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Review article

What is new in the exposome?

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ABSTRACT

The exposome concept refers to the totality of exposures from a variety of external and internal sources including chemical agents, biological agents, or radiation, from conception onward, over a complete lifetime. It encompasses also "psychosocial components" including the impact of social relations and socio-economic position on health. In this review we provide examples of recent contributions from exposome research, where we believe their application will be of the greatest value for moving forward. So far, environmental epidemiology has mainly focused on hard outcomes, such as mortality, disease exacerbation and hospitalizations. However, there are many subtle outcomes that can be related to environmental exposures, and investigations can be facilitated by an improved understanding of internal biomarkers of exposure and response, through the application of omic technologies. Second, though we have a wealth of studies on environmental pollutants, the assessment of causality is often difficult because of confounding, reverse causation and other uncertainties. Biomarkers and omic technologies may allow better causal attribution, for example using instrumental variables in triangulation, as we discuss here. Even more complex is the understanding of how social relationships (in particular socio-economic differences) influence health and imprint on the fundamental biology of the individual. The identification of molecular changes that are intermediate between social determinants and disease status is a way to fill the gap. Another field in which biomarkers and omics are relevant is the study of mixtures. Epidemiology often deals with complex mixtures (e.g. ambient air pollution, food, smoking) without fully disentangling the compositional complexity of the mixture, or with rudimentary approaches to reflect the overall effect of multiple exposures or components.

From the point of view of disease mechanisms, most models hypothesize that several stages need to be transitioned through health to the induction of disease, but very little is known about the characteristics and temporal sequence of such stages. Exposome models reinforce the idea of a *biography-to-biology transition*, in that everyone's disease is the product of the individual history of exposures, superimposed on their underlying genetic susceptibilities. Finally, exposome research is facilitated by technological developments that complement traditional epidemiological study designs. We describe in depth one such new tools, adductomics. In general, the development of high-resolution and high-throughput technologies interrogating multiple -omics (such as epigenomics, transcriptomics, proteomics, adductomics and metabolomics) yields an unprecedented perspective into the impact of the environment in its widest sense on disease.

The world of the exposome is rapidly evolving, though a huge gap still needs to be filled between the original expectations and the concrete achievements. Perhaps the most urgent need is for the establishment of a new generation of cohort studies with appropriately specified biosample collection, improved questionnaire data (including social variables), and the deployment of novel technologies that allow better characterization of individual environmental exposures, ranging from personal monitoring to satellite based observations.

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1. What is the exposome?

According to several definitions, the exposome concept refers to the totality of exposures from a variety of external and internal sources including chemical agents, biological agents, radiation, and more general exposures and determinants from conception onward, over a complete lifetime (Wild, 2005, 2012; Rappaport and Smith, 2010; Vineis et al., 2017; Miller Gary and Jones, 2014; Dagnino, 2019). Under this definition, the exposome includes biologically active chemicals induced in response to both external environmental stimuli, as well as the internal chemical environment, and is considered to offer a conceptual leap in studying the role of the environment in human diseases. It encompasses also "psychosocial components" including the impact of social relations and socio-economic position on both specific exposures and directly on health.

After the first wave of demonstrator projects, the utility of exposomic approaches to environmental research has become clearer. In this review we provide examples of recent contributions from exposome research, which demonstrate where we believe their application will be of the greatest value for moving forward.

2. Six good reasons to conduct exposome research

1. *Beyond hard and rare outcomes* - Epidemiological research on the health effects of environmental stressors has mainly focused on hard outcomes, such as mortality, disease exacerbation and hospitalizations. However, as Fig. 1 shows, there are many more outcomes (including clinical and sub-clinical symptoms) that can be related to environmental exposures, such as air pollution. Investigations in these areas are facilitated by an improved understanding of internal biomarkers of exposure and response, through the application of omic technologies.
2. *Where causality is uncertain*. Though we have a wealth of studies on environmental pollutants, the assessment of causality is often difficult because of confounding, reverse causation and other uncertainties. For example, an International Agency for Research on Cancer Working Group classified ambient air pollution as carcinogenic to humans (IARC, 2016), but for other exposures such as many pesticides epidemiological studies are limited, both quantitatively and qualitatively. Biomarkers and omic technologies allow better causal attribution, for example using instrumental variables in triangulation, as we will discuss below.
3. *Time matters* – All models of disease imply a temporal sequence of events, from disease induction to death. For most non-communicable diseases, models have been proposed in which latent genetic susceptibilities present at birth are influenced by multiple behavioural and environmental exposures, inducing acquired susceptibility that modulates the risk of disease: for example, in cancer via toxicant induced mutations, epigenetic events, and the other "hallmarks of cancer". Most models hypothesize that several stages need to be transitioned through health to the induction of disease, but very little is known about the characteristics and temporal sequence of such stages. These models reinforce the idea of a *biography-to-biology transition*, in that everyone's disease is the product of their individual history of exposures, superimposed on their underlying genetic susceptibilities.
4. *Social-to-biological interactions*. Even more complex is the understanding of how social relationships (particularly socio-economic differences) influence health and imprint on the fundamental biology of the individual. The importance of socio-economic factors to exposome research has been previously highlighted (Smith et al., 2015; Juarez et al., 2014), particularly with regard to exposure distributions and individual susceptibility. However, the identification of molecular changes that are intermediate between social determinants and disease status is a way to fill the gaps in the mechanisms of biological embodiment (Vineis et al., 2009), particularly for less tangible pathways such as psychological and social stress.
5. *Complex, multiple and concurrent exposures* – Epidemiology has mainly dealt with single exposures (treating others as confounders or effect modifiers), or with complex mixtures (e.g. ambient air pollution, food, smoking). In the latter case this was often done without (a) fully disentangling the compositional complexity of the pollutant aerosol, or diet, and (b) with rudimentary approaches to reflect the overall effect of multiple exposures or components.
6. *New tools* – Exposome research is facilitated by technological developments (e.g. array technology) and provides new tools for better exposure assessment and for the investigation of the early molecular initiating events that precede disease onset, and sequential changes in biology that determine disease severity and outcome. These tools and the identification of molecular fingerprints of exposure and response complement traditional epidemiological study designs.

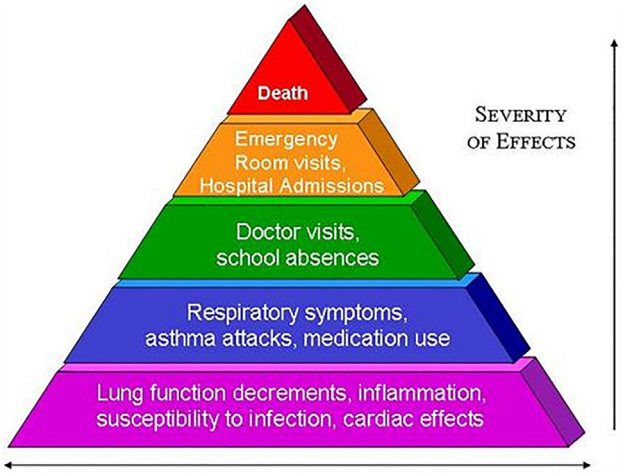

Fig. 1. Pyramid of Ambient Air Pollution Adverse Human Health Effects (courtesy of George Thurston, NYU).

Within this review we will focus on the integration of omic markers in epidemiological studies. The development of high-resolution and high-throughput technologies interrogating multiple -omics (such as epigenomics, transcriptomics, proteomics, adductomics and metabolomics) yields an unprecedented perspective into the impact of the environment in its widest sense on disease, for example by identifying biomarkers of exposure, or early molecular events in the pathways leading to disease. Incorporation of validated biomarkers in population studies has the potential to strengthen causal inferences by offering multi-level evidence, whilst highlighting the importance of exposure timing, duration, intensity, the reversibility of the observed changes and the influence of individual susceptibility. More generally, features of an exposome approach include combining experimental studies with epidemiological observations, and interdisciplinarity.

