OPINION

‘Omics’ and endocrine-disrupting chemicals — new paths forward

Carmen Messerlian, Rosie M. Martinez, Russ Hauser and Andrea A. Baccarelli

Abstract | The emerging field of omics — large-scale data-rich biological measurements of the genome — provides new opportunities to advance and strengthen research into endocrine-disrupting chemicals (EDCs). Although some EDCs have been associated with adverse health effects in humans, our understanding of their impact remains incomplete. Progress in the field has been primarily limited by our inability to adequately estimate and characterize exposure and identify sensitive and measurable outcomes during windows of vulnerability. Evolving omics technologies in genomics, epigenomics and mitochondriomics have the potential to generate data that enhance exposure assessment to include the exposome — the totality of the lifetime exposure burden — and provide biology-based estimates of individual risks. Applying omics technologies to expand our knowledge of individual risk and susceptibility will augment biological data in the prediction of variability and response to disease, thereby further advancing EDC research. Together, refined exposure characterization and enhanced disease-risk prediction will help to bridge crucial gaps in EDC research and create opportunities to move the field towards a new vision — precision public health.

Omics — defined as fields and technologies that use large-scale data-rich biology[1] — offer promising new methods to advance our understanding of the impact of endocrine-disrupting chemicals (EDCs) on human health[2,3]. EDCs are substances found in our environment, food and everyday consumer products that interfere with the endocrine system by altering the synthesis, release, transport, metabolism or action of endogenous hormones[4–6]. Although we lack toxicity data for most of the countless chemicals produced today, among those that we have studied, many have been identified as endocrine disruptors[7]. EDCs comprise several classes of compounds including bisphenols, ortho-phthalates, polybrominated diphenyl ethers (PBDEs), perfluorinated and polyfluorinated chemicals and polychlorinated biphenyls (PCBs), among others[5]. Animal and human studies have linked EDCs with myriad adverse health effects such as obesity, diabetes mellitus and metabolic disease, female and male reproductive alterations (including infertility), behavioural and developmental disorders, and hormone-sensitive cancers[8]. The estimated economic and health burden associated with exposure to EDCs exceeds US$340 and US$217 billion annually in the USA and Europe, respectively[9].

A recent Endocrine Society Statement called for more mechanistic research and recommended greater consideration of genetic diversity and population differences to expand our knowledge of the health effects of EDCs[8]. By adapting a concept underlying the White House Precision Medicine Initiative[10], we propose that data-driven omics have the potential to bridge the exposure-assessment gap by taking into account individual variability in exposure, dose, biological response and disease risk. In this Opinion article, we discuss three relevant omics approaches — genomics, epigenomics and mitochondriomics — and describe how they can be applied to characterize and estimate exposure and to identify individuals at risk of developing EDC-related diseases and disorders (FIG. 1).

Omics and EDC research

In the past decade, mapping of the human genome has inspired the parallel concept of mapping the ‘exposome’ — the totality of exposure over the life course[11]. One of the inherent challenges in EDC research is the difficulty in accurately measuring exposure during critical sensitive windows or extending exposure assessment to measure the exposome over the life course[11]. For many disease end points that develop over time and/or have long latency and preclinical phases, exposure estimates are needed months, years or even decades before the outcome. Moreover, the frequent lack of biological samples during relevant periods along with the need to study EDCs with short half-lives further complicates exposure assessment. Herein, we consider genomics, epigenomics and mitochondriomics, which have overlapping or emerging roles in relation to EDC research. These technologies might ultimately be applied to create unique molecular ‘fingerprints’ that represent personal exposure, dose, biological response and susceptibility (FIG. 2). By incorporating new large-scale data-rich approaches with more accurate exposure assessment and improved risk prediction, EDC research has the potential to move towards novel and tailored public health prevention strategies or precision public health[12].

Individual-level variability in biological measures generated by each omics approach is the primary determinant of its potential application in EDC research. Although the DNA sequence is static and rarely altered by environmental exposures, including EDCs, which are typically non-mutagenic, the DNA sequence can be used to identify individuals whose genetic background makes them more or less susceptible to adverse effects of environmental chemicals[13–18]. Conversely, expression levels of proteins, metabolites and RNA — used in proteomics, metabolomics and transcriptomics, respectively — are highly sensitive and dynamic metrics and exhibit profound and rapid changes immediately after common, frequent experiences such as eating or physical activity, or following

