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LUND UNIVERSITY

DOCTORAL DISSERTATION

Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Medicine at Lund University to be publicly defended on January 27th, 2023 at 08.00 in Segerfalksalen Hall, BMC Hus A, Sölvegatan 17, 223 62 Lund, Sweden

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Title and subtitle: Placental molecular mechanisms as pathways linking prenatal exposure to ambient air pollution to preeclampsia and fetal growth

Abstract:

Background: PE is a “multifactorial syndrome” in which ambient air pollution may contribute to the etiology of PE. However, the underlying mechanism of this association is not clearly elucidated. This dissertation aims at understanding the placental molecular mechanism underlying the relationship of prenatal exposure to ambient air pollution with preeclampsia and fetal growth by characterizing and evaluating the mediator role of placental aging using mitochondrial DNA content, telomere length, DNA methylation and gene expression analyses.

Methods: Scania in Sweden and Barcelona in Spain are the two main study areas. Maternal Air Pollution in Southern Sweden (MAPSS) is a population-based cohort consisting of 43,688 singleton pregnancies recorded from 2000 to 2009 in Scania, Sweden. For placental molecular studies, we selected a sub-cohort of 361 mother-child pairs from the Barcelona Life Study Cohort (BiSC), a prospective cohort consisting of 1080 mother-child pairs recruited between 2018 and 2021 as well as 137 participants from unique biobank designed to study PE at Lund University. Each individual-level air pollutant concentrations were estimated either by a high-resolution dispersion model or by a passive sampling methods. Placental mtDNAcn and TL were measured using qPCR. Placental DNAm and gene expression were analyzed with EPIC array and RNA-seq, respectively. Linear, logistic regression and linear mixed effects models were used on SPSS and R statistical programs.

Results: In a setting with fair air quality in Scania, the increased risk of PE and SGA were associated with both linear exposure trend and quartile-specific exposure of ambient particles and NO_x. In a setting with moderate air quality in Barcelona, early pregnancy exposure to ambient NO_2 measured by home-outdoor and personal sensors were associated with lower birth weight and increased odds of having SGA neonate. Although the mediatory role of placental mtDNAcn and telomere length were not confirmed, prenatal exposure to ambient NO_x and NO_2 affect the placental aging process differently depending on the exposure concentration and exposure period during pregnancy. We observed reduced mtDNAcn and shorter TL in placenta in relation to exposure during first trimester, whereas increased mtDNAcn in relation to NO_2 exposure during third trimester. Prenatal exposure to ambient NO_x during first trimester affected differential placental DNA methylation in PE and NO_x combined groups, resulting in placental age deceleration and showing sexual dimorphism.

Conclusion: Placental senescence and aging may be the underlying molecular mechanism linking the association between prenatal exposure to ambient air pollution and pregnancy complications including preeclampsia and small-for-gestational age foetuses and neonates.

Key words: Ambient air pollution, Placenta, Preeclampsia, Small-for-gestational age, Mitochondrial DNA content, Telomere length, Global DNA methylation, Gene expression, Epigenetic age

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To Grigorios

and our Anu, Vasileios and Maximos

Table of Contents

Abstract

PE is a “multifactorial syndrome” in which ambient air pollution may contribute to the etiology of PE. However, the underlying mechanism of this association is not clearly elucidated. This dissertation aims at understanding the placental molecular mechanism underlying the relationship of prenatal exposure to ambient air pollution with preeclampsia and fetal growth by characterizing and evaluating the mediator role of placental aging using mitochondrial DNA content, telomere length, DNA methylation and gene expression analyses.

Scania in Sweden and Barcelona in Spain are the two main study areas. Maternal Air Pollution in Southern Sweden (MAPSS) is a population-based cohort consisting of 43,688 singleton pregnancies recorded from 2000 to 2009 in Scania, Sweden. For placental molecular studies, we selected a sub-cohort of 361 mother-child pairs from the Barcelona Life Study Cohort (BiSC), a prospective cohort consisting of 1080 mother-child pairs recruited between 2018 and 2021 as well as 137 participants from unique biobank designed to study PE at Lund University. Each individual-level air pollutant concentrations were estimated either by a high-resolution dispersion model or passive sampling methods. Placental mtDNAcn and TL were measured using qPCR. Placental DNAm and gene expression were analyzed with EPIC array and by RNA-seq, respectively. Linear, logistic regression and linear mixed effects models were used on SPSS and R statistical programs.

In a setting with fair air quality in Scania, the increased risk of PE and SGA were associated with both linear exposure trend and quartile-specific exposure of ambient particles and NO_x. In a setting with moderate air quality in Barcelona, early pregnancy exposure to ambient NO_2 measured by home-outdoor and personal sensors were associated with lower birth weight and increased odds of having SGA neonate, respectively. Although the mediatory role of placental mtDNAcn and telomere length were not confirmed, prenatal exposure to ambient NO_x and NO_2 affect the placental aging process differently depending on the exposure concentration and exposure period during pregnancy. We observed reduced mtDNAcn and shorter TL in placenta in relation to exposure during first trimester, whereas increased mtDNAcn in relation to NO_2 exposure during third trimester. Prenatal exposure to ambient NO_x during first trimester affected differential placental DNA methylation in PE and NO_x combined groups, resulting in placental age deceleration and showing sexual dimorphism.

Placental senescence and aging may be the underlying molecular mechanism linking the association between prenatal exposure to ambient air pollution and pregnancy complications including preeclampsia and small-for-gestational age foetuses and neonates.

Populärvetenskaplig sammanfattning

Luftföroreningar är ett allvarligt miljöproblem som bidrar till förtida dödsfall över hela världen. Luftföroreningar delas in i grova, fina och ultrafina partiklar baserat på deras storlek i förhållande till aerodynamiken. Detta är en liknande process som det fallande äpplet från trädet som inspirerade Newton att upptäcka gravitationen. Storleken på partiklarna har störst betydelse när det gäller hälsoeffekter. Ju mindre partikelstorlek (dvs ultrafina partiklar), desto mer signifikant blir den negativa effekten på hälsan. Tidigare studier har funnit att ultrafina partiklar kan färdas djupt ner till alveolerna i lungan där gasutbytet av inandat syre och utandad koldioxid äger rum.

Dessutom kan det transporteras till det systemiska blodflödet och orsaka inflammation i olika organsystem i vår kropp. Vid det här laget har du förmodligen upptäckt att ultrafina partiklar kan leta sig in i livmodern genom moderkakan. Det har bevisats i djurexperimentella studier, men dess effekt på människors hälsa visas av stora epidemiologiska studier där barn föds för tidigt eller med låg födelsevikt när deras mödrar bodde i de mycket förorenade områdena under graviditeten. Men innan de påverkar det växande barnet i livmodern, påverkar luftföroreningar hälsan hos en gravid kvinna som orsakar graviditetsinducerad hypertoni.

Ungefär en av sex mödrar dör av graviditetsinducerad hypertoni på ett år världen över. Särskilt tonårsmödrar (<20 år gamla) löper större risk än äldre kvinnor i låg- och medelinkomstländer. I min doktorsavhandling undersökte jag risken för havandeskapsförgiftning och småhet hos bebisar i relation till luftföroreningar i Sverige och Spanien. Vi utförde också placenta molekylära studier för att förstå de underliggande biologiska effekterna av denna association.

I en miljö med rättvis luftkvalitet i Skåne var prenatal exponering för luftföroreningar associerad med den ökade risken för havandeskapsförgiftning och spädbarn som är små för graviditetsåldern. I en miljö med måttlig luftkvalitet i Barcelona var tidig graviditetsexponering för omgivande NO_2 uppmätt med sensorer i hemmet och utomhus och personliga sensorer associerad med lägre födelsevikt respektive ökade chanser att få barn små för graviditetsåldern. Prenatal exponering för luftföroreningar påverkar också placentans åldrandeprocess genom DNA-metylering, mitokondriell stress och telomerförkortning.

Sammantaget har resultaten av dessa studier ett antal viktiga implikationer för framtida praktik. De reproduktiva hälsoeffekterna som behandlas i båda studiemiljöerna med rättvis och måttlig luftkvalitet stöder starkt WHO:s rekommendationer för uppdaterade globala gränsvärden för luftkvalitet.

List of Papers

Paper I

Mandakh Y, Rittner R, Flanagan E, Oudin A, Isaxon C, Familari M, Hansson SR, Malmqvist E. (2020) Maternal Exposure to Ambient Air Pollution and Risk of Preeclampsia: A Population-Based Cohort Study in Scania, Sweden. International Journal of Environmental Research and Public Health. 17(5):1744. https://doi.org/10.3390/ijerph17051744

Paper II

Mandakh Y, Oudin A, Erlandsson L, Isaxon C, Hansson SR, Broberg K and Malmqvist E (2021) Association of Prenatal Ambient Air Pollution Exposure With Placental Mitochondrial DNA Copy Number, Telomere Length and Preeclampsia. Front. Toxicol. 3:659407. doi: 10.3389/ftox.2021.659407

Paper III

Engström K, Mandakh Y, Garmire L, Masoumi Z, Isaxon C, Malmqvist E, Erlandsson L, Hansson SR. (2021) Early Pregnancy Exposure to Ambient Air Pollution among Late-Onset Preeclamptic Cases Is Associated with Placental DNA Hypomethylation of Specific Genes and Slower Placental Maturation. Toxics. 9(12):338. https://doi.org/10.3390/toxics9120338

Paper IV

Laura C.Gómez Herrera, Yumjirmaa Mandakh, Dries S. Martens, Tim S. Nawrot, Ebba Malmqvist, Mariona Bustamante, Payam Dadvand, María Dolores Gómez-Roig and Jordi Sunyer. (2022) The mediatory role of placental stress, ageing, and function in the association between air pollution exposure and fetal growth. (in manuscript)

Author's contribution to the papers

Paper I

Yumjirmaa is a sole first author. I contributed to conceptualization, methodology, validation, formal analysis, investigation, writing of original draft preparation, review and editing.

Paper II

Yumjirmaa is a sole first author. I contributed to conceptualization, methodology (laboratory work: placental DNA/RNA extraction and qPCR of relative mitochondrial DNA copy number and telomere length), formal analysis, investigation, writing of original draft, review and editing.

Paper III

Yumjirmaa is a shared first author. I contributed to formal analysis, investigation, methodology (laboratory work: placental DNA/RNA extraction), and writing of original draft, review and editing.

Paper IV

Yumjirmaa is a shared first author. I contributed to formal analysis, investigation, methodology, visualization, writing of original draft, review and editing.

Abbreviations

BC	Black carbon
BiSC	Barcelona Life Study Cohort
BMI	Body Mass Index
CEU	Utah Residents with Northern and Western European Ancestry
CI	Confidence Interval
COVID-19	Coronavirus Disease
CPC	Control Placental Clock
CPR	Cerebroplacental Ratio
DAG	Directed Acyclic Graph
DEGs	Differentially Expressed Genes
DMPs	Differentially Methylated Positions
DMRs	Differentially Methylated Regions
DNAm	DNA Methylation
FC	Fold Change
2logFC	Binary Logarithmic Fold Change
FDR	False Discovery Rate
FGR	Fetal Growth Restriction
GA	Gestational Age
GO	Gene Ontology
IQR	Interquartile Range
ISGlobal	Barcelona Institute for Global Health
ISSHP	International Society for the Study of Hypertension in Pregnancy
KEGG	Kyoto Encyclopedia of Genes and Genomes
LBW	Low Birth Weight
MAF	Minor Allele Frequency
MAPSS	Maternal Air Pollution Southern Sweden
mtDNAcn	Mitochondrial DNA Copy Number
NO2	Nitrogen Dioxide

NO	Nitric Oxide
NOx	Nitrogen Oxides
O3	Ozone
OR	Odds Ratio
PC	Principal Component
PE	Preeclampsia
PM2.5	Particles with aerodynamic diameters smaller than 2.5 □m
PM10	Particles with aerodynamic diameters smaller than 10 □m
qPCR	Quantitative Real-Time Polymerase Chain Reaction Assay
RIN	RNA integrity number
RNA-seq	RNA Sequencing
ROS	Reactive Oxygen Species
Rp	Pearson Correlation
Rs	Spearman Correlation
R2	R-Squared (coefficient of determination)
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SES	Socioeconomic Status
SGA	Small-for-gestational age
SNP	Single-nucleotide Polymorphism
STB	Syncytiotrophoblast
SVD	Singular Value Decomposition
TF	Transcription Factor
TL	Telomere Length
UtA	Uterine Artery
UA	Umbilical Artery
WHO	World Health Organization

Introduction

Definitions

Preeclampsia

Preeclampsia (PE) is a persistent de novo hypertension occurring at or after 20 weeks of gestation accompanied either with proteinuria or fetal growth restriction or maternal organ dysfunction including HELLP syndrome (Hemolysis, Elevated Liver enzymes, Low Platelets) (Brown et al., 2018). Although it affects 3% to 8% of all pregnancies, PE is one of the three leading causes of direct maternal mortality worldwide ("Geographic variation in the incidence of hypertension in pregnancy. World Health Organization International Collaborative Study of Hypertensive Disorders of Pregnancy," 1988; Ghulmiyyah & Sibai, 2012; Rath & Tsikouras, 2018) It is classified into early-onset and late-onset PE based on its development before or after 34 weeks of gestation (Tranquilli et al., 2013). Although PE was recorded as moderate and severe PE in the Swedish medical birth register and mild and severe PE in the Spanish medical birth register, in this dissertation, total PE as a combination of these classifications were used as an outcome variable. In addition, PE is often associated with adverse birth outcome, such as preterm birth, small-for-gestational age neonates and fetal growth restriction.

Small fetus

Low birth weight

Babies born weighing less than 2500 grams is termed as low birth weight (LBW) (WHO, 2004).

Small-for-gestational age

Smallness of fetus and neonates are defined as small-for-gestational-age (SGA) if their birth weight fell below the 10^{th} centile (Figueras et al., 2008).

Fetal growth restriction

Small neonates can be classified into low-risk SGA and true fetal growth restriction (FGR) based on estimated birthweight centile, doppler velocimetry of uterine artery (UtA) and cerebroplacental ratio (CPR) measured at routine third trimester fetal echosonography. The CPR is calculated as the ratio of middle cerebral artery (MCA) to umbilical artery (UA) pulsatility index (PI) values, measured by Doppler ultrasound (Baschat & Gembruch, 2003). Low-risk SGA is diagnosed when small babies present any of the following: SGA in between 3rd-9th centile or CPR doppler index more than 5th centile or UtA pulsatility index less than 95th centile. Those presenting any of the following: SGA less than 3rd centile or CPR doppler index less than 5th centile or UtA pulsatility index more than 95th centile, were classified as true FGR (Figueras & Gratacós, 2014).