The review has two limitations: it is mainly focused on research that took place in Europe in recent years, and thus does not incorporate new international initiatives that are still on-going. For this we refer to other review papers (Vermeulen et al., 2020). And, second, it does not fully address all the new tools for the measurement of the external exposome (including sensors), being more focused on molecular approaches.

3. Beyond hard and rare outcomes: Molecular changes as outcomes

So far epidemiology has dealt with hard and relatively rare outcomes like cancer or cardiovascular diseases (CVD), that occur in later life, but non-communicable diseases (NCD) have very long latency periods (decades), which makes classical epidemiological studies

unsuitable for early preventive measures. However, the population is affected at younger ages by a multiplicity of health problems that go far beyond hard outcomes, but which themselves may contribute to chronic disease progression (Fig. 1). These range from exposures to air pollution during childhood, or poor diet resulting in sub-optimal development, to the impacts of stress on young or very young workers. The latter may be due to changes in the job market, such as the evolution of the "gig economy", with its associated job instability and shift work. These external environmental stresses may be associated with altered immune status and oxidative stress, manifesting metabolic and transcriptional responses in adverse outcome pathways linked to disease, or features of accelerated ageing (as will be discussed later). Identification of these early molecular changes by 'omic technologies offers the possibility of identifying novel response biomarkers that can be employed much earlier in the population surveillance for hazard identification and risk assessment (National Academies of Sciences Engineering and Medicine, 2017). For example, epidemiological case-control studies have suggested an association between exposure disinfection by-products (DBP), present in drinking water and swimming pools, and cancers of the colon and bladder, but causality is difficult to establish because of misclassification of exposures and confounding (Grellier et al., 2015). As proof-of-principle, we performed metabolomics on blood samples and examined RNA changes in an experimental setting involving a group of swimmers, in which exposure levels of DBPs were measured in exhaled breath (van Veldhoven et al., 2018). In this study a total of 6471 metabolic features were detected in blood, with 293 features associated with at least one DBP detected in the swimmers exhaled breath. From the 293 significant features, the molecular identity of 20 features was established, corresponding to 13 metabolites including compounds in the tryptophan metabolism pathway (after adjustment for physical activity). When we measured RNA transcripts and miRNA expression, we identified 1778 genes and 23 microRNAs that were significantly associated with exposure to at least one DBP. After eliminating previously reported transcripts associated with physical activity, many hits remained associated with DBP exposure. Among these, 9 had been previously linked with bladder and 31 with colon cancer. Concordant microRNA/mRNA expressions were identified in association with DBP exposure for target genes RCOR1 and TLR4 (Espín-Pérez et al., 2018a). Other examples of this approach can be found in the literature, such as metabolic profiling of trichloroethylene exposure (Walker et al., 2016) and multiple pollutants (Maitre et al., 2018). Whether these early molecular changes are predictive of later disease still needs to be confirmed, but the early findings are encouraging, clearly demonstrating that environmental exposures can leave molecular marks that are detectable for application in epidemiological studies. Other examples of the use of molecular targets of exposure in experimental settings are described below in relation to air pollution. Later we will expand on the "meet-in-the-middle" principle that has been proposed to enhance the association of early molecular signatures of adverse responses to environmental stressor to the development of chronic disease.

4. Causality

4.1. Exercises in triangulation

Observational biomedical research often struggles to identify genuine causal relationships, especially when exposures to potential causal agents are highly correlated. One proposal to strengthen causal inference in biomedicine is through "triangulation" (Lawlor et al., 2016; Munafo and Davey Smith, 2018). This represents the "strategic use of multiple approaches to address one question", with each approach employing unrelated assumptions, and having different strength and weaknesses. The logic for this method to improve causal inference, is that if results agree across multiple and contrasting methodologies, they are less likely to be artefactual (Lawlor et al., 2016). Ideally, results from more than two approaches, which have different and unrelated key sources of potential biases, are compared (Lawlor et al., 2016). An additional feature is the mixing of qualitative and quantitative types of evidence that bring breadth and depth to the same research question. Triangulation has become a popular method in epidemiology by using genetic instrumental variables, i.e. through "Mendelian randomization", which is highlighted in our first example below. In particular, 2-sample Mendelian randomisation, where the association of genetic variants with the exposure and outcome can be investigated in separate populations, has provided an unprecedented opportunity to study causal effects of variables and clinical outcomes that are not available in the same data collection. Often, the same investigation in conventional observational settings would have taken years. However, Mendelian randomization is not the only example of triangulation, and we propose two others that pertain to exposome research.

4.1.1. First example: Mendelian randomization

Mendelian randomization (MR) is analogous to a randomized trial; in fact, it is a natural experiment with observational data based on the use of gene variants as instrumental variables. Gene variants are a special case of instrumental variables since they are randomly assorted at meiosis, i.e. they are neither affected by confounders, nor are subject to reverse causation (genetic information is constant over life), in contrast with many exposure variables that are not inherited. An association between a genetic variant that is tightly correlated with the risk factor and the outcome could be indicative of a possible causal effect of the risk factor on the outcome (Fig. 2). An example of MR applied to the impact of socio-economic status on CVD risk has been

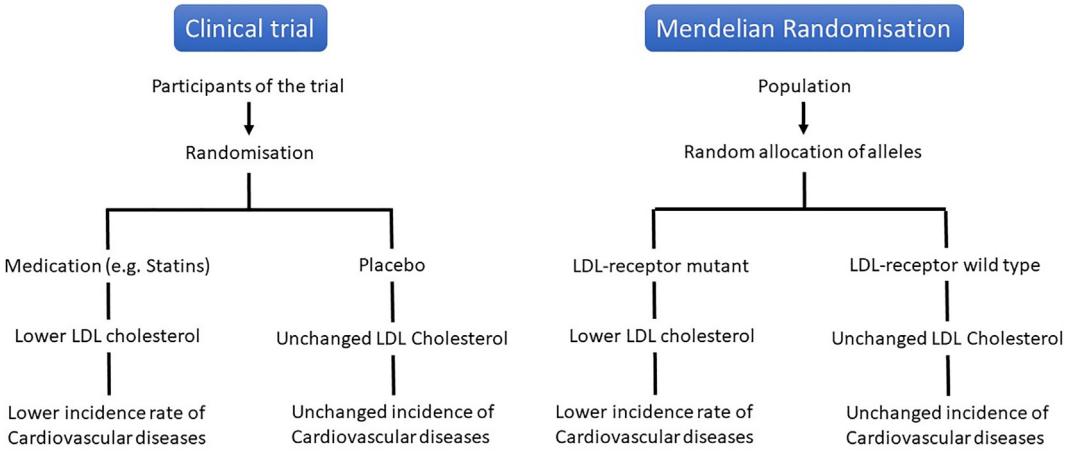

Fig. 2. Schematic representation of mendelian randomization.

