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Systematic Review

SYSTEMATIC REVIEW

Systematic Review and Meta-analysis of Testicular Germ Cell Tumors Following In Utero Exposure to Diethylstilbestrol

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Abstract

Background: Early exposure to estrogen-like compounds has been implicated in the etiology of testicular cancer, but individual level epidemiologic data addressing this hypothesis are scarce. The synthetic estrogen diethylstilbestrol (DES) was administered during pregnancy from 1948 to 1971, but sequelae of in utero exposure have been more extensively characterized in females than in males.
Methods: By systematic review, we sought to identify all epidemiologic research relating testicular cancer to a history of in utero exposure to diethylstilbestrol. Identified studies were critically appraised to assemble a set of nonredundant data in which any in utero exposure to DES was compared between men with incident testicular cancer and cancer-free men. These data were synthesized using random effects meta-analysis to estimate the summary association between in utero DES exposure and testicular cancer.
Results: By meta-analysis of data from the six qualifying studies, the summary odds ratio estimate of the in utero DES-testicular cancer association was 2.98 (95% confidence interval = 1.15 to 7.67).
Conclusions: Results of this comprehensive meta-analysis accord with a threefold increase in testicular cancer risk among men who were exposed in utero to DES, implicating early hormonal exposures in etiology of testicular cancer. Because use of DES ceased in 1971, this work may provide the most comprehensive estimate of this association that will be made.

The synthetic estrogen diethylstilbestrol (DES) was synthesized and chemically characterized in the early 1930s (1). Beginning in 1947, DES was prescribed to pregnant women in the United States in an effort to prevent threatened and recurrent miscarriage. This practice began after abnormally low urinary levels of steroid hormones were observed in women who experienced toxemia of pregnancy, premature delivery, or fetal demise (2). Although research conducted in the 1950s found that DES was ineffective in preventing either miscarriage or premature birth (3–5), DES continued to be used in pregnancy until suspicion of harmful effects arose in the early 1970s. An estimated 1.8 to 10 million Americans were exposed to DES either as pregnant women or during in utero development (6,7) before the US Food and Drug Administration ultimately banned use in pregnancy in 1971 (8).

The harbinger of reproductive harms to women who had been exposed in utero, now termed *DES daughters*, was a report in 1970 describing six women diagnosed with vaginal clear cell adenocarcinoma (CCAC) diagnosed at the uncharacteristically young ages of 14 to 21 years. All six were daughters of women to whom DES had been administered in pregnancy (9); DES was immediately implicated because CCAC is extremely rare and previously had primarily affected elderly women. Observational studies published in the subsequent 3 years (10–13) documented

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a pronounced excess of CCAC in adolescent and young adult women who had been exposed in utero.

Active screening of DES daughters began in 1972 with the immediate objective of early detection of CCAC. Unexpectedly, this effort revealed high prevalence of vaginal adenosis and numerous structural abnormalities of the reproductive tract (6). Consequent review of autopsy material established elevated prevalence of vaginal adenosis even in DES-exposed female fetuses and live born infants who did not survive the neonatal period (14), heightening suspicion that key pathologic processes had occurred before birth. By 1979 these non-neoplastic conditions and CCAC had been shown to be most strongly associated with DES exposure by 7 weeks of gestation in a dose-dependent fashion (15), and in utero exposure to DES was widely believed to cause a constellation of abnormalities of the female reproductive tract. This interpretation was strengthened by two additional types of research: experimental studies demonstrated that female mice exposed in utero to DES develop many abnormalities of DES daughters (16), and cancer surveillance studies documented CCAC epidemics in birth cohorts of women born in the years DES was prescribed (17,18).

