Supplementary Online Content

Martens DS, Cox B, Janssen BG, et al. Prenatal air pollution and newborns' predisposition to accelerated biological aging. *JAMA Pediatr*. Published online October 16, 2017. doi:10.1001/jamapediatrics.2017.3024

This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods 1. Cord blood and placental tissue collection.

Umbilical cord blood was drawn immediately after delivery in BD Vacutainer® plastic whole blood tubes with spray-coated K2EDTA (BD, Franklin Lakes, NJ, USA). Samples were centrifuged at 3200 rpm for 15 min, plasma was separated and the remainder with the buffy coats was stored at −80°C. Placentas were collected and stored at −20°C within 10 minutes after delivery. Four different placental biopsies (1 to 2 cm^3) were taken directly underneath the chorioamniotic membrane for DNA extraction at the fetal side at approximately 4 cm from the umbilical cord and were stored at -80°C as described previously.[1] Care was taken by visual examination and dissection to avoid chorioamniotic membrane contamination. As the average within variation of the placental telomere length was low, i.e. 11.7% (measured for 4 biopsies in 14 placentas), only one biopsy taken to the right of the main artery was used for placental telomere length measurement.[2]

eMethods 2. Secondary and sensitivity analyses methods.

In a secondary analysis, we used traditional linear regression to model average $PM_{2.5}$ exposure during the three trimesters (entered in one model) as well as average entire-pregnancy exposure (actual pregnancy duration), hereafter referred to as "average exposure models". These models were adjusted for the same variables as in the main analysis (including the DLNM cross-basis for weekly mean temperature). Sex-specific estimates were obtained by including an interaction between the cross-basis for $PM_{2.5}$ and an indicator variable for newborn sex in the main DLM models. Effect modification by newborn sex was formally tested by comparing models with and without the interaction term (likelihood ratio test on 5 df). We also examined the shape of the dose-response relationship through the use of natural cubic splines.

In a first sensitivity analysis, we used a dummy parameterization assuming constant distributed lag effects along the strata of lags 1-10, 11-20, 21-30, and 31-40. Secondly, we used an unconstrained DLM to define the lag structure, that is, a model in which the weekly mean exposures (n=40) are entered as separate variables.[3,4] Because of the correlation between air pollution concentrations for contiguous weeks, the unconstrained DLM may result in unstable estimates for the individual lags, but this model is more flexible and less prone to bias for the estimate of the overall effect.[3] We also adjusted our model for month of delivery instead of season of delivery. Finally, we tested whether results were robust after the exclusion of newborns from non-European origin, mothers with low education, current or former smokers, mothers with complications during pregnancy, and those with cesarean section.

eTable 1. Newborn and parental predictors of telomere length at birth.

Variable	Cord blood telomere length (n=641)		Placental telomere length (n=641)	
	Percentage change (95% CI)	*P* Value	Percentage change (95% CI)	*P* Value
Maternal age, years	-0.06 (-0.45, 0.43)	0.82	-0.14 (-0.72, 0.45)	0.65
Paternal age, years	0.24 (-0.16, 0.64)	0.24	0.16 (-0.32, 0.64)	0.51
Maternal BMI, kg/m^2	-0.33 (-0.65, -0.02)	0.040	-0.36 (-0.74, 0.03)	0.070
Gestational age, weeks	1.13 (-2.95, 5.38)	0.59	7.53 (2.30, 13.03)	0.0045
Ethnicity				
Non-European Caucasian	Ref		Ref	
European Caucasian	-4.55 (-9.49, 0.17)	0.060	-7.65 (-13.84, -1.79)	0.010
Newborn sex				
Boys	Ref		Ref	
Girls	8.07 (5.05, 11.17)	<0.0001	7.20 (2.30, 13.03)	<0.0001
Smoking				
Non-smoking	Ref		Ref	
Former smoker	-0.17 (-3.54, 3.31)	0.92	0.91 (-3.20, 5.20)	0.67
Current smoker	0.27 (-4.24, 4.99)	0.91	0.38 (-5.06, 6.13)	0.89
Parity				
1	Ref		Ref	
2	-1.46 (-4.50, 1.69)	0.36	1.42 (-2.36, 5.35)	0.47
≥3	-2.58 (-7.56, 2.67)	0.33	0.78 (-5.42, 7.39)	0.81
Maternal education				
Low	Ref		Ref	
Middle	1.75 (-3.14, 6.88)	0.49	-0.68 (-6.43, 5.42)	0.82
High	4.81 (-0.39, 10.28)	0.071	2.26 (-3.86, 8.76)	0.48
Season delivery				
Winter	Ref		Ref	
Spring	1.97 (-6.80, 11.57)	0.67	5.40 (-5.48, 17.53)	0.34
Summer	11.68 (0.78, 23.76)	0.035	11.89 (-1.20, 26.71)	0.078
Autumn	5.65 (-4.81, 17.24)	0.30	8.97 (-3.94, 23.61)	0.18

