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Epigenome-wide association study of pregnancy exposure to green space and placental DNA methylation

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ABSTRACT

Green space exposure during pregnancy has been associated with lower risk of adverse birth outcomes, but the biological mechanisms remain unclear. Epigenetic changes, such as DNA methylation (DNAm), may contribute to this association. The placenta, crucial for foetal development, has been understudied in relation to prenatal green space exposure and DNAm on a genome-wide scale. Here, we aimed to investigate the association between green space exposure during pregnancy and epigenome-wide placental DNAm in 550 mother-child pairs from the Barcelona Life Study Cohort (BiSC) in Spain. Green space exposure was assessed as (i) residential surrounding greenness (satellite-based Normalized Difference Vegetation Index (NDVI) in buffers of 100 m, 300 m and 500 m), (ii) residential distance to the nearest major green space (meters), (iii) use of green space (hours/week), and (iv) visual access to greenery through the home window (≥half of the view). Placental DNAm was measured with

Abbreviations: ADCY4, Adenylate Cyclase 4 gene; AIP, Aryl Hydrocarbon Receptor Interacting Protein; BiSC, Barcelona Life Study Cohort; BMIQ, Beta-mixture quantile; BN, Bonferroni; Bp, base pairs; CpG, Cytosine-phosphate-guanine; COPD, chronic obstructive pulmonary disease; DMP, Differentially methylated position; DMR, Differentially methylated region; DNAm, DNA methylation; IQR, Interquartile range; eQTM, Expression quantitative methylated regions; ESCAPE, European Study of Cohorts for Air Pollution Effects; Enh, Enhancer; EWAS, Epigenome-wide association study; gDMR, Germline differently methylated region; GPS, Global Positioning System; GS, Green space; GO, Gene Ontology; HUGE-F, Human Genome facility; ICGC, Cartographic and Geology Institute of Catalonia; KEGG, Kyoto Encyclopedia of Genes and Genomes; LUR, Land Use Regression Models; mQTL, methylation Quantitative Trait Loci; mtDNAc, mitochondria DNA content; PC, Principal Component; PM2.5, Particulate matter with aerodynamic diameter 2.5 microm; PMD, Partially methylated domain; QC, Quality control; R, Red values; MAF, minor allele frequency; NIR, near infrared values; NDVI, Normalized Difference Vegetation Index; SLC25A10, Solute carrier family 25 member 10 gene; SNPs, single nucleotide polymorphisms; SES, socioeconomic status; TSS, transcription start site; TssA, active transcription start site; TssAFlink, flanking active transcription start site; UCSC, University of California Santa Cruz; UTR, untranslated region.

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the EPIC array. Differentially methylated positions (DMPs) were identified using robust linear regression models adjusted for covariates, while differentially methylated regions (DMRs) were identified using the *dmrff* method.

After Bonferroni correction, cg14852540, annotated to *SLC25A10* gene, showed an inverse association with residential greenness within 500 m buffer. Additionally, 101 DMPs were suggestively significant (p-values $<1 \times 10^{-5}$) and annotated to genes involved in glucocorticoid-related pathways, inflammatory response, oxidative stress response, and oocyte maturation. No DMRs were identified.

Overall, we identified an association between residential greenness and DNAm levels at one CpG in the *SLC25A10* gene. Larger studies are needed to validate these findings and understand the biological pathways.

1. Introduction

Exposure to green space has been associated with health benefits over the entire life-course (Hu et al., 2021; Yuan et al., 2020; Zare Sakhvidi et al., 2023). Specifically, prenatal exposure to green space has been associated with lower risk of adverse birth outcomes such as low birthweight (Nieuwenhuijsen et al., 2019; Torres Toda et al., 2022). Mechanistically, potential pathways underlying these associations include capacity instoration (e.g., encouraging physical activity and increasing social cohesion), harm mitigation (e.g., improving air quality, reducing noise, and dissipating heat, reducing exposure to air pollution, noise, and heat), capacity restoration (e.g., reducing stress and renewing attention) and immunity improvement (e.g., by enriching the microbiome) (Bowyer et al., 2022; Cruells et al., 2024; Markevych et al., 2017).

Epigenetic changes may be one of the molecular mechanisms contributing to the association between prenatal green space exposure and lower risk of adverse birth outcomes, either directly or indirectly through the aforementioned pathways. The epigenome comprises all modifications to DNA, or to DNA-associated RNA and proteins, that permit interpretation of the genome to instruct cell identity and function (Hemberger et al., 2020). These modifications ultimately influence gene expression. Among all epigenetic marks, DNA methylation (DNAm), the addition of a methyl group to the C5 position of the cytosine within a cytosine-guanine (CpG) dinucleotide, is the most widely investigated in epidemiological settings due to its relative stability with storage and the multitude of technical platforms available for analysis (Maccani and Marsit, 2009).

Recently, two genome-wide studies have explored the association between prenatal exposure to green space and DNAm in cord blood. One study, involving 538 neonates from Belgium, identified one significant CpG and 147 DMRs in association with exposure to green space (Alfano et al., 2023). The second study, involving 2988 mother-infant pairs from seven European birth cohorts, found four DMRs associated with this exposure (Aguilar-Lacasaña et al., 2024). However, to better understand the intrauterine environment, it is crucial to investigate the placenta. This essential organ regulates nutrient and oxygen transfer and hormone and immune supply, and may act as a barrier to environmental exposures, playing a key role in foetal growth and development (Griffiths and Campbell, 2015; Mortillo and Marsit, 2022). To date, only a candidate gene study has explored the association between green space and placental DNAm, where the methylation status of the serotonin receptor *HTR2A* was positively associated with pregnancy green space exposure (Dockx et al., 2022). Thus, it is important to explore the association between maternal green space exposure during pregnancy and DNAm in placenta not only in specific genes but also on a genome-wide scale in relatively large samples sizes. Moreover, the aforementioned studies have mainly relied on residential surrounding greenness and/or proximity to a green space to assess green space exposure, overlooking other important aspects of this complex exposure such as use of green spaces or visual access to green space.

Our study aimed to fill these gaps by investigating the association between different aspects of maternal green space exposure during pregnancy and genome-wide placental DNAm. In addition to assessing residential greenness and proximity to green spaces, we also used data on the time spent in green spaces during pregnancy and the visual access to greenery through home windows.

2. Material and methods

2.1. Study population

The Barcelona Life Study Cohort (BiSC) is a prospective cohort study of 1080 pregnant women, their offspring and partners in Barcelona that aims to identify early environmental and genetic causes of normal and abnormal growth, development and health from foetal life until young adulthood (Dadvand et al., 2024). Briefly, the enrolment of the BiSC participants was carried out between October 2018 and April 2021 at three tertiary university hospitals in Barcelona, Spain. Participants were recruited and had their first data collected at the end of the first trimester of pregnancy, with follow-ups in the second and third trimesters of pregnancy, delivery, and months 1, 2, 6, 8, 12, 18, 24, and 48 postnatally. Pregnant women aged 18–45 with singleton pregnancies were included. Exclusions applied to those residing outside the catchment area, not being able to communicate effectively in Spanish, Catalan or English, or with fetuses having known congenital anomalies. The study was approved by the ethical committees of the centers involved in the study, and written informed consent was obtained from all the participants. The detailed description of the recruitment of the study participants, data collection, and follow-ups are reported elsewhere (Dadvand et al., 2024). The total number of mother-child pairs in BiSC was 1032. The subset with available data on indicators of green space and placental DNAm included in this study is shown in Fig. 1.

2.2. Indicators of exposure to green space

We characterized four aspects of exposure to green space: (i) residential surrounding greenness, (ii) residential distance to nearest major green space as a proxy for access to green spaces, (iii) use of green space, and (iv) visual access to green space through the home windows.

First, to characterize residential surrounding greenness, Normalized Difference Vegetation Index (NDVI) based on high-resolution (1×1 m) aerial image (2020) prepared by Cartographic and Geology Institute of Catalonia was used (Cartographic and Geologic Institute of Catalonia, 2021). NDVI was obtained as a ratio between the red (R) and near infrared (NIR) values in traditional fashion: $(NIR - R)/(NIR + R)$. Its values range between -1 and 1, with higher numbers indicating more greenness (Tucker, 1979). For each participant, residential surrounding greenness was assessed as the average NDVI in buffers of 100 m, 300 m and 500 m around the residential address(es) of the participant during pregnancy (Gascon et al., 2016). For those participants who moved home during pregnancy, we calculated an average of these values for all residential addresses weighted by the time spent in each address during pregnancy.

Second, residential distance to nearest major green space (>5,000 m^2) was evaluated using the 2018 land use dataset from the Cartographic and Geology Institute of Catalonia (ICGC), which is based on 1 m resolution imagery (Cartographic and Geologic Institute of Catalonia, 2018). The land use categories included urban, agricultural and natural green spaces.

Third, the use of green space referred to the amount of time (in hours per week) spent in green spaces during the free time in pregnancy. This indicator was self-reported and collected using questionnaires. Information about the first trimester was collected at week 12, while data on the second and third trimesters was collected at week 32 of pregnancy. The question was: "*In a typical week, during your current pregnancy, on average, how many hours of your free time do you spend in the following green spaces? (i) public parks, (ii) forest and other natural green spaces, and (iii) private garden (at home)*". To calculate the average number of hours per week that women spent in green spaces during pregnancy, we first summed the hours spent per week in various green spaces for each trimester, including public parks, forests, other natural green spaces, and private gardens at home. Then, we computed the average across the three trimesters. A minimum of data from one trimester was required.

