Nutrigenomics in Cardiovascular Medicine

Dolores Corella, PhD; Jose M. Ordovas, PhD

Nutrigenomics represents a suitable approach to cardiovascular medicine, potentially enabling both, better prevention and treatment of cardiovascular diseases (CVD) through optimization of individuals’ dietary intakes. However, nutrigenomics is still developing its research methodology and learning from its achievements and its shortcomings. Its foundations have been laid, allowing us to validate its theoretical basis and, from there, to pursue research aimed to obtain a higher level of scientific evidence needed for its effective translation to clinical practice. This review discusses these aspects and summarizes the literature pertaining to gene-diet interactions related to intermediate and final CVD phenotypes.

Despite their multifactorial complexity, CVD have been the group of diseases in which most progress has been made on the knowledge of their genetic risk factors, both in identifying candidate genes through the classic approach based on the protein function and through the recent genome-wide association studies. Current knowledge regarding CVD genetic factors is summarized in several recent reviews.1–5 This has been possible due to the previous characterization of multiple intermediate phenotypes linked to those diseases, among which are plasma lipid concentrations, plasma glucose and related parameters, markers of inflammation and endothelial damage, oxidative stress, blood pressure, anthropometric measurements, and even phenotypes obtained by means of noninvasive imaging techniques such as measuring the intima-media thickness of artery walls.6,7 The relative ease of measuring these intermediate phenotypes and the specific understanding of them as risk factors has allowed many studies to be carried out aimed at identifying gene and genetic variants related to each of them and so obtaining a more detailed knowledge of the numerous genes involved in the final phenotypes of CVD (ie, coronary heart diseases, cerebrovascular diseases, and peripheral CVD).

Despite the spectacular advances made over recent decades in the discovery of genes and gene variants involved in the intermediate and final phenotypes of CVD, we still have a very incomplete knowledge of all genes and genetic variants that are providing such genetic susceptibility. Moreover, in that search for genetic susceptibility, the interaction with environmental factors must be taken into account. Therefore, a genetic variant will not always present a greater risk of disease, but its effects will be modified by the environmental factors (ie, tobacco smoking, physical activity, and dietary intake) that interact with it. Among the environmental factors, diet may be the most directly involved in the genetic modulation of the different intermediate and final phenotypes of CVD. However, we still need to find out how certain dietary components may modulate the risk conferred by genetic susceptibility due to variation in one or more genes involved in the etiology of CVD. This knowledge is not only crucial for contributing to better primary prevention of CVD but also for increasing the effectiveness of the treatment once the altered phenotypes have been diagnosed. Furthermore, from the Public Health point of view, the understanding of really important gene-diet modulations could help to profile the general dietary recommendations for each population.

Diet and CVD

It should be borne in mind that diet has traditionally been considered as one of the main risk factors in the etiology of CVD, a significant percentage of the increase of the incidence of these diseases being attributed to the harmful changes toward less healthy diets that has taken place in recent decades in different geographical areas.8,9 However, despite various initiatives carried out by different national and international organizations, focusing on making nutritional recommendations to the population to improve food intake patterns, success has not been forthcoming in reducing the risk of those diseases. There are many factors involved in this failure, among them, the inherent difficulties in changing behaviors, fashions, mass media pressure, sedentarism, deficient interventions in nutritional education, etc.10,11 What has also had an impact on the creation of a certain degree of skepticism among the general public is the absence of clear scientific evidence on which is the best diet to prevent or treat the different altered phenotypes that are involved in CVD.10 It must be admitted that often contradictory nutritional recommendations, based on the results of studies with little external validity and on different kinds of commercial interest, have been formulated over recent decades.12–15

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Therefore, an intense debate has been taking place on the best composition of macronutrients in the diet to prevent or treat CVD, especially as regards the percentage provided by total fat and its different fatty acids,\textsuperscript{16–21} and even on the origin of those fatty acids; as the monounsaturated fatty acids derived from olive oil as opposed to monounsaturated fatty acids derived from meat and other foods of animal origin.\textsuperscript{21,22}

Likewise, there is much controversy over the best origin and type (omega-6 and omega-3 series) of polyunsaturated fatty acids (PUFA)\textsuperscript{21,23} in preventing or treating CVD. Especially lively has been the debate on the virtues of a low-carbohydrate, high-fat diet as opposed to a low-fat, high-carbohydrate diet in controlling body weight, and related cardiovascular risk factors.\textsuperscript{24,25} Faced with this diversity of effects and recommendations, it is not surprising that various authors insist that there is no perfect diet,\textsuperscript{10} but this diet may vary depending on individual’s characteristics and the results that the individual wishes to achieve, and, hence, it is necessary to delve deeper into the study of these interindividual differences.\textsuperscript{26}

**Interindividual Differences in Response to Diet and Nutritional Genomics**

The different responses to diet depending on the particular characteristics of an individual is not a new observation but has already been widely documented in dozens of studies.\textsuperscript{27,28} For example in 1965, Keys et al,\textsuperscript{29} in their study on the effects of diet in plasma concentrations of cholesterol, stressed the dramatic differences between individuals, concluding that it was the “intrinsic characteristics” of the individual that motivated the different lipid responses to the same dietary intervention. Having admitted that each individual (or in practice, each group of individuals) may respond differently to the same diet, it becomes crucial to identify the factors defining such differential response. Among the many potential factors, genetic variability could play a significant role.\textsuperscript{30,31}

Accordingly, studies have been undertaken to determine whether genetic variants, mainly single-nucleotide polymorphisms (SNP), can explain those differences. These gene-diet modulations may also help to explain the different phenotypes observed for a given genotype, such as those observed in some monogenic forms of CVD.\textsuperscript{32,33} Thus, the same gene variant may be associated with wide spectrum of clinical manifestations, ranging from no symptoms at all to severe CVD. Therefore, in addition to potential epistasis, other nongenetic factors (among which we could emphasize diet) may be important in modifying the clinical phenotype, either by exacerbating or protecting against the diseased phenotype.

Nutritional genomics came into being at the beginning of the 1990s.\textsuperscript{31} The main goal was to gain knowledge about the interaction between dietary factors and the genome that modulate phenotypic expression. This knowledge could explain the genetic basis for the interindividual response to diet and the reasons for the different clinical phenotypes observed for the same genetic variant. This discipline has been gathering momentum for the last 2 decades to become a significant player in cardiovascular research. Table 1 shows the main reviews published on the subject, stressing their particular emphasis,\textsuperscript{26,31,33–59} such as basic concepts of the discipline; potential applications in different areas of disease prevention, and treatment; Public Health perspective; training needs for dieticians and their potential involvement; advantages and drawbacks of dietary individualization; consumers perceptions; the role of the food industry; and the ethical and legal aspects associated with this new field.

