Published in final edited form as:

Curr Environ Health Rep. 2017 March ; 4(1): 89–98. doi:10.1007/s40572-017-0126-3.

The Exposome Research Paradigm: An Opportunity to Understand the Environmental Basis for Human Health and Disease

Germaine M. Buck Louis, Ph.D., M.S.[1], **Melissa M. Smarr, Ph.D.**[2], and **Chirag J. Patel, Ph.D.**[3]

[1]Office of the Director, Division of Intramural Population Health Research, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, 6710B Rockledge Drive, Room 3148, Rockville, MD 20852, USA, 301-496-6155

[2]Office of the Director, Division of Intramural Population Health Research, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, Rockville, MD 20852, USA, 301-435-4964

[3]Chirag J. Patel, Department of Biomedical Informatics, Harvard Medical School, 10 Shattuck St., Boston, MA 02115

Abstract

Purpose of review—This paper presents an overview of the exposome research paradigm with particular application to understanding human reproduction and development, and its implications for health across the lifespan.

Recent findings—The exposome research paradigm has generated considerable discussion about its feasibility and utility for delineating the impact of environmental exposures on human health. Early initiatives are underway, including smaller proof-of-principle studies and larger concerted efforts. Despite the notable challenges underlying the exposome paradigm, analytic techniques are being developed to handle its untargeted approach and correlated and multi-level or hierarchical data structures such initiatives generate, while considering multiple comparisons. The relatively short intervals for critical and sensitive windows of human reproduction and development seem well suited for exposome research, and may revolutionize our understanding of later onset diseases.

Summary—Early initiatives suggest that the exposome paradigm is feasible, but its utility remains to be established with applications to population human health research.

Keywords

Design; epidemiology; exposome; methods; reproduction

Correspondence to: Germaine M. Buck Louis.

Conflict of Interest

Germaine M. Buck Louis, Melissa M. Smarr, and Chirag J. Patel declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

Introduction

The term ‘exposome’ ignites considerable discussion amongst some scientific disciplines, largely because of its ambitious attempt to assess environmental exposures simultaneously using untargeted analytic methods. However, controversy has given way to early initiatives aimed at assessing the feasibility and utility of the exposome paradigm for environmental research. As such, the exposome research paradigm has the potential to shed new light on the origin and mechanisms underlying environmental impacts on human health. While there are at least three sides to the exposome discussion – those in favor, opposed, or uncertain – a number of commentaries have been published in the last decade describing its potential utility and feasibility, while cautiously urging moving forward [1–4]. We offer that the exposome research paradigm will be an impactful contribution to population health scientists who wish to understand human health and disease in the context of how people are exposed and respond to environmental agents and stressors. With a commitment from the National Institute of Environmental Health Sciences to support exposome research (e.g., Children’s Health Exposure Analysis Resource (CHEAR) http://www.niehs.nih.gov/news/newsletter/2016/6/feature/feature2-children/index.htm), the timing appears right for moving beyond generalized discussion to proof-of-principle and more formalized research initiatives aimed at answering the many lingering data gaps about the impact of environmental exposures on human health.

An underlying tenet of the exposome paradigm is the importance of environmental exposures from conception onward. As such, this paper focuses on the application of the exposome during the critical and sensitive windows of human reproduction and development. While essential, these windows are challenging to capture in light of our limited ability to observe and measure these earliest stages (e.g., conception, implantation) at the population level. Understanding the exposome during these early windows may offer insight for exposome research focusing on later developmental stages, such as childhood, adolescence, adulthood, and senescence. This paper is written from a population health perspective, and is presented in three parts: 1) an overview of the development of the exposome research paradigm and its potential implications for furthering our understanding of human health and disease; 2) a summary of unique methodologic considerations; and 3) a discussion of emerging analytic techniques for moving forward.

Evolution of the Exposome

The exposome was introduced by Wild in 2005 and defined as the “…totality of environmental exposures from conception onward…” [5]. Subsequently, Wild expanded his original definition to delineate the internal and external exposomes, while continuing to emphasize the importance of the exposome for complementing our understanding of the human genome [6]. Specifically, the internal exposome is conceptualized as representing the individual’s response to environmental stimuli or his/her physiologic and biologic responses needed for maintaining homeostasis, which encompass inflammation, metabolic and stress pathways among others. The external exposome is further categorized as general and specific. Under this conceptualization, macro level exposures such as those involving the physical or social environment (e.g., air and water, built environment, climate change, noise,

social support) comprise the *general* external exposome, while individual level exposures (e.g., bathing, cosmetic use, diet, lifestyle, physical activity, sleep) represent the *specific* external exposome. A notable strength of the exposome research paradigm is the ability to look at a hierarchical array of exposures from macro to micro level irrespective of the availability of biomarkers. One notable shortcoming of the definition is the lack of recognition of "deconvolving" endogenous indicators of the external environmental factors and their associated phenotypic responses. While some authors argue for a top down approach for moving exposome research forward, defined as using biospecimens to quantify endogenous and exogenous chemicals to identify important signals for more targeted research, others argue for a bottom up approach where chemicals in physical environment are measured to identify important signals for exogenous exposures [7]. For example, the top-down approach suggests measuring the internal chemical environment reflecting not only chemicals but also homeostatic responses to external exposures. The bottom-up approach suggests measuring environmental exposures from various external sources, such as residential, occupational or lifestyle pathways. Irrespective of approach, diseased and non-diseased individuals are compared to identify potential signals for more targeted research. The research question will direct the choice of approach or a hybrid combination, and either strategy offers the promise of discovery.

