**Title:** Particulate matter exposure *in utero* is associated with childhood saliva DNA methylation at ages 9 and 15

**Supplemental figures and tables**



**Supplemental Figure 1.** Fragile Families and Child Wellbeing study sample flow chart. Boxes with solid lines on the left are included observations and individuals. Boxes with dashed lines on the right are excluded observations and individuals.



**Supplemental Figure 2.** Analytic samples by age and particulate matter type in the Fragile Families and Child Wellbeing.

**Supplemental Table 1.** Descriptive statistics of the included and excluded study samples from the Fragile Families and Child Wellbeing study.

|  | **[ALL]  N=1811** | **Excluded sample  N=269** | **Included sample  N=1542** | **p-value** | **N** |
| --- | --- | --- | --- | --- | --- |
| Child or teen visit |  |  |  | 0.517 | 1811 |
| Child | 886 (48.9%) | 137 (50.9%) | 749 (48.6%) |  |  |
| Teen | 925 (51.1%) | 132 (49.1%) | 793 (51.4%) |  |  |
| Child sex |  |  |  | 0.688 | 1811 |
| Female | 899 (49.6%) | 130 (48.3%) | 769 (49.9%) |  |  |
| Male | 912 (50.4%) | 139 (51.7%) | 773 (50.1%) |  |  |
| Child race/ethnicity |  |  |  | <0.001 | 1811 |
| Non-Hispanic White | 314 (17.3%) | 58 (21.6%) | 256 (16.6%) |  |  |
| Non-Hispanic Black | 965 (53.3%) | 97 (36.1%) | 868 (56.3%) |  |  |
| Hispanic | 410 (22.6%) | 104 (38.7%) | 306 (19.8%) |  |  |
| Other | 50 (2.76%) | 6 (2.23%) | 44 (2.85%) |  |  |
| Multi-Racial | 72 (3.98%) | 4 (1.49%) | 68 (4.41%) |  |  |
| Age at DNA methylation measure | 12.5 (3.09) | 12.8 (3.22) | 12.4 (3.07) | 0.115 | 1780 |
| Missing | 31 (100%) | 31 (100%) | 0 (.%) | . | 31 |
| Maternal income/poverty at birth | 2.24 (2.45) | 2.07 (2.17) | 2.27 (2.49) | 0.179 | 1811 |
| Maternal race/ethnicity |  |  |  | <0.001 | 1811 |
| Non-Hispanic White | 336 (18.6%) | 62 (23.0%) | 274 (17.8%) |  |  |
| Non-Hispanic Black | 999 (55.2%) | 97 (36.1%) | 902 (58.5%) |  |  |
| Hispanic | 416 (23.0%) | 104 (38.7%) | 312 (20.2%) |  |  |
| Other | 60 (3.31%) | 6 (2.23%) | 54 (3.50%) |  |  |
| Maternal marital status |  |  |  | 0.579 | 1807 |
| Married | 423 (23.4%) | 58 (21.9%) | 365 (23.7%) |  |  |
| Not Married | 1384 (76.6%) | 207 (78.1%) | 1177 (76.3%) |  |  |
| Missing | 4 (100%) | 4 (100%) | 0 (.%) | . | 4 |
| Maternal city of residence at birth |  |  |  | . | 1811 |
| Oakland | 116 (6.41%) | 2 (0.74%) | 114 (7.39%) |  |  |
| Austin | 127 (7.01%) | 127 (47.2%) | 0 (0.00%) |  |  |
| Baltimore | 107 (5.91%) | 10 (3.72%) | 97 (6.29%) |  |  |
| Detroit | 335 (18.5%) | 23 (8.55%) | 312 (20.2%) |  |  |
| Newark | 56 (3.09%) | 5 (1.86%) | 51 (3.31%) |  |  |
