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Development and application in the EuroCHILD Cohort Network of an integrated early-life exposome model

Work package 3- Task 3.3 – Deliverable:

D3.6 Report on exposome models based on proof-of-principle studies. (Mo54)

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D3.6 Report on exposome models based on proof-of-principle studies. (Mo54)

Background and Objectives

The exposome represents the totality of life course environmental exposures (including lifestyle and other non-genetic factors), from the prenatal period onwards. This holistic concept of exposure provides a new framework to advance the understanding of complex and multifactorial diseases. Prospective pregnancy and birth cohort studies provide a unique opportunity for exposome research as they are able to capture, from prenatal life onwards, both the external (including lifestyle, chemical, social and wider community-level exposures) and the internal (including inflammation, metabolism, epigenetics, and gut microbiota) domains of the exposome.

As part of WP3, we developed protocols for analysis of associations of a fully integrated early-life stressor model (the early-life exposome) with lifecycle health outcomes and tested these in proof-of-principle studies. Proof-of-principle datasets were those of the EU-FP7 funded HELIX project (www.projecthelix.eu) including six cohorts across Europe (n=30,000 for entire cohorts, n=1300 for subcohort). These studies were used to evaluate model performance in cohorts of different sizes and depths. We first linked the early-life stressors to health and disease outcomes using the following steps: (1) a first explorative Environment-Wide Association Analysis (ExWAS) including independent models for each of the stressors accounting for multiple comparisons via false discovery rate (FDR) correction of p-values; (2) variable selection algorithms based on regression equations that account for a potential joint action of multiple exposures on health. Simulation studies conducted by the HELIX and EXPOSOMICS (www.exposomicsproject.eu) projects have identified iterative model search algorithms (DSA) and the Bayesian variable selection approaches (GUESS) as those that provide the highest sensitivity and the lowest FDR; (3) methods aimed at capturing joint effects of exposures by dividing study participants into groups (clusters) sharing similar exposure profiles (i.e. similar exposomes). Following the proof-of principles, the next steps within LifeCycle will be to include other LifeCycle cohorts and implement the exposome analyses through DataSHIELD.

1 PROTOCOL: Applying the exposome concept in birth cohort research

In a review of statistical approaches (Santos et al., 2020), we describe the steps required for applying an exposome approach, describe the main strengths and limitations of different statistical approaches and discuss their challenges, with the aim to provide guidance for methodological choices in the analysis of exposome data in birth cohort studies. An exposome approach implies selecting, pre-processing, describing and analyzing a large set of exposures.

Several statistical methods are currently available to assess exposome-health associations, which differ in terms of research question that can be answered, of balance between sensitivity and false discovery proportion, and between computational complexity and simplicity (parsimony). Assessing the association between many exposures and health still raises many exposure assessment issues and statistical challenges.

The following considerations are involved, as described in the publication (Santos et al., 2020):

- Selecting and pre-processing exposures
    - Handling missing data in the exposures
    - Dealing with exposure values below the limits of detection and quantification
    - Correcting for measurement error of the exposures
- Describing the exposome
    - Correlation structure of the exposome
    - Dimensionality of the exposome
- Variability of the exposome
- Assessing the determinants of the exposome
- Assessing exposome-health associations
    - Single exposure approaches
    - Variable selection techniques
    - Dimension reduction techniques
- Incorporating omics into exposome research (Figure 1)
- Sample size in an exposome context

