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Age at menarche in Seveso daughters exposed in utero to 2,3,7,8-tetrachlorodibenzo-p-dioxin

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Background: In utero exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is associated with delayed pubertal development in animal studies. No epidemiologic study has investigated this association. We examined the relationship of in utero exposure to TCDD with reported age at onset of menarche in female children born to a unique cohort of TCDD-exposed women resulting from an explosion in Seveso, Italy, on 10 July 1976.

Methods: In 2014, nearly 40 years after the explosion, we enrolled postexplosion offspring, 2 to 39 years of age, in the Seveso Second Generation Study. Age at onset of menarche (years) was collected for 316 daughters by maternal report or self-report at interview. In utero TCDD exposure was defined by maternal TCDD serum concentrations extrapolated to the pregnancy.

Results: At interview, 287 daughters were postmenarche and reported age at menarche averaged 12.1 years (±1.3 years). Overall, we found no change in risk of menarche onset with a 10-fold increase in in utero TCDD exposure (hazard ratio [HR] = 0.86; 95% confidence interval [CI] = 0.71, 1.04). When we considered maternal menarche status in 1976 as a potentially sensitive developmental exposure window, in utero TCDD (log_{10}) was associated with later age at menarche among daughters whose mothers were premenarche (HR = 0.71; 95% CI = 0.52, 0.97) but not postmenarche (HR = 0.89; 95% CI = 0.71, 1.12) at the time of the explosion (*P* int = 0.24).

Conclusions: These results suggest that in utero TCDD exposure may alter pubertal timing among daughters of women who were prepubescent at the time of the Seveso accident.

Keywords: Dioxin; 2,3,7,8-Tetrachlorodibenzo-p-dioxin; In utero exposure; Menarche; Seveso

Introduction

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the most toxic member of a class of planar halogenated aromatic hydrocarbons; it is a ubiquitous environmental contaminant with potent endocrine-disrupting effects.[1–3] Given its decade-long half-life, TCDD can be detected in the lipid stores of humans, and fetal exposure has been shown to occur through transplacental transfer.[4–6] In animal studies, perinatal dioxin exposure alters offspring pubertal development and ovarian function. In rodents, in utero and lactational TCDD exposure is associated with delays in pubertal development (e.g., delayed vaginal opening, altered vaginal estrous cyclicity[7,8]) and effects on ovarian function.[7,9] A similar spectrum of effects has been reported in studies with in utero exposure to other dioxin-like compounds, including polychlorinated dibenzo-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs).[10–13]

To date, no prior epidemiologic studies have examined the association of in utero TCDD exposure with age at menarche of offspring of those exposed. Three studies, however, have examined the relation of dioxin-like compounds exposure to pubertal development, with inconsistent conclusions. Perinatal PCDD/PCDF exposure (measured in breast milk) was associated with delayed initiation of breast development but no difference in age at menarche in the Netherlands birth cohort.[14] In utero PCB exposure (as measured by maternal serum PCB levels extrapolated to pregnancy) was not associated with age at menarche in daughters of the Michigan anglers cohort.[15] Finally, in utero exposure to 2,2,4,4,5,5 hexabromobiphenyl (as measured by maternal serum polybrominated biphenyl (PBB) levels extrapolated to pregnancy) was associated with earlier age at menarche of daughters of Michigan women who had consumed PBB-contaminated food in 1973 (Michigan PBB cohort).[16]

On 10 July 1976, an explosion at a chemical factory near Seveso, Italy, resulted in a toxic plume that exposed nearby downwind residents to high levels of TCDD.[17–19] The Seveso

What this study adds

In the second generation of the Seveso Women's Health Study cohort, in utero 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) exposure was associated with later age at menarche among daughters whose mothers were still prepubescent at the time of the Seveso accident. Epigenetic mechanisms potentially could explain the observed intergenerational effects and apparent multiple susceptible windows of exposure and warrant further investigation. This is important given that effects of early life exposures on age at menarche can have important implications for conditions diagnosed later in life, such as breast cancer and cardiovascular disease.