Fourth, visual access to green space through the home windows was self-reported and collected using questionnaire at enrolment (week 12 of pregnancy). If they changed homes, mothers were asked to respond this question again at week 32. Given the difficulty of averaging two categorical variables, we examined week 12 (first trimester) and week 32 (third trimester) separately. The question was: "*How much greenery (trees, grass, flowers, etc) can you see through the living room window? 1 = No greenery/no window; 2 = A quarter; 3 = Half; 4 = Three quarters; 5 = all is green*". We dichotomized the answers in: 1 and 2 as visual access to greenery in less than half of the view from the living room, and 3, 4 and 5 as visual access to greenery in equal or more than half of the view from the living room window.

2.3. Placental biopsy and DNA extraction

Out of the 1032 mother-child pairs that were followed until birth, 611 placentas were collected based on maternal consent and the feasibility of collection at the hospital. Biopsies were obtained by trained gynaecologists following a harmonized protocol across hospitals. Briefly, placenta biopsies of around 2.5 cm (from the maternal to the foetal side) and 1 cm width were obtained from two opposite quadrants at a distance of around 3–4 cm from site of cord insertion. Then, these biopsies were cut in two, giving a total of 4 biopsies of 2.5 × 0.5 cm. Two of them (one from each quadrant) were directly frozen in liquid nitrogen and transferred to −80 °C. The other two biopsies were treated with RNAlater and sent to the laboratory where they were divided in four pieces of 0.5 × 0.5 cm, corresponding roughly to the foetal membranes, the upper foetal villi, the lower foetal villi and the maternal decidua. Finally, all the biopsies were stored at −80 °C for future use.

Before DNA extraction, placenta samples were completely randomized and the distribution of main design and biological variables was checked across batches. For genomic DNA extraction, a fragment of approximately 5–6 mm^3 (30–40 mg) was dissected from the foetal villi biopsy below the foetal membranes collected in RNAlater. All the dissection process was done in liquid nitrogen to avoid the tissue thawing. Then, the tissue was disrupted/homogenized using a bead mill (bead beater) at 4 °C for 26 s. Genomic DNA was then isolated using the AllPrep®DNA/RNA/miRNA Universal Kit, (Qiagen, CA, USA). DNA was eluted in 80 μl and stored in different aliquots at −80 °C. DNA quality was evaluated on a NanoDrop spectrophotometer (Thermo Scientific, Waltham, MA, USA) and additionally 500 ng of DNA was run on 1% agarose gels to confirm that samples did not present visual signs of degradation.

2.4. Methylation data acquisition, quality control and normalization

DNAm was assessed in 624 placental DNA samples (including 589 unique individuals and 35 technical duplicates) with the commercial Infinium MethylationEPIC BeadChip from Illumina, following manufacturer's protocol in the Human Genome facility (HUGE-F) at the

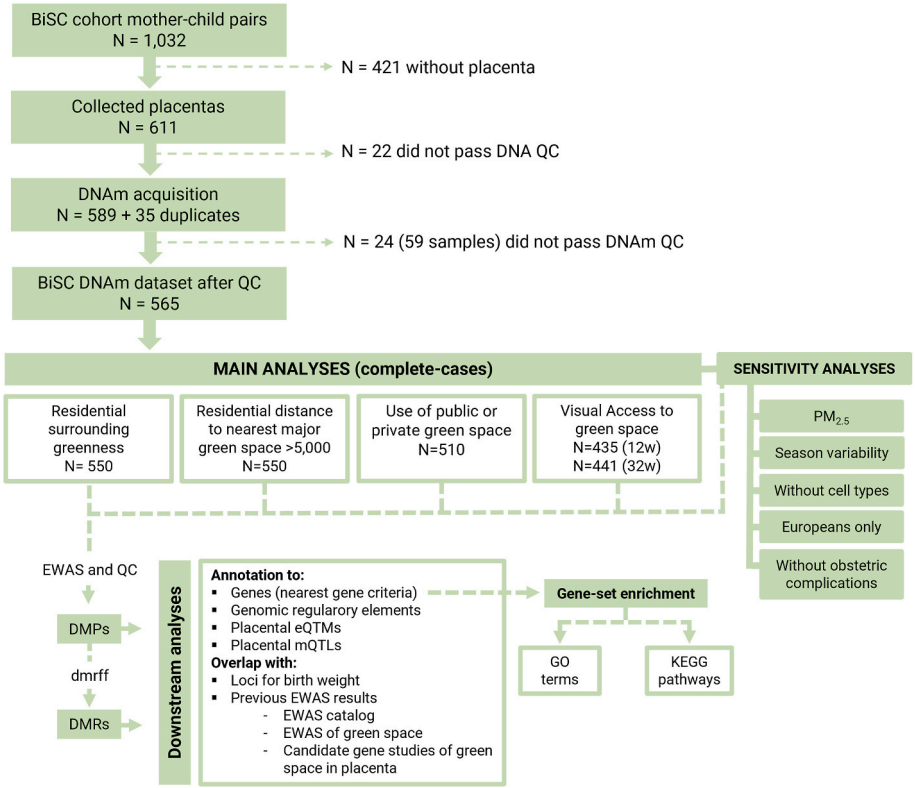

Fig. 1. Analysis scheme. 12w: week 12 of pregnancy 32w; week 32 of pregnancy; CpG: cytosine-guanine dinucleotide; DMPs: differentially methylated positions; DMRs: differentially methylated regions; DNAm: DNA methylation; eQTM: Expression quantitative trait methylation; EWAS: Epigenome-wide association study; GO: Gene Ontology terms; KEGG: Kyoto Encyclopedia of Genes and Genomes; mQTLs: methylation quantitative trait loci; QC: quality control; $PM_{2.5}$: particulate matter with an aerodynamic diameter <2.5 μg/m^3; PMD: partially methylated domains.

Erasmus Medical Centre core facility. The array contains 850,000 CpG probes, that primarily target gene promoter regions. Additionally, a small number of these probes target FANTOM enhancers and regulatory elements (Campagna et al., 2021).

Briefly, 750 ng of DNA were bisulfite-converted using the EZ 96-DNAm kit following the manufacturer's standard protocol, and DNAm measured using the Infinium protocol.

The methylation data was pre-processed using the PACEAnalysis R package (v.0.1.9) (https://www.epicenteredresearch.com/). The pre-processing pipeline consists of probe quality control, sample quality control, normalization, batch correction, winsorization of outlier values and estimation of cell type proportions. Detection p-values were estimated using out-of-band array hybridization as implemented in the SeSAMe R package (Zhou et al., 2018). Probe values were masked if the intensity values were zero, estimated based on less than three beads, and/or if they had a detection p-value>0.05. Based on these criteria (probe call rate <95%), we flagged probes that failed among at least 5% of samples for removal prior to downstream analysis (N = 63,158). Then, fifty-nine samples corresponding to 24 individuals were discarded according to: low methylated and unmethylated signal intensities overall (N = 5), sample call rate <95% (N = 3), sex inconsistencies (N = 9), samples with indication of substantial contamination with maternal DNA or with DNA from another participant in the study (N = 11) (Heiss and Just, 2018), duplicates identified by clustering samples based on their genetic similarity using the single nucleotide polymorphisms (SNPs) included in the array (N = 29) and siblings (N = 2). Exclusion of one of the duplicate pairs was done randomly. A total of 565 samples remained after the sample quality control.

After removal of problematic samples, we performed pre-processing of the remaining arrays. Signal intensities were pre-processed by performing linear dye bias correction followed by single-sample background correction based on Normal-exponential convolution using out-of-band Infinium I probes (ssNoob) (Fortin et al., 2017; Triche et al., 2013). Unwanted between-array variation was minimized by applying functional normalization using the control probes (Fortin et al., 2014). Beta-mixture quantile (BMIQ) normalization was then used to correct for the bias of Type-2 probe values (Teschendorff et al., 2013). After that, we explored the clustering of the data through Principal Component Analysis and tested the association of the 12 first principal components (PCs) with technical variables (plate, array, extraction batch, time to placental storage, DNA concentration and 260/280 and 260/230 ratios), design variables (hospital of birth and SARS-CoV-2 confinement) and biological variables (child's sex and ethnic origin). PC1 explained 30.36% of variability, PC2 explained 7.40%, and the rest accounted for less than 3%. The array variable was associated with all the PCs from 1 to 10, and thus, we decided to apply the ComBat method implemented in the sva Bioconductor R package to eliminate its effect (Johnson et al., 2007). Additionally, sex, ethnicity, gestational age, SARS-CoV-2 confinement and hospital of birth were associated with some of the first PCs and were included as covariates in the statistical models (see section 2.6.1).

Following these pre-processing steps, we removed the probes flagged as problematic among the study population. Finally, to correct for the possible outliers, we winsorized the extreme values to the 1% percentile (0.5% in each side), where percentiles were estimated with the empirical beta-distribution. DNAm values are expressed as beta values, where 0 means un-methylation and 1 complete methylation.

Cell type proportions of six main placenta populations (trophoblasts, syncytiotrophoblast, nucleated red blood cell, Hofbauer cells, endothelial cells, and stromal cells) were estimated from DNAm using the reference panel from term placentas implemented in the planet R package (Yuan et al., 2021). Finally, a DNA contamination score was calculated using SNP probes included in the EPIC array. The score is defined as the average log odds from the SNP posterior probabilities from the outlier component; capturing how irregular the SNP beta-values deviate from the ideal tri-modal distribution (Heiss and Just, 2018).

