

# Gender, a Significant Factor in the Cross Talk Between Genes, Environment, and Health

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## ABSTRACT

**Background:** Although men and women share most genetic information, they have significantly different disease susceptibilities that go well beyond the expected gender-specific diseases. Sex influences the risk of nearly all common diseases that affect both men and women, including atherosclerosis and diabetes and their preceding risk factors (eg, hyperlipidemia, insulin resistance, and obesity).

**Objective:** The goal of this article was to examine the interplay between genes, gender, and disease susceptibility, and assess it in the context of the added complexity of environmental factors (ie, dietary habits, smoking, alcohol consumption) in the modulation of the balance between health and disease.

**Methods:** Original and review articles published by the author were reexamined for evidence of gene-gender interactions.

**Results:** Evidence from some key factors in lipid metabolism (apolipoprotein E [*APOE*]) and obesity (perilipin [*PLIN*]) indicates that the interplay between genes, gender, and environmental factors modulates disease susceptibility. In the Framingham Heart Study, complex interactions have been shown between a promoter polymorphism at the apolipoprotein A1 gene, gender, and dietary polyunsaturated fatty acid intake that modulate plasma concentrations of high-density lipoprotein cholesterol. Likewise, highly and clinically relevant interactions have been observed between the *APOE* gene common alleles *APOE2*, *APOE3*, and *APOE4*, gender, and smoking that determine cardiovascular disease risk. Most interesting is the gender-dependent association between common polymorphisms at the *PLIN* locus and obesity risk that has been replicated in several populations around the world.

**Conclusions:** These data support the idea that gender-specific differences in morbidity and mortality may be mediated in part by genetic factors and by their differential response to the environment. The new knowledge generated by a more careful and complete elucidation of the complex interactions predisposing to common diseases will result in an increased ability to provide successful personalized behavioral recommendations to prevent chronic disorders. (*Gend Med.* 2007;4[Suppl B]:S111-S121) Copyright © 2007 Excerpta Medica, Inc.

**Key words:** nutrigenomics, gender, cardiovascular disease, diet therapy, obesity, lipoproteins.

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## INTRODUCTION

Although men and women share most genetic information, they have dramatically different disease susceptibilities that go well beyond the expected gender-specific diseases (ie, cervical or prostate cancer).<sup>1</sup> Sex influences susceptibility to nearly all common diseases that affect both men and women, including atherosclerosis and diabetes and their preceding risk factors (eg, hyperlipidemia, insulin resistance, and obesity). These are all known to be highly complex and multifactorial in their origin, involving genetic factors but also a myriad of environmental and behavioral factors that interact with the genetic component, which itself is highly polygenic. This complexity underlies the poor replication obtained for most candidate gene association studies examining common diseases and their predisposing risk factors.<sup>2–4</sup>

For many years, scores of studies have found gender-specific associations with risk factors and their associated diseases.<sup>5</sup> More attention should be paid to these enticing and relevant observations. Better understanding and greater recognition of the significance of specific disease-associated genetic polymorphisms in the context of sex is of critical public health and clinical importance. The goal of this article is to highlight this interplay between genes, gender, and disease susceptibility, and assess it in the context of the additional complexity associated with other environmental factors (ie, dietary habits, smoking, alcohol consumption) in the modulation of the balance between health and disease. Rather than conducting an extensive review of the literature about gender interactions, this article focuses on a few examples that provide strong support for the concept of high-level interactions involving genes, gender, and environment.

## METHODS

A compilation of original and review articles published by the author was searched for evidence of gene–gender interactions.