The definition of the exposome continues to evolve from the perspective of other authors. Ho and colleagues conceptualized the internal and external exposome as the "interactome" [8], while Miller and Jones noted the importance of including associated biological responses throughout the lifespan into the definition [9]. Miller further characterized the exposome as including lifetime external forces, arising from many possible sources at the individual (e.g., environment, lifestyle) and macro level (e.g., societal, economic), which act upon the genome [10]. Even Wikipedia offers a definition for the exposome https://en.wikipedia.org/wiki/Exposome. Irrespective of definition, there are key facets that are explicit or implicit in just about all definitions. They are: 1) need to measure a multitude of (nongenetic) exposures during sensitive windows of human development (e.g., folliculogenesis; spermatogenesis, periconception, implantation) and across the lifespan (e.g., childhood, adolescence, early and late adulthood); 2) need for hierarchical data collection to capture both individual and macro level environmental exposures and accompanying modeling approaches for exposures including those without specific biomarkers; and 3) recognition of the dynamic nature of exposures and the bodily responses and phenotypic changes they elicit. New technologies such as mobile health applications ("apps") and analytic methods grounded within informatics and data science disciplines [11] offer promise for overcoming these challenges.

Potential utility of the exposome paradigm for human reproductive health and disease

The exposome paradigm is responsive to two bodies of evidence that have evolved over the past few decades. The first body of evidence is that which gave way to the early origins of health and disease (DOHaD) research paradigm, which posits that exposures during critical and sensitive windows of human reproduction and development have the ability to (re)program the developing conceptus/embryo/fetus for postnatal life [12]. To this end, adverse exposures early during development have potential implications for health and

disease across the lifespan and underscore the developmental plasticity of the embryo and fetus. For example, infants exposed to inadequate nutrition during mid- to late-pregnancy during the Dutch Famine in 1944-1945 had smaller birth sizes than unaffected infants, whereas infants with early pregnancy exposure had an earlier (≈3 years) onset of coronary artery disease [13]. Considering the early origins of reproductive health, male fetuses exposed to *in utero* cigarette smoke were reported to have earlier pubertal onset and diminished semen quality in adulthood relative to unexposed offspring [14]. A second body of evidence consistent with exposome research is epigenetics, defined as the molecular alterations in gene expression or phenotype without concomitant changes in DNA sequence. Epigenetic mechanisms control both cellular and tissue differentiation, which result in the expression or inactivation of genes at specific developmental stages [8]. Notable epigenetic reprogramming occurs during the earliest reproductive windows of gametogenesis and early embryogenesis [15]. Some epigenetic changes can be transitory while others are passed directly through the germline to the offspring [16]. Thus, human reproduction and development is more than the unfolding of the rigid genome and encompasses a series of highly timed and integrated processes that are intended to prepare the developing organism for postnatal life even in the context of associated structural or functional changes.

Within the overarching framework of the DOHaD paradigm in which epigenetics is mechanistically grounded, two paradigms have been offered to help conceptualize how early exposures affect reproductive health, which in turn affects health and disease across the lifespan and, possibly, future generations. Both paradigms provide a framework for exposome-related research whose goal is to better delineate successful reproduction and development as a means for ensuring health across the lifespan. The two paradigms are: testicular dysgenesis syndrome (TDS) and the ovarian dysgenesis syndrome (ODS). While an in-depth description of both is beyond the scope of this paper, the TDS and ODS paradigms posit that environmental exposures during critical and sensitive windows of human development may manifest in a variety of adverse structural or functional changes, in part, depending upon the exposure(s), susceptibility and underlying mode of action(s). A key point of this premise is that an exposure(s) may result in a spectrum of endpoints depending upon timing or dose, genetic predisposition or the constellation of other concomitant environmental exposures among other considerations. For TDS, exposures that disrupt normal testicular and genital development may manifest as genital-urinary malformations (e.g., hypospadias, cryptorchidism), diminished sperm counts or testes cancer [17]. Leydig cell dysfunction and androgen insufficiency are believed responsible for the spectrum of adverse reproductive outcomes. Similarly, the ODS posits that alterations in ovarian development and function arising from exposures during early development may affect folliculogenesis and steroidogenesis resulting in a spectrum of adverse reproductive outcomes such as polycystic ovarian syndrome, premature ovarian insufficiency or failure, alterations in menstruation, ovulation or time-to-pregnancy (TTP), pregnancy loss, and reproductive site cancers [18]. A key aspect of both the TDS and ODS is the recognition that early life environmental exposures including endocrine disrupting chemicals affect reproductive health, which in turns affect health and disease across the lifespan. For example, men born with genital-urinary malformations are at greater risk of testes cancer [19], while women with endometriosis are at greater risk of ovarian and other reproductive

site cancers [20]. In fact, two recent population based studies have found diminished semen quality to be associated with earlier mortality [21, 22] supporting the premise that reproductive health is a marker for overall health and, possibly, longevity. Also, various urologic and gynecologic diseases are now suspected to have an *in utero* origin [23]. There is recent evidence of fetal endometriosis [24] underscoring the importance of understanding environmental exposures during critical and sensitive windows. Moreover as our understanding of human reproduction and development moves beyond the unfolding of the rigid genome to one that appreciates developmental plasticity [25], the exposome paradigm offers promise for understanding lifetime health.