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Women's Health Study (SWHS), initiated in 1996, is a cohort of 981 female residents who were exposed to a high dose of TCDD during or before their childbearing years.[20,21] Previously in SWHS, we found that serum TCDD levels were not associated with age at onset of menarche in the 282 women who were prepubescent at the time of the 1976 explosion.[22] Among those who were younger than 5 years old, however, serum TCDD levels were nonsignificantly associated with earlier age at menarche.[23] In 2014, nearly 40 years after the explosion, we followed up the SWHS cohort and enrolled their postexplosion offspring in the Seveso Second Generation Study. Here, we examine the relationship of in utero TCDD exposure with reported age at onset of menarche in daughters in the Seveso Second Generation cohort.

Methods

Study population

Details of the SWHS and the Seveso Second Generation Study have been previously presented.[21,24] Briefly, the SWHS cohort, initiated in 1996, includes 981 women who were newborn to 40 years of age in 1976, resided in the most highly contaminated areas (zone A or B) at the time of the explosion, and had adequate stored sera collected soon after the explosion in which to measure individual-level TCDD exposure. Enrollment and data collection in the Seveso Second Generation Study took place between May 2014 and June 2016. Eligible participants included SWHS women and their children who were born after the explosion (10 July 1976) and were at least 2 years of age. Questionnaire data were complete for a total of 677 children (341 females, 336 males) born to 438 mothers. Of the 341 female children (75.3% of 453 eligible), 25 were less than 8 years and therefore not asked about menarche, leaving an analysis sample of 316 daughters, ranging in age from 8 to 39 years at interview, born to 271 mothers.

Procedure

The study was approved by the Institutional Review Boards of participating institutions. Before data collection, we obtained written informed consent from children 18 years or older and mothers of children less than 18 years, written assent from children who were 13 to 17 years, and oral assent from children who were 7 to 12 years of age. Data collection for all second-generation participants included a fasting blood draw, anthropometric measurements, interview, and medical record abstraction. Children 18 years or older completed a structured personal interview; for children younger than 18 years, the mother completed a structured personal interview which included questions about the health of her children including puberty. Children 10 to 17 years also completed an online self-administered questionnaire, which included questions about puberty status. Information collected during the interview included sociodemographic and lifestyle characteristics as well as reproductive and medical histories. Age at menarche was determined from the question "At what age (in years) did you (your daughter) get your (her) first menstrual period?"

In utero TCDD exposure

In utero TCDD exposure was defined by maternal TCDD serum concentrations estimated at pregnancy. For all SWHS participants, TCDD was measured in archived sera collected soon after the explosion by high-resolution gas chromatography/high-resolution mass spectrometry methods.[25] Details of 1976 serum TCDD measurements are presented elsewhere.[20,21] For those SWHS participants who reported a live birth between 1994 and 2014, TCDD was also measured in archived sera (n = 312) collected at the 1996 or 2008 follow-up study by high-resolution gas chromatography/isotope-dilution high-resolution mass spectrometry methods.[26] Details of TCDD concentrations measured in 1996 or 2008 serum are presented elsewhere.[24,27] All values are reported on a lipid weight basis as picograms per gram lipid or parts per trillion (ppt).[28] Nondetectable values were assigned a value of one half the detection limit.[29]

We estimated in utero TCDD exposure by extrapolation from the maternal serum TCDD concentration nearest to but preceding the pregnancy (1976, 1996, 2008) using a first-order kinetic model with a half-life that varied with initial dose, age, and other covariates.[24,27] For the study sample, maternal TCDD at pregnancy is based on extrapolation from maternal serum TCDD concentrations measured in samples collected in 1976, 1996, and 2008 for 230, 83, and 3 daughters, respectively.

Statistical analyses

In utero TCDD exposure was $\log_{10}$-transformed to reduce the influence of outliers. We used Cox proportional hazards models to evaluate the relationship between in utero TCDD exposure and age at onset of menarche; all participants entered the risk pool at age 7, and failed at age at menarche or were censored at age at interview if they had not reached menarche. We evaluated the appropriateness of a Cox proportional hazards model by testing the proportional hazards assumption for the overall models, as well for the TCDD exposure terms. We also evaluated the shape of the exposure-response curves using generalized additive models (GAMs) with a 3-degree of freedom cubic spline. The proportional hazards model was not violated for the overall models or the TCDD exposure term. As we did not find evidence of nonlinearity, all models presented use linear regression with continuous exposures.