Research into nutritional genomics is a leading subject in numerous calls for national and international projects, and relevant research networks, such as the Nutrigenomics Organization, have been launched. The Nutrigenomics Organization is a European-funded Network of Excellence, the full title of which is “The European Nutrigenomics Organization: linking genomics, nutrition and health research,” which is carrying out cutting-edge research into all the omics related to nutrition and health as well as into the ethical aspects derived. In the rise of nutritional genomics, it is also important to stress the creation of numerous research institutes in various countries dedicated to this new discipline. Among them, for example are as follows: The Cornell Institute for Nutritional Genomics at Cornell University, Ithaca, NY; the Center of Excellence in Nutritional Genomics, Calif, which is funded by an award from the National Center for Minority Health and Health Disparities; or the recently launched Salk Center for Nutritional Genomics in La Jolla, Calif. Besides these institutes, programs or laboratories devoted to research into nutritional genomics have been created in most nutrition research centers. As investment and scientific training in this discipline has increased, so too has the number of scientific publications centered on that field and in an exponential way.

Nonetheless and despite the huge promises made in numerous articles on this subject, we need to underscore that nutritional genomics is a discipline still in its infancy, and more progress needs to be done before practical tools can be developed for the prevention and treatment of CVD. Given the novelty of this discipline, there is still some confusion about the delimitation of its concepts, as often the terms of nutritional genomics, nutrigenetics, and nutrigenomics, are used as synonyms. Nutritional genomics implies greater generalization referring to the joint study of nutrition and the genome including all the other omics derived from genomics: transcriptomics, proteomics, and metabolomics. The terms nutritional genomics would be equivalent to the wide ranging term of gene-diet interaction. Within the wide framework of the concept of nutritional genomics, we can distinguish 2 subconcepts: nutrigenetics and nutrigenomics. Currently, there is a wide consensus on considering nutrigenetics as the discipline that studies the different phenotypic response to diet depending on the genotype of each individual. The term nutrigenomics is subject to a greater variability in its delimitation, but it seems that there is a certain consensus in considering nutrigenomics as the discipline which studies the molecular mechanisms explaining the different phenotypic responses to diet depending on the genotype, studying how the nutrients regulate gene expression, how they affect polymorphisms in this regulation, and how these changes are interrelated with proteomics and metabolomics. However, despite this specific delimitation, there is also a widespread line of thought that uses the term nutrigenomics as equivalent
to nutritional genomics. This wider interpretation of the nutrigenomics concept is the one that we shall use in this review.

**The Present Application of Nutrigenomics in CVD**

Notwithstanding the numerous studies published in which statistically significant gene-diet interactions have been found determining different intermediate or clinical CVD outcomes (Table 2) or clinical CVD outcomes (Table 3), the main problem that nutrigenomics faces is the lack of replication of the initially reported gene-diet interactions. This precludes its present application in the prevention and treatment of CVD. Hence, it is now essential to increase the consistency of results by implementing gene-diet interaction replication studies in various populations. When the results are available it is also essential to carry out quantitative meta-analyses of the gene-diet interactions to check the homogeneity or heterogeneity of the effect and to obtain overall association measurements. It
Table 2. Selected Studies Showing Statistically Significant Gene-Diet Interactions in Determining Intermediate CVD Phenotypes