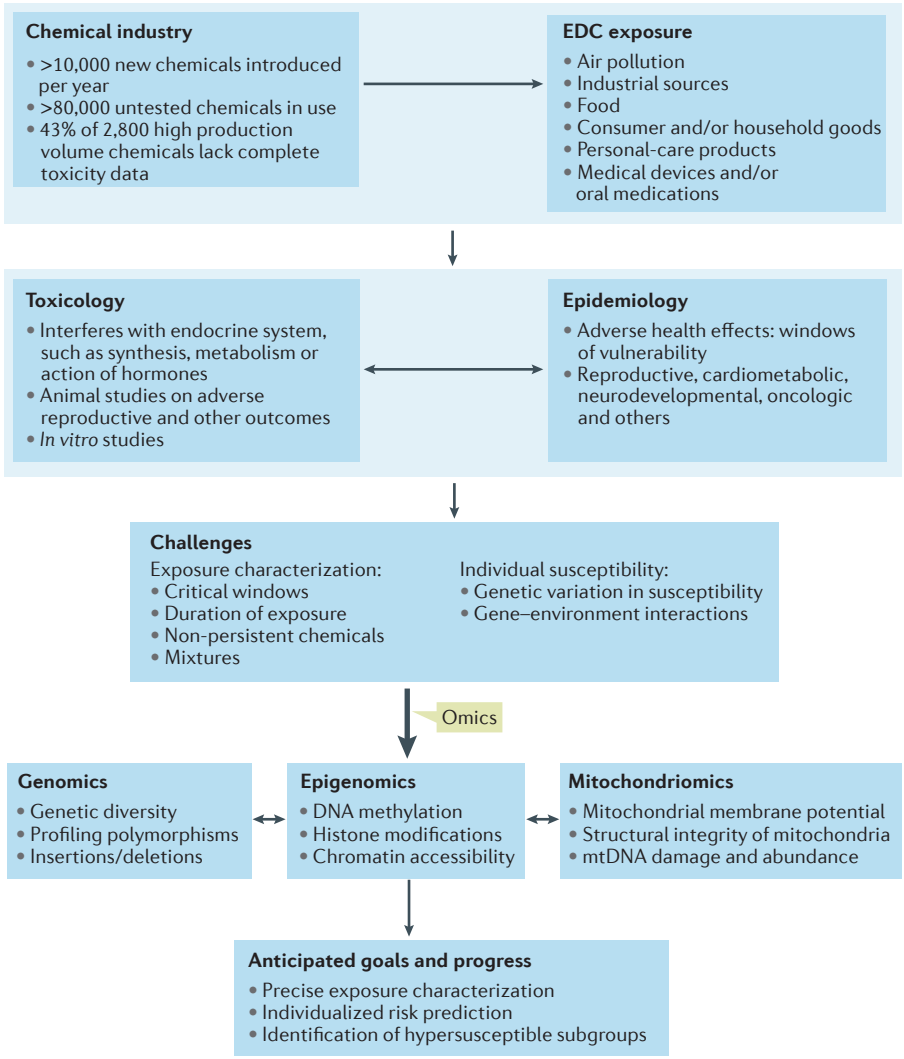

Figure 1 | **Challenges and opportunities of using omics in EDC research.** Schematic representation of the sequence of steps from production of chemicals to endocrine-disrupting chemical (EDC) exposure, from toxicological research to human studies, and from challenges with exposure characterization and risk prediction to the anticipated goals and opportunities of applying genomics, epigenomics and mitochondriomics to the field of EDC research. mtDNA, mitochondrial DNA.

diurnal cycles. Although their high temporal variability might be leveraged to identify the impact of current or recent exposure, they are probably less useful to characterize long-term, prior exposure. In EDC research, we are particularly interested in the long-term exposures and the application of temporally stable omics technologies that accumulate and reflect the influence of these exposures. Finally, other omics have intermediate sensitivity and timing of response to changes such as DNA methylation, an epigenetic mechanism that has been shown to be modified by environmental factors such as EDCs[3]. At least some of these molecular changes can persist over time even if the environmental factor that caused them is removed, thus reflecting a form of biological memory[19–25]. Individual methylation modifications in the DNA methylome have a wide array of temporal variability, ranging from minutes (for example, genes related to immune function need to change expression rapidly to respond to antigen and microbial threats) to years, and some stay stable over the entire lifetime, including the developmental marks that are established *in utero* during embryonic development. This temporal variability demonstrates that DNA methylation has the flexibility to operate over different time frames and can be particularly useful to generate a molecular fingerprint of past exposures.

If exposure to chemicals induces molecular fingerprints that are specific and reflect the dose, duration and time since cessation of exposure, this information could be vital to assess past and cumulative exposures, and be equally important in predicting the risk of future disease. In the following sections, we propose a conceptual model (FIG. 2) to describe how genomics, epigenomics and mitochondriomics can be applied to predict EDC exposure and to identify individuals at risk of EDC-related diseases.

Established omics technologies

Genomics. Genomics is a well-established field that investigates the genome or complete set of DNA of an organism, including all its genes[26]. In past decades, the field has substantially evolved, largely as a result of increased access to technologies that have enabled sequencing the human genome in its entirety, or more commonly of the exome; that is, all the expressed genes in the genome. The genome that each of us inherits is virtually unchanged across our lifespan; however, variation does exist between individuals including that in the form of single-nucleotide polymorphisms (SNPs) and insertions/deletions (INDELs). The genetic variation in SNPs and INDELs among individuals can be measured to generate large-scale data that might serve to signal risk of common chronic diseases[27] and be used as a marker for risk prediction. Gene–environment interactions (GxEs) — the interplay between the environment and the human genome[28] — represent the concept that the genetic make-up of an individual determines their susceptibility or resistance to adverse effects in certain environments[29]. A well-known clinical example of a GxE is that of patients with phenylketonuria, who have a mutation in the gene encoding phenylalanine hydroxylase, the enzyme that metabolizes phenylalanine (an essential amino acid obtained from dietary sources). This genetic mutation leads to an accumulation of high levels of phenylalanine and consequently to neurotoxicity with concomitant mental retardation[30].

