How Nutrigenetics Can Help Prove that Nutrient-Based Interventions Reduce Disease Risk

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Key messages

- Current designs for nutrition intervention studies often yield inconsistent results because they aggregate nutrient-sensitive and nutrient-insensitive people, creating "noisy" data that can obscure the effects of the intervention.
- The reasons why people are metabolically different are only just being elucidated, and underlying mechanisms include genetic and epigenetic variation and microbiome differences.
- The tools for measuring genetic variation are the most developed, and they can help reduce some of the variance in data that makes it hard to prove that nutrient-based interventions reduce disease risk.

Why is it so hard to prove that nutrient-based interventions reduce risk for disease?

All too often, a report that a nutritional intervention reduced disease risk is followed shortly thereafter by another publication observing that, in a different population, the effect of the nutritional treatment could not be replicated. For example, a meta-analysis of clinical trials concluded that vitamin D_3 decreased mortality in elderly women who are in institutions and dependent care.[1] Subsequently, other investigators observed that, in critically ill patients with vitamin D deficiency, administration of high-dose vitamin D_3 compared with placebo did not reduce mortality.[2] Inconsistent results fueled confusion about whether vitamins A or E lower the risk for developing lung cancer. Some clinical trials produced results suggesting efficacy, but other large randomized trials reported that these vitamins increased lung cancer risk.[3–6] There are many other examples of what appear to be nutritional contradictions.

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Understanding nutrition and metabolism requires complicated systems biology, and it is always possible that inconsistent results from clinical trials are due to confounding variables that were not properly controlled for. For example, many studies do not provide a sufficiently low intake of the nutrient for the control group, or they do not provide adequate intake of other essential nutrients needed for the test nutrient to manifest its effect. A more common reason for inconsistent effects of a nutrition intervention is that nutrition studies are inherently "noisy", having relatively large variance in measurements compared to the magnitude of the effect size of the nutrient intervention. This noise makes it difficult to detect significant effects. Some of this noise derives from measurement error (e.g., assessment of dietary intake is challenging and prone to measurement error),[7] but a good deal of this noise is due to metabolic variation between people.

> "A good deal of the 'noise' in nutrition studies is due to metabolic variation between people"

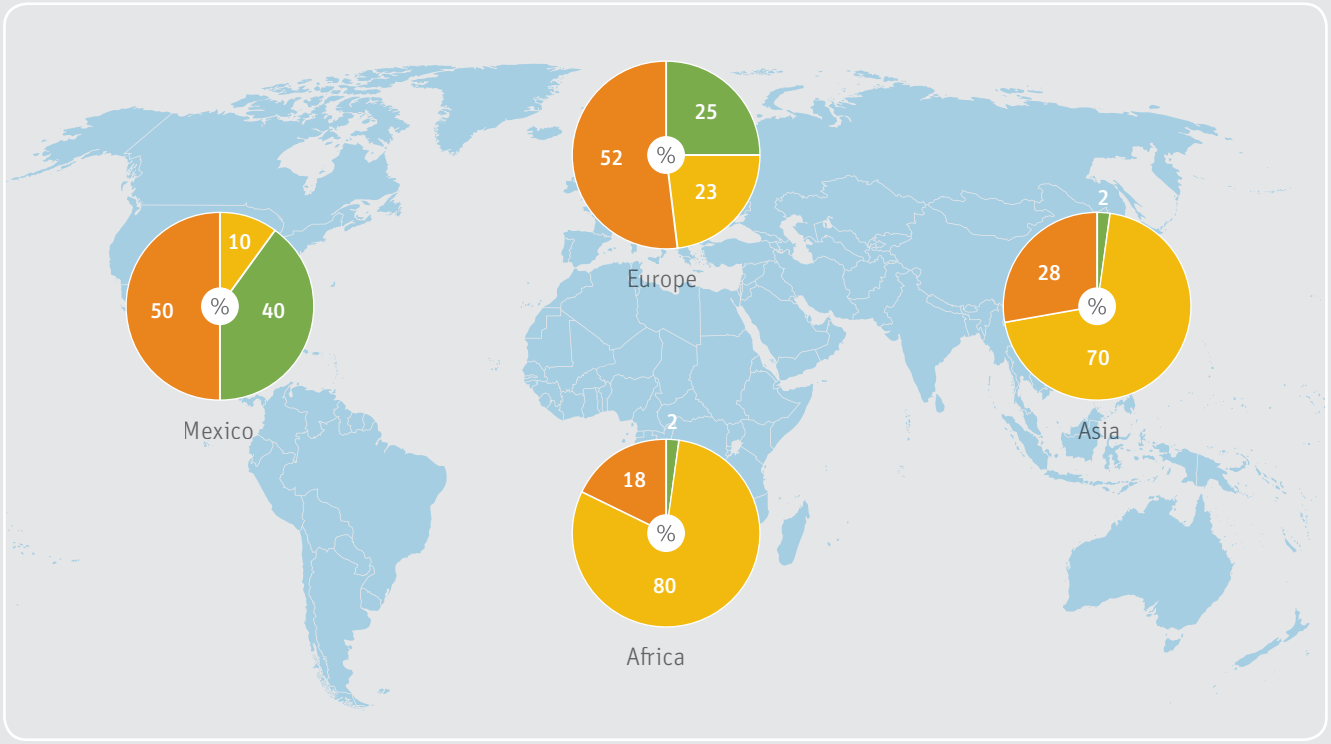
Each person has approximately 50,000 common genetic single nucleotide polymorphisms (SNPs; differences in the

