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Fatty acid interactions with genetic polymorphisms for cardiovascular disease

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Abstract

Purpose of review—The number of studies investigating interactions between genes and nutrients for cardiovascular disease continues to grow, and holds tremendous potential for reducing disease risk at the level of the individual genotype. However, understanding the limitations and challenges of interaction studies, whether of observational or interventional design, is essential for critical evaluation of these studies.

Recent findings—Nutrient-gene interactions for cardiovascular disease both parallel and extend nutrition studies, encompassing both traditional and novel cardiovascular risk factors. Fatty acid quality, lipid metabolism, inflammation, post-prandial metabolism, fatty liver and macronutrient-gene interactions for obesity and metabolic syndrome represent a subset of the major areas of recent focus. With few exceptions, however, reports of gene-nutrient interactions are limited to a single population.

Summary—Gene-nutrient research will continue to expand as genome wide association studies uncover new sources of genetic variability associated with cardiovascular risk. However, in addition to investigation of newly discovered variants, continuing efforts must focus on the confirmation of previously reported genetic associations and interactions in additional populations.

Keywords

nutrient-gene interactions; cardiovascular disease; fatty acids

Introduction

Analyses of the role of gene-nutrient interactions in the risk of cardiovascular disease (CVD) address the fundamental question of inter-individual variation in the response to diet, and lay the groundwork for construction of a genetically-based framework for tailoring diets to minimize disease risk. The predominant analysis approach relies on candidate gene association and interaction studies, in which genes are selected based on known functions of the proteins they encode, and evaluated for their associations with outcomes in populations. In the case of interaction studies, modulation of a genetic association by nutrients (usually intake, but also biomarkers reflecting intakes and metabolism), energy intake or a

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phenotype, such as obesity, are evaluated for specific outcomes. Phenotypic outcomes are usually intermediate risk factors (eg, lipids, glucose and insulin, anthropometrics) rather than vascular end-points (eg, myocardial infarction, atherosclerotic lesion), and the following review is organized by outcome. Regardless of the specific outcome, however, individual genetic effect sizes tend to be small, and obtaining adequate sample size to capture relatively small effects is a frequent challenge. False positive results are another potential pitfall associated with multiple testing of metabolic outcomes or variants. In response to these challenges, replication of gene-nutrient interactions in independent populations has become critical but is still relatively rare. While collaborative data sharing arrangements have provided greater opportunity for replications, heterogeneity introduced by genetic and cultural factors in populations of differing ethnicities further complicates analyses, as many of the included studies illustrate.

Myocardial Infarction/Atherosclerosis

Investigation of gene-nutrient interactions for endpoints such as myocardial infarction (MI) or atherosclerosis is less common due to the complexity of the outcome, but these outcomes have been examined in a few recent studies (1,2,3). Pro-inflammatory leukotrienes, which are derived from arachidonic acid (AA) in the 5-LO (lipoxygenase) pathway, appear to play a proatherogenic role in people. A 5-LO promoter polymorphism was shown to be associated with carotid atherosclerotic lesions, and to interact with dietary fatty acids in a US population (n=470) which included non-Hispanic whites, Hispanics, Asian or Pacific Islanders, Blacks, and others. In this cohort, AA and linoleic acid were associated with increased atherosclerosis (intima-media thickness) in carriers of the variant allele, and omega-3 fatty acids were protective in carriers (3). More recently, the same group demonstrated a similar interaction in a Costa Rican population (n=3770) for the related outcome of MI (2). Replication of interactions between 5-LO genotype and fatty acids in two independent populations of different ethnicities supports the plausibility of a biological relationship between fatty acids and 5-LO genotype. While a previous study had failed to reproduce the association for the outcome of MI in a smaller (n=688) Spanish population, this study did not evaluate the role of dietary factors, which differ considerably among US, Costa Rican and Spanish populations. (4).

