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Original Contribution

Maternal Smoking During Pregnancy and Timing of Puberty in Sons and Daughters: A Population-Based Cohort Study

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Because early puberty has been linked to diseases later in life, identification of modifiable causes of early puberty is of interest. We explored the possible associations between maternal smoking during pregnancy and pubertal development in sons and daughters. Between 2012 and 2017, 15,819 children from the Danish National Birth Cohort, born during 2000–2003, provided half-yearly information on puberty from the age of 11 years. We estimated adjusted age differences (in months) at attaining various pubertal milestones, including Tanner stages, per 10 daily cigarettes smoked in the first trimester of gestation. In sons, exposure to smoking in utero was associated with earlier genital development (Tanner 2, −1.3 months, 95% confidence interval (CI): −2.5, 0.0; Tanner 5, −3.7 months, 95% CI: −5.3, −2.0), pubic hair development (Tanner 2, −1.8 months, 95% CI: −2.9, −0.6; Tanner 5, −2.9 months, 95% CI: −4.2, −1.7), and voice break (−2.4 months, 95% CI: −3.6, −1.3). In daughters, maternal smoking was associated with earlier breast development (Tanner 2, −3.4 months, 95% CI: −5.3, −1.5; Tanner 5, −4.7 months, 95% CI: −6.5, −2.9), pubic hair development stages 3–5 (Tanner 5, −2.5 months, 95% CI: −4.1, −1.0), and menarche (−3.1 months, 95% CI: −4.0, −2.3). Fetal exposure to tobacco smoke might advance timing of puberty in boys and girls.

maternal exposure; menarche; prenatal exposure delayed effects; puberty; sexual development; sexual maturation; smoking; tobacco smoking

Abbreviations: BMI, body mass index; DNBC, Danish National Birth Cohort.

The timing of puberty has become earlier during the last century in girls, but it remains unsettled whether this also happened to boys (1, 2). A decline is a potential source of concern, given that early puberty is related to several adult diseases, such as obesity, diabetes mellitus, cardiovascular disease, breast cancer, and testicular cancer (3–6). Modifiable causes of early puberty might, therefore, provide another avenue for prevention of some chronic diseases.

Maternal smoking during pregnancy might be such a modifiable cause of early puberty. Maternal smoking during pregnancy has been related to other markers of reproductive health in sons and daughters, such as poor semen quality and reduced fecundability (7). Likewise, maternal smoking during pregnancy could advance timing of puberty through one or more mechanisms. First, tobacco smoke contains several toxic compounds (8), which might result in androgenization of the fetal hormonal milieu (9–11), leading to altered timing of puberty in rodents (12, 13), but results from observational studies have been conflicting (14–16). Second, these toxic tobacco compounds might also alter expression of genes involved in neuronal development due to changes in DNA methylation observed in fetuses of women who smoke during pregnancy (17). Because genes involved in timing of puberty are expressed mainly in the neural tissue, this provides a second potential mechanism for advanced timing of puberty (18). Third, maternal smoking during pregnancy might also advance timing of puberty through low birth weight and childhood obesity, which are both associated with advanced pubertal development (19–22).

A widely used marker of pubertal development in girls, age at menarche, has been reported to occur earlier in daughters of smoking mothers than in daughters of nonsmoking mothers in some studies (16, 23–31), whereas other studies reported no association (29, 32, 33) or even later age at menarche (34–36). Only a few studies in sons have been published,

and some have indicated younger age at voice break, regular shaving, first ejaculation, and acne, although these studies were either low in power or prone to recall bias of the pubertal milestones (37–39). Other important markers of puberty are less well studied. These markers include breast and pubic hair development in daughters (26, 37, 40) and genital and pubic hair development in sons (39, 41).

In this cohort study, we used detailed information on various markers of puberty, collected half-yearly during puberty, and detailed information on smoking collected during pregnancy. The aim was to investigate whether maternal smoking during pregnancy is associated with earlier timing of puberty in their sons and daughters.

METHODS

Study population

This population-based cohort study is based on the Puberty Cohort, a subcohort of the Danish National Birth Cohort (DNBC). The DNBC holds information on approximately 92,000 mothers and their children born during 1996–2003 (42). Mothers were interviewed twice during pregnancy and at 6 and 18 months postpartum. Additionally, they completed questionnaires when the children were 7 years and 11 years of age.

