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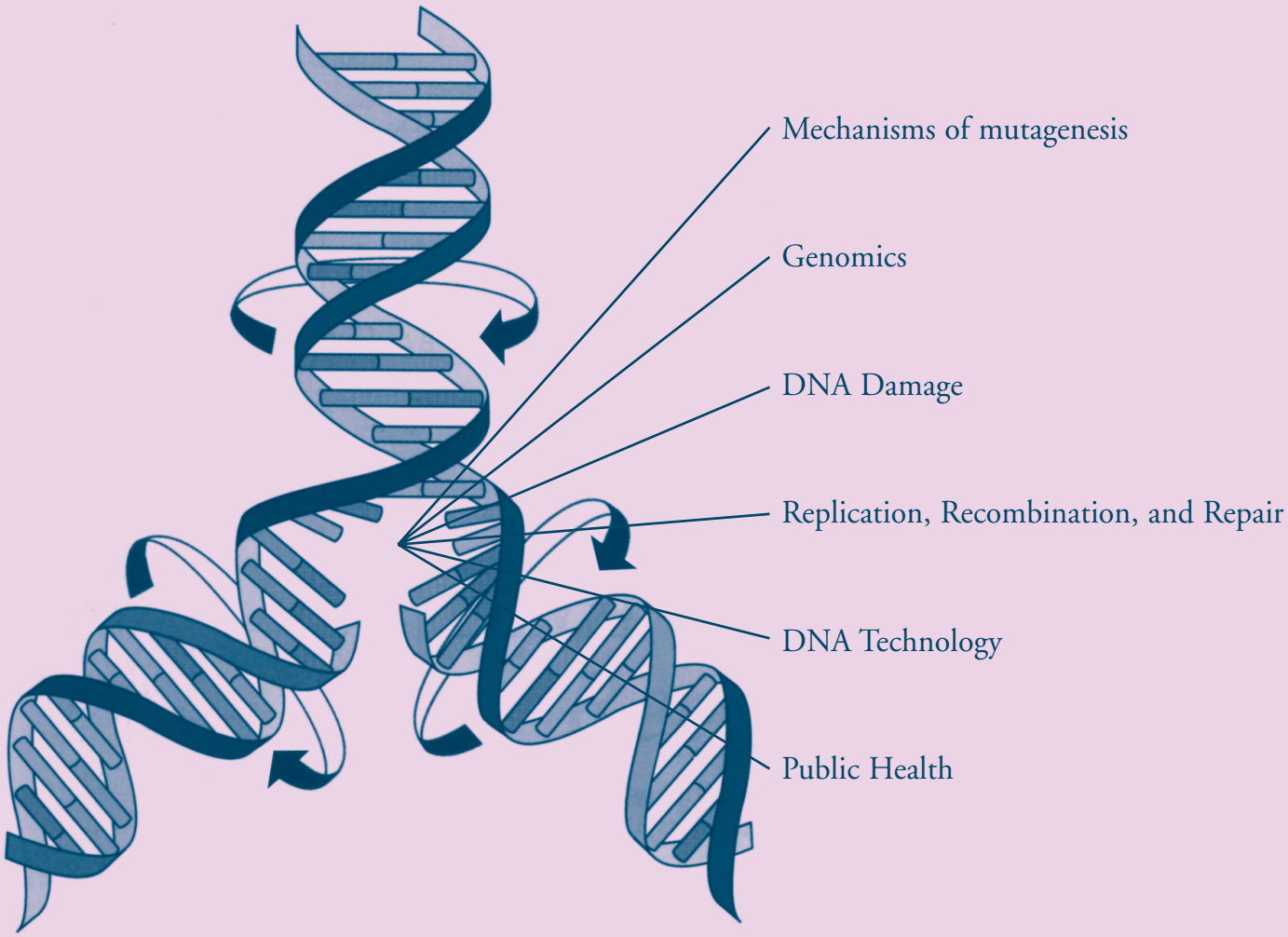

In this issue: Martyn T. Smith, winner of the 2014 Alexander Hollaender award, and co-workers present a commentary on the use of exposomics in cumulative risk assessment.

