Genetic influences on blood lipids and cardiovascular disease risk: tools for primary prevention

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ABSTRACT

Genetic polymorphism in human populations is part of the evolutionary process that results from the interaction between the environment and the human genome. Recent changes in diet have upset this equilibrium, potentially influencing the risk of most common morbidities such as cardiovascular diseases, obesity, diabetes, and cancer. Reduction of these conditions is a major public health concern, and such a reduction could be achieved by improving our ability to detect disease predisposition early in life and by providing more personalized behavioral recommendations for successful primary prevention. In terms of cardiovascular diseases, polymorphisms at multiple genes have been associated with differential effects in terms of lipid metabolism; however, the connection with cardiovascular disease has been more elusive, and considerable heterogeneity exists among studies regarding the predictive value of genetic markers. This may be because of experimental limitations, the intrinsic complexity of the phenotypes, and the aforementioned interactions with environmental factors. The integration of genetic and environmental complexity into current and future research will drive the field toward the implementation of clinical tools aimed at providing dietary advice optimized for the individual’s genome. This may imply that dietary changes are implemented early in life to gain maximum benefit. However, it is important to highlight that most reported studies have focused on adult populations and to extrapolate these findings to children and adolescents may not be justified until proper studies have been carried out in these populations and until the ethical and legal issues associated with this new field are adequately addressed.

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INTRODUCTION

The major public health concerns in the developed world (ie, cardiovascular disease, cancer, diabetes, and obesity) have both genetic and environmental causes. The interface between public health and genetics consists of working toward an understanding of how genes and the environment act together to cause these diseases and how the environment (eg, diet) might be modified in a more personalized fashion to help prevent or delay the onset of disease. As such, cardiovascular disease (CVD), the leading cause of mortality in most industrialized countries, is a multifactorial disease that is associated with nonmodifiable risk factors, such as age, sex, and genetic background, and with modifiable risk factors, including dyslipidemia, obesity, hypertension, and insulin resistance, all of them components of the metabolic syndrome, as well as other more novel risk factors related to inflammation (ie, C-reactive protein). Although considerable strides have been made toward controlling all these risk factors, the area in which we have gained more knowledge has been in relation to dyslipidemia.

Lipoproteins are macromolecular complexes of lipids and proteins that originate mainly from the liver and intestine and are involved in the transport and redistribution of lipids in the body. Lipid and lipoprotein metabolism can be viewed as a complex biological pathway containing multiple steps. Lipid homeostasis is achieved by the coordinated action of a large number of nuclear factors, binding proteins, apolipoproteins, enzymes, and receptors involving hundreds of genes. Lipid metabolism is also closely linked with energy metabolism and is subjected to many hormonal controls that are essential for adjustment to environmental and internal conditions.

In view of the accumulated evidence linking lipoprotein metabolism with the development of atherosclerosis, the major emphasis for CVD prevention and therapy has been placed on lowering serum cholesterol concentration, with the clinical standards being defined by the National Cholesterol Education Program–Adult Treatment Panel convened by the National Heart, Lung, and Blood Institute (2, 3). Since the publication of the National Cholesterol Education Program–Adult Treatment Panel III guidelines, several large-scale clinical trials of cholesterol lowering have been conducted, the findings of which have the potential to further refine current clinical practice standards (4, 5). Although most of the beneficial evidence from lowering serum LDL-cholesterol values in reducing CVD morbidity and mortality comes from pharmacologic interventions, the National

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Cholesterol Education Program has emphasized that therapeutic lifestyle change should be the primary treatment for lowering cholesterol values, with drug therapies reserved for cases in which lifestyle modification is ineffective. The modifications advocated include dietary changes, increased physical activity, and weight management. The recommended dietary changes include restriction of the amount of saturated fat to <7% of calories and of cholesterol to <200 mg/d and an increase in viscous fiber (10–24 g/d) and plant stanols/sterols (2 g/d) to enhance LDL lowering (3). However, it is not known how many individuals can achieve the recommended concentrations of serum lipids by using this approach because of our current inability to predict individual plasma lipid response to dietary changes (6).