Children eligible for participation in the Puberty Cohort were live-born singletons from the DNBC, born during 2000–2003, whose mothers had participated in the first pregnancy interview and had not withdrawn from the DNBC before May 2012 (n = 56,641). We sampled 22,439 children and invited them to give half-yearly information on puberty through web-based questionnaires from the age of 11.5 years to full maturity (defined as Tanner stage 5 for both pubic hair development and breast or genital development) or 18 years of age, whichever came first. From August 2012 to March 2017, 14,756 children returned at least 1 questionnaire. Furthermore, 10,665 of the 22,439 invited children gave information on puberty in the DNBC's 11-year follow-up, which had similar questions on puberty to the Puberty Cohort. When this information was added, a total of 15,819 children (7,696 sons and 8,123 daughters) participated in the Puberty Cohort (participation rate 70%) and returned on average 5.3 (range, 1–11) questionnaires (Figure 1). In total, 83,810 questionnaires were returned. The participants were by March 2017 between 14 and 17 years of age.

Maternal smoking during pregnancy

The main exposure was smoking during the mother's first trimester of pregnancy. In the first 3 interviews in the DNBC, the women were asked: "Did you smoke during pregnancy?", "Do you smoke now?", "Did you have periods during your pregnancy where you did not smoke (for at least one week)?", "In which weeks of gestation did you not smoke?" (yes/no for each of week 1 through 42), and "How much did you smoke on average?" (number of daily cigarettes). From this information, we derived the variables that described average daily smoking in the first trimester (gestational week 1–12) and throughout pregnancy: continuous and categorical (nonsmoker, light-smoker (1–10 daily cigarettes), heavy-smoker (>10 daily cigarettes)).

Timing of puberty

The outcome was age at attaining various pubertal milestones. We used a translated version of the questionnaire used in the British Avon Longitudinal Study of Parents and Children (41). The questionnaire included the following items: menarche (yes/no; if yes, then year and month), first ejaculation of semen (yes/no; if yes, then year and month), voice break (yes—sometimes, yes—definitive changes, no), axillary hair (yes/no), and acne (yes/no). The children also provided information on their current pubertal stage in terms of Tanner stages 1–5 for each of the following: pubic hair and genital development for boys and pubic hair and breast development for girls (43, 44). To collect information on Tanner stages, we used the Sexual Maturation Scale, which uses illustrations of each of the 5 Tanner stages assisted by a short description of each stage (45). The children were asked to indicate which of the 5 illustrated Tanner stages best represented their current pubertal stage. The questionnaire is available in Danish (46).

Covariates

Identification of potential confounders was guided by directed acyclic graphs (47). The potential confounders considered were prepregnancy body mass index (BMI), alcohol consumption in the first trimester, time to pregnancy (including assisted reproductive technology), parity, maternal age at delivery, maternal age at menarche, highest social class of parents, and cohabitation of parents during pregnancy. Confounders were categorized as shown in Table 1. We retrieved parity and maternal age at delivery from the Danish Medical Birth Registry and highest social class of parents from Statistics Denmark; the latter was based on the International Standard Class of Occupation and Education codes (ISCO-88 and ISCED). Information on all other potential confounders were retrieved from the DNBC provided by the women during pregnancy. Paternal smoking in the first trimester, duration of exclusive breastfeeding, exposure to postnatal smoking (defined as maternal smoking in the first 6 postnatal months), and childhood BMI at 7 years were retrieved from the DNBC, and birth weight was retrieved from the Danish Medical Birth Registry.

Statistical analysis

Analyses were performed using Stata MP, version 13.1 (StataCorp LLC, College Station, Texas). Because we had half-yearly information on puberty, the outcomes were either left, interval, or right censored; the outcome was left censored when the milestone was already attained by the first questionnaire, interval censored when the pubertal milestone was attained between 2 questionnaires, and right censored when the milestone was not attainted by the last questionnaire (Web Table 1, available at https://academic.oup.com/aje). Therefore, we fitted a multivariable regression model for interval censored data, assuming normally distributed residuals, using Stata's intreg package. The exposure, maternal smoking in the first trimester, was first included in categories, with nonsmoking as the referent, and plotted on a graph to visually inspect a potentially dose-dependent pattern. Then, maternal smoking in the first trimester