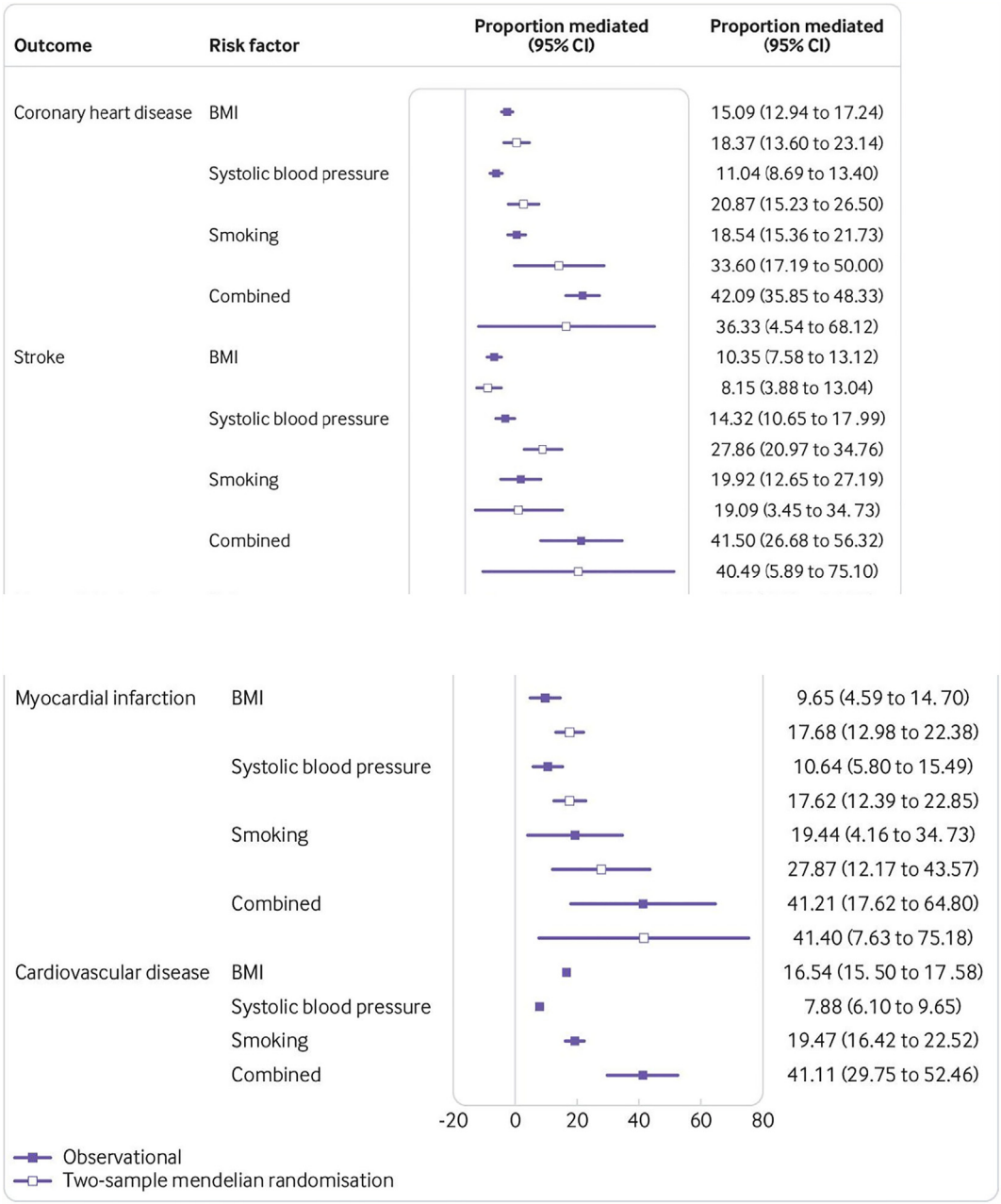

Fig. 3. Effect of education on CVD explained by the risk factors. Around 40% of the effect of education on CVD is explained by the three risk factors combined, both in observational and mendelian randomisation analyses. Over half of the effects of education remain unexplained in these analyses.

published using a two-stage MR method (Carter et al., 2019). Gene variants associated with education were used as instrumental variable (Fig. 3). In this study a high degree of consistency was seen between the observational study and the MR approach, with around 40% of the effect of education on CVD explained by three major risk factors combined (smoking, BMI and blood pressure), in both the observational and mendelian randomisation analyses. This figure is consistent with the previous investigations on the role of socio-economic position and health, in particular from the Lifepath consortium (Stringhini et al., 2017).

In addition to Mendelian randomization based on exposures and outcomes (plus genetic instrumental variables), future exposome research can also exploit the results of GWAS done with metabolomics, proteomics (Sun et al, 2019), microRNAs (Nikpay et al., 2019) and eQTL data in GTeX, all resources that can be used in MR analysis.

4.1.2. *Second example: Environmental genomics*

The second example is again taken from genomics, but this time refers to acquired mutational spectra, not inherited variants. There is no doubt that tobacco smoke is carcinogenic to humans. What is striking about the vast body of research on the adverse effects of tobacco is the great consistency across layers of evidence: from toxicity observed in cell culture to in vivo effects in experimental animals (including pet animals exposed to second-hand smoke), to human biomarker and epidemiological studies. Fig. 4 (Alexandrov et al., 2016) shows that the biopsies of lung or larynx cancer patients who were smokers show a spectrum of mutations that is different from that observed in other organs also affected by tobacco carcinogenesis, and that the spectrum in the lung, in particular, is almost entirely overlapping with the mutations observed in cell cultures in vitro treated with benzo(a)pyrene. This example serves several purposes: (a) it demonstrates the *triangulation* of evidence on mutational spectra in the lung and larynx cancers

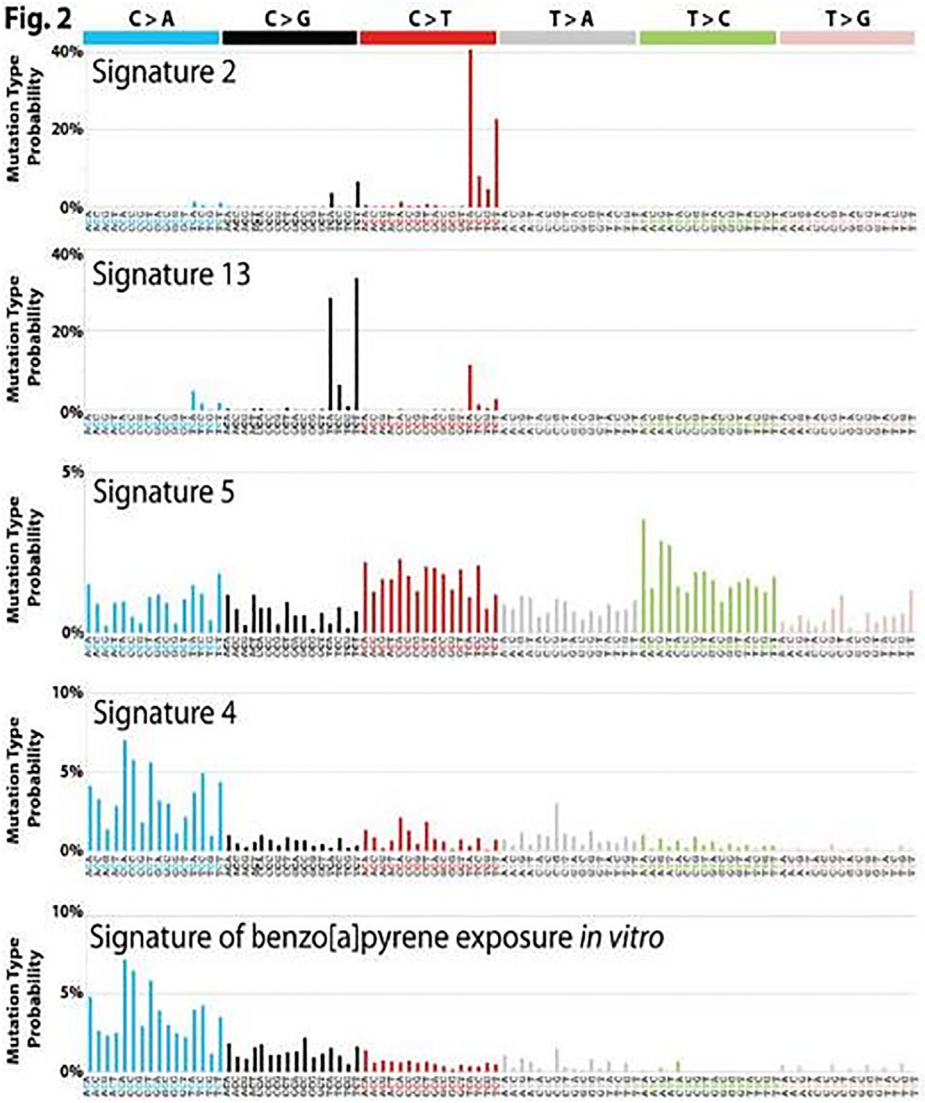

Fig. 4. Environmental genomics: mutational fingerprints of smoking suggest that lung cancer and larynx cancer biopsies from smokers have a mutational fingerprint that is very similar to that left in calls in culture by benzo(a)pyrene (Alexandrov et al., 2016).

