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Review

Nutrition in the genomics era: Cardiovascular disease risk and the Mediterranean diet

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The effect of dietary changes on phenotypes (*i.e.*, plasma lipid measures, body weight and blood pressure) differs significantly between individuals. This phenomenon has been more extensively researched in relation to changes in dietary fat and plasma lipid concentrations for the prevention of cardiovascular disease (CVD) compared to other pathological conditions. Although common knowledge associates low fat diets with reductions in total and plasma LDL cholesterol, the clinical evidence shows dramatic inter-individual differences in response that are partially due to genetic factors. The discovery of the cardioprotective and other healthy properties of the Mediterranean diet has popularized the consumption of Mediterranean products such as olive oil. Molecular, clinical, and epidemiological studies have begun to shed some light about how various components of this diet may protect the cardiovascular system and to decrease the risk of other diseases such as cancer. However, it is also possible that the right combination of genetic, cultural, socioeconomic factors is needed to achieve full benefit. It has been proposed that the Mediterranean diet may be closer to the ancestral foods that were part of human development and our metabolism may have evolved to work optimally on such a diet rather than with the current diets richer in saturated fat and highly refined and processed foods. Therefore, it is possible that alleles that are associated with increase disease risk may be silenced in the presence of that more ancestral and traditional diet and lifestyle. This knowledge may provide the basis for successful public health as well individual approaches for disease prevention.

Keywords: Mediterranean diet / MUFA / Nutrigenetics / Single nucleotide polymorphism

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1 Introduction

The effect of dietary changes on phenotypes (*i.e.*, plasma lipid measures, body weight and blood pressure) differs significantly between individuals [1–3]. Some individuals appear to be relatively insensitive (hyporesponders) to dietary intervention, whereas others have enhanced sensitivity (hyperresponders) [3]. This phenomenon has been more extensively researched in relation to changes in dietary fat and plasma lipid concentrations for the prevention of cardiovascular disease (CVD) compared to other pathological conditions. Although common knowledge associates low fat diets with reductions in total and plasma low-density lipoprotein cholesterol (LDL-C), the clinical evidence shows dramatic inter-individual differences in response and may be one of the underlying causes of the limited success of dietary recommendations in the prevention of diseases observed by recent randomized clinical trials [4].

A growing body of data supports the hypothesis that the inter-individual variability in response to dietary modification is determined by genetic factors, especially for lipid and lipoprotein phenotypes [5]. Indirect evidence comes from the general observation that the phenotypic response to diet is determined partly by the baseline value of the phenotype that is itself affected by genetic factors [3]. The main chal-

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Abbreviations: CVD, cardiovascular disease; **FABP**, fatty acid binding protein; **HOMA-IR**, homeostasis model assessment insulin resistance index; **LDL-C**, LDL cholesterol; **LXR**, liver X receptor; **MTHFR**, methylenetetrahydrofolate reductase; **PPAR**, peroxisome proliferator-activated receptor; **SNP**, single nucleotide polymorphism; **TAG,** triacylglyceride lenges are (i) how to uncover and elucidate the many potential gene-diet interactions, and (ii) how potential epistatic (gene–gene) interactions caused by differing ancestral background effect these gene-diet interactions.

Several studies have found specific genes to be associated with the variability in response of LDL-C levels in response to changes in dietary fat but so far, the findings have been highly inconsistent. These conflicting outcomes reflect both the complexity of the mechanisms involved in dietary responses and the limitations of the current experimental designs used to address this problem. In addition to their effects on plasma LDL-C levels, low fat diets can result in reduced plasma HDL and/or increased triacylglyceride (TAG) concentrations [2] that may be particularly harmful for some persons. For example, it has been shown that individuals with a predominance of small, dense LDL particles (subclass pattern B), a phenotype that is associated with an increased risk of coronary heart disease, benefit more from a low-fat diet [6] than do those with the subclass pattern A (larger LDL). A significant proportion of the latter group unexpectedly exhibited a more atherogenic pattern B subclass after consuming a low-fat diet. Intervention studies are increasingly focusing on the inter-individual differences in response to diet rather than on the mean effect analyzed for a population. Moreover, new evidence indicates that the variability in response is an intrinsic characteristic of the individual, rather than being the result of different dietary compliance with the experimental protocols. Jacobs *et al*. [7] found that individual TAG responses to a high-fat or to a low-fat diet were vastly different, suggesting that many patients with hypertriglyceridemia are not treated optimally if general advice for either a low-fat or a high-fat diet is given. Studying the reasons for this variation will allow us to better identify individuals who can benefit from a particular dietary intervention. Obviously, this is not an easy task and some authors have already proposed different statistical algorithms in attempts to better predict the response of individuals to different diets [8].