Estimates provided as a percentage change (95% CI) in mean relative cord blood or placental telomere length for a one unit increment of continuous variables or compared with the reference (Ref) group for categorical variables. Estimates derived from fully adjusted models and are therefore independent of each other.

eTable 2. Sex-specific estimates for the association between $PM_{2.5}$ exposure and newborn telomere length.

	Boys (n=323)	Girls (n=318)	
Tissue	**Percentage change (95% CI)**	**Percentage change (95% CI)**	***P* Value[a]**
Cord blood	-8.9 (-14.9, -2.5)	-8.9 (-15.0, -2.3)	0.96
Placenta	-14.9 (-21.6, -7.6)	-11.2 (-18.3, -3.4)	0.69

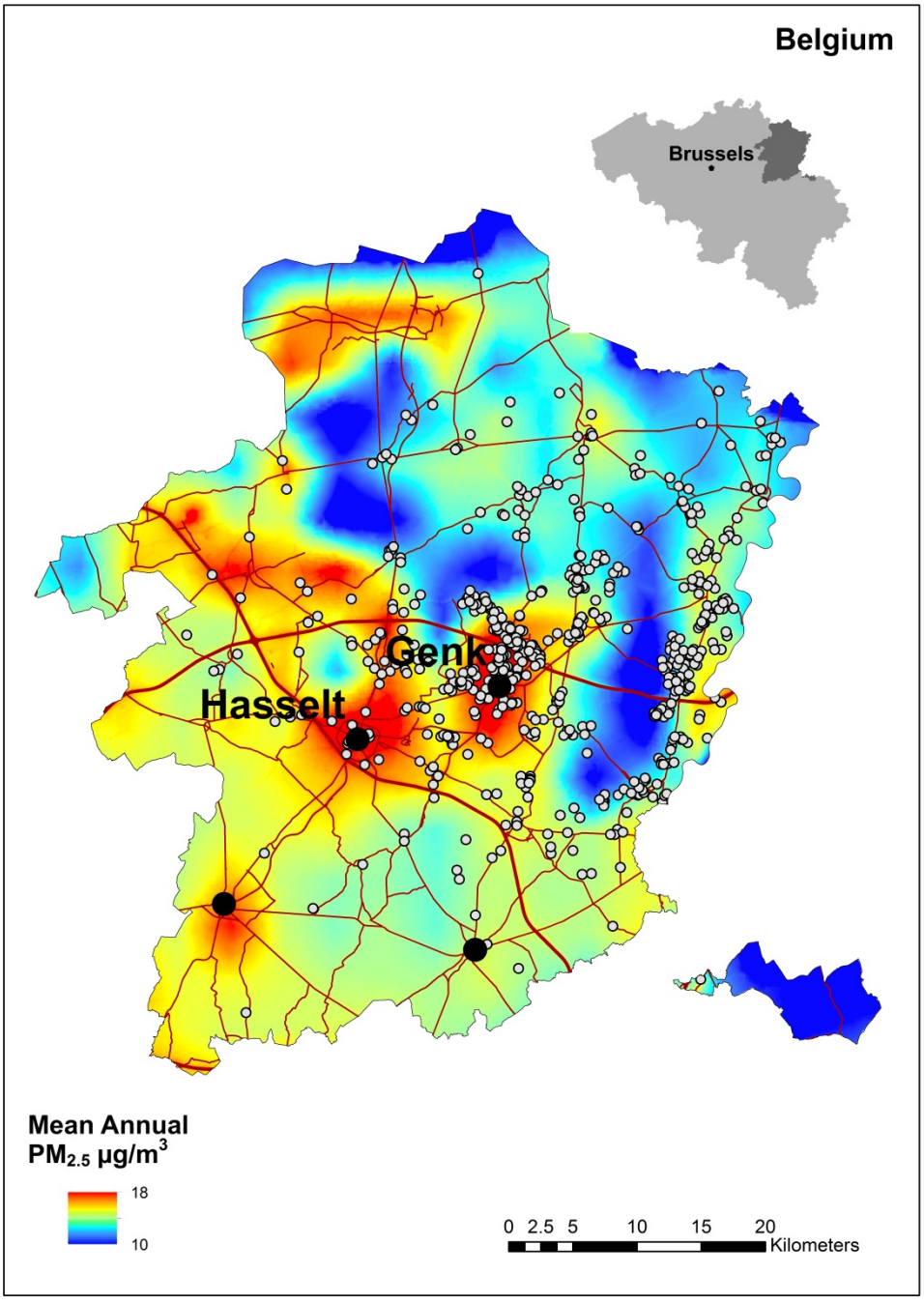
Estimates provided as a percentage change in mean relative telomere length for a 5 $\mu g/m^3$ increment in $PM_{2.5}$, estimated by distributed lag models (DLM) including an interaction between the cross-basis for $PM_{2.5}$ and an indicator variable for newborn sex.
Models adjusted for date of delivery, gestational age, maternal BMI, maternal age, paternal age, newborn sex, newborn ethnicity, season of delivery, parity, maternal smoking status, maternal education, pregnancy complications and ambient temperature.
[a]P Value obtained by comparing models with and without the $PM_{2.5}$ cross-basis by sex interaction (likelihood ratio test on 5 df).