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Commentary

Using Exposomics to Assess Cumulative Risks and Promote Health

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Under the exposome paradigm all nongenetic factors contributing to disease are considered to be 'environmental' including chemicals, drugs, infectious agents, and psychosocial stress. We can consider these collectively as environmental stressors. Exposomics is the comprehensive analysis of exposure to all environmental stressors and should yield a more thorough understanding of chronic disease development. We can operationalize exposomics by studying all the small molecules in the body and their influence on biological pathways that lead to impaired health. Here, we describe methods by which this may be achieved and discuss the application of exposomics to cumulative risk assessment in vulnerable populations. Since the goal of cumulative risk assessment is to analyze, characterize, and quantify the combined risks to health from exposures to multiple agents or stressors, it seems that exposomics is perfectly poised to advance this important area of environmental health science. We should therefore support development of tools for exposomic analysis and begin to engage impacted communities in participatory exposome research. A first step may be to apply exposomics to vulnerable populations already studied by more conventional cumulative risk approaches. We further propose that recent migrants, low socioeconomic groups with high environmental chemical exposures, and pregnant women should be high priority populations for study by exposomics. Moreover, exposomics allows us to study interactions between chronic stress and environmental chemicals that disrupt stress response pathways (i.e., 'stressogens'). Exploring the impact of early life exposures and maternal stress may be an interesting and accessible topic for investigation by exposomics using biobanked samples. Environ. Mol. Mutagen. 56:715–723, 2015. © 2015 Wiley Periodicals, Inc.

Key words: exposome; risk assessment; biomarkers; stress; early life exposure

2014 ALEXANDER HOLLAENDER AWARD

The Environmental Mutagenesis and Genomics Society conferred this award to Dr. Martyn T. Smith for his outstanding contributions to the field of environmental toxicology. Dr. Smith's research has focused on the mechanisms by which environmental agents, such as benzene, pesticides, and arsenic, exert genotoxic effects relevant to cancer. Many of the major advances in understanding the adverse effects of benzene have been derived from Dr. Smith's research. He has been a pioneer in the use of genomic, proteomic and epigenomic approaches to fully characterize changes occurring in workers exposed to environmental toxicants and in promoting the exposome paradigm. Dr. Smith has contributed to public health protection through his efforts on advisory panels and expert working groups and his research contributions have resulted in public health-protective regulatory actions around the world.

THE EXPOSOME AND THE NEW FIELD OF EXPOSOMICS

Several definitions of the exposome now exist. Wild originally defined the "exposome" as representing all environmental exposures (including those from diet, lifestyle, and endogenous sources) from conception onwards,

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as a quantity of critical interest to disease etiology [Wild, 2005]. His goal in doing so was to articulate the need for new tools to assess environmental exposures from all sources for studies of adverse gene-environment interactions as causative factors in chronic disease.

As toxicologists we recognize that adverse effects on the body's tissues and organs are related to the concentration of chemical agents circulating in the biofluids that bathe the tissues, notably the blood plasma and lymph. This internal dose of the chemical or drug is directly related to the toxicity and biological effects at given concentrations. Thus, when Rappaport and Smith considered how Wild's original exposome concept could be measured, they concluded that this could best be achieved by monitoring the internal chemical environment of the human body during critical windows of exposure (i.e., measuring "snapshots") [Rappaport and Smith, 2010]. They also recognized that all chemical and nonchemical stressors mediate effects on the body via signaling of small molecules that alter cellular activity and physiological processes. For example, during emotional stress our adrenal glands release adrenaline (also known as epinephrine) and other hormones into the bloodstream that increase breathing, heart rate, and blood pressure. Thus, if one wants to consider all nongenetic factors that influence health, it is reasonable to consider the "environment" as the body's internal chemical environment and "exposures" as the amounts of biologically active chemicals (small molecules) in this internal environment that stem from both exogenous and endogenous sources.

The new field of exposomics should therefore attempt to measure as many small molecules as possible in human bodily fluids. A million molecule exposome is a potential goal that is not too unrealistic. Further, it should attempt to link the presence of these small molecules with functional changes in biology leading to chronic illnesses. The internal measurements made in exposomics could be of individual chemicals, groups of chemicals or the totality of chemicals acting on a particular receptor or biological pathway in a functional assay. Hence, exposomics can be operationalized by studying all the small molecules in the body and their influence on biological pathways that lead to impaired health. This concept of exposomics fits with the revised definition of the exposome proposed by Miller and Jones that explicitly incorporates the body's response to environmental influences [Miller and Jones, 2014]. They argue that the exposome and biology are interactive and that changes in biology due to the environment may change one's vulnerability to subsequent exposures. Further, Miller and Jones argue that by studying the effects of exposures we may gain insight into past chemical exposures as they may leave a molecular fingerprint. Thus, through linking exposures to specific biological responses, exposomics could serve as an approach to gain insight into the mechanistic connections between a culmination of exposures and risk of adverse health outcomes that occur over a lifetime.

