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The Pregnancy Exposome

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Abstract The exposome concept takes a holistic approach facilitated by new and emerging technologies to describe 'the totality of human environmental (i.e. non-genetic) exposures from conception onwards, complementing the genome'. It provides a framework to advance the environmental epidemiology field that has until now focused almost exclusively on single-exposure health effects. The exposome includes an external domain, measured by methods including geo-spatial modelling, questionnaire and biomonitoring of external exposures while the internal domain is commonly assessed through molecular omics platforms. The internal domain, in part, reflects the biological response to the external domain. New statistical frameworks are required to integrate and assess exposome-health effects. The pregnancy period is a key starting point to describe the dynamic exposome, due to its heightened sensitivity and potential lifetime impact. A handful of studies have started to move towards an exposome approach in assessing the effects of the multiple exposures during pregnancy on child development. New research projects are underway to test the exposome approach on a large scale.

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Introduction

The idea of an 'exposome' was first proposed by Wild [1] as a call to arms for health research to complement the impressive advances made in measuring the human genome with similar strides in measuring the environmental component of disease aetiology. Like genomics and other rapidly proliferating '*omics*' fields, the key focus of the exposome concept is to take a holistic approach facilitated by new and emerging technologies to describe 'the totality of human environmental (i.e. non-genetic) exposures from conception onwards, complementing the genome'. The hope is that through the use of data-driven approaches pioneered in the genomics fields, advances can be made in the environmental epidemiology field that has until now focused almost exclusively on single-exposure health effects. The exposome has been delineated to include three overlapping and complimentary domains [2]: a general external domain including macro-level factors such as climate, social and economic context and psychosocial factors; a specific external domain including agents such as environmental pollutants, diet and drugs; and a specific internal domain including inflammation, metabolism and the gut microflora (Fig. 1). How to incorporate the hierarchical structure of the interlinked domains (if at all) into a putative exposome analysis is just one complexity still to be addressed.

In this review, we first discuss initial attempts to put the exposome concept into practice and some of the challenges that are being faced, before discussing the importance of the pregnancy exposome. We then review the handful of studies that have so far addressed the relationship between the external and internal domains of the pregnancy exposome and child

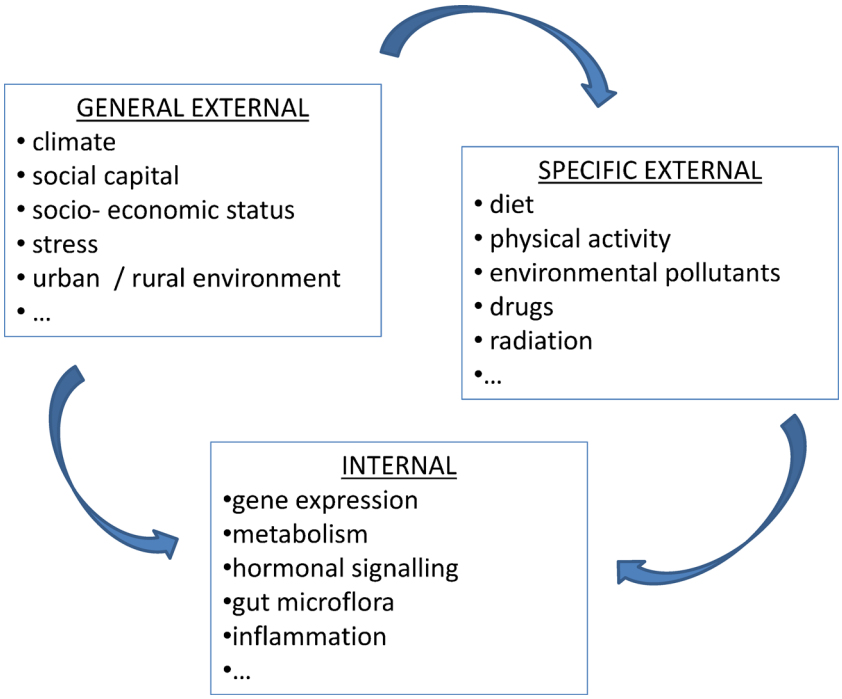

Fig. 1 The three domains of the exposome as defined by Wild [2], with some examples of each domain

health and development. We conclude with a discussion on future perspectives.