So how might the exposome foster discovery and advance scientific thinking, particularly in the area of human reproduction and development? While it can be daunting when thinking about the multitude of exposures that require measurement in exposome-related work particularly when considering the lifespan, a unique feature of reproduction and development is their relatively short sensitive windows for exposure characterization and quantification. To this end, epidemiologic research could be designed to capture a multitude of environmental exposures and the bodily changes they induce relative to well-defined endpoints. For example, delineating the environmental determinants of male and female fecundity, as measured by folliculogenesis or spermatogenesis, within the exposome research paradigm becomes possible. Table 1 presents the estimated windows for key reproductive and developmental endpoints, ranging from a matter of hours as in the case of the ovulation or fertilization to days for pregnancy. Still, two important points are noteworthy when contemplating exposome research. First, it is important to appreciate that human reproduction and development is a continuum comprising highly interrelated and conditional states, in that the early conceptus may or may not reach the embryonic or fetal stages. With approximately 30% of pregnancies resulting in incident pregnancy loss [26, 27], it is important to recognize the conditional nature of gestation in addition to understanding the exposome underlying pregnancy loss. A second point is our inability to measure the earliest endpoints such as conception for which there is no biomarker, implantation of the blastocyst and early embryonic development at the population level. This challenge can be overcome to a certain extent by focusing on specific population subgroups, such as couples seeking assisted reproductive technologies where gametes are handled and manipulated outside the body allowing for the visualization of early developmental stages prior to implantation. Use of such clinical populations does, however, have important implications for external validity with regard to nonclinical populations in terms of underlying fecundity status and access to care. Improvements in obstetric ultrasonology such as 3D and 4D measurements offer promise for capturing post-implantation pregnancy and related development including supporting structures such as the placenta.

In essence within approximately one year, it is possible to define the exposome for male, female and couple fecundity and fertility as well as any associated impairments for couples planning pregnancies. For example, exposome research could identify environmental exposures that are (un)favorably associated with fecundity endpoints such as steroidogenesis, spermatogenesis and semen quality, menstrual and ovulation, conception, implantation, and pregnancy maintenance. As for fertility outcomes, it would be informative to know if there is an exposome associated with sex selection, plurality, gestation, or birth

size. If research is designed and implemented properly, an exciting aspect is the ability to assess the male, female and couple exposome(s) relative to a spectrum of endpoints in keeping with the interrelated and conditional nature of human reproduction. Research involving couples undergoing ART is truly exciting and provides opportunities to define the exposome during the earliest stage of human development, or from fertilization through the 2- to 8-cell stage and beyond. And, these stages can be observed and measured for this population subgroup. This strength is important as the genome is activated during the 4- to 8-cell stages. Researchers have completed comparative microarray analysis of cDNA for viable and non-viable blastocysts leading to the identification of over 7,000 transcripts expressed only in blastocysts resulting in pregnancies [28]. Other promising avenues of exposome-related research include using spent culture media to measure the embryonic metabolome with the goal of identifying embryonic quality and viability [29, 30]. Irrespective of *in utero* exposure, increasing evidence highlights the importance of DNA methylation in mediating potential effects [31]. Also notable research opportunities now exist for understanding the placental exposome, given that it is a dynamic organ with an ever-changing molecular structure and function over the course of pregnancy [32].

Unique Methodologic Challenges

There are a number of challenges in executing exposome-based research in light of its untargeted analysis of a totality of exposures approach. However, it promises to be a game changer in terms of how epidemiologists and other population health scientists have traditionally designed and implemented research. Most notably, exposome research embraces all exposures (or at least as many as possible) rather than focusing on a particular exposure or class of environmental chemicals. The rationale is to model human health endpoints consistent with the manner in which people are exposed, viz., to a multitude of exposures that vary over time. The identification of potential signals informative for health and disease still require affirmation in more targeted research, which means the exposome is informative for more traditional approaches.

So what are some of the important methodologic challenges that need consideration for moving exposome research forward? While a complete listing is beyond the scope of this paper, we present considerations in keeping with the epidemiologic method. As such, they include specifying the research question, study population, exposures, and health and disease endpoints all while building a hierarchical data management structure that is capable of accommodating multi-level exposures. Of special note is the importance of adhering to strong epidemiologic methods for exposome research, meaning the agnostic search for signals should not ignore fundamental study design and methodologic considerations. Table 2 illustrates that both traditional and exposome (i.e., agnostic) methods require careful attention in specifying the study question, selection of study cohort/sample, choice of exposures/biospecimens and study outcomes, development of an analytic plan and implementation of analytic methods appropriate for the question and methods, and careful interpretation of the findings (e.g., spurious findings or reverse causation). A few comments relative to epidemiologic principles and methods as tailored for exposome research follow.

Framing the Research Question

All research needs a well-developed and formulated question from which all methods are built. Even with agnostic exposome type observational studies, it is important to formulize the question even if it is something vague like identifying signals of disease onset for a particular study population. The question directs the type of data/biospecimens to be collected including when and how and from whom, along with the operational definition for all variates and study outcomes. For example, research focusing on a totality of exposures-based approach means moving away from *a priori* defined exposures of concern to the measurement of mixtures particularly during sensitive windows. To some extent, the scientific expert steps aside for agnostic computation approaches that identify signals. The ambitious goal of the exposome is a holistic approach for capturing the nature of human exposure. Analytic methods capable of handling mixtures (e.g., more than one exposure in tandem) are needed to empirically identify 'signals' and to aid in the interpretation of results in light of the multitude of comparisons made. Thus, extending epidemiologic methods (e.g., directed acyclic graphs) to guide the development of exposome-related research may help ensure the relevancy of the findings for population health.

Study design

Study design is another key consideration with prospective cohort designs with longitudinal data collection being ideal to ensure the timing of the exposure-outcome relationship and, thereby, minimize reverse causation or misclassification bias. Time and cost considerations often prompt investigators to seek abbreviated approaches, but the use of cross-sectional designs requires extreme caution to avoid erroneous conclusions including reverse causation. While cross-sectional findings may be interesting, alone they are insufficient for setting public health direction until a body of evidence supports policy related action. While exposome research relying on biomonitoring data and other cross-sectional health outcomes have been successfully completed [33], cautious interpretation is needed as the temporal ordering cannot be established making it difficult to determine if biomarkers are 'causal' or a reflection of disease onset. However as with all observational research, exposome-based research as described will be correlational and subject to potential biases, such as confounding. With regard to choice of study population/sample, there is growing pressure to move away from probability-based sampling to more convenience-based sampling frameworks such as with internet-based recruitments. Considerable discussion has addressed this topic as provided elsewhere [34], and suffice to say externally validated findings are still critical for impacting population health globally. Other emerging techniques involve crowdsourcing [35] that require extreme care to ensure the correct capture of people for research purposes or reality mining where wireless devices are used to identify patterns of human behavior [36]. An example of this latter approach is a study involving 100 mobile phones over nine months, which demonstrated the ability to define relationships as well as individual and group behaviors based upon patterns of mobile use data [37]. The increasing power of mobile devices will continue to offer opportunities for capturing a multitude (e.g., physical activity, sleep, social interactions) of exposure data that can be utilized in exposome research, but computational methods to infer across exposomic and mobile app information remain elusive.