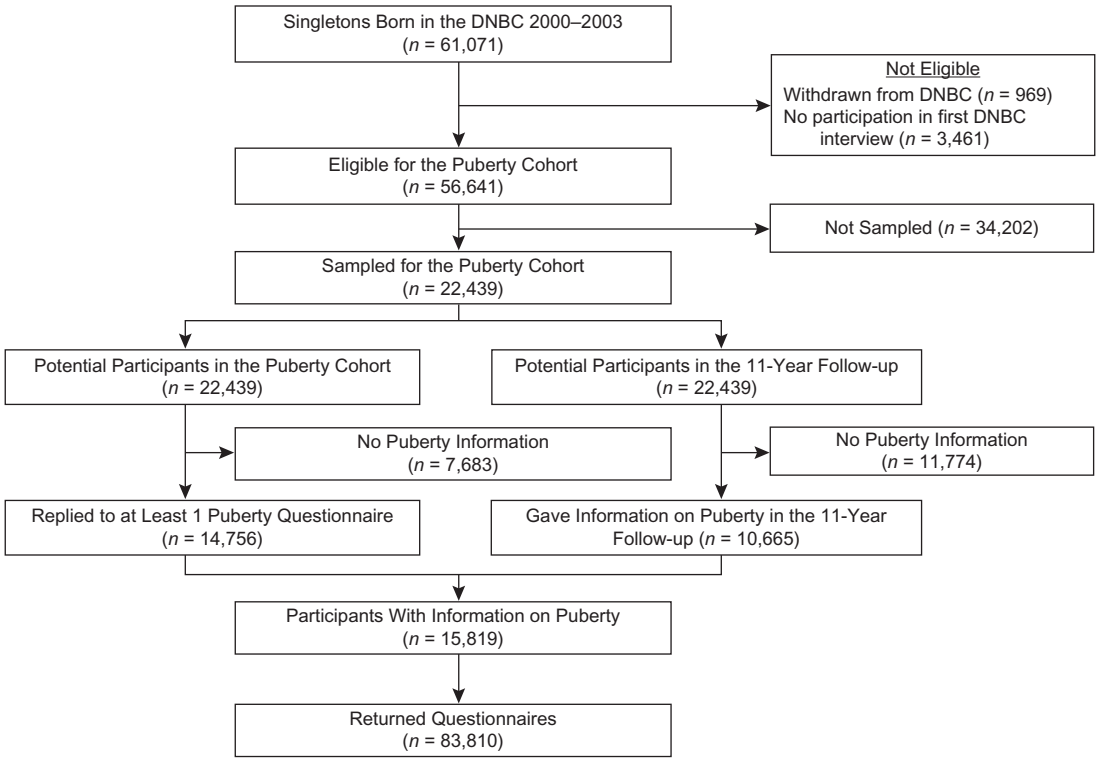

Figure 1. Flow diagram of participants in the Puberty Cohort, Danish National Birth Cohort (DNBC), Denmark, 2000–2017.

was included as a continuous variable in units of 10 daily cigarettes to estimate the difference in age at attaining each pubertal milestone (in months) per 10 daily cigarettes smoked by the mother in the first trimester as a test for trend. Maternal age was introduced as a second-order polynomial variable to allow for departure of linearity.

We performed 6 subanalyses. First, a maternal-paternal comparison was performed, where paternal smoking was intended to be a negative control (48). This was performed by using an exposure variable consisting of 4 groups of parental smoking during first trimester: "No parent smokes," "only mother smokes," "only father smokes," and "both parents smoke." Second, we conducted a multidimensional bias analysis for unmeasured confounding under different scenarios for age at voice break in sons and age at menarche in girls (for a detailed description see Web Appendix 1) (49). Third, we analyzed smoking throughout the entire pregnancy (continuous), rather than solely in the first trimester, as the exposure of interest. Fourth, fifth, and sixth, we further adjusted for childhood BMI, exposure to postnatal smoking, and duration of exclusive breastfeeding. To evaluate the risk of bias due to missing information on these 3 variables, we also restricted the main analysis to having nonmissing information on any of these 3 variables and compared the results with those from the main analysis.

Normality of the interval-censored residuals was checked in R x64, version 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria), by fitting a stepwise cumulative incidence function, using the nonparametric distribution estimator in the icenReg package. The nonparametric distribution was compared visually with the cumulative incidence function based on the normal distribution. Further, we stratified the plots by levels of covariates to check that the mean and standard deviation of the residuals were independent of the covariates.

