na	na	na*
Total musculoskeletal defects	9	18.9	12	25.2	0.75	0.33	1.71
Oral clefts							
Choanal atresia	2	4.2	1	2.1	2.00	0.18	22.06
Cleft lip with and without cleft palate	3	6.3	2	4.2	1.50	0.25	8.98
Cleft palate without cleft lip	2	4.2	4	8.4	0.50	0.09	2.73
Total oral defects	7	14.7	7	14.7	1.00	0.32	2.41
Ear or eye defects							
Aniridia	0	0.0	0	0.0	na	na	na
Anophthalmia/microphthalmia	0	0.0	1	2.1	na	na	na
Anotia/microtia	0	0.0	0	0.0	na	na	na
Congenital cataract	0	0.0	1	2.1	na	na	na
Total birth defects	225	471.9	175	367.0	**1.29**	**1.07**	**1.54*****

[a]Rate: birth defects rate per 10,000 live births.
[b]Significant ($P < .05$) relative risk and confidence interval are indicated in bold type face, those that cannot be calculated are indicated with na.
[c]*P* value is calculated by McNemar test.
*$P = .050–.100$.
**$P = .010–.049$.
***$P < .010$.

may be etiologically related to the development of the male reproductive system and therefore tend to be more prevalent (Lary and Paulozzi, 2001).

Females in the opposite-sex twin population were found to have a higher risk of congenital hip dislocation compared with their twin brothers. This difference may again result from higher testosterone levels in the developing male fetus, which increases the collagen content and fibril diameter of the hip joint (Hama et al., 1998).

The RRs in obstructive genitourinary defects, pyloric stenosis, and congenital hip dislocation were greater in opposite-sex twins than in their counterparts in the general population, although none of these differences were statistically significant.

Sex differences have also been observed before the initiation of gonadal differentiation—for example, in embryonic weight and somite number (Scott and Holson, 1977; Seller and Perkins-Cole, 1987). In this study the male twin was found to have a higher risk of diaphragmatic hernia than his twin sister (8.4/10,000 vs. 0, $P = .04$). This defect usually occurs during weeks 3–7 of gestation. This difference may reflect different susceptibilities to teratogens due to the developmental speed difference between male and female fetuses (Lary and Paulozzi, 2001; Dott et al., 2003).

Male and female twins in the opposite-sex twin population we studied had similar risks of having an atrial or ventricular septal defect, pulmonary valve atresia, and stenosis. This finding suggests that both sexes have a similar cardiac developmental procession and/or similar susceptibility to teratogens.

In a study of curly tail mutant mice, Brook et al. (1994) observed that males were advanced in growth and development relative to their female littermates during neurulation, but the rates of growth and development did not differ. They suggested that the differences in some specific aspects of the neurulation process increase the susceptibility of females for developing neural tube defects. We observed that each of the central nervous system defects affected more females than males (except for encephalocele, which did not occur in either sex), but the difference in the total number of central nervous system defects was marginally significant ($P = .08$).

To our knowledge, this is the first study to observe sex differences in birth defects between opposite-sex twins. By design, our study attempted to exclude all confounding factors that may affect fetal development through the mother. The possibility remains, however, that the intraamniotic environment may differ, because the twins grow in separate amniotic cavities with separate placentas. The impact of this difference on our results is unknown. A major limitation of this study is its small sample size. We examined 6 Florida birth cohorts, totaling more than 1 million infants; however, since the prevalence of opposite-sex twins is ~1% in the general population, the number of birth defects detected was still small, especially for some rare defects. Therefore, differences between the male and female twin siblings for those defects may not have been detected, or the RRs may have been exaggerated. Another limitation is that we only included birth defects detected in the first year of life. Because the detection of birth defects decreases dramatically after 1 year of age, the impact of our cutoff point on the results would likely be small. A third limitation is that the FBDR included only live births. The impact of excluding twins who did not survive is related to the sex ratio of birth defects among stillbirths and abortions. In the general population, this sex ratio (males to females) is close to unity in spontaneously aborted human embryos (Byrne and Warburton, 1987) and stillbirths (McKeown and Lowe, 1951; Naeye et al., 1971; Machin, 1975). However, very little information is available about the sex ratio in birth defects among aborted or stillborn opposite-sex twins. If this sex ratio is close to that in the general population, or if the number of live births is much higher than the number of abortions and stillbirths, the impact on the result would be negligible. A fourth limitation is that many dizygotic twins have a familial propensity to develop birth defects, and therefore the birth defects could be biased by genetic influence. However, the impact of this limitation is expected to be small because we excluded sex chromosomal defects and compared twin siblings to balance the genetic influence of autosomal chromosomes.

In conclusion, this study shows that there are sex differences in birth defects between opposite-sex twins.