Lipids

Genetic associations (5) and fatty acid-gene interactions continue to be widely explored for lipid outcomes (6,7). A growing body of studies focus on peroxisome proliferator-activated receptor (*PPAR*) variants, a series of nuclear receptors which are activated by long chain fatty acids. *PPARα* regulates genes involved in glucose and lipid metabolism as well as atherosclerotic lesion formation, and responds to triglyceride (TG)-lowering medications, such as fibrates, as well as long chain polyunsaturated fatty acids. One widely studied single nucleotide polymorphism (SNP), *PPARα* L162V, was shown in earlier studies to be associated with lipids and CVD risk, and recently reported to interact with PUFA in a US White population for plasma TG concentrations (8). Evaluation of fatty acids interaction for this SNP and 8 others was performed in two US populations comprising the ARIC study (n=10,134 Whites and n=3480 Blacks) (9). In spite of the large samples in the current study, particularly for Whites, the previously reported L162V interaction with PUFA was not reproduced. Of 8 other SNPs tested, two showed interaction with PUFA, one (3'UTR G→A) interacted with omega-6 PUFA to modulate total cholesterol and low density lipoprotein cholesterol (LDL-C) only in Whites and another (3'UTR C→T) interacted with omega-3 PUFA to modulate the same lipids only in Blacks. Detection of interactions for two different SNPs and two types of PUFA in the two populations is not unexpected, given large differences in minor allele frequencies (MAF) for the SNPs in the two groups, combined

with significant differences in omega-3 and omega-6 intakes in the Blacks and Whites studied. Interaction *P* values were nominal (0.02–0.03) in the context of large samples used to evaluate a large number of outcomes and SNPs, suggesting that additional studies in ethnically appropriate populations would be useful.

Although the previous study reported omega-3 fatty acid modulation of cholesterol, omega-3 fatty acids have been most widely investigated for their modulation of TG. Omega-3 fatty acids lower TG, and intra-individual variability in this response may be related to genetic variability. *CD36* encodes a class B scavenger receptor which interacts with a wide variety of ligands including long chain fatty acids and oxidized LDL-C, and which has been implicated in atherosclerosis and insulin resistance. Evaluation of potential relationships between four *CD36* SNPs, omega-3 fatty acids and lipids was performed in a UK population of healthy, middle-aged White men (n=111) (10). *CD36* genotype modulated fish oil-associated changes in TG such that for three of the four SNPs, TG was significantly lower only in homozygous carriers of the G allele, which was minor in three SNPs and major in the other. This study did not replicate associations for non-esterified fatty acids (NEFA) demonstrated in the larger study from which the SNPs were selected (11). Additional studies with larger populations would be useful in confirming the novel interactions reported here.

The preceding interactions focused on fasting lipids, but recognition of the critical impact of postprandial lipemia on CVD risk has led to a large number of association and interaction studies designed to assess the impact of genotype on the response to oral fat loads (12). Hepatic lipase (*LIPC*) plays a key role in the lipid metabolism, especially for high density lipoprotein cholesterol (HDL-C). While the *LIPC* -514 C/T variant has been widely studied, several other promoter variants including *LIPC* -250G/A are in linkage disequilibrium with this SNP in Whites. In a small group of healthy male Spanish students (GG genotype n=30 and A carrier genotype n=21), the presence of the A allele for the -250 G/A SNP was associated with a greater postprandial response, which was most pronounced for small TG-rich lipoproteins (13). The same group previously demonstrated a lower postprandial response for carriers of the variant allele for *LIPC* -514 (14), which raises questions about the functional nature of each SNP and potential interaction among *LIPC* SNPs.