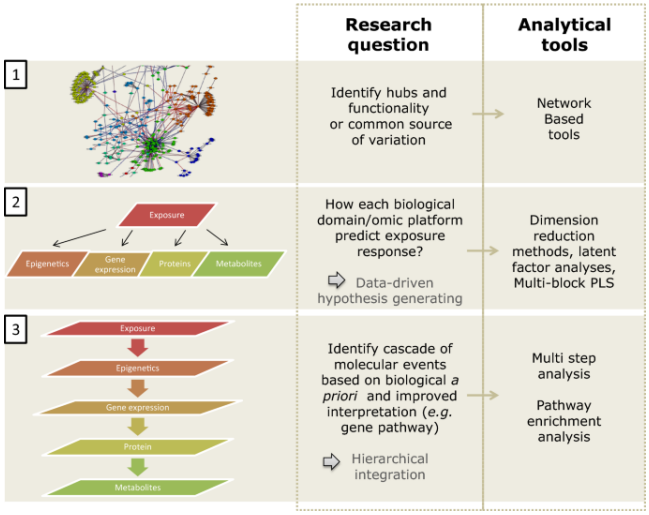


Figure 1. Specific omics tools that can be used in exposome research

2 Proof-of-principle studies using the HELIX database

Data from the EU-FP7 funded HELIX project (www.projecthelix.eu) including six cohorts across Europe (entire cohorts n=30,000, and subcohort n=1300) were used for proof-of-principle exposome studies, as these were the only exposome datasets available at the start of LifeCycle. This dataset includes 5 LifeCycle cohorts (BiB, EDEN, INMA, MoBa, RHEA). Now, work is ongoing to implement the methods in other LifeCycle cohorts.

2.1 ExWAS studies and health outcomes

Two examples were recently published on the exposome-wide associations (ExWAS) between the prenatal and childhood exposome and behaviour problems and cognitive function. In both studies, two sets of modeling approaches were employed: (1) single exposure model, (2) variable selection models: DSA or LASSO and (3) multiple exposure models.

2.1.1 Early-life environmental exposure determinants of child behavior in Europe: A longitudinal, population-based study (Maitre et al., 2021)

Highlights
- A wide range of pre- and postnatal environmental exposures affects child behavior.
- The exposome includes outdoor, indoor, chemical, lifestyle and social exposures.
- Maternal tobacco smoke and car traffic increased behavioural problems in children.
- Child sleep, healthy diet and higher family social capital reduced symptoms.
- Child exposure to lead, copper, indoor air pollution, increased symptoms.

Background: Environmental exposures in early life influence the development of behavioral outcomes in children, but research has not considered multiple exposures. We therefore aimed to investigate the impact of a broad spectrum of pre- and postnatal environmental exposures on child behavior.

Methods and findings: We used data from the HELIX (Human Early Life Exposome) project, which was based on six longitudinal population-based birth cohorts in Europe. At 6-11 years, children underwent a follow-up to characterize their exposures and assess behavioral problems. We measured 88 prenatal and 123 childhood environmental factors, including outdoor, indoor, chemical, lifestyle and social exposures. Parent-reported behavioral problems included (1) internalizing, (2) externalizing scores, using the child behavior checklist (CBCL), and (3) the Conner's Attention Deficit Hyperactivity Disorder (ADHD) index, all outcomes being discrete raw counts. We applied LASSO penalized negative binomial regression models to identify which exposures were associated with the outcomes, while

adjusting for co-exposures. In the 1287 children (mean age 8.0 years), 7.3% had a neuropsychiatric medical diagnosis according to parent's reports. During pregnancy, smoking and car traffic showing the strongest associations (e.g. smoking with ADHD index, aMR:1.31 [1.09; 1.59]) among the 13 exposures selected by LASSO, for at least one of the outcomes. During childhood, longer sleep duration, healthy diet and higher family social capital were associated with reduced scores whereas higher exposure to lead, copper, indoor air pollution, unhealthy diet were associated with increased scores. Unexpected decreases in behavioral scores were found with polychlorinated biphenyls (PCBs) and organophosphate (OP) pesticides.

Conclusions: Our systematic exposome approach identified several environmental contaminants and healthy lifestyle habits that may influence behavioral problems in children. Modifying environmental exposures early in life may limit lifetime mental health risk.

2.1.2 Early life multiple exposures and child cognitive function: A multi-centric birth cohort study in six European countries (Julvez et al., 2021)

Highlights

- Previous evidence for environmental risk factor associations with neurodevelopment is based on analyses of single exposures.
- We systematically analysed multiple environmental exposures in relation to child neurodevelopment.
- The findings describe a list of outcome-related exposures: Diet, house crowding, indoor air pollution and tobacco smoke.
- The findings show methodological complexities of analysing multiple exposures and their associations with neurodevelopment.