We used inverse probability weights to account for both the sampling approach applied in the Puberty Cohort (see Web Appendix 2 and Web Table 2 for detailed description) and potential selection bias due to nonparticipation. In short, we sampled participants for the Puberty Cohort from 12 prenatal exposures hypothesized to be important for the timing of puberty, including maternal smoking during pregnancy, supplemented with a random sample of 8,000 children. From the sampling fractions for each exposure group, we derived sampling weights corresponding to the inverse probability of being sampled. To account for selection bias due to nonparticipation, we created selection weights (50), which estimate the inverse probability of participation and were estimated using a multivariable logistic regression model. The variables used to estimate the selection weights were a priori believed to be important for participation: maternal smoking and alcohol consumption in the first trimester, prepregnancy BMI, paternal smoking in the first trimester, parity, maternal age at delivery, maternal age at menarche, highest social class of parents, and cohabitation of parents during pregnancy. Finally, the selection weights were multiplied by the sampling weights and included in the analyses. All models were fitted using robust standard errors to take into account the weighting approach and clustering of siblings. Estimates were computed with 95% confidence intervals.

Table 1. Background Characteristics According to Maternal Smoking in the First Trimester of Pregnancy for 15,766[a] Children in the Puberty Cohort, Danish National Birth Cohort, Denmark, 2000– 2017

Background Characteristic	Smoking in the First Trimester							
	Nonsmoker (n = 11,347)		1–10 Daily Cigarettes (n = 3,512)		>10 Daily Cigarettes (n = 907)		Missing	
	No.	%	No.	%	No.	%	No.	%
Maternal characteristics								
Prepregnancy BMI[b]							217	1.4
<18.5	683	6.1	303	8.8	69	7.8		
18.5–24.9	6,973	62.2	2,139	62.0	504	56.8		
25.0–29.9	2,414	21.5	679	19.7	205	23.1		
≥30.0	1,139	10.2	331	9.6	110	12.4		
Alcohol in the first trimester, units/week[c]							4	0.0
0	5,780	51.0	1,864	53.1	502	55.3		
0.1–1.0	3,724	32.8	988	28.1	204	22.5		
1.1–3.0	1,339	11.8	441	12.6	116	12.8		
>3.0	500	4.4	219	6.2	85	9.4		
Paternal smoking in the first trimester							9	0.1
No	8,927	78.7	1,674	47.7	349	38.5		
Yes	2,413	21.3	1,837	52.3	557	61.5		
Time to pregnancy (including ART)							44	0.3
0 month	2,312	20.4	574	16.4	137	15.1		
1–2 months	2,232	19.7	586	16.8	121	13.4		
3–5 months	1,870	16.5	565	16.2	109	12.0		
6–12 months	1,460	12.9	440	12.6	132	14.6		
>12 months	825	7.3	312	8.9	102	11.3		
ART	1,186	10.5	240	6.9	37	4.1		
Not planned	1,434	12.7	781	22.3	267	29.5		
Parity							0	0.0
First child	5,571	49.1	1,989	56.6	372	41.0		
Second child or later	5,776	50.9	1,523	43.4	535	59.0		
Maternal age at delivery, years[d]	30.8 (4.2)		29.9 (4.7)		30.7 (5.0)		6	0.0
Maternal age at menarche							123	0.8
Earlier than peers	2,826	25.1	934	26.8	241	26.7		
Same time as peers	6,413	56.9	2,019	58.0	524	58.2		
Later than peers	2,022	18.0	528	15.2	136	15.1		
Highest social class of parents							31	0.2
High-grade professional	2,953	26.1	632	18.0	98	10.8		
Low-grade professional	3,992	35.3	978	27.9	198	21.9		
Skilled worker	2,925	25.8	1,110	31.6	307	33.9		
Unskilled worker	1,199	10.6	680	19.4	261	28.8		
Student	205	1.8	90	2.6	16	1.8		
Economically inactive	48	0.4	18	0.5	25	2.8		
Cohabitation of parents							9	0.1
Did not live together	118	1.0	137	3.9	71	7.8		
Lived together	11,224	99.0	3,373	96.1	834	92.2		
Child's characteristics								

Table continues

Table 1. Continued

Background Characteristic	Smoking in the First Trimester							
	Nonsmoker (n = 11,347)		1–10 Daily Cigarettes (n = 3,512)		>10 Daily Cigarettes (n = 907)		Missing	
	No.	%	No.	%	No.	%	No.	%
Birthweight, grams[d]	3,571 (586)		3,448 (596)		3,319 (597)		57	0.4
Duration of exclusive breastfeeding, months							2,298	14.6
0	486	5.0	189	6.4	72	9.4		
<4	2,193	22.5	1,026	34.8	351	45.8		
≥4	7,072	72.5	1,736	58.8	343	44.8		
Exposure to postnatal smoking[e]							2,346	14.9
No	9,507	97.8	1,071	36.4	95	12.5		
Yes	214	2.2	1,868	63.6	665	87.5		
Child's BMI at age 7 years[d]	15.6 (1.7)		15.9 (1.8)		16.1 (2.1)		4,755	30.2

Abbreviations: ART, assisted reproductive technology; BMI, body mass index.
[a] 15,766 of 15,819 children with nonmissing information on maternal smoking in the first trimester (53 missing).
[b] BMI calculated as weight (kg)/height (m)^2.
[c] 1 unit = 12 g of pure alcohol.
[d] Values are expressed as mean (standard deviations).
[e] Exposure to postnatal smoking defined as maternal smoking during the first 6 months after birth.