The influence of DES on males exposed in utero, or *DES sons*, has been less thoroughly investigated, although such exposure has been implicated in testicular germ cell tumors (TGCT) and at least two associated conditions: cryptorchidism (19) and hypospadias (20,21). Increasing TGCT incidence in successive birth cohorts of the early twentieth century has long implicated in utero exposure to unrecognized exogenous compounds (22), and the specific hypothesis that in utero exposure to DES could be a cause was articulated by 1977 (23) and addressed in a series of epidemiologic studies. Several reported more frequent DES exposures in utero among men diagnosed with TGCT than among cancer-free men, but no result achieved statistical significance. Detection of excess TGCT attributable to DES would a priori require studies far larger than those that implicated DES in CAAC of young women, because TGCT occurred in adolescent and young adult males even before DES was prescribed. Thus, results of the epidemiologic studies of TGCT could plausibly reflect either inadequate statistical power to detect a positive association between DES exposure and TGCT predicted by the DES hypothesis, or DES having no etiologic role. Results of two other lines of research accord with plausibility of the DES hypothesis. Epidemiologic studies identified associations between DES exposure in utero and both cryptorchidism and hypospadias (24–26). And elegant molecular studies targeting expression of the hormone insulin-like 3 defined a mechanism whereby DES administered early in development can cause cryptorchidism in rodent models (27,28).

Testicular cancer incidence rates continue to rise, and although progress toward identifying environmental causes has been limited, several lines of reasoning have implicated exposure, including estrogen-like compounds, early in development (29). If DES exposure in utero were indeed a cause of testicular cancer, the broader hypothesis that in utero exposure to exogenous estrogens can cause TGCT would warrant further investigation. We therefore sought anew to learn whether DES sons experienced elevated risk of testicular cancer. We systematically reviewed epidemiologic studies of testicular cancer that might have enrolled DES-exposed males, critically appraised identified reports, and conducted meta-analysis of relevant data. Because most men born before 1971 have now surpassed ages when TGCT is characteristically diagnosed, this synthesis likely represents most if not all epidemiologic data that will address this question.

Methods

We used the Population, Intervention, Comparison, Outcome method to develop the focused research question addressed in the primary meta-analysis. We defined population as adolescent and young adult men, the sex- and age- group among whom TGCT arises; intervention as in utero exposure to DES; comparison group as cancer-free men and outcome as incident TGCT. For a secondary meta-analysis, described in Supplemental Materials (available online), we redefined intervention as in utero exposure to either DES or other exogenous hormones. A study providing original data was approved by the University of Southern California Institutional Review Board, and the systematic review was exempt from institutional review board oversight owing to the de-identified, summary nature of published data. The review and analysis were implemented according to Meta-analyses of Observational Studies in Epidemiology guidelines (30).

Systematic Review

We queried PubMed without language restrictions from inception to August 1, 2018, to find articles identified by any of the keywords “DES,” “diethylstilbestrol,” or “stilbestrol” together with any of “testis cancer,” “testis neoplasm,” “testicular cancer,” or “testicular neoplasm.” We sought additional data sources by querying presentations at scientific conferences, catalogued theses, and dissertations and monographs published by the International Agency for Research on Cancer of the World Health Organization and by surveying established TGCT researchers. Reports meeting inclusion criteria described below were subjected to review of cited references and citation searches implemented using Web of Science, whether identified by the initial search or additional procedures. Identified reports were captured in an Excel spreadsheet, with those identified by multiple searches reduced to unique entries. The search was designed and overseen by an epidemiologist experienced in systematic review.

Title and abstract of each report were reviewed independently by a PhD epidemiologist and an MD to identify studies that could potentially satisfy inclusion criteria. Minimum requirements were 1) inclusion of men with documented occurrence of incident TGCT and a cancer-free comparison group and 2) assessment of whether participants were exposed in utero to DES. We excluded studies without human subjects and case reports. Two investigators independently reviewed full text of the remaining reports to identify those that met inclusion criteria and to eliminate redundant inclusion of data presented in multiple reports. All discrepancies were resolved by consensus.

Original Data Analysis

Data from a population-based case-control study described elsewhere (31) were provided by Dr Leslie Bernstein and analyzed separately to estimate the DES-TGCT association for inclusion in the meta-analysis. In brief, we conducted a case-control study of testicular cancer in which cases who were 18–35 years of age when diagnosed with their cancer were individually matched on age and race and/or ethnicity to cancer-free controls. Following in-person interviews, participants for whom it was feasible, and who agreed, provided their mother’s contact information so that their mothers could be interviewed by

telephone. Participating mothers (87 case mothers and 139 control mothers) responded to a structured questionnaire providing information about their exposures during the index pregnancy, including whether they had used DES during any part of that pregnancy (yes or no), their reproductive history, and their own and their sons' health history.

