

# Perilipin Gene Variation Determines Higher Susceptibility to Insulin Resistance in Asian Women When Consuming a High-Saturated Fat, Low-Carbohydrate Diet

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**OBJECTIVE** — To investigate the association between genetic variation in the adipocyte protein perilipin (*PLIN*) and insulin resistance in an Asian population as well as to examine their modulation by macronutrient intake.

**RESEARCH DESIGN AND METHODS** — A nationally representative sample (Chinese, Malays, and Indians) was selected in the Singapore National Health Survey following the World Health Organization–recommended model for field surveys of diabetes. A total of 1,909 men and 2,198 women (aged 18–69 years) were studied. Genetic (*PLIN* 11482G→A and 14995A→T), lifestyle, clinical, and biochemical data were obtained. Homeostasis model assessment of insulin resistance (HOMA-IR) was used to evaluate insulin resistance. Diet was measured by a validated food frequency questionnaire in one of every two subjects.

**RESULTS** — We did not find a significant between-genotype difference in insulin resistance measures. However, in women we found statistically significant gene-diet interactions (recessive model) between *PLIN* 11482G→A/14995A→T polymorphisms (in high linkage disequilibrium) and saturated fatty acids (SFAs;  $P = 0.003/0.005$ ) and carbohydrate ( $P = 0.004/0.012$ ) in determining HOMA-IR. These interactions were in opposite directions and were more significant for 11482G→A, considered the tag polymorphism. Thus, women in the highest SFA tertile (11.8–19%) had higher HOMA-IR (48% increase;  $P$  trend = 0.006) than women in the lowest (3.1–9.4%) only if they were homozygotes for the *PLIN* minor allele. Conversely, HOMA-IR decreased (–24%;  $P$  trend = 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 three ethnic groups.

**CONCLUSIONS** — *PLIN* 11482G→A/14995A→T polymorphisms modulate the association between SFAs/carbohydrate in diet and insulin resistance in Asian women.

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**Abbreviations:** HOMA-IR, homeostasis model assessment of insulin resistance; LD, linkage disequilibrium; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

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A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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**D**ramatic increases of diabetes prevalence are seen in some parts of Asia (1). Socioeconomic development, associated with changes in dietary patterns, and abdominal obesity may be key underlying factors for the development of insulin resistance and diabetes (2). The relation between adipocyte metabolism and insulin resistance suggests that some of the genetic components involved in insulin resistance may be related to genes primarily expressed in adipocytes (3). Perilipin is the predominant protein associated with adipocyte lipid droplets and has a key role in regulating adipocyte lipid storage and body fat accumulation (4–8). Consistent with those functional observations, we have found significant associations between perilipin (*PLIN*) genetic variants and body weight in several populations (9–11). However, their relation to insulin resistance and diabetes was not analyzed.

The connection between perilipin, body fat, and insulin resistance has been shown in knockout mouse models, which displayed reduced body fat and, paradoxically, an increased risk of glucose intolerance and peripheral insulin resistance (7). In addition, dietary factors play a role in the relationship between perilipin and body weight. Thus, in the same mouse models, ablation of *PLIN* expression was associated with differential sensitivity to obesity induced by a high-fat diet compared with wild-type mice (7). Moreover, we found that a *PLIN* polymorphism modulates weight loss in response to a low-energy diet (12). Therefore, our objectives were 1) to estimate the association between genetic variation at the *PLIN* locus and diabetes and insulin resistance–related measures in a large multiethnic Asian cohort, including Chinese, Malays, and Indians and 2) to examine if dietary intake modulates the associations between the *PLIN* locus and insulin resistance in this population.

## RESEARCH DESIGN AND METHODS

Participants were recruited in the framework of the 1998 Sin-

gapore National Health Survey. The detailed methodology of this survey of a nationally representative sample has been described elsewhere (13). Briefly, the survey protocol was based on the World Health Organization–recommended model for field surveys of diabetes. A stratified and systematic sampling was used to select individuals aged 18–69 years from the National Database on Dwellings, with oversampling of the minority groups (Malays and Indians) to ensure that prevalence estimates were reliable. The ethnic composition of the sample was 64% Chinese, 21% Malays, and 15% Asian Indians and included 4,723 individuals. The study was approved by the Ministry of Health in Singapore, and informed consent was obtained from all participants. We included in our analyses 4,107 subjects (1,909 men and 2,198 women) who had DNA and *PLIN* genotypes successfully determined. Demographic, clinical, biochemical, genetic, and lifestyle data (alcohol and tobacco consumption and physical activity) were obtained in all these participants as previously reported (11,13). There were no differences in these variables between participants and subjects in which genetic analysis was not available.