Based on our review of the literature, we considered the following variables collected at interview as potential confounders: maternal age at menarche, maternal age at explosion, maternal age at pregnancy, maternal smoking at pregnancy, maternal education, household socioeconomic status including maternal or primary wage earner education, occupation, income, and marital status, child birthweight (low/normal), birth decade, childhood adiposity (child body mass index [BMI]). The final set of covariates was determined using a directed acyclic graph (DAG) (eFigure 1; http://links.lww.com/EE/A103) and included maternal age at menarche (continuous) and primary wage earner's education (categorical, less than or equal to required, secondary school, greater than secondary). We considered effect modification by maternal menarche status at the time of the explosion, a potentially sensitive developmental window of exposure[30–32] by performing stratified analyses, as well as performing formal tests for interaction using cross-product terms. Interactions with P values <0.2 were considered significant.

We performed several sensitivity analyses. In some preliminary models, maternal age at menarche violated the proportional hazards assumption. Thus, we considered several alternate methods of parameterizing the variable: as either a time-varying covariate or a cubic spline or by stratifying the baseline hazard. We reran analyses using parametric survival regressions with a 2-parameter Weibull distribution, and others using a discrete-time survival model.[33] We repeated the final models including the following additional covariates in separate models: year of birth (as a continuous variable or a cubic spline), cohort birth period (1976–1984, 1985–1994, 1995–2008), child BMI category at interview, and maternal education (instead of primary wage earner's education). Finally, we limited the analysis to the subset of daughters (N = 200) who were the first-born child after the explosion. For all models, standard errors were estimated using the robust Huber-White sandwich estimator and a clustered sandwich estimator of variance was used to account for nonindependence of sibling clusters. All statistical analyses were performed using STATA 15.0 (StataCorp L.L.C., College Station, TX).

RESULTS

Select characteristics of the 316 daughters born postexplosion to 271 Seveso mothers are presented in Table 1. The majority (228, 84%) of mothers had one daughter in the study population, 41 (15%) had two daughters, and 2 (0.7%) had three daughters. Almost two thirds (63%) of daughters in the sample were the first born after the explosion. At the time of the explosion in 1976, mothers were an average age of 15.6 years (±7.8 years) and about one third were still premenarche. Maternal age at menarche averaged 12.5 years (±1.6 years) but was higher among mothers who were premenarche (12.9 ±1.7 years) compared with postmenarche (12.3 ±1.5 years) at the time of the explosion. Maternal age at pregnancy averaged 29.5 years (±4.9 years), 10% of women reported smoking during the pregnancy, and 7% of births were low birthweight. At the time of interview, daughters were an average of 24.2 years (±8.9 years), ranging from 8 to 39 years. About half (51.3%) of the households' primary wage earners had more than the required education. Maternal education was similar, with about half exceeding the required education.

Table 1.

Select characteristics of Seveso daughters exposed in utero to TCDD, Seveso Second Generation Study, Italy, 1976–2016.

Characteristic	N (%)
Total mothers	271 (100.0)
Maternal age at explosion (years)	
0–10	68 (25.1)
11–15	70 (25.8)
16–20	58 (21.4)
21–33	75 (27.7)
Maternal menarche status at explosion	
Premenarche	95 (35.1)
Postmenarche	176 (64.9)
Total daughters	316 (100.0)
Postexplosion birth order	
1	200 (63.3)
2	103 (32.6)
3+	13 (4.2)
Maternal age at pregnancy (years)	
<25	45 (14.2)
25–29	116 (36.7)
30–34	102 (32.3)
35+	53 (16.8)
Maternal smoking during pregnancy	
No	283 (89.6)
Yes	33 (10.4)
Birthweight	
≥2,500 g	293 (92.7)
<2,500 g	23 (7.3)
Age at interview (years)	
8–12	42 (13.3)
13–17	49 (15.5)
18–24	67 (21.2)
25–29	50 (15.8)
30–34	54 (17.1)
35–39	54 (17.1)
Menarche status at interview	
Premenarche	29 (9.2)
Postmenarche	287 (90.8)
Reported age at menarche (years)[a]	
<12	90 (31.4)
12–13	147 (51.2)
≥14	50 (17.4)
Primary wage earner education	
Less than or equal to Required (less than High School)	154 (48.7)
Secondary (High School/Technical School)	146 (46.2)
Greater than Secondary (Bachelors or higher)	16 (5.1)
Maternal education	
Less than or equal to Required (less than High School)	158 (50.0)
Secondary (High School/Technical School)	148 (46.8)
Greater than Secondary (Bachelors or higher)	10 (3.2)

[a]Of postmenarche daughters n = 287.