Epidemiological studies mostly focus on single environmental exposures. This study aims to systematically assess associations between a wide range of prenatal and childhood environmental exposures and cognition.

The study sample included data of 1298 mother-child pairs, children were 6–11 years-old, from six European birth cohorts. We measured 87 exposures during pregnancy and 122 cross-sectionally during childhood, including air pollution, built environment, meteorology, natural spaces, traffic, noise, chemicals and life styles. The measured cognitive domains were fluid intelligence (Raven's Coloured Progressive Matrices test, CPM), attention (Attention Network Test, ANT) and working memory (N-Back task). We used two statistical approaches to assess associations between exposure and child cognition: the exposome-wide association study

(ExWAS) considering each exposure independently, and the deletion-substitution-addition algorithm (DSA) considering all exposures simultaneously to build a final multiexposure model.

Based on this multiexposure model that included the exposure variables selected by ExWAS and DSA models, child organic food intake was associated with higher fluid intelligence (CPM) scores (beta = 1.18; 95% CI = 0.50, 1.87) and higher working memory (N-Back) scores (0.23; 0.05, 0.41), and child fast food intake (−1.25; −2.10, −0.40), house crowding (−0.39; −0.62, −0.16), and child environmental tobacco smoke (ETS) (−0.89; −1.42, −0.35), were all associated with lower CPM scores. Indoor PM2.5 exposure was associated with lower N-Back scores (−0.09; −0.16, −0.02). Additional associations in the unexpected direction were found: Higher prenatal mercury levels, maternal alcohol consumption and child higher perfluorooctane sulfonic acid (PFOS) levels were associated with better cognitive performance; and higher green exposure during pregnancy with lower cognitive performance. (Figure 2)

This first comprehensive and systematic study of many prenatal and childhood environmental risk factors suggests that unfavourable child nutrition, family crowdedness and child indoor air pollution and ETS exposures adversely and cross-sectionally associate with cognitive function. Unexpected associations were also observed and maybe due to confounding and reverse causality.

