IMPACT VAN BLOOTSTELLING AAN FIJN STOF OP DNA-METHYLATIE IN DE PLACENTA

Gezondheidseffecten over generaties door (beroepsmatige) blootstelling?

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DEVELOPMENTAL ORIGINS OF HEALTH AND DISEASE

Epigenetics

Nutrition  Vitamins  Drugs  Estrogen Disruptors  Herbicides Pesticides  Particulate Matter (PM)

Epigenetics

Reproductive Disorders  Growth Disorders  Pediatric Disorders  Imprinting Disorders  Cancer  Inflammatory Neurological Disorders  Cardiovascular Disorders

Adapted with permission of Wim Vanden Berghe
ENVIRONAGE BIRTH COHORT

Birth cohort

N = 600 and counting

Exposures:
- Fine dust
- Toxic metals
- Smoking

Placenta

Cord blood

Maternal matrices

Health Evaluation

Biomarkers:
- mtDNA-content
- DNA methylation
- Telomeres
**Questions to Answer**

1. Is placental **global DNA methylation** associated with air pollution *in utero*?

2. Is placental **mitochondrial DNA content** and **mitochondrial DNA methylation** sensitive to exposure of air pollution *in utero*?
GLOBAL DNA METHYLATION AND AIR POLLUTION IN ADULT POPULATIONS

Changes in DNA Methylation Patterns in Subjects Exposed to Low-Dose Benzene

Valentina Bollati,1 Andrea Baccarelli,1,2 Lifang Hou,3 Matteo Bonzini,1 Silvia Fustinoni,1 Domenico Cavallo,4 Hyang-Min Byun,5 Jiayi Jiang,5 Barbara Marinelli,1 Angela C. Pesatori,1 Pier A. Bertazzi,1 and Allen S. Yang5

Rapid DNA Methylation Changes after Exposure to Traffic Particles

Andrea Baccarelli1-2, Robert O. Wright2-3, Valentina Bollati1, Letizia Tarantini1, Augusto A. Litonjua3, Helen H. Suh2, Antonella Zanobetti2, David Sparrow4, Pantel S. Vokonas4, and Joel Schwartz2

Influence of ambient air pollution on global DNA methylation in healthy adults: A seasonal follow-up

Sofie De Prins a,b,*, Gudrun Koppen a, Griet Jacobs a, Evi Dons a,c, Els Van de Mieroop d, Vera Nelen d, Frans Fierens e, Luc Int Panis a,c, Patrick De Boever a,f, Bianca Cox f, Tim S. Nawrot f, Greet Schoeters a,b
### DNA Methylation and Particle Exposure — Some Examples

<table>
<thead>
<tr>
<th>+/-</th>
<th>Genes</th>
<th>Type</th>
<th>Tissue</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑</td>
<td>APC &amp; p16</td>
<td>Human</td>
<td>Peripheral Blood Leukocytes</td>
<td>Hou et al. 2011</td>
</tr>
<tr>
<td>↓</td>
<td>p53 &amp; RASSF1A</td>
<td>Human</td>
<td>Leukocytes</td>
<td></td>
</tr>
<tr>
<td>↓</td>
<td>Global (LINE1 &amp; ALU)</td>
<td>Human</td>
<td>Buffy Coat</td>
<td>Tarantini et al. 2008</td>
</tr>
<tr>
<td>↓</td>
<td>iNOS</td>
<td>Human</td>
<td>Blood</td>
<td>Baccarelli et al. 2009</td>
</tr>
<tr>
<td>↓</td>
<td>Global (LINE1)</td>
<td>Human</td>
<td>Blood</td>
<td></td>
</tr>
<tr>
<td>↓</td>
<td>CYP1A1</td>
<td>Human</td>
<td>Placenta</td>
<td>Suter et al. 2010</td>
</tr>
<tr>
<td>↓</td>
<td>Global (ELISA)</td>
<td>Human</td>
<td>Cord serum</td>
<td>Guerrero-Preston et al. 2010</td>
</tr>
<tr>
<td>↑</td>
<td>Global (ALU)</td>
<td>Human</td>
<td>Placenta</td>
<td>Wilhelm-Benartzi et al. 2011</td>
</tr>
</tbody>
</table>

#### Environmental Exposure
(traffic-related) PM

#### Personal Exposure
Maternal tobacco smoke
Does exposure to particulate air pollution (PM$_{2.5}$) during pregnancy affect global DNA methylation in placental tissue of newborns?
Epigenetic marks may be particularly vulnerable during the very early stage of development, which is a crucial period for establishing and maintaining epigenetic marks.
PLACENTAL BIOPSIES