Extending the preceding studies are those investigating the effects of background dietary intakes of fatty acids on genetically based differences in the postprandial response. Variants of the transcription-factor 7-like 2 (*TCF7L2*) gene have been associated with impaired insulin secretion, metabolic syndrome traits, and altered postprandial lipoprotein response, although results have not been consistent (15,16) and nutrient interactions for postprandial lipid metabolism are unexplored for this gene. Warodomwicht et. al. hypothesized that differences in diet among populations might influence baseline metabolic associations for *TCF7L2*, as well as postprandial responses (17). In a study of 1083 US Whites, they showed that the *TCF7L2* variant rs12255372 interacted with dietary PUFA for fasting very low density lipoproteins (VLDL), and the postprandial response (TG, chylomicrons, VLDL and large VLDL). For each lipid outcome, a more atherogenic profile was associated with the variant allele (T) only in the context of high PUFA intake. While these data provide a plausible explanation for inconsistent results from previous association studies, the authors note that a large number of tests were performed in a single population, and recommend confirmation in additional populations.

While the preceding studies have evaluated fatty acid interactions using dietary intakes, concentrations of specific plasma fatty acids which may interact with genes are determined both by both intake and metabolism (18–23). Specifically, the activity of key enzymes (eg, desaturases) encoded by genes in the *FADS* cluster contribute to PUFA concentrations. A

recent genome-wide association study using the Italian InCHIANTI population (n=1075) (24••) which evaluated signals associated with plasma PUFA reported strong associations for the *FADS1*, *FADS2*, and *FADS3* clusters as well as for the *ELOVL2* gene which encodes enzymes involved in the elongation of PUFA. Five SNPs were chosen for replication in the US GOLDN population (n=1076 Whites). Of the five SNPs, one *FADS1* SNP (rs174537) showed patterns similar to those in InCHIANTI for linoleic acid, AA, alpha linolenic acid (ALA), eicosapentanoic acid (EPA) as well as total cholesterol and LDL-C. A second *ELOVL2* SNP (rs953413) was also strongly associated with docosapentanoic acid (DPA) in the GOLDN population and DHA in both populations. Although not an interaction study, this confirmation study by Tanaka et al. identifies sites at which genetic variability in enzymes metabolizing essential fatty acids interacts with diet to alter specific plasma fatty acid concentrations, potentially modulating genetic risks in subsequent metabolic pathways.

Obesity

Lipids represent one of the earliest CVD risk factors to be investigated genetically, but recognition of genetic susceptibility to obesity and its contribution to CVD risk via lipid and glucose metabolic dysregulation is growing (25–29). Obesity is highly heritable, but may be modifiable through dietary composition as well as energy intake. Two recent studies have reported interactions between genetic variants and monounsaturated fatty acids (MUFA) for obesity-related outcomes (30•,31•). Variants of the adipocyte-derived protein adiponectin have been previously linked to obesity and insulin resistance, but association studies among different ethnic groups have not been consistent. In a population of 1083 US Whites, the –A allele of the adiponectin 11391 G>A variant was protective against obesity independently of dietary intake and also under conditions of high MUFA intake, but this relationship was reversed (eg, the A variant was associated with increased obesity risk and BMI) in subjects consuming low intakes of MUFA (30•). Additional interactions for PUFA intake were observed for the same SNP, but did not achieve significance after correction for multiple testing, suggesting that a larger sample might yield additional interactions.

MUFA intake also interacted significantly with variants of *WDTCL1*, the human ortholog of the *Adipose* gene, which was shown to be associated with obesity in *Drosophila sp.* (31•). Although *WDTCL1* was associated with obesity in two populations, US Whites (n=1115) and Caribbean Hispanic Americans (n=935), the patterns were slightly different and reached significance only in women in the Whites. Further, MUFA was protective for AA subjects for the i22835A>G variant and associated with increased BMI for GA and GG subjects only in the Caribbean Hispanics. The partial replication demonstrated for obesity in the two populations likely reflects not only genetic heterogeneity, but also the wide phenotypic and dietary variability documented in these two groups.

