WEB MATERIAL

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

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Web Appendix 1: Bias Analysis of Unmeasured Confounding

To explore the potential impact of unmeasured confounding, we conducted a multidimensional bias analysis using a simplified approach suggested by VanderWeele and Arah 2011 (1). This approach assumes a binary confounder, U, with constant prevalence difference, δ, between exposure categories across strata of all confounders, and with constant effect, γ, on the outcome across strata of all confounders and the exposure. The bias factor is then the product, $\delta\gamma$, which is interpreted as the magnitude of bias due to unmeasured confounding. Estimates are then corrected by subtracting the bias factor, $\delta\gamma$, from point estimates and 95% confidence intervals (CI). By choosing a range of different plausible values for δ and γ, we obtain multiple estimates under different scenarios. This is referred to as a multidimensional bias analysis.

In the present study, we conducted the bias analysis on the estimates from Table 2 for voice break in sons and age at menarche in daughters. In this setting, δ is the prevalence difference of, U, per 10 daily cigarettes, and γ is the effect on age at voice break or menarche in months. To specify realistic parameters for δ, we assessed the prevalence difference for measured confounders in Table 1. The prevalence difference between light-smokers and non-smokers (as well as between heavy-smokers and light-smokers) were 5 % for most measured confounders, but 10% for a few confounders, such as unplanned pregnancy and highest educational class of parents. As the categorical smoking variable used in Table 1 was based on groupings per 10 daily cigarettes, it is reasonable to assume similar prevalence difference for the continuous smoking variable, which is also in units of 10 daily cigarettes. To have both realistic and more extreme scenarios, we specified δ to be 5%, 10% and 20%. We specified the confounder effect on the pubertal milestones (γ) to 0, 3, and 6 months difference based on a range of potential effect sizes for other exposures on pubertal development observed in other studies (2, 3).

The results are shown in Web Table 3 for age at voice break and Web Table 4 for age at menarche. In the present study, an important unmeasured confounder may be “unhealthy lifestyle of the family” which may well have higher prevalence among smokers and may well advance the timing of puberty through unhealthy diet or increased exposure to endocrine disrupting chemicals. Thus, our focus is in the lower left corner of Web Table 3 and 4. Even under an unrealistically high prevalence difference of the confounder (δ = 20%) and a high confounder effect on the outcome (γ = –6 months) only part of the associations for age at voice break and menarche were explained, and the results remained statistically significant.

As an extreme scenario, we assessed the strength of the confounder to completely explain the observed associations. In sons, it would require a confounder prevalence difference of 20% and a confounder effect on age voice break of –12 months to explain the association (bias adjusted age difference in months: 0.0 (95% CI: -1.2, 1.1)). In daughters, it would require a confounder prevalence difference of 20% and a confounder effect on age at menarche of –15 months to explain the association (bias adjusted age difference in months: -0.1 (95% CI: -1.0, 0.7) month). These numbers seem highly unrealistic.

Studies on smoking are considerably prone to residual confounding and residual confounding are most likely present in the present study too. However, the multidimensional bias analysis suggested that our results could only be partly explained by unmeasured confounding under realistic scenarios. To completely explain the observed association by residual confounding from unmeasured confounders, these unmeasured confounders should be extraordinarily strong.

References

1. Vanderweele TJ, Arah OA. Bias formulas for sensitivity analysis of unmeasured confounding for general outcomes, treatments, and confounders. *Epidemiology* 2011;22(1):42-52.

2. Monteilh C, Kieszak S, Flanders WD, et al. Timing of maturation and predictors of Tanner stage transitions in boys enrolled in a contemporary British cohort. *Paediatr Perinat Epidemiol* 2011;25(1):75-87.

3. Maisonet M, Christensen KY, Rubin C, et al. Role of prenatal characteristics and early growth on pubertal attainment of British girls. *Pediatrics* 2010;126(3):e591-600.

Web Appendix 2: Sampling Procedure and Sampling Weights in the Puberty Cohort

The Puberty Cohort was created by sampling participants from 12 different perinatal exposures hypothesized to be important for timing of puberty and a random sample from the Danish National Birth Cohort (DNBC). This sampling strategy increased the exposure contrasts in the study population and thereby increased the efficiency of the statistical analyses. As each individual was not sampled at random from the DNBC, this sampling strategy produced a study population that was not representative of the source population from which we sampled. Hence, we needed to take the sampling strategy into account in the analyses.

The *source population* will in the following refer to the population of all children eligible for sampling to the Puberty Cohort. Children eligible for participation were live born, singletons from the Danish National Birth Cohort (DNBC) born during 2000 through 2003, whose mothers had not withdrawn from the DNBC by May 2012 and had replied to the first telephone interview during pregnancy. The source population comprised a total of 56,641 children. From this source population, we first sampled from 12 exposures. As some exposures were categories with more than two values, we sampled from a total of 27 exposure subgroups. We also drew a random sample of 8,000 from the entire source population. Thus, we sampled from 28 different overlapping subgroups, which we will refer to as *sampling frames*. In total, we sampled 22,439 potential participants to constitute the Puberty Cohort. Web Table 1 shows the 28 sampling frames, together with the number of potential participants in each frame, number of persons sampled within the frame, the sampling fraction and the total number of persons sampled from the frame.

Each person could be part of more than one sampling frame as the sampling frames were overlapping. This implied, that the same person could be sampled more than once, eg, the person could be sampled during sampling for smoking and then afterwards for alcohol consumption. However, the person entered the Puberty Cohort only once.