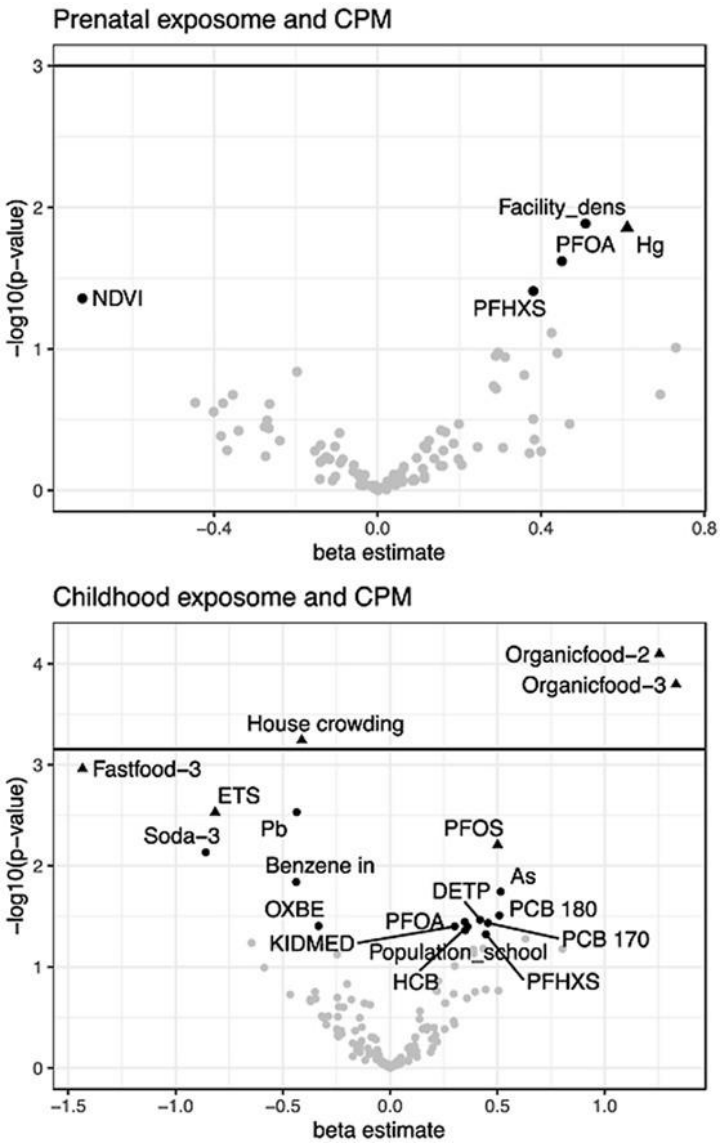


Figure 2. Exposome-wide associations with child cognitive functions (single-exposure models)

2.2 Urban exposome studies and health outcomes

These studies were based on the larger exposome and outcome datasets with the sample size varying between 4,279 and 11,673. The populations were younger, aged 4-5 years (before HELIX follow-up), and based on harmonized cohort datasets. The statistical analyses were similar to the above mentioned ExWAs studies with an additional step where urban environment exposure clusters were created and associated with the health outcomes.

2.2.1 Urban environment during early-life and blood pressure in young children (Warembourg et al., 2021)

Highlights

- Environmental stressors in urban settings are multiple.
- Exposure to air pollution during early life was associated with higher blood pressure.
- Ambient temperature was inversely associated with blood pressure in children.
- Noise, SES-area level, and features of the built environment were also predictors of blood pressure.
- Designing cities that promote healthy environments is of high importance.

Background

The urban environment is characterized by many exposures that may influence hypertension development from early life onwards, but there is no systematic evaluation of their impact on child blood pressure (BP).

Methods

Systolic and diastolic blood pressure were measured in 4,279 children aged 4–5 years from a multi-centre European cohort (France, Greece, Spain, and UK). Urban environment exposures were estimated during pregnancy and childhood, including air pollution, built environment, natural spaces, traffic, noise, meteorology, and socioeconomic deprivation index. Single- and multiple-exposure linear regression models and a cluster analysis were carried out.

Results

In multiple exposure models, higher child BP, in particular diastolic BP, was observed in association with higher exposure to air pollution, noise and ambient temperature during pregnancy, and with higher exposure to air pollution and higher building density during childhood (e.g., mean change [95% confidence interval] for an interquartile range increase in prenatal NO2 = 0.7 mmHg[0.3;1.2]). Lower BP was observed in association with higher temperature and better street connectivity during childhood (e.g., temperature = -1.1[-1.6;-

0.6]). Some of these associations were not robust in the sensitivity analyses. Mother-child pairs were grouped into six urban environment exposure clusters. Compared to the cluster representing the least harmful urban environment, the two clusters representing the most harmful environment (high in air pollution, traffic, noise, and low in green space) were both associated with higher diastolic BP (1.3[0.1;2.6] and 1.5[0.5;2.5]).

Conclusion
This first large systematic study suggests that living in a harmful urban environment may impact BP regulation in children. These findings reinforce the importance of designing cities that promote healthy environments to reduce long-term risk of hypertension and other cardiovascular diseases.

2.2.2 Urban environment during early-life and obesity in young children (Fossati *et al.* manuscript in preparation)

Aim
To determine which prenatal and postnatal outdoor exposures are associated with childhood BMI, and overweight/obesity risk, and weight and height at age 3-4 years.

Methods
11,673 children aged 3-4 years from the HELIX multi-centre European cohort (France, Greece, Lithuania, Norway, Spain, and UK) were included.

Outcomes: Height, weight, BMI (weight/height^2) age-and-sex–standardized z-scores (WHO).
Urban environment exposures during pregnancy and year before included:

- Air pollution
- Built environment
- Green spaces (including a measure of vegetation, Normalized Difference Vegetation Index (NDVI), categorized in tertiles due to its non linear relation with the outcomes)
- Traffic
- Noise
- Meteorology
- Socioeconomic deprivation index.

