Supplementary Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.
Maternal and paternal tobacco use

Maternal tobacco use at enrollment was assessed in the first questionnaire by asking whether the mother smoked during pregnancy. In the second and third questionnaires (mid- and late pregnancy), mothers were asked whether they had smoked in the last 2 months. Mothers who reported no smoking or to have smoked until pregnancy was known in the first questionnaire, but acknowledged in the second or third questionnaire to have smoked during pregnancy were categorized to ‘continued smoking during pregnancy’. When information was missing on maternal smoking at enrollment, information from the second and/or third questionnaire was used to classify the mothers into non-smokers or continued smokers. The frequency of tobacco use for the smoking mothers was categorized into ‘less than 1 cigarette a day’, ‘1-2 cigarettes a day’, ‘3-4 cigarettes a day’, ‘5-9 cigarettes a day’, ‘10-19 cigarettes a day’, and ‘20 or more cigarettes a day’.

For paternal tobacco use, the question for mothers was ‘In the months preceding this pregnancy, did he (the biological father) smoke cigarettes, cigars or shag’; the question for participating partners was ‘In the two months before the pregnancy of your partner, have you smoked’.

Neuroimaging

Parameters used for T1-weighted image sequence: GE option BRAVO, TR = 8.77 ms, TE = 3.4 ms, TI = 600 ms, flip angle = 10°, matrix size = 220 × 220, field of view = 220 mm × 220 mm, slice thickness = 1 mm, number of slices = 230, ARC acceleration factor = 2.

Cortical reconstruction smoothing: thickness maps for each subject were smoothed with a 10 mm full-width half-maximum Gaussian kernel, and local gyrification index (LGI) maps were smoothed using a 5 mm full-width half-maximum Gaussian kernel.

Statistical analysis

Multiple comparison correction for surface-based analyses: clusterwise p-values were Bonferroni corrected for the two hemispheres (p<0.025), and a cluster forming threshold (CFT) of p=0.001 was selected for significance testing because it has shown high correspondence with actual permutation testing at the smoothing kernels used.¹
eFigure 1. Flowchart for Study Population Selection

- 9778 women recruited
  - 246 twin births, 104 fetal deaths (including induced abortion), and 45 lost to follow-up
  - 9506 live singletons
  - 1484 without information on maternal tobacco use during pregnancy
  - 8022 with information on maternal tobacco use during pregnancy
    - 1770 were not invited or did not participate at age 9-11 years due to withdraw or loss to follow-up
  - 6252 were invited and agreed to participate at age 9-11 years
    - 2659 did not visit neuroimaging research center; and 210 did not undergo brain MRI assessment
  - 3383 visited neuroimaging research center and underwent brain MRI assessment with consent
    - Missing T1-weighted images (n=22); heterogeneous scanning parameters (n=20); major incidental findings (n=22); braces (n=77); and data of insufficient quality (n=538)
- 2704 with usable brain MRI data

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### eTable 1. Nonresponse Analysis

| Characteristics                              | Respondents<sup>a</sup> (n=2704) | Non-respondents (5318) | P value<sup>b</sup> |
|---------------------------------------------|----------------------------------|------------------------|---------------------|
| **Maternal**                                |                                  |                        |                     |
| Age at enrollment, mean (SD), years         | 31.1 (4.9)                       | 29.3 (5.5)             | <.001               |
| Ethnicity, n (%)                             |                                  |                        |                     |
| Dutch                                       | 1587 (58.7%)                     | 2449 (46.5)            | <.001               |
| Non-Dutch Western                           | 233 (8.6%)                       | 464 (8.8)              |                     |
| Non-Dutch non-Western                       | 884 (32.7%)                      | 2350 (44.6)            |                     |
| Marital status (with partner), n (%)        | 2407 (89.0)                      | 4355 (83.6)            | <.001               |
| Pre-pregnancy BMI, mean (SD)                | 23.4 (4.1)                       | 23.7 (4.5)             | .03                 |
| Parity (multipara), n (%)                   | 1090 (40.3)                      | 2374 (45.4)            | <.001               |
| Psychopathology score, mean (SD)<sup>c</sup> | 0.3 (0.4)                        | 0.3 (0.4)              | <.001               |
| Educational level, n (%)                    |                                  |                        |                     |
| Primary or below                            | 174 (6.4)                        | 690 (13.5)             | <.001               |
| Secondary                                   | 1114 (41.2)                      | 2521 (49.3)            |                     |
| Higher                                      | 1416 (52.4)                      | 1907 (37.3)            |                     |
| Alcohol use during pregnancy, n (%)         |                                  |                        |                     |
| Never                                       | 1105 (40.9)                      | 2623 (53.1)            | <.001               |
| Until pregnancy was known                   | 392 (14.5)                       | 639 (12.9)             |                     |
| Occasionally                                | 949 (35.1)                       | 1383 (28.0)            |                     |
| Frequently<sup>d</sup>                      | 258 (9.5)                        | 293 (5.9)              |                     |
| Household net income, €/month, n (%)        |                                  |                        |                     |
| Less than 1200                               | 420 (15.5)                       | 896 (23.8)             | <.001               |
| 1201-2000                                   | 435 (16.1)                       | 765 (20.3)             |                     |
| More than 2000                              | 1849 (68.4)                      | 2108 (55.9)            |                     |
| Smoking during pregnancy, n (%)             |                                  |                        |                     |
| Never                                       | 2102 (77.7)                      | 3793 (71.3)            | <.001               |
| Until pregnancy was known                   | 238 (8.8)                        | 442 (8.3)              |                     |
| Continued                                   | 364 (13.5)                       | 1083 (20.4)            |                     |
| **Child**                                   |                                  |                        |                     |
| Sex (male), n (%)                            | 1334 (49.3)                      | 2718 (51.1)            | .13                 |