Measuring Exposures

With regard to environmental exposures captured as a part of exposome research, important considerations are needed at the planning stage. Perhaps, the greatest methodologic challenge is in realistically capturing the multitude of exposure possibilities in the context of their underlying correlations or variability. And, few exposures can be measured without error, particularly exposures with notable variation over time. Moreover, some exposures may not have established measurement methods (e.g., climate change representing various facets beyond temperature) or lack valid and reliable biomarkers. Unlike the stable inherited genome, the exposome is highly variable. Still, various tools have arisen for measuring exposures either geographically or individually through apps, wearable devices and sensors [37, 38], and new unthinkable technologies are likely to emerge before long. High-throughput technologies continue their promise for measuring exposures and biologic responses, even in the absence of an exposure type assay ‘chip’. While exposure concerns can seem challenging, others remind us to embrace correlations between exposures as they may hint at exposure pathways or reveal synergistic or interactive effects [39]. Concerns about multiple testing are accounted for using a number of methods. The traditional approach involves correcting the family-wise error rate, such as the Bonferroni correction. The Bonferroni correction is known to be less powerful as it adjusts the nominal p-value threshold (e.g., 0.05) by the number of tests, or the number of exposures being considered with respect to a phenotype. Alternative more powerful approaches include estimation of the “Benjamini-Hochberg” (BH) false discovery rate (FDR). The FDR is an estimate of the number of associations that are false positives at a given significance level [40]. However, both the family-wise error rate and the BH version of the FDR assume each test, or exposure, is independent from one another, which is not always the case [41]. Alternative approaches for estimating the FDR under exposome dependence include estimating the number of effective variables [41, 42] or considering alternative estimates of the FDR, such as permutation of the outcome variable [43, 44] as prescribed by Efron [45]. For research needing to recreate historical exposures, novel technologies suggest such approaches are feasible for some exposures, such as the use of teeth for the detection of specific exposures over time [46].

Study Outcome

Choice of study outcomes is largely determined by the research question and with sufficient forward thinking, it may be possible to position exposome research for continual discovery or the assessment of a spectrum of health states. For example, exposome research is well suited to assessing a spectrum of endpoints over the lifespan and to reveal potentially shared pathways and opportunities to prevent later onset diseases - why are women with polycystic ovarian syndrome at increased risk of type 2 diabetes or metabolic disease and similarly women with endometriosis at risk for reproductive site cancers? This approach would require measuring both the external (e.g., phthalates) and internal (e.g., inflammation and stress biomarkers) chemicals to identify signals that could be followed with more targeted research. It is important not to lose sight that the exposome provides an opportunity to study and understand health in the context of underlying biology, and not just the absence of diagnosed disease. Perhaps through such investigation, our understanding of health will

change as we learn the exposure scenarios associated with well-being in every sense of the word.

A primary challenge of exposomic research includes discovering exposures that were previously unmeasured or unobserved to be associated with disease phenotypes or health related traits. There are examples of statistical approaches developed for exposome research relative to human health endpoints [44, 47, 48]. In moving the analytic work forward, one suggestion is to systematically assess a comprehensive set of environmental exposures relative to a phenotype(s) as one might do in a genome-wide association study [49]. Operationally, an “exposome-wide association study” or equivalently “environment-wide association study” (EWAS) [50] may perform analogously to a GWAS, associating a battery of environmental exposures with phenotype simultaneously. We emphasize that the development of methods to identify robust correlations between exposures and phenotypes comprehensively, such as with EWAS techniques, may enable us to discover individual and mixtures of factors associated with phenotypes that consider the complex phenomenon of exposure. Intuitively, the phenomenon of exposure is defined by mixtures, such as the array of multiple nutrients found in similar diets or a matrix of pollutants found in air (e.g., heavy metals, hydrocarbons). One challenge in ascertaining the role of multiple exposures with respect to a phenotype is dealing with the number of possible correlations, many of which are known to be “dense” as many exposures are correlated with many others [39, 41, 51]. Given an exposure identified from an EWAS, the independent association is a challenge to deconvolve [52, 53].

Analysis

While the analytic challenges are many, statistical methods are being developed as recently described [54–56]. Some options include the use of data dimension reduction (e.g., discriminant analysis, principle component analysis) techniques, or partial least squares regression (sPLS). The latter method accounts for correlations between multiple exposures and also between exposures and outcomes. A recent study used sPLS techniques to assess various classes of persistent endocrine disrupting chemicals and male reproductive function and reported that 8/10 chemicals identified overlapped with single pollutant models [57]. To this end, the exposome paradigm offers promise for corroborating findings of key predictors of adverse health outcomes from research focusing on a smaller set of exposures that have characterized much of epidemiologic research to date. Moreover, analytic methods are capable of handling multi-level exposures or hierarchical structures along with spatial and temporal variation, while considering correlational structures and multiple comparisons. In addition, there are agnostic or data driven approaches for summarizing masses of data, such as graph theory or combinatorial analysis. Also, multi-modal analytic approaches may help exposome research that moves beyond an individual unit of analysis to include the use of families, including dyads (couples, mother-child) or triads (father-mother-child) [58], as often the case in reproductive and perinatal epidemiology and genetic epidemiology.