2.5. Covariates

The following variables were used as descriptive variables, confounders or in the sensitivity analyses (see section 2.6): Data on maternal age at recruitment (years), maternal education (no university/university), child's ethnicity (European/Latino-American/other), maternal smoking during pregnancy (no/yes), and parity (nulliparous/multiparous) were self-reported at enrolment (week 12 of pregnancy). Child's biological sex was retrieved from clinical records. Gestational age at birth was calculated from the date of the last menstrual period using estimates based on the first ultrasound examination (about 12th week of gestation). Preterm was defined as a gestational age at birth <37 completed weeks. Foetal growth restriction was defined as a birth weight percentile <3 or a birth weight percentile <10 and cerebroplacental ratio pulsatility index percentile at 32w < 5 or a birth weight percentile <10 and uterine artery pulsatility index percentile at 32w > 95. Birth weight percentiles were estimated according to the BCNatal curves (Figueras et al., 2008; Figueras and Gratacós, 2014). Pulsatility indexes were measured through Doppler and percentiles calculated using reference curves described elsewhere (Baschat and Gembruch, 2003; Gómez et al., 2008). Information on obstetric complications (pre-eclampsia/eclampsia, gestational hypertension, placental abruption, placenta previa, oligohydramnios, polyhydramnios and chorioamnionitis) was obtained from medical records. Placental DNA contamination and cellular composition were obtained as described in section 2.4.

Season of conception (autumn/spring/summer/winter) was calculated by subtracting the gestational days of pregnancy (last menstrual period) from the date of birth. A variable describing if pregnancy took place during the COVID-19 confinement was created based on the dates of conception, delivery, and confinement in Spain [pre-confinement (entire pregnancy before confinement), confinement (part of the pregnancy during confinement), and post-confinement (entire pregnancy after confinement)].

As a proxy of neighbourhood socioeconomic status (SES) we used the annual average household income (euros) at census tract level obtained from the 2020 Standard of Living and Living Conditions survey conducted by the Spanish National Institute of Statistics (INE), which is based on fiscal data including wages, pensions, unemployment benefits, other benefits, and other income (Spanish National Institute of Statistics, 2023).

We calculated maternal exposure to particulate matter with an aerodynamic diameter <2.5 μm ($PM_{2.5}$) using land use regression (LUR) models and air pollution environmental data collected in the BiSC campaigns, following the guidelines established by the European Study of Cohorts for Air Pollution Effects (ESCAPE) (Dadvand et al., 2024). We obtained 2 $PM_{2.5}$ exposure measures for the pregnancy period, one referring to the home address(es) and the other referring to the average of three environments (home, workplace, and commuting route).

2.6. Statistical analyses

2.6.1. Differentially methylated positions (DMPs)

Robust linear regression models were fitted to evaluate the association between each maternal exposure to green space during pregnancy (predictor) and genome-wide placental DNAm (outcome) using the PACEanalysis R package (https://github.com/epicenteredresearch/PACEanalysis).

Main models were adjusted for *a priori* selected confounders based on previous literature: maternal age at recruitment (Roustaei et al., 2020), maternal education, annual average household income child's ethnicity and maternal smoking during pregnancy (Everson et al., 2021; Martin et al., 2020). Hospital of birth and COVID-19 confinement were also added into the main model for being potential confounders. The

catchment areas of the hospitals differed in the background socio-demographic structure and although we applied a protocol to harmonize the collection of placenta samples across recruiting hospitals, minor differences might have existed among the hospitals with regards to sample collection. Additionally, the COVID-19 confinement had an impact on the daily life of the participants as well as on the sample collection in the hospitals. Finally, main models were also adjusted for all the following precision variables: child's sex, gestational age, parity, DNA methylation score, and placental cellular composition (Houseman et al., 2015; van Rooij et al., 2019).

We performed the quality control of the EWAS results for each model using the EASIER R package (*ISGlobal-Brge/EASIER: Tools for Methylation Data Analysis*, 2022) (Table S2). This included examining inflation and the distribution of effect estimates, standard errors and p-values. We excluded control probes, non-CpG probes, probes that mapped to X/Y chromosomes, probes with poor base pairing quality (lower than 40 on 0–60 scale), probes with non-unique 30bp 3′-subsequence (with cross-hybridizing problems), Infinium II probes with SNPs of global minor allele frequency (MAF) over 1% affecting the extension base, probes with a SNP in the extension base that causes a color channel switch from the official annotation and probes with any SNP of global MAF over 1% and within 5 bp from the extension base (Zhou et al., 2017) and probes that have shown to be unreliable in a recent comparison of the Illumina 450K and EPIC BeadChips (Fernandez-Jimenez et al., 2019). The percentage of probes removed was 13.98% (Appendix A: Table S2).

Continuous green space indicators (residential surrounding greenness, residential distance to nearest major green space and use of green space) were standardized by dividing them by its interquartile range (IQR). Effect sizes are reported as the difference in DNA m unit by IQR of continuous exposures, or by exposure group of the categorical variables. Multiple testing was accounted for by applying Bonferroni correction (BN) for 694,380 tests (p-value $<7.2 \times 10^{-8}$). Significance was considered suggestive for p-values $<1 \times 10^{-5}$. After multiple testing correction, visual inspection of the results was performed through scatter plots of the exposure versus the DNAm levels at the BN DMPs. If the pattern of the DNAm appeared bi-modal or tri-modal, which was indicative of the presence of a SNP, then, the BN DMP was excluded.

2.6.2. Differentially methylated regions (DMRs)

DMRs were identified using the *dmrff* R package (Suderman et al., 2018). This method identifies candidate DMRs by screening the EWAS results for genomic regions each covered by a sequence of CpG sites with effects in the same direction, p-values <0.05, and <500 bp gaps between consecutive CpG sites. We considered statistically significant DMRs those detected with a BN p-value <0.05 and with a minimum of three consecutive CpGs within the DMR. After multiple testing correction, visual inspection was performed again to check the robustness of the DMR. If the pattern of the DNAm appeared bi-modal or tri-modal, then, the DMR was excluded.

2.6.3. Sensitivity analyses

Five sensitivity analyses were conducted by repeating the DMP and DMR analyses to test the robustness of the results and additional hypotheses. First, given that in some settings air pollution could be a confounder of the health effects of green space exposure (Markevych et al., 2017), we ran an additional model adjusted for the average exposure to $PM_{2.5}$ as a proxy of air pollution during pregnancy. Models of residential exposure to green space were adjusted for residential exposure to $PM_{2.5}$, while models of the use of green space were adjusted for the average of the $PM_{2.5}$ exposure in three environments (home, workplace, and commuting route). Second, to investigate seasonal variability, we adjusted the models using the sine and cosine transformations based on the date of the last menstrual period. These transformations represent time as a continuous circular variable, ensuring smooth modeling of seasonal trends, with one full oscillation per year. Multiplying by 2π correctly maps the annual cycle onto a periodic function, capturing regular seasonal fluctuations (Bhaskaran et al., 2013). Third, differences in placenta cellular composition might arise due to technical noise, but they also can change in response to the exposure (Houseman et al., 2015; van Rooij et al., 2019). Because of this, we ran an additional model unadjusted for cellular composition. Fourth, we repeated the main EWAS in participants of European ethnicity only, which was the largest ethnic subgroup. Finally, given the impact of pregnancy complications on placental DNAm patterns (Cruz et al., 2020; Lee et al., 2021), we decided to run an additional model excluding samples of babies born preterm or with growth restriction or with obstetric complications (see section 2.5).

To assess differences between the main and sensitivity models, we tested the Pearson's correlation of the effect sizes between these models, both for genome-wide results and for the subset of BN DMPs.

2.7. Downstream analyses

First, we tested the overlap of the BN and suggestive (p-value $<1 \times 10^{-5}$) DMPs and DMRs with placental chromatin states (Ernst and Kellis, 2017), placenta germline differently methylated regions (gDMRs) (Hamada et al., 2016), and placenta partially methylated domains (PMDs) (Schroeder et al., 2013). Second, to assess whether the methylation levels of these DMPs and DMRs were associated with the expression levels of nearby genes, we examined previously identified Expression Quantitative Trait Methylation (eQTMs) in the placenta (Delahaye et al., 2018; Deyssenroth et al., 2020). Third, we checked whether the BN and suggestive DMPs and CpGs within the significant DMRs had previously been associated with exposures or health traits using the information from the EWAS Catalog (Battram et al., 2022b). Fourth, we compared the list of BN and suggestive DMPs and DMRs with previously reported studies investigating the association between exposure to green space during pregnancy and DNAm in cord blood (Aguilar-Lacasaña et al., 2024; Alfano et al., 2023) and a candidate gene study in placenta (Dockx et al., 2022).

As DNAm is partially regulated by genetic variants, we explored whether the DMPs and CpGs in the DMRs found in our study had any methylation Quantitative Trait Loci (mQTLs). Foetal *cis* mQTLs (± 0.5 Mb, 1 Mb window) were identified in the BiSC study ($n = 408$) by applying a linear regression for each CpG-SNP pair adjusting for child's sex, child's gestational age at birth, child's ancestry based on 5 genetic PCs, 6 placental cell type proportions estimated from DNA methylation data using the TensorQTL tool (Ongen et al., 2016). Genome-wide significance was established at a p-value $< 5 \times 10^{-8}$. Placental DNAm was measured with the EPIC array as described in section 2.3, and foetal genome-wide genotyping (placenta or cord blood) was conducted using the Infinium Global Screening (GSA) array v.3.0 array and imputed with the Haplotype Reference Consortium (HRC) panel.

