Nutrient selection through nutrigenomic approaches

Jim Kaput

Department of Surgery, University of Illinois Chicago, Chicago, Illinois

THE ABUNDANCE AND VARIETIES OF NUTRIENTS IN DEVELOPED COUNTRIES AND INCREASINGLY IN URBAN AREAS OF TRANSITIONAL AND DEVELOPING COUNTRIES PRESENTS MODERN HUMANS WITH A CONUNDRUM: WHAT ARE THE BEST COMBINATIONS OF MACRONUTRIENTS THAT PROMOTE HEALTH AND LONGEVITY IN EACH INDIVIDUAL? WHY AND HOW DOES AN INDIVIDUAL SELECT AMONG FOODS OF DIFFERENT COMPOSITIONS AND AMOUNTS WHEN THEY ARE IN SURPLUS? IS FOOD SELECTION ONLY DUE TO PARTICULAR TASTE AND ODOR RECEPTORS (5) OR ARE THERE CONNECTIONS AMONG TASTE AND METABOLIC NEEDS FOR ENERGY OR REGULATORY PROCESSES? THAT IS, IS THERE A GENETIC BASIS FOR FOOD INTAKE? THESE BASIC SCIENCE QUESTIONS HAVE SIGNIFICANT IMPLICATIONS FOR INDIVIDUALS AND SOCIETY SINCE OVER 60% OF THE US POPULATION IS OBESE OR OVERWEIGHT, AND WEIGHT PROBLEMS IN POPULATIONS OF OTHER COUNTRIES WILL SOON HAVE OR EXCEED THIS PREVALENCE.

Several chromosomal loci associated with total energy and fat intakes in humans were identified in the Heritage Family study by a genome-wide linkage scan (4). Identifying causative genes in these regions in humans is challenging because power calculations would suggest the need for much larger study populations (18). Laboratory animals are more amenable for mapping strategies because strains can be bred to identify and test loci associated with specific traits if that trait is expressed differently among two or more inbred strains. Smith et al., (32) showed that nine inbred strains of mice preferred food with fat and no carbohydrate over carbohydrate without fat and two strains preferred the carbohydrate diet when both diets were available. Quantitative trait loci (QTL) can exploit differences in physiological, behavioral, or molecular processes to identify loci associated with measurably different phenotypes (6). The Smith group interbred CAST/Ei (carbohydrate preferring) and C57BL/6J (fat preferring) to produce an F1 generation (33). The F1 mice were backcrossed to each of the parental strains that produced an F2 generation differing in preference for fat (F/P diet: 78% calories from fat, 22% from protein) or carbohydrate (CP diet: 78% calories from carbohydrate, 22% from protein) in otherwise balanced (vitamin, mineral) diet compositions. Each F2 mouse has preferences based on the combination of chromosomal segments inherited from the two parental strains. Since the experimental feeding period was only 10 days, the essential fatty acid deficiencies of the C/P diet, which may also be true of the F/P diet, would not presumably affect preferences for macronutrients or total calories (27).

Two QTLs for energy intake (Kcal1 and Kcal2), three for macronutrient fat intake preference (Minf1−Minf3), and three for macronutrient carbohydrate preference intake (Minc1−Minc3) were identified in the initial screening (33). This study was the first to identify genetic loci associated with intakes of total energy and macronutrients and demonstrated that nutrient selection was a complex trait; that is, multiple genes were involved in the process. QTL mapping is a powerful tool but has limitations since it identifies relatively large chromosomal regions and the QTL are specific to the two parental strains. That is, different strains may identify the same, additional, or different QTLs depending on the complexity of the biological process and which causative alleles are inherited among the parental strains (and, therefore, F2 mice). Hence, it is crucial to identify QTLs affecting the phenotype in different strain combinations or to confirm the QTL by other genetic or molecular means.

In this issue of AJP, the Smith team (14a) exploited another advantage of laboratory animal genetics: they used marker-assisted breeding (i.e., speed breeding) to introgress ~60 Mbp containing the chromosome 17 QTL for carbohydrate (Minc1) and total energy (Kcal2) intake, traits associated with the CAST/Ei strain, into C57BL/6J mouse. Analyses of congenic mice with one segment of CAST/Ei in an otherwise B6 genetic background vs. the B6 control (i.e., the parental strain) can confirm the presence of the QTL, assuming that epistatic (gene-gene interactions) and gene X environment interactions are not required for expression of the contributing gene(s) in the QTL. The congenic strain produced (B6.CAST-17) consumed 27% more carbohydrate and 17% more total energy compared with a C57BL/6J littermate with the chromosome 17 region from B6 mice. The data confirm the effect of the chromosome 17 QTL. The difference in magnitude suggests that one or more genes in the B6 background alter some aspects of carbohydrate selection or metabolism and total energy intake through epistatic or gene-environment interactions.

