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Birth Defects in the Sons and Daughters of Women who were Exposed *in utero* to Diethylstilbestrol (DES)

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Abstract

Background—Prenatal exposure to diethylstilbestrol (DES) is associated with adverse health outcomes, including anatomic anomalies of the reproductive tract in women and of the genitourinary tract in men. The mouse model, which replicates many DES-related effects seen in humans, suggests that prenatal DES exposure causes alterations that may affect the next generation of offspring.

Methods—Women participating in a large multi-center study of prenatal DES exposure were asked to report birth defects occurring among 4,029 sons and 3,808 daughters (i.e., the third generation). A subcohort of 793 third generation daughters were also queried for birth defects. We used logistic regression models to generate odds ratios and 95% confidence intervals for the association between prenatal DES exposure in the mother and birth defects in the offspring.

Results—Based on the mothers' reports, overall birth defects were elevated in the sons (OR = 1.53; 95% CI = 1.04, 2.23) and in the daughters (OR = 2.35; 95% CI = 1.44, 3.82). Most estimates of association were imprecise, but daughters appeared to have an excess of heart conditions (OR = 4.56; 95% CI = 1.27, 16.34.

Conclusions—. Our data suggest a possible association between the mother's prenatal DES exposure and birth defects in their offspring, particularly in daughters. We cannot, however, rule-out the possible influence of reporting bias. In particular, the exposed daughters' elevated risk of cardiac defects may be due to the underreporting of these conditions by unexposed mothers.

Keywords

Diethylstilbestrol; Prenatal exposure; Maternal exposure; Birth defects; Epigenetic alterations

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Introduction

Diethylstilbestrol (DES) is a nonsteroidal estrogen that was used to prevent pregnancy losses and complications, but was subsequently found to be ineffective. From about 1940 through the early 1970s, DES was given to at least two million pregnant women in the US alone (Noller, 1988). The adverse effects of *in utero* DES exposure in women include infertility (Bibbo, *et al.*, 1977; Palmer, *et al.*, 2001; Senekjian, *et al.*, 1988), tissue and structural anomalies of the reproductive tract (Bibbo, *et al.*, 1977; Kaufman, *et al.*, 1980; Senekjian *et al.*, 1988), pregnancy loss, premature delivery (Barnes, *et al.*, 1980; Goldberg & Falcone, 1999; Kaufman *et al.*, 1980; Kaufman, *et al.*, 2000), and a rare vaginal clear cell adenocarcinoma (Herbst, *et al.*, 1971). Possible increased risks of breast cancer in women over age 40 (Palmer, *et al.*, 2006) and squamous neoplasia of the cervix (Hatch, *et al.*, 2001) have also been reported. In prenatally exposed men, associated outcomes include genitourinary anomalies (Bibbo, *et al.*, 1977; Coscrove, *et al.*, 1977; Gill, *et al.*, 1979; Wilcox, *et al.*, 1995) and possibly an increased risk of infertility (Perez, *et al.*, 2005; Wise, *et al.*, 2007) and testicular cancer (Strohsnitter, *et al.*, 2001).

Molecular studies of reproductive tract tissues in female mice indicate that exposure to DES during a critical developmental window results in persistent epigenetic alterations; i.e., changes in gene expression (Mclachlan, *et al.*, 2001; Nelson, *et al.*, 1994). Studies also show a higher frequency of tumors in the male and female offspring of mice that were exposed prenatally to DES (Newbold, *et al.*, 1998; Newbold, *et al.*, 2000; Turusov, *et al.*, 1992; Walker, 1984). A question with widespread implications for environmental contaminants is whether prenatal DES exposure in humans causes epigenetic changes that may be transmitted to subsequent generations (Jablonka & Lamb, 1995).

In the present report, we describe birth defects affecting the offspring of prenatally DES-exposed and unexposed women participating in a large, multi-center cohort study. Our data arise from the only study of third generation outcomes in which the prenatal DES exposure status of the mother was verified by the medical record.

Methods

The DES Follow-up study was approved by the institutional review boards at all participating study centers and at the U.S. National Cancer Institute (NCI).