We fit a multivariable logistic regression model to the data, estimating the odds ratio (OR) and its 95% confidence interval (CI) for the association between use of DES during the index pregnancy and incident testicular cancer. We addressed missing values of DES exposure by several standard methods (removing the relevant participants, scoring them as unexposed, assigning exposure status of matched case); from estimates of the DES-TGCT association calculated by each method, we selected for the primary meta-analysis the value nearest 1.0, as a conservative measure. The analytic model included terms for strata defined by race and/or ethnicity (Hispanic white, non-Hispanic white), and sons' age (5-year intervals) at time of cases' TGCT diagnosis and for the proposed or demonstrated TGCT risk factors of maternal age at sons' birth, sons' diagnosis of cryptorchidism, and history of TGCT in a first-degree relative.

Harmonization of Data

Incident TGCT was the outcome variable used in all studies included in the meta-analysis. Histologic subtype was not available for most studies found to meet inclusion criteria, so all histologies were considered together. Ever vs never use of DES during pregnancy was the only measure of DES exposure commonly reported for all studies, so we used any use of DES during the pregnancy to define the exposure of interest. Ratio estimates of the association between DES exposure in pregnancy and incident TGCT were extracted from published reports, when available, or estimated in univariate form from tabular data extracted from published reports or in multivariate form for original data as described above. Inputs for the meta-analysis were point and 95% confidence interval estimates, as appropriate for each contributing study, for the hazard ratio, incidence rate ratio, or odds ratio association between any in utero exposure to DES and incident TGCT of any histologic type. These and additional data describing study characteristics were independently extracted by the reviewers who subsequently compared all entries and resolved discrepancies by discussion and consensus.

Meta-Analysis

We initially analyzed data from contributing studies using a fixed effect model. This allowed us to implement from tabular data the inverse opposite sample size continuity correction (32) required for the two studies with a zero cell count (33,34) (Table 1). We carried forward point and interval estimates into a re-analysis of data from the six contributing studies using a random effects (DerSimonian-Laird) model, and then confirmed compatibility of results from both models. To display results from the random effects model, we created a forest plot stratified on study design (longitudinal randomized trial and cohort studies; case-control studies) displaying each study's contribution to summary estimates. Heterogeneity within these strata and overall was characterized using appropriate *P* values and I^2 statistics (35). A second stratified analysis was performed to compare summary results from a synthesis of univariate vs adjusted estimates of original data. To gauge the likelihood of

Table 1. Studies included in the meta-analysis

Studies	Location	Study design	Years of birth	Years of TGCT diagnosis	Age at TGCT diagnosis, y	TGCT cases controls				Covariates* addressed	Ratio estimate (95% CI)
						DES +	DES -	DES +	DES -		
Vessey 1983 (33)	London, UK	RCT	1952–1953	Until 1982	Unrestricted	1	0	137	126	BY, CO	2.76 (0.11 to 68.37)
Strohsnitter 2001 (41) (Mayo)	Rochester, MN	Cohort	1940–1960	Until 1994	Unrestricted	5	1	655	591	BY, AG, CO, BW, PA, MB	4.53‡ (0.63 to 107.9)
Strohsnitter 2001 (41) (Horne,WHS†)	Boston, MA Portland, ME Hanover, NH	Cohort	1940–1960	Until 1994	Unrestricted	1	1	499	614	BY, AG	1.17‡ (0.03 to 45.76)
Depue 1983 (34)	Los Angeles County	Pop-based Case-control	1943–1963	1973–1979	16-30	2	106	0	107	BY, AG, RE	4.50 (0.21 to 94.82)
Moss 1986 (42)	Northern California	Mixed Case-control	1940 or later	1976–1981	18 or older	4	216	2	222	AG, RE	2.06 (0.37 to 11.34)
Bernstein original data	Los Angeles County	Pop-based Case-control	1951–1973	1986–1991	18-35	5	82	2	137	BY, AG, CO, RE	3.96‡(0.72 to 21.87)
Totals						18	410	1295	1798		

*Covariates address in design or analysis. AG = age; BW = birth weight; BY = birth year; CI = confidence interval; CO = cryptorchidism; DES = diethylstilbestrol MB = mother's history of breast cancer; PA = parity at index birth; TGCT = testicular germ cell tumor; RE = race and/or ethnicity.
†Women's Health Study
‡Estimated by multivariate analysis.

