

Commentary

Molecular mechanism-based model to enhance outcomes of dietary intervention studies for disease prevention

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Abstract

Advances in “omics”-based fields have produced an explosion of new information, fueling high expectations for improved public and individualized health. Unfortunately, there exists a widening gap between basic biochemistry and “omics”-based population research, with both disciplines failing to translate their

full potential impact to human health applications. A paucity of comprehensive study systems is one of the many roadblocks faced by translational research today. This commentary will highlight the current status of such research, particularly emphasizing the role of nutrigenomics.

The “omics” age has the potential to revolutionize the diagnosis and management of various diseases by offering a comprehensive view of the molecular underpinnings of pathology. However, a decade after publication of the complete sequence of the human genome, the promise of genomics for improved treatment and better disease prevention is far from being fulfilled. For example, among the 3,000 single-gene disorders for which the responsible gene has been identified, only a handful (<1%) have had this knowledge translated directly into a new therapy. The full picture, which also includes multi-gene disorders, is even more disappointing as single-gene disorders are estimated to account for only 9% of childhood mortality and less than 2% of overall hospital admissions in the United States (Korf & Mikhail 2007). The vast preponderance of health care costs are devoted to the treatment of common complex disorders, such as coronary artery disease, stroke, diabetes, hypertension, and cancer, which all appear to have large, heritable components (Ginsburg 2011). The National Institutes of Health (NIH) has made “translational” or “bench-to-bedside” research a priority, forming specialized centers and launching the Clinical and Translational Science Award (CTSA) program in 2006 (Woolf 2008). More recently, President Obama signed a spending bill that includes a provision to establish the National Center for Advancing Translational Sciences (NCATS) within the NIH. As a result, basic biomedical research and genome-wide association studies (GWAS), the

two ends of the translational paradigm, are booming. But their impacts, in terms of new therapies and intervention strategies, are growing at a far more modest pace (Laurence 2012). Prevention, in particular, has suffered in the United States because of the use of a traditional “medical” model rather than a “health” model. Financing, medical education, and research support have favored disease treatment over prevention. This is ironic since, historically, the greatest gains in physical well-being have come from preventive rather than curative activities (Frank 1996). However, this relative failure to date should not be a cause for resignation or despair, but rather a stimulus to redouble our efforts.

Nutrigenomics has the potential to lead to evidence-based dietary intervention strategies for restoring health and fitness and preventing disease. It may be viewed as an off-shoot of chemical biology, where nutrients are seen as signals or chemicals that trigger a sequence of reactions leading to changes in gene expression and other biological effects (together termed the “phenotype”) within a specific cell in the body. Recent advances in nutrigenomics are due to the completion of the human genome project and the new biomics technologies that provide the means for simultaneous determination of the expression of many thousands of genes at the mRNA (transcriptomics), metabolite (metabolomics), and protein (proteomics) levels. Genomic and transcriptomic studies are mostly conducted by DNA/RNA microarray technologies, but

proteomics and metabolomics do not yet have standardized large-scale procedures. Proteome analysis is usually performed by two-dimensional gel electrophoresis and liquid chromatography-mass spectrometry, while metabolome analysis can be carried out by gas/liquid chromatography-mass spectrometry and liquid chromatography-nuclear magnetic resonance spectroscopy. These technologies are usually applied in a “differential display” mode, that is, by comparing phenotypes in diseased versus healthy subjects to help achieve better association of clinical phenotypes with the corresponding genotypes. Nutrigenomics data are typically generated on a massive scale and require computational analyses to derive mechanistic understanding of the disease under study (Go et al. 2003).

Although “omics” technologies are booming, the lack of a robust model system to test nutrigenomics principles prior to their application in public health is a serious impediment to progress. Relying on risk factor assessment, the evidence-based path - from basic discovery to effective prevention strategy - seems long, arduous, and confounding. For example, the identification of sequence polymorphisms regulating gene expression is important for understanding human variation in response to dietary intake (Go et al. 2003, Ginsburg 2011). *In vitro* experimental designs for nutrient-gene interactions using human cell lines offer a controlled study environment. Individual dietary components can be tested *in vitro* for a limited number of phenotypes in a dose- and time-dependent manner. However, to evaluate the role of a single nucleotide polymorphism (SNP), the SNP has to be artificially engineered into the cells, making the same experiment a three-way study. In principle, modeling patient genotypes by introduction of gain-of-function or loss-of-function disease mutations (both common in many forms of cancer) into any endogenous gene locus of human cells is possible. However, data obtained from such a study will be difficult to interpret in the context of human populations, where a SNP acts in synergy with other genetic and environmental factors. Fortunately, the option of using an *in vivo* model, such as rodents or higher-order mammals, also exists. The greatest advantage of a mammalian *in vivo* model is the ability to control induction of the disease phenotype (clinical signs), allowing measurements of the preventative or curative effects of any nutrient. Moreover, the diet, environment, and other potentially influential factors can be controlled, monitored, and measured. However, knowledge about conserved gene regulatory elements, such as SNPs, across species is limited at the present time, making data obtained from *in vivo* models not always translatable to the human condition.