## APOLIPOPROTEIN E: POSTER CHILD FOR HIGHLY COMPLEX GENE–GENDER–ENVIRONMENT INTERACTIONS

Apolipoprotein E (*APOE*) is associated in serum with chylomicrons, very-low-density lipoproteins, and high-density lipoproteins (HDLs), and serves as a ligand for the low-density lipoprotein (LDL) receptor and the LDL receptor-related protein.<sup>6,7</sup> The most commonly studied genetic variation at the *APOE* locus results from 3 common alleles in the population: *E4* (representing genotypes *E3E4* and *E4E4*), *E3* (representing *E3E3*), and *E2* (representing *E2/E3* and *E2/E2*), with frequencies among whites of ~0.15%, 0.77%, and 0.08%, respectively, but with significant allelic differences in other racial and ethnic groups.<sup>8</sup>

Population studies have shown that plasma total cholesterol (TC), LDL cholesterol (LDL-C), and apolipoprotein B (APOB) concentrations are highest in individuals carrying the *E4* allele, intermediate in those with the *E3* allele, and lowest in those with the *E2* allele.<sup>9,10</sup> Based on a large body of evidence, it has been estimated that these *APOE* alleles may account for up to 7% of the variation in TC and LDL-C concentrations in the general population, with this effect being substantially greater in women than in men.<sup>10,11</sup> In view of these data, variation at the *APOE* locus may be associated with an individual's cardiovascular disease (CVD) risk. To assess this potential association, the Framingham Offspring Study investigated the relationship between the *APOE* genotype and CVD.<sup>11</sup> Period prevalence of CVD between examinations 1 and 5 (1971–1994) (366 events) was related to *APOE* genotype. Age-adjusted period prevalence of CVD in men was 19% for *E4*, 13% for *E3*, and 18% for *E2* ( $P = 0.004$ ); in women, these rates were 10%, 7%, and 5%, respectively ( $P = 0.04$ ). After adjusting for all CVD risk factors, the relative odds in men with the *E2* allele compared with men with the *E3E3* allele were 1.94 ( $P = 0.004$ ), and in men with *E4* this number was 1.51 ( $P = 0.03$ ). Therefore, the presence of the *E2* or *E4* allele in men was associated with significantly greater CVD risk.

Whereas the *E4* allele was also associated with increased CVD prevalence in women, the *E2* allele was found to be protective, unlike its presence in men.

The gender-*APOE*-disease interaction was also supported by another study testing the hypothesis that the risk of ischemic heart disease (IHD) differs as a function of *APOE* genotype in women and men.<sup>12</sup> These investigators determined the genotypes of 9241 white women and men in the general population and 940 white women and men with IHD. After adjusting for age and other conventional CVD risk factors, the odds ratios (ORs) were 0.38 (95% CI, 0.18–0.79) for women with *E2E3*, 1.35 (95% CI, 1.02–1.78) for men with *E3E4*, and 1.58 (95% CI, 0.80–3.08) for men with *E4E4*. ORs for *E3E4* and *E4E4* versus *E3E3* women and for *E2E3* versus *E3E3* men were nonsignificant. Therefore, the presence of the *E2E3* genotype was protective in women (9% lower relative risk of IHD) compared with *E3E3*, whereas in men, *E3E4* and *E4E4* were associated with increased risk (8% and 2%, respectively). Thus, the Framingham Heart Study<sup>11</sup> and the Copenhagen City Heart Study<sup>12</sup> showed gene-gender interaction for CVD and IHD, respectively. Moreover, the gender specificity of some of the reported associations was not restricted to the traditional *E2*, *E3*, and *E4* alleles, but extended to other single nucleotide polymorphisms (SNPs) in the *APOE* locus.<sup>13</sup>

In addition to the described gender-specific effects, the *APOE*-related CVD risk varies from population to population, being relatively high in those with clustering of CVD risk factors (atherogenic diet, sedentary lifestyle, smoking, and obesity) and almost negligible in those with low metabolic stress. Plasma lipid responses to dietary therapeutic intervention have been reported to be greater in subjects carrying the *E4* allele; however, this has not been a consistent finding.<sup>4,14</sup> Important differences among studies could account for some of the discrepancies observed: they differed in gender, age, and baseline lipid concentrations, all of which are known to play an important role in the vari-

ability of dietary response. Moreover, it has been shown that the *APOE*-dependent mechanism may be specific for large, buoyant LDL particles.<sup>15,16</sup> Consequently, baseline LDL particle distribution, which is also gender dependent,<sup>17</sup> also plays a significant role in the outcome of different studies.

