

SUPPLEMENT

Proceedings of the IUNS 20th Congress of Nutrition

Personalized Nutrition and Cardiovascular Disease Prevention: From Framingham to PREDIMED^{1–3}

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ABSTRACT

Diet is considered the cornerstone for the prevention of age-related diseases, and a low-fat diet has been considered for decades as the most suitable alternative to achieve this goal. However, mounting evidence supports the efficacy of other alternatives, such as the Mediterranean diet. Nevertheless, it is well known that people present a dramatic range of responses to similar environmental challenges, and it has been shown that some of this variability is rooted in the genome. In fact, this knowledge is driving the field of nutrigenetics. The finding of interactions between diet and genetic variants has led to intense research and debate about the effectiveness of personalized nutrition as a more suitable tool for the prevention of chronic diseases than the traditional 1-size-fits-all recommendations. Here, we provide some of our own examples that illustrate the progression of nutrigenetics through the years, from the initial studies within the Framingham Heart Study, to the most recent use of large consortia, such as the Cohorts for Heart and Aging Research in Genomic Epidemiology, and ending up with large dietary intervention studies, such as the PREDIMED (Prevención con Dieta Mediterránea) study. These recent approaches are providing more robust and clinically relevant gene–diet interactions. Therefore, although the current evidence level of applying genomic information to tailoring is at its early stages, the prospect of widespread incorporation of nutrigenetics to the clinical practice is encouraging. *Adv. Nutr.* 5: 368S–371S, 2014.

Introduction

The world is growing older. The positive aspect to this statement is that we are living longer. However, the negative side is that this increase in life expectancy is not balanced with similar gains in quality years. The latter is primarily due to the concurrent increases in the prevalence of age-related chronic diseases, such as diabetes, obesity, cancer, neurologic disorders, and cardiovascular diseases (CVD)⁷. Whereas age is

a major factor associated with the risk of these diseases, their expression is modulated by the complex interactions between intrinsic (nature) and environmental or behavioral (nurture) factors that ultimately define an individual's aging trajectory. Among the behavioral factors, nutrition is an obligated exposure that determines our health status at all ages. Therefore, dietary guidelines have been developed to guide the population toward healthy dietary habits aimed to prevent or delay the onset of age-related diseases.

Traditionally, such dietary guidelines were conveyed to the general population using easy-to-follow visual aids, such as the USDA Food Guide Pyramid or the more recent MyPlate (1), placing the emphasis on the daily recommended servings of certain foods and food groups. However, these global recommendations cannot address the individual needs and preferences driven by genetic and cultural factors. To address some of the intercultural and ethnic differences, the health characteristics of several regional dietary patterns (i.e., Mediterranean, Asian, and Nordic diets) are being investigated. Along these lines, the Mediterranean dietary pattern is the 1 that has been investigated more in depth, and the current knowledge supports its beneficial role in the context of primary and secondary prevention (2).

¹ Published in a supplement to *Advances in Nutrition*. Presented at the International Union of Nutritional Sciences (IUNS) 20th International Congress of Nutrition (ICN) held in Granada, Spain, September 15–20, 2013. The IUNS and the 20th ICN wish to thank the California Walnut Commission and Mead Johnson Nutrition for generously providing educational grants to support the publication and distribution of proceedings from the 20th ICN. The contents of this supplement are solely the responsibility of the authors and do not necessarily represent official views of the IUNS. The supplement coordinators were Angel Gil, Ibrahim Elmadfa, and Alfredo Martinez. The supplement coordinators had no conflicts of interest to disclose.

² Supported by NIH/National Heart, Lung, and Blood Institute grant P50HL105185, NIH/National Institute on Aging grant P01AG023394, a research grant from Merck Serono, and the USDA under agreement 58-1950-0-014. Any opinions, findings, conclusion, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the USDA. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

³ Author disclosures: V. Konstantinidou, L. A. D. Ruiz, and J. M. Ordovas, no conflicts of interest.

⁷ Abbreviations used: APO, apolipoprotein; *CLOCK*, clock circadian regulator; CVD, cardiovascular disease; *LPL*, lipoprotein lipase; miR, micro-RNA; PREDIMED, Prevención con Dieta Mediterránea; SNP, single nucleotide polymorphism; *TCF7L2*, transcription factor 7-like 2; T2D, type 2 diabetes.

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Whereas these regional alternatives to the global recommendations may facilitate the adherence of the particular populations to a traditional, balanced, and healthy diet, they still fall short of addressing the more specific individual requirements. To address this gap, nutrition researchers have adopted genetics to gain additional understanding about the mechanisms associated with the interindividual variability in dietary response and to develop more personalized dietary recommendations.

Nutrigenetics and Individual Variability in Dietary Response

Although the average response to a dietary intervention can be predicted for groups of individuals, the specific response for each individual in a group varies dramatically. It was suggested that these interindividual differences may be partially engrained in an individual's genetic makeup. The science that pursues the identification of informative genetic variants responsible for these gene–diet interactions is known as nutrigenetics.