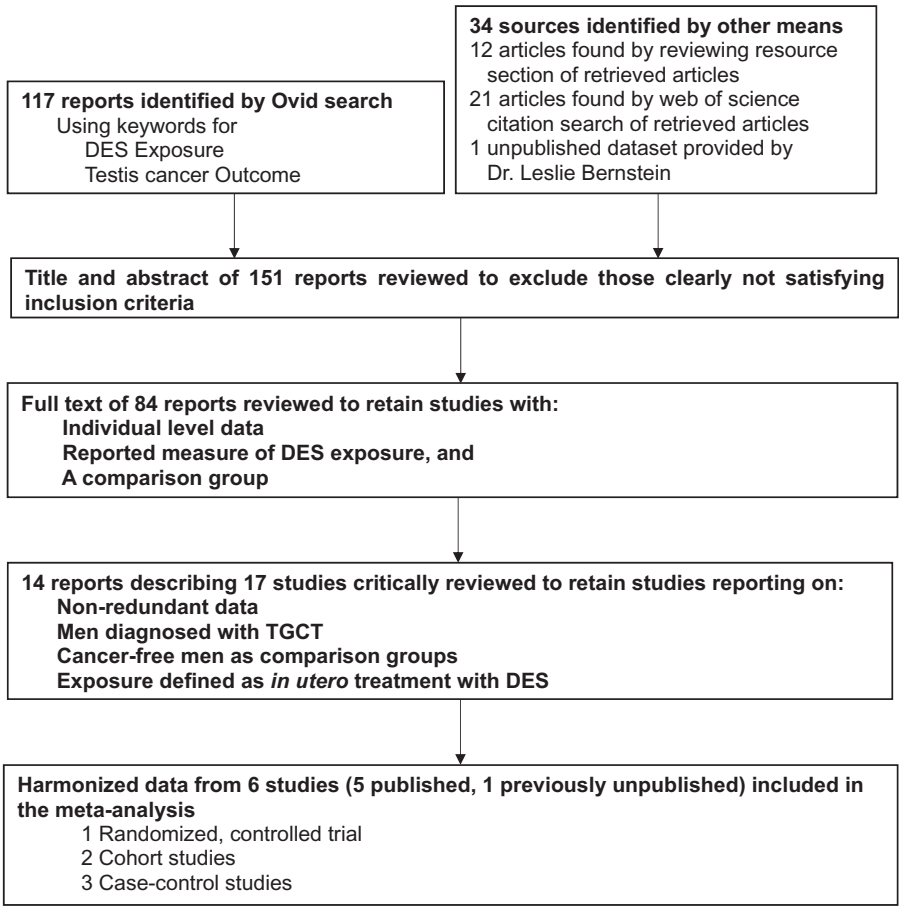

Figure 1. Flow of information through the systematic review and meta-analysis. DES = diethylstilbestrol; TGCT = testicular germ cell tumor.

publication bias, we generated a funnel plot and conducted a cumulative meta-analysis ordered on the publication year of each report. To examine the impact of individual studies on summary estimates, we conducted a cumulative meta-analysis ordered on study weight (largest to smallest) and an influence analysis in which summary estimates were calculated after individually excluding data from each study. Finally, to explore sensitivity of the summary estimate to preferential recall of DES exposure by mothers of men diagnosed with TGCT, we conducted sensitivity analyses in which we recoded a DES-exposed case from each case-control study as unexposed and recalculated the summary estimate after substituting results from the recoded data, one by one into the meta-analysis. All analyses were implemented using Stata 14.2 (College Station, TX).