An Exposure-wide association study (ExWAS) was performed using multiple single exposure linear regression models and accounting for multiple testing using a Bonferroni-type correction adjusted for age, sex, city, maternal age at birth, maternal education, maternal height/weight/BMI, ethnicity. Postnatal models were further adjusted for birth weight.

2.2.3 Urban environment during early-life and cognitive and motor function in young children (Binter *et al.* manuscript in preparation)

Highlights

- Evidence suggest adverse effects of exposure to air pollution on neurodevelopment.
- However, the other urban exposures are poorly studied to date.
- We estimated 13 urban exposures and cognitive and motor function in 5,403 children.
- Built environment indicators are negatively associated with verbal abilities.
- PM2.5 mediate the positive association of greenness and verbal abilities.

Background: The urban exposome may influence neurodevelopment from conception onwards, but there is no evaluation of the impact of multiple exposures. We investigated the association between early-life urban exposome and cognitive and motor function in children.

Methods: We used data from 5,403 mother-child pairs from four population-based birth-cohorts (UK, France, Spain, and Greece). We estimated thirteen urban home exposures during pregnancy and childhood, including: built environment, natural spaces, and air pollution. Verbal, non-verbal, gross motor, and fine motor functions were assessed using validated tests at five years old. We ran adjusted multi-exposure models using the Deletion-Substitution-Addition algorithm.

Results: Higher greenness exposure within 300m during pregnancy was associated with higher verbal abilities (1.5 points (95% confidence interval 0.4; 2.7) per 0.20 unit increase in greenness). Higher connectivity density within 100m and land use diversity during pregnancy were related to lower verbal abilities. Childhood exposure to PM2.5 mediated 74% of the association between greenness during childhood and verbal abilities. Higher exposure to PM2.5 during pregnancy was related to lower fine motor function (-1.2 points (-2.1; -0.4) per 3.2 µg/m3 increase in PM2.5). No associations were found with non-verbal abilities and gross motor function. (Figure 3)

Discussion: This study suggests that built environment, greenness, and air pollution may impact child cognitive and motor function at five years old. This study adds evidence that well-designed urban planning may benefit children's cognitive and motor development.

Next steps:

We aim to replicate and expand our results to 12 European cohorts as part of LifeCycle, and 3 Canadian cohorts (n~125,000 in total) using the DataSHIELD solution. We also plan to include

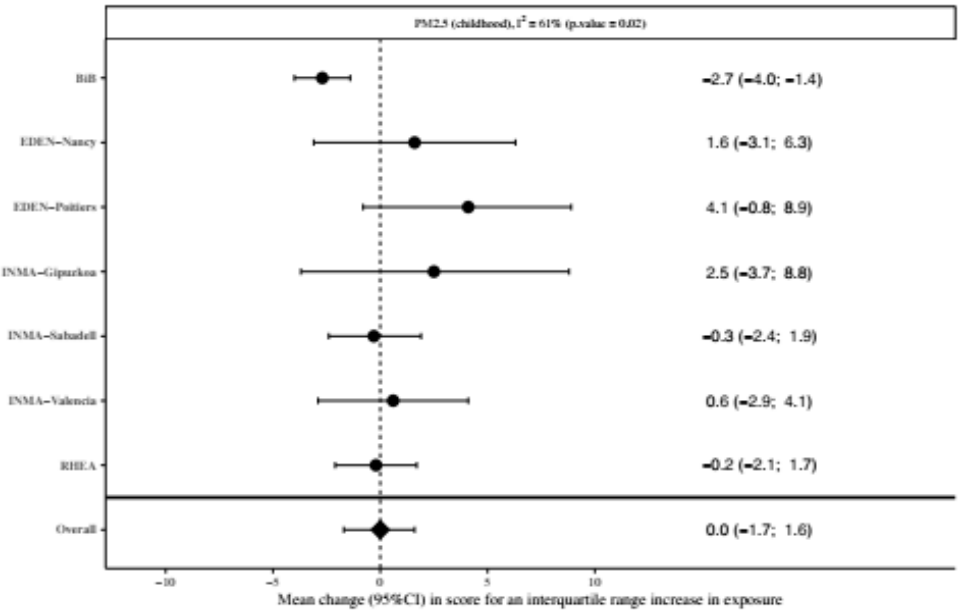
follow-ups at older ages. We will include new exposures where possible (noise, temperature), and we will investigate the potential mediation/moderation effect of air pollution and noise with a 4-way decomposition approach.