A significant diet-*APOE* gene interaction has been shown in studies of men alone.<sup>4,14</sup> In other studies that included men and women, significant effects were also noted only in men, again suggesting a significant gene-sex interaction. Another difference between those studies reporting significant *APOE* gene-diet interactions and those that failed to identify such interactions was related to the baseline lipid concentrations of the subjects. Studies reporting significant associations often included subjects who were moderately hypercholesterolemic and/or had significant differences in baseline TC and LDL-C concentrations among the *APOE* genotype groups, suggesting that the significant gene-diet interaction is apparent only in individuals who are susceptible to hypercholesterolemia. With regard to differences in dietary interventions, significant interactions were more commonly observed in studies in which total dietary fat and cholesterol were modified. It is possible that dietary cholesterol may have a significant effect in this gene-diet interaction. Some studies have found that cholesterol absorption, a significant determinant of plasma cholesterol concentration, is related to *APOE* genotype. An *APOE*-gender interaction modulating the lipid response to hypolipemic drugs (ie, atorvastatin) has also been observed.<sup>18</sup>

Another report from the Framingham Offspring Study investigated the interaction between alcohol consumption and LDL-C concentration.<sup>19</sup> In this study, men who reported themselves to be nondrinkers did not show the traditional association between the *APOE* alleles and LDL-C concentrations (*E2*<<*E3*<*E4*). Conversely, in those classified as drinkers, the *E4* allele was associated with significantly elevated LDL-C concentrations, resulting in a significant *APOE*-drinking-LDL-C interaction ( $P <$

0.001) after adjusting for age, body mass index (BMI), smoking, and diet. However, no such interaction was observed in women—those who reported drinking alcohol generally had lower LDL-C concentrations independent of *APOE* genotype. This interaction was also examined in Spain.<sup>20</sup> The expected effect of the *APOE* genotype on LDL-C concentration was observed in both men and women. However, women drinkers who carried either the *E2* or the *E4* allele had lower LDL-C concentrations than did nondrinkers.

Smoking has also been shown to be a potentially important modulator of the effect of *APOE*.<sup>21</sup> Based on the evidence, data from the Framingham Offspring Study were reanalyzed, specifically examining *APOE*–smoking interactions.<sup>22</sup> No such interactions were found in women, but in 1668 men (316 CVD events) the overall hazard ratio (HR) for smoking was 1.95 (95% CI, 1.52–2.50) compared with nonsmokers. Using *E3E3* as the reference group, in nonsmokers the HRs for *E2* carriers (1.04 [0.61–1.76]) and *E4* carriers (1.04 [0.70–1.54]) showed no major risk increase. In smokers, the HRs were 1.96 (1.26–2.78) for men with *E3E3*, 3.46 (2.14–5.60;  $P = 0.09$  for interaction) for men with *E2*, and 3.81 (2.49–5.84;  $P = 0.01$  for interaction) for men with *E4*, with a significant interaction between daily cigarette consumption and *APOE* genotype on risk ( $P = 0.03$ ).

Overall, the data suggest significant interactions between the *APOE* gene and behavioral factors. However, the fact that several of these factors have the potential to interact and that they may be distributed differently among populations may result in one of the factors (ie, drinking alcohol, smoking) having more weight in some populations than in others. In this regard, Lussier-Cacan et al<sup>23</sup> examined data from a sample of ~1700 men and women who participated in the Quebec Heart Health Survey. In this population, there was no evidence that the *APOE* gene affected the LDL-C and HDL-C associations with alcohol after adjusting for age and BMI. However, in women carriers of the *E4* allele, the authors found a significant alcohol–

BMI interaction on TC, LDL-C, HDL-C, *APOA1*, and *APOB* (all,  $P < 0.01$ ). Furthermore, this interaction was influenced by smoking. Taken together, these reports highlight the complexity of the interactions, and the gender and context dependency of the influence of alcohol on lipid metabolism and of smoking on CVD risk.