In addition to cholesterol lowering, other pharmacologic approaches to CVD risk reduction have been investigated. These include the lowering of serum triglycerides and the raising of HDL-cholesterol concentrations. The most commonly used drugs to lower triglyceride concentrations are the fibrates. These drugs also raise HDL cholesterol and improve small dense LDL cholesterol and so would be expected to have large beneficial effects. Their use in the management of lipoprotein disorders has a history dating back to the mid-1960s. However, their prominence has lessened over the years because of unimpressive results in major clinical trials, safety concerns (7), and the emergence of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins). Moreover, the general trial results with these agents have been confusing, with varying cardiovascular benefits (8–10).

The epidemiologic, experimental, and other circumstantial evidence implicating low HDL-cholesterol concentrations as a major CVD risk has focused considerable attention on this lipoprotein fraction as a potential therapeutic target to further reduce CVD risk beyond what can be achieved with the use of statins. However, doubts about the clinical benefit achievable with treatments enhancing plasma HDL-cholesterol concentrations have been raised by the premature termination of a large phase III trial with torcetrapib, the most potent and most developed HDL-cholesterol-raising compound, because of excess mortality in patients receiving the drug (11). The causes of torcetrapib failure are presently unknown and may be related to the drug mode of action, to off-target toxic effects of the drug, or to a mixture of these. Torcetrapib failure does not mean that the concept of targeting HDL in CVD prevention is definitely ruled out. Other HDL-cholesterol-raising therapies, which act through disparate molecular mechanisms, are in various stages of preclinical and clinical development.

The considerable interindividual variation observed in monitoring these therapies in terms of lipid response, cardiovascular event response, and adverse events is bringing considerable attention to the concept of more targeted therapies based on genetic information. Pharmacogenomics (pharmacogenetics) involves the search and identification of genetic variants that influence response to drug therapy. Over the past decade, some progress has been made in our understanding of the variability associated with statin therapy (12). However, the ethical challenges in conducting the necessary research, including concerns about vulnerability and issues of consent, especially in children (13), the scientific validity of the studies, and the larger policy question of priority setting are important limitations that need to be resolved before its clinical application.

Despite the efficacy of drug therapy to normalize plasma cholesterol concentrations and the demonstrated benefits on CVD events and mortality, it is important to emphasize that diet and behavioral modification should be the first line of defense against CVD, especially in children. Regarding the connection between diet and plasma cholesterol concentrations, during the first half of the 20th century several studies showed that serum cholesterol could be modified by the composition of dietary fat (14, 15), and studies by Keys et al (16) and Hegsted et al (17) provided the first quantitative estimates of the relative effects of the various classes of dietary fatty acids and the amount of cholesterol on serum cholesterol changes. Other predictive algorithms have been developed during the ensuing years, including predictions of response for LDL and HDL cholesterol (18–20). These relations between dietary changes and serum lipid changes are well founded and predictable for groups; however, a striking variability in the response of serum cholesterol to diet between subjects was reported as early as 1933 (21), and this variability has been the subject of multiple reports (19, 22–26). Studies in experimental animal models and in humans have shown that this variability in response to dietary manipulation has a significant genetic component (27–32). Such genetic variability could have a significant effect on the success of public health policies and individual therapeutic interventions. Moreover, it could be partially responsible for the apparent lack of definite endpoint benefits shown by many dietary studies aimed at decreasing CVD (33–36).

As indicated above, the success of CVD risk–reducing strategies has been traditionally measured on the basis of their effect on plasma lipids and, more specifically, on lipoprotein concentrations. Heritability estimates for blood lipids are high, including ≈40–60% for HDL cholesterol, ≈40–50% for LDL cholesterol, and ≈35–48% for triglycerides (37), and genetic variability at candidate genes involved in lipoprotein metabolism has been clearly associated with abnormal lipid metabolism and plasma lipoprotein profiles that may contribute to the pathogenesis of atherosclerosis; however, most of the studies focus primarily on white adult populations. Likewise, there is already copious literature about how variants at candidate genes involved in lipid metabolism may affect dietary response and potentially cardiovascular risk, but again, most of the information concentrates on adults with less information available on children and adolescents, who are at stages of life that may be optimal for effective, targeted primary prevention. This review synthesizes some of the information available on children with the major emphasis being placed on APOE, the gene for which more knowledge has been gathered.