| Philadelphia | 125 (6.90%) | 5 (1.86%) | 120 (7.78%) |  |  |
| Richmond | 160 (8.83%) | 17 (6.32%) | 143 (9.27%) |  |  |
| Corpus Christi | 112 (6.18%) | 19 (7.06%) | 93 (6.03%) |  |  |
| Indianapolis | 93 (5.14%) | 1 (0.37%) | 92 (5.97%) |  |  |
| Milwaukee | 87 (4.80%) | 6 (2.23%) | 81 (5.25%) |  |  |
| New York | 36 (1.99%) | 6 (2.23%) | 30 (1.95%) |  |  |
| San Jose | 84 (4.64%) | 5 (1.86%) | 79 (5.12%) |  |  |
| Boston | 19 (1.05%) | 1 (0.37%) | 18 (1.17%) |  |  |
| Nashville | 27 (1.49%) | 2 (0.74%) | 25 (1.62%) |  |  |
| Chicago | 82 (4.53%) | 9 (3.35%) | 73 (4.73%) |  |  |
| Jacksonville | 22 (1.21%) | 2 (0.74%) | 20 (1.30%) |  |  |
| Toledo | 102 (5.63%) | 15 (5.58%) | 87 (5.64%) |  |  |
| San Antonio | 38 (2.10%) | 7 (2.60%) | 31 (2.01%) |  |  |
| Pittsburgh | 48 (2.65%) | 7 (2.60%) | 41 (2.66%) |  |  |
| Norfolk | 35 (1.93%) | 0 (0.00%) | 35 (2.27%) |  |  |
| PM2.5 exposure at birth | 28.0 (7.04) | 29.7 (6.77) | 27.9 (7.04) | 0.018 | 1634 |
| Missing | 177 (100%) | 177 (100%) | 0 (.%) | . | 177 |
| PM10 exposure at birth | 15.0 (3.05) | 14.9 (2.89) | 15.0 (3.06) | 0.808 | 1515 |
| Missing | 296 (100%) | 179 (100%) | 117 (100%) | . | 296 |
| PM2.5 exposure at age 1 | 25.7 (5.29) | 24.0 (4.91) | 25.9 (5.29) | <0.001 | 1677 |
| Missing | 134 (100%) | 46 (100%) | 88 (100%) | . | 134 |
| PM10 exposure at age 1 | 14.3 (3.16) | 12.3 (3.10) | 14.6 (3.05) | <0.001 | 1677 |
| Missing | 134 (100%) | 44 (100%) | 90 (100%) | . | 134 |
| PM2.5 exposure at age 3 | 26.4 (7.56) | 24.4 (6.12) | 26.7 (7.72) | <0.001 | 1627 |
| Missing | 184 (100%) | 47 (100%) | 137 (100%) | . | 184 |
| PM10 exposure at age 3 | 14.0 (3.27) | 12.4 (2.80) | 14.2 (3.28) | <0.001 | 1636 |
| Missing | 175 (100%) | 47 (100%) | 128 (100%) | . | 175 |
| PM2.5 methylation score (raw) | 0.00 (1.00) | 0.30 (1.86) | -0.05 (0.75) | 0.003 | 1811 |
| PM10 methylation score (raw) | 0.00 (1.00) | 0.48 (2.23) | -0.08 (0.51) | <0.001 | 1811 |
| NO2 methylation score (raw) | 0.00 (1.00) | 0.10 (1.47) | -0.02 (0.89) | 0.207 | 1811 |
| Percent saliva immune cells | 93.7 (13.7) | 92.6 (14.2) | 93.9 (13.6) | 0.187 | 1811 |
| Percent saliva epithelial cells | 6.33 (13.7) | 7.38 (14.2) | 6.15 (13.6) | 0.187 | 1811 |
| Site specific DNA methylation |  |  |  |  |  |
| cg00905156 | 2.69 (3.23) | 3.89 (7.45) | 2.48 (1.52) | 0.002 | 1811 |
| cg06849931 | 73.1 (13.6) | 71.2 (14.1) | 73.4 (13.5) | 0.019 | 1811 |
| cg15082635 | 2.16 (3.59) | 3.59 (9.00) | 1.91 (0.77) | 0.002 | 1811 |
| cg18640183 | 5.20 (4.06) | 7.37 (9.86) | 4.82 (1.21) | <0.001 | 1811 |
| cg20340716 | 92.4 (3.95) | 90.3 (9.45) | 92.8 (1.37) | <0.001 | 1811 |
| cg24127244 | 2.68 (3.00) | 3.94 (7.52) | 2.46 (0.65) | 0.001 | 1811 |