ENVIRONMENTAL STRESSORS, EXPOSURE ASSESSMENT, AND THE EXPOSOME

Another, entirely different approach to examine the relationships between environmental exposures and disease is to measure exposures to various environmental stressors through wearable and regional sensors and survey instruments. These are being used, for example, to measure exposure to air pollution and drinking water contaminants; to better assess the diet through smartphone capture of dietary habits; and, to evaluate exercise through pedometers and other devices. This is how measurement of the exposome was conceptualized in a NAS committee report on exposure science and was expanded to the term eco-exposome so as to include wildlife as well as humans [Committee on Human and Environmental Exposure Science in the 21st Century and Board on Environmental Studies and Toxicology, 2012]. Sensors and 21st century exposure science tools are, of course, useful for improving exposure assessment in targeted epidemiology studies of specific risk factors, such as physical exercise, diet, and air pollution and for avoiding known risks through smartphone applications and other mechanisms. These exposure science tools are limited, however, in their ability to identify novel environmental causes of disease, but in combination with internal exposomics tools they could be a powerful approach to assessing an individual or community's exposome.

TOWARDS MEASUREMENT OF A COMMUNITY'S EXPOSOME AT THE INDIVIDUAL AND GROUP LEVELS

Measuring environmental pollutants has become a subset of an even broader initiative termed the "Public Health Exposome", coined by Juarez, which captures an assessment of risk at the community level, including the influences of the natural, built, social, and policy environment [Juarez et al., 2014]. The natural environment includes chemicals in air, water, soil, and food. The built environment includes quality of the workplace, educational centers, places of worship, and playgrounds as well as access to commercial businesses and public transportation. The social environment includes rates of discrimination, poverty, crime, unemployment in the surrounding area and moderating factors such as social networks, capital, and integration. Lastly, the policy environment represents local rules and regulations that influence the quality of public health services and exposures.

This public health exposome approach incorporates exposures at the ecological-level to determine the impact on the overall health of a population within a particular

TABLE I. Exposomics in the Context of the "Public Health Exposome" [Juarez et al., 2014]

Environment type	Examples	Biological response	Health impact
Natural	• Quality of air, water, soil, food • Chemical contamination	• Inflammation, reactive oxygen species, protein/DNA adducts, • Methylation and gene expression changes	Chronic diseases including cancer and diabetes
Built	• Quality of workplace and housing • Presence of educational centers, places of worship, playgrounds • Access to fresh produce, commercial businesses, public transportation, greenery • Proximity to roadways	• Increased responsiveness to cortisol and "stressogen" on the glucocorticoid receptor • Changes in sex hormone levels and receptor responses	Stress and chronic health issues induced by poor living quality, and lack of resources and social interaction
Social	• Rates of discrimination, poverty, crime, violence, unemployment, gentrification, de facto segregation • Access to capital, loans, social services, law enforcement, education, and health care	• Increased adrenaline, resting heart rate, and blood pressure (vasoconstriction) • Altered brain function, structure and plasticity • Increased pro-inflammatory cytokine secretion	Psychological effects due to unsafe settings and turbulent activities near the home coupled with a lack of economic and community support
Policy	• Impacts of state and federal regulations and laws • Restrictive city ordinances • Local rules • Voting rights • Housing laws • Evident corruption • Voice within town council	• Changes in concentrations of neurotransmitters (ie. dopamine, serotonin, GABA)	Emotional insecurity and feelings of hopelessness due to inequality, disenfranchisement and lack of political representation

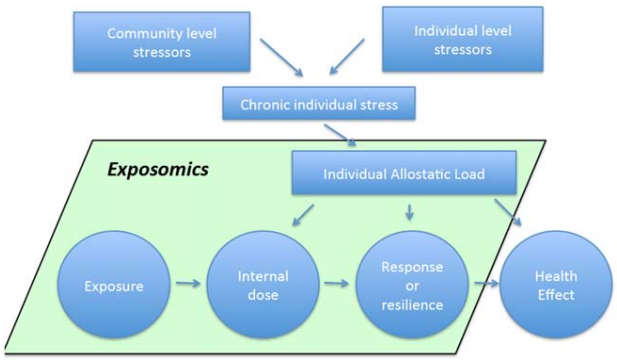

Fig. 1. Cumulative risk framework including the exposome (based on [Morello-Frosch and Shenassa, 2006]).

region. One of the first studies on the public health exposome, included over 600 variables for counties throughout the US to better understand determinants of preterm birth [Kershenbaum et al., 2014]. Interestingly, a unique clustering method distinguished between "resilient counties" with low preterm birth rates nestled within high-risk regions. Hence, identification of resilient versus susceptible sub-groups may be key in deciding optimal target populations for comparison or intervention studies in exposomics.