A total of 287 (90.8%) daughters had reached menarche based on self-report (n = 275) or maternal report (n=12) at interview. The reported age at menarche averaged 12.1 years (±1.3 years) with a range from 9 to 16 years, comparable to other studies in Northern Italy.[34–36] Almost one third (31.4%) of daughters had early menarche (younger than 12 years) and 17% had late menarche (14 years of age or older). Average time to recall was 13.4 years (±8.0 years), but there was no correlation between length of recall and age at menarche ($r = -0.04$, $P = 0.50$). Maternal age at menarche was moderately correlated with daughter's age at menarche ($r = 0.26$, $P < 0.001$). Consistent with the secular decline in age at menarche reported in Italy and other countries,[36,37] the mean difference in age at menarche between mothers and daughters was 0.26 years.

With birth years spanning from 1976 to 2008, in utero TCDD exposure (median, interquartile range) based on maternal estimated TCDD at pregnancy was 15.5 ppt (6.6, 35.2 ppt) for the daughters, but with a wide range (0.9, 679 ppt). Maternal estimated TCDD at pregnancy was higher among daughters whose mothers were postmenarche (median = 21.6 ppt) than among those whose mothers were premenarche at the time of the explosion (median = 7.9 ppt). Maternal estimated TCDD at pregnancy was also higher among daughters whose mothers were older at the time of the explosion and who were oldest at interview (30+ years) because they were born sooner after the explosion (within one half-life) but did not differ by other factors.

Adjusted hazard ratios (HR) from Cox proportional hazards models for age at menarche in Seveso daughters by in utero TCDD exposure, overall and stratified by maternal menarche status at explosion, are presented in Figure 1. Overall, we found no change in risk of menarche onset with a 10-fold increase in in utero TCDD exposure (adjusted HR = 0.86; 95% confidence interval [CI] = 0.71, 1.04). When we considered effect modification by maternal menarche status at exposure, in utero TCDD ($\log_{10}$) was associated with later age at menarche among daughters whose mothers were premenarche (HR = 0.71; 95% CI = 0.52, 0.97) but not postmenarche (HR = 0.89; 95% CI = 0.71, 1.12) at the time of the explosion (P int = 0.24).

Results of sensitivity analyses are presented in eTable 1; http://links.lww.com/EE/A103. Although the magnitude of the hazard ratios changed across models (especially when using discrete-time survival models or restricting the sample to the first-born child), the overall findings were unchanged.

DISCUSSION

Overall, we found no association between in utero TCDD exposure and reported age at onset of menarche of daughters in the Seveso Second Generation cohort. When we considered maternal menarche status at the time of the 1976 explosion, however, we found associations with later age at menarche in daughters whose mothers were premenarche, but not postmenarche, at the time of the explosion. These results suggest that in utero TCDD exposure may alter pubertal timing among daughters of women who were prepubescent at the time of the Seveso accident.

To our knowledge, this is the first epidemiologic study to examine the relationship of in utero TCDD exposure with age at onset of menarche. Our overall finding of no association with age at menarche is generally consistent with previous studies of in utero exposure to other dioxin-like compounds, PCDD/PCDFs[14] and PCBs,[15] but not to PBBs.[16] Previous studies did not examine maternal menarche status or other developmental windows of maternal exposure.

