Using the Blood Exposome to Discover Causes of Disease

Technical Overview

Clinical Research

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Introduction

Of the 52.8 million world-wide deaths in 2010, approximately two-thirds were caused by chronic diseases, mainly cardiovascular disease (> 15 million) and cancer (> 7 million) [1]. Thus, it is reasonable to ask whether chronic diseases are attributable to genetic factors, exposures, or some combination of the two. Data compiled by the Swedish Family-Cancer Database indicate that genetic (G) risks for 15 common cancers were 10% or less [2]. This suggests that approximately 90% of cancer risks result from exposures (E) or G×E interactions.

Despite the relatively small genetic risks for cancer and other chronic diseases, exquisite tools are available to investigate G factors in studies of human diseases. In fact, genome-wide-association studies (GWAS) currently measures more than one million single nucleotide polymorphisms in 2,000–20,000 subjects. In contrast, individuals' exposures are still inferred from personal interviews or self-administered questionnaires [3] much as they were a century ago. Since this disparity in characterizing G and E makes it impossible to thoroughly investigate the GxE matrix, technologies and methods for data-driven analysis of exposures must be developed [4].

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The exposome represents the sum of all chemical exposures received by an individual over a lifetime from both exogenous sources (food, pollutants, ionizing radiation, drugs, lifestyle factor, infections) and endogenous sources (human and microbiota metabolism, oxidative stress, lipid peroxidation, infections, and preexisting disease). "As such, the exposome is everything that is 'not the genome' and serves as an umbrella for all of the traditional omes" [5]. Figure 1 illustrates the placement of the exposome in the continuum between states of human health and disease.

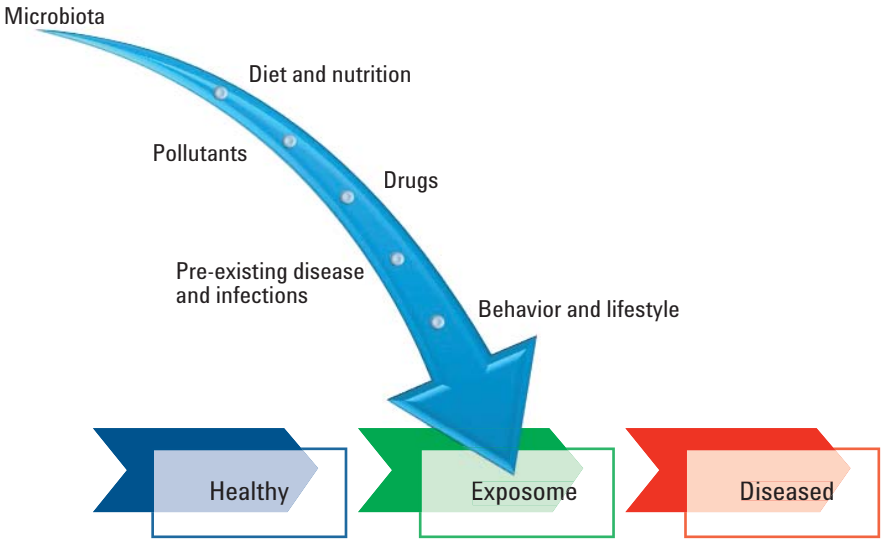


Figure 1. Exposure contributions to chronic human disease.

A Two-stage Strategy for Discovering and Targeting Causal Exposures

By focusing upon all circulating chemicals in the human body, the blood exposome motivates studies of nongenetic causes of chronic diseases in much the same manner that GWAS explores genetic causes [6,7,4]. To this end, Stephen M. Rappaport, Director of the Center for Exposure Biology at the University of California, Berkeley, describes a two-phase approach for interrogating the blood exposome to discover important exposures in disease cases and controls and then to target these exposures in follow-up studies with large populations [7].

Professor Rappaport functionally defines the exposome as roughly 200,000 circulating chemicals in human blood, including metals, small molecules, proteins, and foreign DNA. By comparing untargeted profiles of blood exposomes between diseased and healthy subjects, he suggests that we perform exposome-wide association studies (EWAS) to pinpoint discriminating chemicals [7]. After identifying these key chemicals and verifying their disease associations in independent samples of cases and controls, the chemicals can be used as biomarkers of exposures or disease progression in targeted analyses of blood from large populations. Thus, a successful strategy for discovering and reducing harmful exposures requires an initial data-driven investigation (EWAS), to find promising biomarkers, followed by knowledge-driven studies that use the biomarkers to elucidate exposure-response relationships (biochemical epidemiology), sources of exposure and human kinetics (exposure biology) and mechanisms of action (systems biology). Prof. Rappaport stresses that this two-step strategy will lead to reduced exposures, improved public health, early diagnosis of diseases, and personalized medical interventions. Figure 2 illustrates the two-step strategy of EWAS followed by targeted studies.