Measuring the Exposome

The exposome has also been divided by how each part can be feasibly measured [3••]: The external exposome includes both those factors encountered outside that are principally characterized by geo-spatial modelling and location (e.g. air pollution, noise and the built environment) and those external exposures assessed on an individual basis through questionnaire or biomonitoring (e.g. diet, smoking, persistent organic pollutants (POPs) and pesticides). The internal exposome is then assessed through high-throughput molecular omics methodologies including methylomics, transcriptomics, proteomics and metabolomics and are expected to reflect parts of the measured external exposome [4]. Furthermore, some investigators [5, 6] limit the exposome to what can be internally measured in biological tissues or fluids arguing that, for a given exposure to have a health consequence, there must be a measurable component or response and that this 'top-down' approach is most likely to yield novel risk factors. However, many technical challenges still need to be overcome, and for some important exposures, no biomarkers are available [7].

There are still few practical applications of the exposome. Patel et al. [8] applied the genome-wide association study (GWAS) analytical approach of multiple testing corrected for false discovery to U.S. National Health and Nutritional Examination Survey biomonitoring data of 266 environmental factors, which provided a relatively wide coverage of the external exposome. They identified environmental risk factors for type 2 diabetes with similar effect size to genes commonly indentified through GWAS. This is an important proof of concept, although its cross-sectional nature limits the conclusions that can be drawn. Nieuwenhuijsen et al. [9] developed and tested a personal monitoring kit, worn by volunteers, that continuously measures multiple exposures encountered outside. While promising for future exposome studies, its practicality and cost in its current form limit its use in larger studies. Chadeau-Hyam [10] tested the 'meet-in-middle' concept for linking both external exposures and disease to the same internal exposome markers. In a small pilot study, they identified urinary metabolic markers associated with dietary fibre intake in pre-clinical samples from the European Prospective Investigation into Cancer and Nutrition study that were also associated with later development of colorectal cancer, demonstrating the utility of using internal exposome markers to demonstrate mechanistic links between the external exposome and disease. In a large prospective study, Wang et al. [11] took an internal exposome approach and applied agnostic metabolome-wide measurement of serum and identified novel metabolites, choline, trimethylamine N-oxide and betaine, that predicted cardiovascular disease and could be traced, through careful experiment, back to their dietary source, phosphatidylcholine. This key study demonstrated that it is possible to trace internal exposome markers back to their external exposure origins and identified a novel aetiological pathway of cardiovascular disease risk. Overall, available studies show the complexities of comprehensively

measuring and analysing the exposome; although none has achieved 'complete' exposome coverage (or is ever likely to), they share a similar holistic, rather than single exposure, approach that defines them as exposome studies.

The Dynamic Exposome

A further important feature of the exposome is its longitudinal and dynamic nature, in contrast to the static and fixed genome. Over the course of their lifetime, an individual is exposed to levels of different environmental factors that vary on an hourly to yearly basis, with certain exposures being greatest during certain periods of life such as occupational exposures during the working portion of a lifetime [12]. Since continuous exposome monitoring would be impossible, it is widely envisaged that the longitudinal or 'life course exposome' would be built up from cross-sectional snapshots at key life periods such as in utero, early childhood, adolescence, adulthood and old age. The choice of time point at which the exposome will be surveyed is crucial since the health effects of exposures vary over time for two main reasons. Firstly, sensitivity to the exposome will vary according to age and developmental stage, with key development windows in utero particularly influenced by the exposome which may then have lifetime impacts [13]. Secondly, the effects of certain agents may be altered when co-exposed to other time-varying agents through synergistic or interactive effects. For instance, exposure to the widely distributed pollutant, dichlorodiphenyltrichloroethane (DDT) alters expression of hepatic metabolic enzymes, thereby enhancing susceptibility to other environmental toxicants [14]. The improved understanding of the combined health effects of exposure mixtures generally encountered in reality is one of the promises of the exposome.