Metabolic Syndrome and Diabetes

As obesity prevalence has increased globally, rising rates of related conditions such as metabolic syndrome and diabetes have stimulated exploration of associated genetic risks (32,33). Two recent studies have reported interactions between fatty acid intake and genetic variants for regulators of lipid metabolism, the PDZ-Interacting domain of the Scavenger Receptor Class B Type I and $\delta 6$ -desaturase (encoded by *FADS2*) (34,35). In a study of 1000 US Whites, the T allele of the PDZK1_i33968C>T variant was associated with metabolic syndrome, but the relationship was differentially modified by intake of PUFA and carbohydrate, depending on obesity status (34). Replication of the complex relationships between gene, diet and obesity observed in this overweight population with metabolic syndrome prevalence of 30% is desirable.

In a second study performed in a Costa Rican population (n=1815) with metabolic syndrome prevalence of 36%, the prevalence ratio of metabolic syndrome decreased in subjects with increasing quintiles of adipose tissue ALA (35). This effect trended towards interaction ($P=0.08$) with the *FADS2* rs3834458 genotype. Mechanistically, the reported interaction is plausible although novel, since $\delta 6$ -desaturase mediates the conversion of ALA and linoleic acid to longer chain PUFA, and the same group previously demonstrated lower long-chain PUFA in Costa Rican carriers of the variant *FADS2* allele (36). Exploring the role of fatty acids which compete with ALA for $\delta 6$ -desaturase, such as linoleic acid, and metabolic syndrome in other populations may yield additional relevant data, since the study authors note that Costa Rican intake of linoleic acid is low compared to other groups.

In addition to metabolic syndrome, investigators have examined gene-nutrient interactions for the components of the syndrome including impaired glucose metabolism. Dietary fat quality may alter glucose metabolism such that saturated fat is associated with increased insulin resistance while unsaturated fats are associated with improved insulin sensitivity. Plasma adiponectin concentration is also positively associated with insulin sensitivity, but whether adiponectin and diet interact to modify glucose metabolism is unexplored (37). In a cross-over intervention examining three test diets in healthy Spanish students (n=59 men and women), dietary fat interacted with adiponectin genotype. In men only, homozygotes for the C allele of the -11377 C>G variant demonstrated less insulin resistance following a carbohydrate-rich or MUFA-rich diet compared to the saturated fat-rich diet. (38•). Results from previous studies evaluating the -11377 C>G variant and insulin sensitivity have been mixed, and results from the current study suggest that dietary factors may be one source of variability. However, the small sample size and the limitation of the observed relationship to men in the dietary trial warrant additional investigation in larger groups.

Gender-specific interactions were also reported for omega-3 fatty acids and glucose and fatty acid metabolism outcomes, according to genotype of the *PPAR γ* variant Pro12Ala. (39). *PPAR γ* modulates lipid metabolism and adipocyte differentiation through control of gene expression. Omega-3 fatty acids and *PPAR γ* each appear to play roles in glucose metabolism, and the current study explored potential interactions in 571 Finns. In women, fish and omega-3 intake interacted with *PPAR γ* for postprandial 2-hour glucose whereas in men plasma DHA interacted with *PPAR γ* for serum free fatty acid concentration. In both instances interaction *P* values were nominal in the context of a large number of tests, and the metabolic outcomes were observed for different genotypes in men and women (eg, male Pro homozygotes benefited from high DHA but in women high fish intake was beneficial for Ala carriers). Gender differences for both *PPAR γ* associations and long-chain omega-3 fatty acid metabolism have been previously reported (40,41) but additional studies would help to confirm the reported interactions.