<sup>a</sup> Statistics of the first imputed dataset are reported. Percentages have been rounded and may not total 100.

<sup>b</sup> Continuous variables were compared using t-test or Wilcoxon test; categorical variables were compared using chi-square test.

<sup>c</sup> Scores range from 0 to 4, with higher scores indicating more clinically relevant psychological symptoms.

<sup>d</sup> Defined as ‘one or more glasses of alcohol per week in at least two trimesters’.
**eTable 2. Association of Maternal Smoking During Pregnancy With Cortical Morphology in 10-Year-Old Children**

| Cortical metrics  | Brain region                  | Size (mm²)  | Coordinates | Mean coefficient | Clusterwise p-value |
|-------------------|--------------------------------|-------------|-------------|------------------|---------------------|
|                   |                                |             | X          | Y          | Z          |                   |
| **All children (n=2455)** |                                |             |     |     |     |                   |
| Thickness         | Left inferior parietal         | 301.6       | -32.0      | -85.6     | 12.7      | 0.05               | .0009              |
|                   | Left middle temporal           | 1081.9      | -32.7      | -77.5     | 39.9      | -0.03              | .0001              |
|                   | Left lateral occipital         | 987.1       | -52.5      | -32.8     | -10.3     | -0.02              | .0001              |
|                   | Right inferior parietal        | 313.8       | -11.6      | -96.5     | -10.2     | -0.03              | .02                |
|                   | Right pericalcarine            | 1674.3      | 43.2       | -74.9     | 21.6      | -0.04              | .0001              |
|                   | Gyrification                   | 1633.5      | 15.2       | -92.5     | -4.0      | -0.04              | .0001              |
|                   | Left postcentral               | 1017.2      | -58.1      | -9.4      | 16.8      | -0.08              | .0001              |
| **Children of prenatally included women (n=2268)** |                                |             |     |     |     |                   |
| Surface area      | Left middle temporal           | 762.5       | -53.6      | -33.9     | -10.2     | -0.02              | .0001              |
|                   | Left inferior parietal         | 661.3       | -39.7      | -79.0     | 24.8      | -0.03              | .0003              |
|                   | Right pericalcarine            | 1473.3      | 16.0       | -92.8     | -4.1      | -0.04              | .0001              |
|                   | Right inferior parietal        | 1284.9      | 43.4       | -75.5     | 21.5      | -0.04              | .0001              |
|                   | Gyrification                   | 789.3       | -57.7      | -8.9      | 16.5      | -0.08              | .0004              |

* Cortical metrics, including thickness, surface area and gyrification, in 10-year-old children born to women who continued smoking during pregnancy compared to those born to women who never smoked during pregnancy (2091 vs. 364 for analyses in all children, and 1918 vs. 350 for analyses in children of prenatally included women, respectively). Vertex-wise linear regression was used, and the presented model was adjusted for child sex and age at brain assessment and maternal ethnicity, age at enrollment, marital status, educational level, psychopathology score, alcohol use during pregnancy, and household income. All these clusters survived a clusterwise (Monte Carlo simulation with 5000 iterations) correction for multiple comparisons (p < .001). The corresponding brain regions are visualized in Figure 2.
eTable 3. Demographic Information of Children With DNA Methylation (n = 784)⁴