Linking the Exposome to Health

The exposome concept is defined in terms of its relationship to health, and as such a crucial part of putting the exposome into practice is developing the necessary statistical frameworks to link these data to health outcomes. Recent reviews have outlined a number of modelling methodologies [15•, 16] that address the statistical challenges, such as the multiple testing burden and dense correlations structure, inherent in exposome analysis. Related methodological issues of exposome analysis such as varying confounding sets, dose-response relationships and differential measurement error will also need to be addressed. Some of these issues are discussed in recent multiple air pollution analyses [17, 18], and it is increasingly recognized within air pollution research that multi-pollutant analyses are needed to better reflect reality and improve public health interventions [19]. Billionnet et al. [20] reviewed the air pollution literature to identify studies that have moved towards the multi-pollutant approach and the statistical methodologies used. They indentified 18 studies with a range of methodologies including hierarchical Bayesian approaches, data reduction and clustering methods and more traditional multivariate regression models. The multivariate regression approach is applicable when co-linearity is not too large and individual effects are strong. Otherwise, methods that describe the 'typology' of air pollution are more suitable, and the authors suggested better understanding of the biological pathways through which toxicity is exerted may allow more appropriate grouping of pollutants. Within the literature examining suspected endocrine disrupting contaminants, Lenters et al. [21] recently applied a novel exposomic approach to 18 exposures in four xenobiotic classes (non-essential metals, organochlorines, perfluoroalkyl acids and phthalates) and its relation to male reproductive function. Using sparse partial least squares, a combined data reduction and variable selection method that is able to incorporate correlated exposures, they were able to extract statistically robust associations between certain organochlorines and phthalate metabolites and reproductive parameters. It is apparent that different statistical approaches will be needed for the different objectives of exposome analyses which can include identifying the most important individual exposures (crucial for regulatory policy), describing exposure mixture or exposome patterns (as most normally encountered in reality) and indentifying synergistic or interactive effects, which mainly due to issues of power, have been relatively unexplored in the epidemiological literature. Lampa et al. [22] performed a simulation study using real environmental exposure data and found that the boosted regression trees method is suitable for identifying a range of interaction scenarios. Although the method is susceptible to identifying spurious interactions, it appears a relatively powerful approach for epidemiological study.

The Pregnancy Exposome

The developing fetus is especially vulnerable to effects of environmental exposures [13] since its organs are rapidly growing and developing, its metabolism is immature and the received dose may be greater relative to its body weight [23]. In utero, exposure to environmental stressors during critical windows can disrupt developmental processes and permanently alter body structure, metabolism and physiology, leading to chronic pathologies in later life. There is now good evidence for the effect of prenatal exposure to environmental contaminants including air pollution, metals and industrial agents on fetal growth [24, 25], neurological development [24, 25] and the respiratory system [25, 26]. Evidence is also growing for effects on metabolic signalling [27]. Under The Developmental Origin of Health and Disease hypothesis,

these effects may be expected to have a lifetime impact [28, 29]. Therefore, the 'pregnancy exposome' is a key both as a starting point to develop a lifetime exposome and due to the heightened impact of the exposome during the in utero period. Figure 2 illustrates a typical external exposome profile of environmental contaminants during pregnancy and continuing into early life indicating the dynamic nature of the exposome.