Results

Systematic Review

The keyword search identified citations for 117 unique articles. By review of titles and abstracts, we determined that 51 addressed original data involving humans. We retrieved corresponding articles and identified 12 additional reports of potential relevance in cited references. A citation search of these 63 articles identified 21 additional publications. By full text review of these 84 articles, we identified 14 reports of 17 studies in which history of DES exposure in utero had been assessed in men who developed TGCT and a comparison group. Data described in 10 of these reports (Supplementary Table, available online) (23,24,36–41,53,54) were excluded from the primary meta-analysis, in full or in part, for one or more of the following reasons. We suspected (23) or confirmed (37,41) that three articles were redundant reports of data published elsewhere. Four reports described studies without any occurrence of TGCT (23,24,41,53). Two studies used cancer patients rather than cancer-free men as the comparison group (36,38). Four papers did not report on use of DES separately from use of other drugs or hormones during pregnancy; of these, one publication (39) specified that the exposure measures could not be further separated, and our efforts to communicate directly with authors of the other three (36,40,54) confirmed that this was also so for two others (36,54). The remaining four reports (33,34,41,42) described five studies for which data were judged to meet inclusion criteria. Estimates of the DES-TGCT association from these studies were carried forward to the meta-analysis, together with a sixth estimate calculated from original data (L. Bernstein, unpublished) that satisfied inclusion criteria (Table 1). Movement of published and unpublished data through the search, appraisal, and primary meta-analysis is illustrated in Figure 1.

Among studies selected for the primary meta-analysis, one is a randomized trial that had been conducted to assess efficacy of DES for prevention of miscarriage and premature birth, two are cohort studies that had been conducted to learn of possible harms of DES exposure in utero, and three are case-control studies of testicular cancer in which mothers were asked

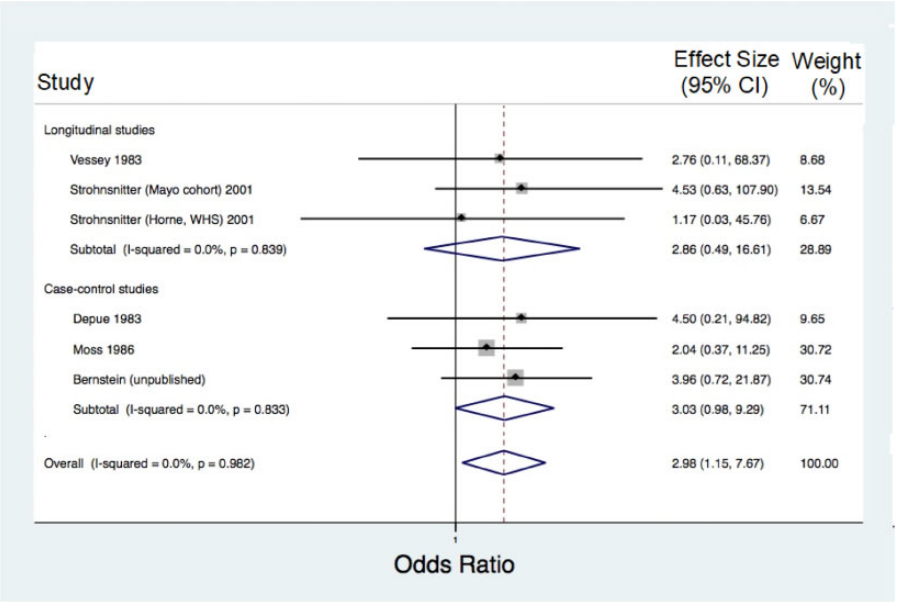

Figure 2. Forest plot displaying results from random effects meta-analysis of six studies of the association between history of in utero exposure to DES and incident TGCT, stratified by study design and ordered within stratum by year of publication. **Centers of squares** and **horizontal bars** through each indicate point and 95% CI estimates of individual studies. **Area of squares** indicate relative weights of individual studies. **Vertical and horizontal apices of diamonds** indicate point and interval estimates of stratum-specific and overall summary estimates. CI = confidence interval; DES = diethylstilbestrol; TGCT = testicular germ cell tumor; WHS = Women's Health Study.

whether they took DES during pregnancies that produced the participating men.