<table>
<thead>
<tr>
<th>Reference</th>
<th>Phenotype</th>
<th>Description of the Gene-Diet Interaction</th>
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<tbody>
<tr>
<td>Lopez-Miranda et al&lt;sup&gt;64&lt;/sup&gt;</td>
<td>Postprandial LDL-C</td>
<td>The −756/A APOA1 SNP influenced the postprandial LDL-C response to MUFA. After consumption of a high MUFA diet, significant increases in LDL-C were noted in carriers of the A allele but not in G/G subjects.</td>
</tr>
<tr>
<td>Jansen et al&lt;sup&gt;67&lt;/sup&gt;</td>
<td>Postprandial LDL-C</td>
<td>Postprandial LDL-C response to dietary fat is influenced by the 347Ser mutation of APOA4. Carriers of the 347Ser allele presented a greater decrease in LDL-C when they were switched from the SFA to the NCEP type 1 diet than homozygous the 347Thr allele.</td>
</tr>
<tr>
<td>D’Angelo et al&lt;sup&gt;68&lt;/sup&gt;</td>
<td>Plasma homocysteine</td>
<td>The C677T SNP in the MTHFR gene interacted with folate and vitamin B12 levels in determining plasma homocysteine concentrations.</td>
</tr>
<tr>
<td>Campos et al&lt;sup&gt;69&lt;/sup&gt;</td>
<td>VLDL and HDL-C</td>
<td>The APOE genotype interacted with saturated fat in determining VLDL and HDL-C concentrations (higher VLDL and lower HDL-C in E2 carriers with a high fat).</td>
</tr>
<tr>
<td>Luan et al&lt;sup&gt;70&lt;/sup&gt;</td>
<td>BMI and fasting insulin</td>
<td>An interaction was found between the PUFA:saturated fat ratio and the Pro12Ala PPAR&lt;sub&gt;G&lt;/sub&gt; polymorphism for both BMI and fasting insulin. With a low ratio, the BMI in Ala carriers was greater than that in Pro homozygotes, but when the dietary ratio was high, the opposite was seen.</td>
</tr>
<tr>
<td>Corella et al&lt;sup&gt;71&lt;/sup&gt;</td>
<td>Fasting plasma LDL-C</td>
<td>Alcohol intake interacted with the APOE SNP in determining LDL-C in men. In E2 subjects, LDL-C was significantly lower in drinkers than in nondrinkers but was significantly higher in drinkers than in nondrinkers in E4 subjects.</td>
</tr>
<tr>
<td>Leeson et al&lt;sup&gt;72&lt;/sup&gt;</td>
<td>Endothelium-dependent, flow-mediated brachial artery dilatation (FMD) and endothelium-independent dilatation response</td>
<td>An endothelial nitric oxide synthase (eNOS) SNP (Glu298Asp) interacted with dietary omega-3 in determining endothelial responses. Omega-3 was positively related to FMD in Asp298 carriers but not in Glu298 homozygotes.</td>
</tr>
<tr>
<td>Ordovas et al&lt;sup&gt;73&lt;/sup&gt;</td>
<td>HDL-C concentrations and HDL particle size</td>
<td>The −514C&gt;T LIPC polymorphism interacted with dietary fat in determining HDL-related measures. T allele was associated with significantly greater HDL-C concentrations and large HDL size only in subjects consuming &lt;~30% of energy from fat.</td>
</tr>
<tr>
<td>Brown et al&lt;sup&gt;74&lt;/sup&gt;</td>
<td>Total cholesterol and LDL-C</td>
<td>The T-455C, T-625del SNP in the APOC3 gene interacted with saturated fat intake in determining total cholesterol and LDL-C. In homozygotes for the APOC3–455T-625T alleles, saturated fat intake was associated with an increase in total and LDL-C. No association was found among carriers of the APOC3–455C-625del allele.</td>
</tr>
<tr>
<td>Memisoglu et al&lt;sup&gt;75&lt;/sup&gt;</td>
<td>Body mass index</td>
<td>The Pro12Ala SNP in the PPAR&lt;sub&gt;G&lt;/sub&gt; gene interacted with fat intake in determining BMI. Among Pro/Pro individuals, those in the highest quintile of total fat intake, had significantly higher BMI compared with those in the lowest quintile whereas among 12Ala carriers there was no association between dietary fat intake and BMI. In contrast, MUFAs intake was not associated with BMI among Pro/Pro women but was inversely associated with BMI among 12Ala carriers.</td>
</tr>
<tr>
<td>Dwyer et al&lt;sup&gt;76&lt;/sup&gt;</td>
<td>Carotid-artery intima-media thickness, and markers of inflammation</td>
<td>Increased dietary arachidonic acid significantly enhanced the apparent atherogenic effect of the 5-lipoxygenase genotype, whereas increased dietary intake of n-3 fatty acids blunted the effect, suggesting that omega-6 promote, whereas marine omega-3 inhibit, leukotriene-mediated inflammation.</td>
</tr>
<tr>
<td>Dedoussis et al&lt;sup&gt;77&lt;/sup&gt;</td>
<td>Plasma homocysteine</td>
<td>The Mediterranean diet score was not significantly associated with homocysteine concentrations. However, a gene-diet interaction with the C677T SNP in the MTHFR was found. Higher adherence to the Mediterranean diet was associated with reduced homocysteine concentrations in carriers of the T allele but not in those with the CC genotype.</td>
</tr>
<tr>
<td>Tai et al&lt;sup&gt;78&lt;/sup&gt;</td>
<td>Fasting triglycerides and apolipoprotein C-III (apoC-III)</td>
<td>The L162V polymorphism at the PPAR&lt;sub&gt;A&lt;/sub&gt; gene interacted with dietary PUFA intake in determining fasting triglycerides and apoC-III concentrations. The 162V allele was associated with greater TG and apoC-III concentrations only in subjects consuming a low-PUFA diet.</td>
</tr>
<tr>
<td>Zhang et al&lt;sup&gt;79&lt;/sup&gt;</td>
<td>Blood pressure levels and hypertension</td>
<td>The angiotensin I-converting enzyme insertion-deletion polymorphism (ACE I/D) interacted with dietary salt intake. In the ID+II genotype, hypertension was increased by high salt intake, whereas in the DD genotype it was not. The interaction was more prominent in the overweight group.</td>
</tr>
<tr>
<td>Corella et al&lt;sup&gt;80&lt;/sup&gt;</td>
<td>Insulin resistance</td>
<td>The PLIN 11482G-&gt;A/14995A-&gt;T polymorphisms (in high linkage disequilibrium) interacted with saturated fat and carbohydrates in determining HOMA-IR in Asian women. These interactions were in opposite directions. Women in the highest SFA tertile had higher HOMA-IR than women in the lowest only if they were homozygotes for the PLIN minor allele.</td>
</tr>
<tr>
<td>Robitaille et al&lt;sup&gt;81&lt;/sup&gt;</td>
<td>Plasma lipid, blood pressure, waist circumference</td>
<td>64 SNP were studied. The Pro12Ala polymorphism in the PPAR&lt;sub&gt;A&lt;/sub&gt;, interacted with fat intake in determining waist circumference. The APOE genotype in interaction with fat intake determined diastolic and systolic blood pressure. The ghrelin Leu72Met polymorphism also interacted with dietary fat in its relation to waist circumference and triglycerides.</td>
</tr>
<tr>
<td>Corella et al&lt;sup&gt;82&lt;/sup&gt;</td>
<td>Body mass index and obesity</td>
<td>The −1131T&gt;C APOA5 SNP interacted with fat intake in determining BMI and obesity risk. In subjects homozygous for the −1131T major allele, BMI increased as total fat intake increased. Conversely, this increase was not present in carriers of the −1131C minor allele.</td>
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**Table 2. Continued**

<table>
<thead>
<tr>
<th>Reference</th>
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<th>Description of the Gene-Diet Interaction</th>
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<tbody>
<tr>
<td>Sofi et al97</td>
<td>Plasma lipoprotein (Lp) (a)</td>
<td>The LPA 93C-&gt;T SNP interacted with dietary fish intake in determining Lp(a) concentrations. The decreasing effect of fish consumption on Lp(a) concentrations was higher in TT subjects.</td>
</tr>
<tr>
<td>Pérez-Martínez et al98*</td>
<td>Plasminogen activator inhibitor type 1 concentrations</td>
<td>The plasminogen Activator Inhibitor Type 1 (PAI-1) – 675 4G/5G polymorphism interacted with dietary saturated fat in determining PAI-1 concentrations. Carriers of the 4G allele showed a decrease in PAI-1 concentrations after the MUFA diet, compared with the SFA-rich and carbohydrate-rich diets.</td>
</tr>
<tr>
<td>Norat et al99</td>
<td>Blood pressure</td>
<td>The M235T polymorphism in the AGT gene interacted with dietary salt intake in determining blood pressure. The regression coefficient for systolic blood pressure associated with each unit of sodium for each of the MT and TT genotypes was approximately double that for the MM homozygotes.</td>
</tr>
<tr>
<td>Nettleton et al80</td>
<td>Plasma HDL-C</td>
<td>A common SNP in the angiopoietin-like 4 gene (ANGPTL4[E40K]) interacted with carbohydrates in determining plasma HDL-C concentrations. In men, the inverse association between carbohydrate and HDL-C was stronger in A allele carriers than noncarriers.</td>
</tr>
<tr>
<td>Kanoni et al81</td>
<td>Plasma zinc, interleukin-6 (IL-6) and interleukin-8 (IL-8)</td>
<td>The −174G/C polymorphism in the IL-6 gene interacted with a dietary zinc score in determining IL-6 concentrations. A higher zinc score was associated with significantly higher plasma IL-6 levels in GG individuals.</td>
</tr>
</tbody>
</table>

