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**Supplemental Material**

Prenatal and Childhood Traffic-Related Air Pollution Exposure and Telomere Length in European Children: The HELIX Project

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**Table of Contents**

**Table S1.** General characteristics of the complete case study population stratified by cohort.

**Table S2.** Exposure characteristics of the complete case study population stratified by cohort.

**Table S3.** Association between leukocyte telomere length and traffic-related air pollution exposure and distance to nearest road.

**Table S4.** Sensitivity analyses.

**Table S5.** Leukocyte telomere length in association with categorized pre- and postnatal ambient air pollution.

**Figure S1.** Distribution of the prenatal and 1-year childhood NO₂ exposure levels across the different HELIX cohorts.

**Figure S2.** Distribution of the prenatal and 1-year childhood PM₂₅ exposure levels across the different HELIX cohorts.
Figure S3. GAM models show the linear relation between (A) Prenatal NO$_2$ exposure ($\mu$g/m$^3$) during the entire pregnancy and child leukocyte telomere length, (B) 1-year childhood NO$_2$ exposure ($\mu$g/m$^3$) and child leukocyte telomere length, and (C) PM$_{2.5}$ exposure ($\mu$g/m$^3$) during the entire pregnancy and child leukocyte telomere length (D) 1-year childhood PM$_{2.5}$ exposure ($\mu$g/m$^3$) during the entire pregnancy and child leukocyte telomere. Models were adjusted for child’s age, sex, qPCR batch, maternal age, maternal education, maternal smoking status during pregnancy, child ethnicity, child BMI, and parental smoking at 8 year.
### Supplemental Table S1. General characteristics of the complete case study population stratified by cohort

|                           | INMA (n = 428) | MOBA (n = 213) | BIB (n = 205) | RHEA (n = 199) | KANC (n = 202) | EDEN (n = 149) |
|---------------------------|----------------|---------------|--------------|----------------|----------------|----------------|
| **Children**              |                |               |              |                |                |                |
| **Sex**                   |                |               |              |                |                |                |
| Girls                     | 206 (48.13)    | 98 (46.0)     | 93 (45.37)   | 89 (44.72)     | 92 (45.54)     | 65 (43.6)      |
| Boys                      | 222 (51.87)    | 115 (54.0)    | 112 (54.63)  | 110 (55.28)    | 110 (54.46)    | 84 (56.4)      |
| **Ethnicity**             |                |               |              |                |                |                |
| African                   | 5 (1.17)       | 0 (0.0)       | 7 (3.41)     | 0 (0.0)        | 0 (0.0)        | 0 (0.0)        |
| Asian                     | 2 (0.47)       | 6 (2.9)       | 13 (6.34)    | 0 (0.0)        | 0 (0.0)        | 0 (0.0)        |
| White European            | 380 (88.32)    | 204 (95.7)    | 89 (43.41)   | 199 (100.0)    | 202 (100.0)    | 149 (0.0)      |
| Mixed native American     | 11 (2.57)      | 2 (1.0)       | 0 (0.0)      | 0 (0.0)        | 0 (0.0)        | 0 (0.0)        |
| Other                     | 4 (0.93)       | 1 (0.4)       | 17 (8.82)    | 0 (0.0)        | 0 (0.0)        | 0 (0.0)        |
| South-Asian               | 0 (0.0)        | 0 (0.0)       | 79 (38.54)   | 0 (0.0)        | 0 (0.0)        | 0 (0.0)        |
| White not European        | 26 (6.07)      | 0 (0.0)       | 0 (0.0)      | 0 (0.0)        | 0 (0.0)        | 0 (0.0)        |
| **Gestational age**       | 39.9 ± 1.4     | 40.1 ± 1.7    | 39.7 ± 1.8   | 38.4 ± 1.4     | 39.4 ± 1.3     | 39.8 ± 1.7     |
| **Age at mtDNA content and telomere length assessment, years** | 9.02 ± 0.65    | 8.5 ± 0.5     | 6.6 ± 0.2    | 6.5 ± 0.3      | 6.5 ± 0.5      | 10.8 ± 0.6     |
| **Mothers**               |                |               |              |                |                |                |
| **Age at delivery**       | 31.5 ± 4.2     | 32.8 ± 3.7    | 28.6 ± 5.8   | 30.9 ± 4.8     | 28.57 ± 5.0    | 30.7 ± 5.0     |
| **Missings**              | 1 (0.2)        | 6 (2.8)       | 1 (0.5)      | 2 (1.0)        | 2 (1.0)        | 0 (0.0)        |
| **Education**             |                |               |              |                |                |                |
| Low                       | 99 (23.1)      | 0 (0.0)       | 88 (42.9)    | 9 (4.5)        | 12 (5.9)       | 11 (7.4)       |
| Middle                    | 174 (40.7)     | 41 (19.2)     | 31 (15.1)    | 111 (55.8)     | 69 (34.2)      | 55 (36.9)      |
| High                      | 141 (32.9)     | 164 (77.0)    | 64 (31.2)    | 79 (39.7)      | 116 (57.4)     | 83 (55.7)      |
| Missings   | 14 (3.3) | 8 (3.8) | 22 (10.7) | 2 (1.0) | 5 (2.5) | 3 (2.0) |
|------------|----------|---------|-----------|---------|---------|---------|
| **Active smoking during pregnancy** |          |         |           |         |         |         |
| Yes        | 109 (225.46) | 9 (4.4) | 25 (12.2) | 43 (21.6) | 13 (6.44) | 31 (20.8) |
| No         | 311 (72.66)  | 198 (91.6) | 157 (76.6) | 156 (78.4) | 184 (91.09) | 118 (79.2) |
| Missings   | 8 (1.87) | 9 (4.4) | 23 (11.2) | 1 (0.5) | 5 (2.5) | 0 (0.0) |
| **Parity** |          |         |           |         |         |         |
| 1          | 230 (53.7) | 93 (43.7) | 83 (40.5) | 74 (37.2) | 84 (41.6) | 71 (47.7) |
| 2          | 165 (38.6) | 86 (40.4) | 52 (25.4) | 85 (42.7) | 59 (29.2) | 51 (34.2) |
| ≥3         | 28 (6.5) | 28 (13.1) | 56 (27.3) | 35 (17.6) | 54 (26.7) | 27 (18.1) |
| Missings   | 5 (1.2) | 6 (2.8) | 14 (6.8) | 5 (2.5) | 5 (2.5) | 0 (0.0) |