Additionally, we assessed the overlap with genomic regions of previously identified SNPs for birth weight in the largest genome-wide association study (GWAS) to date (Juliusdottir et al., 2021). The overlap was investigated using the GenomicRanges R package (Lawrence et al., 2013) and by defining 1 Mb windows (± 0.5 Mb) surrounding each of the autosomal SNPs identified in the GWAS.

Then, the DMPs and DMRs were annotated to genes using the Illumina's annotation file, which annotates CpGs located within a distance of 1500 bp from the transcription start site (TSS). If the CpG did not have a gene annotated within this distance, then the University of California, Santa Cruz (UCSC) Genome Browser was referenced to identify the nearest gene using the matchGenes() function from the bumphunter R package (Jaffe et al., 2012). We conducted functional enrichment analyses for Gene Ontology (GO) terms and pathways of the Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa, 2000) of genes annotated to the suggestive DMPs (p-value $<1 \times 10^{-5}$) and CpGs within the BN DMRs, using the missMethyl method (Phipson et al., 2016) as implemented in EASIER R package, considering pathways with at least two genes. This method controls for probe bias by adjusting for the

number of CpG sites associated with each gene, which is used to estimate the prior probability of gene selection in the enrichment analysis.

3. Results

3.1. Study population

A total of 550 participants were included in the study. A detailed description of our study population characteristics is presented in Table 1 and Appendix A: Table S1**.** The median age of the mothers was 34.4 years old, around two thirds had university education and 7.0% smoked during pregnancy. The median gestational age at birth was 40.0 weeks, half of the offspring were girls, 67.5% of them were Europeans, 28.9% Latino-American, and the rest from other ethnicities. Median residential NDVI value was 0.2, the median residential distance to a green space was 129.9 m and a median of 3 h per week were spent in public and/or private green spaces. Furthermore, around one-third of the mothers had visual access to greenery in more than half of the living room window during the first and third trimesters. The correlation coefficients across the different indicators of residential surrounding greenness (NDVI 100 m, 300 m and 500 m) were high, especially between the 300 m and 500 m buffers. For visual access, the correlation between the first and third trimesters was also very high. We found inverse moderate correlations across residential surrounding greenness and residential distance to a major green space. The correlation coefficients across all green space exposures are shown in Appendix B: Fig. S1.

Table 1
Characteristics of the BiSC study population (N = 550).

	Median (IQR)/n (%)
Pregnancy green space indicators	
Residential greenness	
NDVI 100 m buffer	0.21 (0.18–0.24)
NDVI 300 m buffer	0.22 (0.19–0.24)
NDVI 500 m buffer	0.22 (0.20–0.25)
Residential distance to nearest major green space >5000 m^2 (meters)	129.90 (63.22–227.94)
Use of public and private green space (hours/week) (n missing = 47)	3.17 (1.00–6.33)
Visual acces to greenery through living room window	
First trimester (12w) (n missing = 115)	
Less than half	288 (66.21)
Half or more	147 (33.79)
Third trimester (32w) (n missing = 109)	
Less than half	285 (64.63)
Half or more	156 (35.37)
Maternal characteristics	
Maternal age (years)	34.38 (31.25–37.74)
Maternal education	
No university	183 (33.27)
University	367 (66.73)
Maternal smoking during pregnancy	
No	511 (92.91)
Yes	39 (7.09)
Child characteristics	
Child sex	
Girls	273 (49.64)
Boys	277 (50.36)
Child ethnicity	
European	371 (67.45)
Latino-American	159 (28.91)
Other	20 (3.64)

12w: week 12 of pregnancy 32w; week 32 of pregnancy; NDVI: satellite-based Normalized Difference Vegetation Index; $PM_{2.5}$: Particulate matter with aerodynamic diameter <2.5 μm.

3.2. Green space during pregnancy and placental DNAm

3.2.1. DMPs

The lambda inflation factors for the main models ranged from 0.84 to 1.26 (Appendix A: Table S2; Appendix B: Fig. S2). After BN correction, one DMP (cg14852540) annotated to the *Solute carrier family 25 member 10* (*SLC25A10)* gene was significantly associated with residential surrounding greenness in 500 m buffer. The visual inspection of the DNAm levels of this DMP did not show any bi-modal or tri-modal pattern, indicative of the presence of underlying SNP (Appendix B: Fig. S3). At this DMP, for an IQR increase of NDVI in 500 m, DNAm was 0.3% lower. The effect at this site remained consistent in both the 100 m and 300 m buffers, although not statistically significant (Appendix A: Table S3). No DMPs were significantly associated with residential distance, use or visual access to green space. At suggestive significance (p-value $<1 \times 10^{-5}$), 101 unique DMPs were associated with at least one of the green space indicators (Fig. 2A; Appendix A: Table S3). Pearson correlation coefficients of the effect estimates of all genome-wide CpGs and the BN DMP across the EWAS results from the green space indicators are shown in Appendix A: Table S4 and Appendix B: Fig. S4.

In terms of sensitivity analyses, the most notable difference in effect size among the BN DMP was observed when we restricted the analyses to Europeans (n = 371), leading to an 83.6% decrease in the effect estimate (Fig. 2B). Effect sizes did not change substantially in the rest of sensitivity analyses. The percentage differences in absolute values in the effects between the main and the sensitivity results were as follows: a decrease of 1.6% for $PM_{2.5}$ adjustment, a decrease of 0.5% for seasonality adjustment, an increase of 0.6% for cell type proportion unadjusted model and an increase of 5.5% when excluding obstetric complications (Fig. 2B; Appendix A: Table S5).

3.2.2. DMRs

Ten unique DMRs, annotated to 10 genes, were associated with pregnancy exposure to different green space indicators after BN correction (Appendix A: Table S6). However, all were excluded after visual inspection, as each of the CpGs within the DMR showed a bimodal distribution of the DNAm values indicative of the presence of a SNP in the probe or around the CpG (Appendix B: Fig. S5). For some DMRs, the SNP was identified using the illumina annotation (Appendix A: Table S6).

3.3. Downstream analyses

We searched whether the BN and suggestive DMPs (p-value $<1 \times 10^{-5}$) (102 in total) were located in specific genomic regulatory elements of the placenta. Thirty-eight percent (39/102) were located in active regions: active TSS (TssA), flanking active TSS (TssAFlink), strong transcribed states (Tx), transcribed states at the 5′ and 3′ end of the genes (TxFlnk) and enhancers (Enh and EnhG). Moreover, 34 suggestive DMPs overlapped with 32 unique placental PMDs, regions known to contain relevant genes for placental function (Schroeder et al., 2013). Additionally, two suggestive DMPs (cg04965297; cg10753638) overlapped with placental gDMRs (chr19:13135070–13136459; chr21:41883646–41884725) (Appendix A: Table S3).

Regarding gene expression, none of the DMPs overlapped with the eQTMs identified in the placenta (Appendix A: Table S3). According to genetic variation, the BN DMP (cg14852540) and 13 suggestive DMPs were close to foetal SNPs associated with birth weight (Juliusdottir et al., 2021). Finally, 19 suggestive DMPs have a placental cis-mQTL (Appendix A: Table S3).

We, then, took the BN and suggestive DMPs and conducted gene-set enrichment analyses. Although not significant after multiple testing correction, top pathways included glucocorticoid synthesis and secretion (cortisol), inflammatory response regulation of response to reactive oxygen species, and progesterone-mediated oocyte maturation (nominal p-value <0.1) (Appendix A: Tables S7–S8).

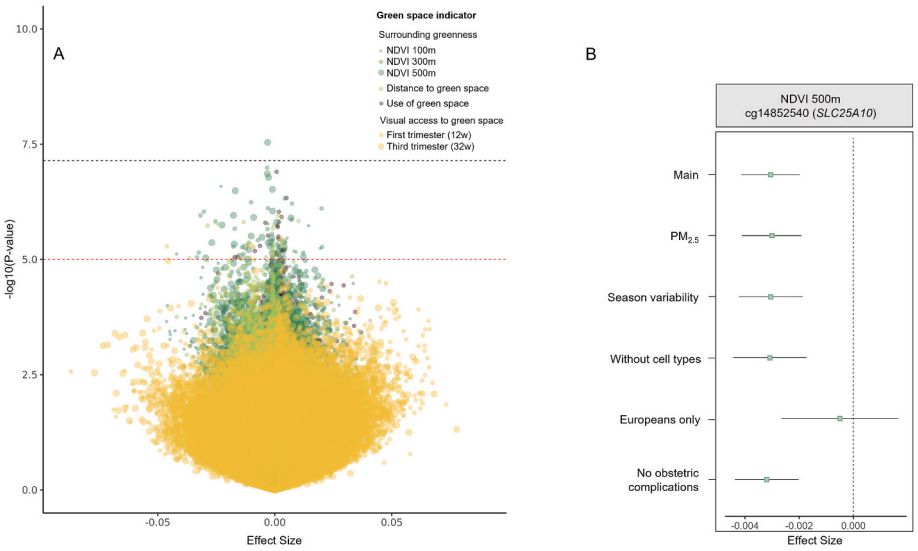

Fig. 2. (A) Volcano plot showing the effect sizes on the x-axis and the ($-\log_{10}$) p-values on the y-axis for the association between all pregnancy green space indicators and placental DNAm. Each dot represents the association of one CpG with one exposure variable. The color of the dot indicates the exposure: Surrounding greenness (dark green), distance to green space (light green), use of green space (purple) and visual access to green space (yellow). Dot sizes correspond to the sizes of the NDVI buffers and time window of visual access (week 12 and week 32 of pregnancy). The black line represents the BN p-value threshold (7.2×10^{-8}) and the red line represents the suggestive p-value (1×10^{-5}). (B) Forest plot showing the effect size and 95% confidence interval of the BN significant DMP in the main model compared with the sensitivity analyses: 1) adjusted for $PM_{2.5}$ during pregnancy; 2) adjusted for seasonality; 3) without adjusting for cell type proportions; 4) restricted to European ethnicity, and 5) without obstetric complications (preterm births, cases of FGR and pregnancy complications). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Neither the BN nor the suggestive DMPs found in this study overlapped with previous findings of green space exposure and DNAm in cord blood (Aguilar-Lacasaña et al., 2024; Alfano et al., 2023) or in placenta (Dockx et al., 2022) (Appendix A: Table S9).