The data from the present study were based on two sources: 1) mothers' reports of birth defects affecting their offspring (the third generation) and 2) birth defects self-reported by a subset of adult daughters participating in a third generation study.

The DES Combined Cohort Follow-Up Study

In 1992, the US NCI established the DES Follow-Up Study, a combined cohort study of DES health effects. The study combined four cohorts of second generation women; i.e., women who were exposed or unexposed *in utero* to DES as indicated by the medical record. Three of these cohorts had been previously followed and the fourth cohort comprised women whose mothers had participated in a study of health outcomes associated with DES exposure during pregnancy (Troisi, *et al.*, 2007).

In 1994, the first combined cohort questionnaires were mailed to 6,551 second generation women, including 4,459 exposed to DES *in utero*, and 2,092 unexposed. Completed questionnaires were returned by 5,707 women (88% of the exposed and 84% of the unexposed). The mothers' reports of birth defects are based on responses to an open-ended question in the 1997 follow-up questionnaire, which queried second generation women for

birth defects occurring in their offspring, whether living or deceased. The 1997 questionnaire was completed by 3,763 (95.0%) of the parous women enrolled in the combined cohort, including 2,517 (95.5%) exposed, and 1,246 (94.0%) unexposed. These women had 4,029 sons (2,640 exposed, 1,389 unexposed) and 3,808 daughters (2,449 exposed, 1,359 unexposed).

Based on the mothers reports, genitourinary anomalies affecting the sons included horseshoe-shaped kidney, renal agenesis, born with one kidney; penile/testicular defects included hypospadias and testicular atrophy; skeletal anomalies included scoliosis, club foot, polydactyly, torticollis, and hip dysplasia; heart defects included heart murmur, ventricular septal disease, tetralogy of fallot, atrial septal defect, and pulmonic stenosis; neurological anomalies included cerebral palsy, ptosis, and autism; muscle or tissue anomalies included cleft palate, hernia, and torticollis; chromosomal/hereditary syndromes included Down's Syndrome, chrondodystrophy, and adrenoleukodystrophy; eye conditions included amblyopia, cataract, and strabismux; hearing loss was unspecified; gastrointestinal defects included trache-oesophageal fistula/atresia, and intestinal or gall bladder anomalies; miscellaneous conditions (defined as conditions affecting fewer than 5 sons) included benign tumors, cysts, fistulas, skin anomalies, and blood disorders.

Based on mothers' reports, skeletal anomalies affecting the daughters included hip dysplasia, scoliosis, club foot, missing limbs, and extra digits; heart defects included atrial septal defect, and ventricular septal defect; chromosomal/heritable conditions included Down's syndrome, Noonan's syndrome, and Williams syndrome; neurological anomalies included cerebral palsy, and anencephalus; genitourinary anomalies primarily involved the kidney and included double kidney, horseshoe shaped kidney, renal agenesis and dysgenesis, and born with one kidney; skin anomalies included hemangioma; miscellaneous conditions (defined as conditions affecting fewer than 5 daughters) included benign tumors, cysts, cleft palate, anomalies of the eye/vision or ear/hearing, learning disabilities, blood disorders, muscle or musculoskeletal anomalies, and gastrointestinal abnormalities.

The DES Third Generation Cohort Study

In 2001, the NCI established a third generation cohort consisting of adult (age ≥18) daughters of second generation women who had participated in the combined cohort study (Titus-Ernstoff, *et al.*, 2006). A review of parity records at all five study centers identified 763 exposed and 577 unexposed mothers who had 966 exposed and 815 unexposed age-eligible daughters. About half of the mothers, 414 (54.3%) of the exposed and 297 (51.5%) of the unexposed, gave permission to contact 515 (53.3%) exposed and 383 (47.0%) unexposed daughters. Compared to mothers who did not grant permission, those who did had more education and older daughters, but were similar with regard to history of infertility or cancer.