| Characteristics                          | Descriptive statistics⁵ |
|------------------------------------------|--------------------------|
| **Maternal**                             |                          |
| Age at enrollment, mean (SD), years      | 32.1 (4.0)               |
| Marital status (with partner), n (%)     | 752 (95.9)               |
| Pre-pregnancy BMI, mean (SD)              | 23.0 (3.8)               |
| Parity (multipara), n (%)                | 294 (37.5)               |
| Psychopathology score, mean (SD)⁶        | 0.2 (0.2)                |
| Educational level, n (%)                 |                          |
| Primary or below                         | 8 (1.0)                  |
| Secondary                                | 241 (30.7)               |
| Higher                                   | 535 (68.2)               |
| Alcohol use during pregnancy, n (%)      |                          |
| Never                                    | 200 (25.5)               |
| Before pregnancy was known               | 113 (14.4)               |
| Occasionally                             | 357 (45.5)               |
| Frequently⁷                               | 114 (14.5)               |
| Household net income, €/month, n (%)     |                          |
| Less than 1200                           | 16 (2)                   |
| 1201-2000                                | 76 (9.7)                 |
| More than 2000                           | 692 (88.3)               |
| Smoking during pregnancy, n (%)          |                          |
| Never                                    | 617 (78.7)               |
| Until pregnancy was known                | 78 (9.9)                 |
| Continued                                | 89 (11.4)                |
| **Child**                                |                          |
| Age at neuroimaging, mean (SD), years    | 10.2 (0.6)               |
| Sex, number (%)                          |                          |
| Male                                     | 388 (49.5)               |
| Female                                   | 396 (50.5)               |

¹ Only children of European ancestry were included.
² Statistics of the first imputed dataset are reported. Percentages have been rounded and may not total 100.
³ Scores range from 0 to 4, with higher scores indicating more clinically relevant psychological symptoms.
⁴ Defined as ‘one or more glasses of alcohol per week in at least two trimesters’.

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eFigure 2. Mediation Analysis of Methylation Risk Score

A

Maternal smoking during pregnancy (continued smoking vs. non-smoking)

Methylation risk score at birth, calculated using 5645 CpG sites with FDR significance

Direct effect: -3.40 (95% CI: -26.34, 18.49)
Indirect effect: -0.27 (95% CI: -7.23, 5.72)

Child total brain volume at age 10 years

b = 0.28 (95% CI: 0.21, 0.35)

B

Maternal smoking during pregnancy (continued smoking vs. non-smoking)

Methylation risk score at birth, calculated using 405 CpG sites annotated to genes involving in brain development with FDR significance

Direct effect: -3.14 (95% CI: -26.39, 18.95)
Indirect effect: -0.63 (95% CI: -5.30, 4.30)

Child total brain volume at age 10 years

b = 0.02 (95% CI: 0.02, 0.03)

b = -1.99 (95% CI: -23.66, 19.69)

b = -27.38 (95% CI: -206.60, 151.85)
Legends: Based on findings from primary analyses, 681 (n=86 exposed to continued maternal smoking during pregnancy, and n=595 without exposure) of the 756 children of European ancestry with a methylation risk score and complete information on four principal genetic components were included in the analysis investigating whether DNA methylation risk score at birth mediates the association between maternal smoking during pregnancy and child brain volumes (here we used total brain volume as an example). Panel A illustrated results of methylation risk scores based on 5643 CpG sites that survived a false positive rate (FDR) correction; panel B illustrate results of methylation risk scores based on 405 CpG sites annotated to brain development pathway (http://amigo.geneontology.org/amigo/term/GO:0007420) that survived an FDR correction. Analyses were adjusted for child sex and age at neuroimaging and maternal age at enrollment, maternal educational level, cell types, plate number, and four genetic principal components.
eTable 4. Association of Maternal Smoking During Pregnancy With Regional Brain Volumes in 10-Year-Old Children, Inverse Probability Weighteda