We used *sampling weights* (SW) to account for the sampling procedure in the Puberty Cohort. Sampling weights are *inverse probability weights* and represents the inverse probability of being sampled. As an example, if a person has 25 % chance of being sampled, this person has a weight of 4 (because 1/0.25 = 4). As a result, this person is weighted up corresponding to 4 copies of himself; thus, he will not only represent himself but also the 3 other persons similar with regards to all aspects, that were not sampled. By using sampling weights, we create a *pseudo population* that is representative of the entire source population of 56,641 children. Robust standard errors were used to account for the weighing approach.

The following shows how we calculated sampling weights for the sampling procedure in the Puberty Cohort.

Suppose we have a *source population* of n persons. Then we randomly sample x_1 persons of n_1 persons with the criteria $A_1 = 1$ ($A_1 = 1$ for persons with that criteria; $A_1 = 0$ for persons without that criteria). Thus, the *first sampling frame* constitutes all persons in the source population with criteria $A_1 = 1$. The number of persons in

the first sampling frame is n_1, and from this number, we sample x_1 persons. The *sampling fraction* is then x_1 / n_1. Let j denote a person in the source population. We define an indicator variable S_{1j} for whether or not the person j will be sampled in the first sampling frame ($S_{1j} = 1$ if person j is sampled; $S_{1j} = 0$ if person j is not sampled). Then, the probability that person j is sampled in the first sampling frame given person j is in the first sampling frame, $P(S_{1j} = 1|A_{1j} = 1)$, is simply the sampling fraction (x_1 / n_1):

$$P(S_{1j} = 1|A_{1j} = 1) = x_1 / n_1,$$

whereas the probability that person j is sampled during the first sampling frame given person j is *not* in the first sampling frame is of course 0:

$$P(S_{1j} = 1|A_{1j} = 0) = 0$$

For ease of notation, we write this as:

$$P(S_{1j} = 1|A_{1j}) = x_1 / n_1 \times A_{1j}$$

Then we randomly sample x_2 persons of n_2 persons with the criteria $A_2 = 1$ from the entire source population. The probability that person j is being sampled during the second sampling frame is:

$$P(S_{2j} = 1 |A_{2j}) = x_2 / n_2 \times A_{2j}$$

We repeat the sampling procedure for k different sampling frames. The probability for being sampled during the lth sampling frames is:

$$P(S_{ij} = 1|A_{ij}) = x_i / n_i \times A_{ij} \qquad \text{(Equation 1)}$$

First, it is important to note, that whether or not a person was sampled in a specific sampling frame did not affect the probability of the person being sampled during sampling in the other sampling frames. Thus, the different samplings were *independent*. Second, we drew a random sample of 8,000 of the source population so that all persons in the source population have had the opportunity to be sampled at least once. Finally, a person was considered sampled for the study if the person was sampled in at least one sampling frame.

We can then calculate the probability for person j of being sampled at least once during the k sampling rounds. The calculation is based on basic rules from probability calculus:

$$P(A) + P(\text{not } A) = 1 \qquad \text{(Equation 2)}$$

This implies that:

$$P(A) = 1 - P(\text{not } A) \quad \text{(Equation 3)}$$

$$P(A \text{ and } B) = P(A) \times P(B), \text{ given A and B are independent} \quad \text{(Equation 4)}$$

$$P(A \text{ or } B) = P(A) + P(B) - P(A \text{ and } B) \quad \text{(Equation 5)}$$

And equation 5 can be rewritten as *(De Morgan's law):*

$$P(A \text{ or } B) = 1 - P(\text{not A and not B}) \quad \text{(Equation 6)}$$

Equation 4 and 6 implies that:

$$P(A \text{ or } B) = 1 - P(\text{not A}) \times P(\text{not B}), \text{ given A and B are independent} \quad \text{(Equation 7)}$$

Combining equation 3 and 7 gives:

$$P(A \text{ or } B) = 1 - (1 - P(A)) \times (1 - P(B)), \text{ given A and B are independent} \quad \text{(Equation 8)}$$

We can now easily extend equation 8 to three *independent* events (A, B and C)

$$P(A \text{ or B or C}) = 1 - (1 - P(A)) \times (1 - P(B)) \times (1 - P(C)) \quad \text{(Equation 9)}$$

Instead of referring to event P(A), P(B) and P(C), we can refer to the probability of being sampled for person *j* in each of the *k* sampling frames $P(S_{1j} = 1|A_{1j})$, $P(S_{2j} = 1|A_{2j}),\ldots,P(S_{kj} = 1|A_{kj})$. Note some of these sampling probabilities may be zero for individuals that are not in the particular sampling frame. For example, if a person *j* are not member of the *l*th sampling frame, $A_{ij} = 0$, the sampling probability $P(S_{ij} = 1| A_{ij} = 0) = 0$. By extending Equation 9 we get the probability that person *j* will be sampled at least once, $P(S_j = 1|A_{1j},\ldots,A_{kj})$, for each person in the population:

$$\begin{aligned} &P(S_j = 1|A_{1j},\ldots,A_{kj}) \\ &= P(S_{1j} = 1 \text{ or } S_{2j} = 1 \text{ or } \ldots \text{ or } S_{kj} = 1|\, A_{1j},\ldots,A_{kj}) \\ &= 1 - (1 - P(S_{1j} = 1|A_{1j})) \times (1 - P(S_{2j} = 1|A_{2j})) \times \ldots \times (1 - P(S_{kj} = 1|A_{kj})) \end{aligned}$$

or more compactly:

$$\mathrm{P}(S_j = 1|A_{1j}, \ldots, A_{kj}) = 1 - \prod_{i=1}^{k}(1 - P(S_{ij} = 1|A_{ij})) \quad \text{(Equation 10)}$$