Questionnaire mailings to the third generation women began in August 2000 and were completed in April 2003. Questionnaires were returned by 793 (88%) of the 898 women whose contact information was provided by their mothers, including 463 (90%) exposed and 330 (86%) unexposed. The third generation questionnaire queried women for demographic information, hormonal and reproductive factors, and health conditions, including birth defects. Self-reported birth defects were skeletal anomalies including hip dysplasia and missing forearm; congenital heart conditions including heart murmur and atrial septal defect; chromosomal conditions included Down's syndrome and cystic fibrosis; neurological conditions included cerebral palsy and hemiparesis; miscellaneous conditions (defined as conditions affecting fewer than 5 daughters) included anomalies of the eye, ear, skin, and/or blood, and pyloric stenosis.

Agreement between mothers' and daughters' reports

Agreement between the mothers' and daughters' reports of birth defects was assessed using a Kappa coefficient. Ten daughters who participated in the third generation study were omitted from the agreement analysis because their mothers did not respond to the 1997 questionnaire mailing which queried mothers for birth defects in offspring. Consequently, the analysis of agreement between the two sources of reports on birth defects involved 783 (460 exposed, 323 unexposed) third generation study participants and their mothers. For this analysis, each daughter was represented only once. Mothers were represented multiple times according to the number of daughters in the analysis. We considered mother-daughter dyads in concordance if they agreed on at least one condition. In two dyads, the daughter and mother reported different defects; these were included with the counts of daughters reporting a defect and mothers not reporting a defect. Of the 29 mothers reporting a birth defect in a daughter, 21 (72.4%) daughters reported a similar birth defect. Of the 52 daughters reporting a birth defect, 21 (40.4%) mothers reported a similar condition. The apparent underreporting by mothers, relative to their daughters, was evident even for severe defects. A Kappa test indicated only fair overall agreement between the mothers and daughters (K = 0.49; 95% CI = 0.36–0.63). Agreement was better among the exposed (K = 0.55) than the unexposed (K = 0.38).

Statistical analysis of birth defects

We assessed the presence of birth defects in third generation sons, daughters, and in the combined offspring based on the mothers' reports. We also assessed birth defects as self-reported by daughters participating in the third generation study. Outcomes included any birth defect and groupings comprising the most frequently reported birth defects and those that have been observed in prenatally exposed offspring. We used logistic regression to generate odds ratios (OR) and 95% confidence intervals (CI) for the association between the mothers' prenatal DES exposure status and birth defects in the offspring. These models assumed independence among the study participants, although about a fourth of the mothers had more than one offspring. OR were adjusted for cohort and year of birth, which were identified as potential confounders in preliminary analyses (ORs changed by more than 10%). other's age at the time the child was born was assessed as a potential confounder but did not change the estimates. For the ancillary analysis of tracheo-esophageal fistula or tracheo-esophageal atresia (TEF/atresia), we used an exact binomial test (two-sided) to compare rates among exposed offspring in our study, based on mothers' reports, to the average rates in white live born babies reported by four longstanding birth defects registries in the US: the Virginia CARES program (2.1/10,000 for the birth years 1989–1998) (Virginia CARES, 2006) the California Birth Defects Monitoring program (3.0/10,000 for the birth years 1983–1990) (California Birth Defects Monitoring Program, 2006); the Michigan Birth Defects Registry (3.1/10,000 for the birth years 2000–2002) (Copeland, 2005); and the Texas Birth Defects Registry (2.4/10,000 for the birth years 1999–2002) (Texas Birth Defects Registry, 2005).

Results

Mothers' reports of birth defects in their offspring

Twelve percent of the offspring were less than 10 years of age, 78% were between ages 10 and 29, and 10% were age 30 or more. The age distribution was similar for the sons and daughters, and the exposed tended to be older than the unexposed. The mothers reported 159 sons affected by birth defects, including 115/2,640 (4.4%) sons of prenatally exposed women and 44/1,389 (3.2%) sons of the unexposed (Table 1) Prenatally exposed mothers reported 9 sons affected by more than one birth defect and unexposed mothers reported 2 such sons. The cohort and birth year adjusted OR was 1.53 (95% CI: 1.04, 2.23) for the