"spelling" of the genetic code of a gene), and some of these SNPs occur in genes that control metabolism and result in metabolic inefficiencies that alter nutritional requirements or responses.

The nutritional requirements for choline

To illustrate this concept, it is useful to examine the nutritional requirements for choline, which was designated as an essential nutrient for humans in 1998.[8] In carefully controlled clinical studies testing the importance of dietary choline, most (~80%) of men and postmenopausal women developed organ dysfunction (fatty liver, liver damage, or muscle damage) when deprived of choline. In contrast, fewer than half of the premenopausal women in the study became sick.[9,10] Depending on the composition of the population studied, investigators could have concluded that choline was, or was not, an essential nutrient. Multiple factors contributed to the differences in the symptomology of dietary choline deficiency. For example, people have the ability to biosynthesize choline (as phosphatidylcholine) through a series of reactions that involve an enzyme coded for by the *PEMT* gene. Expression of *PEMT* is regulated by estrogen, and this explains why young women (with higher concentrations of estrogen) have a reduced dietary requirement for choline compared to men and postmenopausal women.[10,11] Why, then, did almost half of young women still need to eat choline?[10] These women had SNPs that interfered with the estrogen response element in *PEMT*, making the gene unresponsive to estrogen[11–14] and making the young women with this genetic variant reliant on diet for their choline needs. More than 70% of US women from North Carolina have 1 variant allele, and > 20% have two variant alleles for this *PEMT* SNP.[12] It is important to realize that people inherit SNPs from their ancient ancestors; therefore, prevalence of SNPs can vary greatly between populations. **Figure 1** illustrates how one of the functional SNPs in *PEMT* is distributed around the world. Thus, it is easy to understand why nutrition studies could yield different results depending on which populations are studied. Nutrigenetic analyses are needed to enable the identification of choline-sensitive (responders) and choline-insensitive (non-responders) subgroups of people.

FIGURE 1: The prevalence of a functional single nucleotide polymorphism in the gene PEMT (rs12325817) varies in different populations.



The PEMT (rs12325817) polymorphism involves a substitution of a C for a G in the genetic code. Having a C substituted in this gene results in diminished induction of gene expression by estrogen, and having both copies of the gene substituted with a C abrogates the response to estrogen almost completely.

Using data from the 1000 Genomes database (www.1000genomes.org), it is clear that the proportion of people with CC alleles (green), GC alleles (orange), and GG alleles (yellow) is very different in people of different genetic heritage.