Interpreting findings from observational exposome research requires careful thought and caution. Bias remains a concern that requires careful attention at all stages including the interpretation of findings. With increasing data sharing efforts, researchers can utilize larger

N relative to earlier years but this does not necessarily eliminate bias. Choice of sampling framework coupled with participation and completion rates are critical aspects when interpreting findings. Similarly, translating findings to the public or their health care providers is another challenge, but leveraging what we have learned from literature focusing on communicating risk and uncertainty including for endocrine disrupting chemicals [59] will likely help the delivery of knowledge. And finally, the exposome paradigm will not matter if the general population does not engage or value its relevancy. An evolving literature is addressing acceptability aspects of novel research tools such as crowdsourcing including for public health [35], concerted efforts should be taken to determine how best to use these innovative methods for understanding and promoting population health.

Moving Forward

Pioneer initiatives using the exposome research paradigm are already underway, and others will likely follow. Researchers in the European Union (EU) were early adopters and have launched the Human Early Life Exposure (HELIX) project to measure the internal and external exposome among mother-child pairs allowing for the eventual characterization of children's exposomes as they grow and develop [60]. The Health and Environment-Wide Associations Based on Large Population Surveys (HEALS) is another initiative whose goal is to developed refined methods for integrating analytical and computation tools for EWAS that can be applied to different exposure scenarios [61]. Another EU initiative is EXPOsOMICS, which is developing new methods including personal exposure monitors for assessing environmental exposures, viz., as air pollution and water contaminants [62].

Early pioneering initiatives in the United States are evolving, and include the HERCULES project whose goal is to create resources, such as new measurement tools and educational opportunities (e.g., short courses and workshops), for exposomic-related research [63]. As noted above, CHEAR is an initiative that represents a network of laboratories along with a data and analytic center and coordinating center with the overarching goal of creating the Human Exposome Project [64].

Examples of early research that is using exposome like methods to identify preconception and prenatal exposures associated with pregnancy outcomes or child health have been reported, including for gravid diseases and mortality [65], preterm birth [66] and communication impairments for 9-year old children [67]. Other examples of such work include efforts to delineate the pregnancy exposome [68] and the placenta exposome [69].

Conclusion

In conclusion, the exposome research paradigm offers an exciting avenue of research in our quest to identify environmental exposures that impact health and disease across the lifespan. The overarching goal is to delineate underlying mechanisms that promote health while preventing (or delaying) disease onset at the population level. Understanding the exposome will complement the human genome as Wild originally conceived [5], and potentially help identify new markers of health and disease by an eventual understanding of the potential biologic modifiers of gene by environment interactions [70], and the functional modifiers of

the genome [71]. To this end, such research may help inform underlying mechanisms and the long-term implications of epigenetics. The exposome will continue to evolve in terms of its conceptual and methodologic framework and its utility will be in its ability to promote population health.

Acknowledgments

The Intramural Research Program of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development. Dr. Patel was funded by the National Institute of Environmental Health Sciences (1R00ES023504, 1R21ES025052), and a gift from Agilent Technologies, Inc.

References

Papers of particular interest, published recently, have been highlighted as:

•Of importance

••Of outstanding importance

1. Buck Louis GM, Yeung E, Sundaram R, Laughon SK, Zhang CL. The exposome – exciting opportunities for discoveries in reproductive and perinatal epidemiology. 2013; 27:229–236. DOI: 10.1111/ppe.12040
2. Buck Louis GM, Sundaram R. Exposome: time for transformative research. Stat Med. 2012; 31:2569–2575. DOI: 10.1002/sim.5496 [PubMed: 22969025]
3. Cui Y, Balshaw DM, Kwok RK, Thompson CL, Collman GW, Birnbaum LS. The exposome - embracing the complexity for discovery in environmental health. Environ Health Perspect. 2016; 124(8):A137–A140. DOI: 10.1289/EHP412 [PubMed: 27479988]
4. Lioy PJ, Rappaport SM. Exposure science and the exposome: an opportunity for coherence in the environmental health sciences. Environ Health Perspect. 2011; 119:A466–A467. DOI: 10.1289/EHP412 [PubMed: 22171373]
5. Wild CP. Complementing the genome with an “exposome”: The outstanding challenge of environmental exposure measurement in molecular epidemiology. Cancer Epidemiol Biomarkers Prev. 2005; 14:1847–1850. DOI: 10.1289/ehp.1104387 [PubMed: 16103423]
6. Wild CP. The exposome: from concept to utility. Int J Epidemiol. 2012; 41:24–32. DOI: 10.1158/1055-9965.EPI-05-0456 [PubMed: 22296988]
7. Rappaport SM. Implications of the exposome for exposure science. J Expo Sci Environ Epidemiol. 2011; 21(1):5–9. DOI: 10.1038/jes.2010.50 [PubMed: 21081972]
8. Ho S-M, Johnson A, Tarapore P, Janakiram V, Zhang X, Leung Y-K. Environmental epigenetics and its implication on disease risk and health outcomes. ILAR Journal. 2012; 53(3–4):289–305. DOI: 10.1093/ilar.53.3-4.289 [PubMed: 23744968]
9. Miller GW, Jones DP. The nature or nurture: refining the definition of the exposome. Toxicol Sci. 2014; 137:1–2. DOI: 10.1093/toxsci/kft251 [PubMed: 24213143]
10. Miller, GW. The Exposome: A Primer. Academic Press Elsevier, Inc.; Waltham, MA: 2014.
11. Manrai AK, Cui Y, Bushel PR, Hall M, Karakitsios S, Mattingly C, et al. Informatics and data analytics to support exposome-based discovery for public health. Ann Rev Public Health. 2016; 38(1)
12. Gluckman PD, Hanson MA. Living with the past: evolution, development, and patterns of disease. Science. 2004; 305:1733–1736. DOI: 10.1126/science.1095292 [PubMed: 15375258]
13. Painter RC, de Rooij SR, Bossuyt PM, Simmers TA, Osmond C, Barker DJ, et al. Early onset of coronary artery disease after prenatal exposure to the Dutch famine. Am J Clin Nutr. 2006; 84:322–327. [PubMed: 16895878]
14. Ravnborg TL, Jensen TK, Andersson AM, Toppari J, Skakkebaek NE, Jørgensen N. Prenatal and adult exposures to smoking are associated with adverse effects on reproductive hormones, semen