association between the mother's DES exposure and any birth defect in the son (Table 2). The most frequently reported anomalies in sons were genitourinary anomalies, skeletal anomalies and heart defects (Table 1). The cohort and birth year adjusted OR was 2.10 (95% CI: 0.89, 4.94) for a genitourinary defect in relation to the mothers' DES exposure. Most of the cases were penile and testicular anomalies, which have been associated with prenatal DES exposure (Bibbo *et al.*, 1977;Coscrove *et al.*, 1977;Gill *et al.*, 1979;Wilcox *et al.*, 1995); the OR was 1.68 (95 % CI: 0.71, 3.99) for penile and testicular anomalies in the third generation sons. The OR for heart defects in sons was 1.05 (95% CI: 0.43, 2.53).. The OR for skeletal defects was 1.70 (95% CI: 0.68, 4.24).

Based on the mothers' reports, 110 daughters were affected by birth defects, including 86/2,449 (3.5%) daughters of prenatally exposed women and 24/1,359 (1.8%) daughters of the unexposed (Table 1). Of the daughters reported to have more than one birth defect, 7 were born to exposed mothers, and one was born to an unexposed mother. The cohort and birth year adjusted OR was 2.35 (95% CI: 1.44, 3.82) for the association between DES exposure in the mother and any birth defect in the daughter (Table 2) The most frequently reported birth defects in daughters were skeletal and heart anomalies (Table 1). The OR for skeletal defects was 2.56 (95% CI: 0.99, 6.57). The OR was 4.56 (95% CI: 1.27, 16.34) for the association between the mother's DES exposure and a heart defect in the daughter. No other associations were noted between categories of defects and the mothers' prenatal DES exposure, although small numbers of outcomes limited our ability to conduct meaningful analyses.

Combining the mothers' reports of sons and daughters, the overall cohort and birth year adjusted OR for any birth defect in the offspring was 1.82 (95% CI: 1.35, 2.45) based on 201/5,089 exposed and 68/2,748 unexposed cases. The adjusted OR was 1.84 (95% CI: 0.91, 3.72) for the association between the mother's DES exposure status and heart defects in the combined offspring based on 38 exposed and 12 unexposed cases. The OR for skeletal defects in the combined offspring was 2.08 (95% CI: 1.08, 4.01). Because an association with TEF/atresia has been reported previously, we assessed this condition in the combined sons and daughters. TEF/atresia occurred in 3 exposed individuals (2 sons, 1 daughter) but in none of the unexposed. The average rate of TEF/atresia in whites based on the four birth defects registries was 2.7 per 10,000 live births. In our data, the observed rate of TEF/atresia in the offspring of exposed women was about twice that expected (i.e., 5.9/10,000), but the difference was not statistically significant ($p = 0.20$).

Birth defects self-reported by third generation women

Most of the third generation study participants (52%) were between ages 18 and 24, 27% were between ages 25 and 29, and 21% were 30 years of age or more. Of the 793 third generation study participants, 52 (6.6%) women, including 34/463 (7.3%) daughters of the exposed and 18/330 (5.5%) daughters of the unexposed, self-reported birth defects (Table 1). Of the 5 women reporting more than one birth defect, 2 were daughters of exposed mothers, and 3 were daughters of unexposed mothers. The OR for any birth defect was 1.46 (95% CI: 0.76, 2.81) (Table 2). As in the mothers' reported data, the most commonly self-reported birth defects among daughters were skeletal and heart anomalies (Table 1). The OR for skeletal defects was 1.13 (95% CI: 0.40, 3.14). The OR for heart defects was 1.39 (95% CI: 0.39, 5.02).

Discussion

Studies in mice indicate that the adverse reproductive tract outcomes associated with prenatal DES exposure may be mediated by alterations in the expression of genes involved in estrogen signaling/regulation or patterning of the reproductive tract (McLachlan *et al.*,

2001; Nelson *et al.*, 1994). Mouse studies also suggest that epigenetic alterations may be transmitted to the next generation, although the effects in the prenatally exposed females may differ from those in their daughters. For example, an excess of uterine adenocarcinoma is seen in the prenatally exposed mice and in their daughters, but infertility affects only the prenatally exposed females (Newbold, *et al.*, 1998).