The GxE concept can be similarly applied to the EDC context, in which genetic differences might make individuals more susceptible to the effects of environmental chemicals. For example, evidence exists suggesting that genetic polymorphisms modify the antiandrogenic effect of dioxin exposure through differential activation of the aryl hydrocarbon receptor, which results in male reproductive disturbances[29]. In 2016, GxE for PCB exposure and autism risk were investigated[31]. Specifically, the researchers identified genome-wide, PCB-associated

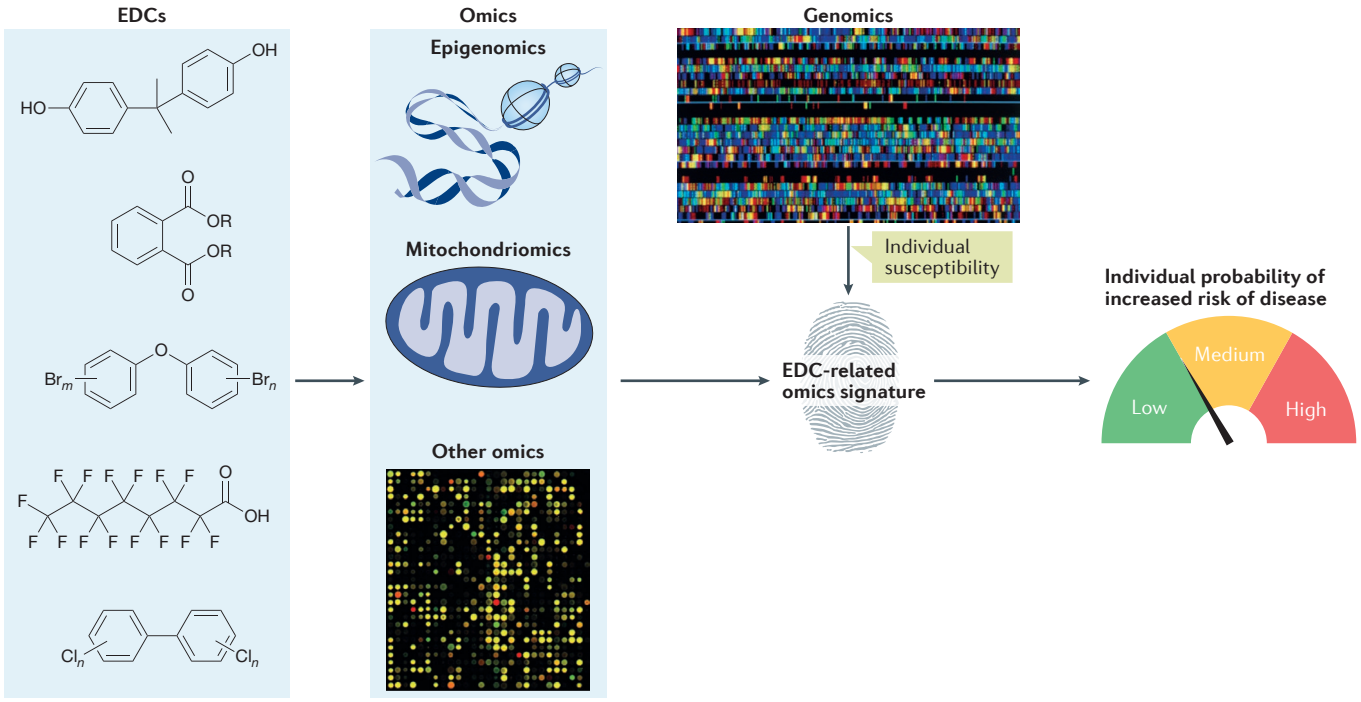

Figure 2 | **Role of omics in identifying molecular fingerprints in EDC research.** Schematic representation of the overall paradigm of using genomics, epigenomics, mitochondriomics and other omics technologies in endocrine-disrupting chemical (EDC) research to create unique molecular 'fingerprints' that represent personal exposure, dose, biological response and susceptibility to EDCs.

methylation changes and used these to investigate genetic interactions; specific genes involved in autism spectrum disorder (ASD) with altered PCB-related methylation were identified. The researchers concluded that gene-specific epigenetic vulnerability to both genetic and environmental challenges are important in identifying different ASD aetiologies and further suggested that such knowledge could be used to develop targeted, individual treatment options[31]. Although the application of GxE to EDC research is still new, this salient example demonstrates the utility of omics methods to identify at-risk groups on the basis of genetic and EDC profiles and highlights the potential of using such data towards precision medicine for an outcome with a large public health burden. However, enthusiasm about GxE studies over the past decade has been replaced with caution and scepticism given the inconsistencies in results and null findings.

As technologies continue to evolve, the strengths of both developed and emerging omics methods will need to be considered in light of their inherent limitations. In TABLE 1, we present relevant omics methods and outline the strengths and limitations to their application in EDC research. For a more extensive discussion on genomics and the role of genetics in determining susceptibility to toxicants, including EDCs, see REFS 32–35.