External Pregnancy Exposome Studies

A Pubmed search ((pregnancy or in utero) and (exposome or multiple pollutant)) identifies only a small number of studies of health outcomes that take an external exposome or multiple pollutant approach. These early examples of 'pregnancy exposome' studies are described in the following section and in Tables 1 and 2. Warren et al. [30] jointly examined the effects of two air pollutants, $PM_{2.5}$ and ozone, continuously measured throughout pregnancy on the incidence of preterm birth (PTB) in TX, USA. Using a hierarchical Bayesian framework, they identified critical windows of effect that differed between the pollutants. $PM_{2.5}$ had the stronger effect overall with exposure in weeks 4 to 22 most relevant while ozone was found to have the greatest impact on PTB in weeks 1 to 5, illustrating the importance of understanding the temporal nature of the pregnancy exposome. Le et al. [31] examined the effects of air pollutants CO, SO_2, NO_2 and PM_{10} on PTB and term small for gestational age (SGA) births in Detroit, USA, taking into account interactive effects with social context. Due to the diverse pollutant sources in the area, correlations between exposures were modest allowing the use of multi-pollutant regression models to separate the effects of each pollutant individually. Significant effects at various critical windows were found for SGA births for CO (first and second trimester), SO_2 (all trimesters), NO_2 (first month and all trimesters) and PM_{10} (first month and first trimester). For PTB births, odds ratios were elevated during only first month SO_2 and NO_2 exposures. Effect sizes were greater among mothers with lower educational level and black ethnicity, while there was heterogeneity in effects by pollutant between smokers and non-smokers. Positive associations with SGA were observed for among smokers for NO_2, but associations with CO and PM_{10} were only observed among non-smokers. Dadvand et al. [32•] extended their analysis of term low birth weight (LBW) in Barcelona, Spain, to include additional components of the community level external exposome including the built environment (proximity to major roads), temperature, noise, air pollutants and the buffering effects of roadside tree planting. Living within 200 m of a major road while pregnant was associated with a 46 % increased risk of LBW, and measured air pollutants and heat together explained around one third of this association. There was some evidence for attenuation of the effect size with increasing tertiles of tree cover. Vafeidi et al. [33] measured serum levels of multiple POPs, including pesticides, polychlorinated diphenyls and polybrominated diphenyl ether (PBDE)-47, among pregnant women during the first

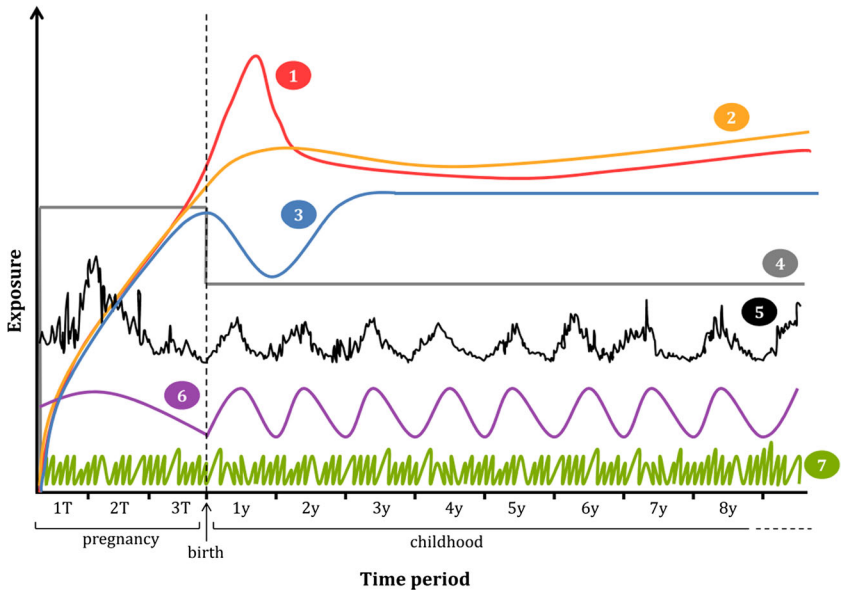

Fig. 2 The dynamic pregnancy and early-life external exposome [12]. Representative of the temporal variation of environmental measurements of a child who is breastfed, is exposed to maternal smoking during pregnancy and childhood, and does not change address. *1. Red line*: persistent organic pollutants, including organochlorines, brominated and perfluorinated compounds (breastfeeding). *2. Orange line*: mercury and lead (cumulative during pregnancy). *3. Blue line*: arsenic (not cumulative and reduced exposure during breastfeeding). *4. Grey line*: environmental tobacco smoke. *5. Black line*: air pollution, noise, and green spaces. *6. Purple line*: ultraviolet radiation, organophosphates pesticides, benzophenone-3 (seasonal exposure). *7. Green line*: non-persistent pollutants, including phthalates, phenols, and triclosan, and water disinfection by products