Another behavioral factor that has been receiving increased and well-deserved attention relates to physical activity. Obtaining reliable information about this variable in large population studies is usually difficult, especially when the studies have not been specifically designed to have physical activity as a major variable. Despite those limitations, at least 2 studies have shown a reproducible interaction between *APOE* genotype and the effect of physical activity on plasma lipid concentrations. The first study reported that the association between HDL-C concentration and physical activity (energy expenditure) was *APOE* dependent in a Spanish population.<sup>20</sup> This interaction was confirmed and examined in more detail by Bernstein et al,<sup>24</sup> who investigated this interaction in a population-based cross-sectional survey that included 1708 men and women aged 35 to 74 years. Similar to those described for alcohol consumption, smoking, and BMI, the findings were gender dependent. For men, increased physical activity had a greater protective effect in *E4* carriers compared with *E3* homozygotes and *E2* carriers in terms of increases in HDL-C concentrations and decreases in triglyceride concentrations. In women, the protective effect of exercise on *E4* carriers was limited to HDL-C, and it was significant only for the difference versus carriers of the *E2* allele. There also appears to be a significant interaction between exercise training and *APOE* genotype.<sup>25</sup>

Several other *APOE*–gender associations have been reported for a variety of other diseases, including those of neurologic origin,<sup>26,27</sup> cancer,<sup>28</sup> osteoporosis,<sup>29</sup> vision,<sup>30</sup> and overall longevity.<sup>31,32</sup> However, the potential modulation of these associations by diet and other behavioral aspects remains mostly unexplored.

### **APOLIPOPROTEIN A1: ANOTHER REVEALING EXAMPLE OF COMPLEX INTERACTIONS INVOLVING GENES, GENDER, AND CVD-RELATED TRAITS**

APOE was mentioned previously as an example of the multiple interactions that exist between genes, gender, and environment that modulate a number of risk factors and disease outcomes. Another such example is the interaction between the apolipoprotein A1 (*APOA1*) gene, dietary fat, and gender-specific modulation of HDL-C concentration.

*APOA1* is the major protein of HDL, an in vivo activator of the enzyme lecithin-cholesterol acyltransferase, and constitutes a key component of the reverse cholesterol transport process. The *APOA1* gene is clustered with the *APOC3*, *APOA4*, and *APOA5* genes on the long arm of human chromosome 11. This DNA region has been extensively analyzed, and multiple reports have shown associations between some of the common genetic variants and lipid abnormalities, as well as increased coronary heart disease risk,<sup>33</sup> although the results have been inconsistent. Moreover, several rare genetic abnormalities at this locus have been associated with severe HDL deficiency and with premature coronary atherosclerosis.<sup>34</sup>

A common variant due to adenine (A)-to-guanine (G) transition (G/A) was described more than 2 decades ago 75 bp upstream from the *APOA1* gene transcription start site. Several studies have reported that individuals with the A allele, which occurs at a frequency of 0.15% to 0.20% among whites, have higher concentrations of HDL-C than do individuals who are homozygous for the most common G allele.<sup>35</sup> However, the magnitude of the effects reported has differed among studies. A meta-analysis examined the associations of this SNP with plasma lipid profiles and concluded that there may be a mild association with plasma *APOA1* concentration that is more apparent in men than in women. Therefore, although dietary fatty acids and the -75G/A *APOA1* SNP can each influence plasma lipoprotein concentrations, studies examining the effect of each

factor separately have produced conflicting results.

In particular, evidence regarding the effect of dietary polyunsaturated fatty acids (PUFAs) on HDL-C concentration is conflicting. Increased dietary PUFA was believed to lower plasma HDL-C concentration; however, analysis of allelic variations in the *APOA1* gene and dietary PUFA in a subset of the Framingham Offspring Study challenged this association.<sup>36</sup> In this analysis of 755 men and 822 women for whom complete genetic, biochemical, and dietary information was available, the association of higher dietary PUFA with lower HDL-C concentration was observed for female G/G homozygotes, but not for female carriers of the A allele (A/A or G/A). Because the homozygous G/G genotype accounts for ~70% of the general population, it is not surprising that studies examining only the association between PUFA and HDL-C concentrations, without considering *APOA1* genotype, would sometimes fail to detect an HDL-lowering effect of PUFA.