quality, final height and body mass index. Hum Reprod. 2011; 26(5):1000–1011. DOI: 10.1093/humrep/der011 [PubMed: 21335416]

15. Obata Y, Kaneko-Ishino T, Koide T, Takai Y, Ueda T, Domeki I, et al. Disruption of primary imprinting during oocyte growth leads to modified expression of imprinted genes during embryogenesis. Development. 1998; 125(8):1553–1560. [PubMed: 9502736]
16. Owen CM, Segars JH. Imprinting disorders and assisted reproductive technology. Semin Reprod Med. 2009; 27:417–428. DOI: 10.1055/s-0029-1237430 [PubMed: 19711252]
17. Skakkebaek NE, Rajpert-DeMeyts E, Main KM. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. Hum Reprod. 2001; 16:972–978. DOI: 10.1093/humrep/16.5.972 [PubMed: 11331648]
18. Buck Louis GM, Cooney MA, Peterson CM. The ovarian dysgenesis syndrome. J Dev Origins Health Dis. 2011; 2(1):25–35. DOI: 10.1017/S2040174410000693
19. Trabert B, Zugna D, Richiardi L, McGlynn KA, Akre O. Congenital malformations and testicular germ cell tumors. Int J Cancer. 2013; 133(8):1900–1904. DOI: 10.1002/ijc.28207 [PubMed: 23580254]
20. Mogensen JB, Kjaer SK, Mellemkjaer L, Jensen A. Endometriosis and risks for ovarian, endometrial and breast cancers: A nationwide cohort study. Gynecol Oncol. 2016 Jul 15. pii: S0090-8258(16)30964-7. doi: 10.1016/j.ygyno.2016.07.095. [Epub ahead of print].
21. Eisenberg ML, Li S, Behr B, Cullen MR, Galusha D, Lamb DJ, et al. Semen quality, infertility and mortality in the USA. Hum Reprod. 2014; 29(7):1567–1574. DOI: 10.1093/humrep/deu106 [PubMed: 24838701]
22. Jensen TK, Jacobsen R, Christensen K, Nielsen NC, Bostofte E. Good semen quality and life expectancy: A cohort study of 43,277 men. Am J Epidemiol. 2009; 170(5):559–565. [PubMed: 19635736]
23. Buck Louis, GM. Early Origins of Endometriosis: Role of Endocrine Disrupting Chemicals. In: Giudice, L.Evers, JLH., Healy, D., editors. Endometriosis: Science and Practice. Oxford, UK: Wiley-Blackwell Publishers; 2012. p. 153-163.
24. Schuster M, Mackeen DA. Fetal endometriosis: A case report. Fertil Steril. 2015; 103(1):160–162. DOI: 10.1016/j.fertnstert.2014.09.045 [PubMed: 25450297]
25. Latham KE. Epigenetic modification and imprinting of the mammalian genome during development. Curr top Dev Biol. 1999; 43:1–49. [PubMed: 9891882]
26. Wang X, Chen C, Wang L, Chen D, Guang W, French J. Conception, early pregnancy loss, and time to clinical pregnancy: a population-based prospective study. Fertil Steril. 2003; 79:577–584. DOI: 10.1016/S0015-0282(02)04694-0 [PubMed: 12620443]
27. Buck Louis GM, Sapra KJ, Schisterman EF, Lynch CD, Maisog JM, Grantz KL, et al. Lifestyle and pregnancy loss in a contemporary cohort of women recruited prior to conception, LIFE Study. Fertil Steril. 2016; 106(1):180–188. DOI: 10.1016/j.fertnstert.2016.03.009 [PubMed: 27016456]
28. Jones GM, Cram DS, Song B, Kokkali G, Pantos K, Trounson AO. Novel strategy with potential to identify developmentally competent IVF blastocysts. Hum Reprod. 2008; 23(8):1748–1759. DOI: 10.1093/humrep/den123 [PubMed: 18477572]
29. Katz-Jaffe M, Gardner DK. Can proteomics help to shape the future of human assisted conceptions? Reprod Biomed Online. 2008; 17:497–501. [PubMed: 18854102]
30. Scott R, Seli E, Miller K, Sakkas D, Scott K, Burns DH. Noninvasive metabolomics profiling of human embryo culture media using Raman spectroscopy predicts embryonic reproductive potential: a prospective blinded pilot study. Fertil Steril. 2008; 90(1):77–83. DOI: 10.1016/j.fertnstert.2007.11.058 [PubMed: 18281045]
31. Green BB, Kappil M, Lambertini L, Armstrong DA, Guerin DJ, Sharp AJ, et al. Expression of imprinted genes in placenta is associated with infant neurobehavioral development. Epigenetics. 2015; 10(9):834–841. DOI: 10.1080/15592294.2015.1073880 [PubMed: 26198301]
32. Green BB, Marsit CJ. Select prenatal environmental exposures and subsequent alterations of the gene-specific and repetitive element DNA methylation in fetal tissues. Curr Environ Health Rep. 2015; 2(2):126–136. DOI: 10.1007/s40572-015-0045-0 [PubMed: 26231362]