APOLIPOPROTEIN E

Apolipoprotein E (apo E) in serum is associated with chylomicrons, VLDL cholesterol, and HDL cholesterol and serves as a ligand for the LDL receptor and the LDL receptor–related protein (38–41). When apo E deficiency is present, there is marked accumulation of cholesterol-enriched lipoproteins of a density <1.006 g/mL containing apo B-48 and apo A-IV, as well as apo B-100 (42). Moreover, in this disorder there is delayed clearance of both apo B-100 and apo B-48 within triacylglycerol-rich lipoprotein. Genetic variation at the APOE locus results from 3 common alleles in the population, E4, E3,
and E2, with frequencies in white populations of \( \approx 15\%, 77\% \), and \( 8\% \), respectively (41). Population studies, mostly in adult cohorts, have shown that plasma cholesterol, LDL-cholesterol, and apo B concentrations are highest in subjects carrying the APOE4 isoform, intermediate in those with the APOE3 isoform, and lowest in those with the APOE2 isoform (43, 44). It has been suggested that APOE allelic variation may account for \( \leq 7\% \) of the variation in total and LDL-cholesterol concentrations in the general population (45). This relation between LDL-cholesterol concentrations and APOE genetic variation is not independent of environmental and ethnic factors, and less is known about how these effects track from birth into adulthood.

The association of the APOE4 isoform with elevated serum cholesterol concentrations is greater in populations consuming diets rich in saturated fat and cholesterol than in other populations. These data indicate that the higher LDL-cholesterol concentrations observed in subjects carrying the APOE4 isoform are manifested primarily in the presence of an atherogenic diet characteristic of certain societies and that the response to dietary saturated fat and cholesterol may differ among individuals with different APOE phenotypes. Many studies have been conducted to prove this hypothesis (31). Some investigators reported greater plasma lipid responses in subjects carrying the APOE4 allele, whereas others failed to find significant associations between APOE genotype and plasma lipid response (31, 46). Important differences exist among these studies that could account for some of the discrepancies observed. Studies differed in subject sex, age, and baseline lipid concentrations, and all of these variables are known to play an important role in the variability of dietary response.

The APOE gene also has been implicated as one of the genetic factors modulating variability in postprandial lipemia response. The APOE2 isoform is considered to decrease remnant clearance because of impaired affinity for the receptors. Conversely, the APOE4 isoform should induce a faster clearance. However, studies that have compared postprandial triglyceride responses across different APOE genotypes have produced conflicting results, especially regarding the effects associated with the APOE4 allele (47–53). Postprandial response was examined at 4 and 8 h by Boerwinkle et al (54) in a sample of individuals taking part in the Atherosclerosis Risk in Communities Study, following a single high-fat meal containing vitamin A (used as a marker for intestinal lipoprotein synthesis). Postprandial plasma retinyl palmitate response was significantly different among APOE genotypes, with delayed clearance in subjects carrying the APOE2 allele, compared with E3/3 and E3/4 subjects; however, measurements of other lipid variables, such as triglyceride concentration and triglyceride-rich lipoprotein were not sensitive enough to detect these effects. Another study by Nikkilä et al (50), carried out in CVD cases and controls, showed that in CVD patients with the APOE2/3 phenotype, triglyceride concentrations were highest and still increasing after 7 h, reflecting delayed chylomicron remnant clearance. The same effect also was observed in normotriglyceridemic patients with non-insulin-dependent diabetes (55) and in nondiabetic normolipemic subjects (56), although, in this report, the delayed chylomicron remnant was observed only in E2/2 individuals.

The findings associated with the APOE4 allele have been more discordant. In an earlier report, heterozygosity for this allele was associated with lower lipemic response in relation to other phenotype groups (49); however, in another study the E4 allele was associated with prolonged postprandial responses of lipids and apolipoproteins in triacylglycerol-rich lipoprotein (57).

Several mechanisms have been proposed to explain these apo E–related differences in individual response to dietary therapy. Some studies have shown that intestinal cholesterol absorption is related to APOE phenotype, with APOE4 carriers absorbing more cholesterol than non-APOE4 carriers. Other mechanisms, such as different distribution of apo E on the lipoprotein fractions, LDL apo B production, bile acid and cholesterol synthesis, and postprandial lipoprotein clearance, may also be involved.

Other genetic variants within the APOE locus have been investigated in relation to the association with lipid phenotypes and response to dietary intervention. The current evidence suggests that variability in the APOE promoter region is associated independently of the traditional E2, E3, and E4 alleles with plasma, lipid concentrations (58), dietary response (59–61), and CVD risk (62).