in smokers with evidence obtained in vitro in cells treated with benzo (a)pyrene, demonstrating the same mutational signature; (b) it suggests that benzo(a)pyrene is involved in some cancer sites associated with smoking, but not in all of them (in fact, there is evidence that aromatic amines are involved in bladder cancer); and finally (c) it provides a nice example of how biomarkers (in this case derived from genomic investigations) can contribute to the understanding of mechanisms, since the mutational signature complements a wealth of information on the ability of benzo(a)pyrene to induce cancer in animals, and its ability to form DNA adducts.

4.1.3. *Third example: multi-layered approach to social inequalities in health*

Causality is difficult to assess in biological sciences and even more so in social sciences, especially when attempting to address biography-to-biology transition. From the Lifepath consortium we have reported the association of socio-economic disadvantage with: (a) long-term health outcomes, before and after taking risk factors into account; (b) biological intermediaries that increase susceptibility to disease, such as childhood obesity; (c) intermediate circulating biomarkers and omic-based measurements (transcriptomics, DNA methylation, inflammatory proteins, allostatic load); and (d) immunity (Vineis et al., 2019). These analyses have been performed in several cohort studies, countries, and at different stages of the life course in up to 1.7 million subjects. This approach was taken to test the assumption that each layer is characterized by different types of bias and confounding, and that consistency across layers reinforces causality. The findings from this study showed associations of social disparities with unfavourable health outcomes at all levels, spanning inflammatory biomarkers, DNA or RNA-based markers, infection, indicators of physical functioning and mortality. Although each of these associations was subject to a different set of confounders, a dose-response relationship was nevertheless consistently observed, thus showing the power of the multi-layered approach adopted for this work (Vineis et al., 2019).

4.2. *The meet-in-the-middle concept*

Investigations on the effects of air pollution and other environmental pollutants in the past either considered biomarkers as an outcome of exposure (usually only reflective of relatively short-term exposure periods), or – alternatively - examined chronic health outcomes in relation to long-term exposures. None has used the “meet-in-the-middle” (MITM) approach we have proposed to bridge the gulf between early biomarkers of response to the development of chronic disease. The MITM approach consists in measuring intermediate biomarkers

(often in agnostic 'omic investigations) and relating them retrospectively to measurements of external exposure, and prospectively to the ultimate health outcome. In this way we create a potential pathway to disease that can be further validated. In addition, this approach refines one of Bradford Hill's guidelines for causality assessment in epidemiology, i.e. biological plausibility. MITM is only feasible now through parallel technological developments in omics, improved methods for exposure assessment, and by the existence of long-term longitudinal population cohorts with biological samples stored over many years.

An example of the application of the MITM approach is the work on cerebro-cardiovascular disease (CCVD) performed as part of the EXPOsOMICs network. All newly diagnosed cases of CCVD and revascularization, that arose during 12.2 years of follow-up on average were identified in a large cohort, with biomarker analyses (inflammatory proteins, genome-wide DNA methylation, metabolites) performed using prospectively collected and archived blood samples. Using these samples, we demonstrated enrichment of altered DNA methylation in "reactive oxygen species/Glutathione/Cytotoxic granules" and "Cytokine signalling" pathways related genes, associated with both air pollution and CCVD risk. Interleukin-17 was associated with higher exposure to NO_2, NO_x, and CCVD risk (OR = 1.79; CI 1.04–3.11, when comparing extreme tertiles) (Fiorito et al., 2018). In the subsequent metabolomics investigation, that was extended to asthma (Jeong et al., 2018), we observed perturbation of the linoleate metabolism pathway, that was associated with both air pollution exposure and the two disease outcomes, CCVD and asthma. Linoleate is involved in the modulation of IL-8, and thus also in the immune response. In summary, we identified both epigenetic and metabolomic signals that were intermediate between air pollution exposure and disease outcomes, an example of MITM and of pathways to disease that deserve further exploration.

5. Time matters: Establishing temporality in pathways leading to disease

In the EXPOsOMICs consortium we also tried to agnostically identify specific molecular changes associated with air pollution exposures. Table 1 shows the main results, for single omic signals identified to date. Several signals replicated across studies: IL-8 in three studies (including linoleate, that is involved in IL-8 activation), the carnitine shuttle in three studies, and phosphatidylcholines in two studies. These signals suggest that pathways commonly perturbed after exposure to air pollution involve immunity, inflammation and oxidative stress. In contrast, DNA methylation results did not replicate across studies. Only few genes have been found so far to be consistently hypo- or hypermethylated in relation to environmental exposures, except for tobacco smoke (notably hypomethylation of the aryl-hydrocarbon receptor repressor, Guida et al., 2015).

It is likely that the pathways associated with disease aetiology and progression involve multiple molecules, and that perturbations in these response biomarkers are organized in a temporal sequence. A research program within the exposome community is currently trying to reconcile the exposure-associated pathways with "hallmarks" of disease or toxicant exposures. For example, Lopez-Otin et al. (2013) have proposed nine 'hallmarks of ageing'. Similarly, hallmarks of cancer have been put forward, displaying a partial overlap with those for ageing (Hanahan and Weinberg, 2011), and also similar "key characteristics of carcinogens" have been proposed (Smith et al, 2016c). When we examined signals corresponding to hallmarks of cancer or key characteristics of carcinogens in the EXPOsOMICs project (Table 1), we detected significant perturbation of the following response categories: miRNA expression; cytokine signalling; reactive oxygen species (oxidative stress); glycosphingolipids (apoptosis, cell growth, senescence, cell cycle control); NOTCH1 (cell cycle control); and carnitine shuttle and acylcarnitines (implicates as a response to oxidative stress). Immunity, inflammation, oxidative stress and miRNA are also involved in respiratory and cardiovascular diseases. Oxidative stress pathways are compatible with our observations on the oxidative potential of air pollutants as measured on filter associated particulate matter (Gulliver et al., 2018).

However, the concept of hallmarks remains inadequate because it does not specify their temporal sequence. For example, hallmarks of cancer in the original concept put forward by Hanahan and Weinberg are cross-sectional, i.e. they are a description of the cancer phenotype rather than a reconstruction of the causal sequence of events (Demetriou et al., 2018).