Figure 3. Associations between the urban exposome (multi-exposures) and verbal cognition at 4-5 years in 4 birth cohorts. **A.** and **B.** results of the multi-exposure models using first the Deletion/Substitution/Addition algorithm (DSA) then an adjusted linear regression model with all the exposure selected by the DSA. **C.** results of the causal mediation analysis.

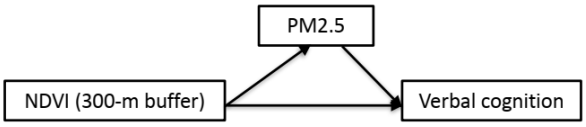
A.

	Pregnancy		Childhood	
	Pooled analysis	Meta-analysis	Pooled analysis	Meta-analysis
Urban environment				
Connectivity density in 100m buffer (Δ 192 intersections/km²)	-0.8 (-1.4; -0.2)	-0.8 (-1.4; -0.1)		
Land use SEI (Δ 0.1 units)	-0.9 (-1.4; -0.3)	-0.8 (-1.4; -0.3)	-0.6 (-1.1; 0.0)	-0.3 (-1.1; 0.4)
NDVI in 500m buffer (Δ 0.2 units)	1.5 (0.4; 2.7)	1.6 (-0.1; 3.4)		
Air pollution				
PM2.5 (Δ 3.2 µg/m³)			-1.2 (-2.1; -0.4)	0.0 (-1.7; 1.6)

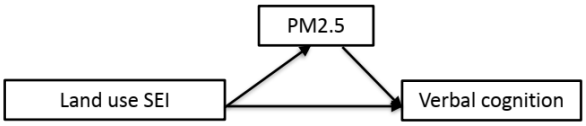
B.



C.



	Estimate (95%CI)
Natural direct effect	0.5 (-1.4; 2.4)
Natural indirect effect	1.5 (0.6; 2.4)
Total effect	2.0 (0.5; 3.7)



	Estimate (95%CI)
Natural direct effect	-1.7 (-2.6; -0.8)
Natural indirect effect	0.1 (0; 0.1)
Total effect	-1.6 (-2.5; -0.7)

2.3 ExWAS and internal signatures

The exposome can also be associated to internal molecular signatures, such as accelerated ageing markers, able to capture subtle exposome effects in children before the appearance of clinical symptoms. Here we present one study on an ExWAS and epigenetic age acceleration in 1,173 children.

The early-life exposome and epigenetic age acceleration in children (Prado-Bert et al. Environ Int 2021)

Highlights

- Early life is an important window of susceptibility to environmental exposures.
- Environmental exposures can influence on biological aging.
- Biological aging can be evaluated using epigenetic clocks.
- Pregnancy and childhood exposure to tobacco smoke might accelerate epigenetic
- aging.
- Childhood exposure to indoor PMabs is related to accelerated epigenetic aging.

The early-life exposome influences future health and accelerated biological aging has been proposed as one of the underlying biological mechanisms.

We investigated the association between more than 100 exposures assessed during pregnancy and in childhood (including indoor and outdoor air pollutants, built environment, green environments, tobacco smoking, lifestyle exposures, and biomarkers of chemical pollutants), and epigenetic age acceleration in 1,173 children aged 7 years old from the Human Early-Life Exposome project. Age acceleration was calculated based on Horvath's Skin and Blood clock using child blood DNA methylation measured by Infinium HumanMethylation450

BeadChips. We performed an exposure-wide association study between prenatal and childhood exposome and age acceleration.