2 How nutrients communicate with genes

Before presenting some of the current nutrigenetic evidence in the area of lipid metabolism and CVD, it is helpful to gain an understanding of how nutrients and other chemicals in the diet may influence gene expression and drive gene-diet interactions. The study of these interactions is the subject of nutrigenomics, which seeks to understand gene-diet interactions in the context of the total genetic makeup of each individual. Technological limitations in the past restricted the investigator to a piecemeal approach: one gene, one gene product and one nutrient at a time. Conceptual and technological advances are changing the playing field. For the first time, researchers can cast a wide net in the form of microarrays that can potentially capture the information about each one of the genes expressed in a specific cell or tissue of interest. Despite these advances, the challenges are not trivial given the chemical complexity of food, and our incomplete knowledge about the various bioactive components present in food grown in different climates at different times of the year. This is clearly the case with regard to the composition of olive oil, a key element of the Mediterranean diet [9, 10]. Moreover, our ability to carry out mechanistic studies in humans gets impaired by our inability to assay gene expression in the most appropriate target tissues in humans and by the challenge of controlling for or determining many lifestyle factors that also influence expression of genetic information.

Regulation of expression of genes involved in fatty acid metabolism occurs when a dietary fat or metabolite binds to and activates specific fatty acid transcription factors. These dietary chemical-regulated transcription factors are members of the nuclear receptor super family. This gene family consists of 48 mammalian transcription factors that regulate nearly all aspects of development, inflammation, and metabolism. Two subclasses, the peroxisome proliferator-activated receptors (PPARs) and liver X receptors (LXRs), are lipid-sensing receptors that have critical roles in lipid and glucose metabolism [11–13]. PPARs are among the best-studied fatty acid-regulated nuclear receptors [14, 15]. After uptake into target cells, subsets of fatty acids or their metabolites are transported to the nucleus in association with fatty acid-binding proteins (FABPs), which facilitates their interaction with PPARs. Several PPAR subtypes have been described [16]. PPAR-alpha (PPARA) plays a key role in lipid oxidation and inflammation, whereas PPAR-gamma (PPARG) is involved in cell (adipocyte) differentiation, glucose lipid storage and inflammation. PPAR-delta (PPARD, also known as PPAR-beta) may play an important role in development, lipid metabolism and inflammation.

In addition to the potential effects of the fatty acids present in olive oil on gene expression [17], it should be noted that PUFA n-3, present in fish and nuts, and also part of the traditional Mediterranean diet, could play a relevant role in providing the health promoting effects of such dietary culture. Moreover, other minor components present in extra virgin olive oil could regulate gene expression [18] and this regulation could be affected in subjects with different alleles at key genes [19]. In addition to fatty acids, pharmacological agonists have been developed for each receptor: PPARA binds fibrates, PPARD binds lipophilic carboxylic acids, and PPARG binds glitazones. The fibrates are used to treat hyperlipidemia. The glitazones are used to manage plasma glucose levels in patients with insulin resistance [20]. Pathway analyses using the MetaDrug workflow software (www.genego.com), which searches and builds an interaction network based on known pathways as well as reports of direct or indirect effects of a compound to a network object based from published data obtained in human or human cell culture studies. The results showed that rosiglitazone (a member of the glitazone drug family) and 15-deoxy-prostaglandin J2 (15-deoxy-PGJ2, a metabolite of dietary fatty acids) had three identical targets but also and 43 common cellular elements [21]. Elements (or network objects) are complexes, genes, proteins, or enzyme activities. Although many of these elements were components of the insulin response or control pathways, a substantial number of "common targets" were in pathways regulating other cellular processes, such as genes involved in apoptosis and regulation of detoxifying enzymes. Pathway analyses demonstrated that drugs and dietary components affect more than one target, that diet would likely alter responsiveness to drugs through multiple pathways, and the practical importance of assessing dietary intakes for clinical medicine.