6. A new solution for an old problem: Biological ageing and socio-economic position

Anecdotal evidence has been put forward on the discrepancy between chronological age and biological age, i.e. how individuals of the same age look and perform differently. The problem was already described by Benjamin (1947) who proposed a list of early indicators of biological age based on his work. The list is a mixed bag of susceptibility factors (including metabolism), plus disease outcomes. It's now possible to deploy a much larger number of indicators to understand the discrepancy between chronological age and biological age, including the availability of large cohorts and a broad array of biomarkers. Once again, omic approaches are particularly useful to develop new biological age markers because they allow agnostic, i.e. hypothesis-free investigations. One marker that has been extensively investigated in relation to ageing is telomere length. Telomeres are the repetitive nucleotide sequences capping the ends of eukaryotic chromosomes that maintain chromosomal integrity. Telomere length decreases with aging, resulting from the rounds of cell division, but it is also impacted by the biochemical environment. Telomere shortening has important functional consequences: short telomeres lead to genomic instability and cellular senescence (e.g. short telomeres in leukocytes lead to the secretion of pro-inflammatory cytokines). The evidence on the ability of TL to predict non-communicable diseases is equivocal, and TL itself is hard to measure, with substantial technical variability. The studies on socio-economic position and TL tend to show an association between telomere shortening and a low SE position, but there are still relatively few investigations in this area (Robertson et al., 2013).

Several studies have been published on another omic marker, epigenetic ageing. The recent literature describes two different mechanisms contributing to age-related DNA methylation (DNAm) changes: 'epigenetic drift' and 'epigenetic clock', that sometimes are used as synonyms even if they describe different molecular mechanisms (Gentilini et al., 2015). Although both are related to aging, epigenetic drift represents the trend of increasing global DNAm variability over time not involving specific DNA regions. On the contrary, the epigenetic clock refers to specific CpG sites whose DNAm levels constantly increase or decrease during aging and can be used to predict chronological age with high accuracy (Jylhävä et al., 2017). Accordingly, two popular measures of epigenetic clocks have been developed, and the concept of epigenetic aging acceleration (EAA) has been introduced as the difference between predicted DNAm age and chronological age through Horvath EAA (Horvath, 2013) and Hannum EAA (Hannum et al., 2013) clocks. Epigenetic AA has been associated with all-cause mortality, cancer risk/incidence and neurodegenerative disorders, as well as non-communicable disease risk factors like obesity, poor physical activity, unhealthy diet, cumulative lifetime stress and infections (Declerck and Vanden Berghe, 2018). Recently, Levine and colleagues introduced a 'next generation epigenetic clock' that is based on a set of CpGs associated with a complex of clinical measures of individuals' "phenotypic age" (Levine et al., 2018), which is thought to represent the physical manifestation of one's biological age (Ferrucci et al., 2018). Levine EAA strongly outperforms previous measures with regard to prediction of a variety of aging outcomes, including all-cause mortality,

Table 1
Main findings (FDR significance) of EXPOsOMICs studies by category (Mostafavi et al., 2018, 2015; Vineis et al., 2017; Fiorito et al., 2018; Jeong et al., 2018; Espin-Perez et al., 2018b; van Veldhoven et al., 2019; Plusquin et al., 2018).

Study and reference	Pollutant	Omics Approach	Molecules
Experimental short term studies			
Oxford Street Study	Traffic related air pollution	miRNome	20 miRNA hits in the Oxford Street UK cohort and 73 miRNA hits in the TAPAS cohort. Common affected miRNAs: Hsa-miR-197-3p, hsa-miR-29a-3p, hsa-miR-15a-5p, hsa-miR-16-5p and hsa-miR-92a-3p.
Oxford Street Study	NO_2	Metabolome	Phenylalanine Caffeine Acyl-carnitine (various molecules): Acyl-carnitine (6:0-OH) (1), Acyl-carnitine (6:0-OH) (2), Acyl-carnitine (phenyl-acetyl carnitine), Acyl-carnitine (10:3) (1), Acyl-carnitine (10:2) (2), Acyl-carnitine (11:1),
TAPAS Study (25)	$PM_{2.5}$	Metabolome	Phosphatydilcholines: PC(16:0) PC(20:2)
Personal exposure monitoring panels			
Personal exposure monitoring in EXPOsOMICs	$PM_{2.5}$	DNA Methylome	Methylation of KNDC1 and FAM50B
Personal exposure monitoring in EXPOsOMICs	$PM_{2.5}$	Proteome	Increase in CCL22 serum concentration
Personal exposure monitoring in EXPOsOMICs (unpublished)	$PM_{2.5}$	miRNome	61 miRNA
Long-term studies			
Cerebro-Cardiovascular Disease Risk in EPIC	NO_2	Proteome	IL-8, IL-17, EGF, and eotaxin plasma concentrations
Cerebro-Cardiovascular Disease Risk in EPIC	$PM_{2.5}$	DNA Methylome	Enriched pathways: "Cytokine Signalling" and "Reactive Oxygen Species/Glutathione/Cytotoxic granules"
Cerebro-Cardiovascular Disease Risk in EPIC	NO_2	DNA Methylome	Enriched pathway: "Cytokine Signalling"
Asthma and Cerebro-Cardiovascular Disease Risk in EPIC and SAPALDIA	$PM_{2.5}$	Metabolome	Pathways associated to PM2.5 in air pollution in both SAPALDIA and EPIC Italy: Linoleate metabolism (regulator of IL-8) (confirmed molecule(s): Linoleate) Fatty acid activation (confirmed molecule(s): Linoleate, Octanoic acid)
Asthma and Cerebro-Cardiovascular Disease Risk in EPIC and SAPALDIA	UFP	Metabolome	Pathways associated to UFP in air pollution in both SAPALDIA and EPIC Italy: Linoleate metabolism (regulator of IL-8) (confirmed molecule(s): Linoleate) Glycerophospholipid metabolism (confirmed molecule(s): Linoleate) Glycosphingolipid metabolism (confirmed molecule(s): Sphingosine)
Asthma and Cerebro-Cardiovascular Disease Risk in EPIC and SAPALDIA	NO_2	Metabolome	Pathways associated to NO_2 in air pollution in both SAPALDIA and EPIC Italy: Carnitine shuttle (confirmed molecule(s): L-Carnitine) Pyrimidine metabolism
Children of ALSPAC and EXPOsOMICs Birth Cohort Consortium	PM_{10}	DNA Methylome	Enriched pathways: GABA-ergic synapse p53 signaling NOTCH1signalling
ESCAPE cohorts	Traffic related air pollution (NOx)	Proteome	IL-8

cancers, disease-free survival and physical functioning. Fig. 5 shows results from a study performed within the Lifepath consortium in which we meta-analyzed 18 cohorts with data on risk factors for NCD, socio-economic position and methylation measurements. Socio-economic position was associated (with a dose-response gradient) with all of the age acceleration markers, but particularly with Levine's. Also, the strength of association was similar (though generally smaller) to what has been observed for the main risk factors for NCDs (Fiorito et al., 2019).

Wild originally proposed aging to form part of the internal domain of the exposome. Further research in this area, including the continued development of aging biomarkers (Robinson et al., 2020; Lu et al., 2019) may elucidate how multiple exposures can act cumulatively to "accelerate" the aging process and increase risk of a diverse range of age-associated diseases, and equally how aging may provide the "receptive environment" required for the onset of environmentally determined disease (Sierra, 2016).