The public health exposome can uncover plausible sources of social determinants of health that contribute to the internal exposome. In Table I, we expand upon this framework proposed by Juarez et al. by providing examples of biological mechanisms disrupted through various community level exposures. Exposomics would allow detection of these biological responses and, furthermore, assessment of the overall health impacts (Table I). This may be a particularly novel approach for assessing cumulative risk in the community setting.

USING EXPOSOMICS TO ASSESS CUMULATIVE EXPOSURES AND CUMULATIVE RISK

From this discussion, one can see that the health of a given community, and the individuals within it, is dependent on a variety of environmental and social factors. The EPA defines cumulative risk assessment as, "Combined risks from aggregate exposures to multiple agents or stressors, where agents or stressors may include chemical and nonchemical stressors" [US EPA, 2003]. This is essentially the exposome paradigm where all non-genetic environmental stressors are considered. Therefore, cumulative risk assessment, where the impact of all stressors on a population is assessed, could be operationalized by exposomics (Fig. 1).

There has been little effort so far to examine the totality of both chemical and nonchemical stressors on a population. Initial observations of low-income, race, and other socioeconomic factors exacerbating the effects of individual chemical exposures have been reported [Shankardass et al., 2009; Zota et al., 2013; Vishnevetsky et al., 2015]. New agnostic methods can be applied to identify candidate chemicals that exacerbate disease risk via interaction with effects of the social environment. Exposomics could be used for the discovery of environmental chemicals which interfere with stress response pathways that are chronically activated by adverse social environments.

Bruce McEwen was the first to propose that prolonged activation of these stress response pathways causes "wear and tear" on regulatory mechanisms, adjusting the homeostatic set point of various physiological systems [McEwen, 1998]. This cumulative burden on the body is referred to as the allostatic load and is quantified using a cumulative index of physiologic deregulation of the cardiovascular, inflammatory, and endocrine systems (Fig. 1). While there is evidence that increased allostatic load and stressful life-experiences enhance vulnerability to the adverse health and behavioral effects of chemicals [Shankardass et al., 2009; Zota et al., 2013; Vishnevetsky et al., 2015], it is unclear how these "natural" and "social" environments work in concert to cause disease.

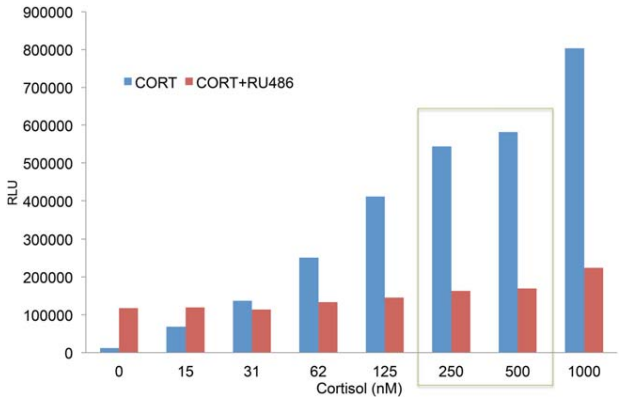

Fig. 2. Bioassay modeling disrupted glucocorticoid receptor (GR) signaling. Cortisol is a GR agonist. RU486 is a chemical antagonist that inhibits cortisol activation of GR. The box represents the endogenous cortisol range of 193–690 nM. Environmental stressogens may act as an agonist like cortisol or as antagonists like RU486.

An exposomics approach would quantify endogenous primary mediators found in the blood, such as cortisol and adrenaline, to obtain a measurement of "allostatic load". Cortisol, secreted by the adrenal gland in response to stress, activates the glucocorticoid receptor (GR) and has systemic effects on the endocrine, metabolic, cardiovascular, immune, reproductive, and central nervous systems [Sapolsky et al., 2000]. Environmental chemicals that mimic cortisol can disrupt stress response pathways through altered GR signaling [Odermatt et al., 2006; Odermatt and Gumy, 2008]. We define these environmental chemicals that alter stress response pathways as "stressogens."

Within the context of exposomics, it is essential to obtain a measure of the total stressogen burden within subject samples. Using a functional bioassay that measures GR activity, we have identified a number of stressogens, including the morning-after pill, RU486, that perturb the stress response by exerting either agonistic or antagonistic effects on GR (Fig. 2). We are now applying this assay to identify additional environmental chemicals that may act as stressogens and to measure the totality of chemicals acting on GR in an individuals blood plasma. Exposomic classification of stressogens and detection of endogenous stress response mediators moves us one step closer to developing more holistic models of attributable risk factors of disease.