Nonalcoholic Fatty Liver Disease

While the outcomes discussed above contribute to CVD risk, nonalcoholic steatohepatitis (NASH) has been shown to predict risk independently of other factors (42). Mechanisms for this relationship are not well understood, but plasma adiponectin appears to play a major role in postprandial lipemia, NASH and CVD risk (43). In the current study, Musso et.al. examined adiponectin variants 45TG and 276GT for their relationship to NASH and postprandial lipemia following oral fat load in 70 NASH cases and 70 matched controls (44). Genotypes 45TT and 276GT/TT were more common in patients with disease, associated with greater disease severity, with lower adiponectin in controls, with higher postprandial response and with lower postprandial adiponectin compared to other genotypes. While the study did not evaluate nutrient gene interactions, and may have lacked statistical power to do so, subjects with NASH showed higher intake of saturated fat and lower intake

of PUFA than controls. A third study by the same group identified a relationship between PUFA and saturated fatty acid ratio, *TCF7L2* genotype and the postprandial glucose-dependent insulinotropic peptide response in NASH (45). In light of these observations and those reported for fatty acids and adiponectin variants for obesity and insulin sensitivity (6,30), investigation of modulation of adiponectin-related genetic risk by diet may suggest ways to ameliorate disease severity in people with NASH.

Conclusions

Gene-nutrient interaction studies have the potential to reduce longstanding knowledge gaps in potential relationships between nutrients, genetic factors and cardiovascular disease phenotypes. Not only do these studies elucidate the reasons for inconsistencies of prior genetic association studies and support mechanistic relationships between genetic and nutritional factors, but they also provide a framework on which rational preventive nutritional strategies may eventually be based. In spite of this wide-ranging potential, consistent challenges emerge from many of the preceding studies. Addressing these limitations will require replications in larger sample sizes, more extensive genotyping along with deeper, standardized phenotyping, and analyses of interactions in populations of varying ethnicities.

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References

1. Martinelli N, Girelli D, Malerba G, et al. FADS genotypes and desaturase activity estimated by the ratio of arachidonic acid to linoleic acid are associated with inflammation and coronary artery disease. *Am J Clin Nutr*. 2008; 88(4):941–949. [PubMed: 18842780]
2. Allayee H, Baylin A, Hartiala J, et al. Nutrigenetic association of the 5-lipoxygenase gene with myocardial infarction. *Am J Clin Nutr*. 2008; 88(4):934–940. [PubMed: 18842779]. This study confirms a previously described nutrient-gene interaction for a similar phenotype and includes functional investigation of the examined variants.
3. Dwyer JH, Allayee H, Dwyer KM, et al. Arachidonate 5-lipoxygenase promoter genotype, dietary arachidonic acid, and atherosclerosis. *N Engl J Med*. 2004; 350(1):29–37. [PubMed: 14702425]
4. Gonzalez P, Reguero JR, Lozano I, et al. A functional Sp1/Egr1-tandem repeat polymorphism in the 5-lipoxygenase gene is not associated with myocardial infarction. *Int J Immunogenet*. 2007; 34(2):127–130. [PubMed: 17373938]
5. Lindi V, Schwab U, Louheranta A, et al. The G-250A polymorphism in the hepatic lipase gene promoter is associated with changes in hepatic lipase activity and LDL cholesterol: The KANWU Study. *Nutr Metab Cardiovasc Dis*. 2008; 18(2):88–95. [PubMed: 17327141]
6. Perez-Martinez P, Lopez-Miranda J, Cruz-Teno C, et al. Adiponectin gene variants are associated with insulin sensitivity in response to dietary fat consumption in Caucasian men. *J Nutr*. 2008; 138(9):1609–1614. [PubMed: 18716158]
7. Goyenechea E, Collins LJ, Parra D, et al. CD36 gene promoter polymorphisms are associated with low density lipoprotein-cholesterol in normal twins and after a low-calorie diet in obese subjects. *Twin Res Hum Genet*. 2008; 11(6):621–628. [PubMed: 19016618]
8. Tai ES, Corella D, Demissie S, et al. Polyunsaturated fatty acids interact with the PPARAL162V polymorphism to affect plasma triglyceride and apolipoprotein C-III concentrations in the Framingham Heart Study. *J Nutr*. 2005; 135(3):397–403. [PubMed: 15735069]