Some of the recent findings in children and adolescents regarding the association of genetic variability at the APOE locus and plasma lipid concentrations are shown in Table 1 (63–84). In general, the data suggest that the influence of the APOE gene on plasma lipid concentrations manifests early in life, and it may track during the lifespan. Therefore, this genetic predisposition could be easily detected and the information used to implement early disease prevention with dietary and behavioral education and modification. However, several issues need to be considered before taking this apparently obvious path to disease prevention, the first one being ethical. APOE may be a marker for CVD risk and dietary response, but the association between APOE4 and Alzheimer’s (85) needs to be taken into consideration. Guidelines are needed before such approach can be put in place (86) because individuals being tested for one condition may not want to know their risk for another condition, especially when there is no successful therapy. The second issue is more physiologic and relates to the fact that APOE genetic variability may have significant prebirth effects, and some of these have been proven to be quite paradoxical. Thus, it has been shown that in a Scottish cohort of perinatal deaths (87), the APOE2 allele was over-represented as compared with healthy newborns, which suggests that this allele may be detrimental to pregnancy outcome. In contrast, the prevalence of APOE4 was higher in healthy liveborn infants compared with stillbirths and with adults. In addition, an intriguing cross-talk between the maternal and fetal alleles may affect lipoprotein metabolism in the mother through placental mechanisms (88). These investigators took advantage of the fact that placental proteins are encoded from the fetal genome and examined the associations between lipids of 525 pregnant women and the presence, in their newborns, of the APOE genetic polymorphism. After adjustment for maternal polymorphisms, newborn APOE2 (compared with APOE3/E3) was associated, again paradoxically, with higher maternal LDL cholesterol. This significant association was independent of the APOE alleles carried by the mothers and of lipid concentrations in newborns. Such findings support the active role of APOE in the metabolism of maternal lipoproteins and suggest that fetal genes may modulate the risk of problems related to maternal dyslipidemia (preeclampsia, pancreatitis, and future cardiovascular disease). Therefore, contrary to the negative implications of APOE4 later in life, it appears that the APOE2 allele may
TABLE 1
Genetic associations between the APOE locus and lipid-related traits in children