Nutritionally relevant single nucleotide polymorphisms (SNPs)

Many other pathways of nutrient metabolism are affected by genetic variation, and these nutritionally relevant SNPs are present in many people. Homozygosity for the variant *C677T* allele in the methylenetetrahydrofolate reductase gene *(MTHFR)* creates a metabolic inefficiency that increases the dietary requirement for folate.[15] Twenty percent of Mexican Americans[16] and 14% of Caucasian Americans[17] have two variant alleles for this SNP. Thus, genotyping can be used to identify likely responders and non-responders to folic acid treatment. Similarly, the metabolic consequences of common SNPs may well have contributed to the different interpretations of vitamin D_3 clinical trials discussed earlier: vitamin D is activated by hydroxylation reactions catalyzed by the products of the *CYP2R1* and *CYP27B1* genes. Common SNPs in either of these genes reduce enzyme throughput and hence the availability of activated vitamin D.[18,19] Activated 1,25-$(OH)_2$-vitamin D_3 must bind to the vitamin D receptor (VDR), and common SNPs in the VDR gene that codes for this receptor further diminish the response to vitamin D.[18,20] People with these SNPs are 2.5 times more likely to be vitamin D insufficient.[18] Knowledge of individuals' genetic variations and associated metabolic inefficiencies could help identify responders and non-responders to vitamin D interventions. These are just a small selection of the many common nutritionally relevant SNPs that result in metabolic variation between individuals. There is a great deal more research that needs to be done before we have identified all of these SNPs, and the expansion of the catalog of nutritionally relevant SNPs is the goal of the research center that I direct.

Nutritionally relevant SNPs probably only result in a phenotype in people who are nutritionally challenged. If people can eat enough of the relevant nutrient, a metabolic inefficiency may not cause a problem. However, metabolic inefficiencies become very important when the availability of the nutrient is marginal (and sometimes they can become important when the diet intake of a nutrient is excessive if the metabolic inefficiency alters removal of the nutrient or its metabolites). This highlights an important concept: investigators need to assess dietary intake as well as genotype when they try to identify nutritionally relevant SNPs. Two conditions may be necessary before an abnormal phenotype exists: presence of an SNP that causes a metabolic inefficiency, and low (or high) dietary intake of the relevant nutrient. Analyses using genetic data alone can miss nutritionally relevant SNPs because when people with high and low dietary intake of the nutrient are lumped together, the effect of the SNP is obscured. Genome-wide association studies (GWAS) miss important nutritionally relevant SNPs because these studies usually do not also consider the SNPs' interactions with dietary intake.

The role of the gut microbiome

Genetic variations are not the only reason that people differ metabolically; they are just the easiest to measure. Differences in the gut microbiome also change how people process nutrients and metabolize them. For example, when fecal microbiota from obese or lean twins were transplanted into mice, the mice that received the obese person's microbiota developed an obesity-associated metabolic phenotype while the lean twin's microbiota transmitted a lean metabolic phenotype.[21] Recently, an interesting study demonstrated that eating artificial sweeteners induced changes in the gut's microbial populations, and this altered microbiome resulted in perturbed glucose metabolism in the host.[22]

Reducing the "noise" in nutrition research

In summary, there is a fundamental problem with the idea that humans are metabolically the same, and with the resulting assumption that responses to nutrients should be distributed along a normal bell-shaped curve. Rather, there is biologically determined metabolic variation that results in discrete subpopulations that are nutrient-sensitive (responders) and nutrient-insensitive (non-responders) to nutrient interventions. Current experimental designs for nutrition intervention studies often yield inconsistent results because they lump together these responders and non-responders, creating "noise" that obscures the effects of the intervention. Identification and recruitment of high-risk vs. low-risk subjects is standard practice for clinical trials of pharmaceuticals, where risk factors (obesity, *BRCA1* mutations) for particular diseases (diabetes, breast cancer) are well known. The challenge for nutrition researchers is to build the catalog of mechanisms whereby metabolic variation can be predicted in people. The next generation of nutrition intervention studies should use this information to identify and recruit nutrient-sensitive subjects, and thereby avoid obscuring therapeutic effects by combining data from this subgroup with data from non-responders. When lacking this knowledge, designs for nutrition trials need to include genetic, microbiome and other measures that allow for *post-hoc* identification of responders, and use this information to build the metabolic-variation knowledge base needed so that investigators can reduce the "noise" in their nutrition research.

> "There is a fundamental problem with the idea that humans are metabolically the same"

Conflict of Interest Disclosures

The author has completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Dr Zeisel reports hono-

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