Inborn variation in metabolism

Vamsi K Mootha & Joel N Hirschhorn

Advances in analytical biochemistry have recently made it possible to obtain global snapshots of metabolism. A new study couples such technology with genome-wide genetic analysis to explore inherited variation in human metabolism.

Inborn errors of metabolism refer to single-gene disorders wherein loss of the function of a single enzyme results in altered levels of linked reaction metabolites, generally substrates or products of biochemical reactions. The term was coined by Archibald Garrod, who first elucidated the biochemical basis of disorders such as alkaptonuria over 100 years ago. Since then, hundreds of inborn errors of metabolism have been characterized. Garrod predicted that inborn errors were "merely extreme examples of variations of chemical behavior which are probably everywhere present in minor degrees[1]" and that this "chemical individuality [confers] predisposition to and immunities from the various mishaps which are spoken of as diseases"[2]. Indeed, the levels of many metabolites do show substantial interindividual variation, some of which is likely to be influenced by genetic variation. On page 137 of this issue, Karsten Suhre and colleagues report an early implementation of a strategy to systematically explore the genetic basis of chemical individuality[3].

Genetic and metabolite profiling

Genome-wide association (GWA) studies have been successful in spotlighting loci that influence the risk of human disease phenotypes. Recently the same approach has been extended to uncover the genetic basis of variation of plasma metabolites that are routinely measured in clinical laboratories. For example, investigators have identified dozens of common variants associated with lipid and cholesterol levels in plasma[4,5]. On pages 105 and 142 of this issue, investigators from the MAGIC consortium report multiple loci influencing glucose levels in plasma during fasting[6] and 2 hours after an oral glucose challenge[7]. Several groups have recently combined the power of genome-wide genetic analysis with more global measures of metabolism that are now possible thanks to advances in analytical chemistry. To date, however, most of these studies have been focused on plants[8] and rodents[9], and there have been few such studies in humans.

Illig *et al.*[3] now build on their previous similar but smaller study[10] in reporting GWA for plasma metabolite levels in 1,809 human participants from the KORA study (a German population-based study). They used a mass spectrometry–based platform to measure the abundance of 163 polar metabolites and lipid species, including amino acids, acylcarnitines and phospholipids. They tested for association of common genetic variants with individual metabolite levels and also with all ~26,000 pairwise ratios of metabolites.

Why use metabolite ratios? In their previous study[10], the authors reported that the association *P* values for metabolite ratios were more significant than those for individual metabolites and speculated that this might more accurately reflect variation in kinetic properties of enzymes. However, this might also be accounted for by technical differences (for example, better normalization of mass spectral peaks) or might be a trivial consequence of testing $\sim n^2$ versus n hypotheses.

Using this approach, the authors identify in their current study 15 variants that reach a level of significance (3.64×10^{-12}) sufficient to account for having tested over 500,000 SNPs for association with over 26,000 phenotypes. In a replication study including 422 women from the TwinsUK cohort, 9 of the 15 loci showed compelling evidence of replication and 5 showed suggestive evidence. The authors may have missed additional valid associations by using a sequential replication design rather than the better-powered joint analysis[11].

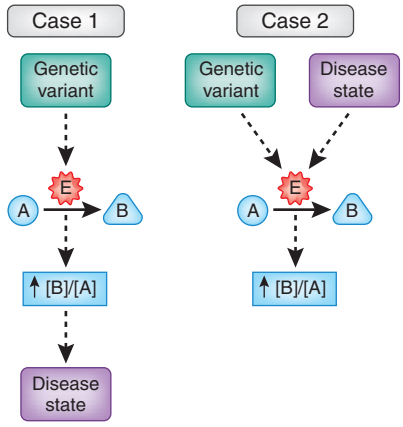

Figure 1 Mendelian randomization experiment to test whether genetic alteration of a metabolic pathway plays a causal role in disease. Shown are two simple scenarios for the relationship between metabolites (A and B, related by the activity of an enzyme, E), the ratio of the metabolite levels (A/B), genetic variation and a disease state. The dotted arrows indicate causality. In each case, the metabolite (A and B) levels are correlated with disease risk and are statistically associated with a variant in a gene whose function is closely related to the metabolic pathway. If the metabolite levels have a causal role in the disease (case 1), a Mendelian randomization experiment would show an association between the genetic variant and disease risk. In case 2, the genetic variant does not show association with disease risk, and the metabolite levels are correlated but not causal for the disease.