Many of the previously published nutrigenetic [*i.e.*, single gene/single nucleotide polymorphism (SNP)] studies focused on genes that are the subject of regulation by PPARs and other nuclear receptors [22]. Polymorphisms in promoter regions of these genes may disrupt or at least alter the communication with these transcription factors, which would have significant consequences in a person's response to dietary factors that are ligands (*i.e.*, PUFAs) of the transcription factors. It is also obvious that polymorphisms within the transcription factors themselves will have a significant impact in the way that each one of us responds to dietary factors. The evidence for gene-diet interactions between common SNPs at candidate genes and dietary factors related to lipid metabolism is increasing and some have implicated the consumption of a Mediterranean diet [19]. However, caution is needed before applying these results to clinical practice for three primary reasons: (i) the meaning of "statistically significant results" is subject to differing interpretations and often depends upon the study design, (ii) many initial gene-nutrient-phenotypes associations are not replicated in subsequent studies, and (iii) gene variations may influence phenotypes differently in individuals from different ancestral backgrounds due to gene-gene (epistatic) interactions.

3 Results from interventional studies

Interventional studies in which subjects receive a controlled dietary intake provide the best approach for conducting gene-nutrient-phenotype association studies. However, these well-controlled feeding studies have several important logistical limitations, most importantly the small number of participants and the brief duration of the interventions. Scores of interventional studies examining gene-diet interactions on different parameters of lipid metabolism have been published. However, the level of replication among studies analyzing the same genetic variation tends to be low. The lack of replication is most likely due to the different characteristics (ethnicity, physical condition, age, lifestyle differences) of study subjects, length of intervention, sample size, and heterogeneity in the design. In a systematic review (from 1966 to 2002), Masson *et al*. [23] identified 74 relevant articles including dietary intervention studies that had measured the lipid and lipoprotein response to diet in different genotype groups and 17 reviews on gene-diet interactions. After a comparative analysis of the individual findings, they concluded that there is evidence to suggest that (i) variations in the *APOA1*, *APOA4*, *APOB*, and *APOE* genes contribute to the heterogeneity in the lipid response to dietary intervention, and (ii) all of these genes are regulated directly or indirectly by PPARA or other nuclear receptors. However, the evidence suggested by Masson *et al*. [23] in relation to the above genes comes from meta-analyses of the published data and described the average effect. It should be noted that there is not total consistency of results among individual studies.

More recently, one of our groups [24–26] reviewed this topic extensively and included additional studies reported after 2002. The median for the sample sizes in these more recent studies was in the range of 60 subjects. These small sample sizes highlight one of the traditional problems for lack of reproducibility, specifically, the statistical power is low. In addition, the composition of the dietary intervention in these studies varied considerably. We propose that the design of future intervention studies should be standardized for key dietary intake variables and phenotype measurements. A minimum set of variables would include patients' physical and genetic characteristics (including genetic ancestry analyses), medications, composition and length of the dietary treatment, and sample size. Such standardization would allow better comparison among studies and the possibility of conducting meta-analyses, which is not possible under current experimental conditions.

The genetic modulation of a Mediterranean diet has not been widely investigated in intervention studies. Some studies are currently being carried out that will provide key information in this regard [27–29] but at the present time, most studies are based on very small number of subjects [30, 31] or they have focused on the postprandial phase, which is discussed below.