### Dietary intake

A previously validated food frequency questionnaire (14) was administered to a systematic random sample of the 4,723 participants (1 in 2). This random sample did not differ in any characteristic from the initial sample. A list of 159 individual food items grouped into 23 main food types and 25 subfood types were included in this questionnaire. Each food group was carefully considered for ensuring the representatives of listed foods in the three ethnic groups. Nutrient contents were estimated using the food composition database of the Singapore Ministry of Health. Energy intake, carbohydrate, proteins, total fat, saturated fatty acids (SFAs), mono-unsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs) were computed. To avoid the potential bias caused by the change in habitual dietary patterns in diabetic subjects who know their disease, diet was only analyzed in subjects who initially did not have diagnosis of diabetes. The gene-diet interaction analysis was carried out in 2,154 participants (1,001 men and 1,153 women).

### Anthropometrical and biochemical measurements

Weight, height, total cholesterol, and triglycerides were determined as previously described (11,13). Fasting glucose (Boehringer Mannheim, Mannheim, Germany) and fasting insulin (immunoassay using an Abbott AxSYM; Abbott Laboratories, Chicago, IL) were determined in all participants (13). After the fasting sample collection, a 75-g oral glucose tolerance test was taken for all subjects except diabetic subjects on medication ( $n = 3,884$ ). A total of 75 g glucose dissolved in 250 ml of water was ingested. After 2 h, a second blood sample was obtained, and glucose (2-h glucose) and insulin (2-h insulin) were measured. Insulin resistance (fasting glucose  $\times$  fasting insulin/22.5) was calculated using the homeostasis model assessment of insulin resistance (HOMA-IR) method (15). Initially, previously diagnosed diabetes was assessed by questionnaire. In addition, following biochemical determinations, subjects with fasting glucose  $\geq 7.0$  mmol/l or 2-h glucose  $\geq 11.1$  mmol/l were also classified as having diabetes (16).

### Genotyping

DNA was isolated from blood, and, based on our previous results, two common *PLIN* polymorphisms (*PLIN* 11482G $\rightarrow$ A and *PLIN* 14995A $\rightarrow$ T) were determined by multiplex PCR on an ABI Prism 3100 genetic analyzer using Genotyper Version 3.7 (Applied Biosystems, Foster City, CA) as previously described (11).

### Statistical analyses

A general inheritance model was fitted and a recessive model was finally used based on observed effects. Multiple linear regression models with interaction terms were used to analyze the associations and interactions between genotypes and insulin resistance. Estimated means were adjusted for age, BMI, ethnicity, smoking, alcohol consumption, and exercise. Additional adjustment for energy intake and other macronutrients (total fat or carbohydrate) was included in the dietary analysis as indicated. Dietary fat (total, SFAs, MUFAs, or PUFAs) and carbohydrate intake were first treated as categorical according to the population tertiles. In addition, the SFA-to-carbohydrate ratio was computed as continuous. Logarithm transformation was done for fasting glucose, fasting insulin, 2-h glucose, 2-h insulin, and HOMA-IR before statistical testing to improve the normality. SAS

(Version 8.0 for Windows) was used to analyze data. Hardy-Weinberg equilibrium, linkage disequilibrium (LD), and haplotype analyses were carried out using the HelixTree program. Haplotypes were inferred for each individual, and combined haplotypes in a recessive model were also considered.

**RESULTS**— General characteristics of participants ( $n = 4,107$ ) by sex and ethnic group (2,763 Chinese, 746 Malays, and 598 Asian Indians) are presented in Table 1. Initially, 147 subjects (77 men and 70 women) were previously diagnosed as having diabetes. From those, 133 were on diabetes medication. Biochemical data (fasting glucose and 2-h glucose) obtained during the study revealed that 245 subjects (105 men and 140 women) could be adjudicated as newly diagnosed diabetic subjects. Thus, 392 participants (182 men and 210 women) were finally classified as diabetic subjects. Both *PLIN* genotypes were in Hardy-Weinberg equilibrium. Frequencies of the variant alleles were lower in Asian Indians than in the other groups. There was strong ( $D' = 0.80$ ,  $r^2 = 0.77$ ,  $P < 0.0001$ ) pairwise LD between *PLIN* 11482G $\rightarrow$ A and *PLIN* 14995A $\rightarrow$ T across all three ethnic groups.