The two-stage model of preeclampsia

The early-onset and late-onset PE evolve in different pathways in the first stage and converges on syncytiotrophoblast stress with placental dysfunction at the onset of the maternal syndrome of PE caused by generalized vascular inflammation in the second stage (Redman et al., 2022). The incomplete remodelling of the spiral arteries along with focal oxidative stress in the vicinity of the spiral artery causes malplacentation and later on placental malperfusion in the first stage of early-onset PE with concomitant FGR. In the first stage of late-onset PE, with normal placentation, progressive placental hypoxia and fetal stress along with massive growth of chorionic villous tree persists from the beginning of the second half of pregnancy without any known well-established cause (Redman et al., 2022). The second stage of PE is syncytiotrophoblast stress featuring with oxidative stress, endoplasmic reticulum stress, mitochondrial stress, apoptosis, autophagy, necrosis and syncytial knots as well as producing large amount of vascular endothelial growth factor-1 (VEGF-1), also known as the soluble fms-like tyrosine kinase-1 (sFlt-1), and placental growth factor (PIGF).

Ambient air pollution

Ambient particulate air pollution is the fourth leading risk factor for attributable deaths for both males and females accounting for 2.92 million and 3.75 million deaths of all female and male deaths in 2019, respectively (Murray et al., 2020).

Nitrogen oxides (NO_x) is a toxic gas in the atmosphere. It includes nitric oxide (NO), a colorless and odorless gas, and nitrogen dioxide (NO_2), a reddish-brown gas with a strong odor. Total NO_2 concentrations are composed of primary NO_2 emission

from diesel exhaust (up to 25%), secondary NO_2 formed by the reactions of NO and ozone (O3) (70%) and regional background (Casquero-Vera et al., 2019). Particulate matter is a mixture of solid particles and aerosols varying in its aerodynamic diameter: being less than 10 μm (PM_{10}) and 2.5 μm ($PM_{2.5}$). $PM_{2.5}$ is one of the three major and rapidly increasing risk exposures globally in 2019 (Murray et al., 2020). 90% of $PM_{2.5}$ pollution is generated from diesel engines in cities (Bernasconi et al., 2022). Other emission sources include the combustion of coal, oil, and gasoline. WHO 2021 update on global air quality guidelines recommends a maximum level of 5 $\mu g/m^3$ for $PM_{2.5}$, 15 $\mu g/m^3$ for PM_{10} and 10 $\mu g/m^3$ for NO_2 for preventing the global community from health effects related to long-term exposure (World Health Organization, 2021).

Oxidative stress has been proposed to be the major mechanism of the adverse health effect of air pollution exposure. Each interquartile range increase in prenatal exposure to ambient $PM_{2.5}$ during the first trimester was statistically significantly associated with increased maternal serum malondialdehyde (MDA), a biomarker of systemic lipid peroxidation, and total antioxidant capacity (Zhang et al., 2020). On the other hand, higher plasma levels of haematocrit, MDA and nitrates during first trimester were statistically significantly associated with the later development of PE in twin pregnancies (Ramiro-Cortijo et al., 2020). In a mouse experimental study, chronic $PM_{2.5}$ exposure induce systemic cellular inflammatory response through Toll-like receptor (TLR)4/NADPH oxidase-dependent mechanism (Kampfrath et al., 2011). Monocytes in the peripheral blood from early-onset PE had higher TLR4 expression when compared with late-onset PE (Romão-Veiga et al., 2020). A recent meta-analysis showed that short-term exposure to ambient $PM_{2.5}$ was associated with increased serum levels of MDA (Li et al., 2020).

PE is a “multifactorial syndrome” in which ambient air pollution may contribute to the etiology of PE (Gyselaers, 2020; Pedersen et al., 2014). Systematic reviews suggest that early pregnancy exposure to ambient air pollution is associated with increased risk of PE (Bai et al., 2020; Pedersen et al., 2014; Yu et al., 2020). However, the underlying mechanism of this association is not clearly elucidated, numerous studies have attempted to explain how external and internal factors influence the risk of hypertensive disorder of pregnancy by using the pregnancy exposome approach (Hu et al., 2020; Robinson & Vrijheid, 2015).

Placental molecular mechanisms underlying the effects of long-term exposure to ambient air pollutants

Placental mitochondrial DNA content

Mitochondria are cell organelles where oxidative phosphorylation to produce adenosine triphosphate occurs. Due to high energy electron transfer during the energy metabolism, free radicals capable of oxidation is contained within mitochondrial double membrane. When mitochondria are under stress, these free radicals are released to the cytoplasm and damage cell molecules causing oxidative stress. Mitochondrial DNA (mtDNA) is susceptible to oxidative stress damage due to its structure of two strands without histone shield (Lee & Wei, 2000). MtDNA copy number (mtDNAcn) vary by each mitochondrion, cell type, tissue and organ. MtDNA is inherited only from mother. Prenatal exposure to ambient air pollution has been found to be associated with reduced placental mtDNAcn, whereas placentas from pregnancies complicated with early-onset PE had increased mtDNAcn when compared with normotensive controls (Clemente et al., 2017; Janssen et al., 2012; Vishnyakova et al., 2016).

Placental telomere length

Human telomeres consists of tandem repeats of "TTAGGG" and are located at the end of each chromosome (Blackburn, 1991). Telomere-driven replicative cell senescence is a critical end point of a cell going to senescence and apoptosis due to telomere shortening from successive cell replication process (Hayflick & Moorhead, 1961; Pańczyszyn et al., 2020). Such high guanine content renders telomeres sensitive to reactive oxygen species (ROS) and thus leads to accelerated telomere shortening (Pańczyszyn et al., 2020; Richter & Zglinicki, 2007). Previous studies investigating the association between air pollution exposure and telomere length showed inconsistent findings. Increase in annual mean exposure to NO_x, NO_2, $PM_{2.5}$ and PM_{10} were associated with longer telomere (Walton et al., 2016). Maternal exposure to traffic-related air pollution and lack of green environment surrounding the residence were associated with shorter placental telomere length (Bijnens et al., 2015). Telomere was shorter in placentas from pregnancies complicated with PE, FGR and PE with FGR (Tal Biron-Shental et al., 2010).

Placental DNA methylation

DNA methylation (DNAm) is defined as an addition of methyl group at the 5' position of cytosine in CpG dinucleotides (CpGs) in CpG islands located abundantly in gene promoters and repetitive DNA elements in human genome (Rakyan et al., 2011). CpGs in gene-promoters are mostly unmethylated whereas in gene bodies and in repetitive DNA elements are mostly methylated (Suzuki & Bird, 2008). The main roles of DNAm is to regulate gene expression and to protect genome integrity by suppressing transposable element transcription which could otherwise integrate

into new chromosome by translocations (Levin & Moran, 2011). DNAm is the most stable epigenetic marker which is heritable during cell division. It also plays a critical role in cell differentiation and epigenetic reprogramming during embryonic development during first trimester (Collas et al., 2007). Environmental exposures during early pregnancy can potentially induce additional DNAm and alter gene expression resulting in increased susceptibility to disease (Jirtle & Skinner, 2007; Saenen et al., 2019).

The villous syncytiotrophoblast is the major cell type in the placenta (Gude et al., 2004) and has been suggested to explain the hypomethylation of the placental genome across gestation (Bianco-Miotto et al., 2016) due to its low levels of methylated cytosine residues (5'mC) and *de novo* methylation enzymes, namely DNMT 3a and 3b (Fogarty et al., 2015). Furthermore, the underlying differentiating cytotrophoblast cells with methylated cytosine residues (5'mC) constantly fuse to syncytiotrophoblast (Mayhew et al., 1999). Therefore placental DNA methylation has been reported to be largely dependent on the methylation profile of cytotrophoblast cells (Grigoriu et al., 2011). Moreover, number of Hofbauer cells and especially dendritic cell-specific ICAM-grabbing non-integrin (DC-SIGN) expressing Hofbauer cells in the placental villous core were found to be decreased in PE placenta (Tang et al., 2013; Yang et al., 2017). DC-SIGN positive Hofbauer cells are suggested to be involved in the immune tolerance during pregnancy (Reyes & Golos, 2018). Placental DNA methylation may be one of the underlying mechanistic links connecting prenatal exposure to ambient air pollution and PE.

Placental gene expression

DNA methylation along with other epigenetic modifications regulate gene expression (Bianco-Miotto et al., 2016), however, the findings are inconsistent among the following few multi-omics studies that have analyzed both methylome and transcriptome of the same placentas from preeclamptic and normotensive pregnant women. Xuan et al. (2016), Sundrani et al. (2013) and (Blair et al., 2013) have identified negative correlation, whereas Leavey et al. (2018) have found no association between DNA methylation and gene expression. Moreover, inhibin was found to be the only biomarker for preeclampsia by multi-omics approach (Benny et al., 2020). Nevertheless, there are only few studies investigating integrated multi-omics concerning the exposure to ambient air pollution exposure (Alfano et al., 2018). Long term exposure to ambient NO_x was associated with hypomethylation and 9 enriched pathways relating to immune regulation (Plusquin et al., 2017).

Aim

This dissertation aims at understanding the placental molecular mechanism underlying the relationship of prenatal exposure to ambient air pollution with preeclampsia and fetal growth by characterizing and evaluating the mediator role of placental aging using mitochondrial DNA content, telomere length, DNA methylation and gene expression analyses.

Paper I

The major objective of this study was to investigate the association between prenatal exposure to ambient air pollutants (black carbon, NO_x, $PM_{2.5}$, PM_{10}) and preeclampsia in Scania, Sweden

Paper II

The aim of this study was to identify how prenatal exposure to ambient NO_x was associated with preeclampsia by analysing the possible mediation effects of placental relative telomere length and mitochondrial DNA content.

Paper III

This paper investigates placental DNA methylation pattern and gene expression in relation to prenatal exposure to ambient NO_x among preeclamptic cases and normotensive controls.

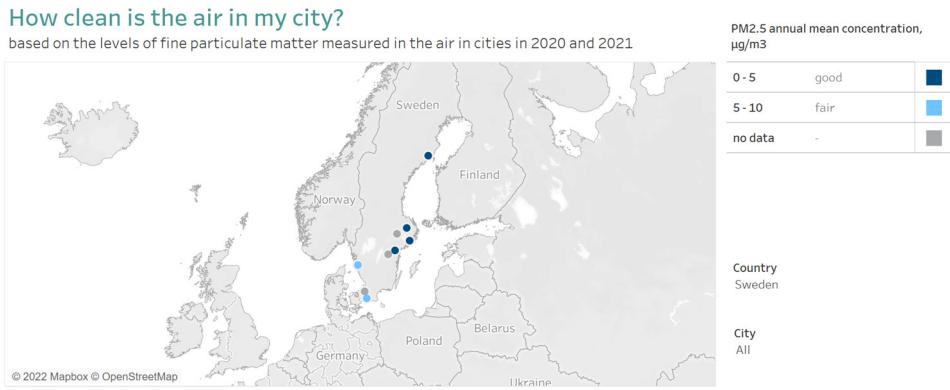
Paper IV

The main aim of this investigation was to assess the several aspects of fetal growth indicators associated with prenatal exposure to ambient NO_2 measured by personal, home-outdoor and home-indoor sensors as well as to determine whether placental senescence measured by telomere length and mitochondrial DNA content explains the underlying mechanism of the association.

Material and methods

Study population

Scania in Sweden and Barcelona in Spain are the two main study areas. Scania is the southernmost and the second most densely populated province of Sweden with 1.41 million inhabitants in total and 127 people per square kilometre (km^2) (Regionfakta, 2022). As can be seen from Figure 1, Scania's largest city, Malmö, ranked at 59 with fair air quality based on its $PM_{2.5}$ annual mean concentration of 8.2 $\mu g/m^3$ in the past two calendar years (European Environment Agency, 2022).



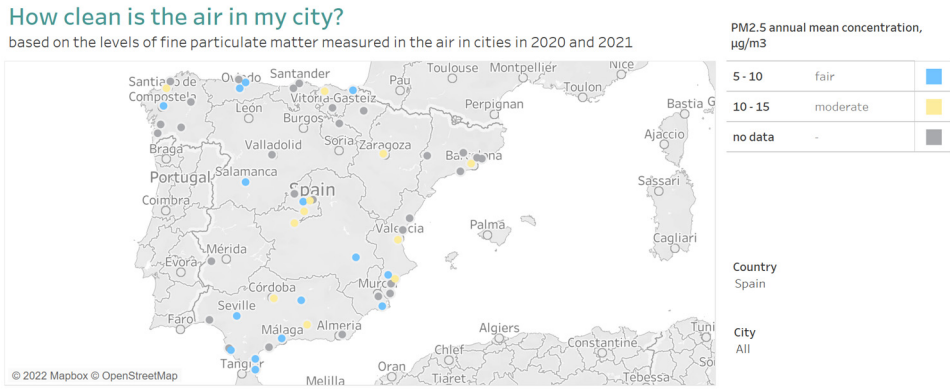
Air in European cities – from the cleanest to the most polluted

City name	Country	Rank	Fine particulate matter in ug/m3	Population in the city
Umeå	Sweden	1	3.1	125080
Stockholm (greater city)	Sweden	6	4.2	1745766
Uppsala	Sweden	7	4.2	219914
Norrköping	Sweden	11	4.9	140927
Göteborg	Sweden	19	5.8	564039
Malmö	Sweden	59	8.2	333633

Figure 1. Air quality in Sweden (European Environment Agency, 2022)

Barcelona city is the capital of the autonomous community of Cataluña and the second most densely populated metropolitan city in Spain, with approximately 1.64 million inhabitants in total and 16094 people per km^2 (Instituto Nacional de Estadística, 2022). Out of 344 European cities, Barcelona ranked at 244 with

moderate air quality based on its $PM_{2.5}$ annual mean concentration of 13.3 µg/m^3 in 2020 and 2021 (Figure 2) (European Environment Agency, 2022).