9. Volcik KA, Nettleton JA, Ballantyne CM, et al. Peroxisome proliferator-activated receptor [alpha] genetic variation interacts with n-6 and long-chain n-3 fatty acid intake to affect total cholesterol and LDL-cholesterol concentrations in the Atherosclerosis Risk in Communities Study. *Am J Clin Nutr*. 2008; 87(6):1926–1931. [PubMed: 18541586]
10. Madden J, Carrero JJ, Brunner A, et al. Polymorphisms in the CD36 gene modulate the ability of fish oil supplements to lower fasting plasma triacyl glycerol and raise HDL cholesterol concentrations in healthy middle-aged men. *Prostaglandins Leukot Essent Fatty Acids*. 2008; 78(6):327–335. [PubMed: 18550349]
11. Ma X, Bacci S, Mlynarski W, et al. A common haplotype at the CD36 locus is associated with high free fatty acid levels and increased cardiovascular risk in Caucasians. *Hum Mol Gen*. 2004; 13(19):2197–2205. [PubMed: 15282206]
12. Rubin D, Helwig U, Nothnagel M, et al. Postprandial plasma adiponectin decreases after glucose and high fat meal and is independently associated with postprandial triacylglycerols but not with --11388 promoter polymorphism. *Br J Nutr*. 2008; 99(1):76–82. [PubMed: 17663805]
13. Jimenez-Gomez Y, Perez-Jimenez F, Marin C, et al. The -250G/A polymorphism in the hepatic lipase gene promoter influences the postprandial lipemic response in healthy men. *Nutr Metab Cardiovasc Dis*. 2008; 18(3):173–181. [PubMed: 17399967]
14. Gomez P, Miranda JL, Marin C, et al. Influence of the -514C/T polymorphism in the promoter of the hepatic lipase gene on postprandial lipoprotein metabolism. *Atherosclerosis*. 2004; 174(1):73–79. [PubMed: 15135253]
15. Kimber CH, Doney AS, Pearson ER, et al. TCF7L2 in the Go-DARTS study: evidence for a gene dose effect on both diabetes susceptibility and control of glucose levels. *Diabetologia*. 2007; 50(6):1186–1191. [PubMed: 17429603]
16. Melzer D, Murray A, Hurst AJ, Weedon MN, Bandinelli S, Corsi AM, Ferrucci L, Paolisso G, Guralnik JM, Frayling TM. Effects of the diabetes linked TCF7L2 polymorphism in a representative older population. *BMC Medicine*. 2006; 4:34. 2006. [PubMed: 17181866]
17. Warodomwicht D, Arnett DK, Kabagambe EK, et al. Polyunsaturated fatty acids modulate the effect of TCF7L2 gene variants on postprandial lipemia. *J Nutr*. 2009; 139(3):439–446. [PubMed: 19141698]
18. Zeman M, Vecka M, Jachymova M, et al. Fatty acid CoA ligase-4 gene polymorphism influences fatty acid metabolism in metabolic syndrome, but not in depression. *Tohoku J Exp Med*. 2009; 217(4):287–293. [PubMed: 19346733]
19. Koletzko B, Demmelmair H, Schaeffer L, et al. Genetically determined variation in polyunsaturated fatty acid metabolism may result in different dietary requirements. *Nestle Nutrition Workshop Series. Paediatric Programme*. 2008; 62:35–44. discussion 44-9. [PubMed: 18626191]
20. Malerba G, Schaeffer L, Xumerle L, et al. SNPs of the FADS gene cluster are associated with polyunsaturated fatty acids in a cohort of patients with cardiovascular disease. *Lipids*. 2008; 43(4):289–299. [PubMed: 18320251]
21. Franks PW, Scheele C, Loos RJ, et al. Genomic variants at the PINK1 locus are associated with transcript abundance and plasma nonesterified fatty acid concentrations in European whites. *FASEB J*. 2008; 22(9):3135–3145. [PubMed: 18495756]
22. Bendlova B, Vejrazkova D, Vcelak J, et al. PPARgamma2 Pro12Ala polymorphism in relation to free fatty acids concentration and composition in lean healthy Czech individuals with and without family history of diabetes type 2. *Physiol Res*. 2008; 57 Suppl 1:S77–S90. [PubMed: 18271689]
23. Ward NC, Tsai IJ, Barden A, et al. A single nucleotide polymorphism in the CYP4F2 but not CYP4A11 gene is associated with increased 20-HETE excretion and blood pressure. *Hypertension*. 2008; 51(5):1393–1398. [PubMed: 18391101]
24. Tanaka T, Shen J, Abecasis GR, et al. Genome-wide association study of plasma ••polyunsaturated fatty acids in the InCHIANTI Study. *PLoS Genetics*. 2009; 5(1):e1000338. [PubMed: 19148276] . Identifies variants in genes encoding PUFA metabolic enzymes, which represent sources of variability in plasma fatty acid concentrations. These variants have the potential to complicate nutrient-gene analyses which typically rely on food frequency questionnaires to estimate nutrient intakes. Results are partially replicated in an independent population.