|  |  |
| --- | --- |
| **A)** | **B.** |

**Supplemental Figure 3.** Distribution of particulate matter exposure levels during the *in utero* period in the Fragile Families and Child Wellbeing Study. A) Particulate matter < 2.5 µM levels measured in µg/m3/day. The red vertical line corresponds to the United States National Ambient Air Quality Standard (https://www.epa.gov/criteria-air-pollutants/naaqs-table). B) Particulate matter < 10 µM levels measured in µg/m3/day.

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**Supplemental Figure 4.** Pearson correlations among air pollution levels (PM2.5, PM10) measured at birth, age 1, and age 3 in the Fragile Families and Child Wellbeing Study.

|  |  |  |  |
| --- | --- | --- | --- |
| **Pollutant** | **Raw DNA methylation scores** | **Probe mean centered DNA methylation scores** | **Probe z-score standardized DNA methylation scores** |
| **PM2.5** | **A.** | **B.** | **C.** |
| **PM10** | **D.** | **E.** | **F.** |
| **NO2** | **G.** | **H.** | **I.** |

**Supplemental Figure 5.** Distribution of air pollution poly DNA methylation scores in the Fragile Families and Child Wellbeing Study. The rows are organized by pollutant: A, B, C) Particulate Matter < 2.5 uM; D, E, F) Particulate Matter < 10 uM; G, H, I) Nitrogen dioxide. The columns are organized by method of calculation of poly DNA methylation scores. A, D, G) Raw DNA methylation values were used. B, E, H) DNA methylation values were centered within the study sample prior to weighting for the scores. C, F, I) DNA methylation values were centered and scaled within the study sample prior to weighting for the scores.

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**Supplemental Figure 6.** Pearson correlations among air pollution poly DNA methylation scores in the Fragile Families and Child Wellbeing Study.

|  |  |  |  |
| --- | --- | --- | --- |
| **Pollutant** | **All participants and observations** | **Age 9** | **Age 15** |
| **PM2.5** | **A.** | **B.** | **C.** |
| **PM10** | **D.** | **E.** | **F.** |

**Supplemental Figure 7.** Bivariate associations between particulate matter exposure and poly DNA methylation score.

**Supplemental Table 2.** Adjusted associations between DNA methylation score for prenatal particulate matter exposure and levels of prenatal particulate matter exposure in the Fragile Families and Child Wellbeing Study. All ages models are mixed effects regression models with random intercepts for participants. Age stratified models are linear regression models. Models are adjusted for age at DNA measurement, child sex, maternal race, maternal income to poverty ratio, proportion epithelial cells, proportion immune cells, and postnatal age 1 air pollution exposure.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  | Raw DNA methylation | | |  | Centered DNA methylation | | |  | Centered & scaled DNA methylation | | |  |
| Exposure | Age | Nindiv | Nobs | ß | Lower confidence interval | Upper confidence interval | P-value | ß | Lower confidence interval | Upper confidence interval | P-value | ß | Lower confidence interval | Upper confidence interval | P-value |
| PM2.5 | All | 738 | 1454 | -0.0173 | -0.0706 | 0.0360 | 0.5234 | -0.0173 | -0.0706 | 0.0360 | 0.5234 | -0.0055 | -0.0471 | 0.0361 | 0.7956 |
| PM2.5 | 9 | 710 | 710 | -0.0101 | -0.0682 | 0.0480 | 0.7323 | -0.0101 | -0.0682 | 0.0480 | 0.7323 | -0.0028 | -0.0503 | 0.0447 | 0.9075 |
| PM2.5 | 15 | 744 | 744 | -0.0058 | -0.0630 | 0.0513 | 0.8411 | -0.0058 | -0.0630 | 0.0513 | 0.8411 | 0.0045 | -0.0424 | 0.0515 | 0.8502 |
| PM10 | All | 682 | 1342 | -0.2422 | -0.4546 | -0.0298 | 0.0255 | -0.2422 | -0.4546 | -0.0298 | 0.0255 | -0.2086 | -0.3997 | -0.0176 | 0.0323 |
| PM10 | 9 | 653 | 653 | -0.0143 | -0.0405 | 0.0118 | 0.2828 | -0.0143 | -0.0405 | 0.0118 | 0.2828 | -0.0137 | -0.0361 | 0.0087 | 0.2307 |
| PM10 | 15 | 689 | 689 | -0.0302 | -0.0556 | -0.0047 | 0.0202 | -0.0302 | -0.0556 | -0.0047 | 0.0202 | -0.0268 | -0.0484 | -0.0052 | 0.0150 |