Air in European cities – from the cleanest to the most polluted

City name	Country	Rank	Fine particulate matter in ug/m3	Population in the city
Sevilla	Spain	148	9.8	884899
Jaén	Spain	151	9.9	112757
Gijón	Spain	155	9.9	271717
Valencia	Spain	163	10.2	1417464
Zaragoza	Spain	176	10.5	681877
Bilbao	Spain	200	11.2	797590
Valdemoro	Spain	212	11.7	77270
Toledo	Spain	215	11.8	85811
Córdoba	Spain	223	12.3	326039
Alicante/Alacant	Spain	224	12.3	337482
Torrejón de Ardoz	Spain	230	12.5	132853
Barcelona	Spain	244	13.3	3755512
Granada	Spain	253	13.6	404809
A Coruña	Spain	259	14.0	247604

Figure 2. Air quality in Spain (European Environment Agency, 2022)

All studies followed the ethics conduct and data protection according to the national rules. Ethics approval was obtained from Lund University Ethical Review Board LU803-2 (2015/14) and (Dnr 696/2014) in Paper I, II and III. The Barcelona Life Study Cohort (BiSC) study was approved by the Ethical Review Board of Parc de Salut Mar (CEIm - Parc de Salut Mar) under the project reference number 2018/8050/I ("Pre-natal exposure to AIR pollution and pre- and post-Natal Brain Development") in Paper 4. All participants gave written informed consent.

Paper I

Maternal Air Pollution in Southern Sweden (MAPSS) is a population-based cohort consisting of 43,688 singleton pregnancies recorded from 2000 to 2009 in Scania, Sweden. MAPSS is based on the local birth register, Perinatal Revision Syd, with 98% coverage of all births as well as individual air pollution exposure concentrations modelled at each maternal residential address within the catchment

area of hospitals in Malmö, Lund and Trelleborg (Figure 3). Additional sociodemographic and socioeconomic factors were obtained from Statistics Sweden and linked to MAPSS based on unique personal identification number.

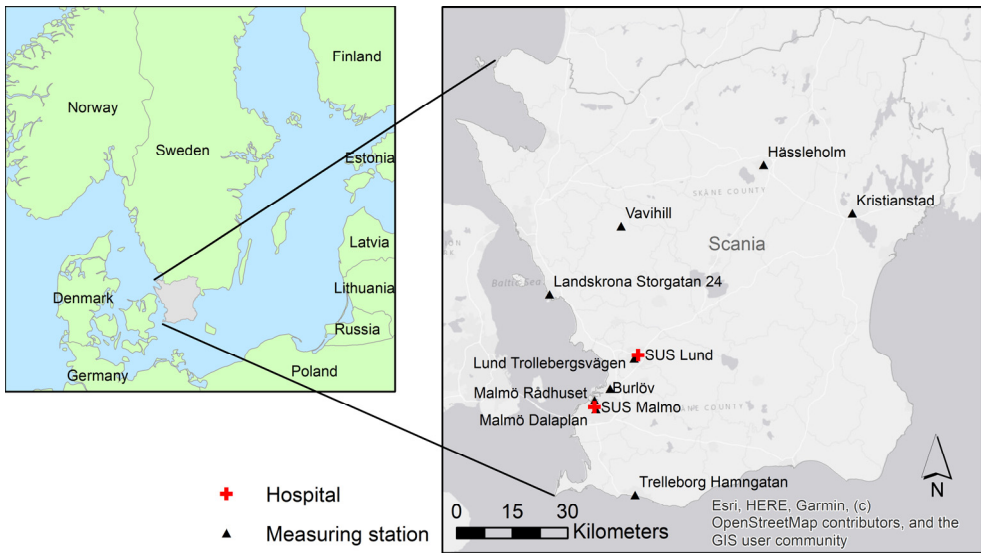


Figure 3. A map of Scania, Sweden
SUS – Skåne University Hospital in Lund and Malmö are marked in red and air quality monitoring stations in Scania are marked in black. Figure created by Kristoffer Mattisson.

Paper II and III

The study population of placental molecular studies, Paper II and III, in Scania were based on the unique biobank data designed to study preeclampsia at the Department of Obstetrics and Gynaecology, Lund University. The study participants were recruited from the catchment areas of Skåne University Hospital in Lund and Malmö (Figure 4).

Paper II was a cross-sectional study of 42 preeclamptic and 95 arbitrarily selected normotensive pregnant women whose prenatal exposure to ambient NO_x were modelled at their residential addresses using the Gaussian plume dispersion modelling in Scania, Sweden.

Study III involved 111 participants in total. For the DNA methylation analysis, between-subjects 2x2 factorial design was employed and therefore subjects were divided into four combined groups based on PE status and prenatal exposure to ambient NO_x: PE cases with low NO_x exposure, PE cases with high NO_x exposure, normotensive controls with low NO_x exposure, and normotensive controls with high NO_x exposure. A total of 17 subjects were included in the analyses of differential gene expression, and therefore we compared PE cases with normotensive controls.

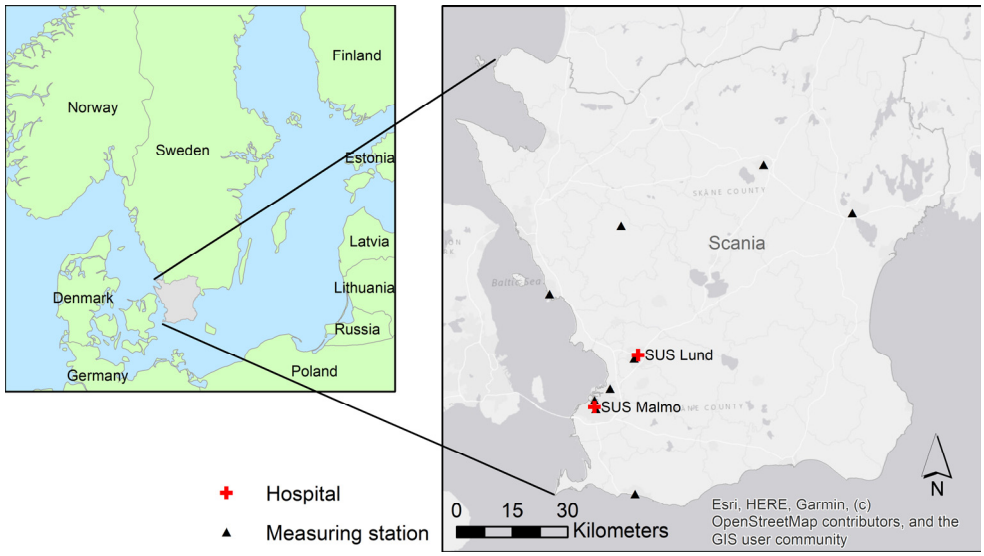


Figure 4. A study area of placental molecular studies in relation to the association between ambient NO_x exposure during pregnancy and preeclampsia in Scania, Sweden
Figure created by Kristoffer Mattisson

Paper IV

The Barcelona Life Study Cohort (BiSC) is a prospective cohort consisting of 1080 mother-child pairs recruited between 2018 and 2021 from three collaborating hospitals (Hospital Sant Joan de Déu, Hospital Clínic-La Maternitat and Hospital de Sant Pau i la Santa Creu) (Figure 5). Criteria for selecting the subjects were as follows: aged between 18 and 45 years, had singleton pregnancy and spoke either Catalan, Spanish or English. Eligible women who matched this inclusion criteria were identified by midwives during the first prenatal visit (between 8-14 weeks of gestation). Exclusion criteria consisted of having high-risk pregnancy, complex chronic conditions or mental illness and permanent residence located outside the boundary of Barcelona city. Of the initial cohort of 1080 mother-child pairs, 361 were included in the analysis based on its complete set of data on ambient NO_2 exposure, placental biomarkers, clinical and covariate data.

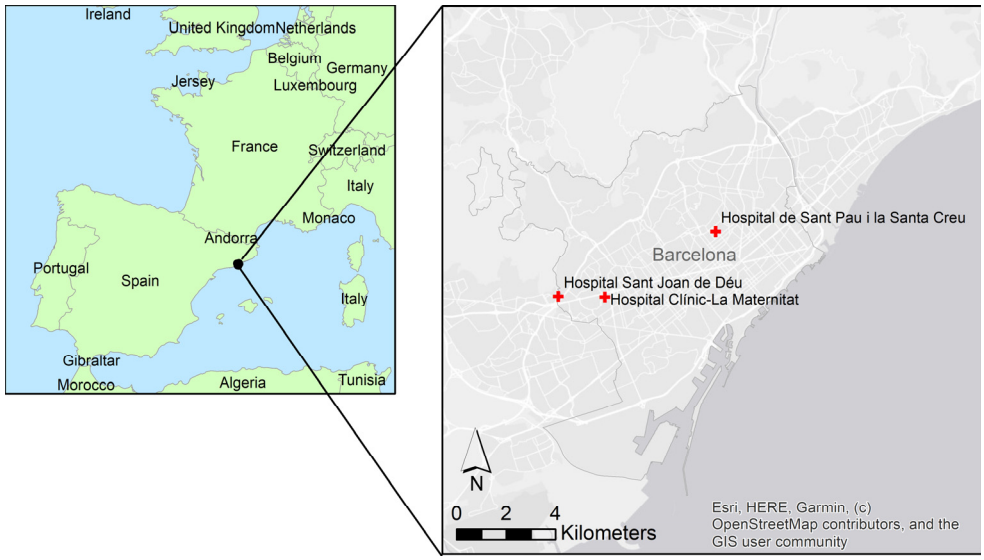


Figure 5. A map of Barcelona city, Cataluña, Spain
Figure created by Kristoffer Mattisson

Air pollution exposure assessment

Unlike the brief description of air pollutants in the introduction, it is necessary to clarify how exposure to air pollution differs from the definition of air pollution. Exposure refers to how much quantity of air pollutant that an individual experience over certain study period. Each individual-level air pollutant concentrations were estimated either by a dispersion model or passive sampling methods in all studies. A Gaussian plume air dispersion model on ENVIMAN software was employed for estimating the individual modelled exposure levels of air pollutants (BC, locally emitted $PM_{2.5}$ and $PM_{10,}$ total $PM_{2.5}$ and PM_{10}, NO_x) of MAPSS birth cohort in Paper I as well as in Paper II and III. Personal exposure to NO_2, home-outdoor and home-indoor NO_2 concentrations were measured for 24 hours and seven days during first and third trimesters by a passive diffusion tube by Gradko Environmental passive dosimeters (Gradko International Ltd., UK) in Paper IV.

To get a glimpse of how air pollution differs between the study settings, Screening for High Emission Reduction Potential on Air (SHERPA) tool allows to understand how the air quality in Malmö and Barcelona are influenced by different emission sources. As shown in Figure 6, a large proportion of total mass of urban $PM_{2.5}$ in Malmö is a transboundary air pollution by spatial allocation as well as traffic and agriculture sectors by sectoral allocation (European Commission Joint Research Centre et al., 2021). In contrast in Barcelona, almost half of urban $PM_{2.5}$ is emitted

in the core city mostly from the industrial and residential sources (Figure 7) (European Commission Joint Research Centre et al., 2021).

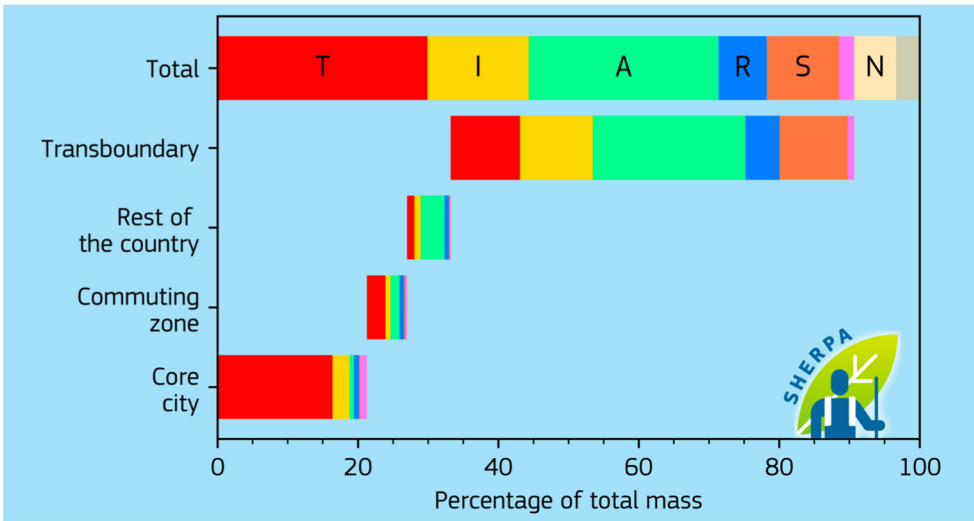


Figure 6. Urban PM2.5 spatial and sectoral allocation of Malmö, Sweden (SHERPA v.2.2.0) (European Commission Joint Research Centre et al., 2021)
Emission sources: T – transport, I – industry, A – agriculture, R – Residential, S – shipping, O – other, N – Natural, E – External.

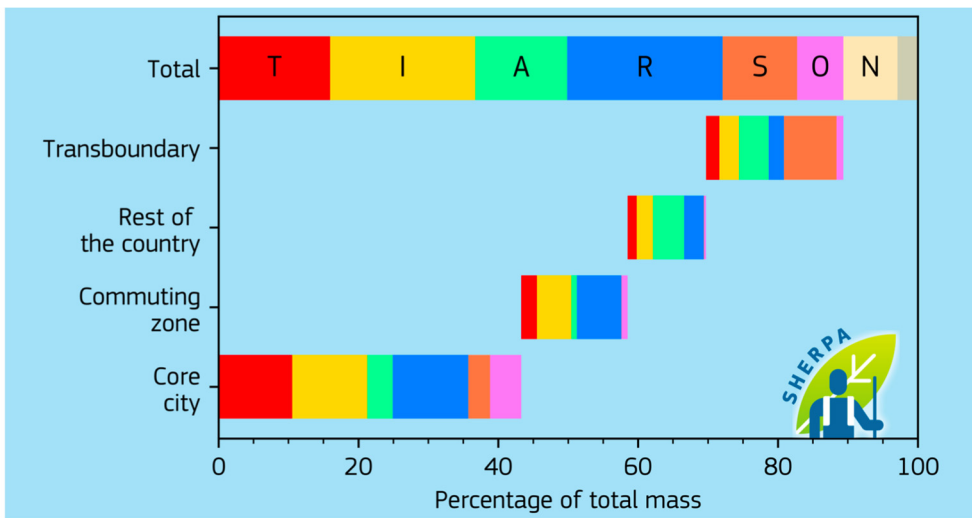


Figure 7. Urban $PM_{2.5}$ spatial and sectoral allocation of Barcelona, Spain (SHERPA v2.2.0) (European Commission Joint Research Centre et al., 2021)
Emission sources: T – transport, I – industry, A – agriculture, R – Residential, S – shipping, O – other, N – Natural, E – External.