4 Results from observational studies

Observational studies have the advantage of large numbers of subjects and the ability to estimate long-term dietary habits. However, the level of evidence of the results obtained from these studies has traditionally been considered to be lower than that of experimental studies. Nevertheless, the level of confidence in such studies can be increased by taking into consideration the principle of Mendelian randomization [32]. This concept reflects the random assortment of alleles at the time of gamete formation. Such randomization results in population distributions of genetic variants that are generally independent of behavioral and environmental factors that confound epidemiological associations between potential risk factors and disease. This topic has been extensively reviewed [24, 26]. The median population size for recent observational studies is approximately 850. This sample size may be informative for traditional genotype-phenotype association studies but, considering the higher measurement error of dietary intake in comparison with experimental studies, it may not have enough statistical power to address properly the complexity of gene-environment interactions. As pointed out for intervention studies, replication of results is still very low. In addition, these findings need the synergy of those studies examining the effects of nutrients on gene expression (nutrigenomics) to provide the mechanistic knowledge that will support the reported statistical associations.

Genotype-nutrient-phenotype analyses may be improved by determining ancestral backgrounds of each study participant. These additional data are necessary since SNPs may be expressed differently among individuals of differing ethnicities because of varying gene-gene and gene-nutrient interactions. Determining the genetic architecture (that is, geographical origin of chromosomal regions) in each study participant may reduce statistical noise caused by mismatching case control [32].

Few observational studies have examined the interaction between SNPs at candidate genes, CVD risk factors and the consumption of a Mediterranean diet or MUFAs. However, some knowledge is starting to emerge about the additional benefits of such diets in subjects with specific alleles. This is the case with the reported interaction between the Pro12Ala SNP at the PPARG locus, type 2 diabetes mellitus (T2DM), and peripheral insulin sensitivity in a population characterized by a high intake of oleic acid [19]. These investigators examined these associations and interactions in a population-based study in Pizarra (Spain). A total of 538 subjects, aged 18–65 years, were randomly selected. Consistent with some previous reports, those subjects with the Ala12 allele had lower risk of diabetes. Moreover, a significant and complex interaction was observed between the homeostasis model assessment insulin resistance index (HOMA-IR), obesity, the PPARG Ala12 allele and the intake of MUFA. This interaction suggests that obese subjects with the Ala12 allele have higher HOMA-IR values in the background of a low intake of MUFA. Along those lines, we have recently reported how MUFA are not associated with increases in BMI and risk of obesity, especially in subjects with certain allele at the APOA5 locus [33]. In this study, our objective was to study whether dietary intake modulates the association between APOA5 gene variation and body weight in a large population-based study. Specifically, we have examined the interaction between the APOA5-1131T > C and 56C > G (S19W) polymorphisms and the macronutrient intake (total fat, carbohydrate, and protein) in their relation to the BMI and obesity risk in men and women participating in the Framingham Study. We found a consistent and statistically significant interaction between the –1131T > C SNP (but not the 56C > G SNP) and total fat intake for BMI. This interaction was dose dependent, and no statistically significant heterogeneity by gender was detected. 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. When specific fatty acid groups were analyzed, MUFA showed the highest statistical significance for these interactions. Therefore, our study showed that the APOA5-1131T > C SNP, which is present in approximately 13% of this population, modulated the effect of fat intake on BMI and obesity risk in both men and women and this effect might be primarily driven by the intake of MUFA characteristic of the Mediterranean diet.

Another interesting observational study has focused on the interaction between oxidative modification of LDL, the methylenetetrahydrofolate reductase (MTHFR) C677T mutation and the Mediterranean diet [34]. The investigators studied demographics, lifestyle, clinical, biochemical and genetic data from 322 men and 252 women free of clinical CVD from the Attica region in Greece. The distribution of MTHFR genotypes was: 41% for homozygous normal (CC) genotype, 48% for heterozygous (CT) and 11% for homozygous mutant (TT) genotype. Ox-LDL levels were higher in TT as compared to CT and CC (71, 64 and 51 respectively). Greater adherence to the Mediterranean diet was inversely associated with ox-LDL levels. However, stratified analysis revealed that adherence to the Mediterranean diet was associated with lower ox-LDL levels in TT and CT individuals, but not in CC. Therefore, the reported gene-to-diet interaction on ox-LDL concentrations may provide a pathophysiological explanation by which a Mediterranean type of diet could influence coronary risk in people with increased oxidative stress.