**Supplemental Table 3.** Adjusted associations between DNA methylation score for prenatal particulate matter exposure and levels of prenatal particulate matter exposure in the Fragile Families and Child Wellbeing Study. All ages models are mixed effects regression models with random intercepts for participants. Age stratified models are linear regression models. Models are adjusted for age at DNA measurement, child sex, maternal race, maternal income to poverty ratio, proportion epithelial cells, proportion immune cells, and postnatal age 3 air pollution exposure.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  | Raw DNA methylation | | |  | Centered DNA methylation | | |  | Centered & scaled DNA methylation | | |  |
| Exposure | Age | Nindiv | Nobs | ß | Lower confidence interval | Upper confidence interval | P-value | ß | Lower confidence interval | Upper confidence interval | P-value | ß | Lower confidence interval | Upper confidence interval | P-value |
| PM2.5 | All | 714 | 1405 | -0.0316 | -0.0893 | 0.0261 | 0.2821 | -0.0316 | -0.0893 | 0.0261 | 0.2821 | -0.0189 | -0.0638 | 0.0260 | 0.4083 |
| PM2.5 | 9 | 684 | 684 | -0.0165 | -0.0793 | 0.0463 | 0.6057 | -0.0165 | -0.0793 | 0.0463 | 0.6057 | -0.0077 | -0.0592 | 0.0438 | 0.7699 |
| PM2.5 | 15 | 721 | 721 | -0.0268 | -0.0883 | 0.0347 | 0.3929 | -0.0268 | -0.0883 | 0.0347 | 0.3929 | -0.0148 | -0.0651 | 0.0354 | 0.5628 |
| PM10 | All | 666 | 1309 | -0.2004 | -0.4204 | 0.0196 | 0.0742 | -0.2004 | -0.4204 | 0.0196 | 0.0742 | -0.1699 | -0.3677 | 0.0279 | 0.0922 |
| PM10 | 9 | 635 | 635 | -0.0036 | -0.0303 | 0.0231 | 0.7930 | -0.0036 | -0.0303 | 0.0231 | 0.7930 | -0.0083 | -0.0314 | 0.0147 | 0.4786 |
| PM10 | 15 | 674 | 674 | -0.0343 | -0.0604 | -0.0082 | 0.0100 | -0.0343 | -0.0604 | -0.0082 | 0.0100 | -0.0249 | -0.0470 | -0.0029 | 0.0268 |

**Supplemental Table 4.** Adjusted associations between DNA methylation score for prenatal particulate matter exposure and levels of prenatal particulate matter exposure in the Fragile Families and Child Wellbeing Study. All ages models are mixed effects regression models with random intercepts for participants. Age stratified models are linear regression models. Models are adjusted for age at DNA measurement, child sex, maternal race, maternal income to poverty ratio, proportion epithelial cells, proportion immune cells, and the co-exposure to particulate matter air pollution (either PM2.5 or PM10).