Continuous covariates expressed by mean and standard deviation ± SD; categorical covariates described by number and frequencies (%); Data are complete for all observations unless otherwise indicated.
## Supplemental Table S2. Exposure characteristics of the complete case study population stratified by cohort

|          | n   | Mean   | SD    | 5th Percentile | 25th Percentile | 50th Percentile | 75th Percentile | 95th Percentile |
|----------|-----|--------|-------|----------------|-----------------|-----------------|----------------|----------------|
| **INMA** |     |        |       |                |                 |                 |                 |                |
| NO₂ Prenatal | 351 | 43.23  | 11.17 | 24.55          | 36.08           | 43.24           | 49.07           | 60.83          |
| NO₂ Postnatal  | 401 | 33.04  | 11.81 | 11.74          | 26.83           | 35.4            | 40.69           | 50.18          |
| PM₁₂.₅ Prenatal | 351 | 15.08  | 1.72  | 12.3           | 14.1            | 14.97           | 15.98           | 17.86          |
| PM₁₂.₅ Postnatal | 401 | 13.31  | 1.72  | 10.47          | 12.66           | 13.3            | 13.88           | 15.7           |
| **MOBA** |     |        |       |                |                 |                 |                 |                |
| NO₂ Prenatal | 206 | 20.51  | 7.67  | 11.17          | 14.49           | 18.52           | 25.24           | 36.31          |
| NO₂ Postnatal  | 207 | 26.2   | 5.41  | 19.35          | 22.72           | 25.44           | 29.59           | 33.6           |
| PM₁₂.₅ Prenatal | 207 | 12.06  | 2.22  | 8.13           | 10.47           | 12.66           | 13.53           | 15.94          |
| PM₁₂.₅ Postnatal | 207 | 8.12   | 1.61  | 5.95           | 7.06            | 7.77            | 9.06            | 11.13          |
| **BIB** |     |        |       |                |                 |                 |                 |                |
| NO₂ Prenatal | 205 | 20.79  | 3.43  | 15.66          | 18.43           | 20.61           | 23.12           | 26.71          |
| NO₂ Postnatal  | 205 | 31.6   | 3.93  | 26.68          | 28.61           | 31.29           | 33.67           | 38.11          |
| PM₁₂.₅ Prenatal | 205 | 14.37  | 1.78  | 11.49          | 13.28           | 14.18           | 15.48           | 17.5           |
| PM₁₂.₅ Postnatal | 205 | 14.39  | 1.2   | 12.66          | 13.58           | 14.23           | 15.12           | 16.44          |
| **RHEA** |     |        |       |                |                 |                 |                 |                |
| NO₂ Prenatal | 199 | 12.14  | 4.21  | 8.34           | 9.28            | 11.19           | 12.8            | 21.83          |
| NO₂ Postnatal  | 199 | 10.99  | 3.47  | 7.66           | 6.87            | 10.09           | 12.05           | 18.72          |
| PM₁₂.₅ Prenatal | 199 | 14.49  | 1.24  | 12.95          | 12.95           | 14.39           | 15.26           | 16.99          |
| PM₁₂.₅ Postnatal | 199 | 14.09  | 1.86  | 11.71          | 12.83           | 13.63           | 15.12           | 17.47          |
| **KANC** |     |        |       |                |                 |                 |                 |                |
| NO₂ Prenatal | 195 | 18.53  | 3.74  | 13.42          | 15.94           | 17.83           | 20.67           | 24.79          |
| NO₂ Postnatal  | 194 | 13.99  | 2.51  | 10.05          | 12.51           | 13.99           | 15.21           | 17.8           |
| PM₁₂.₅ Prenatal | 195 | 17.61  | 2.44  | 13.49          | 15.78           | 17.98           | 19.09           | 20.93          |
| PM₁₂.₅ Postnatal | 194 | 18.29  | 1.6   | 15.28          | 17.58           | 18.28           | 19.34           | 20.68          |