TARGETED AND UNTARGETED METHODS TO MEASURE SNAPSHOTS OF THE EXPOSOME

To measure snapshots of the exposome, we must be able to quantify exposure to and the impacts of all nongenetic factors including chemicals, drugs, dietary components and supplements, psycho-social stress, infection, and ionizing radiation during critical stages in the life course. This is clearly a major challenge but seemingly not an impossible one. By focusing on classes of

TABLE II. Current Techniques for Exposomics

- Metabolomics: ~30,000 small molecules in untargeted analysis; targeted analysis of 100–500 compounds.
- Targeted mass spectrometry: Can measure low levels of environmental pollutants.
- Adductomics: measures electrophiles binding to blood proteins.
- Hormone receptor activation in cell based assays: measure endocrine disruptors.
- AhR cell based assay: measures totality of persistent organic pollutants (POPs) and short-term transient activators.
- Mass spectrometry and speciation of metals: ~20 easily measured.
- Antibody arrays and subtractive sequencing: measures current and past exposure to infectious agents.
- Assays of telomere length, telomerase activity, CD28 cells, cortisol, amylase: measures stress.
- Oxidative stress markers: isoprostanes etc. (panel).
- Markers of inflammation: cytokines, C-reactive protein (panel).
- Early biomarkers of response/resilience: transcriptome, methylome, cellular immune response, etc.

chemicals with probable effects such as electrophiles and chemicals that target specific receptors we may be able to assess the impacts of many of the chemicals in commerce (Table II). Further, modern mass spectrometry now allows us to measure pharmaceuticals, vitamins, and other dietary components with relative ease and is being expanded to untargeted methods which measure thousands of molecular ions (Table II). Psycho-social stress could be measured by various markers including telomere length, cortisol and amylase levels and activity through stress response pathways such as GR (Table II). It is also important to measure current and prior exposures to infectious agents, as they can play an important role in chronic disease development.

There is some debate over the best strategies to use for exposomics research, given the limitations of both targeted and untargeted methods. While untargeted methods provide promise in examining thousands of molecules simultaneously, some sensitivity is sacrificed in measurement of low abundance compounds. It has been previously observed in the literature that the majority of pollutants are at 100–1000 times lower concentration than drugs and dietary components [Rappaport et al., 2014]. While this begets the need for targeted methods with improved sensitivity, it is important to incorporate both in exposomics research.

The advantage of untargeted methods is the potential for discovery of novel analytes while measuring hundreds to thousands of compounds simultaneously. This technique has been demonstrated successfully in previous cases [Wang et al., 2011a,b], however, given the statistical limitations of these methods, the likelihood of obtaining reproducible findings still remains small. To improve upon characterization of "biologically active" molecules in the blood by metabolomics, the method could be paired with other assays to quantify the net potential effect of endogenous and exogenous compounds in human serum. These preliminary screening methods may allow discrimination between analytes of interest and background noise that are measured using untargeted approaches (i.e., metabolomics). An example of such methods is use of receptor-binding reporter assays in responses to chemicals in human blood samples. Currently we are using sensitive CALUX receptor-based reporter bioassays, which measure the overall net effect of both endogenous and exogenous molecules acting on a particular receptor simultaneously (e.g., This includes the GR receptor activity from stressogens, as described earlier.) This high-throughput and inexpensive method of detecting total endocrine activity of serum against a particular receptor can be scaled-up, as previously done for purposes of chemical screening within ToxCast and Tox21.

Several methods are being explored to isolate the candidate active agonistic/antagonistic compounds from serum. For example, the serum can be fractionated by HPLC, and then the fractions can be applied separately to the receptor assays to measure activity of endogenous hormones versus exogenous chemicals [Bonefeld-Jorgensen et al., 2011]. Another method is to use receptor affinity extraction liquid chromatography to first isolate the chemicals that bind to the column and then elute the bound chemicals for further profiling by LC–MS/MS [Hock, 2012]. This has been improved upon by immobilizing the receptor ligand binding domain, which has more stable binding affinity than the entire receptor and still maintains high sensitivity to xenobiotics. While this method was originally demonstrated with ERα [Pillon et al., 2005], it can be expanded to other binding domains as well [US EPA]. Lastly, active molecules could be identified by running the serum in tandem on both the bioassay and an HPLC–MS/MS instrument, and modeling differences in average peak sizes between comparison populations in association with reporter signals. These agnostic methods provide an exposomic approach to detect novel endogenous and exogenous exposures that influence cellular function.