Ethical approval

The Committee for Biomedical Research Ethics in Denmark approved the collection of data in the DNBC ((KF)01-471/94). A written informed consent was obtained from mothers upon recruitment covering both mother's and offspring's participation until the children turned 18 years of age. The present study was approved by the Danish Data Protection Agency (2012-41-0379 and 2015-57-0002) and the Steering Committee of the DNBC (2012-04 and 2015-47).

RESULTS

The prevalence of maternal smoking in the first trimester was 28%. Heavily smoking mothers (>10 daily cigarettes, 6%) were slightly more likely to consume alcohol, to have a smoking partner, to have had an unplanned pregnancy, to have had a longer time to pregnancy, to be parous, to have lower social class, to be living without the father during pregnancy, to have children with lower birth weight, to breastfeed less, and to smoke after pregnancy than nonsmoking mothers (Table 1).

Figures 2 and 3 show the adjusted difference (in months with 95% confidence intervals) in age at attaining the pubertal milestones according to maternal smoking categories (nonsmoker, light smoker, or heavy smoker) in the first trimester. For sons, maternal smoking in the first trimester was consistently associated with earlier age at attaining all pubertal milestones in a dose-dependent manner. For daughters, dose-dependent associations were observed for acne, menarche, and all stages of breast development, but only for Tanner stages 3–5 for pubic hair development.

Table 2 shows the unadjusted and adjusted difference in age at attaining a given pubertal milestone (in months) per 10 daily cigarettes smoked in the first trimester. In sons, maternal smoking in the first trimester was consistently associated with 1–4 months' earlier pubertal development for all milestones per 10 daily cigarettes. In daughters, maternal smoking in the first trimester was associated with 1–4.5 months' earlier age at breast development, pubic hair development (except Tanner stage 2), menarche, and acne per 10 daily cigarettes.

The maternal-paternal comparison showed associations between paternal smoking during pregnancy and timing of puberty in the offspring similar in direction and magnitude as the associations between maternal smoking during pregnancy and timing of puberty (Web Figures 1 and 2).

Under realistic scenarios, the multidimensional bias analysis showed that uncontrolled confounding could explain some but not all of the association between maternal smoking during pregnancy and age at voice break and menarche (Web Appendix 1 and Web Tables 3 and 4). We repeated the analysis with maternal smoking exposure throughout pregnancy (continuous), and the results were slightly attenuated (Web Table 5). When adjusting for childhood BMI at age 7 years, the associations were also slightly attenuated (Web Table 6). When adjusting for exposure to postnatal smoking, the results were essentially the same for sons but were attenuated for daughters (Web Table 7). Adjusting for duration of exclusive breastfeeding did not change the estimates (Web Table 8).

DISCUSSION

In this longitudinal study, we found dose-dependent associations between maternal smoking during pregnancy and earlier timing of puberty in both sons and daughters. All pubertal milestones occurred 1–4 months earlier per 10 daily cigarettes in sons, and in daughters, breast development, pubic hair development (except Tanner stage 2 for pubic