<table>
<thead>
<tr>
<th>No. of subjects (reference)</th>
<th>Population</th>
<th>Ages</th>
<th>Design</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>247 (63) White girls (Project HeartBeat!)</td>
<td>8–14 y at baseline</td>
<td>Mixed longitudinal</td>
<td>At baseline, mean TC values were E2/3 &lt; E3/3 &lt; E3/4 (P &lt; 0.001). The APOE effect on TC and LDL cholesterol and their change during adolescence is strong and may be modified by factors affecting growth, maturation, and reproductive function.</td>
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<tr>
<td>1520 (64) Bogalusa Heart Study</td>
<td>5–14 y at baseline</td>
<td>16-y follow-up</td>
<td>The E2 allele lowered the adulthood LDL-cholesterol concentration to a greater extent than the childhood concentration (P &lt; 0.05). The APOE locus not only influences the concentrations and tracking of certain lipoproteins from childhood to adulthood but also modulates the association between lifestyle-related factors (ie, waist-hip) and lipoproteins.</td>
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<tr>
<td>203 (65) Hungarian and Romani children</td>
<td>~4.3 y of age</td>
<td>Cross-sectional</td>
<td>APOE allele frequencies differed between Hungarian and Romani children as did the association with TC (E2 &lt; E3 &lt; E4 in Hungarians, not significant; and E3 &lt;&lt; E4 ~ E2, P &lt; 0.5 in Romanis).</td>
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<tr>
<td>515 (66) Columbia University Biomarkers Study</td>
<td>Mean age: 9.7 y</td>
<td>Cross-sectional</td>
<td>The APOE2 allele was associated with an antiatherogenic lipid pattern in children.</td>
<td></td>
</tr>
<tr>
<td>60 (67) Columbia University Biomarkers Study</td>
<td>Mean age: 14.0 y</td>
<td>Standardized fat load</td>
<td>APOE4 genotype was not associated with the postprandial triglycerides or retinyl palmitate responses.</td>
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<tr>
<td>36 (68) Special Turku Coronary Risk Factor Intervention Project</td>
<td>13-mo-old children</td>
<td>Intervention</td>
<td>APOE4 children may absorb cholesterol and plant sterols more effectively than the children with APOE 3/3 phenotype without compensatory reduction in endogenous synthesis of cholesterol.</td>
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<tr>
<td>63 (69) Familial hypercholesterolemia</td>
<td>8–17 y</td>
<td>Randomized, double-blind, placebo-controlled clinical trial with simvastatin</td>
<td>The contribution of APOE polymorphism and the dosage of simvastatin to LDL-cholesterol responsiveness is influenced by the nature of the LDLR gene mutation.</td>
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<tr>
<td>1062 (70) Special Turku Coronary Risk Factor Intervention Project</td>
<td>7-mo at baseline</td>
<td>Mixed longitudinal/ intervention</td>
<td>APOE strongly influenced tracking of non-HDL cholesterol and apo B values in early childhood, whereas dietary intervention had no effect on tracking of any of the lipids.</td>
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<tr>
<td>1255 (71) White schoolchildren</td>
<td>6–7 y old</td>
<td>Cross-sectional</td>
<td>The influence of APOE on TC, LDL cholesterol, and apo B concentrations is more evident in girls than in boys. This difference in effect is not due to sex hormones.</td>
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<tr>
<td>933 (72) White schoolchildren</td>
<td>6–8 y old</td>
<td>Cross-sectional</td>
<td>Interaction between APOE genotype and birth weight could be an important determinant of TC, LDL-cholesterol, and apo B concentrations and could influence atherosclerosis development later in life.</td>
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<tr>
<td>81 (73) Special Turku Coronary Risk Factor Intervention Project</td>
<td>6 y old</td>
<td>Randomized prospective trial</td>
<td>Plant stanol esters reduce serum cholesterol concentration in healthy children irrespective of their sex or APOE4 phenotype.</td>
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<tr>
<td>450 (74) Familial hypercholesterolemia</td>
<td>—</td>
<td>Cross-sectional</td>
<td>APOE4 allele was associated with lower HDL-cholesterol concentrations in an affected sib-pair analysis, which strongly suggests that APOE4 influences HDL-cholesterol concentrations in children with familial hypercholesterolemia. Moreover, the strong association suggests that APOE4 carries an additional disadvantage for children with familial hypercholesterolemia.</td>
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<tr>
<td>81 (75) Obese children</td>
<td>Mean age: 9.4 y</td>
<td>Case-control</td>
<td>APOE seems to influence some lipid profile abnormalities associated with obesity in childhood. However, clustering of risk factors and insulin resistance seem not to be dependent on APOE polymorphism.</td>
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<tr>
<td>257 (76) Birth cohort</td>
<td>6.7–8.1 y old</td>
<td>Cross-sectional</td>
<td>APOE and CETP loci have an additive and interactive influence on plasma lipid and lipoprotein concentrations in children.</td>
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<tr>
<td>348 (77) Vietnamese children living in urban and rural areas</td>
<td>7–9 y old</td>
<td>Cross-sectional</td>
<td>Plasma lipid profiles of APOE4 carriers may be a risk factor for atherogenesis in Vietnamese children having Westernized eating habits.</td>
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<tr>
<td>420 (78) Mexicans living in Mexico City</td>
<td>12–16 y old</td>
<td>Cross-sectional</td>
<td>Unlike previous studies in the adult Mexican population, these results in children show that lipid and lipoprotein concentrations are under the influence of APOE polymorphism. The APOE gene may be a genetic determinant for ischemic heart disease in relatively isolated populations.</td>
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(Continued)
be a marker of prenatal risk for the fetus and the mother. This hypothesis has been further supported by Corbo et al (89), who observed that in preindustrial populations, the highest fertility was associated with the APOE4 allele and by Wright et al (90) who showed that the APOE4 isoform may have advantages over those with the E2 or E3 isoforms with respect to early life neuronal/brain development. These findings suggest that some positive selection for increased performance early in life may compromise the ability to cope with the aging process (91). In addition, there is little information about gene-diet interactions in children from controlled intervention studies. However, a recent report has proposed intrauterine gene-diet interactions predisposing for early- and adult-life phenotypes (92).