Paper I, II and III

ENVIMAN is a software with several modules. Data management module consists of emission sources data (traffic count, car models, car exhaust, type of tires etc) and meteorological data (wind speed, wind direction, temperature and global solar radiation). Dispersion modelling module estimates concentrations of NO_x, $PM_{2.5}$ and PM_{10} over the 100m x 100m grid in two years 2000 and 2011. Hourly concentrations of NO_x were calculated according to pregnancy length from the date of birth and backwards. Hourly concentrations of $PM_{2.5}$ and PM_{10} were estimated approximately by calendar months, from January till December, and for the one month prior to the conception date. Using R software, the hourly concentrations were aggregated into monthly mean concentrations for the months of 2000 and 2011.

Furthermore, in ArcGIS program, the monthly mean concentrations of 2000 and 2011 were inserted and by interpolation with atmospheric ventilation index, the individual-level pollutant concentrations of years in between (2001-2009) were estimated at each participant's residential address. Atmospheric ventilation index was calculated at the Department of Environment at City of Malmö for each month of this time period. It estimates how well the atmosphere disperses the smoke from its source in the atmospheric mixed layer on any given day. It is a product of the average wind velocity in the mixed layer and the height of the mixed layer.

Detailed description of the Gaussian plume air dispersion model can be found in Rittner et al. (2020). In brief, $PM_{2.5}$ and PM_{10} concentrations were modelled at seven monitoring stations in Scania, as shown in Figure 3 above. For validation, the measured concentrations at the monitoring stations at Malmö City Hall and Vavihill were compared with the modelled concentrations using R^2 Pearson correlation method. Moderate to strong correlation were found with a variable R^2 values between 0.46 and 0.83 for PM_{10} and 0.44 and 0.86 for $PM_{2.5}$. BC was measured for shorter period and it was unable to validate measured values with the modelled values (Rittner et al., 2020).

NO_x model was adopted from Stroh et al. (2012). There was a good agreement between the measured and modelled concentrations of NO_2 at individual exposure levels ($r_s = 0.8$, $p > 0.001$).

Paper IV

We measured home-outdoor, home-indoor, and personal NO_2 concentrations for 24 hours and seven days a week around 12th week of gestation during first trimester as well as around 32nd week of gestation during third trimester with Gradko Environmental passive dosimeters (Gradko International Ltd., UK). The home-outdoor measuring tube was installed on the most exposed façade in the window or a balcony. Home-indoor tube was placed near the bedside at participant's home. The personal exposure to NO_2 were measured using passive diffusion tubes installed on

the shoulder strap of a backpack. The study participants were asked to carry the backpack during the week-long measurement period. They were allowed to leave the tube hanging at a minimum height of 1.5 m while they are at home, but instructed to put on the backpack whenever they were going outside. All instructions for proper installation (during COVID-19 lockdown) and usage were given by the trained field workers.

Quality control measures were taken using at least two laboratory blank tubes in each batch when samples were sent to the laboratory. As a reference NO_2 monitor, two simultaneous tubes were installed at the urban background reference station of Palau Reial for a week every month. There was a good agreement between the reference NO_2 monitor and passive tubes (R^2=0.88).

Statistical methods

Clinical outcome

Outcome variables including PE and SGA were created as secondary variables in MAPSS on Paper I as well as in Paper II and III in accordance with their definitions.

In Paper IV, the customized birth weight standard for a Spanish population was adopted for estimating birthweight centile (Figueras et al., 2008). For FGR, CPR centiles were estimated using normative reference values developed by Baschat and Gembruch (2003). To following three criteria was adopted to subclassify small babies to low-risk SGA or true FGR: (1) SGA in between 3rd-9th centile or SGA less than 3rd centile; (2) CPR doppler index more than 5th centile or less than 5th centile; and (3) UtA pulsatility index less than 95th centile or more than 95th centile, respectively (Figueras & Gratacós, 2014).

Placental outcome

Paper II and IV

Placental senescence was assessed based on the changes in placental relative mtDNAcn and telomere length. In Study II (N=137 placental samples) and Study IV (N=591 placental samples), placental DNA was extracted from the collected biopsy of placental villious parenchyma using Allprep® DNA/RNA/miRNA Universal kit from Qiagen, following the manufacturer's instructions. Using a real-time quantitative polymerase chain reaction (PCR), the target gene used to determine mtDNAcn was *MT-TL1* encoding the Mitochondrially Encoded TRNA-Leu (UUA/G) 1 in Paper II and *MT-ND1* encoding the Mitochondrial encoded NADH dehydrogenase subunit 1 in Paper IV. For relative telomere length

quantification, *TEL1* and *TEL2* primers were used for Paper II, whereas in Paper IV, *TelC* and *TelG* primers were used. The ratios of both mtDNAcn and telomere native sequences copy numbers to nuclear single-copy reference genes (haemoglobin beta (*HBB)* in Paper II and haemoglobin subunit gamma gene (*HBG1* and *HBG2* primers) in Paper IV) were determined as relative mtDNAcn and telomere length, respectively.

Paper III

Bisulfite treatment was performed on 500 ng DNA extracted from 111 placental villous parenchyma using EZ-96 DNA Methylation kit v 1.1 (Zymo Research, Irvine, CA, USA). Genome-wide DNA methylation analysis was conducted at the Center for Translational Genomics (CTG), Lund University using Infinium MethylationEPIC BeadChip (Illumina, San Diego, CA, USA) targeting over 850,000 CpG sites. Placental DNA methylation preprocessing and data analysis were performed using the statistical software R and R packages ChAMP, minfi, Planet (placental cell type and epigenetic clock), limma, and missMethyl.

Only 17 extracted RNAs of all placental samples had RIN value over 7. At CTG, total RNA library was prepared using the TruSeq® Stranded mRNA Library Prep (20020594, Illumina, San Diego, CA, USA) and quality control was performed using LabChip DNA High Sensitivity Reagent kit (CLS138948, Perkin Elmer, Waltham, MA, USA) and DNA 1K/12K/Hi Sensitivity Assay LabChip (760517, Perkin Elmer). Using the QuantIT® dsDNA HS Assay Kit (Q33120, Thermo Fischer, Waltham, MA, USA), the library pool was quantified and sequenced as paired-end, 75-bp reads on a NovaSeq 6000 (Illumina). Raw data were processed using the FastQC tool on the bcl2fastq software (Illumina, San Diego, CA, USA) and the reference genome sequence from the Ensembl database, the Human GRCh38 as well as the StringTie software.

Data analysis

IBM SPSS Statistics software (IBM, Chicago, IL, USA), version 25 was used for the statistical analyses of Paper I and II. The statistical software R, version 4.0.208 and 4.2.2 (The R Foundation for Statistical Computing) were used for Paper III and IV.

The continuous dependent variables (mtDNAcn, TL and birthweight) were tested for normality first and in case of skewed distribution, it was log2-transformed. Complete case analysis was applied in all studies by excluding all observations with missing values in the categorical predictors. Based on a priori knowledge, maternal age, maternal pre-pregnancy body mass index (BMI), season of birth and fetal sex were the covariates adjusted for all studies (Bartsch et al., 2016; Casquero-Vera et al., 2019; Iodice et al., 2018; Martens et al., 2017; Pedersen et al., 2014; Phillips et

al., 2004; Rudra & Williams, 2005; Silva et al., 2020; Wacker et al., 1998). Directed acyclic graph was employed to select the covariates for adjustment in Paper I (Figure 8). Mothers who had a history of smoking were excluded from the placental molecular studies in Scania due to its known influence on DNA methylation (Lee & Pausova, 2013) as well as its protective effect on PE (Cudmore et al., 2007; Wei et al., 2015). In Barcelona study, we added "smoking anytime during pregnancy" as a covariate for the analysis of association between prenatal NO_2 exposure and fetal growth indicators. Other covariates adjusted for the models, but not exclusively in all studies, include parity, gestational age, maternal country of birth, education level, annual household income, child ethnicity, and maternal comorbidity (diabetes mellitus, gestational diabetes, essential hypertension, and maternal alcohol consumption during pregnancy.

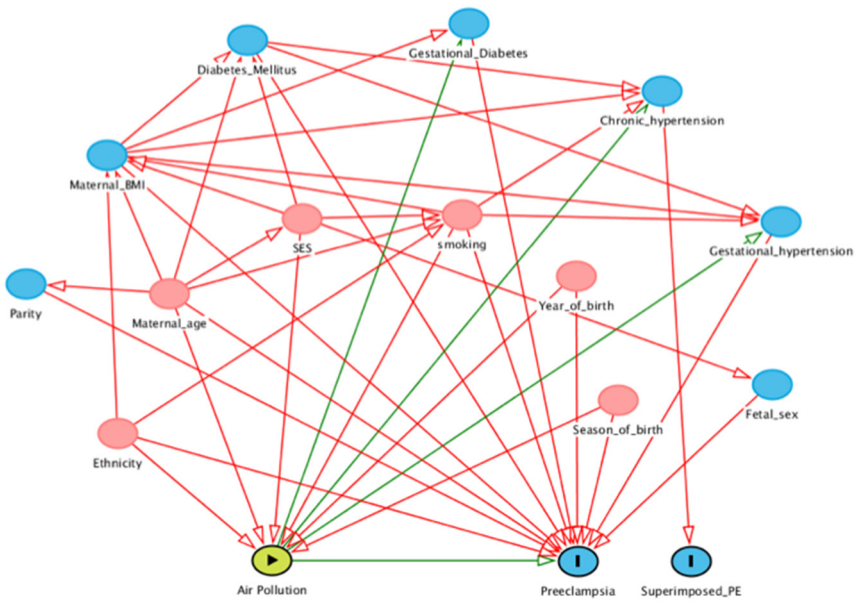


Figure 8. Directed Acyclic Graphs to guide the selection of potential confounders for the association between the ambient air pollution and preeclampsia: (yellow) exposure; (blue with I) outcome; (red dot) common cause; (blue dot) ancestor (Mandakh et al., 2020).

In Paper I, binary logistic regression models were applied in univariate and multivariable analyses for all pollutants during each trimester exposure period as well as entire pregnancy. The effect size was reported in adjusted odds ratio (OR) with 95% confidence interval (CI) for both linear-exposure increase and quartile-specific exposure for all pollutants. In Paper II and III, for the association between prenatal NO_x exposure and risk of PE, adjusted OR with 95% CI were estimated

using logistic regression. For the analysis of prenatal NO_x exposure and placental mtDNAcn or TL, linear regression model with same set of covariates were used to report the regression coefficient with 95% CI as an effect estimate.

In Paper III, to predict gestational age (GA) acceleration or deceleration, "control placental clock" (CPC) was applied using the R package Planet by regressing the predicted GA to the chronological GA (Horvath, 2013). If the residual value is positive, the placenta ages faster than the normal physiological process and therefore accelerating the placental epigenetic clock. In contrast, if it is negative residual value, the placenta ages slower than expected and termed as GA deceleration. Linear regression model was used to determine the GA acceleration/deceleration differences amongst the PE and NO_x combined groups.

Moreover, singular value decomposition (SVD) analysis was performed to identify the covariates, including technical and biological variables, that were associated with the DNAm. ComBat adjustments in ChAMP was applied to remove the technical variations presented in the first and second principal components. From the estimated placental cell type proportions, syncytiotrophoblast and Hofbauer cells were included in the final regression model. In addition, pre-pregnancy BMI, gestational age, fetal sex, and DNA concentration were included as covariates for the final model. Differentially Methylated Positions (DMPs) were reported in M-values (log_2 ratio of methylated and unmethylated probes) using linear regression model adjusted with aforementioned covariates. The *q*-value determined by Benjamin-Hochberg false discovery rate (FDR) method was used for assessing the statistical significance level. In addition, fetal-sex stratified analyses were performed. Furthermore, Interaction network and Gene ontology analyses were performed for genes that had CpGs with $q < 0.1$ in the DMP analyses using ConsensusPathDB and gometh function in the R package missMethyl. The final gene expression analysis consisted of 18,524 genes. The R packages, limma and Limma voom were used for differentially expressed genes analysis.

In Paper IV, the associations between nitrogen dioxide (NO_2), birthweight, SGA and FGR were estimated using linear and logistic regression models adjusted for covariates. For analysis of association between prenatal NO_2 exposure and placental mtDNAcn and TL, linear mixed effects models were employed by considering the random effects, including hospital of delivery and qPCR plate number.

To assess the mediatory role of placental relative mtDNA content or telomere length in the association between prenatal NO_x exposure and PE or prenatal NO_2 exposure and fetal growth indicators in Paper II and IV, the standard Baron and Kenny (1986) method of mediation analysis was employed. The mediation is tested through three step regressions including, (1) the prenatal NO_x or NO_2 exposure predicting the odds of PE or fetal growth indicators, repectively (2) the prenatal NO_x or NO_2 exposure predicting the mediators, namely, placental relative mtDNAcn or telomere length, and (3) prenatal NO_x or NO_2 exposure and the mediators predicting the clinical

outcome, including PE and fetal growth indicators. At first and second steps, the associations should be statistically significant to conduct the third step. If there is complete mediation, prenatal NO_x or NO_2 exposure should not be statistically significant whereas mediators are statistically significant. On the other hand, if there is a partial mediation, the coefficient for prenatal NO_x or NO_2 exposure should be reduced after the mediator is controlled as compared to the coefficient in the first step (Baron & Kenny, 1986).

Summary of results

Paper I

The incidence of PE was 1034 (2.9%) of CCA study population (N = 35,570). Preeclampsia was more prevalent among pregnant women with the following risk factors: obese (BMI ≥ 30), gestational diabetes, and both essential and gestational hypertension. Higher proportion of PE cases were observed for Nordic-born women when compared with mothers born in other countries.

As shown in Table 1, each 1 μg/m^3 increase in locally emitted BC during entire pregnancy was associated with increased risk of PE (AOR 2.14; 95% CI: 1.48, 3.09, $p < 0.01$). Similar patterns were observed for all trimesters as well as for each 5 μg/m^3 increment in locally emitted $PM_{2.5}$ and PM_{10} at all exposure windows. Early pregnancy exposure seems to be more prominent for the association with PE, as can be seen from all statistically significant associations for all pollutants, including long-range transboundary air pollution shown in the total $PM_{2.5}$ and PM_{10} results.