*Intervention study in which short-term diets or specific components have been evaluated. In the other studies habitual dietary intakes were analyzed.

is also crucial to increase the evidence level of the replicated gene-diet interactions found in observational studies by undertaking nutritional intervention studies.

**Major Methodological Considerations in Studies of Gene-Diet Interactions**

To increase the validity of individual studies in nutrigenomics is critical to control the potential information and selection bias that may contribute to hinder replication. In experimental studies, these potential biases are minimized. However, the difficulty of conducting dietary intervention studies in large samples is a current limitation in nutrigenomics. In observational studies (ie, cohort, case-control, cross-sectional), the researcher does not provide the diet and has to gather nutritional information from dietary questionnaires. Therefore, high-quality dietary information in these epidemiological studies is crucial for minimizing information bias. Traditional dietary instruments (ie, diet records, 24-hour recalls, food-frequency questionnaires) and specific biomarkers of intake should be improved and tailored to the specific gene-diet interaction measured. In addition, it is still unclear what kind dietary information is more relevant in nutrigenomics. Should we be using nutrients, foods, or dietary patterns? There may not be a single solution and may depend on the specific aims and hypothesis being investigated. More information related to these methodological issues can be found in some previous reviews.31,33

Bearing in mind the difficulty of measuring diet in observational studies, it would be necessary to standardize the design of new studies in the field of cardiovascular genomics to clearly define the variables to be measured, both for diet and for potential confounding variables (ie, sex, age, tobacco smoking, physical activity, education, obesity, and ethnicity or genetic admixture).

To palliate some of the limitations of classical methods to measure dietary intake, several efforts are currently in place. Some of them focus on standardization of data acquisition across studies. Along these lines, the PHENX85 (consensus measures for phenotypes and exposures) project (https://www.phenx.org/) has been launched by the National Human Genome Research Institute to facilitate the integration of genetics and epidemiological research, which aims to standardized measurement protocols for inclusion in genome-wide association studies and other large-scale genomic studies to enable valid cross-study comparisons and analyses. In addition, other efforts aim to improving existing instruments to more precisely evaluate food intake including the use of new information and communication technologies.86

The clinical characteristics of the populations would also have to be controlled and stratified appropriately: general population, diabetics, dyslipemics, subjects with hypertension, or CVD must be clearly differentiated. Likewise, standardization should also include cut-off points for food intake and/or dietary components. Without this previous standardization, replication of gene-diet interactions will be very difficult to achieve. Although, this seems to be a minor problem, we cannot forget that nutrigenomic studies also require a good quality control and validity of the genetic determinations.

**Consistency of the Results Obtained From Association Studies in Cardiovascular Genomics and Gene-Diet Interactions**

The same consistency demanded of nutrigenomics studies is now being required of association studies between gene variants and intermediate and final CVD phenotypes. Replication of initial findings in various populations is now the most commonly used and demanded tool to demonstrate the validity of genome-wide association studies results. In addition, a number of meta-analyses are appearing that evaluate the effect of one or more genetic variants on the final and intermediate phenotypes of CVD. The recently published meta-analyses on the effects of the classic APOE and CETP polymorphisms in plasma lipids and CVD risk97,98 are some examples of this trend. The first of these included 86 067 individuals for the intermediate phenotypes and 37 850 cases and 82 727 controls for the disease phenotypes. The second of these included 113 833 individuals for the intermediate phenotypes and 27 196 coronary cases and 55 338 controls for the final phenotypes. In both, it was concluded that there
was a very consistent effect of each of the polymorphisms on plasma lipid concentrations (mainly high-density lipoprotein cholesterol [HDL-C] in the case of CETP, and low-density lipoprotein cholesterol [LDL-C] in the case of APOE). None of these meta-analyses evaluated possible gene-diet interactions but both concluded that certain heterogeneity between the studies could be observed. Gene-diet interactions could be one of the causes of heterogeneity observed in the studies involving different populations. In this case, the same genotype may have different effects on intermediate phenotypes or clinical outcomes depending on dietary intakes.

