The Challenging Chase for Nutrigenetic Predictors of Metabolic Responses to Dietary Interventions

Development of the metabolic syndrome (MetS) is influenced by genetic and environmental factors. It is of interest to identify genetic factors associated with changes in MetS in response to dietary interventions to maximize the individual benefits gained from preventive or therapeutic measures (i.e., personalized nutrition). In this issue of *Diabetes Care*, Qi et al. (1) examined the contributions of two genetic variants (rs1522813 and rs2943641) near the insulin receptor substrate 1 (*IRS1*) gene and their interactions with dietary fat intake on the reversion of the MetS using the data of the Preventing Overweight Using Novel Dietary Strategies (POUNDS LOST) study.

POUNDS LOST study—The POUNDS LOST study was a 2-year population-based trial in which 811 overweight and obese adults (64% females) were randomly assigned to one of four diets (2). The percentage of calories derived from the macronutrients varied 20–40% for dietary fat, 15–25% for protein, and 35–65% for carbohydrates. A total of 645 subjects were defined as completers and they lost 4 kg on average with no significant differences among the four dietary groups. MetS was present in 32% of the participants at baseline and this prevalence was reduced to ~20% at 2 years with no differences between diet groups. Participants changed their diets in the direction of the specified macronutrient goals, but these goals were not fully achieved. For instance, the reported fat intake at 2 years differed from the targets by almost 7 percentage points, with the differences being ~7% higher for the 20% dietary fat diet group and ~7% lower for the 40% fat diet group (2).

For reasons not specified in the article, Qi et al. used 738 subjects for the current study, a number that apparently includes almost 100 noncompleters as defined in the original publication. Most of the 738 subjects were white (80%), but 15% were black, 3% Hispanic, and the rest other ancestries. It is not possible to evaluate the influence of the heterogeneity of the study population on the results for the rs2943641 variant as the authors found no significant dietary effects on MetS status by genotype and the data are not shown. However, an influence of the sample ethnic heterogeneity on the findings for the rs1522813 variant cannot be excluded from the data reported by Qi et al. (see Table 1 for the ethnic distribution by genotype).

Main findings of Qi et al.—Qi et al. found that the MetS reversion rate (proportion of subjects with MetS at baseline but reverted at 6 months or 2 years) was higher in the high-fat diet group compared with the low-fat group over the 2-year intervention in A-allele carriers at rs1522813 ($P = 0.002$), while no differences were found between the low- and high-fat diet groups among the GG genotype (1). The age-, sex-, ethnicity-, and body weight changes–adjusted odds ratio for 2-year MetS reversion for the high-fat compared with low-fat group was 2.88 (95% CI 1.25–6.67; $P = 0.01$) for A-allele carriers ($P = 0.04$ for genotype–diet interaction). These results did not change after adjustment for physical activity or changes in insulin resistance. The authors concluded that their findings provide supportive evidence for the notion of personalized dietary intervention in the management of MetS.

The study appears to be the first to examine gene–diet interactions on changes in MetS status in a large, long-term randomized dietary intervention. It represents an example of how trait responses to an intervention may be conditioned by genotype, emphasizing the importance of individual-level compared with population-level data when examining responses to standardized interventions. However, despite the positive findings and strengths of the POUNDS LOST trial, several limitations of the current study warrant further discussion.

Power and sample size—Statistical power and multiple testing are important considerations when evaluating genetic association studies. The authors acknowledged that they likely lacked the statistical power to detect the modest genetic associations and interactions they found for MetS reversion. In the current study, only 342 subjects had MetS at baseline and thus were eligible for analyses involving MetS reversion. Furthermore, they had a maximum sample size of 185 for the GG genotype and 157 for A-allele carriers. There was also an approximately 24% dropout from baseline to 2 years, further reducing the sample size.

The authors tested associations at two variants near *IRS1* but did not account for multiple testing, as a P value ≤ 0.05 was considered statistically significant. Correcting for the fact that two variants were tested would result in a Bonferroni-corrected threshold of $P = 0.025$ for statistical significance. Furthermore, the authors conducted numerous statistical tests across many traits. The results in Table 2 show that 22 statistical tests were performed for the association of rs1522813 genotype with various traits, while Supplementary Table 1 shows 24 additional tests (1). It can be assumed these tests were also performed for the rs2943641 genotype, resulting in approximately 100 total statistical tests and a Bonferroni multiple testing corrected threshold of about $P = 0.0005$. Using this threshold, none of the reported associations in the study would be considered statistically significant.

Association of *IRS1* with metabolic traits—Variants in or near the *IRS1* gene have been associated with MetS and several of its components (3–8). Furthermore, several studies have reported significant genotype–diet interactions between variants in or near *IRS1* and dietary factors on metabolic traits (9–11), including the POUNDS LOST study (12). The rs2943641 and rs1522813 variants tested by Qi et al. are located approximately 500 kb and 660 kb from *IRS1*, respectively, which is quite remote from the associated gene transcript. It is not clear whether these variants are in linkage disequilibrium with *IRS1* variants and/or related to *IRS1* function. Teslovich et al. (8) noted that variation at rs2972146, a variant associated with metabolic traits in recent genome-wide

association studies (GWAS) (6,8), correlated with *IRS1* expression in omental fat, despite being located 495 kb away from the *IRS1* gene.

Recent GWAS reports have identified over 20 loci associated with MetS at the genome-wide level, a list that does not include *IRS1* (13–15). Although loci identified in observational GWAS are not always associated with trait responses to interventions (16), the inclusion of only variants near *IRS1* and the modest effect sizes reported in the current study should be viewed with healthy skepticism. Furthermore, the POUNDS LOST group itself has reported elsewhere that variation in at least six different genes modulates the association between dietary composition and changes in metabolic traits (12,17–21). Thus, it is clear that no single gene or variant is likely responsible for interindividual differences in the response of MetS and its components to dietary interventions.

Outlook for nutrigenetic studies

—The article by Qi et al. represents an opportunity to discuss the conditions necessary to identify genotype–nutrition interaction effects on metabolic traits. Multiple challenges have to be met for nutrigenetic studies to be successful (22). First, the true magnitude of a gene–nutrition interaction effect is not likely to be revealed by observational studies. Only intervention studies in which compliance with the experimental dietary exposure is not in doubt have the potential to uncover the complex relationships between DNA sequence variants in critical genes and a given nutrient or combination of nutrients and their effects on metabolic traits of interest. Second, in studies dealing with differential exposures to macronutrients, it is a major challenge to isolate the macronutrient truly responsible for the nutrigenetic effects, particularly for fat and carbohydrates as they tend to be manipulated in such a way that one is compensated by the other. The problem is thought to be greatly diminished when the dietary exposure pertains to other nutrients. Third, the challenge relating to sample size and statistical power as emphasized above. Based on this issue alone, it is likely that the vast majority of the nutrigenetic findings reported to date are false (see reference 23 for a discussion). It is a challenge to design large-scale human nutrigenetic experiments and an even more daunting task to get them adequately funded in the current environment, but unfortunately there is no true substitute.

In brief, it is possible that calorically restricted high-fat diets designed to induce weight loss are more effective in the management of nascent metabolic disorders compared with low-fat diets in people carrying the A-allele at the rs1522813 polymorphic site in the vicinity of the *IRS1* gene, but the evidence available to that effect remains inconclusive at this time.

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