In this study, based on mothers' reports, the overall proportion of offspring affected by birth defects (3.7%) resembled rates reported for US whites (3.5%) (Texas Birth Defects Registry, 2005). Although our data suggested that birth defects may be elevated in the daughters of prenatally DES-exposed women, we did not observe a pattern of defects consistent with those reported in the prenatally exposed offspring. For example, in the third generation sons, we found limited support for an association between the mothers' prenatal exposure and penile/testicular defects, which may affect the prenatally exposed men (Bibbo *et al.*, 1977; Coscrove *et al.*, 1977; Gill *et al.*, 1979; Wilcox *et al.*, 1995). Although the present study suggested a possible excess of cryptorchidism, this finding was compatible with chance. None of the mothers reported sons affected by epididymal cysts, which may affect men with prenatal DES exposure (Bibbo *et al.*, 1977; Coscrove *et al.*, 1977; Gill *et al.*, 1979; Wilcox *et al.*, 1995). An excess of hypospadias has been reported for sons of prenatally exposed women (Klip, *et al.*, 2001), but DES exposure was unverified in that study, and the association was unconvincing in the NCI combined cohort study (OR: 1.7; 95% CI: 0.4, 6.8) (Palmer, *et al.*, 2005).

None of the mothers reported daughters affected by reproductive tract anomalies, including T-shaped uterus and abnormalities of the cervix, outcomes associated with prenatal DES exposure in women (Shapiro & Slone, 1979). However, for the most part, such conditions would become evident only when the daughter underwent a work-up for infertility or reproductive dysfunction. Less than half of the women in the third generation study had reached age 18 as of 1997, when the mothers were asked to report birth defects in their offspring; consequently, the study population may have been too young to manifest such outcomes, even if an association existed. A clinical study of the adult daughters of prenatally DES-exposed women did not identify gynecological anomalies (Kaufman & Adam, 2002), but the sample was small (n = 28) and certain conditions, such as structural abnormalities of the uterus or fallopian tubes, might not be evident on physical examination. Further study of the third generation will be needed to determine whether the reproductive tract toxicity observed in prenatally DES-exposed women also affects their daughters.

Based on the mothers' reports, our data suggested an association between the mothers' prenatal DES exposure and heart conditions in the daughters. However, this association may be an artifact of underreporting of these conditions by the unexposed mothers. Population-based birth defects registries indicate that rates of cardiac defects are similar for males and females (Texas Birth Defects Registry, 2005). In our data, the proportions affected were similar for the exposed sons (0.7%), unexposed sons (0.6%) and exposed daughters (0.8%). The proportion was markedly reduced, however, in the unexposed daughters (0.2%), consistent with mothers' under-reporting of heart defects in this group. There are no previous reports of cardiac defects in the offspring of women who were exposed prenatally to hormones or specifically to DES. An early cohort study (Heinonen, *et al.*, 1977) and a case-control study based on birth certificate data (Janerich, *et al.*, 1977) suggested a two-fold increase of congenital heart disease in individuals who were prenatally exposed to exogenous female hormones (any type), but the findings were not corroborated by a meta-analysis of prospective studies assessing the effects of prenatal exposure to oral contraceptives (Bracken, 1990). A syndrome involving vertebral anomalies, anal atresia, cardiac defects, TEF, renal anomalies, and limb reduction (VACTERL) has been postulated in relation to prenatal exposure to estrogen/progestogen (Nora & Nora, 1975), but evidence

supporting the association is mixed (Shapiro & Slone, 1979). A recent study of third generation offspring showed an increased risk of TEF in third generation offspring (Felix, *et al.*, 2007), but this association was not clear in our data.