Early nutrigenetic studies focused on the interaction between common gene variants at candidate genes [mostly single nucleotide polymorphisms (SNPs)] and dietary fats modulating the response of traditional CVD risk factors (i.e., plasma lipoprotein concentrations). Most of the candidate genes known during the 1980s and 1990s belonged to the APO family of genes (i.e., *APOA1*, *APOA2*, *APOA4*, *APOB*, *APOC2*, *APOC3*, and *APOE*). The role of APOs in lipoprotein metabolism and heart disease development was studied extensively during those years. Their role in lipid transport was characterized, and deficiencies in different APOs were associated with atherosclerosis, lipid disorders, and CVD. The isolation and characterization of their genes allowed the discovery and the study of several polymorphisms, and some of them were shown to be associated with CVD risk factors (i.e., *APOE* and plasma concentrations of LDL lipoprotein cholesterol). Moreover, this knowledge provided the starting point to investigate whether the reported associations were modified by dietary components, such as FAs and cholesterol.

Among the initial findings, we can highlight those involving functional polymorphisms at the *APOA4* and *APOE* loci, which, in the context of small dietary intervention studies, were found to contribute to the variability in response of plasma lipoprotein concentrations to changes in dietary fat and cholesterol (3).

During the ensuing years, the field of nutritional genomics related to CVD risk expanded to other candidate genes in lipid metabolism, such as those involved in the processing of plasma lipoproteins [i.e., lipoprotein lipase (*LPL*) and hepatic lipase] (4,5). Moreover, the increasing availability of genetic information in epidemiologic studies, such as the Framingham Heart Study, opened the possibility of investigating gene–diet interactions in large cohorts with rich phenotypic and behavioral data. This reached a new scale recently after the creation of research consortia, such as Cohorts for Heart and Aging Research in Genomic Epidemiology consortium (6), which gather tens of thousands of participants.

Although some of the polymorphisms investigated at that time were potentially functional (5,7,8), most of the time,

they did not have a defined function. Therefore, the mechanisms behind statistically significant associations and interactions remained unclear, given our limited knowledge of the genome. In this regard, some of these ambiguities are being answered thanks to the remarkable progress that we are experiencing in the field of genetics. In this regard, we showed recently that the molecular basis for some of the observed effects could involve differential regulation by microRNAs (miRs) (9). Thus, we reported that 1 of the *LPL* polymorphisms shown previously to be associated with plasma lipids and dietary response could exert these effects through the differential binding of miRs. Specifically, the *LPL* rs13702 minor allele had been predicted to disrupt an miR recognition element seed site for miR-410, resulting in highly significant and beneficial associations with plasma TG and HDL cholesterol concentrations. Moreover, meta-analyses demonstrated a significant interaction between rs13702 and dietary PUFA with respect to TGs. These results suggest that rs13702 induces the allele-specific regulation of *LPL* by miR-410 in humans and provides mechanistic explanation for some of the previously reported *LPL* variants associated with plasma lipid phenotypes and gene–diet interactions.

The investigation of gene–diet interactions kept embracing newly discovered candidate genes, including those belonging to the *PPAR* (peroxisome proliferator activated receptor) family of transcription factors (8,10–13), as well as those involved in inflammation-related pathways (14). However, we need to keep in mind that those pioneering studies were performed with suboptimal samples sizes, and, in general, the level of replication of the initial findings was very low, most probably because of the aforementioned low statistical power affecting those studies. In fact, this lack of replication has been of great concern in the field and 1 of the major barriers affecting the progress of nutrigenetics and its translation to clinical applications. More recently, the combination of technologic advances and their application to increasingly larger populations has driven the revitalization of the field, resulting in more consistent findings. One such example is the interaction between a functional *APOA2* gene variant [*APOA2* –265T > C (rs5082)], dietary SFAs, and BMI. We analyzed gene–diet interactions between the *APOA2* –265T > C polymorphism and SFA intake on BMI and obesity in 3462 individuals from 3 American populations: 1) Framingham Heart Study (1454 whites); 2) Genetics of Lipid Lowering Drugs and Diet Network (1078 whites); and 3) Boston–Puerto Rican studies (930 Hispanics of Caribbean origin). We found that the magnitude of the difference in BMI between the CC and TT + TC participants differed with the SFA intake. We observed a mean increase of 6.2% BMI between genotypes when SFA consumption was high (≥ 22 g/d), but this was not the case when the SFA consumption was low. Likewise, the CC genotype was significantly associated with higher obesity prevalence in all populations only in case of high SFA intake (15). This was the first work that consistently replicated a gene–diet interaction influencing BMI and obesity in 3 independent populations. Moreover, this replication was further extended to

other geographical areas and ethnic groups (16). More recently, we showed that this interaction, initially observed through analysis of nutrients (i.e., SFA), can be applied to specific food groups, such as dairy products (17).