7. Complex, multiple and concurrent exposures

7.1. Improving exposure assessment – The external exposome

While effective chemical biomarkers of internal dose represent the holy grail of exposure assessment, this is (a) often unavailable at the population level, (b) not simplistically relatable to commonly employed pollutant metrics used to interrogate health impacts, such as particulate mass, or number concentration, and (c) difficult to relate to the impacts of long-term exposure over many decades. In the current epidemiological literature on the health impacts of poor air quality several separate, though often complementary approaches have been adopted: (a) most simplistically using measures of distance from a point source, i.e. distance to major roads, or some other proxy of traffic exposure; (b) employing short to long-term measurements of air pollution made at monitoring sites, usually situated away from point sources to reflect the average population exposure; (c) enhanced modelling approaches to estimate exposures over discrete periods and geographical domains; and (d) increasingly using personal air pollution monitoring devices. In the ESCAPE project standardized air pollution models for the major criterion pollutants were produced across the European domain, based on Land Use Regression techniques (LUR) (de Hoogh et al., 2013),

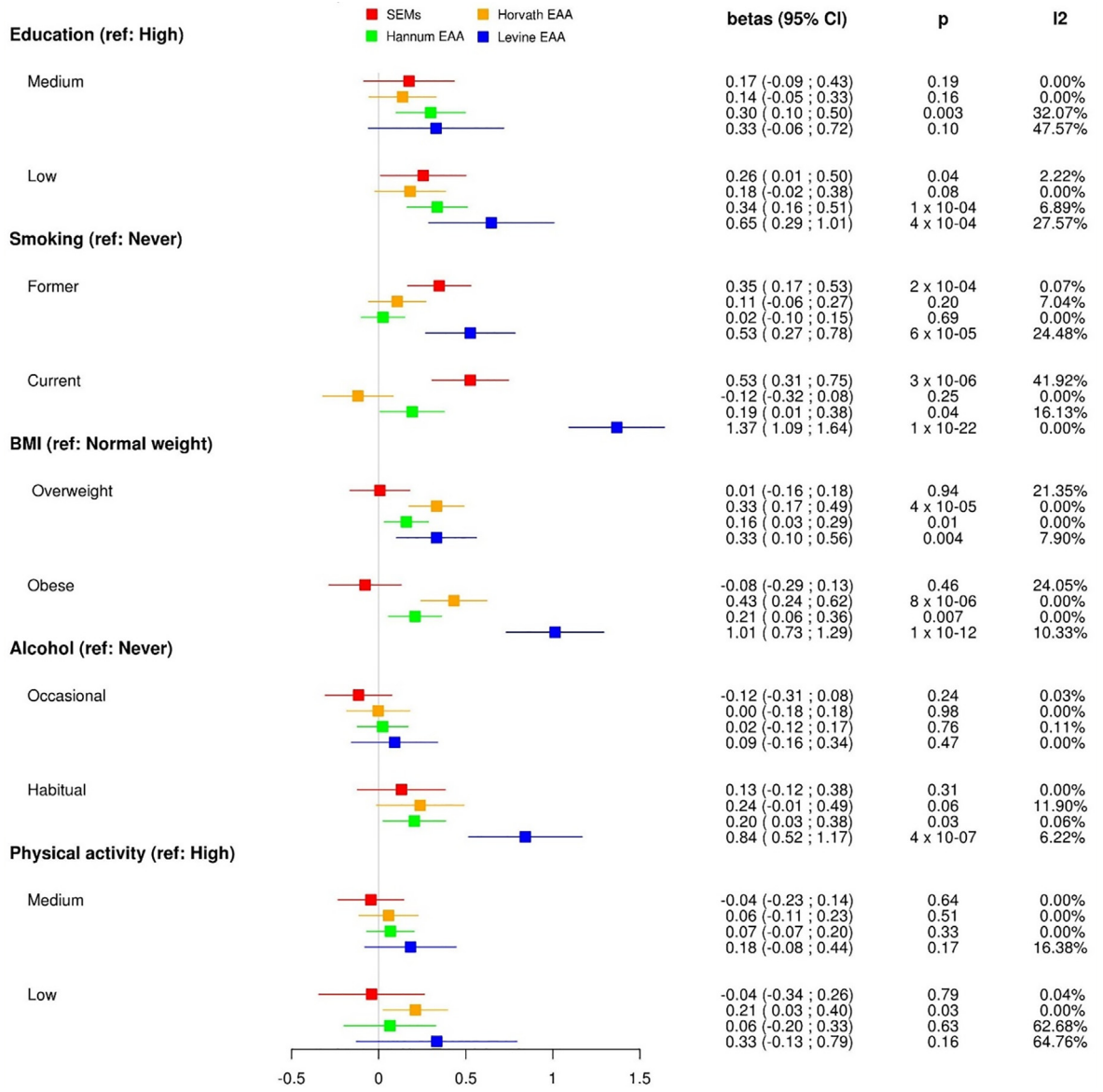

Fig. 5. Meta-analysis of 16,000 people across 18 cohorts), reporting a comparison of effects of leading NCD risk factors on epigenetic ageing. Horvath, 2015 (orange), Hannum et al., 2013 (green) and Levine et al., 2018 (blue) measures of epigenetic age acceleration and stochastic epigenetic mutations (SEMs) (red) were assessed. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

which were then augmented through the EXPOsOMICs project to include models for PM oxidative potential (a measure of their intrinsic pro-oxidant characteristics) (Gulliver et al., 2018) and ultrafine particles (van Nunen et al., 2017), and by others for $PM_{2.5}$ components (Jedynska et al., 2014). These models have been enhanced by the incorporation of satellite observations and dispersion model estimates to provide higher resolution (100 × 100 m) and more temporally resolved exposure estimates for Western Europe (de Hoogh et al., 2018). High resolution models (at a spatial scale of 20x20m) based on data from local atmospheric emission inventories, coupled to meteorological and dispersion models are also available (Beevers et al., 2013), that allow highly resolved exposure estimation for the population and forward air pollution scenario evaluation, i.e. the impact of emission mitigation policies or climate changes actions on projected air quality (Williams et al., 2018). The coupling of these emission-based models to the Community Multiscale Air Quality Model (CMAQ) has further enhanced the estimates to provide more information on a broader range of pollutant species over finer temporal scales (Beevers et al., 2012) and this model has been further developed to reflect exposures within mobile populations, by considering data from personal monitoring campaigns and measurements within discrete transport environments and in the home (Smith et al., 2016a, 2016b). This later development is highly relevant to a consideration of the external exposome, as it moves away from the purely location-based assessment of air pollution exposure, to an individualized assessment of population groups. This has the potential to take learning gained from personal monitoring campaigns in relatively small groups of individuals and apply them to the larger population for epidemiological assessment, providing information to limit the extent of exposure misclassification for pollutants that vary markedly over fine spatial scales, such as particle number concentration and NO_2. The assessment of personal exposures themselves remains highly relevant within the quasi-experimental setting, especially in relating short term physiologic responses and changes in the internal exposome to the actual exposures experienced by volunteers, as exemplified in the work performed as part of the Oxford Street II study (Sinharay et al., 2018). Whilst this is a burgeoning area of research it needs to be acknowledged that all of the technological solutions available for personalized monitoring are not equal and extreme care

needs to be taken in presenting results, especially for difficult to measure, or novel chemical species (Chatzidiakou et al., 2019).

7.2. What is in a mixture

We are all exposed to mixtures, in food, water and air. The contribution of mixtures, either in the round, or in terms of 'key' toxic components, to our exposure history and individual disease biography remains highly problematic. In the case of food, disentangling the health effects of single nutrients has always been a challenge, and even more so for complex effects of dietary patterns or for chemical contaminants of food. In the case of air pollution most studies have focused on the criterion, regulated air pollutants (i.e. $PM_{2.5}$, PM_{10}, NO_2, O_3, benzene, etc), or novel metrics for which there is an emerging literature suggesting associations with adverse health (Ultrafine Particles, oxidative potential etc). This is already a massive oversimplification as air pollution is in fact a mixture of thousands of chemicals, many of which are highly correlated if they are derived from a common source. Whilst the consensus in the literature is that $PM_{2.5}$ is the most harmful component, it is itself compositionally highly variable and there are ongoing discussions over the most harmful components: the smaller particle fractions within it (represented by elemental carbon and UFPs), the primary or secondary organic aerosol, metals such as Nickel or Cadmium, or indeed (within urban settings with a high proportion of diesel vehicles) the relative toxicity of $PM_{2.5}$ versus NO_2. Here we will consider NO_2 in particular, as *"previous reviews of both the toxicologic and epidemiologic literature have concluded that the evidence was not sufficient to infer a causal relationship between long-term exposure to NO_2 and mortality"*, due to a *"lack of consistency in study findings and concerns relating to potential confounding by co-pollutants, especially particles in traffic exhaust"* (Atkinson et al., 2018).