eTable 3. Comparison of the linear and the non-linear dose response associations between $PM_{2.5}$ exposure and newborn telomere length.

	Linear dose-response		Non-linear dose-response		
	Percentage change (95% CI)		Percentage change (95% CI)		
Tissue	**5 µg/m^3 increase**	**AIC**	**15 to 20 µg/m^3**	**35 to 40 µg/m^3**	**AIC**
Cord blood	-8.8 (-14.1, -3.1)	-1418	-9.6 (-15.0, -4.0)	-27.4 (-41.5, -9.8)	-1422
Placenta	-13.2 (-19.3, -6.7)	-1173	-13.2 (-19.3, -6.7)	-49.8 (-61.2, -34.9)	-1195

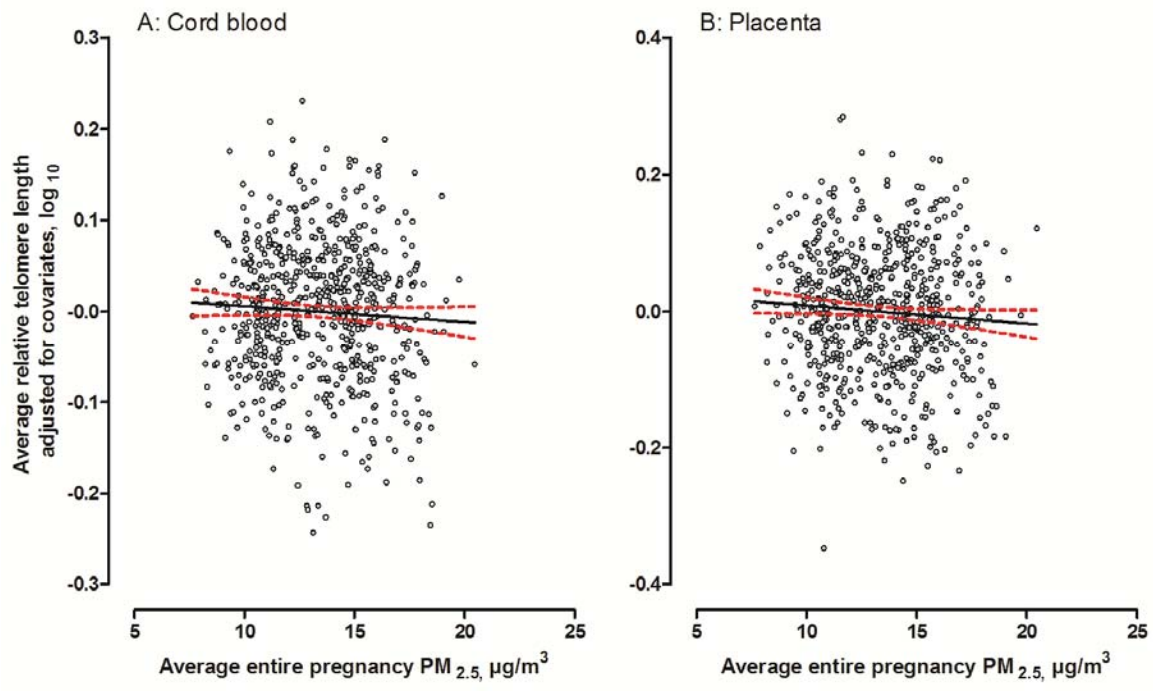
Estimates provided as a percentage change in mean relative telomere length for a 5 µg/m^3 increment in $PM_{2.5}$ (linear-dose response) and for an increment from 15 µg/m^3 (roughly the median) to 20 µg/m^3 and from 35 µg/m^3 (roughly the 95th percentile) to 40 µg/m^3 (non-linear dose-response), estimated by distributed lag models (DLM) using a natural cubic spline with 2 df to model the exposure response association.
Models adjusted for date of delivery, gestational age, maternal BMI, maternal age, paternal age, newborn sex, newborn ethnicity, season of delivery, parity, maternal smoking status, maternal education, pregnancy complications and ambient temperature.