According to the EWAS Catalog, methylation levels at the BN and suggestive DMPs have previously been related to child age, gestational age, autoimmune diseases such as rheumatoid arthritis and respiratory conditions such as chronic obstructive pulmonary disease (COPD). None of these studies were conducted in placenta and most of them were evaluated in blood (see Appendix A: Table S3 for detailed information).

4. Discussion

This study evaluated association of different aspects of maternal exposure to green space during pregnancy and placental DNAm in the BiSC cohort. We found that residential greenness within 500 m was associated with one DMP (cg14852540) annotated to the gene body of the *SLC25A10*. *SLC25A10*, a dicarboxylate carrier, plays a role in metabolic processes by transporting small molecules into or out of the mitochondria thereby providing substrates for metabolic processes including the Krebs cycle and fatty acid synthesis (Freund et al., 2014; Huypens et al., 2011; Mizuarai et al., 2005). Buccal mitochondria DNA content (mtDNAc), a proxy of mitochondrial function (Castellani et al., 2020), has been positively associated with green space exposure (Hautekiet et al., 2022). Furthermore, mitochondrial respiration has been shown to play a role in the invasive and migratory capabilities of trophoblasts, which are necessary for embryo implantation and placental development (Xiong et al., 2024; Yu et al., 2024). Additionally, recent studies found that DNAm at cg09996840, located in the same gene in blood, was associated with body mass index and waist circumference in adolescents (Huang et al., 2022). However, this CpG was not correlated with ours. Finally, the BN DMP identified in our study was located at <0.5 Mb of a loci containing foetal SNPs associated with birth weight (Juliusdottir et al., 2021), which may suggest the involvement of this genomic region in foetal growth. Despite this, none of these variants were an mQTL for this CpG in our data.

Among the top gene-sets and GO and KEGG terms identified with the suggestive DMPs, although not surviving multiple-testing, there were glucocorticoid-related pathways, inflammatory response, regulation of response to reactive oxygen species, and progesterone-mediated oocyte maturation. The genes in the glucocorticoid-related pathways whose DNAm levels were suggestively associated with green spaces in our study were *Adenylate Cyclase 4 (ADCY4)* (cg10588355 with NDVI 300 m and NDVI 500 m nominal p-values of 75×10^{-7} and 9.85×10^{-6}, respectively), *Aryl Hydrocarbon Receptor Interacting Protein (AIP)* (cg21052656 with NDVI 300 m and NDVI 500 m, nominal p-values of 2.8×10^{-6} and $8.91 \times 10^{-6,}$ respectively), and *Phosphodiesterase 8A (PDE8A)* (cg05733361 with NDVI 500 m, nominal p-value of 3.4×10^{-6}) genes. Glucocorticoids have a central role in foetal maturation, but excessive levels can induce foetal growth restriction (Fowden et al., 2016). According to the literature, stress reduction could be one of the mechanisms linking green exposure to improved health outcomes through epigenetics (Nwanaji-Enwerem et al., 2024). Therefore, larger studies with increased statistical power are needed to explore this and other suggestive pathways concerning green space and its potential implications for placental and foetal development.

Taking into account the BN and suggestive DMPs, almost half of the associations between green space exposure and placental DNAm were observed with NDVI in buffers of 300 m and 500 m during pregnancy, while around 9% were found for NDVI within a 100 m buffer. It has been hypothesized that NDVI within a 100 m buffer may better capture immediate environmental features around homes, such as trees, which may particularly influence health by reducing harm (e.g., reducing exposure to air pollution, heat, and noise), as well as green views, which can provide restorative benefits. Alternatively, larger buffers might better reflect the potential influence of green space on recreational physical activity (Markevych et al., 2017). Considering these points, it can be hypothesized that the observed effects on placental DNAm are more closely linked to broader health-promoting mechanisms of green space, such as physical activity and social interactions, which are better captured by larger buffers.

In addition to DMPs, our study also has investigated DMRs. Out of the 10 unique DMRs identified in the study, none of them passed the visual inspection criteria of the DNAm profile. All CpGs within the DMRs

presented bi-model or tri-model distributions suggestive of the presence of underlying SNPs within the probe or in the nearby region. Moreover, the associations of individual CpGs within the DMRs were marginally significant.

To date, two recent studies evaluated the association between residential greenness exposure during the prenatal period, a critical period for perinatal and life-long health, with DNAm in cord blood (Aguilar-Lacasaña et al., 2024; Alfano et al., 2023). None of the BN or suggestive DMPs identified in this study overlapped with the findings from the aforementioned studies. This could be explained by the differences between tissues (Ohgane et al., 2008), but also differences in the exposure and outcome assessment.

Alfano et al., used land cover data to classify green space based on vegetation height, rather than NDVI, to calculate green space exposure. Additionally, the buffer of 100 m was the only one common to both studies. In our analysis, we did not observe any significant DMP or DMR within this buffer. Moreover, the lack of overlap with our previous study (Aguilar-Lacasaña et al., 2024) may be due to differences in the study areas. While the previous study covered various regions of Europe, this study was conducted only in Barcelona. Additionally, differences in the resolution of the aerial images used, with the current study having a much higher resolution (1 × 1 m) compared to the previous study (30 × 30 m), may also contribute to the lack of overlap.

These variations, along with contextual differences in greenness across countries such as the amount of greenness, vegetation types, species, biodiversity, and other quality aspects (Wu et al., 2023), as well as sociodemographic, cultural, behavioural, and genetic factors, could further explain the observed discrepancies. Given these factors, generalization of our findings to other regions should be done with caution, and replication in other settings is necessary.

In placenta, only a candidate gene study (N = 327) has explored the association between green space and DNAm (Dockx et al., 2022). In that study, methylation levels at the promoter of *5-Hydroxytryptamine Receptor 2A (HTR2A)* gene, a serotonin receptor with a potential effect on neurodevelopment function, was positively associated with maternal green space exposure within 1000 m, 2000 m and 3000 m buffers measured using land cover data and stratified into low (<3 m) and high (≥3 m) vegetation. We were unable to assess the same CpGs in our study as they were not included in the EPIC array. Therefore, we examined the direction of the effect of nearby CpGs located in the promoter region (cg27068143 and cg10323433) of *HTR2A*, and although not statistically significant (nominal p-value >0.05), they showed a consistent direction of the effect in our study.

One of the strengths of this study was the analysis of a relatively large number of placental samples, a relevant tissue for foetal development. Second, to characterize residential surrounding greenness, NDVI was derived from high-resolution (1 × 1 m) aerial images and averaged over the entire pregnancy, considering the changes in participants' residences. Moreover, we expanded our analyses beyond surrounding greenness by including other green space indicators such as distance to nearest major green space, use of green spaces and also visual access to greenery through the home window. Third, relying on wealth of available data in BiSC, we were able to conduct sensitivity analyses by further adjusting our analyses for air pollution, seasonality, cellular composition or excluding pregnancy and foetal complications, and we observed that our findings were generally robust after conducting these analyses.

It is important to interpret the results of this study considering its limitations. First, despite being the largest study on placenta evaluating the association of prenatal exposure to green space and DNAm, the sample size is still limited, which may result in low statistical power to detect some associations. For instance, we did not detect any robust association with DMRs and only one DMP was significant after multiple-testing correction. Moreover, the effect size of the significant DMP was reduced substantially in the subset of Europeans, suggesting some residual confounding by ethnic origin not well controlled in the full population. We note that models were adjusted for ethnic origin. Furthermore, we did not examine cell-interacting, sex-specific or sex chromosome associations due to statistical power limitations (Inkster et al., 2023). Future studies with larger samples sizes (e.g. multi-cohort meta-analysis) are needed to explore these questions. Second, our assessment of residential surrounding greenness was based on aerial images taken at a single point of time. We therefore were not able to account for seasonality in our assessed exposure levels, which in turn might have influenced our analyses. However, our previous studies (Dadvand et al., 2012) have shown that the spatial contrast in NDVI remains relatively stable in our study region with a Spearman's correlation coefficient >0.9 between assessed residential surrounding greenness levels in cold and warm seasons. Third, we did not have data in other important aspects of the green space exposure, such as type of vegetation and quality characteristics of the green space. Fourth, the variable measuring the use of green space was obtained from questionnaire, which could have resulted in exposure misclassification. Validating this measure by employing other techniques such as mobile phone Global Positioning System (GPS) data would improve its reliability (Heikinheimo et al., 2020). Fifth, whilst we controlled our analyses for a wide array of covariates, we cannot assume that associations we found are causal. In the same way, we cannot assume that differences in DNAm will affect health outcomes, as epigenetic mechanisms are more complex than what can be discerned from DNAm (Min et al., 2021). Finally, in addition to being unable to explore associations with other epigenetic marks and gene expression, this study, in common with other EWAS studies, covers only a small proportion of the epigenome (less than 5% of the 23 million CpGs for the EPIC array) (Battram et al., 2022a).