Targeted methods of past and current exposures are also useful for examining chemical compounds that are known to be pervasive and/or bioaccumulative in the environment. With improved resolution of instrumentation, smaller volumes are needed than before to assess levels of these chemicals in bodily fluids. For example, Agilent Technologies has recently developed a method using a quadrupole GC–MS/MS system with only 200 μL of plasma/serum to measure more than 60 POPs including PCBs, PBDEs, OCPs, PAHs, furans, and dioxins [Macherone et al., 2015]. This has potential for scale-up to measure even more compounds. Plasma is extracted using chemical denaturation, liquid–liquid extraction, solid-phase cleanup, and reconstituted with isooctane. This targeted GC MS/MS method exemplifies improvements in measuring differential POPs exposure profiles over those previously used by the CDC (NHANES) and others by reducing volumes of precious blood samples by at least 10-fold. The limits of detection are 0.005–0.02 ng/mL for PCB; 0.05–0.15 ng/mL for OCP; 0.0075–0.075 ng/mL for PBDE. Targeted methods like these should be restricted to chemicals such as POPs with known persistence in the environment and association with harmful effects. Interestingly, given the long half-life of these pollutants, previous exposure and migration patterns can be chronicaled, particularly among populations that have migrated from highly exposed to lower exposed areas during their life.

INCLUDING MEASUREMENT OF EXPOSURE TO INFECTIOUS AGENTS IN EXPOSOMICS RESEARCH

New advancements in detecting past and current exposure to infectious agents allows for expansion of this branch of exposomics. Recently, a screening procedure,

called VirScan, has demonstrated extreme sensitivity and specificity for detecting antibodies against previous infections in just 1 μL of serum [Xu et al., 2015]. The VirScan target library is based on the viral proteome sequence database within UniPro [Consortium, 2014] and includes 206 known viral species and over 1000 different strains. Several strategies have been used to discover novel non-human sequences in the human transcriptome including digital transcriptome subtraction [Feng et al., 2008]. To detect such integrated viral sequences, algorithmic methods, such as VirScan, can screen RNA Seq or whole genome data for viruses that map to a viral database [Chen et al., 2013]. Recently, "sequence-based ultrarapid pathogen identification," SURPI, was developed to assess both known and novel bacterial, viral, fungal, and parasitic sequences in human tissue samples [Naccache et al., 2014]. Published NGS data can also be scavenged for discovery of new emerging infectious agents. This was exemplified using metagenomics data from fecal samples of twins and their mothers from a public database to discover a new species of bacteriophage and then validated in a separate target population of over 900 samples [Dutilh et al., 2014]. These new techniques to study current and previous infections in population studies are imperative to understanding their relationship with other exposures and disease onset within the exposome.

Exposomics research relies on understanding the interactions of both past and present exposures to chemical and nonchemical agents, but there are few studies that have examined links between environmental exposures and susceptibility to new or recurrent infection. Previous work has focused on early-life exposure to individual environmental pollutants and increased incidence of viral infections. Associations have been found between early-life exposure to persistent organic pollutants such as PAHs, dioxins, and PCBs and increased risk of flu-like symptoms, and respiratory and ear infections [Winans et al., 2011]. There is also evidence of altered immune function with early-life exposures to heavy metals such as arsenic [Rager et al., 2014], and increased mortality from infection due to arsenic exposure [Smith et al., 2010]. Exposomics has the capacity to expand upon these findings by examining how interactions between numerous chemical and nonchemical stressors increase risk of disease by infectious agents.

IN WHICH POPULATIONS SHOULD WE DO EXPOSOMICS?

If the objective of exposomics is to perform an agnostic search of many different environmental exposures, populations with the highest "totality of exposures" are of primary interest. Attention should focus on vulnerable environmentally-exposed populations, as the risk of chronic illnesses are higher than in the general population. Examples of these "at risk" groups in the U.S. are undisputedly minority populations living in urban or agricultural settings. This is exemplified by the CalEnviroScreen 2.0 [Faust et al., 2014], which maps scores by county based on the pollution burden and population characteristics of the region. Counties with the highest (most severe) scores are invariably concentrated in low-income regions of urban city centers or the agricultural Valleys of California. Thus, these populations could be sampled and compared to adjacent populations with lower CalEnviroScreen scores.

Another population that may be well-suited to exposomic analysis is pregnant women and their newborn infants. Biobanked samples of mid-pregnancy maternal blood, cord blood, and Guthrie card blood spots could be used for exposomic analyses in relation to fetal growth, preterm delivery, birth defects, and other early life outcomes. Methods for the rapid analysis of these biobanked samples should be developed and applied in well-controlled epidemiological studies.