Table 1 External pregnancy exposome studies

Authors	Study design	Pollutants	Analysis	Main findings
Warren et al. [30]	American prospective birth registry	$PM_{2.5}$ and ozone	Time varying hierarchical Bayesian	Different critical windows were found for the effect of each pollutant on preterm birth
Le et al. [31]	American prospective birth registry	CO, SO_2, NO_2 and PM_{10}	Multivariate regression	Associations found with small for gestational age for all pollutant during various critical windows; associations with preterm birth found for first month SO_2 and NO_2 only
Dadvand et al. [32•]	Spanish prospective birth cohort	$PM_{2.5}$, $PM_{2.5–10}$, PM_{10}, $PM_{2.5}$ absorbance, NO_2, NO_x, noise, temperature, distance to major roads, tree cover	Multivariate regression, mediation analysis	Proximity to major road associated with low birth weight; air pollution and temperature explained a third of the association; some evidence for buffering effect by tree cover
Vafeidi et al. [33]	Greek prospective birth cohort	10 Persistent organic pollutants	Regression using exposure score	Exposure score inversely associated with birth weight
Braun et al. [34]	American prospective birth cohort	52 Endocrine-disrupting chemicals	Semi-hierarchical Bayesian	Positive associations found for two chemicals with autistic behaviours in childhood
Yorifugi et al. [35]	Faroese prospective birth cohort	Lead and methylmercury	Multivariate regression with interaction terms	Associations between lead and childhood cognitive function observed in lowest methylmercury exposure group
Golding et al. [36]	British prospective birth cohort	1755 Parental, prenatal and neonatal environment and lifestyle factors	Two-stage multiple univariate testing and final regression model	Association between unhappy childhood of the mother and motor skills in the child

References selected from PubMed search (pregnancy or in utero) and (exposome or multiple pollutant) to represent state of the science

trimester in Crete, Greece. Combining these correlated exposures into single model using an overall exposure score, they observed a 42-g decrease in birth weight with unit increase in exposure score, which was robust to adjustment for social and maternal factors including gestational weight gain.

The effect of multiple external pregnancy exposures on child neurodevelopment has also been investigated by a few studies. Braun et al. [34] measured levels of 52 endocrine-disrupting chemicals among 175 pregnant women in a prospective birth cohort in Cincinnati, USA, and examined the relationship with autistic behaviours in children at 4 and 5 years of age using a semi-hierarchical Bayesian analysis to account for co-linearity and multiple sampling. Increasing concentrations of the pesticide *trans*-nonachlor and PBDE-28 were associated with more autistic behaviours; however, the small study size prevented the dismissal of chemicals for which null associations were found. Yorifugi et al. [35] examined the association between cord blood lead concentration and cognitive function at 7 and 14 years of age in a Faroese birth cohort, while accounting for exposure to methylmercury. Clear associations between lead and cognitive function were only observed in the lowest methylmercury exposure group and evidence suggested a less-than additive or antagonistic interaction. This was explained by the toxicants competing to induce the observed effect through similar pathways and demonstrates the importance of assessing co-pollutants when investigating health effects. Golding et al. [36] applied the exposome approach, in the sense of hypothesis-free multiple testing, to examine environmental or lifestyle factors associated with gross motor skills at 8 years old in a British birth cohort. They conducted multiple univariate analyses on 1755 variables covering factors spanning the lives of both parents and then incorporated the most significant variables into a final regression model. They identified the novel association between an unhappy childhood of the mother and motor skills in the child. However, with only minimal adjustment for repeated testing to account for the generation of false positives, it is difficult to draw conclusions from this study.