Continuous variables expressed by mean and standard deviation (SD)
Table S3. Association between leukocyte telomere length and traffic-related air pollution exposure and distance to nearest road

| Exposure                | Pregnancy       | 1-year childhood | p-value |
|-------------------------|-----------------|------------------|---------|
| **NO₂**                 |                 |                  |         |
| Pregnancy               | -0.0066 (0.013 to 0.001) | 0.02             |
| 1-year childhood        | -0.0071 (0.013 to 0.002) | 0.01             |
| **PM₂.₅**               |                 |                  |         |
| Pregnancy               | -0.0022 (0.0078 to 0.003) | 0.3              |
| 1-year childhood        | -0.0061 (0.0129 to 0.001) | 0.08             |
| **Distance to nearest road** |             |                  |         |
| Pregnancy               | 0.00068 (0.0058 to 0.0072) | 0.8              |
| 1-year childhood        | 0.0068 (0.0001 to 0.014) | 0.04             |

Effect size was estimated for each SD increment in ambient air pollution exposure.

Models were adjusted for child’s age, sex, qPCR batch, maternal age, maternal education, maternal smoking status during pregnancy, child ethnicity, child BMI, and parental smoking at 8 year.
### Supplemental S4. Sensitivity analyses

| Prenatal NO₂ | % difference | 95% CI        | P-value |
|--------------|--------------|---------------|---------|
| Overall      | -1.5         | -2.8 to -0.2  | 0.02    |
| Excluding cohort\(^a\) | | | |
| INMA         | -3.2         | -5.9 to -0.4  | 0.02    |
| MOBA         | -1.7         | -3.1 to -0.3  | 0.02    |
| BIB          | -1.6         | -2.9 to -0.2  | 0.02    |
| RHEA         | -1.1         | -2.3 to 0.1   | 0.08    |
| KANC         | -1.6         | -2.7 to -0.4  | 0.01    |
| EDEN         | -1.6         | -3.1 to -0.03 | 0.048   |
| Adjusted for white blood cell type\(^b\) | -1.3 | -2.5 to -0.1 | 0.04    |
| Stratified by moved or not moved\(^c\) | | | |
| Not moved    | -1.5         | -2.8 to -0.2  | 0.15    |
| Moved        | -1.9         | -9.2 to 6.1   | 0.6     |