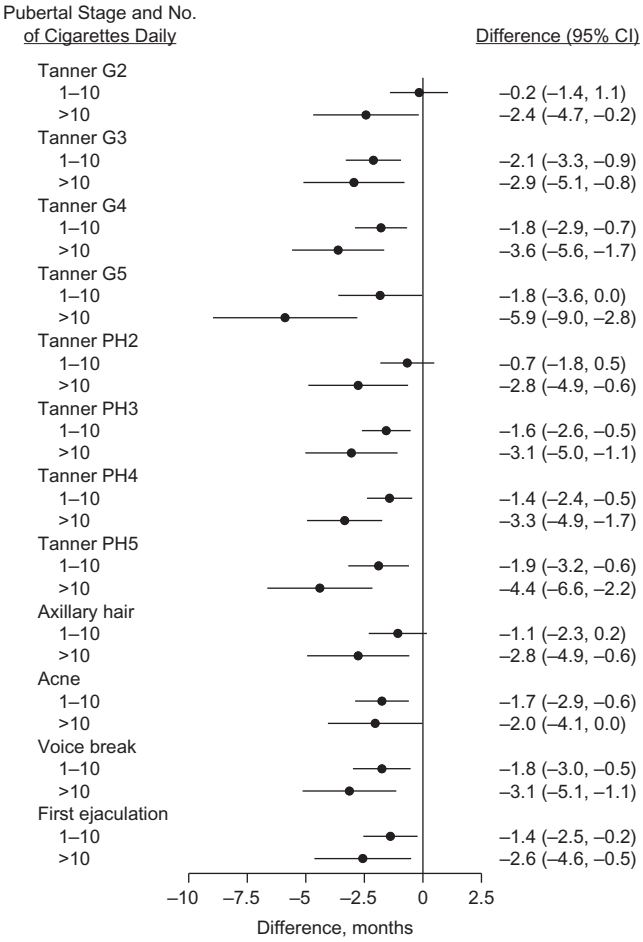

Figure 2. Age difference in timing of puberty among sons in relation to maternal smoking in the first trimester of pregnancy, Puberty Cohort, Danish National Birth Cohort, Denmark, 2012–2017. Estimated age differences in timing of puberty with 95% confidence intervals (CIs). The referent was nonsmoking mothers, and the analysis adjusted for prepregnancy body mass index, alcohol units per week in the first trimester, time to pregnancy (including assisted reproductive technology), highest social class of parents, maternal age at menarche, maternal age at delivery, parity, and cohabitation of parents during pregnancy. G2–5, genital stages 2–5; PH2–5, pubic hair stages 2–5.

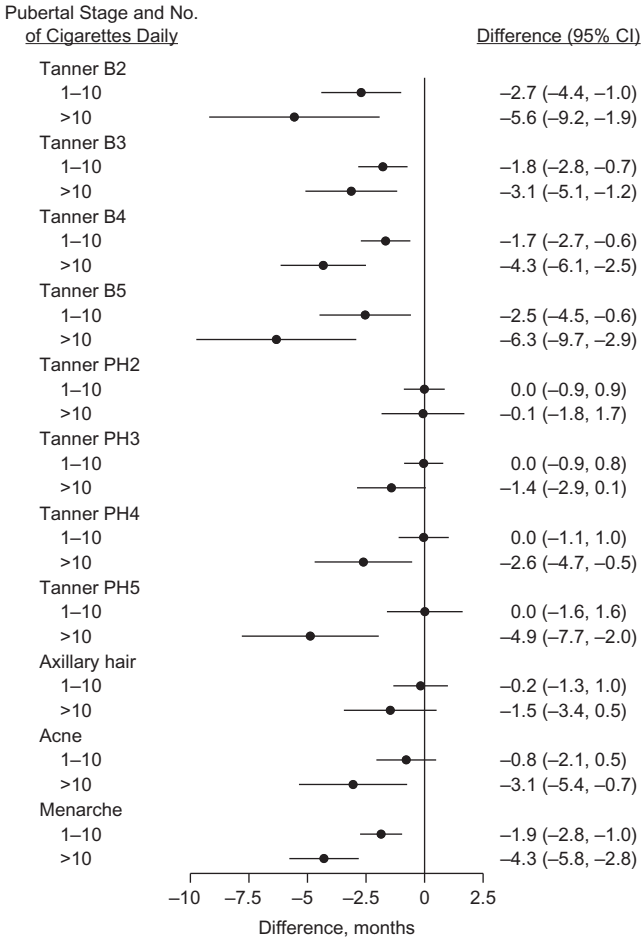

Figure 3. Age difference in timing of puberty among daughters in relation to maternal smoking in the first trimester of pregnancy, Puberty Cohort, Danish National Birth Cohort, Denmark, 2012–2017. Estimated age differences in timing of puberty with 95% confidence intervals (CIs). The referent was nonsmoking mothers, and the analysis adjusted for prepregnancy body mass index, alcohol units per week in the first trimester, time to pregnancy (including assisted reproductive technology), highest social class of parents, maternal age at menarche, maternal age at delivery, parity, and cohabitation of parents during pregnancy. B2–5, breast stages 2–5; PH2–5, pubic hair stages 2–5.

hair), menarche, and acne occurred 1–4.5 months earlier per 10 daily cigarettes.