Epigenomics. Epigenomics is the study of the biological mechanisms that change gene expression. If we consider the DNA sequence to be inheritable and fixed, then epigenetic modifications are the markings of this sequence that alter gene expression. These marks themselves can be persistent and heritable, even though they do not change the actual genetic sequence[36]. In the context of epigenetics, 'persistent' and 'heritable' refer to both the persistence of these marks between parent and daughter cells and the inheritance of these marks between parents and offspring. Therefore, one of the underlying properties of epigenetic modifications is that, once they are established, they do not disappear after the genome is duplicated but instead can propagate and persist through cell division. DNA methylation and histone modifications are the two epigenetic modifications analysed in most human studies. DNA methylation is the addition of a methyl group to a cytosine base commonly followed by a guanosine base, which results in a cytosine–phosphate–guanine (CpG) dinucleotide[37]. Gene silencing is the best known DNA methylation-related mechanism of gene regulation: within the promoter-associated regulatory regions of a gene, the presence of increased methylated CpGs can downregulate expression of that gene[37]. However, DNA methylation is not always associated with gene repression. For instance, within the gene body, high levels of methylation are highly correlated with upregulation of gene expression[38].

Today, genome-scale platforms that measure millions of methylation sites are readily available with choices that balance depth of information per sample with sample size and cost (TABLE 1). DNA methylation has become the most frequently studied epigenetic mark in EDC research because of the availability of robust laboratory methods for its analysis[3]. Large human epidemiologic studies known as epigenome-wide methylation studies often opt for platforms with low costs per sample, such as the Illumina Infinium methylation BeadChip, which in its current configuration measures DNA methylation at ~850,000 methylation sites[39]. Clinical studies with small sample sizes have increasingly used platforms based on deep sequencing, such as candidate-gene pyrosequencing or sequence-specific bisulfite sequencing. For example, in 2015, maternal exposure to PBDEs (a class of flame retardant) and promoter methylation in the tumour necrosis factor (*TNF*) gene was examined in cord blood[40]. Increased maternal serum concentrations of PBDE47 were associated with reduced methylation in the *TNF*

Table 1 | **Strengths and limitations of omics technologies in EDC research**

Type of omics	Biomarker	Methods	Description	Strengths	Limitations	Refs
Genomics	DNA sequencing	• Sanger sequencing • Next-generation sequencing	Determines the order of nucleotides within a DNA molecule and enables full interrogation of the genome, both targeted and global	• Not just limited to nDNA — can be extrapolated to mtDNA • Able to investigate all DNA variants and how they might be related to EDCs	• Can have high cost, especially for whole-genome sequencing • Information limited to the DNA sequence	77
	GWASs	GWASs — microarray	Examines specific genetic variants across the genome in different individuals and can be used to establish associations between these variants and disease or quantitative traits	• Not hypothesis driven — no prior gene information required, thus enables discovery analyses • Well established for investigating outcomes • Enables associations to be examined, especially environmental exposures	Hard to use if certain EDCs target variants other than SNPs or copy number variation	77
Transcriptomics	Gene expression analyses	• RNA-seq • RT-qPCR • Microarray	Examines expression patterns of specific genes, an array of genes or the entire transcriptome, and reveals the presence and quantity of RNA in a biological sample	• Enables associations to be examined between EDCs and specific expressed genes or an array of genes • Primer design enables study of specific a priori genes to be examined, and developed microarrays are readily available • Some toxicology or *in vitro* models in relation to EDCs	• Gene expression varies by tissue type making it more difficult to identify the biological mechanism • Usually represents data at the point in time the sample was collected; limited in reflecting history of exposures over time • Relatively few RNA-seq EDC studies have been conducted in human populations	78–80
Epigenomics	DNA methylation	• Pyrosequencing — microarray • Whole-genome sequencing	Examines the DNA methylome, ranging from gene-specific areas to a microarray of about 850K sites to the entire DNA methylome, which can affect gene expression	• Can examine gene-specific methylation and/or epigenome-wide DNA methylation (up to 95%) • Able to examine associations with both EDC exposures and outcomes • Established methods within epidemiology studies that enable replication of EDC findings • Methods can also be used to measure methylation in mtDNA	• Tissue specific, so might not be the best representation if target tissue is not obtained • Only represents data at the point in time the sample was collected, might not reflect the windows of susceptibility	81,82
	Histone modifications	ChIP–seq	Examines epigenetic marks on histones, including acetylation, phosphorylation, glycosylation, sumoylation, methylation and ADP ribosylation, which can affect gene expression by altering chromatin structure	Previous studies have examined the relationship between histone modifications and environmental exposures (such as nickel, arsenic and a few EDCs)	• Still a relatively understudied field in EDC research • Most studies examining histone modifications are *in vitro* or in toxicology models	28, 83–87
	Chromatin remodelling	• DNAse-seq • MNase-seq • FAIRE-seq • ATAC-seq	Examines the dynamic modification of the chromatin architecture that enables the transcriptional machinery to adhere to the DNA, which can affect gene expression	Provides information on the actual chromatin conformation. Analyses a cellular state intermediate between the epigenetic marks (for example, DNA methylation or histone modifications) and gene expression	• Most assays require high numbers of cells • Still not widely applied in EDC studies	88