5. Conclusion

Overall, we identified an association between residential surrounding greenness and placental DNAm levels at cg14852540, which is annotated to the *SLC25A10* gene and involved in mitochondrial respiration. Additionally, suggestive DMPs were annotated to genes involved in glucocorticoid-related pathways, inflammatory response, regulation of response to reactive oxygen species, and progesterone-mediated oocyte maturation. This points to a potential role of placental epigenetic mechanisms in the effects of green space exposure during pregnancy on birth outcomes and offspring health. However, further research with larger sample sizes is needed to validate these results and further understand the underlying biological pathways.

CRediT authorship contribution statement

Sofía Aguilar-Lacasaña: Writing – review & editing, Writing – original draft, Visualization, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Marta Cosin-Tomas:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization. **Bruno Raimbault:** Writing – review & editing, Methodology, Data curation. **Laura Gómez-Herrera:** Writing – review & editing, Resources, Data curation. **Olga Sánchez:** Writing – review & editing, Resources. **Maria Julia Zanini:** Writing – review & editing, Resources. **Rosalia Pascal Capdevila:** Writing – review & editing, Resources. **Maria Foraster:** Writing – review & editing, Resources. **Mireia Gascon:** Writing – review & editing, Resources. **Ioar Rivas:** Writing – review & editing, Resources. **Elisa Llurba:** Writing – review & editing, Resources. **Maria Dolores Gómez-Roig:** Writing – review & editing, Resources. **Jordi Sunyer:** Writing – review & editing, Supervision, Investigation, Funding acquisition, Conceptualization. **Mariona Bustamante:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization. **Martine Vrijheid:** Writing – review & editing, Supervision, Investigation, Funding acquisition, Conceptualization. **Payam Dadvand:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization.

Ethics approval and consent to participate

From pregnancy up to 18 months' visits were approved by the Clinical Research Ethics Committee of the Parc de Salut Mar project (2018/8050/I), Medical Research Committee of the Fundació de Gestió Sanitària del Hospital de la Santa Creu i Sant Pau de Barcelona (EC/18/206/5272), and Ethics Committee of the Fundació Sant Joan de Déu (PIC-27-18). Before joining the cohort during their regular first trimester hospital visit, participants were informed by a BiSC midwife or nurse about the study's details, duration, and their option to withdraw without penalty. If they agreed to take part, they signed consent forms permitting the collection of biological samples and genetic studies, receiving a copy for themselves. Parents or legal guardians of children also provided informed consent before any study procedures commenced. Specific consent forms for genetic studies were also provided to mothers and parents or legal guardians of children.

Consent for publication

Not applicable.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2025.121286.

Data availability

The authors do not have permission to share data. Individual cohort level data may be available by application to the relevant institution after obtaining required approvals. Genome-wide DNA methylation summarized results can be found at Zenodo repository (https://doi.org/10.5281/zenodo.14975293). The code for the analyses is available in this GitHub repository link: https://github.com/sofiaguilarl/EWAS_GreenSpace_Placenta.git.

References

Aguilar-Lacasaña, S., Fontes Marques, I., de Castro, M., Dadvand, P., Escribà, X., Fossati, S., González, J.R., Nieuwenhuijsen, M., Alfano, R., Annesi-Maesano, I., Brescianini, S., Burrows, K., Calas, L., Elhakeem, A., Heude, B., Hough, A., Isaevska, E., W V Jaddoe, V., Lawlor, D.A., et al., 2024. Green space exposure and blood DNA methylation at birth and in childhood – a multi-cohort study. Environ. Int. 188 (March), 108684. https://doi.org/10.1016/j.envint.2024.108684.

Alfano, R., Bijnens, E., Langie, S.A.S., Nawrot, T.S., Reimann, B., Vanbrabant, K., Wang, C., Plusquin, M., 2023. Epigenome-wide analysis of maternal exposure to green space during gestation and cord blood DNA methylation in the ENVIRONAGE cohort. Environ. Res. 216. https://doi.org/10.1016/j.envres.2022.114828.

Bhaskaran, K., Gasparrini, A., Hajat, S., Smeeth, L., Armstrong, B., 2013. Time series regression studies in environmental epidemiology. Int. J. Epidemiol. 42 (4), 1187–1195. https://doi.org/10.1093/ije/dyt092.

Baschat, A.A., Gembruch, U., 2003. The cerebroplacental Doppler ratio revisited. Ultrasound Obstet. Gynecol. 21 (2), 124–127. https://doi.org/10.1002/uog.20.

Battram, T., Gaunt, T.R., Relton, C.L., Timpson, N.J., Hemani, G., 2022a. A comparison of the genes and genesets identified by GWAS and EWAS of fifteen complex traits. Nat. Commun. 13 (1), 7816. https://doi.org/10.1038/s41467-022-35037-3.

Battram, T., Yousefi, P., Crawford, G., Prince, C., Sheikhali Babaei, M., Sharp, G., Hatcher, C., Vega-Salas, M.J., Khodabakhsh, S., Whitehurst, O., Langdon, R., Mahoney, L., Elliott, H.R., Mancano, G., Lee, M.A., Watkins, S.H., Lay, A.C., Hemani, G., Gaunt, T.R., et al., 2022b. The EWAS Catalog: a database of epigenome-wide association studies. Welcome Open Res. 7 (May), 41. https://doi.org/10.12688/wellcomeopenres.17598.1.

Bowyer, R.C.E., Twohig-Bennett, C., Coombes, E., Wells, P.M., Spector, T.D., Jones, A.P., Steves, C.J., 2022. Microbiota composition is moderately associated with greenspace composition in a UK cohort of twins. Sci. Total Environ. 813, 152321. https://doi.org/10.1016/j.scitotenv.2021.152321.

Campagna, M.P., Xavier, A., Lechner-Scott, J., Maltby, V., Scott, R.J., Butzkueven, H., Jokubaitis, V.G., Lea, R.A., 2021. Epigenome-wide association studies: current knowledge, strategies and recommendations. Clin. Epigenet. 13 (1), 1–24. https://doi.org/10.1186/s13148-021-01200-8.

Cartographic and Geologic Institute of Catalonia, 2018. Land cover map of Catalonia (MCSC) 2018 (version 1.0). https://www.icgc.cat/es/Ambitos-tematicos/Territori-sostenible/Cubiertas-del-suelo.

Cartographic and Geologic Institute of Catalonia, 2021. Normalized difference vegetation index (NDVI) 2020 (version 1.0). https://ide.cat/geonetwork/srv/eng/catalog.search#/metadata/ndvi-v1r0-2020.

Castellani, C.A., Longchamps, R.J., Sun, J., Guallar, E., Arking, D.E., 2020. Thinking outside the nucleus: mitochondrial DNA copy number in health and disease. Mitochondrion 53 (June), 214–223. https://doi.org/10.1016/j.mito.2020.06.004.

Cruells, A., Cabrera-Rubio, R., Bustamante, M., Pelegrí, D., Cirach, M., Jimenez-Arenas, P., Samarra, A., Martínez-Costa, C., Collado, M.C., Gascon, M., 2024. The influence of pre- and postnatal exposure to air pollution and green spaces on infant's gut microbiota: results from the MAMI birth cohort study. Environ. Res. 257 (June), 119283. https://doi.org/10.1016/j.envres.2024.119283.

Cruz, J. de O., Conceição, I.M.C.A., Tosatti, J.A.G., Gomes, K.B., Luizon, M.R., 2020. Global DNA methylation in placental tissues from pregnant with preeclampsia: a systematic review and pathway analysis. Placenta 101 (August), 97–107. https://doi.org/10.1016/j.placenta.2020.09.004.

Dadvand, P., Sunyer, J., Basagaña, X., Ballester, F., Lertxundi, A., Fernández-Somoano, A., Estarlich, M., García-Esteban, R., Mendez, M.A., Nieuwenhuijsen, M.J., 2012. Surrounding greenness and pregnancy outcomes in four Spanish birth cohorts. Environ. Health Perspect. 120 (10), 1481–1487. https://doi.org/10.1289/ehp.1205244.

Dadvand, P., Gascon, M., Bustamante, M., Rivas, I., Foraster, M., Basagaña, X., Cosín, M., Eixarch, E., Ferrer, M., Gratacós, E., Gómez Herrera, L., Jimenez-Arenas, P., Júlvez, J., Morillas, À., Nieuwenhuijsen, M.J., Persavento, C., Pujol, J., Querol, X., Sánchez García, O., et al., 2024. Cohort profile: Barcelona life study cohort (BiSC). Int. J. Epidemiol. 53 (3), 1–9. https://doi.org/10.1093/ije/dyae063.

Delahaye, F., Do, C., Kong, Y., Ashkar, R., Salas, M., Tycko, B., Wapner, R., Hughes, F., 2018. Genetic variants influence on the placenta regulatory landscape. PLoS Genet. 14 (11), e1007785. https://doi.org/10.1371/journal.pgen.1007785.

Deyssenroth, M.A., Marsit, C.J., Chen, J., Lambertini, L., 2020. In-depth characterization of the placental imprintome reveals novel differentially methylated regions across birth weight categories. Epigenetics 15 (1–2), 47–60. https://doi.org/10.1080/15592294.2019.1647945.