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<tr>
<td>Fumeron et al(^82)</td>
<td>MI</td>
<td>Alcohol consumption interacted with the CETP-TaqIB SNP in determining MI. The increasing effect of the B2 allele on plasma HDL-C was absent in subjects drinking (&lt;25) g/d of alcohol but increased commensurably, with higher values of alcohol consumption. Accordingly, the OR for MI of B2B2 subjects decreased from 1 in nondrinkers to 0.34 in those drinking 75 g/d or more.</td>
</tr>
<tr>
<td>Markus et al(^83)</td>
<td>Ischemic cerebrovascular disease</td>
<td>The C677T SNP in the MTHFR gene interacted with serum folate concentrations in determining the risk of ischemic stroke. The TT genotype was a risk when serum folate was low.</td>
</tr>
<tr>
<td>Yoo and Park(^84)</td>
<td>MI</td>
<td>Alcohol consumption interacted with the alcohol dehydrogenase type 3 (ADH3) SNP in determining MI. Moderate drinkers who are homozygous for the slow-oxidizing ADH3 allele (gamma2) have higher HDL levels and a substantially decreased risk of MI.</td>
</tr>
<tr>
<td>Hines et al(^85)</td>
<td>MI</td>
<td>Alcohol consumption interacted with the alcohol dehydrogenase type 3 (ADH3) SNP in determining MI. Moderate drinkers who are homozygous for the slow-oxidizing ADH3 allele (gamma2) have higher HDL levels and a substantially decreased risk of MI.</td>
</tr>
<tr>
<td>Yatos and Lucock(^86)</td>
<td>Thrombotic event</td>
<td>The C677T SNP in the MTHFR gene interacted with B-vitamin nutritional in determining homocysteine levels and risk for a thrombotic event.</td>
</tr>
<tr>
<td>Younis et al(^87)</td>
<td>MI</td>
<td>Significant alcohol-ADH3 genotype interaction on coronary heart disease risk was observed, with gamma2 homozygotes, who were modest drinkers, displaying 78% risk reduction compared to y1 homozygotes.</td>
</tr>
<tr>
<td>Cornelis et al(^88)</td>
<td>MI</td>
<td>A cytochrome P450 1A2 (CYP1A2) polymorphism interacted with coffee intake in determining MI risk. Individuals who are homozygous for the CYP1A2<em>1A allele are “rapid” caffeine metabolizers, whereas carriers of the variant CYP1A2</em>1F are “slow” caffeine metabolizers. Intake of coffee was associated with an increased risk of nonfatal MI only among individuals with slow caffeine metabolism.</td>
</tr>
<tr>
<td>Volck et al(^89)</td>
<td>MI</td>
<td>The PON1 polymorphism interacted with heavy alcohol intake in determining coronary heart disease in black men.</td>
</tr>
<tr>
<td>Yang et al(^90)</td>
<td>MI</td>
<td>The APOE SNP interacted with saturated fat intake in determining MI. E2 and E4 gene variants increase susceptibility to MI in the presence of high-saturated fat diet.</td>
</tr>
<tr>
<td>Ruiz-Narváez et al(^91)</td>
<td>MI</td>
<td>The Pro12Ala PPARG polymorphism interacted with PUFA intake to affect the risk of MI. The protective effect of PUFA intake on MI is attenuated in carriers of the Ala12 allele of PPARG.</td>
</tr>
<tr>
<td>Cornelis et al(^92)</td>
<td>Coronary heart disease</td>
<td>Cruciferous vegetables are a major dietary source of isothiocyanates. Isothiocyanates induce glutathione S-transferases (GSTs), polymorphic genes. The GST genotypes modified the association between cruciferous vegetable intake and the risk of MI. Compared with the lowest tertile of cruciferous vegetable intake, the highest tertile was associated with a lower risk of MI among persons with the functional GSTT1<em>1 allele but not among those with the GSTT1</em>0*0 genotype.</td>
</tr>
<tr>
<td>Allayee et al(^93)</td>
<td>MI</td>
<td>A 5-Lipoxygenase (5-LO) SNP interacted with dietary arachidonic acid (AA) in determining MI. The variant alleles were associated with greater risk of MI in the context of a high-AA diet.</td>
</tr>
<tr>
<td>Jensen et al(^94)</td>
<td>MI</td>
<td>The CETP-TaqIB SNP interacted with alcohol consumption in determining MI risk. Alcohol consumption was associated with lower risk in carriers of the B2 alleles.</td>
</tr>
</tbody>
</table>

All of these were observational studies and habitual dietary intake was analyzed.

Based on results from initial studies, there is support for the notion that APOE locus interacts with dietary saturated fat, increasing LDL-C concentrations, and CVD risk, more in E4 carriers than in others (E2 and E3). However, the evidence level of this interaction in epidemiological studies is low.\(^63,90,99\) Likewise, experimental evidence from animal studies or from gene expression studies is mostly lacking. Therefore, despite the numerous studies carried out, there is currently insufficient epidemiological evidence available to be able to introduce a dietary recommendation on fat consumption based on the APOE genotype. Most of the disturb-
ing heterogeneity in the results of those studies may have its origin in their low statistical power, the lack of accurate measurements of dietary fats, confusion by other gene variants, confusion by other components of the diet such as alcohol or antioxidants, and other selection or information biases. Until all these factors are adequately controlled, it will not be possible to have more definitive conclusions about the presence or not of a gene-diet interaction between the APOE genotype and fat. Therefore, building enough evidence to make dietary recommendations related to total fat intake, types of fatty acids, or dietary patterns (ie, Mediterranean diet) based on APOE genotypes will require new, properly designed, and standardized studies that can be replicated in different populations. The very thought that we have so far been unable to establish a gene-diet interaction that began to be studied more than 10 years is quite remarkable. A significant amount of the blame for this situation is attributable to publication fashions. Nowadays, we have reached the time in which much better designed studies can be undertaken to conclusively characterize those interactions that we have been pursuing since the early 1990s, such as those related to the APOE genotype, dietary fat, and LDL-C. However, it is also true that investigating that interaction is no longer attractive in terms of innovation, and researchers prefer to focus on the recently discovered gene variants that make their research work seem more novel and gives them a greater chance of being published in high-impact journals. However, progress in nutrigenomics needs to come from researching novel gene variants and from revisiting classic interactions to clearly establish and to adequate scientific evidence level whether those gene-diet interactions do occur and to establish their magnitude and relevance.

Similar situation exists for the CETP locus. Initial studies reported a statistically significant interaction between CETP polymorphism and alcohol consumption in determining HDL-C concentrations and CVD risk. However, its current evidence level, as in the case of APOE-fat interaction, is very low. The first report was that of the Fumeron et al\textsuperscript{102} in French men (608 myocardial infarction [MI] patients and 724 controls). In that study, the increasing HDL-C effect of the B2 allele was absent in subjects drinking <25 g/d of alcohol but increased considerable with higher values of habitual alcohol consumption (interaction: \(P<0.001\)). Accordingly, the risk of MI was lower in B2 subjects who had a high alcohol consumption level. Following this observation, there have been differing results published on that interaction.\textsuperscript{94,100} Thus, the Framingham Study did not support such interaction\textsuperscript{103} nor was confirmed by a meta-analysis of Boekholdt et al\textsuperscript{102} in \(\approx 13,000\) individuals.

The APOE locus has also been shown to have statistically significant interactions with habitual alcohol consumption in determining plasma LDL-C\textsuperscript{65} and HDL-C.\textsuperscript{103} Although consistency in studies has not been obtained, there is some evidence showing that moderate habitual consumption of alcohol could be beneficial for E2 carriers by significantly reducing their LDL-C plasma concentrations.\textsuperscript{65} However, for E4 carriers, alcohol consumption could be harmful for 2 reasons. First, alcohol drinking raises their LDL-C concentrations, and second, the expected HDL-C raising effect of alcohol is not seen in E4 carriers.\textsuperscript{103}

Similarly, a statistically significant gene-diet interaction has been published for the CETP locus and habitual dietary fat intake in determining HDL-C.\textsuperscript{104} However, there are hardly any studies that have later examined these interactions and the single one that has done so, did not replicate the findings.\textsuperscript{105}

Taking into consideration the significant contributions of these candidate genes to the variability in lipid levels, it will be highly important to revisit these interactions using more powerful approaches than those available during the last 2 decades, in addition to continue the exploration of newly discovered genes, that generally have less impact over the variability of the lipid traits at the population level.