The primary meta-analysis synthesized data from a total of 3521 men, of whom 428 had been diagnosed with TGCT and 3093 were free of cancer at completion of the respective studies. The Supplementary Table (available online) enumerates reports on DES and TGCT that did not contribute to the meta-analysis, with reasons for their exclusion.

Original Data

By multivariate analysis of original data provided for the study, we found a reported history of having been exposed in utero to DES to be associated with incident testicular cancer, although the estimate did not achieve statistical significance (OR = 3.96, 95% CI = 0.72 to 21.9).

Primary Meta-analysis

We estimated the summary association between any in utero exposure to DES (vs no exposure) and incident TGCT from six studies meeting inclusion criteria (OR = 2.98, 95% CI = 1.15 to 7.67). Estimates were similar for subsets of data defined by study design for longitudinal (OR = 2.86, 95% CI = 0.49 to 16.61) and for case-control (OR = 3.03, 95% CI = 0.98 to 9.29) studies (Figure 2), and for subsets defined by form of model used in original data analysis for univariate (OR = 2.51, 95% CI = 0.65 to 9.71) (33,34,42) and for multivariate (OR = 3.50, 95% CI = 0.93 to 13.17) analyses (41).

The analyses reveal no evidence of influence apart from random error on distribution of study results (I^2 = 0.0%; P = .98, overall). We found no indication that the DES-TGCT association arose from publication bias, because the funnel plot displays no overabundance of positive results among smallest studies (lower-right vs lower-left regions of the funnel; Figure 3), and

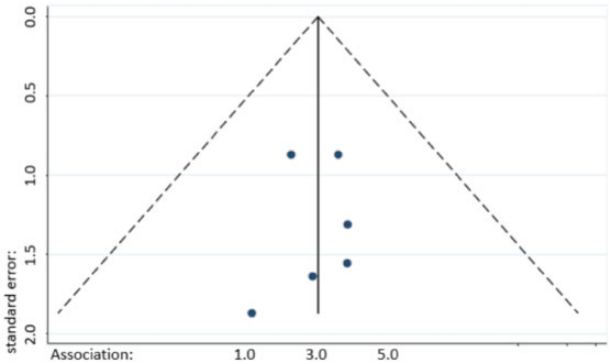

Figure 3. Funnel plot displaying dispersion of data from six studies contributing to the primary meta-analysis. **Dots** indicates point estimates and accompanying standard errors of estimated association between history of in utero exposure to diethylstilbestrol and incident testicular germ cell tumor from the six contributing studies. **Vertical line** indicates summary estimate of the association, and **dotted black lines** indicate pseudo 95% confidence intervals.

point estimates from the meta-analysis cumulated by year of publication are positive at each step of the synthesis (Supplementary Figure 1, available online).

The point estimate from each contributing study was greater than 1.0, but results of individual studies did not achieve statistical significance, likely because of limited size of each. The meta-analysis that cumulated data according to study weight (Supplementary Figure 2, available online) and influence analysis (Supplementary Figure 3, available online) show that the summary estimate is robust to absence of data from any study except the original data. Finally, sensitivity analyses recoding as unexposed the individual exposed cases in case-control studies indicate that modest recall bias is unlikely to explain the positive summary estimate (data not shown).

Conclusions

The meta-analysis reveals a threefold positive association between a history of DES exposure in utero and subsequent occurrence of testicular cancer. Contributing studies, identified by systematic review completed in 2018, may constitute the final set of nonredundant high-quality human data addressing this hypothesis, because most TGCT is diagnosed between 15 and 45 years of age and DES treatment of pregnant women ceased in 1971.