The most relevant study system for dietary interventions is the human organism, and there is a growing trend toward system-wide approaches in public health studies. However, these studies have so far been limited to either large-scale association-observation studies or small-to-medium-scale intervention studies. In these studies, there is a preference for applying an array of technologies to the same sample, allowing physiological changes to be assessed more robustly throughout all the molecular layers, including mRNA, protein, and metabolite. However, a critical assessment of study outcomes reveals uncertainty in data interpretation, knowledge gaps, as well as the need for improved study designs and more comprehensive phenotyping of volunteers before selection for study participation (Wittwer et al. 2011). For example, to investigate preventative responses to a diet influenced by a specific SNP or a combination of SNPs is challenging at multiple levels. For effective diet-based prevention studies, both large-scale and long-term interventions are critical. Screening of a large number of subjects for the presence of one or more SNPs, controlling their dietary intake over a long period of time, minimizing environmental and lifestyle variations of study participants throughout the experimental duration, indefinite timelines for spontaneous disease phenotype occurrence, and compliance issues are just a few examples of the potential roadblocks to generating an optimal study design. Ultimately, because many cases of chronic diseases are influenced by multiple dietary factors, nutrition-genome interactions cannot be identified unless diet and genotype are controlled and crossed over in the experimental design (the same diet with different genotypes or different genotypes with the same diet). Because human intervention studies are costly and difficult to conduct, observational epidemiologic studies (which mostly detect associations, not causal relationships) have continued to be the gold standard and will likely continue to dominate the field of nutrigenomics. However, such observational designs are not appropriate for preventive interventions that rely on an understanding of molecular mechanisms (Wittwer et al. 2011).

A second example from the field of nutrigenomics in which a model study system is urgently needed is the relationship between diet, epigenetic events, and cancer prevention (Go et al. 2003, Ross et al. 2008). Cancer is caused by spontaneous mutations resulting in both abnormal genetic and epigenetic events. Epigenetic events are important mechanisms by which gene function is selectively activated or inactivated. One such event, DNA methylation, which is a hallmark of multiple human malignancies, involves covalent addition of a methyl group to a cytosine resi-

due within CpG dinucleotides. DNA methylation changes gene expression, regulates chromosomal stability, and is modulated by dietary factors (Ross et al. 2008). Another example in which diet can regulate an epigenetic event involves the Polycomb group (PcG) proteins, which function as transcriptional repressors through chromatin modification and contribute to the pathogenesis of cancer (Kashyap et al. 2011). Histone modifications triggered by polycomb repressive complex signaling are important during embryonic stem (ES) cell differentiation. The active vitamin A constituent retinoic acid (RA) is involved in differentiation of various cancer cells in culture and has been found to modify polycomb complex signaling during differentiation of ES cells (Kashyap et al. 2011). Hence, both DNA methylation and chromatin modification events are excellent examples of processes by which environmental factors, including diet, may modify cancer risk and tumor behavior (Ross et al. 2008).

To validate basic research findings in human, such as whether dietary intake of RA can prevent or delay cancer development in relevant high-risk groups, once again, well-designed, long-term, and large-scale studies will be needed. For example, combining the features of a prospective cohort study, a randomized controlled trial, “next-generation” measures of diet and clinical parameters, as well as extensive biospecimen collection and storage for measurement of genetic and epigenetic measurements will be critical. The focus should be on how individual dietary components influence epigenetic events and how this correlates with phenotypic changes and genotype. Very little information currently exists about gene-specific epigenetic changes in human as influenced by biologically active food components. Furthermore, very little information exists to evaluate, in a comprehensive manner, the specificity of individual nutrients, the impact of intakes and exposures, and any acclimation with time and/or tissue specificity. With new technologies and falling costs, it is possible to scan and sequence larger data sets, with improved statistical power to detect a wider spectrum of risk variants. Functional pathway analyses, robust methods for power analyses, as well as recruiting multidisciplinary teams of experts will be critical. Sustainable funding strategies need to be identified, as the costs of such research will be exorbitant. Although, in theory, all of these requirements can be met, in reality, such massive studies are difficult to plan, implement, and sustain, even when conducted on a national scale. A good example of a study of this magnitude is the National Children’s Study (NCS), which is still only an association type of study without any mandated intervention. The NCS was launched in 2000 when Congress directed the NIH to study “the

effects of both chronic and intermittent exposures on child health and human development”. Law-makers specified that the exposures could be biological, chemical, physical, or psychosocial and that the study should address health disparities and monitor US children in all their diversity for 21 years. However, by 2012, after significant investments have already been made, NIH has come to see the study as unsustainable in its current design (Wadman 2012).