1. LC/MS-MS analysis to measure global DNA methylation

2. Real-time qPCR:
   To measure mitochondrial DNA content
EXPOSURE ASSESSMENT: WINDOWS OF SUSCEPTIBILITY

- Kriging interpolation method
  - Measuring stations interpolate air pollution levels to grids
- Exposure calculation at residence

<table>
<thead>
<tr>
<th>Pregnancy</th>
<th>3 m</th>
<th>6 m</th>
<th>9 m</th>
</tr>
</thead>
<tbody>
<tr>
<td>Implantation range</td>
<td>Trimester 1 (week 1-13)</td>
<td>Trimester 2 (week 14-26)</td>
<td>Trimester 3 (week 27-birth)</td>
</tr>
</tbody>
</table>

Measurement at birth!
Placental DNA hypomethylation in association with particulate air pollution in early life

Bram G Janssen¹, Lode Godderis²,³, Nicky Pieters¹, Katrien Poels², Michał Kiciński¹, Ann Cuypers¹, Frans Fierens⁴, Joris Penders⁵,⁶, Michelle Plusquin¹, Wilfried Gyselaers⁶,⁷ and Tim S Nawrot¹,²*

- PM₂.₅, µg/m³
- Global methylation, %

Trimester 1

\[ r = -0.25 \]
\[ p = 0.0001 \]
\[ n = 240 \]
### Placental tissue as a molecular ‘footprint’ for in utero exposure

<table>
<thead>
<tr>
<th>Multi-lag model, PM$_{2.5}^{a, b}$</th>
<th>Relative difference</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trimester 1 (1-13w)</td>
<td>−2.13%</td>
<td>−3.71 to −0.54%</td>
<td>0.009</td>
</tr>
<tr>
<td>Trimester 2 (14-26w)</td>
<td>−0.43%</td>
<td>−1.84 to 0.98%</td>
<td>0.55</td>
</tr>
<tr>
<td>Trimester 3 (27w-delivery)</td>
<td>0.74%</td>
<td>−0.85 to 2.33%</td>
<td>0.36</td>
</tr>
</tbody>
</table>

$^a$All the three trimester exposures were fitted as independent variables in the same regression model. The effect size is a relative difference (95% CI) in mean placental global DNA methylation for each 5 µg/m$^3$ increase of PM$_{2.5}$ exposure (µg/m$^3$).

$^b$Adjusted for newborn’s gender, maternal age, gestational age, parity, maternal education, smoking status, prenatal acetaminophen use, season at conception and trimester-specific apparent temperature.

Janssen BG et al. Particle and Fibre Toxicology 2013
Placental tissue as a molecular ‘footprint’ for in utero exposure

Adjusted for:
- Model 1: Newborn’s gender, maternal age, gestational age, parity, maternal education, smoking status, prenatal acetaminophen use and season at conception and trimester-specific apparent temperature.
- Model 2: Additionally adjusted for the corresponding NO$_2$ and maximum 8-hour average O$_3$ exposure
CONCLUSIONS (1)

We showed that global DNA hypomethylation at birth is associated with air pollution during critical stages of early development, including the critical stages of implantation.
1. Is placental **global DNA methylation** associated with air pollution *in utero*?

2. Is placental **mitochondrial DNA content** and **mitochondrial DNA methylation** sensitive to exposure of air pollution *in utero*?
Mitochondrial DNA content as a potential biomarker of mitochondrial dysfunction?

- Reported in a broad range of human diseases such as:
  - preeclampsia/ IUGR/ Parkinson / diabetes / metabolic diseases / aging / ...
- The premise of this theory is that the mtDNA content of a particular cell type, normally within a healthy range, could change in conditions of oxidative stress.

![Diagram showing the relationship between environmental components, mitochondrial dysfunction, ROS, and mtDNA mutation.](Image)
**MTDNA CONTENT AND PARTICLE EXPOSURE — SOME EXAMPLES**