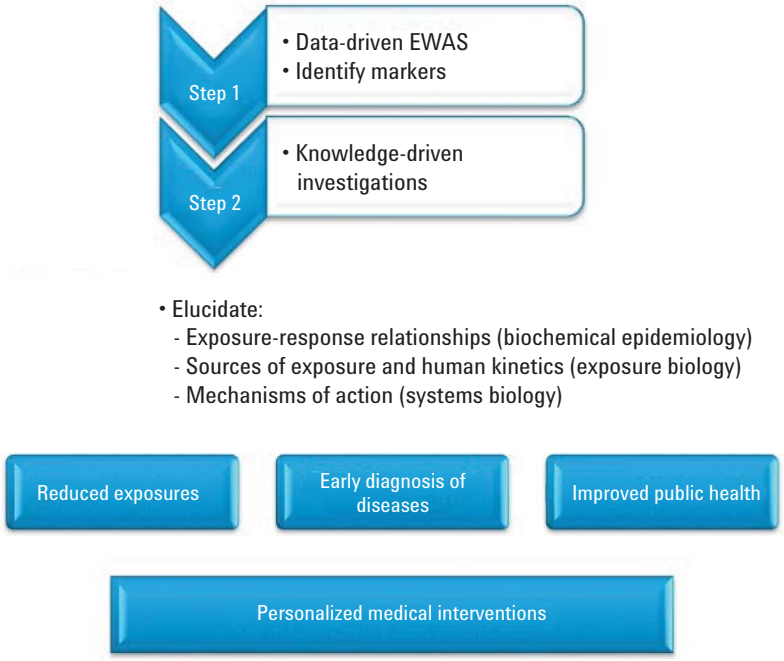
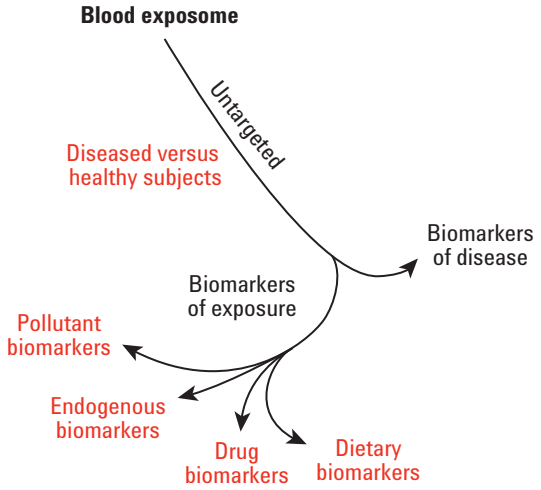


Figure 2. Two-phase EWAS targeted analytical strategy to identify and reduce harmful exposures.

Measuring the Exposome

Enormous technical challenges must be confronted to achieve the necessary combination of extreme multiplexing, sensitivity, and throughput required for EWAS and follow-up studies. It is desired to conduct untargeted EWAS with 10–50 µL of blood, serum, or plasma from each of a few hundred subjects, and to perform targeted analyses of biomarkers in equivalent volumes of blood or serum from thousands of subjects. A relevant analytical platform for many EWAS would wed high-resolution liquid chromatography (LC) or gas chromatography (GC) with a time-of-flight (TOF) mass spectrometer (MS) having excellent sensitivity and mass accuracy. Follow-up investigations of promising biomarkers could then employ robotics and triple quadrupole MS with multiple-reaction monitoring. Through rigorous selection of specimens from prospective cohort studies, it is possible to differentiate biomarkers of causal exposures from biomarkers of disease progression.



Based on:
S. Rappaport, *Biomarkers*, 2012, **17**(6), 48: 3-9

Figure 3. EWAS of blood offers differentiation of biomarkers of exposure (causal pathways) from biomarkers of disease (reactive pathways).

Proof-of-concept studies have already identified unknown biomarkers of exposure for colorectal cancer and cardiovascular disease and biomarkers of disease progression for diabetes [7].

Analytical Strategies

Clearly, dedicated platforms of nanoflow LC/TOF and LC/Triple Quad and GC/TOF, GC/Triple Quad are essential to ensure the necessary sensitivity and precision for both data-driven EWAS and targeted, high-throughput follow-up studies. To this end, Ivanisevic and Zhu, *et al*. (2013) [8] reported the ability to measure more than 30,000 unique features from a single 100 µL sample of serum or plasma in an untargeted analysis using reversed-phase LC and hydrophilic interaction chromatography (HILIC) in both positive and negative electrospray modes. The analytical methodology used an Agilent 1200 Series LC system coupled to an Agilent 6538 UHD Accurate-Mass Q-TOF LC/MS system, and is the approach needed for a small-molecule EWAS.

Figure 4. The Agilent 1200 Series LC system and an Agilent 6500 Series Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) LC/MS system.