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  | Raw DNA methylation | | |  | Centered DNA methylation | | |  | Centered & scaled DNA methylation | | |  |
| Exposure | Age | Nindiv | Nobs | ß | Lower confidence interval | Upper confidence interval | P-value | ß | Lower confidence interval | Upper confidence interval | P-value | ß | Lower confidence interval | Upper confidence interval | P-value |
| PM2.5 | All | 727 | 1425 | -0.0278 | -0.0806 | 0.0249 | 0.3007 | -0.0278 | -0.0806 | 0.0249 | 0.3007 | -0.0119 | -0.0529 | 0.0291 | 0.5690 |
| PM2.5 | 9 | 690 | 690 | -0.0225 | -0.0781 | 0.0332 | 0.4280 | -0.0225 | -0.0781 | 0.0332 | 0.4280 | -0.0111 | -0.0563 | 0.0342 | 0.6309 |
| PM2.5 | 15 | 735 | 735 | -0.0225 | -0.0796 | 0.0346 | 0.4387 | -0.0225 | -0.0796 | 0.0346 | 0.4387 | -0.0067 | -0.0536 | 0.0402 | 0.7783 |
| PM10 | All | 727 | 1425 | -0.1474 | -0.3079 | 0.0131 | 0.0718 | -0.1474 | -0.3079 | 0.0131 | 0.0718 | -0.1453 | -0.2898 | -0.0009 | 0.0486 |
| PM10 | 9 | 690 | 690 | -0.0043 | -0.0239 | 0.0153 | 0.6671 | -0.0043 | -0.0239 | 0.0153 | 0.6671 | -0.0062 | -0.0230 | 0.0105 | 0.4650 |
| PM10 | 15 | 735 | 735 | -0.0231 | -0.0424 | -0.0038 | 0.0191 | -0.0231 | -0.0424 | -0.0038 | 0.0191 | -0.0231 | -0.0394 | -0.0067 | 0.0057 |

**Supplemental Table 5.** Adjusted associations between DNA methylation score for prenatal nitrogen dioxide exposure and levels of prenatal particulate matter exposure in the Fragile Families and Child Wellbeing Study. All ages models are mixed effects regression models with random intercepts for participants. Age stratified models are linear regression models. Models are adjusted for age at DNA measurement, child sex, maternal race, maternal income to poverty ratio, proportion epithelial cells, and proportion immune cells.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  | Raw DNA methylation | | |  | Centered DNA methylation | | |  | Centered & scaled DNA methylation | | |  |
| Exposure | Age | Nindiv | Nobs | ß | Lower confidence interval | Upper confidence interval | P-value | ß | Lower confidence interval | Upper confidence interval | P-value | ß | Lower confidence interval | Upper confidence interval | P-value |
| PM2.5 | All | 787 | 1542 | 0.0292 | -0.0551 | 0.1135 | 0.4969 | 0.0292 | -0.0551 | 0.1135 | 0.4969 | 0.0071 | -0.0608 | 0.0749 | 0.8383 |
| PM2.5 | 9 | 749 | 749 | 0.0518 | -0.0433 | 0.1468 | 0.2853 | 0.0518 | -0.0433 | 0.1468 | 0.2853 | 0.0453 | -0.0322 | 0.1228 | 0.2512 |
| PM2.5 | 15 | 793 | 793 | 0.0306 | -0.0642 | 0.1254 | 0.5265 | 0.0306 | -0.0642 | 0.1254 | 0.5265 | -0.0068 | -0.0836 | 0.0700 | 0.8620 |
| PM10 | All | 728 | 1425 | 1.4166 | 0.7019 | 2.1314 | 0.0001 | 1.4166 | 0.7019 | 2.1314 | 0.0001 | 1.0661 | 0.4878 | 1.6444 | 0.0003 |
| PM10 | 9 | 690 | 690 | 0.1386 | 0.0678 | 0.2095 | 0.0001 | 0.1386 | 0.0678 | 0.2095 | 0.0001 | 0.1095 | 0.0515 | 0.1675 | 0.0002 |
| PM10 | 15 | 735 | 735 | 0.1271 | 0.0520 | 0.2022 | 0.0009 | 0.1271 | 0.0520 | 0.2022 | 0.0009 | 0.0920 | 0.0305 | 0.1534 | 0.0034 |