The Framingham analysis uncovered the differential response to dietary PUFA between female carriers of the A allele (A/A or G/A) and female G/G homozygotes. This study is particularly interesting because it describes a sex-specific differential effect of dietary PUFA on HDL-C concentration. Female G/G homozygotes had higher HDL-C concentrations than did female carriers of the A allele when dietary PUFA was <4% of total energy, but the opposite was true when dietary PUFA was >8% of total energy. In males, the interaction between PUFA and -75G/A polymorphism was statistically significant only when alcohol consumption and smoking were factored into the regression model, confirming the importance of analyzing men and women separately. In this setting, higher PUFA intake benefits female carriers of the A allele by increasing their HDL-C concentration and thus reducing their CVD risk. In addition, no difference was observed between genotypes for moderate dietary PUFA (4%–8% of total energy), which is consistent with previous studies that reported no effect of HDL-C

related to allelic variation.<sup>35</sup> The findings of this study may explain the conflicting evidence from previous studies that did not examine gene–diet interactions in a sex-specific manner.

In summary, the mechanisms responsible for the observed effect are still unknown. This mutation may have a direct effect on liver and/or intestinal *APOA1* gene expression, as suggested in previous studies, or it may be in linkage disequilibrium with a functional mutation in any of the neighboring genes (*APOC3*, *APOA4*, or *APOA5*).

### **Genes, Gender, and Obesity-Related Traits**

The World Health Organization identified obesity as “one of today’s most blatantly visible—yet most neglected—public health problems.”<sup>37</sup> This rising epidemic of overweight and obesity has been termed “globesity” to emphasize that this is a global problem and that, unless action is taken, billions of people will suffer from debilitating conditions associated with this disorder.

Like most other disorders affecting the well-being of the population at large, obesity is a complex condition in which psychological, physiological, and social determinants interact to produce the undesired end point. Obesity is believed to constitute a major risk factor for age-related disorders (eg, diabetes mellitus, CVD, hypertension and stroke, and certain forms of cancer). All of these disorders are associated with an increased risk of premature death and with chronic conditions that reduce overall quality of life.<sup>38,39</sup>

More than 1.1 billion people worldwide are classified as overweight or obese. The question is obvious: How and why did the world become so fat? There is a simple overarching answer—too many calories and too little exercise trigger biochemical and physiological changes that lead to overweight and obesity. However, at the individual level the answer is much more complex, as highlighted by the very limited long-term success achieved by weight-reducing therapies. At the individual level, obesity is

determined by a combination of nurture and nature (ie, a combination of environmental and genetic factors). The major environmental factors are high caloric intake and too little activity. However, we still do not fully understand the genetic factors that predispose some people to obesity, especially in certain ethnic groups. Moreover, we do not have sufficient knowledge of the mechanisms by which gender-related factors may determine the expression of relevant phenotypes and comorbidities.<sup>40</sup>

In general, women have both a higher body fat content and a different fat distribution than do men. Women store more fat in subcutaneous depots, mainly in the gluteal-femoral region, and men store more fat in visceral (abdominal) depots. There are significant sex differences in fat metabolism, some of which are probably due to the different fat distributions in each sex. However, even for the same fat depot, sex-dependent differences in fat metabolism and gene expression have been reported.<sup>40</sup> Despite this general knowledge, and, consistent with the problem raised previously in relation to lipid metabolism, little attention has been paid to the gender-specific differences related to the genetics of obesity.