Table 1 (cont.) | **Strengths and limitations of omics technologies in EDC research**

Type of omics	Biomarker	Methods	Description	Strengths	Limitations	Refs
Mitochondriomics	Mitochondrial copy number	• Multiplex RT-qPCR • Digital-droplet PCR	Examines the number of copies of mtDNA compared with nDNA within a given sample	• mtDNA copy number can be altered by the presence of environmental chemicals • Assay has been optimized for toxicology, *in vitro* and human studies	• Measurements are relative to the controls used, so it can be hard to compare between studies • Still new to the EDC field, and few studies have examined mtDNA copy number in relation to EDCs	59, 63,89
	Mitochondrial lesions	Long-range qPCR and picogreen fluoroescence	Examines the number of DNA lesions within a fragment of mtDNA	• Assay can be used in human studies, enabling reliable and sensitive measures • Low cost • Small amount of DNA needed • As is PCR based, enables easy set up and running of the assay	• Measurements are relative to controls used, so it can be hard to compare between studies • Cannot distinguish the nature or location of DNA damage • Not all types of lesions are captured by this method • Emerging technology; has not been studied with EDCs	90
	Mitochondrial sequencing	• Next-generation sequencing • MiSeq • MitoExome • Sanger sequencing	Similar to genomic sequencing, enables ascertaining the order of nucleotides and full interrogation of the mtDNA genome	• Able to investigate all DNA variants and how they might be related to EDCs • Measures mtDNA heteroplasmy, which can vary over time and is in principle influenced by environmental exposures • mtDNA hypervariable region could be used as a tool for exposure fingerprinting	• Information limited to the mtDNA sequence • Emerging technology; has not been studied with EDCs • mtDNA sequence variation is tissue specific, so mechanisms can be missed if measuring in a different tissue type • Potential co-amplification of nuclear homologues of mtDNA, which can lead to inaccurate measures	91,92

ATAC-seq, assay for transposase-accessible chromatin with sequencing; ChIP–seq, chromatin immunoprecipitation followed by sequencing; DNAse-seq, DNase I hypersensitive sites sequencing; EDCs, endocrine-disrupting chemicals; FAIRE-seq, formaldehyde-assisted isolation of regulatory elements sequencing; GWASs, genome-wide association studies; MNase-seq, micrococcal nuclease sequencing; mtDNA, mitochondrial DNA; nDNA, nuclear DNA; RNA-seq, RNA sequencing; RT-qPCR, real-time quantitative polymerase chain reaction; SNPs, single-nucleotide polymorphisms.

promoter region[40]. This result suggests that maternal exposure to PBDE47 alters CpG methylation in the promoter region, which might lead to altered expression of *TNF*[40]. Similar studies could provide a basis from which additional biomarkers could be developed for the purpose of improving characterization and risk assessment of EDC exposure. Epigenome-wide methylation methods can measure increased numbers of methylation sites and, at higher cost, can even provide complete coverage of all the 28 million methylation sites in the human genome[41]. The main advantage of epigenome-wide methylation profiling lies in its ability to determine absolute levels of DNA methylation (covering ~95% of the DNA methylome). However, this method is subject to high costs, is dependent on technical expertise and has downstream computational requirements[38] (for additional examples of epigenomics methods and their strengths and limitations in EDC research, see TABLE 1).

Although a biomarker reflecting past EDC exposure has yet to be developed and validated, a well-studied example in the tobacco literature provides a potential model. DNA methylation as a result of exposure to tobacco smoke has led to the development of the first known omics biomarker that reflects detailed personal exposure history. Traditionally, assessment of smoking has primarily relied on self-reported data or measures of urinary cotinine, the main metabolite of nicotine; however, personal recall of smoking is prone to bias, and levels of cotinine are largely a reflection of recent tobacco consumption[42]. A seminal study by Joubert and colleagues[43] identified 26 CpG sites in cord blood that differed markedly between mothers who smoked during pregnancy and mothers who were non-smokers. Furthermore, a meta-analysis examining 13 cohorts found 6,073 CpG sites that differed markedly depending on maternal smoking status[44]; these sites also comprised those identified by Joubert[43].

Both studies identified cg05575921 — a CpG locus that maps to the aryl hydrocarbon receptor repressor (*AHRR*) gene and known to be activated by tobacco smoking — as the single most important site[43,44]. Several studies on adult populations have demonstrated that *AHRR* methylation is associated with not only smoking status (current, former or never) but also with the average number of cigarettes smoked, years of smoking, pack-years of smoking and, among those individuals who quit, years since quitting[43,45,46]. Although such a biomarker should be evaluated with similar exposures, including non-tobacco smoke, DNA methylation of the *AHRR* might be a potentially useful biomarker to predict past exposure, a potential that provides motivation for researching DNA methylation biomarkers that are responsive to EDC exposures. Despite the fact that analysis of DNA methylation is a well-developed technology, applying methylation data of specific genes to categorize or predict

exposure to EDCs is still new and requires further study and development. Several large human cohorts that assessed EDC exposures have now also generated epigenome-wide methylation data; we expect a wave of results on associations between EDC exposures and DNA methylation to be forthcoming. Results from these new studies can be expected to enhance our understanding of the inter-relationship between EDCs and DNA methylation and to identify potential biomarkers.