33. Patel CJ, Bhattacharya J, Butte AJ. An environment-wide association study (EWAS) on type 2 diabetes mellitus. PLoS One. 2010; 5(5):e10746.doi: 10.1371/journal.pone.0010746 [PubMed: 20505766]
34. Keiding N, Louis TA. Perils and potentials of self-selected entry to epidemiological studies and surveys. J R Statist Soc A. 2016; 179:1–28. Part. 2.
35. Brabham DC, Ribisl KM, Kirchner TR, Bernhardt JM. Crowdsourcing applications for public health. Am J Prev Med. 2014; 46(2):179–187. DOI: 10.1016/j.amepre.2013.10.016 [PubMed: 24439353]
36. Eagle N, Pentland A. Reality mining: sensing complex social systems. Personal Ubiquitous Comput. 2006; 10(4):255–268. DOI: 10.1007/s00779-005-0046-3
37. Balshaw DM, Kwok RK. Innovative methods for improving measures of the personal environment. Am J Prev Med. 2012; 42(5):558–559. DOI: 10.1016/j.amepre.2012.02.002 [PubMed: 22516500]
38•. Nieuwenhuijsen MJ, Donaire-Gonzalez D, Foraster M, Martinez D, Cisneros A. Using personal sensors to assess the exposome and acute health effects. Int J Environ Res Public Health. 2014; 11(8):7805–7819. This study provides the reader with thoughtful perspectives about measuring exposures including new devices. DOI: 10.3390/ijerph110807805 [PubMed: 25101766]
39•. Patel C, Manrai AK. Development of exposome correlation globes to map out environment-wide associations. Pac Symp Biocomput. 2015; 20:231–242. This paper provides the reader with one of the first systematic maps of pairwise correlations between >250 exposures, displaying their dense interdependencies. DOI: 10.1142/9789814644730_0023
40. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Ser B. 1995; 57(1):289–300.
41. Patel CJ, Ioannidis JPA. Placing epidemiological results in the context of multiplicity and typical correlations of exposures. J Epidemiol Community Health. 2014; 68(11):1096–1100. DOI: 10.1136/jech-2014-204195 [PubMed: 24923805]
42. Nyholt DR. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. Am J Hum Genet. 2004; 74(4):765–769. DOI: 10.1086/383251 [PubMed: 14997420]
43. Patel CJ, Bhattacharya J, Butte AJ. An environment-wide association study (EWAS) on type 2 diabetes mellitus. PLoS One. 2010; 5(5):e10746.doi: 10.1371/journal.pone.0010746 [PubMed: 20505766]
44. Patel CJ, Cullen MR, Ioannidis JPA, Butte AJ. Systematic evaluation of environmental factors: persistent pollutants and nutrients correlated with serum lipid levels. Int J Epidemiol. 2012; 41(3): 828–843. DOI: 10.1093/ije/dys003 [PubMed: 22421054]
45. Efron, B. Large-Scale Inference. Cambridge: Cambridge University Press; 2010.
46. Andra SS, Austin C, Arora M. The tooth exposome in children's health research. Curr Opin Pediatr. 2016; 28:221–227. DOI: 10.1097/MOP.0000000000000327 [PubMed: 26859286]
47. Patel CJ, Rehkopf DH, Leppert JT, Bortz WM, Cullen MK, Chertow GM, et al. Systematic evaluation of environmental and behavioural factors associated with all-cause mortality in the United States National Health and Nutrition Examination Survey. Int J Epidemiol. 2014; 42(6): 1795–1810. DOI: 10.1093/ije/dyt208
48. Patel CJ, Ioannidis JPA, Cullen MR, Rehkopf DH. Systematic assessment of the correlations of household income with infectious, biochemical, physiological, and environmental factors in the United States, 1999–2006. Am J Epidemiol. 2015; 181(3):171–179. DOI: 10.1093/aje/kwu277 [PubMed: 25589242]
49. Pearson TA, Manolio TA. How to interpret a genome-wide association study. JAMA. 2008; 11:1335–44. DOI: 10.1001/jama.299.11.1335
50. McGinnis DP, Brownstein JS, Patel CJ. Environment-wide association study of blood pressure in the National Health and Nutrition Examination Survey (1999–2012). Sci Rep. 2006; 30373doi: 10.1038/srep30373
51. Ioannidis JPA. Exposure-wide Epidemiology: revisiting Bradford Hill. Stat Med. 2016; 35(11): 1749–1762. DOI: 10.1002/sim.6825 [PubMed: 26646432]

52. Patel CJ, Zhongkai Hu TY, Wen Q, Sung J, El-Sayed YY, Cohen H, et al. Investigation of maternal environmental exposures in association with self-reported preterm birth. Reprod Toxciol. 2013; 45:1–29. DOI: 10.1016/j.reprotox.2013.12.005

53. Patel CJ, Ioannidis JPA. Studying the elusive environment in large scale. JAMA. 2014; 311(21): 2173–2174. DOI: 10.1001/jama.2014.4129 [PubMed: 24893084]

54. Chadeau-Hyam M, Campanella G, Jombart T, et al. Deciphering the complex: methodological overview of statistical models to derive OMICS-based biomarkers. Environ Mol Mutagen. 2013; 54(7):542–557. DOI: 10.1002/em.21797 [PubMed: 23918146]

55. Sun Z, Tao Y, Li S, Ferguson KK, Meeker JD, Park SK, et al. Statistical approaches for constructing health risk models with multiple pollutants and their interactions: possible choices and comparisons. Environ Health. 2013; 12:85. http://www.ehjournal.net/content/12/1/85. doi: 10.1186/1476-069X-12-85 [PubMed: 24093917]