Internal Pregnancy Exposome Studies

Analyses investigating the internal exposome and its response to external environmental exposures have also been developing in recent years, using the maturing *omics* platforms.

Table 2 Internal pregnancy exposome studies

Authors	Study design	Pollutants	Analysis	Main findings
Koestler et al. [37]	American prospective birth cohort	Arsenic	450 K DNA methylation array in cord blood	Dose-response linear relationships with methylation of genes encoding oestrogen receptor alpha and a nuclear receptor co-activator
Fry et al. [38]	Thai prospective birth cohort	Arsenic	Microarray of mRNA in cord blood	11 Transcripts highly predictive of arsenic exposure; activation of inflammation, stress and cell death pathways
Rager et al. [39•]	Mexican prospective birth cohort	Arsenic	Microarray of mRNA and miRNA in cord blood	Activation of pathways involved in cytokine activity, immune response, inflammatory response and stress
Ahmed et al. [40]	Bangladeshi birth cohort	Arsenic	Cytokine panel in cord blood and T cell count in placental tissue	Reduction in T cells in placenta and association with cytokine activity
Suter et al. [41]	American prospective birth cohort	Maternal smoking	27 K DNA methylation array and microarray of mRNAs in placenta	Correlation between altered methylation and expression in 428 genes primarily involved in oxidative stress pathways
Joubert et al. [42•]	Norwegian birth cohort	Maternal smoking	450 K DNA methylation array in cord blood	Altered methylation within 10 genes, including AHRR and CYP1A1 involved in detoxification of xenobiotics
Hochstenbach et al. [43]	Norwegian birth cohort	Dioxin-like compounds, estrogenic and androgenic agents, and adducts of acrylamide and glycidamide	Microarray of mRNA in cord blood	Significant differences between sexes in exposure response with activation of TNF-alpha-NF-kB signalling and Wnt-pathway in boys
Maitre et al. [44•]	Greek prospective birth cohort	Metabolomic analysis	^{1}H NMR analysis of first trimester urine samples	Metabolites acetate, formate, trimethylamine and tyrosine as associated with fetal growth restriction. *N*-acetyl glycoprotein resonances associated with induced preterm birth

References selected from PubMed search (see Table 1) and recent reviews [4, 45, 46] (including citing and cited references) to represent state of the science

Studies were identified through the Pubmed search described above and from recent reviews [4, 45, 46] (including citing and cited references). Most studies have looked at single exposures that can be well measured during pregnancy to identify associated internal exposome profiles and improve mechanistic understanding (Table 2). Arsenic is a known human carcinogen and an important public health hazard since millions of people worldwide are chronically exposed to arsenic in their drinking water, while dietary sources, including rice, other grains and fruit juices are also important. A few studies have now looked at the effects of in utero exposure on internal exposome profiles of newborns at various levels of biological organization. Koestler et al. [37] examined the relationship between maternal arsenic exposure and DNA methylation, involved in epigenomic regulation of gene expression, in cord blood of newborns. Although they did not detect global changes in methylation across the genome, a number of differentially methylated CpG dinucleotide sites were identified, predominately with greater methylation in the high-exposed group. Several of these loci exhibited dose-response linear relationships between methylation and arsenic exposure including genes encoding oestrogen receptor alpha and a nuclear receptor co-activator, which given the importance of hormonal signalling in fetal development may have health consequences. A study in Thailand [38] compared RNA transcripts in the cord blood of newborns born to mothers differentially exposed to arsenic using microarray analysis. They found that prenatal arsenic exposure had a strong impact on the transcriptome of the newborn, with a subset of 11 transcripts highly predictive of arsenic exposure. Pathway analysis of gene differentially expressed across the genome identified activation of pathways including inflammation, stress and cell death. Rager et al. [39•] performed a similar analysis in a Mexican birth cohort; however, they also measured levels of miRNAs, small non-coding RNAs involved in regulation of messenger RNA (mRNA) translation. They identified 12