Developing omics technologies

Transgenerational epigenetic inheritance. Transgenerational epigenetic inheritance consists of phenotypic expression that is transmitted across generations via gametes through epigenetic marks but is independent of the genome sequence. Evidence from animal models suggests that environmental stressors, including some EDCs such as pesticides, persistent organic pollutants and others[47,48], can lead to adverse health outcomes among descendants not directly exposed[49–51]. Many examples of transgenerational inheritance occur in rodent models[52–54]. One study examining di-(2-ethylhexyl) phthalate (DEHP) exposure to F0 pregnant female rats led to the identification of the multigenerational inheritance of cryptorchidism (undescended testes)[52]. A statistically significant upregulation of three kinds DNA methyltransferases (DNMT1, DNMT3A and DNMT3B) was seen in progeny F1 and F2 male rat testes compared with unexposed controls[52]. The main effects were shown in the F1 and F2 generations; however, no effect was observed in the F3 and F4 generations. This pattern suggests that the observed phthalate effects might be due to direct exposure of the fetus and its gamete cells *in utero* rather than to genuine transgenerational inheritance. Indeed, the gametes that will generate F2 are already present in the F1 embryo *in utero* and could be reprogrammed at that stage to produce demonstrable effects in F2 progeny.

A rodent study on methoxychlor, an insecticide and pesticide, demonstrated true epigenetic transgenerational inheritance of disease through certain sperm epimutations (differentially methylated regions of DNA)[53]. Only the F0 generation of gestating females was exposed to methoxychlor. Exposure of the F0 gestating females was associated with kidney disease, ovarian dysfunction and obesity among the unexposed F3 generation descendants. This study also compared epigenetic changes in sperm between the control lineage and methoxychlor-exposed lineage F3 rats and found 37 epimutations that were markedly different between the two groups. These 37 sperm epimutations were further compared with epimutations caused by exposure to other chemicals, such as other pesticides (dichlorodiphenyltrichloroethane and *N,N*,diethyl-3-methylbenzamide) and plastics (bisphenol A (BPA) and phthalates); only 4 of the 37 methoxychlor-induced epimutations overlapped with those associated with other compounds. This finding suggests that the transgenerational sperm epimutations found in the F3 generation are exposure specific and induced by methoxychlor[53]. However, transgenerational epigenetic inheritance in humans is difficult to determine and requires demonstration of adverse effects of EDCs in F3 generation offspring or beyond. Despite evidence in animals, the exact mechanisms that are involved in epigenetic transgenerational inheritance are not yet known. Designing and conducting human multigenerational studies is particularly challenging, not only because of the time required to follow up multiple generations but also because of the difficulty of observing exposures and DNA methylation at the appropriate time windows across generations. Further research is therefore needed to understand the extent to which multigenerational epigenetic inheritance operates in humans.

Mitochondriomics. Investigation of the properties of mitochondrial DNA (mtDNA) is a relatively new, yet promising, field in environmental health and EDC research. Each human cell contains thousands of mitochondria, each carrying 2–10 copies of their own genome, a double-stranded circular mtDNA molecule of ~16 kb in length. Mitochondria act as the cell's 'power plant', as they convert energy substrates derived from the breakdown of glucose and fatty acids into ATP[55]. The characteristics of mtDNA differ from those of nuclear DNA (nDNA) and include the lack of histone-wrapping protection and limited repair mechanisms, which makes mtDNA more vulnerable to accumulating damage when exposed to environmental chemicals[56].

Mitochondrial damage can result from many sources, and damage can affect mitochondrial structure or function, as well as the mtDNA sequence. Endogenous reactive oxygen species are a primary agent of mitochondrial damage, and these can be amplified in the presence of an external pollutant source, including many environmental chemicals[57,58]. When reactive oxygen species are produced past the point of homeostatic levels, oxidative stress can lead to alterations in mitochondrial structure and function, including abnormalities in electron transport chain activity, membrane potential, ion transport and apoptotic signalling, which can ultimately lead to cell death[59]. Several studies on sperm function have found that abnormal mitochondria and structural alterations to mitochondria or their sheath are associated with reduced sperm motility[60,61]. Studies on the effects of EDCs on mtDNA are scarce. However, studies that showed effects on mitochondrial membrane potential (MMP), which has been extensively used to document mitochondrial dysfunction[2], suggest that EDCs might affect mitochondria. For instance, one such study found that men with increased concentrations of phthalate in semen had reduced MMP; reduced MMP was further associated with semen quality[58]. In 2015, the Toxicology Testing in the 21st Century (Tox21) programme of the United States Environmental Protection Agency assessed the potential of some environmental chemicals and pharmaceuticals to induce mitochondrial dysfunction by measuring MMP. Among the >8,000 different chemicals that were tested *in vitro*, 11% of these agents decreased MMP, including certain classes of EDCs[2]. Although these studies do not provide information on whether EDCs affect the mtDNA, they do show that mitochondria are a target of EDCs.