Table 1. Adjusted odds ratios (AOR) from the complete case analysis (CCA) on the effects of maternal exposure to ambient particles on risk for preeclampsia associated with a 1 μg/m^3 increase in the concentration of BC and 5 μg/m^3 increase in the concentration of local and total $PM_{2.5}$ and PM_{10} (with the intermediate variables[a] derived from DAGs) (Mandakh et al., 2020).

Exposure	Entire pregnancy AOR[§] (95% CI)	First trimester AOR[§] (95% CI)	Second trimester AOR[§] (95% CI)	Third trimester AOR[§] (95% CI)
Black carbon[†] (n/N)	935/32341 2.14** (1.48, 3.09)	970/33544 1.89** (1.34, 2.65)	976/33475 2.08** (1.45, 2.98)	982/33606 1.77** (1.28, 2.45)
Local PM2.5 [‡] (n/N)	893/30892 2.74** (1.68, 4.47)	962/33086 2.01** (1.34, 3.02)	946/32547 2.11** (1.42, 3.13)	979/33573 1.78** (1.22, 2.61)
Local PM10 [‡] (n/N)	908/31033 1.50** (1.19, 1.89)	973/33400 1.31** (1.09, 1.58)	954/32610 1.32** (1.11, 1.58)	972/33240 1.32** (1.11, 1.57)
Total PM2.5 [‡] (n/N)	742/25050 1.98** (1.27, 3.09)	909/31103 1.31* (1.06, 1.61)	844/28468 1.14 (0.92, 1.42)	827/27659 1.27* (1.03, 1.56)
Total PM10 [‡] (n/N)	909/31039 1.40** (1.13, 1.72)	974/33411 1.24** (1.09, 1.41)	954/32605 1.10 (0.97, 1.26)	972/33240 1.11 (0.98, 1.24)

[a]Intermediate variables are gestational diabetes, essential hypertension, gestational hypertension.

[†]Estimates are for each 1 μg/m^3 increase of black carbon particles. [‡]Estimates are for each 5 μg/m^3 increase of local and total $PM_{2.5}$ and PM_{10}. [§]Adjusted for maternal age, body mass index, parity, smoking, diabetes mellitus, gestational diabetes, essential hypertension, gestational hypertension, maternal country of birth, education level, annual household income, fetal sex, year and season of birth. *p-value < 0.05 as compared to the healthy controls. **p-value < 0.01 as compared to the healthy controls.

Moreover, the association between both linear exposure trend and quartile-specific exposure of BC, local $PM_{2.5}$ and PM_{10}, the risk of PE was pronounced when PE was accompanied with SGA (Table 2).

Table 2. Adjusted analysis for PE with SGA vs. PE without SGA in relation to the exposure quartiles of local $PM_{2.5}$ particles (μg/m^3) during each window of exposure (Mandakh et al., 2020).

	Local $PM_{2.5}$ Adjusted OR[§] (95% CI)	
Range, μg/m^3	**PE with SGA**	**PE without SGA**
Entire pregnancy	193/30192	700/27747
Linear (5 μg/m^3)	4.33 (1.56, 12.04)**	2.56 (1.48, 4.43)**
Q1 (0.13 – 0.96)	Reference	Reference
Q2 (0.96 – 1.50)	1.09 (0.69, 1.73)	1.18 (0.94, 1.49)
Q3 (1.50 – 2.08)	1.42 (0.92, 2.21)	1.24 (0.98, 1.57)
Q4 (2.08 – 7.52)	1.65 (1.06, 2.58)*	1.50 (1.19, 1.90)**
First trimester	209/32783	761/30148
Linear (5 μg/m^3)	2.57 (1.13, 5.82)*	1.96 (1.24, 3.10)**
Q1 (0.09 – 0.89)	Reference	Reference
Q2 (0.89 – 1.42)	1.19 (0.77, 1.83)	1.01 (0.81, 1.25)
Q3 (1.42 – 2.11)	1.36 (0.89, 2.09)	1.21 (0.97, 1.50)
Q4 (2.11 – 10.68)	1.59 (1.03, 2.45)*	1.32 (1.05, 1.65)*
Second trimester	199/31800	747/29230
Linear (5 μg/m^3)	2.87 (1.26, 6.55)*	2.00 (1.28, 3.11)**
Q1 (0.08 – 0.88)	Reference	Reference
Q2 (0.88 – 1.40)	0.77 (0.49, 1.21)	1.03 (0.82, 1.28)
Q3 (1.40 – 2.09)	1.20 (0.80 1.81)	1.02 (0.82, 1.28)
Q4 (2.09 – 10.5)	1.29 (0.84, 1.99)	1.31 (1.04, 1.65)*
Third trimester	206/32800	773/30149
Linear (5 μg/m^3)	2.65 (1.19, 5.92)*	1.65 (1.07, 2.53)*
Q1 (0.10 – 0.83)	Reference	Reference
Q2 (0.83 – 1.35)	1.71 (1.10, 2.65)*	1.20 (0.97, 1.49)
Q3 (1.35 – 2.05)	1.72 (1.10, 2.68)*	1.26 (1.01, 1.57)*
Q4 (2.05 – 9.64)	1.92 (1.21, 3.05)**	1.25 (0.99, 1.58)

[§]Adjusted for maternal age, body mass index, parity, smoking, diabetes mellitus, gestational diabetes, essential hypertension, gestational hypertension, maternal country of birth, education level, annual household income, fetal sex, year and season of birth. *p-value < 0.05 as compared to the healthy controls. **p-value < 0.01 as compared to the healthy controls.

As shown in Table 3 in Paper I, the association between the exposure to locally-emitted $PM_{2.5}$ during the third trimester and the risk of PE showed a clear dose-response relationship between quartiles with AOR (95% CI) being 1.27 (1.05, 1.55) at second quartile, 1.32 (1.08, 1.61) at third quartile and 1.36 (1.10, 1.67) at fourth quartile. Similar pattern was observed with local PM_{10}. When comparing the lowest quartile of exposure to the highest quartile of exposure to ambient NO_x, BC, local $PM_{2.5}$ and PM_{10}, the significant association with increased risk of PE were found for all exposure windows.

Paper II

Those exposed to higher NO_x concentrations during the first trimester (> 11.9 μg/m^3) had increased odds of developing PE (OR 4.0; 95% CI: 1.4, 11.1; $p = 0.008$). Also, higher NO_x exposure during entire pregnancy (> 12.0 μg/m^3) was associated with increased odds of PE (OR 3.7; 95% CI: 1.3, 10.4; $p = 0.012$). Those exposed to higher NO_x concentrations during the first trimester (> 11.9 μg/m^3) had reduced placental relative mtDNAcn (-0.20; 95% CI: -0.36, -0.04; $p = 0.01$). Placental relative mtDNAcn did not appear to mediate the association between prenatal exposure to ambient NO_x and PE during first trimester. No association was observed with placental relative TL with prenatal exposure to ambient NO_x and PE.

Paper III

Six DMPs ($q < 0.05$) were identified when comparing controls with low NO_x exposure to cases with high NO_x exposure. 14 DMPs were identified when comparing PE cases to controls with high NO_x exposure. No differentially expressed genes ($q < 0.05$) were found.

When comparing PE cases with high NO_x exposure to normotensive controls with low NO_x exposure, hypomethylation of *YAP1* was found. It may play a role in trophoblast fusion and proliferation in PE, thereby potentially also contributing to defective villous maturation and impaired placental function.

For those exposed to higher NO_x concentrations (> 12.1 μg/m^3) during early pregnancy, PE cases had more hypomethylated DMP cg26672098, PEBP1 (cancer), MICAL1 (cytokinesis), CD163 (Hofbauer cell in placenta) than controls. This finding is consistent with the previous study in which hypomethylated DMP cg26672098 was found to be associated with early-onset PE.

Placentas of pregnancies with female fetuses presented more DMPs (N = 309) than male-derived placentas (N = 1). Female placentas had more hypomethylated positions than male placentas.

Placentas from PE cases with high NO_x exposure showed significant GA deceleration compared to placentas from controls with low NO_x exposure (beta = −0.61, p = 0.035, model $R^2 = 0.03$).

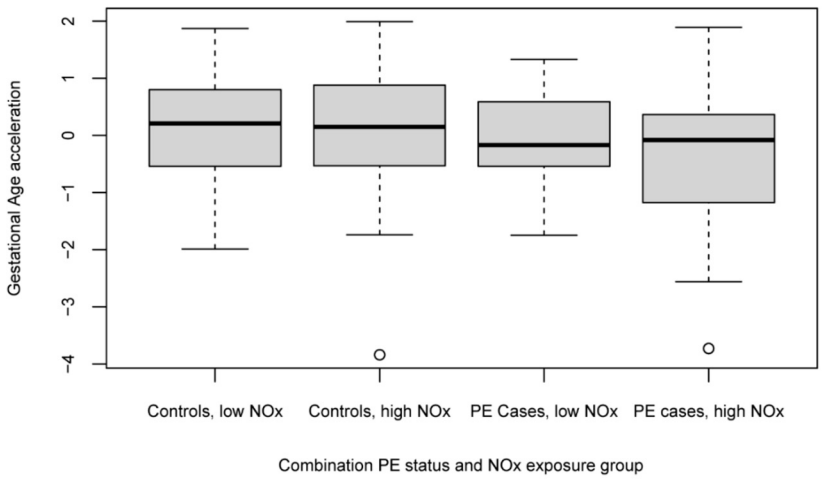


Figure 8. Differences in GA acceleration between combined PE status and NOx exposure groups (Engström et al., 2021)

Paper IV

Each 1 μg/m^3 of home-outdoor NO_2 increase was associated with a decrease in birth weight of 6.05 grams (95% CI: -10.4, -1.7, p=0.006) during entire pregnancy and of 5.3 grams (95% CI: -8.9, -1.6, p=0.005) during the first trimester. Moreover, personal NO_2 exposure during first trimester was associated with increased odds of SGA neonate (adjusted OR 1.033; 95% CI: 1.004, 1.062; p=0.02). Home-outdoor NO_2 exposure during third trimester was associated with increased placental relative mtDNAcn (1.003 fold-change, 95% CI: 1.0002, 1.006, p=0.038). Each 1 μg/m^3 of home-indoor NO_2 increase was associated with placental relative telomere shortening (0.997 fold-change, 95% CI: 0.995, 0.999, p=0.014). The odds of SGA was associated neither with placental relative mtDNAcn (AOR 1.02; 95% CI: 0.52, 2.03; p = 0.94) nor placental relative TL (AOR 1.43; 95% CI: 0.54, 3.91; p = 0.47).

Discussion

In a setting with fair air quality in Scania, the increased risk of PE and SGA were associated with both linear exposure trend and quartile-specific exposure of ambient particles and NO_x. In a setting with moderate air quality in Barcelona, early pregnancy exposure to ambient NO_2 measured by home-outdoor and personal sensors were associated with lower birth weight and increased odds of having SGA neonate, respectively. Although the mediatory role of placental mtDNAcn and telomere length were not confirmed, prenatal exposure to ambient NO_x and NO_2 affect the placental aging process differently depending on the exposure concentration and exposure period during pregnancy. We observed reduced mtDNAcn and shorter TL in placenta in relation to exposure during first trimester, whereas increased mtDNAcn in relation to NO_2 exposure during third trimester. Prenatal exposure to ambient NO_x during first trimester affected differential placental DNA methylation in PE and NO_x combined groups, resulting in placental age deceleration and showing sexual dimorphism.

Our finding of gestational age deceleration in placentas from PE cases with high NO_x exposure indicated the effect of exposure rather than PE on placental pathology. Previous studies have reported that placentas from pregnancies complicated with PE have been found to be associated with accelerated placental aging (Girchenko et al., 2017; Mayne et al., 2017). In contrast, the exposure to ambient nitrogen dioxide was associated inversely with gestational age acceleration (White et al., 2019), whereas $PM_{2.5}$ exposure was positively associated with circadian pathway genes (Nawrot et al., 2018). Growing body of literature on molecular study of placenta found differential DNAm in PE placentas (Cirkovic et al., 2020) as well as in relation to the exposure to ambient air pollution during pregnancy (Neven et al., 2018). Prenatal exposure to ambient NO_2 during first and second trimesters was associated with hypomethylation of two CpGs located at *ADORA2B* (A_{2B} adenosine receptor), a gene suggested to be involved in trophoblast proliferation and invasion in PE (Abraham et al., 2018; Darashchonak et al., 2014).

Placental DNAm findings display sexual dimorphism in the occurrence of PE in relation to early pregnancy exposure to ambient NO_2 in which placentas of pregnancies with a female fetus had more hypomethylated positions than male placentas. In singleton pregnancies, pregnancies with female fetus had higher incidence of PE when compared with those carrying male fetus (Shiozaki et al., 2011). Sexual dimorphic differences in PE have been confirmed by another meta-

analysis, suggesting that preterm PE being more common among pregnancies carrying female fetus than pregnancies with male fetus (Collaboration: et al., 2016).

Returning to the hypothesis posed at the beginning of this dissertation, it is now possible to state that placental aging may be the underlying molecular mechanism linking the association between prenatal exposure to ambient air pollution and pregnancy complications including preeclampsia and small-for-gestational age foetuses and neonates. Due to oxidative stress induced by ambient air pollution, placental DNA differential methylation may lead to syncytiotrophoblast stress whereas changes in placental mitochondrial DNA content and short telomeres trigger placental senescence (Redman et al., 2022; Sultana et al., 2018). Trophoblast senescence is a normal physiologic phenomenon and is expected to advance gradually over the course of pregnancy until full term. However, under prolonged stressors including mitochondrial stress and telomere shortening, increased premature trophoblast senescence were observed for PE and FGR (T. Biron-Shental et al., 2010; Sukenik-Halevy et al., 2016). Taken together, these mechanisms lead to perturbance in placental aging that may contribute to the etiology of preeclampsia and impaired fetal growth.

Several assumptions on confounding must be addressed when linking prenatal exposure to ambient air pollution to preeclampsia and fetal growth. Systematic reviews reported that cigarette smoking during pregnancy is associated with lower incidence of preeclampsia compared to non-smokers (Conde-Agudelo et al., 1999; Wei et al., 2015). One experimental study indicated that nicotine restored the function of human umbilical vein endothelial cells treated with soluble fms-like tyrosine kinase 1 (sFlt1) and soluble endoglin (sEng), the anti-angiogenic factors that induce endothelial dysfunction in PE (Armaly et al., 2018; Mimura et al., 2010). Another possible explanation is that carbon monoxide (CO) in the cigarette smoke lowers sFlt1 and sEng in endothelial cells through heme oxygenase-1 and its metabolite CO pathway (Cudmore et al., 2007). Prenatal chronic exposure to cigarette smoke has been associated with altered DNAm in placental genes encoding hypoxia and oxidative stress pathways (Suter et al., 2011).