Our study is large and used various pubertal milestones including Tanner stages. We accounted for potential sources of selection bias using selection weights (50). Even though we had data on most potential confounders, we cannot rule out confounding from unmeasured factors or residual confounding. Therefore, we performed a maternal-paternal comparison (48), which also showed associations between paternal smoking during pregnancy and timing of puberty in the offspring similar in direction and magnitude as the associations between maternal smoking during pregnancy and timing of puberty. These findings indicate remaining residual confounding or a biological effect of paternal smoking (e.g., through passive smoking or a programming effect on the sperm) (51). Furthermore, if a paternal programming effect exists, we cannot rule out the existence of a maternal programming effect of the ovaries before conception, which might confound the observed associations. To further explore the possibility of residual confounding, we conducted a multidimensional bias analysis for unmeasured confounding for age at voice break and menarche (49). Such an unmeasured confounder might be unhealthy lifestyle in the family that is connected to smoking behavior and could accelerate puberty through diet or exposure to endocrine-disrupting chemicals. Under realistic scenarios, unmeasured confounding explained only part of the observed associations (Web Appendix 1).

Pregnant women tend to underreport their smoking behavior (52). In the present study, information on smoking was

Table 2. Age Difference in Timing of Puberty in Months per 10 Daily Cigarettes in the First Trimester of Gestation for Children in the Puberty Cohort, Danish National Birth Cohort, Denmark, 2012–2017

Pubertal Milestone	No.[a]	Age Difference[b]		
		Unadjusted	Adjusted[c]	
		Mean	Mean	95% CI
Sons				
Tanner genital stage 2	7,446	−1.3	−1.3	−2.5, 0.0
Tanner genital stage 3	7,446	−2.4	−2.1	−3.3, −1.0
Tanner genital stage 4	7,446	−2.6	−2.3	−3.3, −1.3
Tanner genital stage 5	7,446	−4.0	−3.7	−5.3, −2.0
Tanner pubic hair stage 2	7,450	−1.7	−1.8	−2.9, −0.6
Tanner pubic hair stage 3	7,450	−2.4	−2.2	−3.2, −1.2
Tanner pubic hair stage 4	7,450	−2.3	−2.0	−2.9, −1.2
Tanner pubic hair stage 5	7,450	−3.3	−2.9	−4.2, −1.7
Axillary hair	7,455	−2.5	−2.0	−3.2, −0.8
Acne	7,455	−2.2	−1.9	−3.0, −0.8
Voice break	7,253	−3.1	−2.4	−3.6, −1.3
First ejaculation	7,442	−1.6	−1.7	−2.8, −0.6
Daughters				
Tanner breast stage 2	7,866	−4.5	−3.4	−5.3, −1.5
Tanner breast stage 3	7,866	−3.6	−2.6	−3.7, −1.6
Tanner breast stage 4	7,866	−3.6	−2.8	−3.8, −1.8
Tanner breast stage 5	7,866	−5.9	−4.7	−6.5, −2.9
Tanner pubic hair stage 2	7,867	−0.5	−0.1	−1.0, 0.8
Tanner pubic hair stage 3	7,867	−1.3	−0.9	−1.7, −0.1
Tanner pubic hair stage 4	7,867	−1.8	−1.4	−2.5, −0.4
Tanner pubic hair stage 5	7,867	−3.4	−2.5	−4.1, −1.0
Axillary hair	7,872	−1.7	−1.0	−2.1, 0.1
Acne	7,872	−2.8	−2.1	−3.4, −0.9
Menarche	7,864	−4.1	−3.1	−4.0, −2.3

Abbreviation: CI, confidence interval.

[a] Some sons and daughters gave information on some but not all pubertal milestones, so different numbers of observations were used for each outcome.

[b] Change in age (β) in months at attaining pubertal milestones per 10 daily cigarettes in the first trimester with 95% confidence interval.

[c] Adjusted for prepregnancy body mass index, alcohol units per week in the first trimester, time to pregnancy (including assisted reproductive technology), highest social class of parents, maternal age at menarche, maternal age at delivery, parity, and cohabitation of parents during pregnancy.

collected during pregnancy, long before timing of puberty in the children was known, and the resulting misclassification is most likely to be nondifferential, causing bias towards the null. Information on puberty was collected through self-administered questionnaires, which imposes a risk of misclassification but allows for a large sample size and probably less selection bias due to high participation. A high proportion of children had already attained the early pubertal milestones at entry and were, therefore, left censored (Web Table 2). For the estimates related to the early milestones to be unbiased even in the presence of left censoring, the residuals need to be normally distributed. The model check supported this for all later milestones, but for the earliest milestones, this model check is uncertain given that the left part of the distribution was unobserved due to left censoring. In case of skewed residuals, the potential error introduced by left censoring is, however, most likely nondifferential with regard to maternal smoking and cannot possibly explain the associations. This is corroborated by the similar associations for both early and late milestones. The only exception was the null finding for Tanner stage 2 for pubic hair in girls, which is in line with former data on girls of white ethnicity (26, 40). Despite this, we cannot rule out that the null finding for Tanner stage 2 for pubic hair in girls is due to left censoring and a skewed distribution of the residuals for this specific milestone.