miRNAs associated with arsenic exposure, many of which were known to have roles in carcinogenesis, and 334 mRNA transcripts including 28 identified in the study of Fry et al. [38]. They found that a third of miRNA-mRNA pairings, predicted computationally based on target sequences, were correlated in their analysis. At the biological pathway level, analysed using both miRNAs and mRNAs, there was a similar enrichment of genes involved in cytokine activity, immune response, inflammatory response and stress. Interestingly, since arsenic exposure in utero is associated with health outcomes related to a repressed immune system such as increased morbidity during infancy from infectious diseases, they identified genes involved in innate and adaptive immunity. Ahmed et al. [40] also identified inflammatory markers at the proteomic and cellular level in a Bangladeshi cohort associated with high maternal arsenic exposure including reduction in levels of placental T cells and perturbations to cord blood concentrations of cytokines.

Maternal smoking is another exposure that has been relatively well investigated and provides an important model for the use of omics in identifying environmental exposure pathways. Suter et al. [41] used parallel arrays to measure DNA methylation and gene expression in placenta sampled from smoking and non-smoking mothers. They found altered expression of 623 genes and methylation of 1024 CpG sites among smokers, with significant correlation between transcription and methylation in 428 genes primarily in oxidative stress pathways. Altered methylation of six of these genes was significantly associated with birth weight reduction. The study demonstrates that smoking results in site-specific methylation changes resulting in transcriptional disregulation. Joubert et al. [42•] performed an epigenome-wide analysis of methylation changes in cord blood related to maternal plasma cotinine levels, a validated smoking biomarker, in a Norwegian birth cohort. The identified altered methylation in 26 CpG sites mapping to 10 genes that passed conservative correction for multiple testing and were replicated in a smaller American birth cohort. Intriguingly, two of the genes *AHRR* and *CYP1A1* encode proteins involved in detoxification of xenobiotics, while *AHRR* was also identified independently in an epigenome-wide study of lymphoblasts and pulmonary alveolar macrophages in adult smokers [47], demonstrating that in utero, exposure induces epigenetic changes that are also observed in exposed adults.

Hochstenbach et al. [43] took a more global approach to their external exposure assessment in their study of global gene expression changes in cord blood in newborn boys and girls. They measured exposure to dietary and environmental carcinogens using gene reporter assays to determine exposure to dioxin-like compounds, estrogenic and androgenic activity and haemoglobin adducts of acrylamide and glycidamide. They noted significant differences between the sexes in gene expression response with, in particular, male-specific activation of TNF-alpha-NF-kB signalling and the Wnt-pathway, which they suggest may present a mechanistic explanation for the higher rates of childhood leukaemia observed in boys.

Metabolomics, the study of small metabolites present in biological samples, is considered a promising exposomic platform since the metabolome is both environmentally and biologically determined, with a twin study estimating that 60 % of variability observed depends on the environment [48] and is the final expression of all biological processes thereby integrating the environment with all of the biochemical interactions (including with the gut microflora) taking place in the body. A few studies have investigated the pregnancy metabolome with the largest so far conducted by Maitre et al. [44•] on first trimester urine samples collected from a Greek birth cohort. Using ^{1}H NMR spectroscopy that is less sensitive but more robust than other metabolomic platforms, they identified metabolites acetate, formate, trimethylamine and tyrosine as associated with fetal growth restriction. These metabolites were inversely correlated with blood insulin and are associated with activity of the gut microflora and may represent a specific microbial distribution or dietary pattern conducive to such a distribution. *N*-acetyl glycoprotein resonances, thought to reflect inflammation, were predictive of induced preterm birth and correlated with the body mass index of the mother.