Season of birth seems to be another important confounder for the association between air pollution exposure and placental molecular mechanisms as well as preeclampsia and birth weight. In Spain, NO_2 and NO_x ambient concentrations present clear seasonal trends with lower levels during summer compared to winter, partially due to tropospheric ozone formation based on warmer temperatures, extended sunlight and higher emissions of NO_x from primary emission sources including diesel vehicles (Casquero-Vera et al., 2019; Silva et al., 2020). Full-term human placental gene expression analysis found increased expression of circadian clock, vitamin-D receptor and hypoxia-related genes during winter births (Clarkson-Townsend et al., 2020). Birth weight is significantly associated with season of birth indicating higher mean birth weight in the summer months and low birth weight in February (Day et al., 2015). Seasonal variation is also observed in the incidence

rates of hypertensive disorders of pregnancy, particularly increased sunlight before delivery was associated with decreased rates of pregnancy hypertension (Algert et al., 2010).

To study the association between preeclampsia and prenatal exposure to air pollution, central monitoring data have been used to model each individual exposure using the dispersion model. The problem with using the central monitoring data is high spatial variation of air pollutants over a short distance. Within the European Study of Cohorts for Air Pollution Effects (ESCAPE), dispersion model estimates of ambient NO_2 at residence level had a good agreement with land use regression model estimates, probably due to NO_2 traffic sources in the urban areas contributing to small-scale spatial variation (de Hoogh et al., 2014). However, dispersion model estimates for ambient $PM_{2.5}$ and PM_{10} had less agreement with land use regression estimates, suggesting difference in effect size in epidemiological studies employing these models (de Hoogh et al., 2014). Considering this aspect, modelled NO_x concentrations are robust, while the results of prenatal $PM_{2.5}$ and PM_{10} therefore need to be interpreted with caution in Paper I.

Gradkosampler tend to overestimate ambient NO_2 concentrations by 10% to 30% when compared with chemiluminescence analyzers due to interconversion between gas-phase NO_x species in the diffusion tube body (Bush et al., 2001; Heal et al., 1999; Yu et al., 2008). In addition to personal sensor measurement by passive sampling tube, time-activity data was collected with follow-up questionnaire as well as GPS tracking of movement collected with ExpoApp. The limitations concerning this aspect was that it was prone to recall bias and the participants did not answer the follow-up questionnaire completely.

Further research needs to investigate more closely the links between exogenous NO_x exposure and endogenous nitric oxide (NO) generation in pregnancies complicated with PE and FGR. NO is synthesized physiologically by the nitric oxide synthases (NOS) that catalyse the oxidation of L-arginine to L-citrulline and NO (Michel & Vanhoutte, 2010; Palmer et al., 1987). Endothelial NOS (eNOS) is expressed excessively on placental villous vessel endothelium as well as in syncytiotrophoblast of placentas of pregnancies complicated with PE and FGR (Myatt et al., 1997). Moreover, as a marker of eNOS, the total nitrite (NO_2) and nitrate (NO_3) concentrations were significantly increased in umbilical venous serum of PE group when compared with normotensive controls, but not in maternal circulation (Lyall et al., 1995). Oxidative stress and smoking among other factors can down-regulate the release of NO by the endothelium (Michel & Vanhoutte, 2010). In state of increased levels of ROS, NO reacts with superoxide (O_2^-) and generates NO-derived free radicals, such as peroxynitrite ($ONOO^-$), leading to further degradation on bioavailable NO and endothelial dysfunction by nitrosative stress (Leiva et al., 2016). In vitro study on metabolic fate of exogenous NO_x on human placental homogenates found higher NO_x concentrations and the formation of iron nitrosyl (FeNO) species in placental villous tissue collected from

pregnancies complicated with FGR (Mukosera et al., 2020). It would be interesting to assess the effects of ambient NO_x or NO_2 exposure on both oxidative and nitrosative stress in normal and pathologic pregnancies complicated with PE and FGR.

Taken together, the findings of these studies have a number of important implications for future practice. The reproductive health effects addressed at both study settings with fair and moderate air quality strongly support the WHO recommendations on updated global air quality limit values.

Conclusions

In a setting with fair air quality in Scania, Sweden, prenatal exposure to ambient air pollution was associated with increased risk of developing PE with or without SGA;

- The increased risk of PE associated with the linear exposure trend of ambient particles indicates the importance of reducing PM levels.
- Ambient NO_x exposure during the first trimester was associated with reduced placental relative mtDNAcn and an increased risk of PE. Placental relative mtDNAcn did not appear to mediate the association between prenatal exposure to ambient NO_x and PE.
- Early pregnancy exposure to ambient NO_x affected differential placental DNA methylation in PE and NO_x combined groups, resulting in placental immaturity and showing sexual dimorphism.

In contrast, in a setting with moderate air quality in Barcelona, Spain, prenatal exposure to ambient NO_2 was associated with changes in fetal growth indicators as well as in placental biomarkers;

- For home-outdoor sensor, each 1 $\mu g/m^3$ of ambient NO_2 increase was associated with a decrease in birth weight of 6.05 grams during entire pregnancy and of 5.3 grams during the first trimester.
- Personal NO_2 exposure during first trimester was associated with increased odds of SGA neonate.
- Placental relative mtDNAcn was positively associated with home-outdoor NO_2 exposure during third trimester.
- placental relative telomere length was inversely associated with home-indoor NO_2 exposure during first trimester.

Premature placental senescence and placental aging may be the underlying molecular mechanism linking the association between prenatal exposure to ambient air pollution and pregnancy complications including preeclampsia and small-for-gestational age foetuses and neonates. Due to oxidative stress induced by ambient air pollution, placental DNA differential methylation may lead to syncytiotrophoblast stress whereas changes in placental mitochondrial DNA content

and short telomeres trigger placental senescence. Taken together, these mechanisms may contribute to the etiology of preeclampsia and impaired fetal growth.

Considerably more work needs to be done to determine the dose-response relationship of exposure concentrations and the risk of PE and adverse birth outcome to further explore the significant differences in molecular dynamics of placental epigenetic and toxicity markers. Also, more information on both oxidative and nitrosative stress in the placenta would help us to establish a greater degree of accuracy on the association between ambient NO_x and PE or SGA.

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References

Abraham, E., Rousseaux, S., Agier, L., Giorgis-Allemand, L., Tost, J., Galineau, J., Hulin, A., Siroux, V., Vaiman, D., Charles, M. A., Heude, B., Forhan, A., Schwartz, J., Chuffart, F., Bourova-Flin, E., Khochbin, S., Slama, R., & Lepeule, J. (2018). Pregnancy exposure to atmospheric pollution and meteorological conditions and placental DNA methylation. *Environ Int*, *118*, 334-347. https://doi.org/10.1016/j.envint.2018.05.007

Alfano, R., Herceg, Z., Nawrot, T. S., Chadeau-Hyam, M., Ghantous, A., & Plusquin, M. (2018). The Impact of Air Pollution on Our Epigenome: How Far Is the Evidence? (A Systematic Review). *Curr Environ Health Rep*, *5*(4), 544-578. https://doi.org/10.1007/s40572-018-0218-8

Algert, C. S., Roberts, C. L., Shand, A. W., Morris, J. M., & Ford, J. B. (2010). Seasonal variation in pregnancy hypertension is correlated with sunlight intensity. *Am J Obstet Gynecol*, *203*(3), 215.e211-215.e215. https://doi.org/https://doi.org/10.1016/j.ajog.2010.04.020

Armaly, Z., Jadaon, J. E., Jabbour, A., & Abassi, Z. A. (2018). Preeclampsia: Novel Mechanisms and Potential Therapeutic Approaches [Review]. *Front Physiol*, *9*. https://doi.org/10.3389/fphys.2018.00973

Bai, W., Li, Y., Niu, Y., Ding, Y., Yu, X., Zhu, B., Duan, R., Duan, H., Kou, C., Li, Y., & Sun, Z. (2020). Association between ambient air pollution and pregnancy complications: A systematic review and meta-analysis of cohort studies. *Environmental Research*, *185*, 109471. https://doi.org/https://doi.org/10.1016/j.envres.2020.109471

Baron, R. M., & Kenny, D. A. (1986). The moderator-mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. *J Pers Soc Psychol*, *51*(6), 1173-1182. https://doi.org/10.1037//0022-3514.51.6.1173

Bartsch, E., Medcalf, K. E., Park, A. L., & Ray, J. G. (2016). Clinical risk factors for pre-eclampsia determined in early pregnancy: systematic review and meta-analysis of large cohort studies. *Bmj*, *353*, i1753. https://doi.org/10.1136/bmj.i1753

Baschat, A. A., & Gembruch, U. (2003). The cerebroplacental Doppler ratio revisited. *Ultrasound in Obstetrics & Gynecology*, *21*(2), 124-127. https://doi.org/https://doi.org/10.1002/uog.20

Benny, P. A., Alakwaa, F. M., Schlueter, R. J., Lassiter, C. B., & Garmire, L. X. (2020). A review of omics approaches to study preeclampsia. *Placenta*, *92*, 17-27. https://doi.org/10.1016/j.placenta.2020.01.008

Bernasconi, S., Angelucci, A., & Aliverti, A. (2022). A Scoping Review on Wearable Devices for Environmental Monitoring and Their Application for Health and Wellness. *Sensors (Basel)*, *22*(16). https://doi.org/10.3390/s22165994

Bianco-Miotto, T., Mayne, B. T., Buckberry, S., Breen, J., Lopez, C. M. R., & Roberts, C. T. (2016). Recent progress towards understanding the role of DNA methylation in human placental development. *Reproduction*, *152*(1), R23. https://doi.org/10.1530/rep-16-0014

Bijnens, E., Zeegers, M. P., Gielen, M., Kicinski, M., Hageman, G. J., Pachen, D., Derom, C., Vlietinck, R., & Nawrot, T. S. (2015). Lower placental telomere length may be attributed to maternal residential traffic exposure; a twin study. *Environ Int*, *79*, 1-7. https://doi.org/10.1016/j.envint.2015.02.008

Biron-Shental, T., Sukenik-Halevy, R., Sharon, Y., Goldberg-Bittman, L., Kidron, D., Fejgin, M. D., & Amiel, A. (2010). Short telomeres may play a role in placental dysfunction in preeclampsia and intrauterine growth restriction. *Am J Obstet Gynecol*, *202*(4), 381.e381-387. https://doi.org/10.1016/j.ajog.2010.01.036

Biron-Shental, T., Sukenik-Halevy, R., Sharon, Y., Goldberg-Bittman, L., Kidron, D., Fejgin, M. D., & Amiel, A. (2010). Short telomeres may play a role in placental dysfunction in preeclampsia and intrauterine growth restriction. *Am J Obstet Gynecol*, *202*(4), 381.e381-381.e387. https://doi.org/https://doi.org/10.1016/j.ajog.2010.01.036

Blackburn, E. H. (1991). Structure and function of telomeres. *Nature*, *350*(6319), 569-573. https://doi.org/10.1038/350569a0

Blair, J. D., Yuen, R. K. C., Lim, B. K., McFadden, D. E., Von Dadelszen, P., & Robinson, W. P. (2013). Widespread DNA hypomethylation at gene enhancer regions in placentas associated with early-onset pre-eclampsia. *Molecular Human Reproduction*, *19*(10), 697-708. https://doi.org/10.1093/molehr/gat044

Brown, M. A., Magee, L. A., Kenny, L. C., Karumanchi, S. A., McCarthy, F. P., Saito, S., Hall, D. R., Warren, C. E., Adoyi, G., & Ishaku, S. (2018). The hypertensive disorders of pregnancy: ISSHP classification, diagnosis & management recommendations for international practice. *Pregnancy Hypertens*, *13*, 291-310. https://doi.org/10.1016/j.preghy.2018.05.004

Bush, T., Smith, S., Stevenson, K., & Moorcroft, S. (2001). Validation of nitrogen dioxide diffusion tube methodology in the UK. *Atmospheric Environment*, *35*(2), 289-296. https://doi.org/https://doi.org/10.1016/S1352-2310(00)00172-2

Casquero-Vera, J. A., Lyamani, H., Titos, G., Borrás, E., Olmo, F. J., & Alados-Arboledas, L. (2019). Impact of primary NO2 emissions at different urban sites exceeding the European NO2 standard limit. *Science of The Total Environment*, *646*, 1117-1125. https://doi.org/https://doi.org/10.1016/j.scitotenv.2018.07.360

Cirkovic, A., Garovic, V., Milin Lazovic, J., Milicevic, O., Savic, M., Rajovic, N., Aleksic, N., Weissgerber, T., Stefanovic, A., Stanisavljevic, D., & Milic, N. (2020). Systematic review supports the role of DNA methylation in the pathophysiology of preeclampsia: a call for analytical and methodological standardization. *Biol Sex Differ*, *11*(1), 36. https://doi.org/10.1186/s13293-020-00313-8

Clarkson-Townsend, D. A., Kennedy, E., Everson, T. M., Deyssenroth, M. A., Burt, A. A., Hao, K., Chen, J., Pardue, M. T., & Marsit, C. J. (2020). Seasonally variant gene expression in full-term human placenta. *Faseb j*, *34*(8), 10431-10442. https://doi.org/10.1096/fj.202000291R

Clemente, D., Casas, M., Janssen, B., Lertxundi, A., Santa Marina, L., Iñiguez, C., Llop, S., Sunyer, J., Guxens, M., Nawrot, T., & Vrijheid, M. (2017). Prenatal ambient air pollution exposure, infant growth and placental mitochondrial DNA content in the INMA birth cohort. *Environmental Research*, *157*, 96-102. https://doi.org/10.1016/j.envres.2017.05.018

Collaboration:, O. b. o. t. G. P., Schalekamp-Timmermans, S., Arends, L. R., Alsaker, E., Chappell, L., Hansson, S., Harsem, N. K., Jälmby, M., Jeyabalan, A., Laivuori, H., Lawlor, D. A., Macdonald-Wallis, C., Magnus, P., Myers, J., Olsen, J., Poston, L., Redman, C. W., Staff, A. C., Villa, P., . . . Steegers, E. A. (2016). Fetal sex-specific differences in gestational age at delivery in pre-eclampsia: a meta-analysis. *International Journal of Epidemiology*, *46*(2), 632-642. https://doi.org/10.1093/ije/dyw178