Immigrant populations are another exemplary group for exposomics research. These populations were exposed to different environmental and nonchemical stressors in early-life and may have made changes in behavior due to acculturation as compared to the native populations in their new and former residences. This leads to profound differences in disease incidence rates that could be driven by both environmental and genetic factors. Given the unique conditions of immigrant populations, several strategies in study design could be employed to help parse apart environmental from genetic factors. For instance, some populations continually immigrate to the same region for generations, making it possible to measure the exposomics profiles associated with the number of years since emigration as compared to first-generation, nonimmigrant, and nonemigrating populations, all with similar genetic background. Trans-generational effects on the immigrant population can be explored as well. Furthermore, differences in exposomic profiles between countries or regions of emigration could also be used to map genetic and nongenetic contributions to disease onset.

Using the exposomics approach to conduct cumulative risk assessments would be an excellent opportunity to examine differences in disease onset in immigrant populations. For example, this approach may help to resolve enigmas such as the "Hispanic Paradox," which is described as similar rates of health outcomes (including infant mortality, life expectancy, and mortality from CVD and major types of cancer) among immigrant Hispanic populations compared to whites, despite lower socioeconomic status [Markides and Coreil, 1986]. This effect dissipates with acculturation [Burgos et al., 2005]. Taking an exposome approach would incorporate previous observations of differences in early-life nutrients, chemical

exposures, stressogens, and nonchemical stressors into a single study, providing a more comprehensive assessment of exposure.

Another exemplary population for exposomics is the "South Asian Phenotype" of diabetes. This group is deserving of further investigation as South Asians are at fourfold higher risk of type 2 diabetes (T2D) as compared to Caucasian populations and begin to obtain insulin resistance at a relatively lower BMI and younger age of onset than Caucasians (reviewed in [Bakker et al., 2013]). While there has been individual studies to examine effects of low-birth weight, diet, chemical exposure, the *in utero* environment, and even mitochondrial activity in relation to T2D (reviewed in [Bakker et al., 2013]), an exposomics approach would take all these factors into account to explain this unique phenotype in these immigrant populations.

We are currently pursuing exposomic studies in both Hispanic and Indian populations. Specifically, our two study populations of interest are (1) a case–control subset of foreign-born and native Mexican American women from the San Francisco Bay Area Breast Cancer Study, comprising of 5,000 Hispanics, African-Americans, and non-Hispanic whites 2) a cross-sectional study of Indian Asian immigrants and native European whites residing in Greater London and nested within a continuing cohort, called the London Life Sciences Prospective Population (LOLIPOP) Study. While distinct outcomes (breast cancer versus type II diabetes) and populations are considered in these two studies, the exposomics methodology is similar for both. Improved understanding of the role of endogenous and exogenous compounds on endocrine response is imperative for both breast cancer and diabetes. We will examine hormone receptor activation of all small molecules in the serum using luciferase reporter bioassays. Then we will profile subjects with extremes of activity by untargeted high-resolution mass spectrometry (HRMS) of small molecules in the serum to determine which chromatogram peaks may be responsible for the widely-differing levels of receptor activity and are associated with disease onset.

For both of these studies, significant inheritable findings have already led to exciting progress in the respective disease fields. For the Latina population study, a protective SNP variant was identified 5' of the estrogen receptor 1 gene in those of Indigenous American descent [Fejerman et al., 2014]. For the LOLIPOP cohort, six unique genetic variant loci in six separate genes were reported specifically for Indian Asians— three genes were directly linked to insulin sensitivity and pancreatic beta-cell function [Kooner et al., 2011]. Given these strong inheritable components of disease within these susceptible sample populations, genome × exposome interactions will be of great interest once we obtain exposomics data.

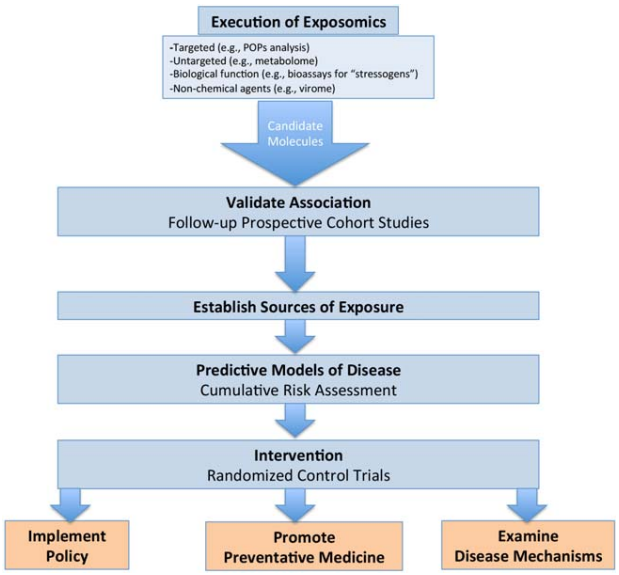

Fig. 3. How exposomics could contribute to disease prevention.