Dockx, Y., Bijnens, E., Saenen, N., Aerts, R., Aerts, J.-M., Casas, L., Delcloo, A., Dendoncker, N., Linard, C., Plusquin, M., Stas, M., Van Nieuwenhuyse, A., Van Orshoven, J., Somers, B., Nawrot, T., 2022. Residential green space in association

with the methylation status in a CpG site within the promoter region of the placental serotonin receptor HTR2A. Epigenetics 17 (13), 1863–1874. https://doi.org/10.1080/15592294.2022.2088464.
Ernst, J., Kellis, M., 2017. Chromatin-state discovery and genome annotation with ChromHMM. Nat. Protoc. 12 (12), 2478–2492. https://doi.org/10.1038/nprot.2017.124.
Everson, T.M., Vives-Usano, M., Seyve, E., Cardenas, A., Lacasaña, M., Craig, J.M., Lesseur, C., Baker, E.R., Fernandez-Jimenez, N., Heude, B., Perron, P., Gónzalez-Alzaga, B., Halliday, J., Deyssenroth, M.A., Karagas, M.R., Íñiguez, C., Bouchard, L., Carmona-Sáez, P., Loke, Y.J., et al., 2021. Placental DNA methylation signatures of maternal smoking during pregnancy and potential impacts on fetal growth. Nat. Commun. 12 (1), 1–13. https://doi.org/10.1038/s41467-021-24558-y.
Fernandez-Jimenez, N., Allard, C., Bouchard, L., Perron, P., Bustamante, M., Bilbao, J.R., Hivert, M.-F., 2019. Comparison of Illumina 450K and EPIC arrays in placental DNA methylation. Epigenetics 14 (12), 1177–1182. https://doi.org/10.1080/15592294.2019.1634975.
Figueras, F., Gratacós, E., 2014. Update on the diagnosis and classification of foetal 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.
Fortin, J.-P., Triche, T.J., Hansen, K.D., 2017. Preprocessing, normalization and integration of the Illumina HumanMethylationEPIC array with minfi. Bioinformatics 33 (4), 558–560. https://doi.org/10.1093/bioinformatics/btw691.
Fortin, J.P., Labbe, A., Lemire, M., Zanke, B.W., Hudson, T.J., Fertig, E.J., Greenwood, C. M.T., Hansen, K.D., 2014. Functional normalization of 450k methylation array data improves replication in large cancer studies. Genome Biol. 15 (11), 1–17. https://doi.org/10.1186/s13059-014-0503-2.
Fowden, A.L., Valenzuela, O.A., Vaughan, O.R., Jellyman, J.K., Forhead, A.J., 2016. Glucocorticoid programming of intrauterine development. Domest. Anim. Endocrinol. 56, S121–S132. https://doi.org/10.1016/j.domaniend.2016.02.014.
Freund, A., Zhong, F.L., Venteicher, A.S., Meng, Z., Veenstra, T.D., Frydman, J., Artandi, S.E., 2014. Proteostatic control of telomerase function through TRiC-mediated folding of TCAB1. Cell 159 (6), 1389–1403. https://doi.org/10.1016/j.cell.2014.10.059.
Gascon, M., Cirach, M., Martínez, D., Dadvand, P., Valentín, A., Plasència, A., Nieuwenhuijsen, M.J., 2016. Normalized difference vegetation index (NDVI) as a marker of surrounding greenness in epidemiological studies: the case of Barcelona city. Urban For. Urban Green. 19, 88–94. https://doi.org/10.1016/j.ufug.2016.07.001.
Gómez, O., Figueras, F., Fernández, S., Bennasar, M., Martínez, J.M., Puerto, B., Gratacós, E., 2008. Reference ranges for uterine artery mean pulsatility index at 11–41 weeks of gestation. Ultrasound Obstet. Gynecol. 32 (2), 128–132. https://doi.org/10.1002/uog.5315.
Griffiths, S.K., Campbell, J.P., 2015. Placental structure, function and drug transfer. Cont. Educ. Anaesth. Crit. Care Pain 15 (2), 84–89. https://doi.org/10.1093/bjaceaccp/mku013.
Hamada, H., Okae, H., Toh, H., Chiba, H., Hiura, H., Shirane, K., Sato, T., Suyama, M., Yaegashi, N., Sasaki, H., Arima, T., 2016. Allele-specific methylome and transcriptome analysis reveals widespread imprinting in the human placenta. Am. J. Hum. Genet. 99 (5), 1045–1058. https://doi.org/10.1016/j.ajhg.2016.08.021.
Hautekiet, P., Saenen, N.D., Aerts, R., Martens, D.S., Roels, H.A., Bijnens, E.M., Nawrot, T.S., 2022. Higher buccal mtDNA content is associated with residential surrounding green in a panel study of primary school children. Environ. Res. 213 (February). https://doi.org/10.1016/j.envres.2022.113551.
Heikinheimo, V., Tenkanen, H., Bergroth, C., Järv, O., Hiippala, T., Toivonen, T., 2020. Understanding the use of urban green spaces from user-generated geographic information. Landsc. Urban Plann. 201 (October 2019). https://doi.org/10.1016/j.landurbplan.2020.103845.
Heiss, J.A., Just, A.C., 2018. Identifying mislabeled and contaminated DNA methylation microarray data: an extended quality control toolset with examples from GEO. Clin. Epigenet. 10 (1), 73. https://doi.org/10.1186/s13148-018-0504-1.
Hemberger, M., Hanna, C.W., Dean, W., 2020. Mechanisms of early placental development in mouse and humans. Nat. Rev. Genet. 21 (1), 27–43. https://doi.org/10.1038/s41576-019-0169-4.
Houseman, E.A., Kelsey, K.T., Wiencke, J.K., Marsit, C.J., 2015. Cell-composition effects in the analysis of DNA methylation array data: a mathematical perspective. BMC Bioinf. 16 (1), 1–16. https://doi.org/10.1186/s12859-015-0527-y.
Hu, C.-Y., Yang, X.-J., Gui, S.-Y., Ding, K., Huang, K., Fang, Y., Jiang, Z.-X., Zhang, X.-J., 2021. Residential greenness and birth outcomes: a systematic review and meta-analysis of observational studies. Environ. Res. 193 (December 2020), 110599. https://doi.org/10.1016/j.envres.2020.110599.
Huang, R.C., Melton, P.E., Burton, M.A., Beilin, L.J., Clarke-Harris, R., Cook, E., Godfrey, K.M., Burdge, G.C., Mori, T.A., Anderson, D., Rauschert, S., Craig, J.M., Kobor, M.S., MacIsaac, J.L., Morin, A.M., Oddy, W.H., Pennell, C.E., Holbrook, J.D., Lillycrop, K.A., 2022. Adiposity associated DNA methylation signatures in adolescents are related to leptin and perinatal factors. Epigenetics 17 (8), 819–836. https://doi.org/10.1080/15592294.2021.1876297.
Huypens, P., Pillai, R., Sheinin, T., Schaefer, S., Huang, M., Odegaard, M.L., Ronnebaum, S.M., Wettig, S.D., Joseph, J.W., 2011. The dicarboxylate carrier plays a role in mitochondrial malate transport and in the regulation of glucose-stimulated insulin secretion from rat pancreatic beta cells. Diabetologia 54 (1), 135–145. https://doi.org/10.1007/s00125-010-1923-5.
Inkster, A.M., Wong, M.T., Matthews, A.M., Brown, C.J., Robinson, W.P., 2023. Who's afraid of the X? Incorporating the X and Y chromosomes into the analysis of DNA methylation array data. Epigenetics Chromatin 16 (1), 1 isglobal-brge/EASIER: Tools for methylation data analysis. (2022). https://github.com/isglobal-brge/EASIER.
Jaffe, A.E., Murakami, P., Lee, H., Leek, J.T., Fallin, M.D., Feinberg, A.P., Irizarry, R.A., 2012. Bump hunting to identify differentially methylated regions in epigenetic epidemiology studies. Int. J. Epidemiol. 41 (1), 200–209. https://doi.org/10.1093/ije/dyr238.
Johnson, W.E., Li, C., Rabinovic, A., 2007. Adjusting batch effects in microarray expression data using empirical Bayes methods. Biostatistics 8 (1), 118–127. https://doi.org/10.1093/biostatistics/kxj037.
Juliusdottir, T., Steinthorsdottir, V., Stefansdottir, L., Sveinbjornsson, G., Ivarsdottir, E. V., Thorolfsdottir, R.B., Sigurdsson, J.K., Tragante, V., Hjorleifsson, K.E., Helgadottir, A., Frigge, M.L., Thorgeirsson, G., Benediktsson, R., Sigurdsson, E.L., Arnar, D.O., Steingrimsdottir, T., Jonsdottir, I., Holm, H., Gudbjartsson, D.F., Stefansson, K., 2021. Distinction between the effects of parental and foetal genomes on foetal growth. Nat. Genet. 53 (8), 1135–1142. https://doi.org/10.1038/s41588-021-00896-x.
Kanehisa, M., 2000. KEGG: Kyoto Encyclopedia of genes and genomes. Nucleic Acids Res. 28 (1), 27–30. https://doi.org/10.1093/nar/28.1.27.
Lawrence, M., Huber, W., Pagès, H., Aboyoun, P., Carlson, M., Gentleman, R., Morgan, M.T., Carey, V.J., 2013. Software for computing and annotating genomic ranges. PLoS Comput. Biol. 9 (8), 1–10. https://doi.org/10.1371/journal.pcbi.1003118.
Lee, S., Kim, Y.N., Im, D., Cho, S.H., Kim, J., Kim, J.-H., Kim, K., 2021. DNA Methylation and gene expression patterns are widely altered in foetal growth restriction and associated with FGR development. Anim. Cell Syst. 25 (3), 128–135. https://doi.org/10.1080/19768354.2021.1925741.
Maccani, M.A., Marsit, C.J., 2009. Epigenetics in the placenta. Am. J. Reprod. Immunol. 62 (2), 78–89. https://doi.org/10.1111/j.1600-0897.2009.00716.x.
Markevych, I., Schoierer, J., Hartig, T., Chudnovsky, A., Hystad, P., Dzhambov, A.M., de Vries, S., Triguero-Mas, M., Brauer, M., Nieuwenhuijsen, M.J., Lupp, G., Richardson, E.A., Astell-Burt, T., Dimitrova, D., Feng, X., Sadeh, M., Standl, M., Heinrich, J., Fuertes, E., 2017. Exploring pathways linking greenspace to health: theoretical and methodological guidance. Environ. Res. 158 (February), 301–317. https://doi.org/10.1016/j.envres.2017.06.028.
Martin, L., White, M.P., Pahl, S., May, J., Wheeler, B.W., 2020. Neighbourhood greenspace and smoking prevalence: results from a nationally representative survey in England. Soc. Sci. Med. 265 (September), 113448. https://doi.org/10.1016/j.socscimed.2020.113448.
Min, J.L., Hemani, G., Hannon, E., Dekkers, K.F., Castillo-Fernandez, J., Luijk, R., Carnero-Montoro, E., Lawson, D.J., Burrows, K., Suderman, M., Bretherick, A.D., Richardson, T.G., Klughammer, J., Iotchkova, V., Sharp, G., Al Khleifat, A., Shatunov, A., Iacoangeli, A., McArdle, W.L., et al., 2021. Genomic and phenotypic insights from an atlas of genetic effects on DNA methylation. Nat. Genet. 53 (9), 1311–1321. https://doi.org/10.1038/s41588-021-00923-x.
Mizuarai, S., Miki, S., Araki, H., Takahashi, K., Kotani, H., 2005. Identification of dicarboxylate carrier Slc25a10 as malate transporter in de Novo fatty acid synthesis. J. Biol. Chem. 280 (37), 32434–32441. https://doi.org/10.1074/jbc.M503152200.
Mortillo, M., Marsit, C.J., 2022. Select early-life environmental exposures and DNA methylation in the placenta. Current Environ. Health Reports 10 (1), 22–34. https://doi.org/10.1007/s40572-022-00385-1.
Nieuwenhuijsen, M.J., Agier, L., Basagaña, X., Urquiza, J., Tamayo-Uria, I., Giorgis-Allemand, L., Robinson, O., Siroux, V., Maitre, L., de Castro, M., Valentin, A., Donaire, D., Dadvand, P., Aasvang, G.M., Krog, N.H., Schwarze, P.E., Chatzi, L., Grazuleviciene, R., Andrusaityte, S., et al., 2019. Influence of the urban exposome on birth weight. Environ. Health Perspect. 127 (4). https://doi.org/10.1289/EHP3971.
Nwanaji-Enwerem, U., McGeary, J.E., Grigsby-Toussaint, D.S., 2024. Greenspace, stress, and health: how is epigenetics involved? Front. Public Health 12 (February), 1–5. https://doi.org/10.3389/fpubh.2024.1333737.
Ohgane, J., Yagi, S., Shiota, K., 2008. Epigenetics: the DNA methylation profile of tissue-dependent and differentially methylated regions in cells. Placenta 29, 29–35. https://doi.org/10.1016/j.placenta.2007.09.011. Suppl. L.
Ongen, H., Buil, A., Brown, A.A., Dermitzakis, E.T., Delaneau, O., 2016. Fast and efficient QTL mapper for thousands of molecular phenotypes. Bioinformatics 32 (10), 1479–1485. https://doi.org/10.1093/bioinformatics/btv722.
Phipson, B., Maksimovic, J., Oshlack, A., 2016. missMethyl: an R package for analyzing data from Illumina's HumanMethylation450 platform. Bioinformatics 32 (2), 286–288. https://doi.org/10.1093/bioinformatics/btv560.
Roustaei, Z., Räisänen, S., Gissler, M., Heinonen, S., 2020. Associations between maternal age and socioeconomic status with smoking during the second and third trimesters of pregnancy: a register-based study of 932 671 women in Finland from 2000 to 2015. BMJ Open 10 (8), e034839. https://doi.org/10.1136/bmjopen-2019-034839.
Schroeder, D.I., Blair, J.D., Lott, P., Yu, H.O.K., Hong, D., Crary, F., Ashwood, P., Walker, C., Korf, I., Robinson, W.P., LaSalle, J.M., 2013. The human placenta methylome. Proc. Natl. Acad. Sci. USA 110 (15), 6037–6042. https://doi.org/10.1073/pnas.1215145110.
Spanish National Institute of Statistics. Atlas of household income distribution 2023. https://www.ine.es/metodologia/metodologia_adrh.pdf. (Accessed 1 July 2024).
Suderman, M., Staley, J.R., French, R., Arathimos, R., Simpkin, A., Tilling, K., 2018. Dmrff: identifying differentially methylated regions efficiently with power and control. bioRxiv 1, 1–26. https://doi.org/10.1101/508556.
Teschendorff, A.E., Marabita, F., Lechner, M., Bartlett, T., Tegner, J., Gomez-Cabrero, D., Beck, S., 2013. A beta-mixture quantile normalization method for correcting probe