And by combining Equation 1 and 10 we get:

$$\mathrm{P}(S_j = 1|A_{1j}, \ldots, A_{kj}) = 1 - \prod_{i=1}^{k}(1 - \mathrm{P}(S_{ij} = 1|A_{ij})) = 1 - \prod_{i=1}^{k}(1 - \left(\frac{x_i}{n_i}\right) \times A_{ij})$$

$$\mathrm{P}(S_j = 1|A_{1j}, \ldots, A_{kj}) = 1 - \prod_{i=1}^{k}(1 - \left(\frac{x_i}{n_i}\right) \times A_{ij}) \quad \text{(Equation 11)}$$

By applying Equation 11 to each person sampled in the Puberty Cohort, we get this person's probability of being sampled. Thus, person *j* has the following sampling weight (*SW*$_j$):

$$SW_j = \frac{1}{\mathrm{P}(S_j=1|A_{1j},\ldots,A_{kj})} = \frac{1}{1-\prod_{i=1}^{k}(1-\left(\frac{x_i}{n_i}\right)\times A_{ij})} \quad \text{(Equation 12)}$$

Equation 12 was then applied to all participants in the Puberty Cohort.

The created pseudo population is representative of the source population given that the sample is big enough. If we let *C* be a row vector ($C_1, C_2, \ldots, C_d$) of all *d* variables in the source population and let P(*C*) be the joint distribution of the *d* variables in the source population. Then the distribution of the *d* variables in the sampled population using sampling weights (P(*C* | *S* = 1) using SW) is an asymptotic approximate estimator of P(*C*).

Web Table 1. Censoring of Pubertal Milestones for Children in the Puberty Cohort, Denmark, March 2017.

	Left-censored		Uncensored		Right-censored		Interval-censored		Total
Pubertal Milestones	No.	%	No.	%	No.	%	No.	%	No.[a]
Sons									
Tanner Genital stage 2	4,987	64.9	0	0.0	555	7.2	2,141	27.9	7,683
Tanner Genital stage 3	1,776	23.1	0	0.0	1,832	23.8	4,075	53.0	7,683
Tanner Genital stage 4	474	6.2	0	0.0	3,208	41.8	4,001	52.1	7,683
Tanner Genital stage 5	74	1.0	0	0.0	5,659	73.7	1,950	25.4	7,683
Tanner Pubic Hair stage 2	4,005	52.1	0	0.0	826	10.7	2,856	37.2	7,687
Tanner Pubic Hair stage 3	1,206	15.7	0	0.0	1,994	25.9	4,487	58.4	7,687
Tanner Pubic Hair stage 4	375	4.9	0	0.0	2,954	38.4	4,358	56.7	7,687
Tanner Pubic Hair stage 5	73	0.9	0	0.0	4,704	61.2	2,910	37.9	7,687
Axillary Hair	1,016	13.2	0	0.0	2,721	35.4	3,956	51.4	7,693
Acne	2,196	28.5	0	0.0	1,553	20.2	3,944	51.3	7,693
Voice break	1,247	16.7	0	0.0	2,304	30.8	3,934	52.6	7,485
First ejaculation	152	2.0	4,358	56.8	3,137	40.9	32	0.4	7,679
Daughters									
Tanner Breast stage 2	6,892	85.0	0	0.0	168	2.1	1,053	13.0	8,113
Tanner Breast stage 3	3,483	42.9	0	0.0	767	9.5	3,863	47.6	8,113
Tanner Breast stage 4	914	11.3	0	0.0	2,095	25.8	5,104	62.9	8,113
Tanner Breast stage 5	104	1.3	0	0.0	5,636	69.5	2,373	29.2	8,113
Tanner Pubic Hair stage 2	4,402	54.3	0	0.0	510	6.3	3,202	39.5	8,114
Tanner Pubic Hair stage 3	1,343	16.6	0	0.0	1,418	17.5	5,353	66.0	8,114
Tanner Pubic Hair stage 4	494	6.1	0	0.0	2,620	32.3	5,000	61.6	8,114
Tanner Pubic Hair stage 5	86	1.1	0	0.0	5,298	65.3	2,730	33.6	8,114
Axillary Hair	2,959	36.4	0	0.0	1,067	13.1	4,094	50.4	8,120
Acne	3,976	49.0	0	0.0	854	10.5	3,290	40.5	8,120
Menarche	0	0.0	5,957	73.4	1,905	23.5	249	3.1	8,111

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

Web Table 2. Overview of Sampling Frames in the Puberty Cohort, Denmark.

Sampling frame	Potential participants in sampling frame	Sampled from sampling frame	Sampling fraction	Drawn in total from sampling frame
Maternal night work	436	436	1.00	436
Maternal evening work	1,162	500	0.43	767
Shift work with night work	3,555	500	0.14	1,696
Shift work without night work	3,867	500	0.13	1,840
Weekly alcohol consumption of 0.5-3.5 units[a]	24,182	1,000	0.04	9,277
Weekly alcohol consumption of 4+ units[a]	1,086	1,000	0.92	1,028
Smoking 1-10 cigarettes daily	6,195	1,000	0.16	3,175
Smoking 11+ cigarettes daily	1,986	1,000	0.50	1,485
Acetaminophen in first trimester only	5,646	500	0.09	2,488
Acetaminophen in second trimester only	3,151	500	0.16	1,516
Acetaminophen in third trimester only	5,975	500	0.08	2,596
Acetaminophen in all trimesters	5,858	500	0.09	2,618
BMI<18.5	2,355	1,000	0.42	1,509
BMI 25-29.9	11,208	1,000	0.09	4,823
BMI 30+	4,894	1,000	0.20	2,528
Agricultural worker	200	200	1.00	200
Gardener	228	228	1.00	228
Painter	184	184	1.00	184
Artificial reproductive treatment	1,209	1,209	1.00	1,209
Small for gestational age	5,502	1,000	0.18	3,036
Gestational age <37+0 weeks	2,573	1,000	0.39	1,695
Work-related stress	8,740	2,000	0.23	4,698
PFAS measured[b]	989	989	1.00	989
Oral contraceptives during pregnancy	758	758	1.00	758
Thyroid diseases	750	750	1.00	750
Infantile autism	153	153	1.00	153
Diabetes mellitus type I & II	456	456	1.00	456
Random sample	56,641	8,000	0.14	22,439
Sampled in total				**22,439**

[a]1 unit = 12 g of pure alcohol

[b]Indicates whether per- and polyfluoroalkyl substances (PFAS) have been measured.