### Gene-phenotype associations

We first examined the association between *PLIN* 11482G $\rightarrow$ A and 14995A $\rightarrow$ T polymorphisms and diabetes (previously reported and newly diagnosed,  $n = 392$ ) in the 4,017 participants. We did not find any significant association between these polymorphisms and diabetes (results not shown) or with insulin resistance–related measures (fasting glucose, 2-h glucose, fasting insulin, 2-h insulin, and HOMA-IR) in any ethnic group or sex. As both polymorphisms were in strong LD and yielded similar results, we only show data for the *PLIN* 11482G $\rightarrow$ A (Table 2). Haplotype analysis did not change the statistical significance of results (not shown).

### Gene-diet interaction in determining insulin resistance

We then analyzed whether macronutrient intake modulates the association between *PLIN* polymorphisms and insulin resistance–related measures in one in every two participants. Subjects with known diabetes were excluded to remove the influence of previously diagnosed disease on dietary patterns. We did not find signifi-

Table 1—Descriptive characteristics of Singapore population by sex and ethnic group

|                                   | Chinese     | Malay       | Indian      |
|-----------------------------------|-------------|-------------|-------------|
| <b>Men</b>                        |             |             |             |
| <i>n</i>                          | 1,263       | 360         | 286         |
| Age (years)                       | 38.2 ± 12.3 | 39.6 ± 12.7 | 41.3 ± 12.1 |
| BMI (kg/m <sup>2</sup> )          | 23.5 ± 3.7  | 24.7 ± 4.0  | 24.6 ± 4.0  |
| Total cholesterol (mmol/l)        | 5.52 ± 1.04 | 5.88 ± 1.13 | 5.72 ± 1.17 |
| Triglycerides (mmol/l)            | 1.69 ± 1.55 | 2.00 ± 1.59 | 2.08 ± 1.78 |
| Current smoker (%)                | 298 (23.4)  | 162 (45.0)  | 87 (30.4)   |
| Alcohol user (%)                  | 749 (59.3)  | 44 (12.2)   | 149 (52.1)  |
| Diabetes (%)*                     |             |             |             |
| Previously diagnosed subjects     | 38 (3.0)    | 15 (4.2)    | 24 (8.4)    |
| Total (newly diagnosed and known) | 91 (7.2)    | 39 (10.9)   | 52 (18.2)   |
| <i>PLIN</i> polymorphisms         |             |             |             |
| 11482G→A (A allele frequency)     | 0.42        | 0.43        | 0.31†       |
| 14995A→T (T allele frequency)     | 0.44        | 0.44        | 0.34†       |
| Dietary intake‡                   |             |             |             |
| Total fat (% energy)              | 26.6 ± 5.3  | 28.1 ± 6.8  | 27.5 ± 5.5  |
| SFAs (% energy)                   | 10.5 ± 2.5  | 12.1 ± 3.6  | 11.4 ± 3.0  |
| PUFAs (% energy)                  | 4.9 ± 1.7   | 4.5 ± 1.8   | 5.5 ± 2.2   |
| MUFAs (% energy)                  | 9.3 ± 2.3   | 9.2 ± 2.7   | 8.3 ± 2.3   |
| Carbohydrate (% energy)           | 60.0 ± 6.3  | 59.9 ± 7.5  | 60.6 ± 6.2  |
| <b>Women</b>                      |             |             |             |
| <i>n</i>                          | 1,500       | 386         | 312         |
| Age (years)                       | 37.8 ± 12.2 | 38.4 ± 12.7 | 40.0 ± 12.1 |
| BMI (kg/m <sup>2</sup> )          | 22.1 ± 3.6  | 26.3 ± 5.6  | 25.6 ± 5.0  |
| Total cholesterol (mmol/l)        | 5.33 ± 1.05 | 5.73 ± 1.17 | 5.33 ± 1.03 |
| Triglycerides (mmol/l)            | 1.16 ± 0.75 | 1.39 ± 0.88 | 1.33 ± 0.68 |
| Current smoker (%)                | 45 (3.0)    | 15 (3.8)    | 1 (0.3)     |
| Alcohol user (%)                  | 494 (32.9)  | 12 (3.1)    | 55 (17.6)   |
| Diabetes (%)*                     |             |             |             |
| Previously diagnosed subjects     | 26 (1.7)    | 22 (5.7)    | 22 (7.1)    |
| Total (newly diagnosed and known) | 99 (6.6)    | 57 (14.8)   | 54 (17.4)   |
| <i>PLIN</i> polymorphisms         |             |             |             |
| 11482G→A (A allele frequency)     | 0.41        | 0.45        | 0.34†       |
| 14995A→T (T allele frequency)     | 0.44        | 0.45        | 0.38†       |
| Dietary intake‡                   |             |             |             |
| Total fat (% energy)              | 26.9 ± 5.4  | 27.8 ± 5.2  | 28.5 ± 5.8  |
| SFAs (% energy)                   | 10.1 ± 2.5  | 11.5 ± 2.8  | 11.7 ± 3.0  |
| PUFAs (% energy)                  | 5.6 ± 2.2   | 5.2 ± 2.2   | 6.3 ± 3.0   |
| MUFAs (% energy)                  | 9.3 ± 2.4   | 9.1 ± 2.4   | 8.2 ± 2.3   |
| Carbohydrate (% energy)           | 59.1 ± 6.4  | 59.2 ± 5.8  | 59.4 ± 6.2  |