5 Gene-diet interactions in the postprandial state

Human beings living in industrialized societies spend most of the waking hours in a non-fasting state because of meal consumption patterns and the amounts of food ingested. Postprandial lipemia, characterized by a rise in TAG after eating, is a dynamic, nonsteady-state condition [35]. Over 25 years ago, Zilversmit [36] proposed that atherogenesis was a postprandial phenomenon as since high concentrations of lipoproteins and their remnants following food ingestion could deposit onto the arterial wall and accumulate in atheromatous plaques. Several studies have investigated the potential interaction between some polymorphisms in candidate genes and diet on postprandial lipids (for review see [26]). In postprandial studies, subjects usually receive a fat-loading test meal that has differing compositions depending on the nutrient(s) to be tested. After the test meal, blood samples are taken to measure postprandial lipids to compare with preprandial levels [35]. Consistency among studies is still very low and replication of findings is a major necessity. Postprandial studies often have a low number of subjects (usually <50) with the added complexity that designs may add even more bias than for other experimental approaches. Those studies that have investigated the interaction between a Mediterranean-like diet or olive oil and the postprandial response have been few and subjected to the sample size limitations indicated above [30, 37, 38] diet and the results suggest that MUFA may provide benefit in terms of the postprandial response to subjects carrying alleles associated with an increased atherogenic profile, but the results are not consistent, probably due to the limitations described above.

6 The roadmap to solidifying the nutrigenomics field

Despite the excitement arising from an increasing number of findings related to nutritional genomics, the progress of the field is hampered by the inadequacy of the current experimental approaches to efficiently deal with the biological complexity of the phenotype(s), the complexity of dietary intakes, differing genetic background among participants, and the limitations of low statistical power of the studies. We and others have proposed that only a comprehensive, international nutritional genomics approach [39, 40] will yield short- and long-term benefits to human health by: (i) revealing novel nutrient–gene interactions, (ii) developing new diagnostic tests for adverse responses to diets, (iii) identifying specific populations with special nutrient needs, (iv) improving the consistency of current definitions and methodology related to dietary assessment, and (v) providing the information for developing more nutritious plant and animal foods and food formulations that promote health and prevent, mitigate, or cure disease. Achieving these goals will require extensive dialogue between scientists and the public about the nutritional needs of the individual vs groups, local food availability and customs, analysis and understanding of genetic differences between individuals and populations, and serious commitment of funds from the public and private sectors. Nutritional genomics' researchers are seeking collaborations of scientists, scholars, and policy makers, to maximize the collective impact on global poverty and health by advancing our knowledge of how genetics and nutrition can promote health or cause disease.

7 Conclusions

Although the current evidence from both experimental and observational nutrigenetics studies is not enough to start making specific personalized nutritional recommendations based on genetic information, there are a large number of examples of common SNPs modulating the individual response to diet as proof of concept of how gene-diet interactions can influence lipid metabolism. It is critical that these preliminary studies go through further replication and that subsequent studies be properly designed with sufficient statistical power and careful attention to phenotype and genotype. The many challenges that lay ahead are evident. This review has examined the vast world of nutrigenetics and nutrigenomics only through the small keyhole of PPARA and dietary fat. Analogous to the use of the X-ray diffraction patterns 50 years ago to determine the structure of DNA, which led to today's progress in sequencing the entire human genome, these initial steps in understanding nutrigenomics will likely lead to fundamental breakthroughs that will both clarify today's mysteries and pave the way for clinical applications. Hopefully, bringing nutrigenetics to the state of becoming a practical and useful tool will not take 50 years. However, to arrive at the point where it is possible to assess the modulation by specific SNPs of the effects of dietary interventions on lipid metabolism, well designed, adequately powered, and adequately interpreted randomized controlled studies (or their equivalent) of greater duration than current studies are needed, with careful consideration given to which patients to include in such studies. Moreover, research must also investigate the potential mechanisms involved in the gene-diet interactions reported by nutrigenetic studies [39]. These imperative needs can be achieved only through the collaboration of experts in the different fields involved, which must include nutrition professionals [40, 41].