| Maternal smoking during pregnancy | Cerebral gray matter volume | Cerebral white matter volume | Cerebellar volume |
|----------------------------------|-----------------------------|------------------------------|-------------------|
|                                  | b (95% CI) | p-value | b (95% CI) | p-value | b (95% CI) | p-value |
| Minimally adjusted modelb |                |     |             |     |             |     |
| Never                           | Reference | NA    | Reference | NA    | Reference | NA     |
| Until pregnancy was known       | 1.4 (-5.2 to 7.9) | .68   | -0.8 (-6.0 to 4.4) | .75   | 0.5 (-1.1 to 2.2) | .52   |
| Continued                       | -15.6 (-21.2 to -10.0) | <.001 | -10.7 (-15.4 to -6.1) | <.001 | -2.4 (-3.8 to -1.0) | <.001 |
| Fully adjusted modelc |                |     |             |     |             |     |
| Never                           | Reference | NA    | Reference | NA    | Reference | NA     |
| Until pregnancy was known       | 0.4 (-6.0 to 6.7) | .91   | -1.3 (-6.7 to 4.0) | .63   | 0.6 (-1.0 to 2.3) | .46   |
| Continued                       | -8.4 (-14.0 to -2.8) | .003 | -6.4 (-11.2 to -1.6) | .01   | -0.9 (-2.3 to 0.5) | .23   |

Abbreviation: NA, not applicable.

a Linear regression was used. The b values represent volumetric differences (in cm³) of the group that smoked until pregnancy was known (n=238) or the group that continued smoking (n=364) compared with the never smoked (reference) group (n=2102).

b Adjusted for child sex and age at brain assessment, and weighted by inverse probability to count for attrition.

c Adjusted for child sex and age at brain assessment and maternal ethnicity, age at enrollment, marital status, educational level, psychopathology score, alcohol use during pregnancy, household income, and weighted by inverse probability to count for attrition.
etTable 5. Association of Maternal Smoking During Pregnancy With Regional Brain Volumes in 10-Year-Old Children, Prenatally Included Only

| Maternal smoking during pregnancy | Cerebral gray matter volume | Cerebral white matter volume | Cerebellar volume |
|----------------------------------|-----------------------------|-------------------------------|-------------------|
|                                  | \( b \) (95% CI)            | p-value                       | \( b \) (95% CI) | p-value |
| Minimally adjusted model<sup>b</sup> |                             |                               |                   |         |
| Never                           | Reference | NA                          | Reference | NA       | Reference | NA         |
| Until pregnancy was known       | 1.5 (-5.3 to 8.4)           | .67                           | -1.4 (-7.2 to 4.5)| .65     | 0.7 (-1.0 to 2.3)| .42        |
| Continued                       | -14.4 (-20.0 to -8.8)       | <.001                         | -9.8 (-14.7 to -5.0)| <.001  | -2.2 (-3.6 to -0.8)| .002       |
| Fully adjusted model<sup>c</sup> |                             |                               |                   |         |
| Never                           | Reference | NA                          | Reference | NA       | Reference | NA         |
| Until pregnancy was known       | 0.2 (-6.5 to 6.8)           | .96                           | -2.1 (-8.0 to 3.7)| .47     | 0.6 (-1.0 to 2.3)| .45        |
| Continued                       | -7.4 (-13.1 to -1.7)        | .01                           | -5.5 (-10.5 to -0.6)| .03     | -0.8 (-2.2 to 0.6)| .24        |

Abbreviation: NA, not applicable.

<sup>a</sup> Linear regression was used. The \( b \) values represent volumetric differences (in cm\(^3\)) of the group that smoked until pregnancy was known (n=224) or the group that continued smoking (n=350) compared with the never smoked (reference) group (n=1929).

<sup>b</sup> Adjusted for child sex and age at brain assessment.

<sup>c</sup> Adjusted for child sex and age at brain assessment and maternal ethnicity, age at enrollment, marital status, educational level, psychopathology score, alcohol use during pregnancy, and household income.
eFigure 3. Association of Maternal Smoking During Pregnancy With Cortical Morphology in 10-Year-Old Children, Prenatally Included Only

Legends: Cortical morphology (i.e., thickness, surface area, and gyrification) in 10-year-old children born to mothers who continued smoked during pregnancy (n=350) compared with those born to mothers who never smoked during pregnancy (i.e., reference, n=1918). Models were adjusted for child sex and age at brain assessment and maternal ethnicity, age at enrollment, marital status, educational level, psychopathology score, alcohol use during pregnancy, and household income. Clusters of red to yellow represent larger surface area or more gyrification; clusters of dark to light blue represent smaller surface area or less gyrification. The cortical differences in the colored clusters all survived a clusterwise (Monte Carlo simulation with 5000 iterations) correction for multiple comparisons (p < .001). CWP, clusterwise p-values; LH, left hemisphere; RH, right hemisphere.
eReference

1. Greve DN, Fischl B. False positive rates in surface-based anatomical analysis. *Neuroimage*. 2018;171:6-1