The location of the peaks Minc1 (12 cM) and Kcal2 (16 cM) overlapped the position of glyoxalase (Glo1) at 16 cM and the glucagon-like peptide 1 receptor (Glp1r) at 18 cM, suggesting they could be candidate genes for producing differences in carbohydrate metabolism and energy intake. Four single nucleotide polymorphisms (SNP) were found between B6 and CAST/Ei Glp1r, including one in the promoter region and a nonsynonymous variant in the coding region (C416Y). One synonymous change was found in Glo1 between these strains. Strain-specific differences in Glp1r expression in hypothalamus and antral stomach were consistent with the differences in macronutrient and energy intake. Glo1 was also upregulated in liver and hypothalamus of homozygous B6.Cast-17 relative to B6 mice.

Since Glo1 and Glp1r are involved in pathways in many tissues, regulation of macronutrients and energy intakes may be linked to differences in flux through carbohydrate and energy metabolic pathways. Hence, nutrient selection may not be driven solely by molecular pathways limited to the hypothalamus (reviewed in Refs. 28 and 31) gastrointestinal tract or through taste and odor receptors (19), although the pathways in these organs are likely to play a role in the complex traits underlying food choice. Since the other QTL involved in macronutrient selection and calorie intake (33) have not been fully characterized, development of QTL-specific congenic strains is likely to provide valuable insights into nutrient intake.
and may provide knowledge for designing better-controlled studies to explore nutrient selection in humans.

While the approach, data, and implications are exciting, the analyses of the B6.CAST-17 locus is not yet complete because the introgressed CAST/Ei region encoded 1138 genes. Many of the genes would not be considered candidates because of their locations within the 60 cM region relative to the position of the QTLs. Nevertheless, there may be other genes near the QTL peak that could be involved in nutrient selection. Identifying causative genes within the QTLs has been a significant limitation for exploiting the power of QTL analyses. Over 2,000 QTLs for various traits in mice and rats have been mapped over a 15-yr period but only 16 genes within these QTL have been identified (6). Another six genes have been identified as susceptibility alleles. Novel methods are needed to speed the analyses of genes within QTLs because of the effort and costs of traditional methods. An example was the gene identified for the Ath1 QTL (23) involved in regulating HDL cholesterol levels. The Ath1 gene was shown to be Tnfsf4 (also called Oxllo or Cd134l) through extensive studies cumulating in fine mapping, gene expression analyses in model systems, targeted gene disruption, and analyses of gene variant associations in humans (36, 37). Clee et al., (3) have recently identified Sorcs1 as the type 2 diabetes 2 (T2dm2) QTL through a combination of genetic and expression analyses.

Analyzing multiple genes within many QTLs requires a higher throughput screening step to select candidates based on experimental evidence. Since many physiological and quantitative traits are influenced by food, candidate genes could be found with strategies that identify genes regulated differentially by nutrients, inbred strain (genotype), and genotype X diet interactions (14). Genes regulated by dietary variables in strains with differing susceptibilities to disease and that mapped to genetic loci linked to the phenotype are better candidates than those identified only by genetic location or differential expression (12, 14, 25). Combining environmental variables, high throughput ‘omics technology, and genetic data from techniques, such as QTL mapping or congenic mice, provides more complete experimental designs and datasets for understanding complex traits. Many phenotypic traits, particularly those involved in metabolic disorders are influenced by diet and other environmental variables (reviewed in Refs. 13 and 21).

Other investigators combined gene expression with QTL analyses (29). Their approach was to analyze mRNA from tissues of F2 mice with oligonucleotide arrays and treat the expression data as quantitative traits. The pattern of expressed QTLs and the genes within them identified new candidate genes for obesity. Gene expression has also been analyzed with RNA from tissues of congenic strains (e.g., see Refs. 1, 16, 20, and 26) to further narrow the choice of candidate genes. Such unsupervised strategies may generate candidates that can then be used in hypothesis-driven experimental designs that are more costly and time consuming (generating transgenic strains).

Epidemiologists (10) and those using expression arrays (8), proteomics (7), and metabolomics (9) are developing best practices for their fields or technologies for improving experimental designs and reliability. Most genetic researchers understand and use the many available genotypes and methods for analyzing the genetic contribution to complex traits. However, environmental variables are often not well controlled. For example, different lots of chow diets have inconsistent fat (15) and phytonutrient (22, 35) levels that will make it difficult to compare expression and physiological results within laboratories or between laboratories. Certain dietary chemicals, such as genistein or metabolites of fatty acids, can alter gene expression directly, through changes in hormone levels, signal transduction, and effects on enzymatic reactions (reviewed in Refs. 11, 13, 21, and 30). Hence, variation in diet composition will alter genetic (such as QTL mapping) and molecular (such as gene expression) analyses. Only recently has QTL mapping incorporated differences in macronutrients as a variable in the experimental design (2). Controlling for nutritional status (i.e., fasted vs. fed) also has been shown to be important in analyzing nutrient responsive genes (17, 24, 34).

A final resource that would aid efforts to understand macronutrient selection and other nutrient–gene interactions would be a centralized database linking experimental data from genomic analyses, transcriptomics, proteomics, and metabolomic data in response to well-characterized differences in nutrient intakes. While the existing databases are well designed for molecular and genetic data, environmental factors known to influence physiology are often not adequately measured or captured for comparative analyses. While these resources would be a challenge to fund and develop, they would be invaluable assets for the scientific community.

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DISCLAIMER
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REFERENCES