One of the first situations where personalized nutrition is likely to be beneficial is with dyslipidemic patients that require special intervention with dietary treatment. It is known that these individuals will display dramatic heterogeneity in response to the currently recommended therapeutic diets and that the recommendations will need to be adjusted individually. This process could be more efficient and efficacious if the recommendations were carried out based on genetic and molecular knowledge. Moreover, adherence to dietary advice may increase when it is supported with information based on nutritional genomics, and the patient feels that the advice is personalized. However, a number of important changes in the provision of health care are needed to achieve the potential benefits associated with this concept, including a teamwork approach, with greater integration among physicians and nutrition professionals. Once more experience is gained from patients and/or individuals at high risk, these approaches could be applied towards primary prevention.

The discovery of the cardioprotective and other healthy properties of the Mediterranean diet has popularized beyond its geographical boundaries the consumption of Mediterranean products such as olive oil. Molecular, clinical, and epidemiological studies have begun to shed some light about how various components of this diet may protect the cardiovascular system and to decrease the risk of other diseases such as cancer. However, still many unknowns remain. Can the same healthy effects of those dietary components be obtained in other regions of the globe? Or alternatively, the right combination of genetic, physico-geographical, socioeconomic and culture is also needed [42]. It has been proposed that the Mediterranean diet may be closer to the ancestral foods that were part of human development. Therefore, our metabolism may have evolved to work optimally on such a diet rather than to the current diets richer in saturated fat and highly refined and processed foods. It is possible that alleles that are associated with increase disease risk may be silenced in the presence of that more ancestral and traditional diet and lifestyle. This knowledge may provide the basis for successful public health as well individual approaches for disease prevention.

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8 References

[1] Jacobs, D. R. Jr., Anderson, J. T., Hannan, P., Keys, A., Blackburn, H., Variability in individual serum cholesterol response to change in diet. *Arteriosclerosis* 1983, *3*, 349–356.

[2] Katan, M. B., Grundy, S. M., Willett, W. C., Should a low-fat, high-carbohydrate diet be recommended for everyone? Beyond low-fat diets. *N. Engl. J. Med.* 1997, *337*, 563–566.

[3] Katan, M. B., Beynen, A. C., de Vries, J. H., Nobels, A., Existence of consistent hypo- and hyperresponders to dietary cholesterol in man. *Am. J. Epidemiol.* 1986, *123*, 221–234.

[4] Prentice, R. L., Caan, B., Chlebowski, R. T., Patterson, R. *et al.*, Low-fat dietary pattern and risk of invasive breast cancer: the Women's Health Initiative Randomized Controlled Dietary Modification Trial. *JAMA* 2006, *295*, 629–642.

[5] Loktionov, A., Common gene polymorphisms and nutrition: emerging links with pathogenesis of multifactorial chronic diseases. *J. Nutr. Biochem.* 2003, *14*, 426–451.

[6] Krauss, R. M., Dietary and genetic effects on low-density lipoprotein heterogeneity. *Annu. Rev. Nutr.* 2001, *21*, 283–295.

[7] Jacobs, B., De Angelis-Schierbaum, G., Egert, S., Assmann, G., Kratz, M., Individual serum triglyceride responses to high-fat and low-fat diets differ in men with modest and severe hypertriglyceridemia. *J. Nutr.* 2004, *134*, 1400–1405.

[8] Parks, E. J., Rutledge, J. C., Davis, P. A., Hyson, D. A. *et al.*, Predictors of plasma triglyceride elevation in patients participating in a coronary atherosclerosis treatment program. *J. Cardiopulm. Rehabil.* 2001, *21*, 73–79.

[9] Angerosa, F., Basti, C., Vito, R., Virgin olive oil volatile compounds from lipoxygenase pathway and characterization of some italian cultivars. *J. Agric. Food. Chem.* 1999, *47*, 836–839.