Web Table 3. Multidimensional Bias Analysis of the Association Between Maternal Smoking During Pregnancy and Age at Voice Break in 7,253 Sons in the Puberty Cohort, Denmark, March 2017.

	Adjusted[a] mean monthly difference in age at attaining voice break per 10 daily cigarettes in first trimester									
	γ^c = -6 months		γ^c = -3 months		γ^c = 0 months		γ^c = +3 months		γ^c = +6 months	
δ^b	Age difference[d]	95% CI	Age difference[d]	95% CI	Age difference[d]	95% CI	Age difference[d]	95% CI	Age difference[d]	95% CI
-20 %	-3.6	-4.8, -2.5	-3.0	-4.2, -1.9			-1.8	-3.0, -0.7	-1.2	-2.4, -0.1
-10 %	-3.0	-4.2, -1.9	-2.7	-3.9, -1.6			-2.1	-3.3, -1.0	-1.8	-3.0, -0.7
-5 %	-2.7	-3.9, -1.6	-2.55	-3.75, -1.45			-2.25	-3.45, -1.5	-2.1	-3.3, -1.0
0%					-2.4	-3.6, -1.3				
+5 %	-2.1	-3.3, -1.0	-2.25	-3.45, -1.15			-2.55	-3.75, -1.45	-2.7	-3.9, -1.6
+10 %	-1.8	-3.0 - 0.7	-2.1	-3.3, -1.0			-2.7	-3.9, -1.6	-3.0	-4.2, -1.9
+20 %	-1.2	-2.4, -0.1	-1.8	-3.0, -0.7			-3.0	-4.2, -1.9	-3.6	-4.8, -2.5

Abbreviations: CI, confidence interval.

[a]Adjusted for pre-pregnancy body mass index, alcohol units per week in first trimester, time to pregnancy including assisted reproductive technology, highest educational class of parents, maternal age at menarche, maternal age at delivery, parity, and cohabitation of parents during pregnancy

[b]δ is the bias parameter describing the difference in prevalence of the confounder, U, per 10 daily cigarettes in first trimester.

[c]γ is the bias parameter describing the difference in age at attaining voice break between levels of the confounder, U.

[d]Change in age (in months) at attaining voice break per 10 daily cigarettes in first trimester.

Web Table 4. Multidimensional Bias Analysis of the Association Between Maternal Smoking During Pregnancy and Age at Menarche in 7,864 Daughters in the Puberty Cohort, Denmark, March 2017.

	Adjusted[a] mean monthly difference in age at attaining menarche per 10 daily cigarettes in first trimester									
	γ^c = -6 months		γ^c = -3 months		γ^c = 0 months		γ^c = +3 months		γ^c = +6 months	
δ^b	Age difference[d]	95% CI	Age difference[d]	95% CI	Age difference[d]	95% CI	Age difference[d]	95% CI	Age difference[d]	95% CI
-20 %	-4.3	-5.2, -3,5	-3.7	-4.6, -2.9			-2.5	-3.4, -1.7	-1.9	-2.8, -1.1
-10 %	-3.7	-4.6,-2.9	-3.4	-4.3, -2.6			-2.8	-3.7, -2.0	-2.5	-3.4, -1,7
-5 %	-3.4	-4.3, -2.6	-3.25	-4.15, -2.45			-2.95	-3.85, -2.15	-2.8	-3.7, -2.0
0%					-3.1	-4.0, -2.3				
+5 %	-2.8	-3.7, -2.0	-2.95	-3.85, -2.15			-3.25	-4.15, -2,45	-3.4	-4.3, -2.6
+10 %	-2.5	-3.4, -1.7	-2.8	-3.7, -2.0			-3.4	-4.3, -2.6	-3.7	-4.6, -2.9
+20 %	-1.9	-2.8, -1.1	-2.5	-3.4, -1.7			-3.7	-4.6, -2.9	-4.3	-5.2, -3.5

Abbreviations: CI, confidence interval.

[a]Adjusted for pre-pregnancy body mass index, alcohol units per week in first trimester, time to pregnancy including assisted reproductive technology, highest educational class of parents, maternal age at menarche, maternal age at delivery, parity, and cohabitation of parents during pregnancy

[b]δ is the bias parameter describing the difference in prevalence of the confounder, U, per 10 daily cigarettes in first trimester.

[c]γ is the bias parameter describing the difference in age at attaining menarche between levels of the confounder, U.

[d]Change in age (in months) at attaining menarche per 10 daily cigarettes in first trimester.

Web Table 5. Age Difference in Timing of Puberty in Months per 10 Daily Cigarettes Throughout Pregnancy for Children in the Puberty Cohort, Denmark, March 2017.