Can an exposome approach address this question? In fact, the question can be decomposed into a few elements: Does NO_2 at ambient levels have any detectable toxicity on the human lung? Which aspects/components of combustion mixtures are responsible for the adverse health effects observed in epidemiological studies? Is NO_2 able to synergise with other pollutants e.g. PM/allergens (act as an effect modifier)? Fig. 6 shows theoretical pathways - including different exposures - that can lead from NO_2 exposure to asthma, and can be interpreted as a source of "Adverse Outcome Pathways (AOP) leading to asthma in humans. These pathways are only partially known, and their components can only be integrated with an exposome approach. In the EXPOsOMICs study we considered air pollutant exposures in volunteers exposed in a randomized cross-over trial to a high diesel site (Oxford Street, London) versus a lower pollution location in Central London (Hyde Park), with detailed assessment of exposures by personal monitoring (Sinharay et al., 2018). Both circulating RNA (mRNA and miRNA, the latter involved in post-transcriptional gene expression modulation) (Espín-Pérez et al., 2018a) and metabolomic profiles (van Veldhoven et al., 2019) derived from these subjects provided signals that correlated with different components of air pollution aerosol at these locations with little apparent overlap, suggesting that each pollutant, though highly correlated in ambient air, may follow a different metabolic or molecular pathways to exert their effects upon inhalation (Fig. 7). This is a critical early observation in that it demonstrates that the acute biologic responses of the individual upon exposure to complex pollutant mixtures may provide discriminatory signatures for key toxicological components in the air. This approach, together with data from comparative in vitro and in vivo toxicity testing, therefore offers the potential to identify the most harmful components of the ambient aerosol, moving beyond the simple reliance on ambient concentrations of PM, to a more evolved consideration of chemical components, which will aid in the development of focused policies to protect public health.

Apart from these examples, the issue of mixtures has been addressed in a more comprehensive way by others, such as a EU report (Bopp et al., 2019). A further discussion would be on how can the overall effect of multiple exposures be assessed on a biological system. Unfortunately, this aspect is still largely underdeveloped in exposome research.

8. Developing new tools: Advancements in metabolomics and adductomics

New tools have recently emerged in environmental research. In general, there has been a rapid development of omic technologies, including for example high resolution mass spectrometry-based

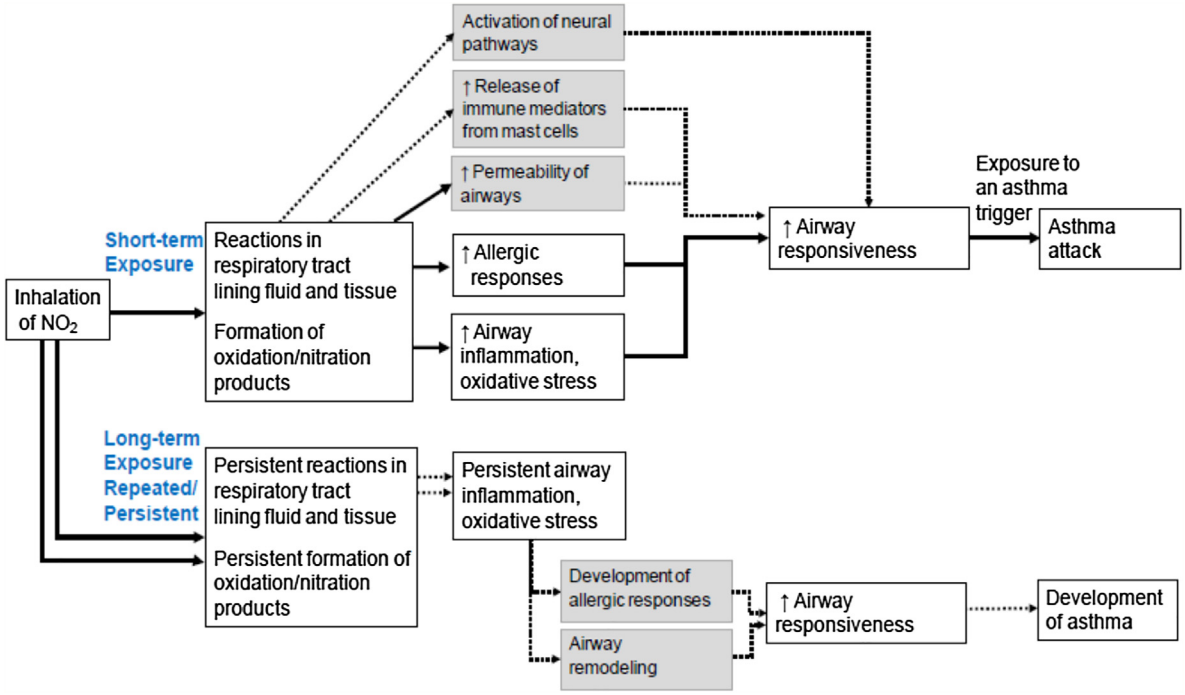

Fig. 6. A theoretical pathway linking exposure to NO2 to asthma.

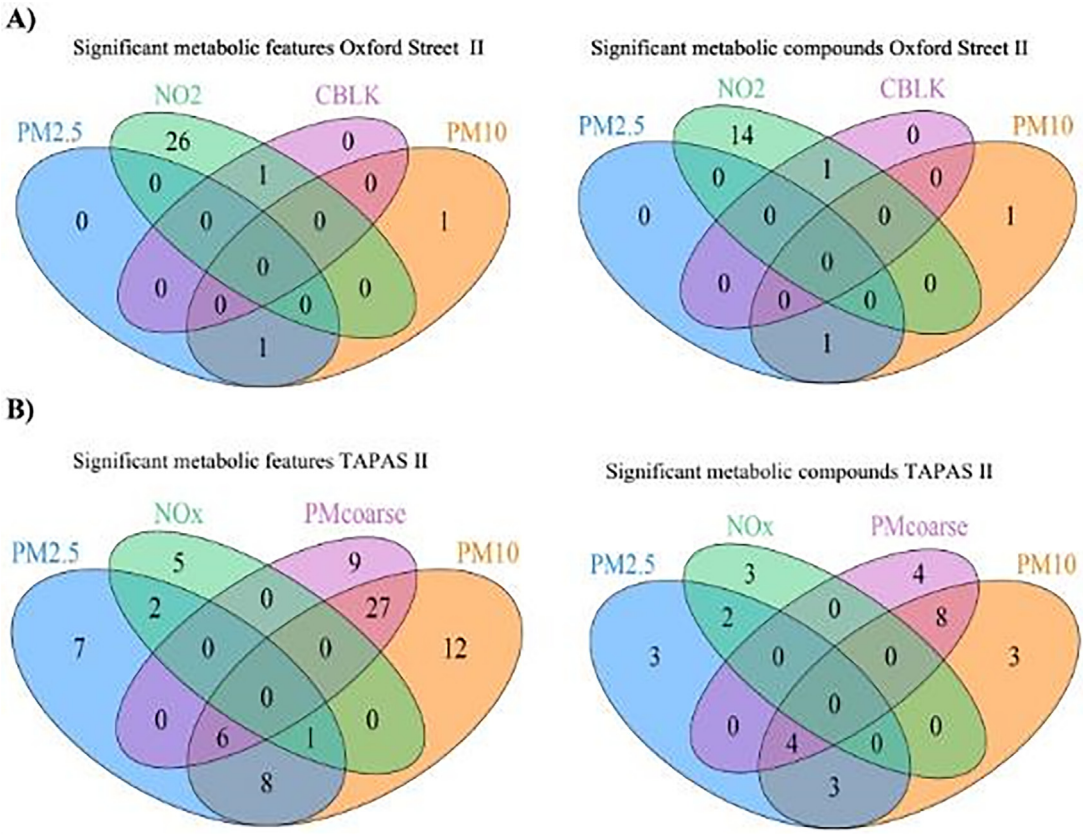

Fig. 7. Associations between metabolic features/compounds in two randomized cross-over studies in the EXPOsOMICs project.