design bias in Illumina Infinium 450 k DNA methylation data. Bioinformatics 29 (2), 189–196. https://doi.org/10.1093/bioinformatics/bts680.

Torres Toda, M., Avraam, D., James Cadman, T., Fossati, S., de Castro, M., Dedele, A., Donovan, G., Elhakeem, A., Estarlich, M., Fernandes, A., Gonçalves, R., Grazuleviciene, R., Harris, J.R., Harskamp-van Ginkel, M.W., Heude, B., Ibarluzea, J., Iñiguez, C., Wv Jaddoe, V., Lawlor, D., et al., 2022. Exposure to natural environments during pregnancy and birth outcomes in 11 European birth cohorts. Environ. Int. 170, 107648. https://doi.org/10.1016/j.envint.2022.107648.

Triche, T.J., Weisenberger, D.J., Van Den Berg, D., Laird, P.W., Siegmund, K.D., 2013. Low-level processing of illumina Infinium DNA methylation BeadArrays. Nucleic Acids Res. 41 (7), e90. https://doi.org/10.1093/nar/gkt090 e90.

Tucker, C.J., 1979. Red and photographic infrared linear combinations for monitoring vegetation. Rem. Sens. Environ. 8 (2), 127–150. https://doi.org/10.1016/0034-4257(79)90013-0.

van Rooij, J., Mandaviya, P.R., Claringbould, A., Felix, J.F., van Dongen, J., Jansen, R., Franke, L., t Hoen, P.A.C., Heijmans, B., van Meurs, J.B.J., 2019. Evaluation of commonly used analysis strategies for epigenome- and transcriptome-wide association studies through replication of large-scale population studies. Genome Biol. 20 (1), 235. https://doi.org/10.1186/s13059-019-1878-x.

Xiong, M., Li, L., Wen, L., Zhao, A., 2024. Decidual stromal cell–derived exosomes deliver miR-22-5p_R-1 to suppress trophoblast metabolic switching from mitochondrial respiration to glycolysis by targeting PDK4 in unexplained recurrent spontaneous abortion. Placenta 153 (December 2023), 1–21. https://doi.org/10.1016/j.placenta.2024.05.131.

Yu, J., Duan, Y., Lu, Q., Chen, M., Ning, F., Ye, Y., Lu, S., Ou, D., Sha, X., Gan, X., Zhao, M., Lash, G.E., 2024. Cytochrome c oxidase IV isoform 1 (COX4-1) regulates the proliferation, migration and invasion of trophoblast cells via modulating mitochondrial function. Placenta 151 (February), 48–58. https://doi.org/10.1016/j.placenta.2024.04.011.

Yuan, V., Hui, D., Yin, Y., Peñaherrera, M.S., Beristain, A.G., Robinson, W.P., 2021. Cell-specific characterization of the placental methylome. BMC Genom. 22 (1), 6. https://doi.org/10.1186/s12864-020-07186-6.

Yuan, Y., Huang, F., Lin, F., Zhu, P., Zhu, P., 2020. Green space exposure on mortality and cardiovascular outcomes in older adults: a systematic review and meta-analysis of observational studies. Aging Clin. Exp. Res., 0123456789 https://doi.org/10.1007/s40520-020-01710-0.

Zare Sakhvidi, M.J., Mehrparvar, A.H., Zare Sakhvidi, F., Dadvand, P., 2023. Greenspace and health, wellbeing, physical activity, and development in children and adolescents: an overview of the systematic reviews. Curr. Opin. Environ. Sci. Health 32, 100445. https://doi.org/10.1016/j.coesh.2023.100445.

Zhou, W., Laird, P.W., Shen, H., 2017. Comprehensive characterization, annotation and innovative use of Infinium DNA methylation BeadChip probes. Nucleic Acids Res. 45 (4), e22. https://doi.org/10.1093/nar/gkw967.

Zhou, W., Triche, T.J., Laird, P.W., Shen, H., 2018. SeSAMe: reducing artifactual detection of DNA methylation by Infinium BeadChips in genomic deletions. Nucleic Acids Res. 46 (20), 1–15. https://doi.org/10.1093/nar/gky691.