**OTHER GENETIC RISK PREDICTORS**

As indicated earlier, cardiovascular disease is very polygenic, and there are many genes, in addition to APOE, involved in shaping that predisposition early in life. However, the amount of research devoted to early detection has been well below what is needed, mostly because of the uncertainties associated with predicting the risk of diseases that will manifest decades later. Nevertheless, several studies, such as the GENESIS (Growth, Exercise and Nutrition Epidemiologic Study In preSchoolers) study, have been conceived with this goal in mind. Associations between adiposity-related phenotypes and genetic variation in peroxisome proliferator–activated receptor γ (PPAR-γ; Pro12Ala and C1431T), as well as PPAR-δ (T+294C) were assessed in 2102 Greek children aged 1–6 y participating in this study (93), and the data revealed that the PPAR-γ Pro12Ala and C1431T polymorphisms were associated with increased adiposity during early childhood in a sex- and age-specific manner and independently of the PPAR-δ T+294C polymorphism.

The PPARγ locus was also investigated in a much smaller study in the context of eating behavior (94). This is a very important issue in the context of the current increases in worldwide obesity. Young children can regulate energy precisely in the short term, which suggests an innate compensation mechanism of eating behavior. However, this precise compensation appears to be attenuated as a function of increasing adiposity, parental feeding style, and age. Polymorphisms in PPARγ and β-adrenergic receptor (ADRB3) genes have been linked to increased body mass index (BMI), obesity, and, more recently, dietary nutrients and preferences. In addition, common variation in ADRB3 interacts with PPARγ to modulate adult body weight. Therefore, Cecil et al (94) investigated whether variants in these genes were associated with child eating behavior in 84 children aged 4–10 y and prospectively selected for variants of the PPARγ Pro12Ala and the ADIPOQ G276T single nucleotide polymorphisms with BMI, HDL cholesterol, and a lower TC/HDL cholesterol ratio compared with Pro/Ala. The PPARγ locus was also investigated in combination with the adiponectin (ADIPOQ) gene (97). The PPARγ Pro12Ala and the ADIPOQ G276T single nucleotide polymorphisms were genotyped in 285 obese children and adolescents. Moreover, the analyses were adjusted for APOE genotype. No associations were found for PPARγ or ADIPOQ polymorphisms with BMI, HDL cholesterol, triglycerides, or insulin resistance. Wild-type carriers of the PPARγ...
Pro12Ala homozygous carriers of the variant allele of ADIPOQ G276T and APOE4 carriers had higher total and LDL-cholesterol concentrations. Therefore, variant genes in candidate genes for insulin resistance were associated with cholesterol concentrations in obese children and adolescents and may offer additional information in the risk assessment of obese children.

Another potentially informative locus is the melanocortin-3 receptor in view of the central role of the melanocortin system in the long-term regulation of energy homeostasis. Santoro et al (98) screened the MC3R coding region in 184 obese girls and boys after 6 and 12 mo of a weight loss program. The C17A (Thr6Lys) and G241A (Val81Ile) were not associated with significant differences in baseline BMI; however, at follow-up, heterozygotes showed a significantly higher BMI. When these children were divided according to the amount of weight lost, a higher prevalence of heterozygotes was observed among subjects who lowered their BMI. Therefore, these results suggest a gene-diet interaction between the MC3R C17A and G241A variants and a weight loss program for the ability to lose weight in childhood obesity.

More recently, and following the current trends in adult populations, the search for genes involved in early risk markers in children has embraced the use of genome scans. Microsatellite markers were typed on 779 white and 444 black siblings participating in the Bogalusa Heart Study (99). These subjects had been examined serially 2–13 times over an average of 22 y from childhood to adulthood. The total and the incremental areas under the growth curves of lipid traits were calculated and used as measures for long-term concentrations and trends. After adjusting for age, sex, and BMI, heritability estimates of the total area values for all lipid variables were higher than those of a single measurement in either childhood or adulthood. In blacks, significant linkage to the low-density lipoprotein cholesterol (LDLC) incremental area was observed on chromosome 1, and suggestive linkage for total area of LDLC was observed on chromosome 19. One suggestive linkage on chromosome 2 was identified in whites for the LDLC incremental area. Other suggestive linkage was noted for LDLc and high-density lipoprotein cholesterol in terms of either total or incremental area on chromosomes 2, 5, 7, and 15 for blacks and whites. Several lipid-related candidate genes such as LDLR, LDL receptor–related proteins 3 and 8, APOE, APOA2, and APOC2 are located in these regions. Linkage evidence found in this community-based study indicates that regions on these chromosomes may harbor genetic loci affecting a predisposition to developing dyslipidemia in childhood. However, these findings need reinforcement from genomewide association studies and replication in large populations.