25. de Luis DA, Aller R, Izaola O, et al. Influence of Ala54Thr polymorphism of fatty acid-binding protein 2 on weight loss and insulin levels secondary, to two hypocaloric diets: a randomized clinical trial. *Diabetes Res Clin Pract.* 2008; 82(1):113–118. [PubMed: 18701184]
26. de Luis DA, Aller R, Izaola O, et al. Influence of Lys656Asn polymorphism of leptin receptor gene on leptin response secondary to two hypocaloric diets: a randomized clinical trial. *Ann Nutr Metab.* 2008; 52(3):209–214. [PubMed: 18544975]
27. Kourlaba G, Pitsiladis YP, Lagou V, et al. Interaction effects between total energy and macronutrient intakes and angiotensin-converting enzyme 1 (ACE) I/D polymorphism on adiposity-related phenotypes in toddlers and preschoolers: the Growth, Exercise and Nutrition Epidemiological Study in preschoolers (GENESIS). *Br J Nutr.* 2008; 100(6):1333–1340. [PubMed: 18479585]
28. Timpson NJ, Emmett PM, Frayling TM, et al. The fat mass- and obesity-associated locus and dietary intake in children. *Am J Clin Nutr.* 2008; 88(4):971–978. [PubMed: 18842783]
29. Qi L, Kraft P, Hunter DJ, et al. The common obesity variant near MC4R gene is associated with higher intakes of total energy and dietary fat, weight change and diabetes risk in women. *Hum Mol Genet.* 2008; 17(22):3502–3508. [PubMed: 18697794]
30. Warodomwicht D, Shen J, Arnett DK, et al. ADIPOQ polymorphisms, monounsaturated • fatty acids, and obesity risk: the GOLDN study. *Obesity.* 2009; 17(3):510–517. [PubMed: 19238139] . Illustrates several common problems in interaction studies include multiple comparisons and dietary variation among populations, and includes the use of bioinformatics analysis to identify potential functional sites.
31. Lai C-Q, Parnell LD, Arnett DK, et al. WDTC1, the ortholog of *Drosophila* adipose gene, •associates with human obesity, modulated by MUFA intakeWDTC1, the ortholog of *Drosophila* adipose gene, •associates with human obesity, modulated by MUFA intake. *Obesity.* 2009; 17(3): 593–600. [PubMed: 19238144] . Confirms similar patterns in two independent, genetically and phenotypically heterogeneous populations.
32. Enhorning S, Leosdottir M, Wallstrom P, et al. Relation between human vasopressin 1a gene variance, fat intake, and diabetes. *Am J Clin Nutr.* 2009; 89(1):400–406. [PubMed: 19056558]
33. Zak A, Jachymova M, Tvrzicka E, et al. The influence of polymorphism of -493G/T MTP gene promoter and metabolic syndrome on lipids, fatty acids and oxidative stress. *J Nutr Biochem.* 2008; 19(9):634–641. [PubMed: 18280132]
34. Junyent M, Arnett DK, Tsai MY, et al. Genetic variants at the PDZ-interacting domain of the scavenger receptor class B type I interact with diet to influence the risk of metabolic syndrome in obese men and women. *J Nutr.* 2009; 139(5):842–848. [PubMed: 19321583]
35. Truong H, DiBello JR, Ruiz-Narvaez E, et al. Does genetic variation in the Delta6-desaturase promoter modify the association between alpha-linolenic acid and the prevalence of metabolic syndrome? *Am J Clin Nutr.* 2009; 89(3):920–925. [PubMed: 19144731]
36. Baylin A, Ruiz-Narvaez E, Kraft P, Campos H. Alpha-Linolenic acid, delta6-desaturase gene polymorphism, and the risk of nonfatal myocardial infarction. *Am J Clin Nutr.* 2007; 85(2):554–560. [PubMed: 17284757]
37. Cnop M, Havel PJ, Utzschneider KM, et al. Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. *Diabetologia.* 2003; 46(4):459–469. [PubMed: 12687327]
38. Perez-Martinez P, Corella D, Shen J, et al. Association between glucokinase regulatory protein (GCKR) and apolipoprotein A5 (APOA5) gene polymorphisms and triacylglycerol concentrations in fasting, postprandial, and fenofibrate-treated states. *Am J Clin Nutr.* 2009; 89(1):391–399. [PubMed: 19056598] . •While not a gene-nutrient interaction analysis per se, the identification of similar associations in eight populations from several continents illustrates the power of collaborative data sharing.
39. Ylonen SK, Salminen I, Lyssenko V, et al. The Pro12Ala polymorphism of the PPAR-gamma2 gene affects associations of fish intake and marine n-3 fatty acids with glucose metabolism. *Eur J Clin Nutr.* 2008; 62(12):1432–1439. [PubMed: 17700648]
40. Crowe FL, Skeaff CM, Green TJ, Gray AR. Serum n-3 long-chain PUFA differ by sex and age in a population-based survey of New Zealand adolescents and adults. *Br J Nutr.* 2008; 99(1):168–174. [PubMed: 17678566]