A novel area of development in the field of personalized nutrition includes the time factor. This is the result of the increasing interest in chronobiology and the demonstration that chronodisruption is associated with the development of chronic diseases. In this regard, recent genome-wide association studies showed associations between *CLOCK* (clock circadian regulator) genes and fasting glucose concentrations, obesity, and metabolic syndrome, highlighting the impact of circadian systems on human disease. Our own research shows that *CLOCK* SNPs (i.e., rs4580704 and rs1801260) were associated with BMI and other variables related to glucose and insulin resistance. Furthermore, we found an interaction between those SNPs and fat intake, such that the associations of these SNPs with plasma glucose, insulin resistance, and anthropometric traits were modulated by the MUFA and SFA intakes (18). Furthermore, variation at the *CLOCK* locus was also associated with energy intake (19).

Nutrigenetics, Mediterranean Diet, and CVD

Numerous studies reported significant gene–CVD associations, but the studies involving gene–diet interactions were limited to reporting on CVD risk factors (i.e., obesity, and plasma lipoprotein and glucose concentrations). However, the fundamental objective is to prevent the disease itself. In this regard, it was shown that the Mediterranean diet may reduce the incidence of CVDs, including stroke in the PREDIMED (Prevención con Dieta Mediterránea) study (20). To those cardioprotective effects seen on the entire cohort, we added that those benefits may be magnified in those participants who are genetically predisposed to suffering diabetes (21).

The transcription factor 7-like 2 (*TCF7L2*) gene is the strongest and most widely replicated locus associated with type 2 diabetes (T2D) (22–28). The *TCF7L2* rs7903146 polymorphism in intron 3 (C > T) is 1 of the most important genetic variants influencing T2D risk (28), with T carriers presenting a higher predisposition toward the disease. At present, the prevalence of the 7903146T allele, associated with higher T2D risk, is lower than that of the C allele in whites. In the PREDIMED study, participants (2993 men and 4025 women) were analyzed at baseline and after a 4.8-y follow-up to assess the interaction of the Mediterranean diet with the rs7903146 SNP. We observed that TT individuals with high adherence to the Mediterranean diet [>9 points according to the Trichopoulou score (29)] did not show the expected higher fasting glucose values when compared with C allele carriers. Conversely, when the adherence to the Mediterranean diet was <9 points, the effects of the SNP on fasting glucose concentrations were as predicted. Most important was the novel finding that, in TT individuals at higher risk of diabetes and stroke, a greater adherence to the Mediterranean diet was able to obliterate their genetic risk, yielding these individuals with a similar risk of stroke

than those study participants who did not have the increased genetic predisposition resulting from the *TCF7L2* gene (21).

Therefore, these results support the notion that the genetic predisposition toward CVD could be quenched by dietary components, specifically by a stricter compliance with the Mediterranean diet pattern. However, CVD genetic predisposition is polygenic, and we have to place this contribution in the context of the much bigger picture needed to accomplish significant clinical impact. In this regard, the PREDIMED study has been adding supporting evidence toward the objective inclusion of personalized dietary advice as a key element of disease prevention.

Whereas the *TCF7L2* rs7903146 SNP was shown to increase CVD risk, other genetic variants were found to be protective. This is the case of a functional SNP (rs3812316, C771G, Gln241His) at the *MLXIPL* (Max-like protein X interacting protein-like). This SNP has been associated with lower plasma TG concentrations; however, it is not known whether this results in actual CVD protection. Based also on data from the PREDIMED study, we replicated previous associations with plasma TG concentrations and shown that this association was modified by the adherence to a Mediterranean diet (30). Thus, the potential CVD protection, resulting for the association of this polymorphism with lower TG concentrations, was enhanced in individuals who were compliant with the Mediterranean diet. Most important was the demonstration that the reduction in CVD risk associated with the Mediterranean diet in the whole PREDIMED population was significantly enhanced in carriers of the minor G allele at the *MLXIPL* locus.

In conclusion, despite the slow progress of the early years of nutrigenetics research, we made significant headway toward the practical use of genetic information to tailor dietary recommendations for the prevention of chronic diseases, especially those related with CVDs. The translational goals of this research were described previously (31) and include the following: 1) widespread use of genetic profiles to guide physicians in the classification of patients according to their disease rather than on their symptoms; and 2) use of those genetic profiles to implement targeted recommendations and therapies aimed to improve prevention. To achieve these goals, the following steps will be required: 1) better legislated and more cost-effective genetic testing; 2) deeper and more comprehensive understanding of gene–environment interactions, supported by computational biology; 3) inclusion of genomic medicine in the training of health professionals; 4) sound and balanced education of the general population on topics related with genetics and personalized medicine; and 5) academic–industry partnerships for the design of personalized diets, including the use of new functional foods and more comprehensive approaches based on family-based behavioral changes. This will be possible through multidisciplinary collaborations between basic and applied scientists, physicians, nutritionists, psychologists, pharmaceutical laboratories, food industries, information technologists, policy makers, and health educators. This collaboration should be present at both sides of the spectrum, from the conception

of new research experimental designs to the final practical implementation of the findings for the benefit of the individual consumer.

Acknowledgments

All authors read and approved the final version of the manuscript.

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