eFigure 1. Contours of long-term $PM_{2.5}$ air pollution (annual mean of 2012) and mothers (n=641) residence (dots) in the recruitment area.



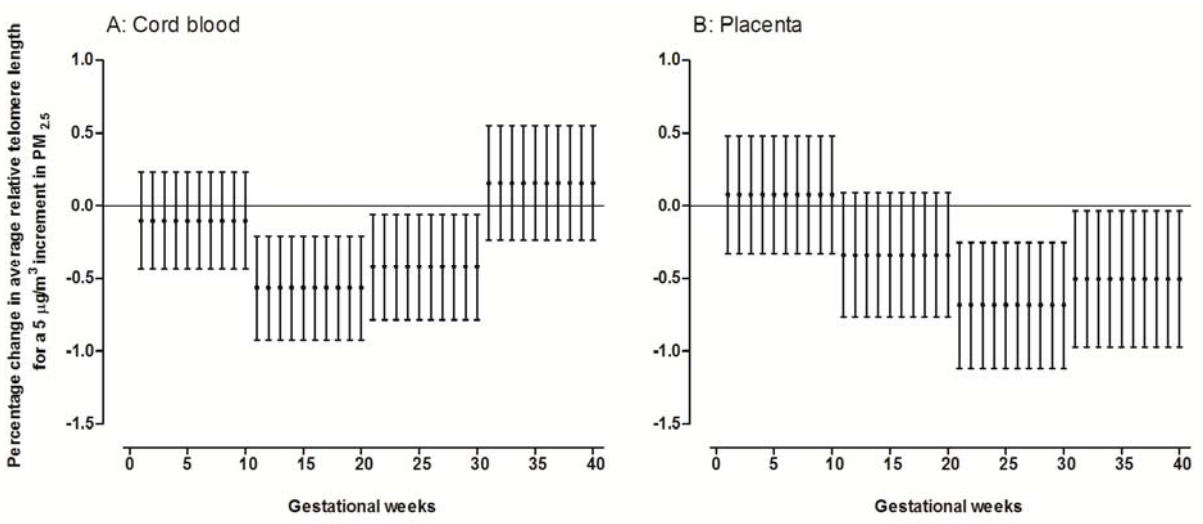
Bold red lines represents high ways, thin red lines major roads.

eFigure 2. Scatterplot showing the association between mean entire pregnancy $PM_{2.5}$ exposure and residualized cord blood (A) and placental (B) telomere length (n=641).



Telomere lengths were residualized by regressing on the covariates (date of delivery, gestational age, maternal BMI, maternal age, paternal age, newborn sex, newborn ethnicity, season of delivery, parity, maternal smoking status, maternal education, pregnancy complications, and ambient temperature).

eFigure 3. Percentage change in relative telomere length (with 95% confidence interval) in cord blood (A) and placenta (B) associated with a 5 µg/m^3 increment in $PM_{2.5}$ exposure, assuming with constant effects within the strata of lags 1-10, 11-20, 21-30, and 31-40.



Models adjusted for date of delivery, gestational age, maternal BMI, maternal age, paternal age, newborn sex, newborn ethnicity, season of delivery, parity, maternal smoking status, maternal education, pregnancy complications, and ambient temperature.

eReferences

1. Janssen BG, Byun HM, Cox B, et al. Variation of DNA methylation in candidate age-related targets on the mitochondrial-telomere axis in cord blood and placenta. *Placenta.* 2014;35(9):665-672.
2. Martens DS, Plusquin M, Gyselaers W, De Vivo I, Nawrot TS. Maternal pre-pregnancy body mass index and newborn telomere length. *BMC Med.* 2016;14(1):148.
3. Schwartz J. The distributed lag between air pollution and daily deaths. *Epidemiology.* 2000;11(3):320-326.
4. Zanobetti A, Wand MP, Schwartz J, Ryan LM. Generalized additive distributed lag models: quantifying mortality displacement. *Biostatistics.* 2000;1(3):279-292.