Another relevant analytical approach involves modifying the Agilent-Fiehn GC/MS methodology [9] to incorporate an Agilent 7890B GC with an Agilent 7200 Q-TOF system for investigating serum extracts derivatized with methoxyamine hydrochloride and MSTFA. In post-acquisition processing, an Agilent retention-time-locked spectral library of over 1,000 chemical entities can be used to annotate unknown features. The data can be used independently or to orthogonally compliment and corroborate LC/MS data.

Figure 5. The Agilent 7890A GC with an Agilent 7200 Q-TOF system.

Once biologically relevant biomarkers are annotated and verified, targeted high-throughput analyses are needed to measure biomarkers of causal exposures and biomarkers of disease progression. A relevant example of such targeted analyses involves measurement of endocrine disrupting chemicals, using the Agilent 7890B GC with an Agilent 7000C Series Triple Quadrupole system [10]. Similar applications of the Agilent GC/MS system have been used in clinical research laboratories to measure estrogen biomarkers in breast cancer studies at extremely low levels (for example, 17β-estradiol can be detected at 0.12 pg/mL (4.4E^{-7} µM)) [11].

Once data are collected, sophisticated bioinformatic software is required to compare biomarker levels across populations and to investigate covariates. Agilent Mass Profiler Professional (MPP) Software provides advanced statistical analysis and visualization tools for GC/MS, LC/MS, CE/MS and ICP-MS data analysis that can be used to identify key features and perform global analyses. The analyst can further map these to biological pathways using the Pathway Architect tools.

Reactive Electrophiles

Reactive electrophiles represent an important class of toxic chemicals that is produced from metabolism of xenobiotic and endogenous precursor molecules, oxidative stress, and lipid peroxidation [12]. Although too reactive to measure directly in blood, levels of these electrophiles can be inferred by measuring adducts from reactions with prominent blood proteins such as human serum albumin (HSA). Prof. Rappaport's laboratory has proposed untargeted MS approaches for profiling adducts of HSA at the nucleophilic hotspot, Cys34, as part of EWAS [13] and for identifying prominent features by high resolution mass spectrometry [14].

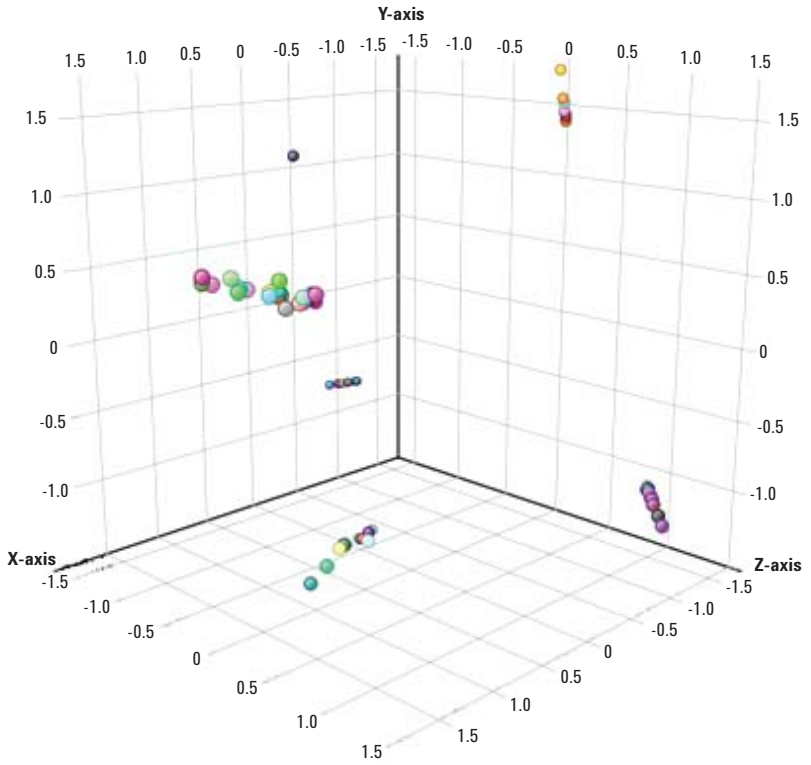


Figure 6. Mass Profiler Professional illustrating separation of different chemotypes from GC/TOF data using PCA analysis of ANOVA results.

Conclusion

Exposomics is a collaborative paradigm that brings together epidemiology, environmental toxicology, analytical chemistry, nutrition, and microbiology in an open access milieu. Since exposomics applies multiple analytical techniques (chromatography, spectrometry, spectroscopy, sensor-array technologies) and bioinformatics to characterize individuals' exposomes, it requires development of novel technologies to achieve the demands of high resolution, high sensitivity and high throughput for EWAS and follow-up investigations. The current state of exposome research could be compared to that of genomics in the early nineties. By integrating several omic technologies (metallomics, metabolomics, proteomics, and metagenomics) with one unified objective, the nascent field of exposomics may well provide the missing links to disease causality and personalized medicine.

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Printed in the USA
September 15, 2015
5991-3418EN

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