| 1-year childhood NO₂ | % difference | 95% CI        | P-value |
|----------------------|--------------|---------------|---------|
| Overall              | -1.6         | -2.9 to -0.4  | 0.01    |
| Excluding cohort\(^a\) | | | |
| INMA                 | -2.4         | -4.8 to 0.2   | 0.07    |
| MOBA                 | -1.7         | -3.1 to -0.3  | 0.02    |
| BIB                  | -1.4         | -2.9 to 0.1   | 0.07    |
| RHEA                 | -1.1         | -2.3 to 0.3   | 0.12    |
| KANC                 | -2.0         | -3.3 to -0.7  | 0.002   |
| EDEN                 | -1.6         | -3.2 to -0.01 | 0.048   |
| Adjusted for white blood cell type\(^b\) | -1.3 | -2.4 to -0.2 | 0.02    |
| Stratified by moved or not moved\(^c\) | | | |
| Not moved            | -1.2         | -2.7 to 0.4   | 0.15    |
| Moved                | -10.0        | -17.9 to -1.3 | 0.03    |

Effect size was estimated as a % difference in LTL for each SD increment in ambient air pollution exposure.

Models were adjusted for child’s age, sex, qPCR batch, maternal age, maternal education, maternal smoking status during pregnancy, child ethnicity, child BMI, and parental smoking at 8 year.

\(^a\) Removing one cohort at the time from the analysis

\(^b\) Model additionally adjusted for white blood cell proportions (CD4+ and CD8+ T-cells, natural killer (NK) cells, monocytes, eosinophiles, neutrophils, and B-cells)

\(^c\) Analysis was stratified by group of children who lived at the same address at both time points versus those who moved between those time points
**Supplemental Table S5.** Leukocyte telomere length in association with categorized pre- and postnatal ambient air pollution.

|                          | % Change (95% CI) | p-value |
|--------------------------|-------------------|---------|
| **Prenatal**             |                   |         |
| NO$_2$ < 20.5 µg/m$^3$   | Ref               |         |
| ≥ 20.5 µg/m$^3$          | -3.0 (-5.2 to -0.8) | 0.008   |
| PM$_{2.5}$ < 15.0 µg/m$^3$| Ref               |         |
| ≥ 15.0 µg/m$^3$          | -0.9 (-3.1 to 1.4)  | 0.43    |
| Distance to nearest road > 150 m | Ref               |         |
| ≤ 150 m                  | -1.7 (-4.6 to 1.3)  | 0.26    |
| **Postnatal**            |                   |         |
| NO$_2$ < 23.5 µg/m$^3$   | Ref               |         |
| ≥ 23.5 µg/m$^3$          | -1.8 (-4.2 to 0.67) | 0.15    |
| PM$_{2.5}$ < 13.3 µg/m$^3$| Ref               |         |
| ≥ 13.3 µg/m$^3$          | -3.0 (-5.3 to -0.62) | 0.01    |
| Distance to nearest road > 150 m | Ref               |         |
| ≤ 150 m                  | -1.9 (-4.0 to 0.31)  | 0.09    |

Effect size was estimated as a % change in LTL for each SD increment in ambient air pollution exposure; SD prenatal NO$_2$ = 13.9 µg/m$^3$, SD postnatal NO$_2$ = 12.2 µg/m$^3$, SD prenatal PM$_{2.5}$ = 2.6 µg/m$^3$, SD postnatal PM$_{2.5}$ = 3.3 µg/m$^3$

Models were adjusted for child’s age, sex, qPCR batch, maternal age, maternal education, maternal smoking status during pregnancy, child ethnicity, child BMI, and parental smoking at 8 year.
Figure S1. Distribution of the prenatal and 1-year childhood NO₂ exposure levels across the different HELIX cohorts.
Figure S2. Distribution of the prenatal and 1-year childhood PM$_{2.5}$ exposure levels across the different HELIX cohorts.
Figure S3. GAM models show the linear relation between (A) Prenatal NO₂ exposure (μg/m³) during the entire pregnancy and child leukocyte telomere length, (B) 1-year childhood NO₂ exposure (μg/m³) and child leukocyte telomere length, and (C) PM₂.₅ exposure (μg/m³) during the entire pregnancy and child leukocyte telomere length (D) 1-year childhood PM₂.₅ exposure (μg/m³) during the entire pregnancy and child leukocyte telomere. Models were adjusted for child’s age, sex, qPCR batch, maternal age, maternal education, maternal smoking status during pregnancy, child ethnicity, child BMI, and parental smoking at 8 year.