[10] Bianco, A., Dezzi, S., Bonadies, F., Romeo, G. *et al.,* The variability of composition of the volatile fraction of olive oil. *Nat. Prod. Res.* 2006, *20*, 475–478.

[11] Li, A. C., Glass, C. K., PPAR- and LXR-dependent pathways controlling lipid metabolism and the development of atherosclerosis. *J. Lipid. Res.* 2004, *45*, 2161–2173.

[12] Pegorier, J. P., Le May, C., Girard, J., Control of gene expression by fatty acids. *J. Nutr.* 2004, *134*, 2444S–2449S.

[13] Jump, D. B., Fatty acid regulation of gene transcription. *Crit. Rev. Clin. Lab. Sci.* 2004, *41*, 41–78.

[14] Clarke, S. D., The multi-dimensional regulation of gene expression by fatty acids: polyunsaturated fats as nutrient sensors. *Curr. Opin. Lipidol.* 2004, *15*, 13–18.

[15] Lapillonne, A., Clarke, S. D., Heird, W. C., Polyunsaturated fatty acids and gene expression. *Curr. Opin. Clin. Nutr. Metab. Care.* 2004, *7*, 151–156.

[16] Kota, B. P., Huang, T. H., Roufogalis, B. D., An overview on biological mechanisms of PPARs. *Pharmacol. Res.* 2005, *51*, 85–94.

[17] Menendez, J. A., Papadimitropoulou, A., Vellon, L., Lupu, R., A genomic explanation connecting "Mediterranean diet", olive oil and cancer: oleic acid, the main monounsaturated fatty acid of olive oil, induces formation of inhibitory "PEA3 transcription factor-PEA3 DNA binding site" complexes at the Her-2/neu (erbB-2) oncogene promoter in breast, ovarian and stomach cancer cells. *Eur. J. Cancer* 2006, *42*, 2425–2432.

[18] Bellido, C., Lopez-Miranda, J., Perez-Martinez, P., Paz, E. *et al.*, The Mediterranean and CHO diets decrease VCAM-1 and E-selectin expression induced by modified low-density lipoprotein in HUVECs. *Nutr. Metab. Cardiovasc. Dis.* 2006, *16*, 524–530.

[19] Soriguer, F., Morcillo, S., Cardona, F., Rojo-Martinez, G. *et al.*, Pro12Ala polymorphism of the PPARG2 gene is associated with type 2 diabetes mellitus and peripheral insulin sensitivity in a population with a high intake of oleic acid. *J. Nutr.* 2006, *136*, 2325–2330.

[20] Berger, J. P., Akiyama, T. E., Meinke, P. T., PPARs: therapeutic targets for metabolic disease. *Trends Pharmacol. Sci.* 2005, *26*, 244–251.

[21] Kaput, J., Perlina, A., Hatipoglu, B., Bartholomew, A., Nikolsky, Y., Nutrigenomics: Concepts and applications to pharmacogenomics and clinical medicine. *Pharmacogenomics* 2007, *8*, 369–390.

[22] Mandard, S., Muller, M., Kersten, S., Peroxisome proliferator-activated receptor alpha target genes. *Cell. Mol. Life Sci.* 2004, *61*, 393–416.

[23] Masson, L. F., McNeill, G., Avenell, A., Genetic variation and the lipid response to dietary intervention: a systematic review. *Am. J. Clin. Nutr.* 2003, *77*, 1098–1111.

[24] Corella, D., Ordovas, J. M., Single nucleotide polymorphisms that influence lipid metabolism: Interaction with dietary factors. *Annu. Rev. Nutr.* 2005, *25*, 341–390.

[25] Ordovas, J. M., Corella, D., Genes, diet and plasma lipids: the evidence from observational studies. *World Rev. Nutr. Diet.* 2004, *93*, 41–76.

[26] Ordovas, J. M., Corella, D., Nutritional genomics. *Annu. Rev. Genomics Hum. Genet.* 2004, *5*, 71–118.

[27] Buttriss, J., Nugent, A., LIPGENE: an integrated approach to tackling the metabolic syndrome. *Proc. Nutr. Soc.* 2005, *64*, 345–347.