		Age difference[b]		
		Unadjusted	Adjusted[c]	
Pubertal milestones	No.[a]	Mean	Mean	95% CI
Sons				
Tanner Genital stage 2	5,861	-0.8	-0.8	-2.1, 0.5
Tanner Genital stage 3	5,861	-1.8	-1.7	-2.9, -0.6
Tanner Genital stage 4	5,861	-1.8	-1.7	-2.8, -0.7
Tanner Genital stage 5	5,861	-2.6	-2.5	-4.1, -0.8
Tanner Pubic Hair stage 2	5,863	-1.5	-1.8	-2.9, -0.6
Tanner Pubic Hair stage 3	5,863	-1.9	-1.9	-3.0, -0.8
Tanner Pubic Hair stage 4	5,863	-1.7	-1.6	-2.5, -0.7
Tanner Pubic Hair stage 5	5,863	-2.4	-2.1	-3.3, -0.9
Axillary Hair	5,867	-1.6	-1.4	-2.5, -0.2
Acne	5,867	-2.2	-2.0	-3.1, -0.9
Voice break	5,706	-2.6	-2.1	-3.2, -0.9
First ejaculation	5,860	-1.2	-1.2	-2.3, 0.0
Daughters				
Tanner Breast stage 2	6,196	-3.7	-3.0	-4.8, -1.1
Tanner Breast stage 3	6,196	-2.9	-2.1	-3.2, -1.0
Tanner Breast stage 4	6,196	-3.4	-2.8	-3.8, -1.7
Tanner Breast stage 5	6,196	-5.1	-4.4	-6.4, -2.5
Tanner Pubic Hair stage 2	6,196	-0.9	-0.6	-1.5, 0.4
Tanner Pubic Hair stage 3	6,196	-1.0	-0.6	-1.4, 0.2
Tanner Pubic Hair stage 4	6,196	-1.8	-1.5	-2.6, -0.4
Tanner Pubic Hair stage 5	6,196	-3.0	-2.3	-3.9, -0.6
Axillary Hair	6,201	-1.7	-1.2	-2.3, 0.0
Acne	6,201	-2.0	-1.3	-2.7, 0.0
Menarche	6,194	-3.3	-2.6	-3.5, -1.8

Abbreviations: CI, confidence interval.

[a]Number of persons in adjusted analysis. No. of persons in this table is less than in Table 2 as there is missing information on smoking history throughout pregnancy.

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

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

Web Table 6. Age Difference in Timing of Puberty in Months per 10 Daily Cigarettes in First Trimester for Children in the Puberty Cohort When Further Adjusting for Childhood BMI at 7 Years, Denmark, March 2017.

		Age difference[b]				
		Unadjusted	Adjusted: Model 1[c]		Adjusted: Model 2[d]	
Pubertal milestones	No.[a]	Mean	Mean	95% CI	Mean	95% CI
Sons						
Tanner Genital stage 2	5,324	-2.0	-1.9	-3.3, -0.4	-1.7	-3.2, -0.3
Tanner Genital stage 3	5,324	-2.9	-2.5	-3.9, -1.1	-2.2	-3.5, -0.8
Tanner Genital stage 4	5,324	-2.9	-2.4	-3.7, -1.2	-2.1	-3.3, -0.8
Tanner Genital stage 5	5,324	-4.7	-4.1	-6.1, -2.2	-3.8	-5.7, -1.9
Tanner Pubic Hair stage 2	5,325	-1.8	-1.7	-3.1, -0.4	-1.5	-2.9, -0.1
Tanner Pubic Hair stage 3	5,325	-2.8	-2.4	-3.6, -1.2	-2.0	-3.2, -0.8
Tanner Pubic Hair stage 4	5,325	-2.7	-2.4	-3.5, -1.4	-2.1	-3.2, -1.1
Tanner Pubic Hair stage 5	5,325	-4.0	-3.4	-4.8, -2.0	-3.0	-4.4, -1.6
Axillary Hair	5,327	-2.5	-2.1	-3.4, -0.7	-1.4	-2.8, -0.1
Acne	5,327	-3.0	-2.8	-4.1, -1.5	-2.5	-3.8, -1.2
Voice break	5,224	-2.8	-2.2	-3.6, -0.9	-1.8	-3.2, -0.5
First ejaculation	5,321	-1.3	-1.3	-2.6, 0.0	-1.0	-2.4, 0.3
Daughters						
Tanner Breast stage 2	5,402	-4.6	-3.1	-5.3, -0.9	-2.6	-4.7, -0.4
Tanner Breast stage 3	5,402	-3.6	-2.3	-3.6, -1.0	-1.8	-3.0, -0.6
Tanner Breast stage 4	5,402	-3.9	-2.8	-4.0, -1.6	-2.3	-3.5, -1.1
Tanner Breast stage 5	5,402	-5.9	-4.6	-6.9, -2.4	-4.0	-6.2, -1.8
Tanner Pubic Hair stage 2	5,402	-0.9	-0.2	-1.3, 0.8	0.0	-1.0, 1.1
Tanner Pubic Hair stage 3	5,402	-1.6	-1.1	-2.1, -0.1	-0.8	-1.8, 0.2
Tanner Pubic Hair stage 4	5,402	-2.2	-1.7	-3.0, -0.3	-1.3	-2.6, 0.1
Tanner Pubic Hair stage 5	5,402	-4.0	-3.0	-4.9, -1.1	-2.5	-4.3, -0.6
Axillary Hair	5,405	-1.7	-0.9	-2.3, 0.5	-0.5	-1.9, 0.8
Acne	5,405	-2.3	-1.3	-2.8, 0.2	-1.1	-2.6, 0.4
Menarche	5,400	-3.9	-2.9	-3.8, -1.9	-2.4	-3.3, -1.4

Abbreviations: CI, confidence interval.

[a]As some sons and daughters gave information on some but not all pubertal milestones, different number of observations were used for each outcome. The number of observations are lower than in Table 2 due to missing information on childhood BMI at 7 years.