Collas, P., Noer, A., & Timoskainen, S. (2007). Programming the genome in embryonic and somatic stem cells [https://doi.org/10.1111/j.1582-4934.2007.00079.x]. *Journal of Cellular and Molecular Medicine*, *11*(4), 602-620. https://doi.org/https://doi.org/10.1111/j.1582-4934.2007.00079.x

Conde-Agudelo, A., Althabe, F., Belizán, J. M., & Kafury-Goeta, A. C. (1999). Cigarette smoking during pregnancy and risk of preeclampsia: A systematic review. *Am J Obstet Gynecol*, *181*(4), 1026-1035. https://doi.org/https://doi.org/10.1016/S0002-9378(99)70341-8

Cudmore, M., Ahmad, S., Al-Ani, B., Fujisawa, T., Coxall, H., Chudasama, K., Devey, L. R., Wigmore, S. J., Abbas, A., Hewett, P. W., & Ahmed, A. (2007). Negative regulation of soluble Flt-1 and soluble endoglin release by heme oxygenase-1. *Circulation*, *115*(13), 1789-1797. https://doi.org/10.1161/circulationaha.106.660134

Darashchonak, N., Koepsell, B., Bogdanova, N., & von Versen-Höynck, F. (2014). Adenosine A2Breceptors induce proliferation, invasion and activation of cAMP response element binding protein (CREB) in trophoblast cells. *BMC Pregnancy Childbirth*, *14*(1), 2. https://doi.org/10.1186/1471-2393-14-2

Day, F. R., Forouhi, N. G., Ong, K. K., & Perry, J. R. B. (2015). Season of birth is associated with birth weight, pubertal timing, adult body size and educational attainment: a UK Biobank study. *Heliyon*, *1*(2), e00031. https://doi.org/https://doi.org/10.1016/j.heliyon.2015.e00031

de Hoogh, K., Korek, M., Vienneau, D., Keuken, M., Kukkonen, J., Nieuwenhuijsen, M. J., Badaloni, C., Beelen, R., Bolignano, A., Cesaroni, G., Pradas, M. C., Cyrys, J., Douros, J., Eeftens, M., Forastiere, F., Forsberg, B., Fuks, K., Gehring, U., Gryparis, A., . . . Bellander, T. (2014). Comparing land use regression and dispersion modelling to assess residential exposure to ambient air pollution for epidemiological studies. *Environment International*, *73*, 382-392. https://doi.org/https://doi.org/10.1016/j.envint.2014.08.011

Engström, K., Mandakh, Y., Garmire, L., Masoumi, Z., Isaxon, C., Malmqvist, E., Erlandsson, L., & Hansson, S. R. (2021). Early Pregnancy Exposure to Ambient Air Pollution among Late-Onset Preeclamptic Cases Is Associated with Placental DNA Hypomethylation of Specific Genes and Slower Placental Maturation. *Toxics*, *9*(12), 338. https://www.mdpi.com/2305-6304/9/12/338

European Commission Joint Research Centre, Wilson, J., Bessagnet, B., Pisoni, E., Thunis, P., De Meij, A., Vignati, E., & Mascherpa, A. (2021). *Urban PM2.5 atlas : air quality in European cities : 2021 report*. Publications Office of the European Union. https://doi.org/doi/10.2760/356670

European Environment Agency, E. (2022). *European city air quality viewer*. Retrieved 2022/11/27 from https://www.eea.europa.eu/themes/air/urban-air-quality/european-city-air-quality-viewer

Figueras, F., & Gratacós, E. (2014). Update on the Diagnosis and Classification of Fetal Growth Restriction and Proposal of a Stage-Based Management Protocol. *Fetal Diagn Ther*, *36*(2), 86-98. https://doi.org/10.1159/000357592

Figueras, F., Meler, E., Iraola, A., Eixarch, E., Coll, O., Figueras, J., Francis, A., Gratacos, E., & Gardosi, J. (2008). Customized birthweight standards for a Spanish population. *Eur J Obstet Gynecol Reprod Biol*, *136*(1), 20-24. https://doi.org/10.1016/j.ejogrb.2006.12.015

Fogarty, N., Burton, G., & Ferguson-Smith, A. (2015). Different epigenetic states define syncytiotrophoblast and cytotrophoblast nuclei in the trophoblast of the human placenta. *Placenta*, *36*, 796-802.

Geographic variation in the incidence of hypertension in pregnancy. World Health Organization International Collaborative Study of Hypertensive Disorders of Pregnancy. (1988). *Am J Obstet Gynecol*, *158*(1), 80-83.

Ghulmiyyah, L., & Sibai, B. (2012). Maternal mortality from preeclampsia/eclampsia. *Semin Perinatol*, *36*(1), 56-59. https://doi.org/10.1053/j.semperi.2011.09.011

Girchenko, P., Lahti, J., Czamara, D., Knight, A. K., Jones, M. J., Suarez, A., Hämäläinen, E., Kajantie, E., Laivuori, H., Villa, P. M., Reynolds, R. M., Kobor, M. S., Smith, A. K., Binder, E. B., & Räikkönen, K. (2017). Associations between maternal risk factors of adverse pregnancy and birth outcomes and the offspring epigenetic clock of gestational age at birth. *Clin Epigenetics*, *9*, 49. https://doi.org/10.1186/s13148-017-0349-z

Grigoriu, A., Ferreira, J. C., Choufani, S., Baczyk, D., Kingdom, J., & Weksberg, R. (2011). Cell specific patterns of methylation in the human placenta. *Epigenetics*, *6*(3), 368-379. https://doi.org/10.4161/epi.6.3.14196

Gude, N., Roberts, C., Kalionis, B., & King, R. (2004). Growth and function of the normal human placenta. *Thrombosis Research*, *114*, 397-407. https://www.thrombosisresearch.com/article/S0049-3848(04)00342-1/fulltext

Gyselaers, W. (2020). Preeclampsia Is a Syndrome with a Cascade of Pathophysiologic Events. *Journal of clinical medicine*, *9*(7), 2245. https://doi.org/10.3390/jcm9072245

Hayflick, L., & Moorhead, P. S. (1961). The serial cultivation of human diploid cell strains. *Exp Cell Res*, *25*, 585-621. https://doi.org/10.1016/0014-4827(61)90192-6

Heal, M. R., O'Donoghue, M. A., & Cape, J. N. (1999). Overestimation of urban nitrogen dioxide by passive diffusion tubes: a comparative exposure and model study. *Atmospheric Environment*, *33*(4), 513-524. https://doi.org/https://doi.org/10.1016/S1352-2310(98)00290-8

Horvath, S. (2013). DNA methylation age of human tissues and cell types. *Genome Biology*, *14*(10), 3156. https://doi.org/10.1186/gb-2013-14-10-r115

Hu, H., Zhao, J., Savitz, D. A., Prosperi, M., Zheng, Y., & Pearson, T. A. (2020). An external exposome-wide association study of hypertensive disorders of pregnancy. *Environ Int*, *141*, 105797. https://doi.org/10.1016/j.envint.2020.105797

Instituto Nacional de Estadística, I.-S. (2022). *Continuous Register Statistics* Retrieved 2022/11/24 from https://www.ine.es/dyngs/INEbase/en/operacion.htm?c=Estadistica_C&cid=1254736177012&menu=ultiDatos&idp=1254734710990

Iodice, S., Hoxha, M., Ferrari, L., Carbone, I. F., Anceschi, C., Miragoli, M., Pesatori, A. C., Persico, N., & Bollati, V. (2018). Particulate Air Pollution, Blood Mitochondrial DNA Copy Number, and Telomere Length in Mothers in the First Trimester of Pregnancy: Effects on Fetal Growth. *Oxid Med Cell Longev*, *2018*, 5162905. https://doi.org/10.1155/2018/5162905

Janssen, B. G., Munters, E., Pieters, N., Smeets, K., Cox, B., Cuypers, A., Fierens, F., Penders, J., Vangronsveld, J., Gyselaers, W., & Nawrot, T. S. (2012). Placental mitochondrial DNA content and particulate air pollution during in utero life. *Environ Health Perspect*, *120*(9), 1346-1352. https://doi.org/10.1289/ehp.1104458

Jirtle, R. L., & Skinner, M. K. (2007). Environmental epigenomics and disease susceptibility. *Nature reviews. Genetics*, *8*(4), 253-262. https://doi.org/10.1038/nrg2045

Kampfrath, T., Maiseyeu, A., Ying, Z., Shah, Z., Deiuliis, J. A., Xu, X., Kherada, N., Brook, R. D., Reddy, K. M., Padture, N. P., Parthasarathy, S., Chen, L. C., Moffatt-Bruce, S., Sun, Q., Morawietz, H., & Rajagopalan, S. (2011). Chronic Fine Particulate Matter Exposure Induces Systemic Vascular Dysfunction via NADPH Oxidase and TLR4 Pathways. *Circulation Research*, *108*(6), 716-726. https://doi.org/doi:10.1161/CIRCRESAHA.110.237560

Leavey, K., Wilson, S. L., Bainbridge, S. A., Robinson, W. P., & Cox, B. J. (2018). Epigenetic regulation of placental gene expression in transcriptional subtypes of preeclampsia. *Clinical Epigenetics*, *10*(1). https://doi.org/10.1186/s13148-018-0463-6

Lee, H. C., & Wei, Y. H. (2000). Mitochondrial role in life and death of the cell. *J Biomed Sci*, *7*(1), 2-15. https://doi.org/10.1007/bf02255913

Lee, K., & Pausova, Z. (2013). Cigarette smoking and DNA methylation [Review]. *Frontiers in Genetics*, *4*. https://doi.org/10.3389/fgene.2013.00132

Leiva, A., Fuenzalida, B., Barros, E., Sobrevia, B., Salsoso, R., Sáez, T., Villalobos, R., Silva, L., Chiarello, I., Toledo, F., Gutiérrez, J., Sanhueza, C., Pardo, F., & Sobrevia, L. (2016). Nitric Oxide is a Central Common Metabolite in Vascular Dysfunction Associated with Diseases of Human Pregnancy. *Curr Vasc Pharmacol*, *14*(3), 237-259. https://doi.org/10.2174/1570161114666160222115158

Levin, H. L., & Moran, J. V. (2011). Dynamic interactions between transposable elements and their hosts. *Nature Reviews Genetics*, *12*(9), 615-627. https://doi.org/10.1038/nrg3030

Li, Z., Liu, Q., Xu, Z., Guo, X., & Wu, S. (2020). Association between short-term exposure to ambient particulate air pollution and biomarkers of oxidative stress: A meta-analysis. *Environmental Research*, *191*, 110105. https://doi.org/https://doi.org/10.1016/j.envres.2020.110105

Lyall, F., Young, A., & Greer, I. A. (1995). Nitric oxide concentrations are increased in the fetoplacental circulation in preeclampsia. *Am J Obstet Gynecol*, *173*(3, Part 1), 714-718. https://doi.org/https://doi.org/10.1016/0002-9378(95)90328-3

Mandakh, Y., Rittner, R., Flanagan, E., Oudin, A., Isaxon, C., Familari, M., Hansson, S. R., & Malmqvist, E. (2020). Maternal Exposure to Ambient Air Pollution and Risk of Preeclampsia: A Population-Based Cohort Study in Scania, Sweden. *Int J Environ Res Public Health*, *17*(5). https://doi.org/10.3390/ijerph17051744

Martens, D. S., Cox, B., Janssen, B. G., Clemente, D. B. P., Gasparrini, A., Vanpoucke, C., Lefebvre, W., Roels, H. A., Plusquin, M., & Nawrot, T. S. (2017). Prenatal air pollution and newborns' predisposition to accelerated biological aging [Article]. *JAMA Pediatr*, *171*(12), 1160-1167. https://doi.org/10.1001/jamapediatrics.2017.3024

Mayhew, T. M., Leach, L., McGee, R., Wan Ismail, W., Myklebust, R., & Lammiman, M. J. (1999). Proliferation, Differentiation and Apoptosis in Villous Trophoblast at 13–41 Weeks of Gestation (Including Observations on Annulate Lamellae and Nuclear Pore Complexes). *Placenta*, *20*(5-6), 407-422. https://doi.org/10.1053/plac.1999.0399

Mayne, B. T., Leemaqz, S. Y., Smith, A. K., Breen, J., Roberts, C. T., & Bianco-Miotto, T. (2017). Accelerated placental aging in early onset preeclampsia pregnancies identified by DNA methylation. *Epigenomics*, *9*(3), 279-289. https://doi.org/10.2217/epi-2016-0103

Michel, T., & Vanhoutte, P. M. (2010). Cellular signaling and NO production. *Pflugers Arch*, *459*(6), 807-816. https://doi.org/10.1007/s00424-009-0765-9

Mimura, K., Tomimatsu, T., Sharentuya, N., Tskitishvili, E., Kinugasa-Taniguchi, Y., Kanagawa, T., & Kimura, T. (2010). Nicotine restores endothelial dysfunction caused by excess sFlt1 and sEng in an in vitro model of preeclamptic vascular endothelium: a possible therapeutic role of nicotinic acetylcholine receptor (nAChR) agonists for preeclampsia. *Am J Obstet Gynecol*, *202*(5), 464.e461-466. https://doi.org/10.1016/j.ajog.2010.01.037

Mukosera, G. T., Clark, T. C., Ngo, L., Liu, T., Schroeder, H., Power, G. G., Yellon, S. M., Parast, M. M., & Blood, A. B. (2020). Nitric oxide metabolism in the human placenta during aberrant maternal inflammation. *J Physiol*, *598*(11), 2223-2241. https://doi.org/10.1113/jp279057

Murray, C. J. L., Aravkin, A. Y., Zheng, P., Abbafati, C., Abbas, K. M., Abbasi-Kangevari, M., Abd-Allah, F., Abdelalim, A., Abdollahi, M., Abdollahpour, I., Abegaz, K. H., Abolhassani, H., Aboyans, V., Abreu, L. G., Abrigo, M. R. M., Abualhasan, A., Abu-Raddad, L. J., Abushouk, A. I., Adabi, M., . . . Lim, S. S. (2020). Global burden of 87 risk factors in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *The Lancet*, *396*(10258), 1223-1249. https://doi.org/10.1016/S0140-6736(20)30752-2