A better understanding of the mechanisms involved in energy metabolism in general and fat metabolism in particular is vital to providing better therapeutic tools to control obesity at both the personal and population levels. A better understanding of the workings of adipocytes may hold the answer to many unknown issues. Beliefs regarding adipocytes have changed dramatically in the past few years. Adipocytes are no longer viewed as passive energy storage tissue; they now are known to produce a number of metabolically and hormonally active substances, collectively termed adipokines or adipocytokines.<sup>41</sup> These adipokines allow the adipocyte to initiate potent feedback actions in the regulation of appetite, food intake, glucose disposal, and energy expenditure. They protect against the establishment of insulin resistance by acting on liver, skeletal muscle, and pancreatic function. They also contribute to the pre-

vention or worsening of atherogenic processes. In addition, some of the factors secreted by adipocytes exert local autocrine and paracrine actions that mainly affect adipose tissue remodeling, adipogenesis, and angiogenesis, and are not found in the blood compartment.

Examples of such significant sex differences in human adipocyte gene expression have been reported for uncoupling protein 2, leptin, resistin, aromatase, agouti signaling protein, and thrombospondin-1.<sup>40</sup> These findings indicate that there is a need for more systematic studies of sex-dependent variability of adipose gene expression. The identification of sex-dependent differences in gene expression may explain the molecular pathways involved in the sex-dependent differences of adipose tissue metabolism.

Perilipin (PLIN) is one of the most abundant proteins in the adipocyte lipid droplet. In the late 1980s, Londos et al<sup>42</sup> first identified a target of protein kinase that they named PLIN A, because of its physical location surrounding lipid droplets. PLIN was found only in adipocytes and in steroidogenic cells. Similar to adipocytes, steroidogenic cells possess intracellular neutral lipid storage deposits that are metabolized by a common enzyme–hormone-sensitive lipase in adipocytes and cholesterol esterase in steroidogenic cells. It was concluded that PLIN A played a critical role in the hydrolysis of neutral lipids.

Common variants at the *PLIN* locus were associated with BMI and obesity risk exclusively in females in 3 studies conducted in European, American, and Asian populations encompassing 4 major ethnic groups (whites, Chinese, Asian Indians, and Malays).<sup>43–45</sup> The first report assessed the association between several polymorphisms at the *PLIN* locus (*PLIN1*: 6209T>C; *PLIN4*: 11482G>A; *PLIN5*: 13041A>G; and *PLIN6*: 14995A>T) and obesity-related phenotypes in 1589 white subjects randomly selected from a general Spanish population.<sup>45</sup> In women (n = 801), the less common alleles of *PLIN1* and *PLIN4*, which were in strong linkage disequilibrium, were significantly associated with lower BMI. Carriers of allele 2 (6209C) at the *PLIN1* locus weighed significantly less (2.2 kg;  $P =$

0.007) than did women homozygous for the normal genotype. The same was true for 11482A carriers at *PLIN4* ( $P = 0.01$ ). Moreover, this *PLIN4* variant was associated with significantly lower waist-to-hip ratio ( $P < 0.03$ ) and plasma glucose ( $P < 0.008$ ) and triglyceride ( $P < 0.005$ ) concentrations. No significant associations with these obesity-related phenotypes were found in men. Supporting these results, statistically significant gene–gender interactions were obtained when the risk of obesity was estimated (281 subjects were obese and 1308 were nonobese). Only in women were *PLIN1* and *PLIN4* variant alleles (6209C and 11482A) associated with a lower obesity risk (OR = 0.58 [95% CI, 0.38–0.93] and OR = 0.56 [95% CI, 0.36–0.89], respectively). This association became stronger when both *PLIN1* and *PLIN4* were combined. In summary, the initial data supported the notion that common alleles at the *PLIN* locus modulate body weight and metabolic variables in humans.

In the second report, the same 4 *PLIN* SNPs were genotyped in 734 white subjects (373 men and 361 women) attending a residential lifestyle intervention program.<sup>44</sup> Baseline anthropometric and biochemical measures were used. Obesity was defined as BMI >30 kg/m<sup>2</sup>. Multivariate analysis demonstrated that in women, 2 of the SNPs, *PLIN5* and *PLIN6*, were significantly associated with percentage of body fat ( $P < 0.016$  and  $P < 0.010$ , respectively) and waist circumference ( $P < 0.020$  and  $P < 0.045$ , respectively). Moreover, haplotype analysis using these 2 SNPs indicated that haplotypes A/T and G/T were both associated with significantly increased obesity risk (OR = 1.76 [95% CI, 1.07–2.90] and OR = 1.73 [95% CI, 1.06–2.82], respectively) when compared with haplotype A/A. No significant associations between *PLIN* variations and obesity were found in men. These data support the hypothesis that the *PLIN* locus may be a significant genetic determinant for obesity risk in whites, and women are more sensitive to the genetic effects of *PLIN* than are men.