Our findings are biologically plausible, as animal studies report delays in pubertal development with in utero TCDD exposure.[7,8] It is unclear why we observe later age at menarche

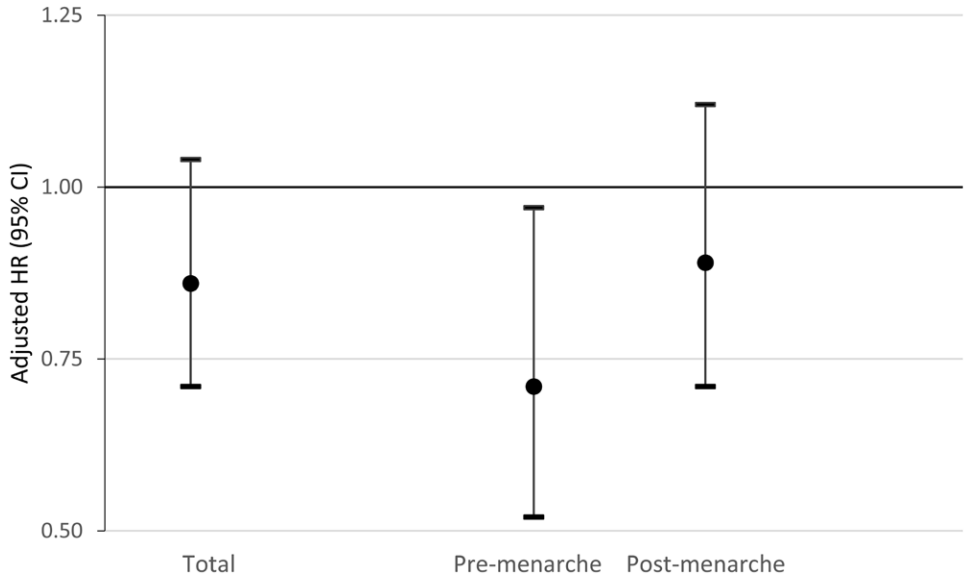

Figure 1. Adjusted hazard ratios from Cox proportional hazard models for age at menarche in Seveso daughters by in utero TCDD exposure (log_{10}) overall and by maternal menarche status in 1976, Seveso Second Generation Study, Italy, 1976–2016. Adjusted for maternal age at menarche and primary wage earner education.

with in utero TCDD exposure only among daughters whose mothers were prepubertal at explosion. Epigenetic mechanisms potentially could explain the observed intergenerational effects and apparent multiple susceptible windows of exposure.[32] There may be differential susceptibility of germ cells, depending on maternal pubertal status. Perhaps the window of susceptibility for "infant" eggs (before puberty) does not have the same body protections against TCDD exposure as after puberty. Alternatively, it could be a spurious finding. Future studies including the SWHS granddaughters could shed light on potential genetic susceptibility and epigenetic mechanisms.

This study has several strengths and limitations. Strengths include the Seveso Second Generation study represents a unique cohort with a relatively large sample. We measured initial TCDD exposure in maternal serum collected near the time of the explosion, and there was a wide range of exposure in the population. We were also able to consider a wide range of potential confounders including maternal age at menarche, and the study population is relatively homogeneous, minimizing potential uncontrolled confounding. Study limitations include in utero TCDD exposure was extrapolated from maternal serum TCDD measurements to the pregnancy, not measured directly, but we expect any exposure misclassification to be nondifferential. We were unable to consider other sources of environmental TCDD exposure,[1] but we expect any exposure misclassification would be nondifferential. Age at menarche was based on self-report or maternal report, and the wide age range of participants led to recall over a variable time period (0 to 29 years); however, we found no correlation between length of recall and age at menarche. Previous studies have found moderate to high agreement of age at menarche as reported in adulthood with the true age at menarche.[38,39] BMI at the time of menarche could be a confounder. Although it was not possible to measure BMI near the time of menarche, a sensitivity analysis that included current BMI as a covariate did not change the results. Finally, the small number of daughters (n = 22) born to Seveso mothers who were younger than 5 years of age at the time of the explosion prevented us from examining this potentially susceptible subgroup separately.[23]

In summary, in the Seveso Second Generation Study, we found that in utero TCDD exposure, as measured by maternal serum TCDD estimated at pregnancy, is not associated with reported age at onset of menarche of daughters. However, among daughters whose mothers were still premenarche at the time of the explosion, in utero TCDD exposure is associated with later age at menarche. This finding is important given that the effect of early life exposures on age at menarche can have important implications for conditions diagnosed later in life, such as breast cancer and cardiovascular disease.[40] Future follow-up of the second generation and the grandchildren of the Seveso Women's Health Study could be informative.

Conflicts of interest statement

The authors declare that they have no conflicts of interest with regard to the content of this report.

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