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

[c]Model 1: restriction to non-missing information on childhood BMI at 7 years and adjustment for pre-pregnancy body mass index, alcohol units per week in first trimester, time to pregnancy including assisted reproductive technology, highest educational class of parents, maternal age at menarche, maternal age at delivery, parity, and cohabitation of parents during pregnancy.

[d]Model 2: adjustment for childhood BMI at 7 years and the same confounders as in Model 1.

Web Table 7. Age Difference in Timing of Puberty in Months per 10 Daily Cigarettes in First Trimester for Children in the Puberty Cohort When Further Adjusting for Postnatal Exposure to Smoking, Denmark, March 2017.

		Age difference[b]				
		Unadjusted	Adjusted: Model 1[c]		Adjusted: Model 2[d]	
Pubertal milestones	No.[a]	Mean	Mean	95% CI	Mean	95% CI
Sons						
Tanner Genital stage 2	6,350	-1.5	-1.4	-2.7, -0.1	-2.5	-4.4, -0.7
Tanner Genital stage 3	6,350	-2.1	-1.9	-3.1, -0.6	-1.9	-3.5, -0.4
Tanner Genital stage 4	6,350	-2.2	-1.8	-2.9, -0.7	-1.2	-2.6, 0.1
Tanner Genital stage 5	6,350	-4.1	-3.8	-5.5, -2.0	-3.0	-5.4, -0.6
Tanner Pubic Hair stage 2	6,353	-1.9	-2.0	-3.2, -0.7	-2.7	-4.3, -1.0
Tanner Pubic Hair stage 3	6,353	-2.2	-2.1	-3.2, -0.9	-2.3	-3.9, -0.8
Tanner Pubic Hair stage 4	6,353	-2.0	-1.7	-2.7, -0.8	-1.2	-2.5, 0.0
Tanner Pubic Hair stage 5	6,353	-3.4	-3.0	-4.3, -1.7	-2.3	-4.0, -0.7
Axillary Hair	6,357	-2.4	-1.8	-3.1, -0.6	-1.4	-3.0, 0.2
Acne	6,357	-2.5	-2.1	-3.3, -0.8	-2.2	-3.8, -0.6
Voice break	6,186	-2.9	-2.1	-3.3, -0.9	-1.4	-3.0, 0.3
First ejaculation	6,349	-1.3	-1.3	-2.5, 0.0	-1.3	-2.9, 0.4
Daughters						
Tanner Breast stage 2	6,685	-4.8	-3.9	-6.0, -1.8	-2.5	-5.2, 0.2
Tanner Breast stage 3	6,685	-3.6	-2.6	-3.8, -1.5	-1.2	-2.8, 0.3
Tanner Breast stage 4	6,685	-3.9	-3.1	-4.2, -2.1	-1.8	-3.2, -0.4
Tanner Breast stage 5	6,685	-6.5	-5.5	-7.5, -3.5	-4.5	-7.1, -1.9
Tanner Pubic Hair stage 2	6,685	-0.7	-0.3	-1.3, 0.7	0.6	-0.8, 1.9
Tanner Pubic Hair stage 3	6,685	-1.3	-1.0	-1.8, -0.1	-0.1	-1.3, 1.1
Tanner Pubic Hair stage 4	6,685	-2.1	-1.8	-3.0, -0.6	-0.6	-2.2, 0.9
Tanner Pubic Hair stage 5	6,685	-3.9	-3.0	-4.7, -1.4	-2.5	-4.6, -0.3
Axillary Hair	6,690	-1.6	-1.0	-2.2, 0.2	-0.2	-1.8, 1.4
Acne	6,690	-2.9	-2.2	-3.6, -0.8	-0.4	-2.2, 1.3
Menarche	6,683	-4.2	-3.3	-4.2, -2.4	-1.8	-3.0, -0.7

Abbreviations: CI, confidence interval.

[a]As some sons and daughters gave information on some but not all pubertal milestones, different number of observations were used for each outcome. The number of observations are lower than in Table 2 due to missing information on exposure to postnatal smoking.

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

[c]Model 1: restriction to non-missing information on exposure to postnatal smoking and adjustment for pre-pregnancy body mass index, alcohol units per week in first trimester, time to pregnancy including assisted reproductive technology, highest educational class of parents, maternal age at menarche, maternal age at delivery, parity, and cohabitation of parents during pregnancy.

[d]Model 2: adjustment for postnatal exposure to smoking and the same confounders as in Model 1.

Web Table 8. Age Difference in Timing of Puberty in Months per 10 Daily Cigarettes in First Trimester for Children in the Puberty Cohort When Further Adjusting for Duration of Exclusive Breastfeeding, Denmark, March 2017.