<table>
<thead>
<tr>
<th>+/-</th>
<th>Exposure</th>
<th>Type</th>
<th>Tissue</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑</td>
<td>Benzene</td>
<td>Human</td>
<td>Whole blood</td>
<td>Carugno <em>et al.</em> 2012</td>
</tr>
<tr>
<td>↑</td>
<td>PM</td>
<td>Human</td>
<td>Buffy coat</td>
<td>Hou <em>et al.</em> 2010</td>
</tr>
<tr>
<td>↓</td>
<td>PAH</td>
<td>Human</td>
<td>Buffy coat</td>
<td>Pieters <em>et al.</em> 2013</td>
</tr>
<tr>
<td>↓</td>
<td>PM and EC</td>
<td>Human</td>
<td>Buffy coat</td>
<td>Hou <em>et al.</em> 2013</td>
</tr>
<tr>
<td>↑</td>
<td>Tobacco smoke</td>
<td>Human</td>
<td>Saliva</td>
<td>Masayesva <em>et al.</em> 2005</td>
</tr>
<tr>
<td>↓</td>
<td>Maternal tobacco smoke</td>
<td>Human</td>
<td>Placenta</td>
<td>Bouhours-Nouet <em>et al.</em> 2005</td>
</tr>
<tr>
<td>↑</td>
<td>Light smokers</td>
<td>Human</td>
<td>Lung tissue</td>
<td>Lee <em>et al.</em> 1998</td>
</tr>
<tr>
<td>↓</td>
<td>Heavy smokers</td>
<td>Human</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Placental Mitochondrial DNA Content and Particulate Air Pollution during in Utero Life

Bram G. Janssen,1 Elke Munters,1 Nicky Pieters,1 Karen Smeets,1 Bianca Cox,1 Ann Cuypers,1 Frans Fierens,2 Joris Penders,3 Jaco Vangronsveld,1 Wilfried Gyselaers,4 and Tim S. Nawrot1,5

1Centre for Environmental Sciences, Hasselt University, Diepenbeek, Belgium; 2Belgian Interregional Environment Agency, Brussels, Belgium; 3Biomedical Research Institute, Hasselt University, Diepenbeek, Belgium; 4Department of Obstetrics, East-Limburg Hospital, Genk, Belgium; 5Department of Public Health, Occupational and Environmental Medicine, Leuven University (KULeuven), Leuven, Belgium

Background: Studies emphasize the importance of particulate matter (PM) in the formation of reactive oxygen species and inflammation. We hypothesized that these processes can influence mitochondrial function of the placenta and fetus.

Objective: We investigated the influence of PM10 exposure during pregnancy on the mitochondrial DNA content (mtDNA content) of the placenta and umbilical cord blood.

- Real-time qPCR: Ratio mtDNA / nDNA
- qBase software (Hellemans et al. 2007)

![Graph showing the relationship between PM10 and placental mtDNA content](image)

Trimester 3

- $r = -0.39$
- $p < 0.0001$
- $n = 174$
Prenatal PM$_{10}$ exposure was associated with placental mitochondrial alterations, which may both reflect and intensify oxidative stress production.

But what about possible mechanism of altered mtDNA content?
IS THERE A LINK BETWEEN DNA METHYLATION AND MITOCHONDRIA?

DNA methyltransferase 1, cytosine methylation, and cytosine hydroxymethylation in mammalian mitochondria


PNAS

Maintaince methylation patterns in nuclear DNA
IS THERE A LINK BETWEEN DNA METHYLATION AND MITOCHONDRIA?

Byun et al. Particle and Fibre Toxicology 2013, 10:18
http://www.particleandfibretoxicology.com/content/10/1/18

RESEARCH

Effects of airborne pollutants on mitochondrial DNA Methylation

Hyang-Min Byun¹,², Tommaso Panni¹,², Valeria Motta¹,³, Lifang Hou⁴, Francesco Nordio¹, Pietro Apostoli⁵, Pier Alberto Bertazzi³ and Andrea A Baccarelli¹

Results: In Study 1, participants with high metal-rich PM₁ exposure showed higher MT-TF and MT-RNR1 methylation than low-exposed controls (difference = 1.41, P = 0.002); MT-TF and MT-RNR1 methylation was significantly associated with PM₁ exposure (beta = 1.35, P = 0.025); and MT-RNR1 methylation was positively correlated with mtDNA copy number (r = 0.36; P = 0.02). D-loop methylation was not associated with PM₁ exposure. We found no effects on mtDNA methylation from air benzene (Study 2) and traffic-derived EC exposure (Study 3).

Conclusions: Mitochondrial MT-TF and MT-RNR1 DNA methylation was associated with metal-rich PM₁ exposure and mtDNA copy number. Our results suggest that locus-specific mtDNA methylation is correlated to selected exposures and mtDNA damage. Larger studies are needed to validate our observations.
Is there a link between DNA methylation and mitochondria?

PM exposure and mitochondrial DNA methylation
steel workers vs. newborns

Byun HM et al. Particulate & Fiber Toxicology 2013

Janssen BG; Byun HM et al. work in progress
**IS THERE A LINK BETWEEN DNA METHYLATION AND MITOCHONDRIA?**

Exploring the functional significance of the association of mtDNA methylation with exposure to PM...