**Gene-Diet Interactions That Do Not Mask the Genetic Associations**

An example of a gene-diet interaction that modulates the effects of a genetic polymorphism on an intermediate CVD phenotype when maintaining a statistically significant association between the SNP and the phenotype, is the case of the interaction between the \(−1131T>C\) SNP in the APOA5 gene promoter and PUFA intake.\textsuperscript{106} This SNP has generally being associated with greater triglyceride concentrations in carriers of the C allele. In the Framingham study, the significant association between the \(−1131T>C\) SNP and fasting triglyceride concentrations was observed in the population as a whole. However, when we examined the potential contribution of the habitual dietary intake on the modulation of that association, we found a statistically significant interaction between this SNP and PUFA intake. We dichotomized dietary PUFA according to the population mean (\(\approx 6\%\)). After multivariate control for potential confounders, we found a statistically significant interaction (\(P<0.001\)) between SNP \(−1131T>C\) and PUFA intake (\(>6\% \text{ or } <6\% \text{ of energy}\)) on triglyceride concentration. In the adjusted model, the \(−1131C\) allele was associated with an increase in fasting triglycerides (21%, \(P=0.002\)) only in subjects consuming \(>6\%\) of energy from PUFA. However, mean fasting triglyceride concentrations were similar in carriers of the \(−1131C\) allele and TT homozygotes when PUFA consumption was low (\(P=0.600\)). We observed similar and significant interactions between PUFA consumption and SNP \(−1131T>C\) on remnant-like particles-triglycerides (\(P<0.001\)) and remnant-like particles-cholesterol (\(P<0.001\)). As observed for fasting triglycerides, remnant-like particles-triglycerides concentrations were higher (34%, \(P=0.005\)) in subjects carrying the \(−1131C\) allele when they consumed \(>6\%\) of energy from PUFAs. More in depth analyses of the dietary data brought up the fact that only omega-6, but not omega-3, PUFAs were associated with statistically significant increases of triglycerides in C-allele carriers.

A more recent example of how a gene-diet interaction can also be present, despite statistically significant effects being observed for the genotype-phenotype association, may be the case of TCF7L2 (transcription factor 7 like 2) gene. Genetic variation at this locus has consistently been associated with higher fasting glucose concentrations and higher diabetes risk...
in carriers of the variant allele.\textsuperscript{107} Despite that, recent studies have found an interaction between some of these variants and habitual dietary intake of carbohydrates in determining the above-mentioned phenotypes.\textsuperscript{108,109} Hence, in a case-control study carried out in the Nurses’ Health Study,\textsuperscript{108} it was found that carbohydrate quality and quantity modified the risk of diabetes associated with \textit{TCF7L2} (rs12255372 G-to-T). Authors used the dietary glycemic load as an indicator of both carbohydrate quality and quantity, and observed that the risk of diabetes associated with the \textit{TCF7L2} TT genotype was greater among individuals consuming a high-glycemic load diet. Compared with the GG genotype, multivariate-adjusted odds ratio (95\% CI) of diabetes associated with the TT genotype were 2.71 (1.64 to 4.47) among individuals in the highest tertile of glycemic load. Corresponding odds ratio among individuals in the lowest tertiles of GL was 1.66 (0.95 to 2.88). Similar results were obtained on considering the glycemic index. From these results, the authors concluded that the greater diabetes risk attributable to the \textit{TCF7L2} T variant was magnified under conditions of increased insulin demand. Despite the potential preventive interest that this gene-diet interaction could have, more studies to replicate that interaction are required before applying these results to clinical practice. This need is supported by the fact that another study reported in the European Prospective Investigation into Cancer and Nutrition-Potsdam Cohort\textsuperscript{109} has also found a statistically significant gene-diet interaction between the rs7903146 C-to-T (in high LDL with the rs12255372 G-to-T) in the \textit{TCF7L2} gene and habitual whole-grain intake in determining diabetes risk; however, the results apparently contradict the findings of Cornelis et al.\textsuperscript{108} In the EPIC Potsdam Cohort, the carriers of the \textit{TCF7L2} variant allele do not present the protective effect of whole grain intake in diabetes risk, which in this case was observed in CC homozygotes.

Once these issues are resolved, the knowledge of gene-diet interactions in cardiovascular genomics could be used to correct altered phenotypes (high fasting glucose and high triglycerides, among others), that are associated with a certain genotype. This could in the near future be included in guidelines and recommendations for clinical practice for those patients with genetic susceptibility and inappropriate dietary behavior. However, as we have reiterated throughout this review, it is essential to increase their level of consistency and scientific evidence by means of further studies.

**Can Gene-Diet Interactions be Responsible for Lack of Association Between Genetic Variants and CVD Intermediate or Final Phenotypes?**

In the case of above-mentioned polymorphisms (\textit{APOE}, \textit{CETP}, \textit{APOA5}, \textit{TCF7L2}), a genetic effect of their genotypes on plasma lipid concentrations (higher LDL-C concentrations in E4 allele carriers; higher HDL-C concentrations in B2 allele carriers; higher TG concentrations in \textit{APOA5} carriers and higher fasting glucose concentrations and diabetes risk in carriers of the variant allele in the \textit{TCF7L2} gene, all of these compared with homozygote subjects for the most common allele) has been widely described. In this situation, diet would act by slightly modulating the effect of these alleles, allowing us to observe genetics effects on a global level.

Nevertheless, not all gene-diet interactions are of this type. Some of them may occur in situations in which a global genetic effect is not even observed. This would involve such a powerful gene-diet interaction that the environmental modulation would neutralize the genetic effects, giving rise to an observed null genetic effect in the population as a whole due to combination of different environmental exposures with opposite effects.