metabolomics or epigenetic chips. As demonstrated by past and recent exposome projects, metabolomics has risen as one of the most prominent technologies for exposome research. Current advances in metabolomics are making it possible, not only to detect molecules related to endogenous processes and metabolism, but also allow the detection of exogenous compounds as well as their metabolites. Initially based on targeted approaches, metabolomics is now moving towards the use of untargeted methods as they do not limit the findings to "a priori" selected targets. The development of new sensitive high-resolution mass spectrometers and the advancement in computational methods for data processing have now make it possible to measure over 100,000 molecules in a single sample (Walker et al., 2019), with the potential to go even further to characterise the "million metabolome" (Uppal et al., 2016). The current limitation of untargeted metabolomics lies in the annotation process, which is the bottleneck of data interpretation. Multiple initiatives are arising to improve annotation of metabolic features, notably the construction of databases that will facilitate the identification such as the metabolites database Metlin and the Human Metabolome Database (Wishart et al., 2007; Guijas et al., 2018). The Exposome-Explorer database created by IARC is aiming at creating a database for dietary and pollutant biomarkers that can be measured with metabolomics, and already includes 908 features (IARC, 2020). Recent efforts have also focussed on computational approaches to improve annotation such as the Mummichog algorithm which allows prediction of pathway activity based on untargeted metabolomics data (Chong and Xia, 2018) or the Rpackage xMSannotator, providing annotation by using a multistage clustering algorithm including metabolic pathway associations in the analysis (Uppal et al., 2017).

Other technical advances are explored within the use of complementary chromatography approaches, such as the analysis by liquid chromatography and gas chromatography on the same sample. These platforms provide complementary analytical capabilities which allow for comprehensive exposome studies (Fiehn, 2016; Walker et al., 2019).

As part of the exposome innovations, it is noteworthy to cite another new technology, that is in its infancy, but might become useful in environmental studies - adductomics. Its main purpose is to measure very low levels of electrophiles. Electrophiles are formed *via* metabolism of endogenous and exogenous molecules. They are highly reactive species which have long been suspected of causing cancer and other diseases. Their reactivity and short half-life make them difficult to measure in human samples, thus new technologies have exploited their capabilities to bind with macromolecules such as DNA or Proteins in order to measure their adducts. Adductomics refers to the systematic measurement of all "adducts" (i.e. products of reaction with macromolecules) of a certain class. For the characterization of the exposome, two main technologies have appeared, the measurement of the adducts of human serum albumin (HSA) and the measurement of DNA-adducts. HSA is the most abundant protein in serum, and has a relatively longer half-life compared to electrophiles themselves. Its cysteine 34 site (Cys34) is highly reactive and acts as a scavenger for small electrophiles. Therefore, Cys34-albumin adducts can be used as an internal "sensor" of a multiplicity of external exposures and internal modifications. The method we have been using recently has been developed by Stephen Rappaport's team at UC Berkeley (Li et al., 2011). This new method is based on the use of high resolution mass spectrometry to agnostically detect putative HSA-electrophile adducts in (biobanked) human serum samples, thus obtaining an adductomic profile for each analysed sample (Grigoryan et al., 2016). This technique has been assimilated to omic technologies with the terminology of "Cys34-Adductomics". Promising results have been detected in early epidemiological studies, where adductomic profiles revealed differences between smokers and non-smokers as well as the offspring of smoking and non-smoking mothers (Yano et al., 2019; Grigoryan et al., 2018). In workers occupationally exposed to benzene, five adducts generated from benzene exposure and metabolism were significantly higher than in the general population (Liu et al., 2018) In the EXPOsOMICs project, this technology was

applied to a subset of the Oxford Street II study (n = 50), where adductomic profiles revealed a perturbation in the glutathione pathway for individuals with chronic obstructive pulmonary disease and ischemic heart disease exposed to air pollution (Liu et al., 2018; Preston et al., 2020). More recently, in a study we conducted on lung cancer the adduct of N-acetyl-cysteine (NAC) was negatively associated to the risk of cancer, years before diagnosis. Differences in adductomic profiles suggested an implication of oxidative stress in cancer onset (Dagnino et al., 2019). This is in line with previous work (including experimental) in humans and animals, suggesting that NAC is a promising molecule for the prevention of this type of cancer (Van Schooten et al., 2002). In another study, colon cancer was negatively associated with albumin adducts formed by products of microflora metabolism (Grigoryan et al., 2019). In both examples blood was drawn many years before the onset of cancer, reinforcing the relevance of adducts for disease prediction. All of these findings indicate the promising applications that can arise from Cys34 adductomics applied to the exposome. Improvements in the method will need to focus on increasing sensitivity to enhance the detection of adducts of environmental exposures present at lower concentrations, and automation of data analysis allowing to apply the method to larger sample sizes.

Alternative adductomics technologies are being developed for the agnostic measure of adducts of DNA and have shown promising results in recent exposome studies. DNA adducts can lead to mutations and adverse health outcomes, such as cancer. DNA adducts formation has long been used to evaluate the genotoxicity and potential carcinogenicity of compounds or mixtures (Food and Drug Administration HHS, 2012). Hence, measuring DNA adducts can contribute to the understanding of the mechanisms involved in the carcinogenic effects of environmental exposures. Recent developments in the field of high-resolution mass spectrometry have allowed great progress in the field. Balbo et al. have developed a new method, which allows the untargeted detection of DNA adducts in human serum (Balbo et al., 2014). Using this technique they were able to identify adducts related to exposure to a chemotherapeutic drug and a bacterial toxin (Stornett et al., 2015; Wilson et al., 2019). However, this technology is still very time consuming and has not yet been applied to a larger exposome concept. Another very recent approach has focused on the measure of DNA adducts in urine. This methodology measures targeted and untargeted DNA adducts in a single urine sample, and, similarly to the Cys34-adductomics approach, it has the ability to measure DNA adducts of exogenous and endogenous processes. By applying this method to in vivo and in vitro studies, Cooke et al. have identified DNA adducts of specific exposures (Methanesulfonate and 5 N-nitrosamines), as well as five new DNA adducts of internal processes induced by the exposure (Chang et al., 2018; Cooke et al., 2018). The use of urine as a measurement matrix and the potential of high-throughput of this method indicate it as a good candidate for future exposome studies (Cooke et al., 2019).

Combined use of omic technologies such as metabolomics and adductomics can provide interesting opportunities to characterize the exposome (Petrick et al., 2020).

9. Conclusions

As we have tried to suggest, the world of the exposome is rapidly evolving, though considerable knowledge gaps still need to be filled between the original expectations and the concrete achievements to date. Technological advancements in several omic technologies and in the development of portable sensors will contribute to filling this gap, though original and thoughtful study designs need to be developed within large cohort investigations to ensure the appropriate application of exposomics approaches. Perhaps the most urgent need is currently the setting up of a new generation of cohort studies with improved (and repeated) biosample collection, improved questionnaire data (including social variables), and the deployment of the enhanced exposure assessment methodologies that allow better characterization of individual true environmental exposures.

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 material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.105887.

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