Conclusions and Future Perspectives

The exposome concept is a broad idea and has generated much discussion of how it should be interpreted. It is part of a wider trend in related fields such as exposure assessment, toxicology, systems biology and public health policy to take a more holistic approach that is more reflective of the exposure mixtures we encounter day to day. Although implementing exposome research remains challenging and expensive, characterization of the ‘pregnancy exposome’ is a logical and feasible area to focus efforts due to its lifetime importance and defined exposure window.

While exposome research is still in its infancy, as we have seen, a number of studies have started to implement the concept during pregnancy through a variety of approaches. As with other *omics* platforms, such in GWAS studies where imputation is required to provide more complete coverage or in metabolomics where coverage will depend on the matrix or analytical platform used, only a partial evaluation of the exposome has so far been achieved. Efforts to move towards wider coverage of the external exposome have been made through measurement of multiple pollutant biomarkers or incorporating geo-spatially derived exposure estimates. Emerging technologies and methods are allowing numerous environmental variables to be measured simultaneously [49];

however, exposure misclassification remains a key concern of exposure assessment, and care must be taken that the exposome analyses do not multiply these problems. Future pregnancy exposome studies must address this issue with, for example, multiple sampling to fully capture exposure to short-lived non-persistent pollutants (a class within which new varieties are continuously produced by the chemical industries) or more detailed information on how mothers move through their external environment [23]. Numerous studies have also characterized the internal pregnancy exposome at various biological levels. To move towards a unified exposome approach, efforts are needed to piece these parts together. Current focus is on linking internal exposome profiles to single or a few external exposures, and it may turn out some of the internal exposome responses emerging are more generalisable to whole classes of external exposures, which may eventually minimize the need for comprehensive external exposure assessment. Alternatively, the internal exposome should be considered as important exposures in their own right rather than external exposome mediators, and 'exposome-wide' health analyses should analyse these domains side by side, with emphasis on interaction between and within the domains. Metabolomics may prove a platform capable of integrating the exposome domains in a single analysis; however, the sensitivity of current machines is still a long way off detecting markers of environmental pollutant exposures alongside currently detectable diet, drug and metabolic profiles [5].

Around the world, research projects are now underway to integrate these parts of the pregnancy exposome within a large number of subjects. In Europe, several projects are ongoing; the HELIX project will measure both the external and internal exposome domains among mother-child pairs and link these to important childhood developmental areas such as pre- and postnatal growth, obesity, behaviour and neurodevelopment, and asthma and allergies [3••]. Among up to 32,000 mother-child pairs, the health effects of the outdoor exposome measured at the community level (air pollution, temperature, noise, UV radiation, green spaces and the built environment) will be analysed, and in a subset of 1200 mother-child pairs, the external exposome to be supplemented with multiple pollutant biomarkers and questionnaire derived variables to build a comprehensive picture of the external exposome. Multiple sampling, personal monitoring and smartphone-derived activity data will also improve external exposome estimates. In parallel, the internal exposome will be measured in the same individuals through multiple omics platforms, which should address some of the questions of how the domains are linked. Statistical methodologies are also being developed to best integrate and analyse the pregnancy and early-life exposome. The Exposomics project [50] uses multiple cohorts covering various life periods and focuses on improved assessment of air and water pollutants and linking these both to acute and chronic internal exposome response. The expectation is that exposome signals from pregnancy exposure measured in archived samples collected in birth cohorts will be replicated in samples from cohorts at different life stages building a life-course exposome picture.

In conclusion, the exposome concept provides an important new framework to integrate and improve knowledge on the environmental component of disease aetiology, with the pregnancy period crucial to full understanding of the human exposome. A more holistic approach, incorporating information on how the various components of the pregnancy exposome co-exist and interact will improve our knowledge of the causes of the complex and multifactoral developmental disorders of childhood, some of which are becoming increasingly common, and ultimately lead to better prevention strategies.

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Compliance with Ethics Guidelines

Conflict of Interest Oliver Robinson and Martine Vrijheid declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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