Damage to mtDNA has also gained attention in the field of mitochondriomics, with biomarkers being used to quantify mtDNA damage and dysfunction. Damaged mtDNA can coexist with normal (undamaged) copies of mtDNA in cells; the influence of these mtDNA alterations ranges from normal to mild to severe, according to the proportion of abnormal mtDNA copies[62]. Biomarkers that measure mitochondrial damage and dysfunction include, among others, mtDNA copy number (a measure of the abundance of mtDNA present compared with nDNA) and mtDNA lesions (the amount of damage present in the mtDNA)[62]. A few studies have examined mtDNA biomarkers in the context of EDCs; one *in vitro* study found that cells treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (the most potent dioxin congener and a known EDC) had an increased number of mtDNA lesions and reduced mtDNA copy number[63]. Another study compared mitochondrial function in lymphoblast cells in relation to BPA exposure between children diagnosed with autism and their unaffected siblings[64]. The researchers examined several different

markers of mitochondrial dysfunction including MMP and copy number of mitochondrial genes. The results of this study suggest that, among genetically susceptible children, BPA exposure might induce mtDNA dysfunction and act as an important environmental risk factor[64].

DNA methylation of the mitochondrial genome is growing in popularity as a potential biomarker in the study of EDCs, although controversies surround the existence and functionality of mtDNA methylation[65]. Earlier studies — performed more than 30 years ago — found no cytosine methylation on mtDNA[66]. More recently (in 2011), the presence of mtDNA methylation was demonstrated, and a possible mechanism of action proposed, through which DNA methyltransferases translocate to the mitochondria following a mitochondrial targeting sequence[67]. However, the unsophisticated structure of the mitochondrial genome has led to the belief that the mtDNA might lack the mechanisms that link DNA methylation with control of gene expression in the nuclear genome.

Although data in human populations are lacking, a 2015 animal study examined the epigenetic effects of low doses of PBDE47, a flame retardant chemical, and demonstrated that prenatally exposed rats have reduced DNA methylation of a specific mitochondrial gene[68]. Even though mtDNA methylation is still largely understudied — partly owing to technical limitations of using standard platforms typically used in nDNA methylation, as well as to lingering doubts about its functionality[62] — other measures of mitochondriomics remain a unique and novel field with potential utility in EDC research. Further development in mitochondriomics technology and an expanded awareness of its potential and its limitations will help to guide and generate new studies in this area (TABLE 1).

Glossary

DNA methylome
The set of methylation modifications in an organism's genome in a particular cell.

DNA methyltransferases
A family of enzymes that catalyse the transfer of a methyl group to DNA.

Epigenomics
The study of heritable changes in gene expression that do not result from changes in actual gene sequences.

Exposome
An individual's lifetime exposure burden.

Gene–environment interactions
(GxEs). The biological interactions between the environment and the human genome.

Genomics
The study of an organism's genome or complete set of DNA, including all its genes.

Histone modifications
Post-translational modifications to histones — referred to as marks — that regulate gene expression.

Metabolomics
The study of the set of metabolites present within an organism, cell or tissue.

Mitochondrial membrane potential
(MMP). The total force driving protons into the mitochondria.

Mitochondriomics
The study of the properties of mitochondrial DNA.

Proteomics
The large-scale study of proteins.

Transcriptomics
The study of transcriptomes and their functions.

Other omics technologies

Transcriptomics, proteomics and metabolomics. The central dogma of molecular biology posits that the information contained in genes flows from DNA sequence to mRNA to proteins[69]. Epigenetic regulation helps to control the flow of information from DNA into mRNA and, indirectly, into proteins. Proteins have many functions in eukaryotic cells, varying from serving as structural components to facilitating the transport and storage of biomolecules and substances within cells. Proteins also serve as enzymes that carry out nearly all the chemical reactions that take place in cells, including all those that transform metabolites. In the past two decades, laboratory technologies, including transcriptomics, proteomics and metabolomics, have increasingly enabled characterization of these interconnected layers of cellular functions. In principle, these technologies — individually or in combination — can be used to determine a biological fingerprint of an EDC exposure[70]. However, a major challenge of transcriptomics, proteomics and metabolomics is that, given that mRNAs, proteins and metabolites are downstream in the flow of information, they are also much more variable over time. Indeed, cells often need to adapt very rapidly in response to environmental conditions and homeostatic signals, and consequently the downstream mechanisms that operate cellular responses are also able to change rapidly. Therefore, although these molecular substrates might reflect current exposures, they are less likely to reflect past exposures. In addition, standardized, easily accessible, high-throughput platforms, while existing for transcriptomics, are not yet readily available for proteomic and metabolomic applications. As a result, these three technologies have not found as much application in EDC research as the methods discussed earlier (TABLE 1). For instance, a PubMed search (conducted on 27 March 2017) on "endocrine disrupting chemicals" yielded 56 papers for "transcriptomics OR mRNA microarray OR mRNA sequencing", 46 papers for "proteomics" and 32 papers for "metabolomics". By comparison, the same search retrieved 126 papers for "epigenetics and epigenomics" and 747 papers for "genetics OR genomics". These search results suggest a limited application of transcriptomic, proteomic and metabolomic technologies in EDC research thus far, despite the fact that they are extremely informative in understanding mechanisms of action and biological effects of EDCs.