A classic example to illustrate this situation could be the common \textit{–75G>A APOAI} polymorphism and its association with HDL-C concentrations. In the Framingham Study,\textsuperscript{110} this polymorphism was not associated either with HDL-C concentrations in the population as a whole. However, in women, a statistically significant interaction was found with habitual PUFA intake as a continuous variable and \textit{APOAI} genotype (\textit{P}=0.005). Thus, when PUFA intake was low (<4\% of energy), G/G subjects had higher HDL-C concentrations than did carriers of the A allele (\textit{P}<0.05). Conversely, when PUFA intake was high (>8\% energy), HDL-C in carriers of the A allele were higher than those of G/G subjects (\textit{P}<0.05).

Another example of this situation is illustrated by the 5-lipoxygenase (5-LO) locus, food intake, and MI\textsuperscript{93} in a Costa Rican case-control study. In this case, a polymorphism in the 5-LO locus was not associated with MI in the population as a whole but was differentially associated depending on the strata of food intake. The 5-LO pathway, which generates leukotrienes from arachidonic acid (AA), has drawn much attention for its role in CVD. 5-LO catalyzes the rate-limiting step of the biosynthesis of proinflammatory leukotrienes from AA and has been associated with atherosclerosis. It was hypothesized that a 5-LO promoter polymorphism consisting of tandem Sp1-binding sites could be associated with greater carotid atherosclerosis and then to higher risk of MI. However, when the population as a whole (1885 cases and 1885 paired controls) was studied, no statistically significant association between the 5-LO promoter polymorphism and MI was found. However, when the amount of habitual dietary intake of AA was considered (low and high AA intake), a statistically significant gene-diet interaction was found. Thus, relative to the common 5 repeat promoter allele, the 3 and 4 alleles were associated with a higher MI risk in the high (≥0.25 g/d) dietary AA group (odds ratio, 1.31; 95\% CI, 1.07 to 1.61) and with a lower risk in the low (<0.25 g/d) AA group (0.77; 0.63 to 0.94; \textit{P} for interaction=0.015). These results support the hypothesis that the effect of the 5-LO promoter repeat polymorphism on higher atherosclerosis risk is exacerbated by high dietary AA but decreased by a low intake of AA.

Taking into account that gene-diet interactions can hide associations of certain genetic polymorphisms on intermediate or final CVD phenotypes, effort should be placed into the reanalysis of data for which no significant associations were found between specific genetic variants and CVD-related phenotypes. For example, most genome-wide association studies have not been considering the effects of dietary factors and they should be reanalyzed (if valid data on food...
Some Relevant Gene-Diet Interactions May Not Be Statistically Significant

Despite current interest for gene-diet interactions, the essential issue of defining and detecting gene-diet interactions remains vague, and the concept of gene-environment interactions is repeatedly used but rarely specified with precision.\textsuperscript{111,112}

In recent years, published gene-diet interactions have largely focused on those that are statistically significant,\textsuperscript{113} meaning that statistical regression models including interaction terms are adjusted and the statistical significance of the interaction term between the dietary exposure and the corresponding genetic variable is estimated. If that interaction term is statistically significant ($P<0.05$), it is concluded that a gene-diet interaction exists. These interactions can include both continuous and categorical variables and predict both dichotomous and continuous outcomes.\textsuperscript{111–113} For example, statistical interaction exists if the degree or direction of the effect of one dietary factor (eg, dietary fiber) differs according to values of a second factor (gene variants). In a simple gene-diet interaction model, in which both the susceptibility genotype at a single locus and the dietary exposure are considered dichotomous in predicting a dichotomous outcome (eg, hypertension), one can fit a logistic regression model incorporating genetic and environmental factors in studying the risk of disease. The statistical interaction of these 2 factors can be measured as a departure from a multiplicative regression model of disease risk.

In addition, it is common to predict continuous variables, such as blood pressure, body mass index, fasting glucose, plasma lipid concentrations, and to analyze continuous variables to measure dietary intake. Then, covariance regression models with interaction terms are commonly used. An example of this is the situations that we mentioned above in Figure 1A and 1B.

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A genetic susceptibility without the interaction term in the mathematical model being statistically significant. Generally speaking, by genetic determinism we mean that situation in which the genotype completely determines the phenotype, that is, the genes alone determine human traits.\textsuperscript{112,116} Assuming then a pure genetic determinism, the intermediate and final CVD phenotypes would only be determined by genetic variability, without the environment being important. In this situation, if a subject had a genetic alteration, such genetic susceptibility would irreversibly determine the CVD phenotype, and that genetic influence would be totally independent of diet. Although it is not well quantified, this genetic determinism situation is thought to be very unusual, the normal situation being that of the modulation of phenotypes by environmental factors. Therefore, it is said that a biological gene-environment interaction situation takes place when there is an environmental factor capable of modifying genetic susceptibility. In other words, a biological gene-environment interaction occurs when a genetic risk factor leads to disorder only under certain environmental conditions or when adverse environmental conditions lead to disorder only for those individuals at genetic risk.\textsuperscript{112} This is not a new concept, it dates back at least to the beginning of the 20th century, before the discovery of DNA. It was Sir Archibald Garrod who in a 1902 landmark article suggested a genetic basis for differences in chemical metabolism and noted that these differences “will readily be masked by the influences of diet.” This concept of biological gene-environment interaction is independent of the statistical significance. In some cases, there are biological gene-environment interactions for which the interaction terms between diet and the genotype are not statistically significant; whereas for others a statistically significant interaction is detected.

To better understand this, let us consider the genetic determinism and the biological gene-environment interaction scenarios (Figure 2). In Figure 2A, subjects carrying the mutant allele have higher plasma concentrations of the “Y” parameter (always in the pathological level) than normal subjects, independently of the dietary intake. The probability value for the dietary effect (\(P_d\)) in subjects carrying the mutant allele is not statistically significant. Figure 2B shows a biological gene-diet interaction for which the probability value for the gene-diet interaction term does not reach the statistical significance. However, depending on the diet, the plasma concentration of the Y parameter in subjects carrying the mutant allele is statistically different, ranging from nonpathological to pathological concentrations (\(P_d<0.05\)). Although the interaction term does not reach the statistical significance, statistical significant differences across dietary strata in genetically susceptible subjects must have to be demonstrated in biological interactions. Figure 2C shows 2 common situations of biological gene-diet interactions that also have statistically significant interaction terms between diet and the genotype.