Vamsi K. Mootha is at the Harvard Medical School Department of Systems Biology, Massachusetts General Hospital, Boston, and the Broad Institute Metabolism Initiative, Cambridge, Massachusetts, USA. Joel N. Hirschhorn is at the Harvard Medical School Department of Genetics, Children's Hospital, Boston, and the Broad Institute Metabolism Initiative, Cambridge, Massachusetts, USA. e-mail: vamsi@hms.harvard.edu or joelh@broadinstitute.org

Variation in biochemical pathways

Several of the gene variants associated with metabolite levels, including those in or near the *FADS* cluster, *ACADS*, *ACADM*, *ELOVL2* and *SLC22A4*, had been previously reported[10,12–14]. As in other GWA studies, each associated variant implicates only a locus, and not necessarily a particular gene at that locus—although in this study, the variants often highlighted obvious candidate genes. In many instances, the associated metabolites are closely related to the activity of an enzyme encoded by a nearby gene (as in the case of *ACADS* and acylcarnitines), and sometimes the metabolites are a few steps removed from the known enzymatic activity (as with *ELOVL2* and diacyl phosphatidylcholines). In other cases, the associated variants have no nearby genes with known roles in metabolism, suggesting that there may be previously unsuspected biochemical or physiological roles for genes at these loci. Of course, the effects of those genes on metabolite levels could be quite indirect.

Several of the identified loci are associated with metabolites that are risk factors for diseases, such as glucose levels or lipid levels[4–7]. Interestingly, some of the loci also have variants associated with risk of common diseases not thought to be directly influenced by metabolite levels: for example, *SLC22A4* and Crohn's disease. However, the *SLC22A4* variant reported in this paper as most strongly associated with carnitine levels is only partially correlated ($r^2 \approx 0.4$) with the variant most strongly associated with Crohn's disease[15], so the overlap does not prove a causal connection between carnitine metabolism and the disease.

Illig *et al.*[3] was limited to the analysis of genetic variants with a high minor allele frequency (here, >10%), typical for current GWA studies. Along these lines, it is notable that eight of the loci include four candidate genes (*ACADS*, *ACADM*, *ACADL* and *ETFDH*) that have also been found to be mutated in rare, inborn errors of mitochondrial fatty acid oxidation. Such disorders are characterized by alterations in plasma metabolites such as glucose, ketone bodies and fatty acid intermediates. As Garrod first postulated, this suggests that the biochemical variation seen in the inborn errors of metabolism represent an extreme end on a continuum. Common variation at the same loci can give rise to more subtle biochemical phenotypes, and variants in the middle of this spectrum could have more significant consequences and yet fall short of causing a recognized inborn error of metabolism.

Human biochemistry and disease

Many biomarkers, including metabolite levels, show correlations with disease, but such correlation does not imply causality. Genetic variants that strongly influence biomarker levels can be tested for association in Mendelian randomization experiments (**Fig. 1**). In such experiments, there are two key elements: a biomarker that is correlated with disease, and a strong association between the biomarker and variation in a gene that is intimately tied to it (such as the protein encoding an enzyme directly involved in the biomarker's metabolism). If the variant shows the expected association with disease, this provides a strong argument in favor of causality, whereas the lack of an association in adequately powered samples that suggests the biomarker is correlated but not causal for disease. If multiple genetic loci associated with disease influence causal metabolites in a single pathway, enzymes in that pathway may emerge as tractable drug targets, and such results may also suggest mechanism-based markers for predicting disease and monitoring its progression.

Most exciting about the current work is the use of two global methodologies—genetic and metabolite profiling—to understand human *in vivo* metabolism. In the future, more comprehensive versions of the profiling technologies could be coupled to perturbations (for example, dietary challenges, drug treatments, aging) or used in combination with isotopic tracers to more directly infer the influence of genetic variation on *in vivo* reaction biochemistry and homeostasis. Just as Garrod's study of inborn errors of metabolism helped write a generation of textbooks on human biochemistry, so, potentially, could comprehensive studies of inborn variation of metabolism inform the next generation.

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Channelopathies converge on TRPV4

Bernd Nilius & Grzegorz Owsianik

Scapuloperoneal spinal muscular atrophy and Charcot-Marie-Tooth disease type 2C are inherited neurodegenerative diseases characterized by sensory defects and muscle weakness. Three new studies demonstrate that they are allelic disorders caused by mutations in the vanilloid transient receptor potential cation-channel gene *TRPV4*.

Normal communication between the central nervous system and skeletal muscles requires intact efferent and afferent neuronal connections between spinal cord and muscle tissue. Functionally, these connections depend on ion channels that maintain action potential propagation, synaptic transmission, plasticity and cell survival. Scapuloperoneal spinal muscle atrophy (SPSMA) and Charcot-Marie-Tooth disease type 2C (CMT2C, or hereditary motor sensory neuropathy type 2, HMSN IIC) are genetically heterogeneous inherited disorders caused by degeneration of peripheral nerves. Individuals with SPSMA show loss and progressive weakness of scapular and peroneal muscle tissue, bone abnormalities and laryngeal palsy. CMT2C, the most common inherited neurological disease, leads to progressive weakness of distal limbs, vocal cords, diaphragm, and intercostal and laryngeal muscles. Sensory deficits often impair hearing and vision. Bladder urgency and incontinence as well as bone abnormalities, such as scoliosis, are also common.

Bernd Nilius and Grzegorz Owsianik are at the Department of Molecular Cell Biology, Katholieke Universiteit Leuven, Leuven, Belgium.
e-mail: bernd.nilius@med.kuleuven.be