Maternal tobacco smoking during pregnancy was nominally associated with increased age acceleration. For childhood exposures, indoor particulate matter absorbance (PMabs) and parental smoking were nominally associated with an increase in age acceleration. Exposure to the organic pesticide dimethyl dithiophosphate and the persistent pollutant polychlorinated biphenyl-138 (inversely associated with child body mass index) were protective for age acceleration. None of the associations remained significant after multiple testing correction.

Pregnancy and childhood exposure to tobacco smoke and childhood exposure to indoor PMabs may accelerate epigenetic aging from an early age.

Table 1. ExWAS of prenatal and childhood exposures vs. age acceleration adjusted for blood cell type proportions (main model).

				ExWAS*	
	Exposure	Exposure family	Units	Estimate (95% CI) [a]	P-value
Prenatal	Maternal tobacco smoking	Tobacco smoke	No vs. Yes	0.14 (0.02, 0.26)	0.025
Childhood	Indoor PM_{abs}	Indoor air	ug/m^3	0.07 (0.02, 0.12)	0.003
	Parental smoking	Tobacco smoke	Neither vs. Both	0.15 (0.01, 0.29)	0.036
	Dimethyl dithiophosphate (DMDTP)	OP Pesticides	Undetected vs. Detected (adjusted for creatinine)	-0.13 (-0.24, -0.02)	0.017
	Polychlorinated biphenyl-138 (PCB-138)	OCs	ng/g (adjusted for lipids)	-0.07 (-0.14, 0.01)	0.037

3 Ongoing studies using the lifecycle cohorts and DataSHIELD platform

The Datashield platform now allows conducting exposome studies with the integration of tools such as ExWAS (see deliverable 3.7 for a description of the tools). This means that exposome studies are being extended to other LifeCycle cohorts. Below is presented two detailed examples and the full list of ongoing papers.

3.1 Environment-wide association study of childhood adiposity

(Ahmed Elhakeem, Deborah Lawlor (UNIVBRIS))

The aim of this study is to examine the associations of many prenatal and postnatal exposures with variation in body mass index (BMI) at four different periods in early life (in infancy (≤12 months), early childhood (age 1 to 5 years), middle childhood (5 to 12 years), and adolescence (12 to 18 years)).

This study consists of 4 steps:

1. Exposure-wide analyses to identify which exposures are associated with BMI at each age period in a UK cohort (the Avon Longitudinal Study of Parents and Children (ALSPAC)
2. Confounder adjusted multivariable regression analyses in the same cohort (ALSPAC) of the associations from step 1 that survive Bonferroni multiple testing
3. Replication of confounder adjusted associations from step 2 in other European origin LifeCycle cohorts
4. One-sample or two-sample Mendelian randomization to explore evidence of causal effects on child BMI for those exposures that replicate in step 3

This proposal is seeking replication in all LifeCycle cohorts that have relevant data.

To be included in the replication study, LifeCycle cohorts should have data on:

- at least one of the exposures identified in step 2 (and child BMI for the same outcome age period)
- data on all the prespecified confounders.

The analyses are almost completed: the main ALSPAC analysis is finished and the replication analysis using DataSHIELD on 8 LC cohorts is nearly all completed. The manuscript is currently being drafted.

3.2 Other ongoing papers of exposome studies in Lifecycle

WP(s)	Title	Researchers involved
WP3, 6	Urban exposome and cognitive and motor function	Anne-Claire Binter, Monica Guxens (ISGLOBAL)
WP3, 6	Urban exposome and emotional and behavioural problems	Anne-Claire Binter, Monica Guxens (ISGLOBAL)

WP4	Environment-wide association study of child BMI	Jane Zhao, Ahmed Elhakeem, Deborah Lawlor (UNIVBRIS)
WP1, 3	Environment and postnatal depression	Tim Cadman, Marie Pedersen, Katrine Strandberg-Larsen, Deborah Lawlor (UNIVBRIS)
WP3 WP6	Urban environment mental health inequalities	Tim Cadman, Marie Pedersen, Katrine Strandberg-Larsen (UCPH)

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