To use a more specific example, let us think about a gene variant, which is associated with higher LDL-C concentrations and subsequent greater risk of MI. However, this situation is not deterministic and can be modulated by the intake of dietary saturated fat, in such a way that a low-saturated fat intake would neutralize the genetic effect in the subjects carrying the mutant allele (\(P<0.05\)), by preventing the increase of their LDL-C concentrations, thus reducing the risk of subsequent MI. This gene-diet interaction does not necessarily have to reach statistically significance, as a lower fat intake can also simultaneously reduce LDL-C concentrations in individuals without the risk genotype. This interaction is a “biological interaction.” It is important to identify biological interactions because doing so provides opportunities for the prevention and treatment of phenotypic alterations in people who are in a situation of greater risk due to their
genotype. That is to say, in this case a lower saturated fat intake would reduce LDL-C concentrations both in subjects with the risk gene variant and in those without. However, nutritional education and motivation efforts for a low-fat intake could focus more on those persons in whom there is a greater risk due to the genotype.

Therefore, biological interaction exists when 2 or more factors influence a phenotype at the same time, but it does not necessarily entail a statistical interaction. Statistical interaction concerns the modeling of combined effects of 2 or more risk factors for a disease.

To better understand this concept; let us illustrate it with 2 real examples of biological and nonstatistically significant gene-diet interaction. The first of these involves the CETP-TaqIB polymorphism and alcohol consumption determining plasma HDL-C concentrations (Figure 3). In the Framingham study, we observed that B1B1 individuals had lower HDL-C concentrations compared with B1B2 and B2B2 individuals.\(^{102}\) Later, we investigated whether there was a gene-diet interaction of this SNP with alcohol consumption.\(^{102}\) Three categories of alcohol intake were considered according to reported daily intake: no alcohol intake, moderate, and high intake. No statistically significant interaction between the CETP-TaqIB genotype and alcohol intake was found \((P=0.7).\)\(^{33}\) However, as depicted in Figure 3A, a biological gene-diet interaction can be observed. If genetically the B1B1 subjects are predisposed to having lower HDL-C and this fact implies that they may present higher CVD risk, we would have to ask if there were some environmental factor (in this case diet) that could make the HDL-C concentrations in B1B1 subjects increase. Figure 3B displays the data for B1B1 extracted from Figure 3A, and we can clearly see that there is no genetic determinism in the association between the CETP-TaqIB SNP and HDL-C concentrations. B1B1 subjects do not always have lower HDL-C concentrations than the other genotypes (genetic effect). Their HDL-C concentrations depend on alcohol consumption (just as in the other genotypes; hence, there is no statistically significant gene-diet interaction). In accordance with Figure 3B, B1B1 subjects with high alcohol consumption present a statistically significant higher HDL-C \((P<0.05)\) than B2B2 subjects who did not consume alcohol. Therefore, in a special situation of low HDL-C in B1B1 subjects, their HDL-C concentrations could be increased by alcohol consumption. This example has been included only to illustrate the biological interaction concept, and we have to be very careful with recommendations on alcohol consumption in CVD prevention as the harmful effects of that consumption must not be overlooked.

The second example of a biological gene-diet interaction with no statistically significant interaction term, involves a \(-765G>C \) polymorphism in the cyclooxygenase-2 gene, a Mediterranean diet intervention and serum concentrations of interleukin-6 and intercellular adhesion molecule-1.\(^{117}\) The \(-765G>C \) cyclooxygenase-2 polymorphism was significantly associated with higher interleukin-6 and intercellular adhesion molecule-1 concentrations in GG individuals. A Mediterranean diet intervention, significantly decreased interleukin-6 and intercellular adhesion molecule-1 concentrations in both genotypes (effect independent from the genotype status that results in a no statistically significant gene-diet interaction). After the dietary intervention with a Mediterranean diet, the GG individuals had interleukin-6 and intercellular adhesion molecule-1 concentrations similar to C carriers before the intervention, so reducing the altered inflammation markers associated with genetic susceptibility. Therefore, a Mediterranean diet may overcome the risk status imposed by genetics.

### Biological Mechanisms Underlying Gene-Diet Interactions

Although remarkable progress has been made in identifying gene-diet interactions in epidemiological studies, the search...
of biological mechanisms underlying gene-diet interactions represents a major challenge. It is generally accepted that cellular processes spanning from gene expression to protein synthesis and degradation can be regulated by dietary components; however, there is a very limited understanding of the nutrient and non-nutrient-related networks. As Panagiotou et al. outlined in their review on nutritional systems biology, a more complete knowledge of network function will further enhance our abilities to study the biological mechanisms underlying the observed differences in response to diet in genetically diverse individuals. In their review, they discuss the way to face and overcome the complexity of nutritional research using appropriate models system and the recent advances in the different x-omics applied to nutritional genomics, in particular nutrigenomics, nutrimentabolomics, and nutritional systems biology, providing some interesting examples. Another review regarding the state-of-the-art and future applications of pathway mapping to the study of CVD is that published by Kanoni et al. discussing results from the Kyoto Encyclopedia of Genes and Genomes pathways “Biosynthesis of unsaturated fatty acids” and “Sphingolipid metabolism,” evidencing multiple changes in gene/lipid levels between low and high cholesterol treatment.

Applications of Nutrigenomics to the Prevention and Treatment of CVD

At present, it is premature to recommend the use of nutrigenomics in the prevention of CVD at the population level. Most probably, the first applications of nutrigenomics in cardiovascular medicine will involve normalization of altered intermediate CVD phenotypes, involving information about statistically significant and biological gene-diet interactions. Thus, it is essential to accumulate a series of well-validated gene variants associated with CVD intermediate phenotypes in the whole population. This will facilitate the identification of genetically susceptible individuals on whom to carefully study dietary interventions aimed to successfully quench their genetic predisposition. In this process, transcriptomics, proteomics, metabolomics in the context of systems biology will contribute to the elucidation of the molecular mechanisms involved in specific gene-diet interactions.

CVD and diet are both very complex and the most plausible scenario is that multiple genes will be used to create genetic profiles or scores. Consequently, attention should be paid to gene-gene interactions and to nutrient-nutrient interactions was well as to the classic gene-diet interaction. Biomedical informatics will help in tackling this complexity.

Although nutrigenomics cannot be rigorously applied to cardiovascular prevention and treatment at this time, important results are being generated that will serve as the basis to achieve the necessary level of evidence. At that point, nutrigenomics will become a reality in dietary personalization, in cardiovascular medicine, and subsequently in optimizing CVD treatment and prevention.

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