		Age difference[b]				
		Unadjusted	Adjusted: Model 1[c]		Adjusted: Model 2[d]	
Pubertal milestones	No.[a]	Mean	Mean	95% CI	Mean	95% CI
Sons						
Tanner Genital stage 2	6,372	-1.4	-1.3	-2.6, 0.0	-1.4	-2.7, -0.1
Tanner Genital stage 3	6,372	-2.1	-1.8	-3.1, -0.6	-1.8	-3.0, -0.5
Tanner Genital stage 4	6,372	-2.1	-1.8	-2.9, -0.7	-1.6	-2.8, -0.5
Tanner Genital stage 5	6,372	-4.1	-3.7	-5.5, -2.0	-3.6	-5.4, -1.8
Tanner Pubic Hair stage 2	6,375	-1.9	-2.0	-3.2, -0.7	-2.0	-3.3, -0.8
Tanner Pubic Hair stage 3	6,375	-2.2	-2.0	-3.2, -0.9	-2.0	-3.1, -0.9
Tanner Pubic Hair stage 4	6,375	-1.9	-1.7	-2.6, -0.7	-1.6	-2.6, -0.7
Tanner Pubic Hair stage 5	6,375	-3.4	-2.9	-4.2, -1.7	-2.9	-4.2, -1.6
Axillary Hair	6,379	-2.4	-1.8	-3.1, -0.5	-1.7	-2.9, -0.4
Acne	6,379	-2.5	-2.0	-3.3, -0.8	-1.9	-3.2, -0.7
Voice break	6,208	-2.8	-2.0	-3.3, -0.8	-2.0	-3.2, -0.7
First ejaculation	6,371	-1.3	-1.2	-2.4, 0.0	-1.3	-2.5, -0.1
Daughters						
Tanner Breast stage 2	6,709	-4.8	-3.9	-6.0, -1.8	-3.8	-5.9, -1.7
Tanner Breast stage 3	6,709	-3.6	-2.6	-3.8, -1.5	-2.6	-3.7, -1.4
Tanner Breast stage 4	6,709	-4.0	-3.2	-4.3, -2.1	-3.1	-4.2, -2.0
Tanner Breast stage 5	6,709	-6.6	-5.5	-7.5, -3.5	-5.4	-7.4, -3.4
Tanner Pubic Hair stage 2	6,709	-0.7	-0.3	-1.3, 0.7	-0.2	-1.2, 0.8
Tanner Pubic Hair stage 3	6,709	-1.3	-1.0	-1.9, -0.1	-0.8	-1.7, 0.1
Tanner Pubic Hair stage 4	6,709	-2.2	-1.8	-3.0, -0.7	-1.8	-3.0, -0.6
Tanner Pubic Hair stage 5	6,709	-4.0	-3.1	-4.7, -1.4	-3.0	-4.6, -1.3
Axillary Hair	6,714	-1.6	-1.0	-2.2, 0.2	-0.9	-2.2, 0.3
Acne	6,714	-2.9	-2.2	-3.6, -0.8	-1.9	-3.3, -0.5
Menarche	6,707	-4.2	-3.3	-4.2, -2.4	-3.0	-3.9, -2.1

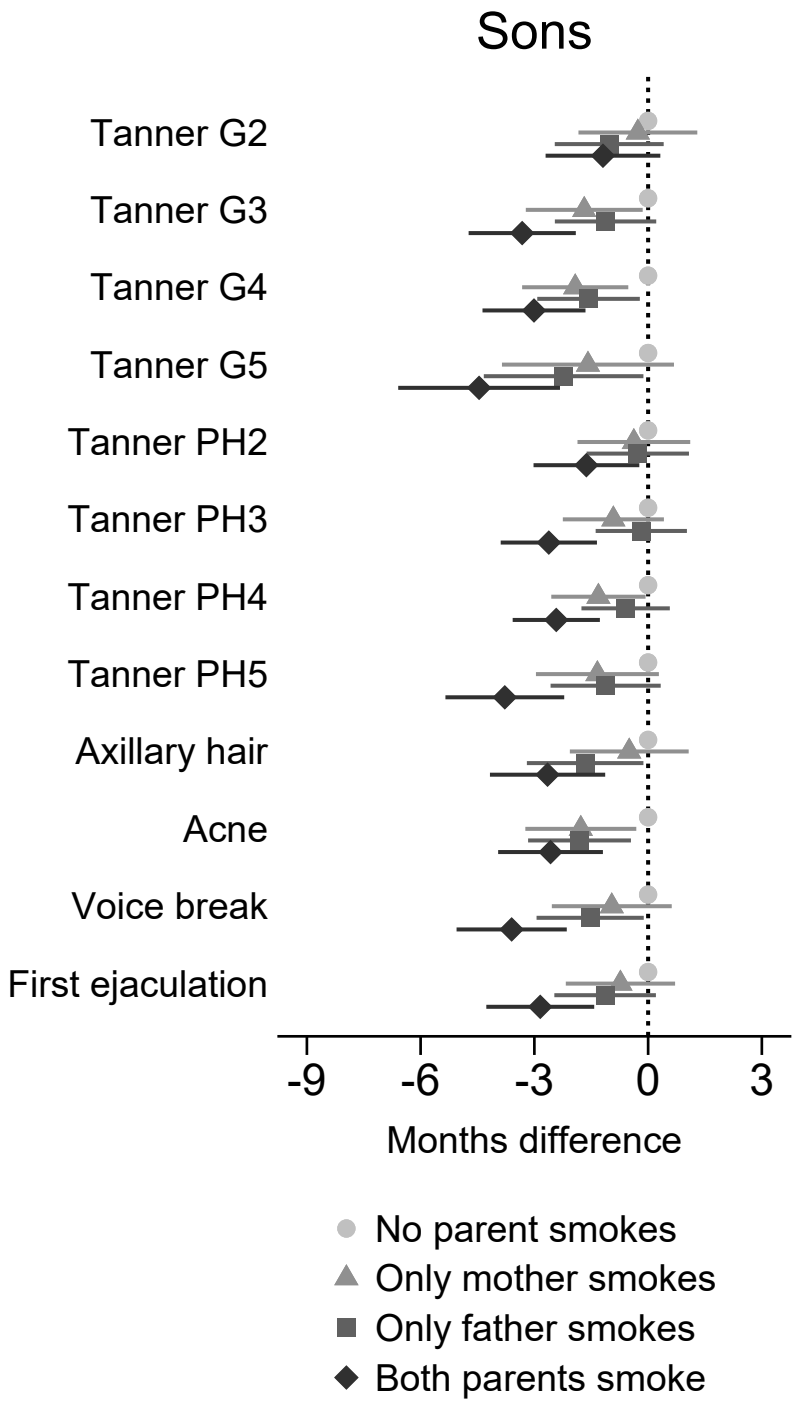
Abbreviations: CI, confidence interval.