41. Gonzalez Sanchez JL, Serrano Rios M, Fernandez Perez C, et al. Effect of the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor gamma-2 gene on adiposity, insulin sensitivity and lipid profile in the Spanish population. *Eur J Endocrinol.* 2002; 147(4):495–501. [PubMed: 12370112]
42. Schindhelm RK, Dekker JM, Nijpels G, et al. Alanine aminotransferase predicts coronary heart disease events: a 10-year follow-up of the Hoorn Study. *Atherosclerosis.* 2007; 191(2):391–396. [PubMed: 16682043]
43. Musso G, Gambino R, Durazzo M, et al. Adipokines in NASH: postprandial lipid metabolism as a link between adiponectin and liver disease. *Hepatology.* 2005; 42(5):1175–1183. [PubMed: 16231364]
44. Musso G, Gambino R, De Michieli F, et al. Adiponectin gene polymorphisms modulate acute adiponectin response to dietary fat: Possible pathogenetic role in NASH. *Hepatology.* 2008; 47(4): 1167–1177. [PubMed: 18311774]
45. Musso G, Gambino R, Pacini G, et al. Prolonged saturated fat-induced, glucose-dependent insulinotropic polypeptide elevation is associated with adipokine imbalance and liver injury in nonalcoholic steatohepatitis: dysregulated enteroadipocyte axis as a novel feature of fatty liver. *Am J Clin Nutr.* 2009; 89(2):558–567. [PubMed: 19141695]