**SUMMARY**

The mechanisms involved in the regulation of plasma lipoprotein concentrations by dietary factors, such as intakes of cholesterol and fatty acids, have been partially elucidated during the past century through the research efforts of scores of investigators, and this knowledge has been translated into therapies to decrease cardiovascular disease. Genetics research has also brought up a number of candidate genes involved in the regulation of the homeostasis of blood lipids. Considering the significant effect of diet on plasma lipids, genetic variation at those loci is expected to explain some of the dramatic interindividual variations in lipoprotein response to dietary change that have been shown to exist among individuals. In fact, the current evidence supports the importance of gene-diet interactions in humans. However, because of conflicting results, more studies will be required to increase our predictive capacity and to reconcile the multiple discordances found in the literature.

At this regard, we have to keep in mind that lipoprotein response to dietary factors is extremely complex, as illustrated by the multiple interrelated pathways and genes discussed here. Therefore, the effects of individual gene variants can be difficult to identify. In fact, the concerted action of differences in gene families may be required to elicit significant interindividual differences in responsiveness to diet. This systems biology approach has been facilitated by the availability of the public information generated by the Human Genome Project and the HapMap (100).

We should also be cautious about the interpretation of studies of the association between allelic variants and common phenotypes. We should direct attention to the population admixture, which can cause an artificial association if a study includes genetically distinct subpopulations, one of which coincidentally displays a higher frequency of disease and allelic variants. Consideration of the ethnic backgrounds of subjects and the use of multiple, independent populations can help avoid this problem.

Another source of concern is multiple-hypothesis testing, which is aggravated by publication bias. Authors who test a single genetic variant for an association with a single phenotype base statistical thresholds for significance on a single hypothesis. But many laboratories search for associations using different variants. Each test represents an independent hypothesis, but only positive results are reported, leading to an overestimate of the significance of any positive associations. Statistical correction for multiple testing is possible, but the application of such thresholds results in loss of statistical power.

An additional caveat about the published literature relates to the fact that most studies were not initially designed to examine gene-diet interactions, but they are a reanalysis of previously obtained data using new information from genetic analysis carried out a posteriori. Future studies need to be carefully designed in terms of sample size, taking into consideration the frequencies of the alleles examined. Moreover, we do not really know the specific dietary factors responsible for most of the effects already reported. Therefore, baseline and intervention diets should be carefully controlled in terms of dietary cholesterol, individual fatty acids, concentrations of fat, as well as fiber and other minor components of the diet such as phytosterols. It is also important to emphasize that some allele effects may be apparent primarily during situations of metabolic stress, such as the postprandial state. Therefore, studies should be designed to test gene-diet interactions in both the fasting and the fed state. As indicated above, the most plausible scenario is that multiple genes will determine the response to dietary manipulation. Consequently, attention should be paid to gene-gene interactions. However, the large number of study subjects required and the subsequent costs involved may make such studies infeasible. Two alternatives for examining these complex interactions in humans are possible: the first selects study participants on the basis of their genetic variants and the second makes better use of the large cohort studies for which dietary information has been or will be collected. Both approaches combined will enable us to take the
concept of gene-diet interactions beyond the research laboratory into the real world. However, we need to keep in mind that most efforts to identify genetic markers of disease and gene-diet interaction are to provide early risk detection and to apply early behavioral changes that will facilitate disease prevention. In this regard, it is important to highlight that most of the information currently available in the literature has been generated by using adult cohorts and that the findings are extrapolated to childhood without adequate testing and confirmation of findings. However, we need to be careful about when and with whom we may implement more personalized prevention because the balance between risk and benefits may be different at different ages. In this regard, the “antagonistic pleiotropy” theory, based in population genetics, proposes that certain alleles that are favored because of beneficial early effects also have deleterious later effects (101). Therefore, we need to make sure that the proper studies are carried out in children to evaluate the balance between optimal early development and healthy aging for individual genomes.

Despite the current uncertainties and limitations, the concept of gene-environment interactions modulating common disease risk factors is well founded and in the future should provide the scientific knowledge to address the major health problems in the population by using molecular and more individually targeted approaches to disease prevention and therapy. (Other articles in this supplement to the Journal include references 102–109.) Therefore, we need to make sure that the proper studies are carried out in children to evaluate the balance between optimal early development and healthy aging for individual genomes.

The author had no conflicts of interest and no disclosures to declare.

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