[a]As some sons and daughters gave information on some but not all pubertal milestones, different number of observations were used for each outcome. The number of observations are lower than in Table 2 due to missing information on duration of exclusive breastfeeding.

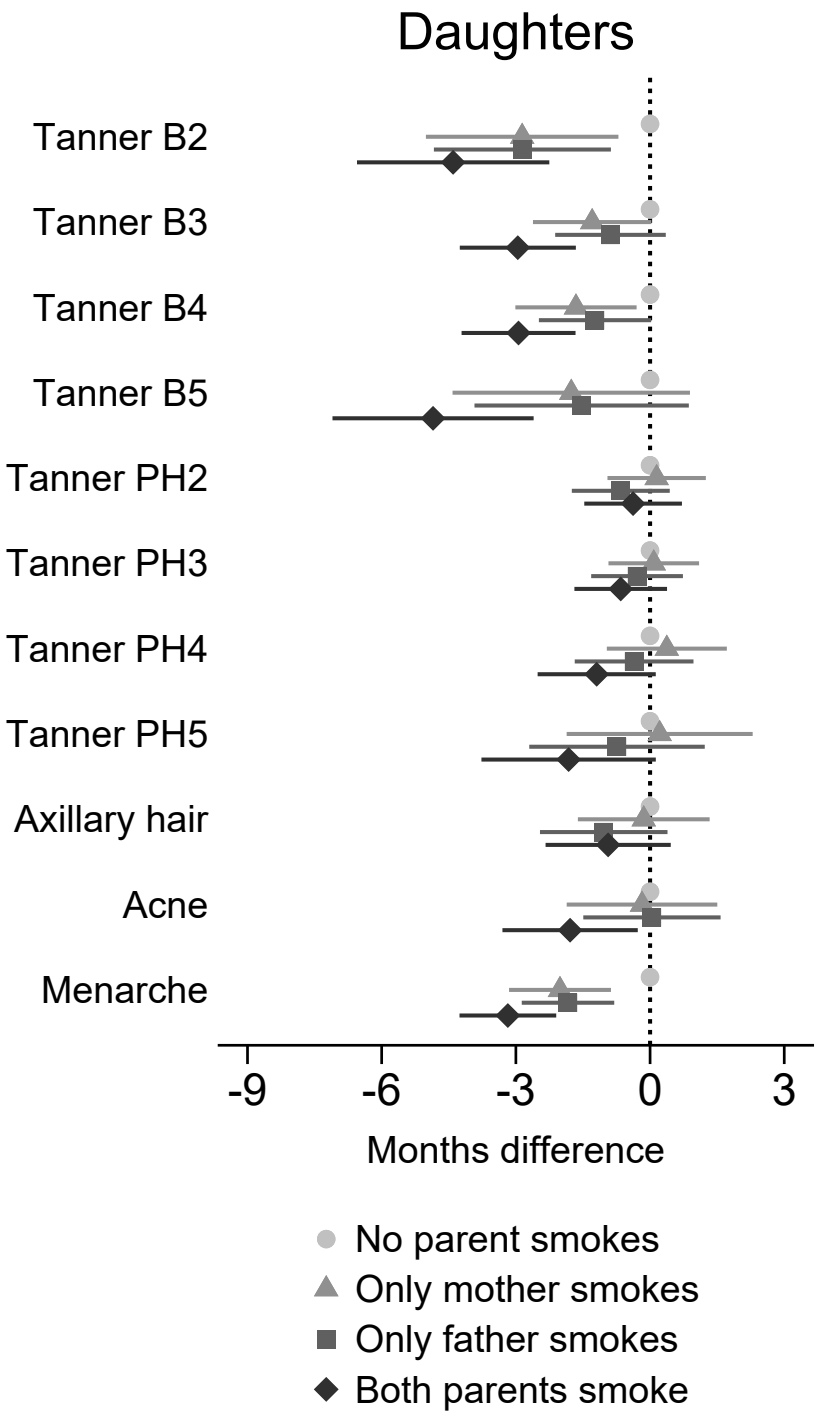
[b]Change in age (β) in months at attaining pubertal milestones per 10 daily cigarettes in first trimester with 95% confidence interval.

[c]Model 1: restriction to non-missing information on duration of exclusive breastfeeding and adjustment for pre-pregnancy body mass index, alcohol units per week in first trimester, time to pregnancy including assisted reproductive technology, highest educational class of parents, maternal age at menarche, maternal age at delivery, parity, and cohabitation of parents during pregnancy.

[d]Model 2: adjustment for duration of exclusive breastfeeding and the same confounders as in Model 1.



Web Figure 1. Adjusted age difference (with 95% CI) in timing of puberty among sons in relation to maternal and paternal smoking, the Puberty Cohort, Denmark, March 2017. Adjusted for pre-pregnancy body mass index, alcohol units per week in first trimester, time to pregnancy including assisted reproductive technology, highest educational class of parents, maternal age at menarche, maternal age at delivery, parity, and cohabitation of parents during pregnancy. Abbreviations: Tanner G2-5, Tanner Genital stage 2-5; Tanner PH2-5, Tanner Pubic Hair stage 2-5.



Web Figure 2. Adjusted age difference (with 95% CI) in timing of puberty among daughters in relation to maternal and paternal smoking, the Puberty Cohort, Denmark, March 2017. Adjusted for pre-pregnancy body mass index, alcohol units per week in first trimester, time to pregnancy including assisted reproductive technology, highest educational class of parents, maternal age at menarche, maternal age at delivery, parity, and cohabitation of parents during pregnancy. Abbreviations: Tanner B2-5, Tanner Breast stage 2-5; Tanner PH2-5, Tanner Pubic Hair stage 2-5.