Future steps

To date, omics studies have been limited to identifying molecular changes that are associated with and/or induced by chemical exposures. Developing a chemical fingerprint requires that a biomarker be sufficiently sensitive to modifications by the exposure of concern; however, sensitivity is a necessary but insufficient criterion for fingerprint development. Given that individuals are not exposed to a single chemical in isolation, but rather to a multitude of chemicals, as well as to other stressors, simultaneously, biomarkers that can serve as molecular fingerprints of exposure need to inherently also be specific. Although a valid and reliable DNA fingerprint in EDC research has yet to be developed, a possible approach might emerge from DNA methylation methods recently developed to predict biological age. For example, Horvath[71] applied DNA methylation arrays to create an algorithm based on an elastic-net machine learning technique to identify 353 age-related CpG methylation sites and combined them to generate a biological measure that highly correlated with chronological age[72]. The value of this measure of DNA methylation age was illustrated in a meta-analysis of 13 large epidemiology studies (comprising 13,089 participants), which showed that individuals who had a positive difference between epigenetic and chronological age at baseline (that is, those who seemed to be epigenetically older than their actual age) had increased mortality during follow-up[73].

Similarly, machine-learning techniques such as those used to construct epigenetic age algorithms might be applied for fingerprinting EDC exposures using not only DNA methylation but also other omics data, individually or in combination. In principle, machine learning might enable the identification of and discrimination between different EDC exposures and yield omics biomarkers that are both sensitive and specific[71]. Indeed, machine learning has emerged in biomedical sciences as a method that can use highly dimensional data to maximize biomarker sensitivity and specificity[74]. However, such applications of machine learning have been sparsely used in environmental health and, to the best of our knowledge, never applied to EDC research. However, particular challenges inherent in EDC research should not be underestimated. Compared with age, EDC exposures might cause smaller biological differences and therefore be associated with weaker biological changes. Furthermore, to develop a fingerprint that is biologically meaningful, omics data should be combined with reliable measures of EDC exposure over time that can accurately characterize current and past exposure profiles. Such data are rarely available in typical human studies and are especially challenging to collect for EDCs with short half-lives, which typically require repeated collection of biological samples for exposure quantification over time. Given the various limitations to machine-learning technology in the context of EDCs, few studies have applied these methods directly to improving exposure measurement; however, these methods are gaining popularity in the field[75,76].

A general challenge in epigenetic studies is tissue specificity. In general, scientists cannot assume, without specific evidence, that the level of an epigenetic mark at a specific locus measured in an easy-to-access surrogate tissue (for example, blood) is correlated with that in a more remote, inaccessible tissue (for example, the brain). Furthermore, even in the presence of correlation between two different tissues in population samples, it cannot be assumed that, when an environmental determinant or a disease changes the levels of the epigenetic mark in one tissue, the second tissue will show the same change. This caveat is a major consideration in epigenetic research that needs to be taken into account when planning and interpreting any epigenetic study. However, for environmental exposures and conditions for which simply having a biomarker might be useful, even just working on easy-to-access tissues might be helpful, provided that no inference is claimed on the biological mechanisms affecting the target tissue of concern. Future research is warranted to combine epigenomics, mitochondriomics and other biomarkers to create novel fingerprints of the human exposome and to incorporate it with genomic data and other individual-level data to predict individual risk of future disease[31].

Conclusions

New and developing technologies in genomics, epigenomics and mitochondriomics are providing new opportunities to bridge the exposome and precision public health in EDC research. Although EDCs have been linked with adverse health effects in experimental and human studies, the field requires improved methods to assess human exposure and biological response across different and multiple life stages. In the absence of costly and time-consuming repeated measures of ambient levels and/or biomarkers in biological samples collected over months or years, reconstructing past exposures remains difficult, if not impossible. Integrating multiple omics technologies will enable improved characterization of past EDC exposures. Application of these technologies will improve our understanding of future risk and might be augmented by the identification of susceptible subgroups. Omics can greatly contribute to developing a comprehensive exposome approach and will help to identify individual risk of disease through targeted biomarkers that reconstruct past exposure and predict future risk. Using large-scale, data-rich biology in tandem with machine learning will enable the development of biologically relevant fingerprints of EDC exposures. These tools will bridge the gap between multiple disciplines and help to further understand the links between EDC exposure — both past and present — and future risk of disease.

Carmen Messerlian and Russ Hauser are at the Department of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, Massachusetts 02115, USA.

Rosie M. Martinez is at the Department of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, Massachusetts 02115, USA; and at the Laboratory of Precision Environmental Biosciences, Department of Environmental Health Sciences, Columbia Mailman School of Public Health, New York, New York 10032, USA.

Andrea A. Baccarelli is at the Laboratory of Precision Environmental Biosciences, Department of Environmental Health Sciences, Columbia Mailman School of Public Health, New York, New York 10032, USA.

Correspondence to A.A.B.
andrea.baccarelli@columbia.edu

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Author contributions

C.M. and R.M.M. researched data for the article and made substantial contributions to discussions of the content. C.M., R.M.M. and A.A.B. wrote the article. C.M., R.M.M., R.H. and A.A.B reviewed and/or edited the manuscript before submission. C.M. and R.M.M. contributed equally to all aspects of the manuscript.

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The authors declare no competing interests.

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