56. Go Y-M, Walker DI, Liang Y, Uppal K, Soltow QA, Tran V, et al. Reference standardization for mass spectrometry and high-resolution metabolomics applications to exposome research. Toxicol Sci. 2015; 148(2):531–543. DOI: 10.1093/toxsci/kfv198 [PubMed: 26358001]

57. Lenters V, Portengen L, Smit LAM, Jönsson BAG, Giwercman A, Rylander L, et al. Phthalates, perfluoroalkyl acids, metals and organochlorines and reproductive function: a multipollutant assessment in Greenlandic, Polish and Ukrainian men. Occup Environ Med. 2015; 72(6):385–93. DOI: 10.1136/oemed-2014-102264 [PubMed: 25209848]

58. Juarcz PD, Matthews-Juarez P, Hood DB, Im W, Levine RS, Kilbourne BJ, et al. The public health exposome: a population-based, exposure science approach to health disparities research. Int J Environ Res Public Health. 2014; 11:12866–12895. DOI: 10.3390/ijerph111212866 [PubMed: 25514145]

59. Buck AJ, Vena JE, McGuinness BM, Cooney MA, Louis GM. Communicating serum chemical concentrations to study participants: follow up survey. Environ Health. 2010 May 4.:9–20. DOI: 10.1186/1476-069X-9-20 [PubMed: 20170506]

60••. Vrijheid M, Slama R, Robinson O, Chatzi L, Coen M, van den Hazel P, et al. The human early-life exposome (HELIX): project rationale and design. Environ Health Perspect. 2014; 122:535–544. This study provides the reader with an example of how one might proceed in designing exposome research. DOI: 10.1289/ehp.1307204 [PubMed: 24610234]

61. HEALS. Health and Environment-wide Associations based on Large population Surveys. 2015. http://www.heals-eu.eu/

62. EXPOSOMICS. 2015. http://www.exposomicsproject.eu/

63. HERCULES. Hercules Exposome Research Center. 2015. http://emoryhercules.com

64. NIEHS. Children's Health Exposure Analysis Resource (CHEAR). 2016. https://www.niehs,nih.gov/research/supported/exosure/chear

65. Oyana TJ, Matthews-Juarez P, Cormier SA, Xu X, Juarez PD. Using an external exposome framework to examine pregnancy-related morbidities and mortalities: implicatons for health disparities research. Int J Environ Res Public Health. 2015; 13(1):ijerph13010013.doi: 10.3390/ijerph13010013 [PubMed: 26703702]

66. Kershenbaum AD, Langston MA, Levine RS, Saxton AM, Oyana TJ, Kilbourne BJ, et al. Exploration of preterm birth rates using the public health exposome database and computational analysis methods. Int J Environ Res Public Health. 2014; 11(12):12346–66. DOI: 10.3390/ijerph111212346 [PubMed: 25464130]

67. Steer CD, Bolton P, Golding J. Preconception and prenatal environmental factors associated with communication impairments in 9 year old children using an exposome-wide approach. PLoS One. 10(3):2–15. e0118701. DOI: 10.1371/journal.pone.0118701

68. Robinson O, Vrijheid M. The pregnancy exposome. Curr Environ Health Rep. 2015; 2:204–213. DOI: 10.1007/s40572-015-0043-2 [PubMed: 26231368]

69. Lewis RM, Demmelmair H, Gaillard R, Godfrey KM, Hauguel-de Mouzon S, et al. The placental exposome: placental determinants of fetal adiposity and postnatal body composition. Ann Nutr Metab. 2013; 63:208–215. DOI: 10.1159/000355222 [PubMed: 24107818]

70. Rappaport SM. Biomarkers intersect with the exposome. Biomarkers. 2012; 17(6):483–489. DOI: 10.3109/1354750X.2012.691553 [PubMed: 22672124]

71. Petronis A. Epigenetics as a unifying principle in the aetiology of complex traits and diseases. Nature. 465:721–727. DOI: 10.1038/nature09230

Table 1

Estimated critical and sensitive windows for specific human reproduction and development

Sensitive Window	Estimated Duration of Sensitive Window
Males	
Steroidogenesis and spermatogenesis	72 days
Females	
Folliculogenesis (selection dominant follicle)	4 months
Menstrual and ovarian cycles	29 days
Ovulation (post surge in luteinizing hormone)	24–36 hours
Fertilization	3–4 hours
Implantation	4–6 days
Early Conceptus	
Zygote	1 day
2-cell stage	1–2 days
4-cell stage	1–2 days
Morula	1–2 days
Blastocyst (formation and implantation)	1–2 days
Pregnancy (post-conception)	266 days 280 days (LMP)
Embryo (post-conception)	56 days
Fetus (post-conception)	30 weeks

NOTE: All times are estimated and vary across individuals and data source. LMP, last menstrual period dating.

Table 2

Comparison of traditional and exposome research methods

Design & Methodologic Considerations	Traditional Research	Exposome Research	Cautionary Notes for Exposome Research
Specify research question	X	X	Question may have many facets & typically agnostic in approach
Specify design	X	X	Prospective cohort designs remain ideal, but timing for lifetime study likely requires novel designs (e.g., pooling existing cohorts)
Specify study population/sample	X	X	Big N can mean big bias depending upon sampling framework (e.g., social media, crowdsourcing) External validity still important for impacting successful population health
Specify exposure(s)	X	X	Hierarchical and correlated exposures Chance of spurious findings with increasing number of exposures
Specify health outcome(s)	X	X	Consider interrelated & conditional nature of health outcomes Consider competing risk scenarios or joint modeling of 2+ health states
Specify analytic plan	X	X	Novel analytic approaches need applications for continued refinement Multiple exposomes (e.g., male, female, couple, fetus)
Interpretation & translation	X	X	Higher chance of false discovery, reverse causation Translating from population to individual health likely challenging