In the third report, research was extended to nonwhite populations and examined 5 common *PLIN* SNPs, which included the 4 previous

variants plus the ethnic-specific 10171A>T, to define their association with obesity risk.<sup>43</sup> The study population involved 4131 subjects in 3 ethnic groups (Chinese, Malay, and Asian Indian) in Singapore. The prevalence of obesity in Malays and Indians was much higher than in Chinese subjects. Moreover, in these 2 groups, the obesity prevalence was 3 times higher in females than in males. Crude analysis indicated that haplotype 11212 (CAAAT) was shared by both Malays and Indians and was significantly associated with increased obesity risk compared with the most common haplotype 21111 (TAGAA) (OR = 1.65 [95% CI, 1.11–2.46] for Malays and OR = 1.94 [95% CI, 1.06–3.53] for Indians). No associations between *PLIN* haplotypes and obesity risk were found in the Chinese subjects. To simplify the haplotype determinations, further haplotype analyses using a subgroup of 3 SNPs (11482G>A, 13041A>G, and 14995A>T) in positive linkage disequilibrium revealed similar associations, indicating that haplotypes XX212 (XXAAT) and XX222 (XXAGT) were associated with increased obesity risk in Malays (OR = 2.04 [95% CI, 1.28–3.25] and OR = 2.05 [95% CI, 1.35–3.12], respectively) and that haplotype XXX212 (XXAAT) was significantly associated with increased obesity risk in Indians (OR = 2.16 [95% CI, 1.10–4.26]), after adjusting for covariates (ie, age, sex, smoking, alcohol consumption, exercise, and diabetes status). Moreover, individual SNP analyses demonstrated that the *PLIN* 14995A>T SNP was the most significant genetic contributor to the observed haplotype association, being significantly associated with increased obesity risk in both Malays (OR = 2.28 [95% CI, 1.45–3.57]) and Indians (OR = 2.04 [95% CI, 1.08–3.84]). These findings support the role of the *PLIN* locus as an ethnic-dependent modulator of obesity risk in humans.

In addition to the associations described previously between *PLIN* genotypes and obesity-related phenotypes, *PLIN*–diet interactions related to weight loss and other metabolic syndrome-related variables were also investigated. The association of the previously reported polymorphisms at the *PLIN* locus with obesity and

weight reduction in response to a low-energy diet in obese patients (mean [SD] BMI, 42 [8] kg/m<sup>2</sup>) was examined.<sup>46</sup> In these patients (n = 150), the minor A-allele at *PLIN4* was associated, as previously reported in the general population, with lower baseline body weight. Moreover, a gene–diet interaction ( $P = 0.015$ ) was found between this polymorphism and weight loss in patients who completed the 1-year dietary follow-up treatment consisting of a low-energy diet. This dietary treatment resulted in significant mean (SD) decreases in body weight (from 114.3 [3.9] kg at baseline to 105.5 [3.5] kg at 1 year;  $P$ -lineal trend [ $P$  value in the body weight trend over time] = 0.020) in G/G patients (n = 33). Conversely, carriers of the minor A-allele (n = 15) did not show significant changes in body weight (from 105.0 [4.6] kg at baseline to 104.3 [4.4] kg at 1 year;  $P$ -lineal trend = 0.985). Thus, *PLIN4* carriers were resistant to weight loss, suggesting that this polymorphism may predict outcome of weight reduction strategies based on low-energy diets.