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REFERENCES

Brook FA, Estibeiro JP, Copp AJ. 1994. Female predisposition to cranial neural tube defects is not because of a difference between the sexes in the rate of embryonic growth or development during neurulation. J Med Genet 31:383–387.

Byrne J, Warburton D. 1987. Male excess among anatomically normal fetuses in spontaneous abortions. Am J Med Genet 26:605–611.

Carter RL, Grove D. 1999. Assessment of case ascertainment accuracy. Tallahassee, FL: Florida Department of Health, Bureau of Environmental Epidemiology.

Davis DL, Gottlieb MB, Stampnitzky JR. 1998. Reduced ratio of male to female births in several industrial countries: a sentinel health indicator? JAMA 279:1018–1023.

del Rio Gomez I, Marshall T, Tsai P, et al. 2002. Number of boys born to men exposed to polychlorinated byphenyls. Lancet 360:143–144.

Dott MM, Wong LY, Rasmussen SA. 2003. Population-based study of congenital diaphragmatic hernia: risk factors and survival in metropolitan Atlanta, 1968–1999. Birth Defects Res A Clin Mol Teratol 67:261–267.

Fernando J, Arena P, Smith DW. 1978. Sex liability to single structural defects. Am J Dis Child 132:970–972.

Francois I, de Zegher F. 1997. Adrenarche and fetal growth. Pediatr Res 41:440–442.

Fukuda M, Fukuda K, Shimizu T, et al. 2002. Parental periconceptional smoking and male: female ratio of newborn infants. Lancet 359:1407–1408.

Garry VF, Harkins ME, Erickson LL, et al. 2002. Birth defects, season of conception, and sex of children born to pesticide applicators living in the Red River Valley of Minnesota, USA. Environ Health Perspect 110(Suppl 3):441–449.

Gittelsohn A, Milham S. 1964. Statistical study of twins—methods. Am J Public Health Nations Health 54:286–294.

Hama H, Yamamuro T, Takeda T. 1998. Experimental studies on connective tissue of the capsular ligament. Influences of aging and sex hormones. J Exp Zool 281:472–481.

Ibanez L, Potau N, Marcos MV, de Zegher F. 1999. Exaggerated adrenarche and hyperinsulinism in adolescent girls born small for gestational age. J Clin Endocrinol Metab 84:4739–4741.

James WH. 2004. Evidence that intrauterine and postnatal androgens affect the development of pyloric stenosis. Birth Defects Res A Clin Mol Teratol 70:37–39.

Lary JM, Paulozzi LJ. 2001. Sex differences in the prevalence of human birth defects: a population-based study. Teratology 64:237–251.

Li SJ, Ford N, Meister K, Bodurtha J. 2003. Increased risk of birth defects among children from multiple births. Birth Defects Res A Clin Mol Teratol 67:879–885.

Lubinsky MS. 1997. Classifying sex biased congenital anomalies. Am J Med Genet 69:225–228.
Maccoby EE, Doering CH, Jacklin CN, Kraemer H. 1979. Concentrations of sex hormones in umbilical-cord blood: their relation to sex and birth order of infants. Child Dev 50:632–642.
Machin GA. 1975. A perinatal mortality survey in south-east London, 1970–73: the pathological findings in 726 necropsies. J Clin Pathol 28:428–434.
McKeown T, Lowe CR. 1951. The sex ratio of stillbirths related to cause and duration of gestation; an investigation of 7,066 stillbirths. Hum Biol 23:41–60.
Naeye RL, Burt LS, Wright DL, et al. 1971. Neonatal mortality, the male disadvantage. Pediatrics 48:902–906.
Rao PV. 1998. Statistical research methods in the life sciences. Belmont, CA: Duxbury Press. pp. 232–234.
Reyes FI, Boroditsky RS, Winter JS, Faiman C. 1974. Studies on human sexual development. II. Fetal and maternal serum gonadotropin and sex steroid concentrations. J Clin Endocrinol Metab 38:612–617.
Schinzel AA, Smith DW, Miller JR. 1979. Monozygotic twinning and structural defects. J Pediatr 95:921–930.
Scott WJ, Holson JF. 1977. Weight differences in rat embryos prior to sexual differentiation. J Embryol Exp Morphol 40:259–263.
Seller MJ, Perkins-Cole KJ. 1987. Sex difference in mouse embryonic development at neurulation. J Reprod Fertil 79:159–161.
Shaw GM, Carmichael SL, Kaidarova Z, Harris JA. 2003. Differential risks to males and females for congenital malformations among 2.5 million California births, 1989–1997. Birth Defects Res A Clin Mol Teratol 67:953–958.
Szathmari M, Vasarhelyi B, Tulassay T. 2001. Effect of low birth weight on adrenal steroids and carbohydrate metabolism in early adulthood. Horm Res 55:172–178.