[28] Vincent-Baudry, S., Defoort, C., Gerber, M., Bernard, M. C. *et al.*, The Medi-RIVAGE study: reduction of cardiovascular disease risk factors after a 3-mo intervention with a Mediterranean-type diet or a low-fat diet. *Am. J. Clin. Nutr.* 2005, *82*, 964–971.

[29] Estruch, R., Martinez-Gonzalez, M. A., Corella, D., Salas-Salvado, J. *et al.*, Effects of a Mediterranean-style diet on cardiovascular risk factors: a randomized trial. *Ann. Intern. Med.* 2006, *145*, 1–11.

[30] Perez-Martinez, P., Ordovas, J. M., Lopez-Miranda, J., Gomez, P. *et al.*, Polymorphism exon 1 variant at the locus of the scavenger receptor class B type I gene: influence on plasma LDL cholesterol in healthy subjects during the consumption of diets with different fat contents. *Am. J. Clin. Nutr.* 2003, *77*, 809–813.

[31] Tomas, M., Senti, M., Elosua, R., Vila, J. *et al.*, Interaction between the Gln-Arg 192 variants of the paraoxonase gene and oleic acid intake as a determinant of high-density lipoprotein cholesterol and paraoxonase activity. *Eur. J. Pharmacol.* 2001, *432*, 121–128.

[32] Campbell, C. D., Ogburn, E. L., Lunetta, K. L., Lyon, H. N. *et al.*, Demonstrating stratification in a European American population. *Nat. Genet.* 2005, *37*, 868–872.

[33] Corella, D., Lai, C. Q., Demissie, S., Cupples, L. A. *et al.*, APOA5 gene variation modulates the effects of dietary fat intake on body mass index and obesity risk in the Framingham Heart Study. *J. Mol. Med.* 2007, *85*, 119–128.

[34] Pitsavos, C., Panagiotakos, D., Trichopoulou, A., Chrysohoou, C. *et al.*, Interaction between Mediterranean diet and methylenetetrahydrofolate reductase C677T mutation on oxidized low density lipoprotein concentrations: the ATTICA study. *Nutr. Metab. Cardiovasc. Dis.* 2006, *16*, 91–99.

[35] Ordovas, J. M., Genetics, postprandial lipemia and obesity. *Nutr. Metab. Cardiovasc. Dis.* 2001, *11*, 118–133.

[36] Zilversmit, D. B., Atherogenesis: a postprandial phenomenon. *Circulation* 1979, *60*, 473–485.

[37] Perez-Martinez, P., Perez-Jimenez, F., Bellido, C., Ordovas, J. M. *et al.*, A polymorphism exon 1 variant at the locus of the scavenger receptor class B type I (SCARB1) gene is associated with differences in insulin sensitivity in healthy people during the consumption of an olive oil-rich diet. *J. Clin. Endocrinol. Metab.* 2005, *90*, 2297–2300.

[38] Dworatzek, P. D., Hegele, R. A., Wolever, T. M., Postprandial lipemia in subjects with the threonine 54 variant of the fatty acid-binding protein 2 gene is dependent on the type of fat ingested. *Am. J. Clin. Nutr.* 2004, *79*, 1110–1117.

[39] van Ommen, B., Stierum, R., Nutrigenomics: exploiting systems biology in the nutrition and health arena. *Curr. Opin. Biotechnol.* 2002, *13*, 517–521.

[40] Kaput, J., Ordovas, J. M., Ferguson, L., van Ommen, B. *et al.*, The case for strategic international alliances to harness nutritional genomics for public and personal health. *Br. J. Nutr.* 2005, *94*, 623–632.

[41] Ordovas, J. M., Nutrigenetics, plasma lipids, and cardiovascular risk. *J. Am. Diet. Assoc.* 2006, *106*, 1074–1081.

[42] Mackenbach, J. P., The Mediterranean diet story illustrates that “why” questions are as important as “how” questions in disease explanation. *J. Clin. Epidemiol.* 2007, *60*, 105–109.