Myatt, L., Eis, A. L., Brockman, D. E., Greer, I. A., & Lyall, F. (1997). Endothelial nitric oxide synthase in placental villous tissue from normal, pre-eclamptic and intrauterine growth restricted pregnancies. *Hum Reprod*, *12*(1), 167-172. https://doi.org/10.1093/humrep/12.1.167

Nawrot, T. S., Saenen, N. D., Schenk, J., Janssen, B. G., Motta, V., Tarantini, L., Cox, B., Lefebvre, W., Vanpoucke, C., Maggioni, C., & Bollati, V. (2018). Placental circadian pathway methylation and in utero exposure to fine particle air pollution. *Environ Int*, *114*, 231-241. https://doi.org/10.1016/j.envint.2018.02.034

Neven, K. Y., Saenen, N. D., Tarantini, L., Janssen, B. G., Lefebvre, W., Vanpoucke, C., Bollati, V., & Nawrot, T. S. (2018). Placental promoter methylation of DNA repair genes and prenatal exposure to particulate air pollution: an ENVIRONAGE cohort study. *The Lancet Planetary Health*, *2*(4), e174-e183. https://doi.org/https://doi.org/10.1016/S2542-5196(18)30049-4

Palmer, R. M. J., Ferrige, A. G., & Moncada, S. (1987). Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature*, *327*(6122), 524-526. https://doi.org/10.1038/327524a0

Pańczyszyn, A., Boniewska-Bernacka, E., & Goc, A. (2020). The role of telomeres and telomerase in the senescence of postmitotic cells. *DNA Repair (Amst)*, *95*, 102956. https://doi.org/10.1016/j.dnarep.2020.102956

Pedersen, M., Stayner, L., Slama, R., Sorensen, M., Figueras, F., Nieuwenhuijsen, M. J., Raaschou-Nielsen, O., & Dadvand, P. (2014). Ambient air pollution and pregnancy-induced hypertensive disorders: a systematic review and meta-analysis. *Hypertension*, *64*(3), 494-500. https://doi.org/10.1161/hypertensionaha.114.03545

Phillips, J. K., Bernstein, I. M., Mongeon, J. A., & Badger, G. J. (2004). Seasonal Variation in Preeclampsia Based on Timing of Conception. *Obstetrics & Gynecology*, *104*(5). https://journals.lww.com/greenjournal/Fulltext/2004/11000/Seasonal_Variation_in_Preeclampsia_Based_on_Timing.21.aspx

Plusquin, M., Guida, F., Polidoro, S., Vermeulen, R., Raaschou-Nielsen, O., Campanella, G., Hoek, G., Kyrtopoulos, S. A., Georgiadis, P., Naccarati, A., Sacerdote, C., Krogh, V., Bas Bueno-de-Mesquita, H., Monique Verschuren, W. M., Sayols-Baixeras, S., Panni, T., Peters, A., Hebels, D. G. A. J., Kleinjans, J., . . . Chadeau-Hyam, M. (2017). DNA methylation and exposure to ambient air pollution in two prospective cohorts. *Environment International*, *108*, 127-136. https://doi.org/https://doi.org/10.1016/j.envint.2017.08.006

Rakyan, V. K., Down, T. A., Balding, D. J., & Beck, S. (2011). Epigenome-wide association studies for common human diseases. *Nature Reviews Genetics*, *12*(8), 529-541. https://doi.org/10.1038/nrg3000

Ramiro-Cortijo, D., de la Calle, M., Rodriguez-Rodriguez, P., Phuthong, S., López de Pablo, Á. L., Martín-Cabrejas, M. A., & Arribas, S. M. (2020). First trimester elevations of hematocrit, lipid peroxidation and nitrates in women with twin pregnancies who develop preeclampsia. *Pregnancy Hypertens*, *22*, 132-135. https://doi.org/https://doi.org/10.1016/j.preghy.2020.09.005

Rath, W., & Tsikouras, P. (2018). [Maternal Deaths Worldwide Falling - But Commonly Preventable]. *Z Geburtshilfe Neonatol*, *222*(4), 143-151. https://doi.org/10.1055/a-0607-2816 (Müttersterbefälle weltweit rückläufig, aber häufig vermeidbar!)

Redman, C. W. G., Staff, A. C., & Roberts, J. M. (2022). Syncytiotrophoblast stress in preeclampsia: the convergence point for multiple pathways. *Am J Obstet Gynecol*, *226*(2, Supplement), S907-S927. https://doi.org/https://doi.org/10.1016/j.ajog.2020.09.047

Regionfakta. (2022). *Skåne län*. Retrieved 2022/11/27 from https://www.regionfakta.com/skane-lan/in-english/population1/population/

Reyes, L., & Golos, T. G. (2018). Hofbauer Cells: Their Role in Healthy and Complicated Pregnancy [Review]. *Frontiers in Immunology*, *9*(2628). https://doi.org/10.3389/fimmu.2018.02628

Richter, T., & Zglinicki, T. v. (2007). A continuous correlation between oxidative stress and telomere shortening in fibroblasts. *Experimental Gerontology*, *42*(11), 1039-1042. https://doi.org/https://doi.org/10.1016/j.exger.2007.08.005

Rittner, R., Gustafsson, S., Spanne, M., & Malmqvist, E. (2020). Particle concentrations, dispersion modelling and evaluation in southern Sweden. *SN Applied Sciences*, *2*(6), 1013. https://doi.org/10.1007/s42452-020-2769-1

Robinson, O., & Vrijheid, M. (2015). The Pregnancy Exposome. *Curr Environ Health Rep*, *2*(2), 204-213. https://doi.org/10.1007/s40572-015-0043-2

Romão-Veiga, M., Bannwart-Castro, C. F., Borges, V. T. M., Golim, M. A., Peraçoli, J. C., & Peraçoli, M. T. S. (2020). Increased TLR4 pathway activation and cytokine imbalance led to lipopolysaccharide tolerance in monocytes from preeclamptic women. *Pregnancy Hypertens*, *21*, 159-165. https://doi.org/10.1016/j.preghy.2020.06.002

Rudra, C. B., & Williams, M. A. (2005). Monthly variation in preeclampsia prevalence: Washington state, 1987–2001. *The Journal of Maternal-Fetal & Neonatal Medicine*, *18*(5), 319-324. https://doi.org/10.1080/14767050500275838

Saenen, N. D., Martens, D. S., Neven, K. Y., Alfano, R., Bové, H., Janssen, B. G., Roels, H. A., Plusquin, M., Vrijens, K., & Nawrot, T. S. (2019). Air pollution-induced placental alterations: an interplay of oxidative stress, epigenetics, and the aging phenotype? *Clinical Epigenetics*, *11*(1), 124. https://doi.org/10.1186/s13148-019-0688-z

Shiozaki, A., Matsuda, Y., Satoh, S., & Saito, S. (2011). Impact of fetal sex in pregnancy-induced hypertension and preeclampsia in Japan. *J Reprod Immunol*, *89*(2), 133-139. https://doi.org/10.1016/j.jri.2010.12.011

Silva, A. V., Oliveira, C. M., Canha, N., Miranda, A. I., & Almeida, S. M. (2020). Long-Term Assessment of Air Quality and Identification of Aerosol Sources at Setúbal, Portugal. *International Journal of Environmental Research and Public Health*, *17*(15), 5447. https://www.mdpi.com/1660-4601/17/15/5447

Stroh, E., Rittner, R., Oudin, A., Ardö, J., Jakobsson, K., Björk, J., & Tinnerberg, H. (2012). Measured and modeled personal and environmental NO2 exposure. *Popul Health Metr*, *10*(1), 10. https://doi.org/10.1186/1478-7954-10-10

Sukenik-Halevy, R., Amiel, A., Kidron, D., Liberman, M., Ganor-Paz, Y., & Biron-Shental, T. (2016). Telomere homeostasis in trophoblasts and in cord blood cells from pregnancies complicated with preeclampsia. *American Journal of Obstetrics & Gynecology*, *214*(2), 283.e281-283.e287. https://doi.org/10.1016/j.ajog.2015.08.050

Sultana, Z., Maiti, K., Dedman, L., & Smith, R. (2018). Is there a role for placental senescence in the genesis of obstetric complications and fetal growth restriction? *Am J Obstet Gynecol*, *218*(2, Supplement), S762-S773. https://doi.org/https://doi.org/10.1016/j.ajog.2017.11.567

Sundrani, D. P., Reddy, U. S., Joshi, A. A., Mehendale, S. S., Chavan-Gautam, P. M., Hardikar, A. A., Chandak, G. R., & Joshi, S. R. (2013). Differential placental methylation and expression of VEGF, FLT-1 and KDR genes in human term and preterm preeclampsia. *Clinical Epigenetics*, *5*(1), 6. https://doi.org/10.1186/1868-7083-5-6

Suter, M., Ma, J., Harris, A., Patterson, L., Brown, K. A., Shope, C., Showalter, L., Abramovici, A., & Aagaard-Tillery, K. M. (2011). Maternal tobacco use modestly alters correlated epigenome-wide placental DNA methylation and gene expression. *Epigenetics*, *6*(11), 1284-1294. https://doi.org/10.4161/epi.6.11.17819

Suzuki, M. M., & Bird, A. (2008). DNA methylation landscapes: provocative insights from epigenomics. *Nature Reviews Genetics*, *9*(6), 465-476. https://doi.org/10.1038/nrg2341

Tang, Z., Buhimschi, I. A., Buhimschi, C. S., Tadesse, S., Norwitz, E., Niven-Fairchild, T., Huang, S.-T. J., & Guller, S. (2013). Decreased Levels of Folate Receptor-β and Reduced Numbers of Fetal Macrophages (Hofbauer Cells) in Placentas from Pregnancies with Severe Pre-Eclampsia. *American Journal of Reproductive Immunology*, *70*(2), 104-115. https://doi.org/https://doi.org/10.1111/aji.12112

Tranquilli, A. L., Brown, M. A., Zeeman, G. G., Dekker, G., & Sibai, B. M. (2013). The definition of severe and early-onset preeclampsia. Statements from the International Society for the Study of Hypertension in Pregnancy (ISSHP). *Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health*, *3*(1), 44-47. https://doi.org/https://doi.org/10.1016/j.preghy.2012.11.001

Vishnyakova, P. A., Volodina, M. A., Tarasova, N. V., Marey, M. V., Tsvirkun, D. V., Vavina, O. V., Khodzhaeva, Z. S., Kan, N. E., Menon, R., Vysokikh, M. Y., & Sukhikh, G. T. (2016). Mitochondrial role in adaptive response to stress conditions in preeclampsia. *Sci Rep*, *6*, 32410. https://doi.org/10.1038/srep32410

Wacker, J., Schulz, M., Frühauf, J., Chiwora, F., Solomayer, E., & Bastert, G. (1998). Seasonal change in the incidence of preeclampsia in Zimbabwe. *Acta Obstetricia et Gynecologica Scandinavica*, *77*(7), 712-716. https://doi.org/10.1080/j.1600-0412.1998.770703.x

Walton, R. T., Mudway, I. S., Dundas, I., Marlin, N., Koh, L. C., Aitlhadj, L., Vulliamy, T., Jamaludin, J. B., Wood, H. E., Barratt, B. M., Beevers, S., Dajnak, D., Sheikh, A., Kelly, F. J., Griffiths, C. J., & Grigg, J. (2016). Air pollution, ethnicity and telomere length in east London schoolchildren: An observational study. *Environ Int*, *96*, 41-47. https://doi.org/10.1016/j.envint.2016.08.021

Wei, J., Liu, C. X., Gong, T. T., Wu, Q. J., & Wu, L. (2015). Cigarette smoking during pregnancy and preeclampsia risk: a systematic review and meta-analysis of prospective studies. *Oncotarget*, *6*(41), 43667-43678. https://doi.org/10.18632/oncotarget.6190

White, A. J., Kresovich, J. K., Keller, J. P., Xu, Z., Kaufman, J. D., Weinberg, C. R., Taylor, J. A., & Sandler, D. P. (2019). Air pollution, particulate matter composition and methylation-based biologic age. *Environ Int*, *132*, 105071. https://doi.org/10.1016/j.envint.2019.105071

WHO. (2004). International statistical classification of diseases and related health problems. In (Second edition ed.). WHO.

World Health Organization, W. (2021). *WHO global air quality guidelines: particulate matter (PM2.5 and PM10), ozone, nitrogen dioxide, sulfur dioxide and carbon monoxide*. World Health Organization. https://apps.who.int/iris/handle/10665/345329

Xuan, J., Jing, Z., Yuanfang, Z., Xiaoju, H., Pei, L., Guiyin, J., & Yu, Z. (2016). Comprehensive analysis of DNA methylation and gene expression of placental tissue in preeclampsia patients. *Hypertens Pregnancy*, *35*(1), 129-138. https://doi.org/10.3109/10641955.2015.1117099

Yang, S. W., Cho, E. H., Choi, S. Y., Lee, Y. K., Park, J. H., Kim, M. K., Park, J. Y., Choi, H. J., Lee, J. I., Ko, H. M., Park, S. H., Hwang, H. S., & Kang, Y. S. (2017). DC-SIGN expression in Hofbauer cells may play an important role in immune tolerance in fetal chorionic villi during the development of preeclampsia. *Journal of Reproductive Immunology*, *124*, 30-37. https://doi.org/https://doi.org/10.1016/j.jri.2017.09.012

Yu, C. H., Morandi, M. T., & Weisel, C. P. (2008). Passive dosimeters for nitrogen dioxide in personal/indoor air sampling: a review. *J Expo Sci Environ Epidemiol*, *18*(5), 441-451. https://doi.org/10.1038/jes.2008.22

Yu, H., Yin, Y., Zhang, J., & Zhou, R. (2020). The impact of particulate matter 2.5 on the risk of preeclampsia: an updated systematic review and meta-analysis. *Environmental Science and Pollution Research*, *27*(30), 37527-37539. https://doi.org/10.1007/s11356-020-10112-8

Zhang, Y., Wang, J., Gong, X., Chen, L., Zhang, B., Wang, Q., Han, B., Zhang, N., Xue, F., Vedal, S., & Bai, Z. (2020). Ambient PM(2.5) exposures and systemic biomarkers of lipid peroxidation and total antioxidant capacity in early pregnancy. *Environ Pollut*, *266*(Pt 2), 115301. https://doi.org/10.1016/j.envpol.2020.115301

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