In our laboratory, we use molecular mechanism-based designs in preclinical and human interventions that represent the breadth of current translational models for nutrient-gene interaction studies. Yet, we are limited in our ability to address all the scientific questions we want to ask. No perfect strategy exists at present to address this technical roadblock to successful utilization of nutrigenomics principles for improvement of public health. Funding agencies such as the NIH are encouraging investigators to seek improvement in research techniques and model systems to better accomplish the touted potential of nutrigenomics. A robust research model, well-controlled, allowing interplay and experimental manipulation of multiple variants and yet physiologically relevant in terms of human health interpretations, is urgently needed.

We are currently optimizing a physiologically more relevant experimental model in our laboratory that may allow manipulation of multiple variants in a controlled manner and fill in some of the existing gaps in nutrigenomics research. This model relies on the availability of biologically relevant human tissue specimens from diseased and healthy individuals. While use of tissue explants *in vitro* is not novel by itself, large-scale experimental platforms utilizing human tissues and/or primary cells are uncommon in nutrigenomics research. The donors are assigned ID numbers, while personal identification information, if present, is removed. Furthermore, appropriate institutional review board permissions are obtained whenever necessary. When a steady supply of relevant tissues is identified along with appropriate accompanying physiological and/or medical records, the genotype of the sample is determined prior to derivation of primary cells from these tissues. Controlled treatment of these cells, representing many of the physiological complexities of the donor, can be subsequently carried out with individual or combinations of dietary factors in a time- and concentration-dependent manner. The resulting molecular events, including cell signaling responses or epigenetic events, and their association with SNPs can be studied in these live cells. Virtually any biological question can be investigated without having to deal with research subject compliance issues, inaccurately controlled experimental parameters, and envi-

ronmental- or lifestyle-based uncertainties. Age-matched populations may be studied, and the potential exists to follow the same subjects for several years. Also, cellular elicitors are used to mimic disease onset in a post-treatment manner to help measure the preventive effects of diet. The knowledge generated from such a study can be directly utilized to design a very specific and small-scale human study. Moreover, since diet-based modifications are generally considered a safer alternative to pharmacological intervention, it is possible that such post-validation in humans will not be necessary at all.

However, even if the proposed experimental model has potential to accelerate progress in nutrigenomics research, we anticipate challenges. Primary cells live for only a short period of time and cannot be passaged beyond a few cycles. Depending on tissue sources, variations in their quality is possible. While they represent larger populations and are ideally obtained through tissue banks, health-care provider networks, and hospitals, batch-to-batch variations will need to be factored into data analyses. Moreover, ready access to human tissues may be limited. Above all, primary cell culture methods are tedious, relatively expensive, and require labor-intensive optimization prior to actual experiments. Finally, characterizing diets or specific nutrients as genome-damaging or genome-protecting using primary cells derived from diseased or healthy tissues may still overlook the variation in benefits that accrue over a lifetime, and in particular, variation relative to the timing of disease onset. Nevertheless, weighing potential benefits versus pitfalls, policy makers should mandate greater human tissue accessibility to researchers.

In conclusion, nutrigenomics holds tremendous potential for providing better nutritional advice to the general public, genetic subgroups, and individuals. Because nutrigenomics requires a deep understanding of nutrition, genetics, bioinformatics, and biochemistry as well as an expanding array of “omics” technologies, it is often difficult, even for professionals, to appreciate the relevance of these disciplines to preventive approaches for optimizing health, delaying the onset of disease, and diminishing disease severity. The findings of molecular biochemistry research can positively impact experimental medicine and dietetics research. For example, identification of major as well as subtle genetic differences is the first step in better understanding human variation in response to diet and environment. Subsequently, an intervention discovery platform may identify potential cellular targets. While conventional *in vitro* and *in vivo* disease model-based research may still be crucial, human specimen acquisition also becomes a critical step. Patient-relevant *in*

vitro disease models have been a missing link in the discovery of novel interventions that are targeted or personalized to the unique genetic mutations that define a patient’s disease type, progression, and, consequently, their inherent or acquired drug sensitivity and resistance profiles. However, since protocols for obtaining and processing human specimens are limited, implementation of human tissue research services will be necessary to ensure reproducible and reliable results. Furthermore, examination of a broad range of human specimens of diverse origin with sufficient numbers for the necessary statistical power is critical. The information obtained from studies with human specimens will greatly facilitate the design of clinical trials, assisting in the identification of the smaller patient population that would most likely benefit from the targeted experimental dietary intervention. This could potentially scale down human intervention studies to save millions of research dollars and take us a step closer to deriving the ultimate benefits of nutrigenomics research.

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Conflicts of Interest

The author declares no conflict of interest with any relevant entity.

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