In this study, the higher prevalence of defects in the exposed offspring, based on the mothers' data, may reflect reporting bias. Participation bias is less likely, as participation was good among the mothers and the question on birth defects was not included in the first combined cohort questionnaire. The overall percent of defects was higher in the daughters' self-reported data than in the mothers' data, which might reflect more thorough reporting by the daughters, or perhaps a tendency of mothers to grant permission to enroll daughters who had birth defects. The proportions of exposed and unexposed women affected by birth defects were more similar in the daughters' self-reported data than in the mothers' data, possibly because reporting bias was minimized in the daughters' data. Twenty-eight percent of the daughters of DES-exposed women were unaware of their exposure, and 60% of the daughters of unexposed women either were not sure of their mothers' exposure or believed their mothers were DES-exposed (Titus-Ernstoff *et al.*, 2006). Finally, prenatal DES exposure in women is associated with infertility and adverse pregnancy outcomes, whereas all of the women who contributed daughters to the Third Generation Study and most of the women reporting on their offspring had at least one live birth. If these women were less impacted by prenatal DES exposure, any potential association between DES and conditions in the next generation might be attenuated.

We found poor agreement between the mothers' and study participants' reports of birth defects, primarily due to fewer reports by the mothers, who omitted even severe birth defects reported by the daughters. Possibly, mothers may have been less likely than daughters to report conditions that were not apparent at the time of birth. Anomalies such as a missing forearm, club foot, and cleft palate, which would have been immediately evident, were among those conditions reported by both the mother and the daughter. In contrast, more than half of daughters' reports of hip anomalies, which might become apparent weeks or months following delivery, were not replicated by the mother. Nevertheless, in the absence of a gold standard, we cannot say for certain whether birth defects were under-reported by the mothers or over-reported by the daughters.

In conclusion, our data raise the possibility that the offspring of prenatally DES-exposed women may have an increased frequency of birth defects. We did not observe a pattern of defects resembling those observed in the prenatally exposed men or women, although reproductive tract changes in third generation women might not become evident until childbearing ages. We cannot exclude the possibility that our findings were distorted by bias. In particular, the excess of cardiac defects in the exposed daughters may reflect an underreporting of such conditions by unexposed mothers rather than a true excess in the daughters of the exposed.

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Table 1

The number of sons and daughters affected by birth defects* as reported by the mothers and self-reported by adult daughters participating in the third generation study.**

	Mothers' Prenatal DES Exposure Status	
Birth Defects	**Exposed**	**Unexposed**
Mothers' Reports		
Sons	**n = 2,640**	**n = 1,389**
Any birth defect	115	44
Genitourinary	32	7
Penile/testicular	27	7
Skeletal	21	7
Heart	18	9
Neurological	10	8
Muscle/tissue	10	5
Chromosomal/hereditary	9	5
Eye	6	2
Hearing loss	4	1
GI	4	0
Miscellaneous	8	1
Daughters	**n = 2,449**	**n = 1,359**
Any	86	24
Skeletal	25	6
Heart	20	3
Mothers' Reports		
Daughters		
Chromosomal/hereditary	12	5
Neurological	8	1
Genitourinary	3	2
Skin anomalies	3	2
Miscellaneous	21	6
Daughters Self-report	**n = 463**	**n = 330**
Any	34	18
Skeletal	11	8
Heart	10	4
Chromosomal/hereditary	2	3
Neurological	5	0
Miscellaneous	8	7

* For more information about specific birth defects, please see methods.

** The number of offspring with specific categories of defects do not sum to the number with any defect because some individuals have more than one defect.

Table 2

The association between the mothers' prenatal DES exposure and the presence of a birth defect in third generation sons and daughters, based on the mothers' reports and on self-reports in the subgroup of adult daughters participating in the third generation study.

	Mothers' Prenatal DES Exposure		
Any Birth Defect	**Exposed**	**Unexposed**	
	n/N (%)	n/N (%)	OR (95% CI)*
Mothers' report			
Sons	115/2640 (4.4%)	44/1389 (3.2%)	1.53 (1.04, 2.23)
Daughters	86/2449 (3.5%)	24/1359 (1.8%)	2.35 (1.44, 3.82)
Sons and Daughters	201/5089 (3.9%)	68/2748 (2.5%)	1.82 (1.35, 2.45)
Self-report			
Daughters	34/463 (7.3%)	18/330 (5.5%)	1.46 (0.76, 2.81)

*OR adjusted for cohort and birth year.