USE OF STRATEGIES FOR GENOMICS ANALYSIS TO INFORM EXPOSOMICS ANALYSIS

Overall, as genomics is the oldest and most advanced omics field, similar strategies employed for GWAS studies could be applied to exposomics. For example, the ease of genome sequencing today has facilitated the study of pleitrophy, defined as a single locus being responsible for multiple phenotypic traits. This is an important concept in studying inheritability of complex diseases (e.g., mental disorders, metabolic syndrome, and cancers)(reviewed in [Yang et al., 2015]). Moreover, pleitrophy in genomics can lend to novel findings of differences in environmental exposures. In a recent study using VARIMED (VARiants Informing MEDicine), a manually curated database of disease–SNP associations, an association was found between gene variants in three genes, gastric cancer and serum magnesium levels [Li et al., 2014]. In a follow-up assessment of medical records, the magnesium levels were altered 1-year prior to gastric diagnosis. We must consider how individual chemicals can have multiple targets in the body simultaneously and can increase risk of multiple phenotypic outcomes. Improved databases, such as ToxCast, that provide evidence of the relationships between chemical exposures and phenotypic traits will help guide the direction of appropriate chemical analysis in exposomics research.

As most chronic illnesses are multi-factorial, it is expected that multiple exposures may be involved with disease onset. The idea that particular exposures can be "inherited" together is an important concept that is likely to be specific per population. In the analysis phase it is important to consider the similarities of particular chemicals in structure and

mechanisms of actions against a given biological target, thus simplifying the combined effects of many exposures. Patel et al demonstrated correlations between particular exposures and the importance of recognizing these clusters [Patel and Manrai, 2014]. The paper draws an analogy to linkage disequilibrium of the genetic code, and how we must not think of every SNP as unique. This comparison could be expanded upon in consideration of other traits at the community level, including social determinants of health as those described by Juarez et al. [2014]

CONCLUSIONS AND RECOMMENDATIONS

Under the exposome paradigm all nongenetic factors contributing to disease are considered to be 'environmental' including industrial chemicals, drugs, infectious agents, and psychosocial stress. It is perhaps best to consider these as environmental stressors.

Exposomics is the comprehensive analysis of exposure to all environmental stressors and should yield a more thorough understanding of chronic disease development. Since exposomics can be performed at the individual as well as the population level it could have a broad impact on personalized preventative medicine, policy changes, and our understanding of disease mechanisms (Fig. 3).

Exposomics can also be used in the context of cumulative risk assessment. Since the goal of cumulative risk assessment is to analyze, characterize, and quantify the combined risks to health or the environment from exposures to multiple agents or stressors, it seems that exposomics is perfectly poised to advance this important area of environmental health science. We should therefore develop and apply exposomics to issue of cumulative risk and support development of tools for exposomic analysis. We should also begin to engage impacted communities and develop the public health exposome concept of Juarez and others. A first step may be to apply exposomics to vulnerable populations already studied by more conventional cumulative risk approaches. Moreover, inferences made from these exposomics studies within the context of cumulative risk assessment may be translated to policymakers for promoting change in environmental exposure regulations.

Exposomics allows us to study interactions between chronic stress and environmental chemicals and to discover environmental chemicals that may disrupt stress response pathways. We have named such chemicals 'stressogens' as they have the ability to influence how our bodies respond to stress. For example, exploring the role of environmental exposures and chronic stress in preterm delivery may be an interesting topic for investigation by an exposomic approach. We further conclude that susceptible groups (migrants, low socioeconomic groups with high environmental exposures, pregnant women) should be the study populations of interest for exposomics. Physicians who work with these populations nationwide and worldwide can use exposomics to work towards earlier identification of high-risk individuals and communities and ultimately disease prevention.

Finally, we highlight the importance of not "reinventing the wheel" when it comes to analysis of large amounts of data that will clearly be generated by exposomics studies. Collaboration with bioinformatists and biostatisticians skilled in analyzing genomics data and other patterns will be essential. This is an exciting time for scientific collaboration across disciplines, and using exposomics research may be transformative in our understanding of the causes of adverse health outcomes in human populations.

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