Previous studies have indicated either no association or earlier timing of puberty in sons of smoking mothers in terms

of voice break (37–39), first ejaculation (38), genital development (39), and pubic hair development (39, 41). However, these studies were limited either by samples being too small (37), lack of confounder adjustment (39), or recalled puberty data in adulthood (38). The first large longitudinal study with detailed confounder adjustment investigated Tanner stages for pubic hair development as the only milestone and did not observe an association between maternal smoking during pregnancy and pubic hair development (41). We overcame the specific limitations mentioned above and found consistent dose-dependent associations between maternal smoking in pregnancy and all pubertal milestones in sons.

In daughters, published results on smoking and pubic hair and breast development have been less consistent (26, 37, 40). One study reported no association with breast development (37), another study reported earlier onset of breast development but not onset of pubic hair development (26), and the third reported earlier onset of pubic hair development but not breast development in daughters of smoking mothers (40). However, the last study found earlier onset of breast development, but not pubic hair development, when only girls of white ethnicity were considered (40), which indicates the presence of heterogeneity of effects by ethnicity. Our results support an association for earlier onset of breast development but not onset of pubic hair development in white girls. Finally, we found earlier age at menarche in daughters of smoking mothers, whereas investigators in other studies have reported conflicting results (16, 23–36). In a recent meta-analysis, maternal smoking during pregnancy was overall associated with slightly earlier age at menarche (53), but the authors also noted heterogeneity of effects between years of birth (53) indicating that important effect modifiers that change over time might be responsible for earlier inconsistent results in the previous literature (16, 23–36). Houghton et al. (54) found heterogeneity according to postnatal growth patterns and suggested that this could be the reason for the inconsistent results in the literature. In an exploratory analysis, we found no evidence of heterogeneity of effect when including a term for interaction between maternal smoking (continuous) and postnatal growth patterns ($P = 0.91$, data not shown) when defined as change in weight z score (continuous, based on United Kingdom–World Health Organization growth reference (55)) between birth and 7 years of age. It should be noted that maternal smoking during pregnancy is most likely not the main driving factor for the decline in age at menarche observed in girls (1, 2), given that the prevalence of smoking during pregnancy has declined over the last decades (56).

Prepubertal childhood obesity and low birth weight might be mediators for the association between maternal smoking during pregnancy and timing of puberty (19–22). We adjusted for childhood BMI at age 7 years but not birth weight because this has been related to severe collider-stratification bias (57, 58). When adjusting for childhood BMI at age 7 years, our results were only slightly attenuated, indicating that other mechanisms are more important.

We further examined the role of exposure to postnatal smoking and breastfeeding by adjusting for these variables. When adjusting for exposure to postnatal smoking, the results remained unchanged in sons but attenuated in daughters, indicating that exposure to postnatal smoking cannot explain the associations. These results should, however, be interpreted cautiously due to the risk of collinearity between maternal smoking during pregnancy and after birth and due to the failure to establish a link between postnatal smoking and timing of puberty so far (23, 34, 54). Maternal smoking during pregnancy might also affect puberty by way of shorter duration of breastfeeding, which has been associated with delayed age at menarche (28), but when adjusting for duration of exclusive breastfeeding, our results remained unchanged.

Because early puberty might be causally related to later diseases in adulthood (3–6), modifiable causes of early puberty need to be identified. Maternal smoking during pregnancy might be such a modifiable cause. If maternal smoking during pregnancy advances the timing of puberty, maternal smoking could have an impact on the incidence of adult diseases. If the estimated associations are true effects, our results could be generalized to Western populations of white origin given that ours is a population-based study, mainly including white persons. The evidence provided in this study could be used by health professionals as an additional argument in the motivation for smoking cessations before or during pregnancy.

In conclusion, maternal smoking during pregnancy was associated with younger age at all pubertal milestones in sons. In daughters, maternal smoking during pregnancy was associated with younger age at breast development, pubic hair development (except Tanner stage 2), menarche, and acne but not with axillary hair. In utero exposure to tobacco smoke might advance the timing of puberty.

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