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Arcas N. (ed.), Arroyo López F.N. (ed.), Caballero J. (ed.), D'Andria R. (ed.), Fernández M. (ed.), Fernandez Escobar R. (ed.), Garrido A. (ed.), López-Miranda J. (ed.), Msallem M. (ed.), Parras M. (ed.), Rallo L. (ed.), Zanoli R. (ed.).
Present and future of the Mediterranean olive sector

Zaragoza: CIHEAM / IOC
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 106

2013
pages 143-151

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I – Introduction

The effect of dietary changes on phenotypes (i.e., plasma lipids, body weight and blood pressure) differs significantly between individuals (Jacobs *et al.*, 1983; Katan *et al.*, 1997; Katan *et al.,* 1986). Some individuals are relatively insensitive (hyporesponders) to dietary intervention, whereas others have enhanced sensitivity (hyperresponders) (Katan *et al.,* 1986). This singularity has been specially investigated in relation to dietary fat, plasma lipid concentrations and prevention of cardiovascular disease (CVD) as compared with other pathological conditions.

Although low fat diets have been associated with reductions in total and plasma low-density lipoprotein cholesterol (LDL-C), the clinical evidence shows dramatic inter-individual differences in response which may be one of the underlying causes of the limited success of dietary recommendations in disease prevention reported by randomized clinical trials (Prentice *et al*., 2006). The current knowledge supports the hypothesis that the inter-individual variability in response to dietary modification is driven by genetic factors (Loktionov, 2003). The main translational challenges 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 genes have been associated with the variability in response of LDL-C levels in response to changes in dietary fat but 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 traditional 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 (Katan *et al.*, 1997) 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 (Krauss, 2001) 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. Therefore, 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.* (2004) 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 basis for this variation will allow us to better identify individuals who can benefit from a particular dietary intervention. Something that some authors already attempted using more traditional approaches (Parks *et al*., 2001).

II – Nutrient-gene communication

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. Nowadays, researchers can cast a wide net in the form of microarrays to 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 (Angerosa *et al*., 1999; Bianco *et al*., 2006). 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 (Li *et al*., 2004; Pegorier *et al*., 2004; Jump, 2004). PPARs are among the best-studied fatty acid-regulated nuclear receptors (Clarke, 2004; Lapillonne *et al*., 2004). 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 (Kota *et al*., 2005). 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 (Menendez *et al*., 2006), 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 (Bellido *et al*., 2006) and this regulation could be affected in subjects with different alleles at key genes (Soriguer *et al*., 2006). 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 (Berger *et al*., 2005). Pathway analyses using the MetaDrug workflow software (www.genego.com), showed that rosiglitazone 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 (Kaput *et al*., 2007). 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 (Mandard *et al*., 2004). 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 so is the understanding of the interactions resulting from the consumption of a Mediterranean diet from recent studies such as the large PREDIMED Study (Estruch *et al*., 2006; Razquin *et al*., 2009, 2010a, 2010b, 2010c; Ortega-Azorín *et al*., 2012; Sotos-Prieto *et al*., 2012) and other smaller intervention studies (Buttriss and Nugent, 2005; Vincent-Baudry *et al*., 2005; Garaulet *et al*., 2011; Sánchez-Moreno *et al*., 2011). 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.

III – 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.* (2003) 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.* (2003) 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 (Corella and Ordovás, 2005; Ordovás and Corella, 2004a,b) 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 using the tools developed by Hamilton *et al.* (2011). 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 the current trend in observational genetic association and gene-diet interaction studies (Hamilton *et al*., 2011).

IV – 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 (Campbell *et al*., 2005). 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 (Ordovás and Corella, 2004a). The median population size for recent observational genetic association studies has increased dramatically in the last few years driven by the formation of large consortia and the implementation of meta-analysis. Current sample sizes amounting to hundreds of thousands of individuals for some common traits are allowing the discovery of an increasing number of loci associated with complex traits, including CVD-risk factors. However, event these very large numbers may just have barely enough statistical power to address genome-wide gene-environment interactions. This is due to multiple reasons including the higher measurement error of dietary intake in comparison with experimental studies. 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. In addition, 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 (Campbell *et al*., 2005).

Some knowledge is starting to emerge about the additional benefits of the Mediterranean diets in subjects with specific alleles (Soriguer *et al*., 2006; Razquin *et al*., 2009; Razquin *et al*., 2010a,b,c; Ortega-Azorín *et al*., 2012; Sotos-Prieto *et al*., 2012; Garaulet *et al*., 2011; Sánchez-Moreno *et al*., 2011). 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 (Soriguer *et al*., 2006). 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 reported how MUFA are not associated with increases in BMI and risk of obesity, especially in subjects with certain allele at the APOA5 locus (Sánchez-Moreno *et al*., 2011; Corella *et al*., 2007). In the Framingham Study (Corella *et al*., 2007), 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. 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 (Pitsavos *et al*., 2006). 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.

V – Gene-diet interactions in the postprandial state

Human beings living in industrialized societies spend most of the waking hours in a non-fasting state. Postprandial lipemia, characterized by a rise in TAG after eating, is a dynamic, nonsteady-state condition (Ordovás, 2001). Over three decades ago, Zilversmit (Zilversmit, 1979) proposed that atherogenesis was a postprandial phenomenon since high concentrations of lipoproteins 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 (Ordovás and Corella, 2004b)]. 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 (Ordovás, 2001). Consistency among studies is still very low and replication of findings is a major necessity, but complicated by the paucity of replication sets available for direct testing or for meta-analysis and the lack of standardization of procedures among the few existing studies. Those studies that have investigated the interaction between a Mediterranean-like diet or olive oil and the postprandial response have been even fewer and subjected to the sample size limitations indicated above (Pérez-Martínez *et al*., 2003; Pérez-Martínez *et al*., 2005; Dworatzek *et al*., 2004). 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 with the limitations described above.

VI – 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 statistical power of the individual studies. We and others have proposed that only a comprehensive, international nutritional genomics approach (van Ommen and Stierum, 2002; Kaput *et al*., 2005) 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, the private sector 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, industry representatives 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.

VII – Conclusions

Although the current evidence from both experimental and observational nutrigenetics studies may not be enough to embark in widespread 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 among other traits (i.e., obesity). It is critical that all 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 dietary fat and CVD risk factors. 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 fill today's gaps 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 and other common risk factors, 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 (van Ommen and Stierum, 2002). These imperative needs can be achieved only through the collaboration of experts in the different fields involved, which must include nutrition professionals (Kaput *et al*., 2005; Ordovás, 2006).

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 (Mackenbach, 2007). 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.

Acknowledgements

This work was supported by NIH grants HL72524, HL54776 and DK075030 and by contracts 53-K06-5-10 and 58-1950-9-001 from the US Department of Agriculture Research Service, and contracts 53-K06-5-10 and 58-1950-9-001 from the US Department of Agriculture Research Service, and by grants from the Ministerio de Ciencia e Innovación and Fondo Europeo de Desarrollo Regional: CIBER CB06/03/0035, PI07-0954, CNIC-06 and AGL2010-22319-C03-03, PFU-AP2007-04663 and the Generalitat Valenciana (GVACOMP2011-151 and AP111/10).

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