Some inconsistent associations with other elements of the metabolic syndrome were observed in addition to the consistent associations between *PLIN* and BMI and obesity. Therefore, the association between *PLIN* variants and insulin resistance, as well as potential modulation by macronutrient intake, was investigated.<sup>47</sup> For this purpose, a total of 1909 men and 2198 women (aged 18–69 years) participating in the Singapore National Health Survey were studied. Homeostasis model assessment of insulin resistance (HOMA-IR) was used to evaluate insulin resistance. Diet was measured by a validated food frequency questionnaire in 1 of every 2 subjects. Although a significant between-genotype difference in insulin resistance measures was not observed, statistically significant gene–diet interactions (recessive model) between *PLIN4/PLIN6* polymorphisms (in high linkage disequilibrium in this population) and saturated fatty acids (SFAs) ( $P = 0.003/0.005$ ) and carbohydrate ( $P = 0.004/0.012$ ) in determining HOMA-IR were found in women. These interactions were in opposite directions and were more significant for *PLIN4*. Women in

the highest SFA tertile (11.8%–19%) had higher HOMA-IR (48% increase;  $P = 0.006$ ) than did women in the lowest tertile (3.1%–9.4%), only if they were homozygous for the *PLIN* minor allele. Conversely, HOMA-IR decreased (24%;  $P = 0.046$ ) as carbohydrate intake increased. These effects were stronger when SFAs and carbohydrate were combined as an SFA-to-carbohydrate ratio. Moreover, this gene–diet interaction was homogeneously found across the 3 ethnic groups. Thus, the *PLIN4/PLIN6* polymorphisms were found to modulate the association between SFAs/carbohydrate in diet and insulin resistance in Asian women. These findings support the notion that dietary recommendations to improve insulin resistance should encompass more personalized guidelines, considering relevant genetic information and gender-specific effects.

### Questions and Challenges

Gender-specific differences in morbidity and mortality may be mediated in part by genetic factors and by their differential response to the environment. Evidence already exists for differing pharmacogenetic responses to lipid-lowering drugs in men and women,<sup>18,48,49</sup> and similar observations exist for dietary responses.<sup>50</sup> However, we still need to replicate interesting findings and to define the functional basis for these gender-specific effects. In fact, a recent review of the literature pertinent to gender differences in genetic association studies concluded that in a sample of prominent claims of sex-related differences in genetic associations, most claims were insufficiently documented or spurious, and claims with documented good internal and external validity were uncommon.<sup>51</sup> However, it should be noted that the review omitted some published papers that have clearly shown and replicated significant gender-related differences.<sup>43–45,47</sup>

In the future, we face multiple challenges:

(1) The recent interest in nutrigenomics is beginning to reveal gene–diet interactions that could be of interest in creating more individualized and effective dietary therapy. From the

emerging knowledge, we are beginning to uncover another layer of complexity involving the presence of 3-way interactions. This would suggest that the differences in response to therapeutic modifications associated with specific genetic mutations may affect men and women differently.

(2) Much of the nutrient information used for nutrigenomic studies in large population studies is obtained from dietary questionnaires. It remains to be determined whether the validity of these data is similar for men and women. If one of the sexes provides less-reliable information on questionnaires than the other does, this would be an important confounder for the outcome of these studies. This underscores the importance of developing reliable biomarkers of dietary intake that are more sensitive and objective than the current instruments.

(3) Gender-related differences in dietary compliance and in adherence to dietary recommendations are not well understood. Again, some consensus is emerging about the need for gender-specific messages to increase the success of dietary modification programs.

(4) Despite the potential confounder of differences in reporting and/or adhering to diets, there is substantial evidence to support intrinsic gender differences in dietary response.

(5) Lastly, we need to remember that women have different dietary needs at different life stages, and that the ultimate goal is to maintain optimal health at all stages. Therefore, recommendations to decrease the risk of a specific age-related disorder (eg, CVD) should take into consideration the effects on other disorders (eg, cancer, osteoporosis, and neurologic disorders).

The resolution of these and other challenges will only be possible through the close interaction and collaboration of researchers and professionals representing a wide range of expertise and knowledge.

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