- Methylation of mitochondrial DNA affects:
  - Mitochondrial transcription and translation
  - The assembly of the mitochondrial ribosome
  - The formation of RNA secondary structures

![Graph showing correlation between placental mtDNA methylation and content](image)

- Placental mtDNA methylation ($RNR1$), %
- Placental mtDNA content (Log10)

$r = -0.63$
$p < 0.0001$

Janssen BG; Byun HM et al. work in progress
Are the effects of PM$_{10}$ exposure during pregnancy on placental mtDNA content mediated by increased mtDNA methylation?

→ Mediation proportion = 80%

Almost all the effects of PM$_{10}$ on mtDNA content are mediated through changes in mitochondrial DNA methylation.
Mitochondrial DNA methylation as a next-generation biomarker and diagnostic tool

Vito Iacobazzi\textsuperscript{a,b,c,*}, Alessandra Castegna\textsuperscript{a}, Vittoria Infantino\textsuperscript{a,d}, Generoso Andria\textsuperscript{e}

Hari Manev* and Svetlana Dzitoyeva

Progress in mitochondrial epigenetics
Mitochondrial epigenome as a biomarker and a putative therapeutic target

Epigenetic modification of liver mitochondrial DNA is associated with histological severity of nonalcoholic fatty liver disease

Carlos Jose Pirola,\textsuperscript{1} Tomas Fernández Gianotti,\textsuperscript{1} Adriana Laura Burguedo,\textsuperscript{1} Manuel Rey-Funes,\textsuperscript{2} Cesar Fabian Loidl,\textsuperscript{2} Pablo Mallardi,\textsuperscript{3} Julio San Martino,\textsuperscript{3} Gustavo Osvaldo Castaño,\textsuperscript{4,5} S Sookoian\textsuperscript{4,5,6}

Environmental exposure and mitochondrial epigenetics: study design and analytical challenges

Hyang-Min Byun · Andrea A. Baccarelli
OVERALL CONCLUSIONS

Placental tissue as a molecular ‘footprint’ for *in utero* exposure

- **Global placental DNA hypomethylation**
  - at birth is associated with exposures early in pregnancy.

- **Placental mtDNA content:**
  - A decreased mtDNA content is associated with exposures late in pregnancy.

- **Placental mtDNA methylation:**
  - Is associated with mtDNA content
  - Probably mechanism through which PM exerts its effects on mtDNA content

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The potential health consequences of altered placental mtDNA content and methylation in early life must be further elucidated
**ENVIRONAGE**

Mother-child pairs recruited

- Collection of maternal blood and urine, placental tissue and cord blood;
  - Questionnaire

Follow-up

- Cardiovascular phenotyping: blood pressure,
  - microvasculature;
  - Questionnaire

Exposure ↔ Biomolecular markers ↔ Endpoints

Carotid Artery

Adventitia (lining of artery)
ACKNOWLEDGEMENT

- Hasselt University
  - Tim Nawrot
  - Wilfried Gyselaers
  - Nelly Saenen / Narjes Madhloum
  - Karen Vrijens / Michelle Plusquin / Kevin Hochstenbach

- Leuven University
  - Lode Godderis
  - Katrien Poels

- Harvard School of Public Health
  - Andrea Baccarelli
  - Hyang-Min Byun
MITOCHONDRIAL DNA CONTENT AS A POTENTIAL BIOMARKER OF MITOCHONDRIAL DYSFUNCTION?

- Reported in a broad range of human diseases such as:
  - preeclampsia/ IUGR/ Parkinson / diabetes / metabolic diseases / aging / ...
- The premise of this theory is that the mtDNA content of a particular cell type, normally within a healthy range, could change in conditions of oxidative stress.
**AND MORE ... UNPUBLISHED DATA**

**Trimester 3**

Maternal tobacco smoke

Placenta log [mtDNA/nDNA] vs. PM$_{2.5}$, $\mu$g/m$^3$

- $r = -0.17$
- $p = 0.0002$
- $n = 474$

Confirms findings of Bouhours-Nouet *et al.* 2005

**Maternal tobacco smoke**

- Non-smoker
- Past-smoker
- Smoker

-2.8% $p = 0.69$
-18.3% $p = 0.01$

$n = 476$
CORD BLOOD AND PLACENTAL mtDNA CONTENT AND PRENATAL PM\textsubscript{10} EXPOSURE

Model adjusted for:

- Newborn’s gender, maternal age, parity, gestational age, ethnicity, smoking status, season, apparent temperature.
- Additionally, umbilical cord blood was adjusted for blood cell count (number of white blood cells, % neutrophils, and number of platelets)

Janssen BG et al. Environ Health Perspect 2012