Strengths of the research are use of this comprehensive collection of relevant data and the practice of strictly defining the independent variable as in utero exposure to DES for the primary analysis. The study is limited by the number of exposed men with TGCT included, small compared to many meta-analyses. Nonetheless, results are statistically robust in that meta-analysis cumulated on study weight shows that only the three largest studies are needed to achieve statistical significance; influence analysis shows that no one study is responsible for the positive direction of the association; and heterogeneity parameters indicate that random error alone can explain the distribution of results of individual studies. The original studies were conducted after some harms associated with DES exposure in utero were known. Nevertheless, recall bias seems unlikely to explain the observed association, not only because stratum-specific analysis shows little difference between summary associations estimated in the retrospective studies in which recall bias can potentially arise, and in the longitudinal studies in which it cannot, but also because a positive summary association persisted in sensitivity analyses designed to attenuate potential influence of recall bias. DES was prescribed in viable pregnancies primarily for threatened or recurrent miscarriage and premature delivery, but also more rarely for other conditions including toxemia and diabetes (43). We are not aware of data implicating the rarer indications in TGCT. Preterm birth has been suggested as a TGCT risk factor, but based largely on sparse data from the DES era (39,44). Yet, to entirely rule out that confounding by this historic indication could explain the DES-TGCT association, preterm birth would need to be evaluated in cohorts born after 1971 using studies that address potential confounding by cryptorchidism, which is associated with both preterm birth and subsequent risk of TGCT (19). Conventional confounding seems an unlikely explanation for the DES-TGCT association, because although analysis of some smaller studies did not include adjustment for covariates in the models, in these studies important potential confounders were addressed by matching or randomization, and because summary estimates were similar for strata containing these studies and studies subjected to multivariate analysis. No evidence of publication bias is shown by the funnel plot or meta-analysis cumulated on year of publication.

Data harmonization did not allow us to consider either histologic subtype of TGCT or details about DES treatment. Regimens of DES treatment during pregnancy varied considerably according to route of administration, dose, duration, and timing (45). The association between in utero DES exposure and cryptorchidism is reportedly due largely to exposure before the eleventh week of gestation (25). If any influence of DES on TGCT risk were similarly confined to a specific period of gestation, our analysis may have underestimated the magnitude of the association of TGCT with DES exposure in the relevant period.

DES is an extensively characterized compound with biological properties that lend plausibility to the hypothesis of testicular carcinogenesis following in utero exposure. In rodents, DES administered prenatally crosses the placenta to preferentially accumulate in the reproductive tract (46), and in a high proportion of male offspring, results in gonadal abnormalities. These include preneoplastic changes and cryptorchidism (43), a condition that is highly associated with TGCT in humans (19). Additionally, in vitro treatment of Syrian hamster embryo cells induces aneuploidy but not DNA damage (47), corresponding to the state of DNA in TGCT nuclei, which unlike that of most other cancers is highly aneuploid with rare somatic mutations (48).

Further observations underscore relevance of the DES-TGCT association to the broader hypothesis that in utero exposure to other estrogens may also cause TGCT. Mice with targeted disruption of the estrogen receptor alpha are not subject to well-described detrimental influences of DES on development of both male and female urogenital structures, indicating that this estrogen receptor mediates at least some teratogenic effects of DES on the reproductive tract (49,50). Moreover, like DES, estradiol has been shown to induce aneuploidy but little DNA damage (47). Finally, historic use of DES in pregnancy, which was documented or reported for exposed participants in studies included in the meta-analysis, points to vulnerability to such influences during the in utero period.

DES has been classified as a group 1 human carcinogen by the International Agency for Research on Cancer expert review starting in 1979 (45,51,52), based on occurrence of breast cancer in women exposed while pregnant and both vaginal and cervical adenocarcinoma in women exposed in utero. Whether DES exposure in utero is associated with subsequent occurrence of TGCT has remained conjectural. Based on fewer sets of human data than those examined here, even the most recent panel (45) concluded that "Because the DES-exposed men now have passed the age of highest risk for testicular cancer, the question of an association is likely to remain unanswered." For the first time, this association has now achieved statistical significance owing to systematic search for scholarly sources, fortuitous access to previously unpublished data, and quantitative synthesis by meta-analysis. We conclude that events in gestation, including exposure to compounds with DES-like properties, may contribute to TGCT etiology.

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