Data are means ± SD for continuous variables or *n* cases (% prevalence) for categorical variables. \*Prevalence of diabetes was estimated considering both known diabetes and newly diagnosed diabetes according to the biochemical analysis. †Frequencies for the minor alleles were statistically lower ( $P < 0.01$ ) in Indians than in Chinese or Malays. No statistically significant differences were found between Chinese and Malays. Haplotype frequencies for the whole population were 0.53 GA, 0.35 AT, 0.07 GT, and 0.04 AA. ‡Diet was determined in a systematically selected (one in every two) random sample of the initial participants. After additional exclusion of 73 previously diagnosed diabetic subjects, the final number was 2,154 participants.

cant gene-by-ethnic group interactions, and the analyses were done including all the three ethnic groups. Conversely, we found significant interaction with sex, and we analyzed men ( $n = 1,001$ ) and women ( $n = 1,153$ ) separately. We observed a recessive effect; therefore, homozygotes and heterozygotes for the

wild-type allele were compared with subjects homozygous for the minor allele. Sex-specific tertiles of macronutrient intakes were calculated. In men, we did not find any significant interaction between any one of the *PLIN* polymorphisms and macronutrients in determining insulin resistance. However, in women we found

statistically significant interactions between the *PLIN* 11482G→A polymorphism and tertiles of total fat, SFAs, and carbohydrate modulating fasting insulin concentrations and HOMA-IR (Table 3). Statistically significant interactions showing similar effects were observed for the *PLIN* 14995A→T polymorphism (Table 3). Thus, in women homozygous for the less common alleles, HOMA-IR increased as total fat, and specifically SFAs, increased. Conversely, carbohydrate intake was inversely correlated with HOMA-IR among these women ( $P$  values for lineal trends were statistically significant only for the 11482G→A polymorphism). Despite some limitations in sample size, due to the low frequency of the GT and AA haplotypes (Table 1), haplotype analysis suggested that *PLIN* 11482G→A was the most significant marker because no statistically significant interactions in determining insulin resistance were found with total fat, SFAs, and carbohydrate ( $P = 0.360$ ,  $P = 0.150$ , and  $P = 0.141$ , respectively) when the haplotype variable was considered. Moreover, no higher effects on insulin resistance were observed in homozygous haplotypes for both polymorphisms (AT/AT) when compared with *PLIN* 11482G→A homozygotes (AX/AX). Therefore, the 11482G→A variant was considered as the tag polymorphism.

Further adjustment of the SFA interaction by the carbohydrate intake did not modify the statistical significance, revealing independent and additive effects. Then, we simultaneously analyzed intakes of SFAs and carbohydrate by calculating the SFA-to-carbohydrate ratio. The interaction term between this variable (as continuous) and the 11482G→A polymorphism was statistically significant ( $P = 0.002$ ) in determining HOMA-IR in the whole population (Fig. 1A). Interestingly, in all the three ethnic groups, HOMA-IR increased in women homozygous for 11482A, as the ratio of SFAs to carbohydrate increased. This increase was statistically significant in Chinese (Fig. 1B) and Malays (Fig. 1C), without reaching the statistical significance in Indians (Fig. 1D) because of their lower prevalence of the 11482A allele. When Malays and Indians were combined to increase the statistical power, the interaction term was statistically significant ( $P = 0.018$ ).

**CONCLUSIONS**— We have found that genetic variation at *PLIN* locus is an important modulator of the effects of ha-

Table 2—Adjusted insulin resistance measures by PLIN 11482G→A genotypes, sex, and ethnicity in a Singapore population

|                          | Men          |              |               |       | P*           | Women        |               |       | P* |
|--------------------------|--------------|--------------|---------------|-------|--------------|--------------|---------------|-------|----|
|                          | GG           | GA           | AA            |       |              | GG           | GA            | AA    |    |
| Chinese                  |              |              |               |       |              |              |               |       |    |
| n                        | 436          | 605          | 222           |       |              | 526          | 728           | 246   |    |
| Fasting glucose (mmol/l) | 5.80 ± 0.05  | 5.80 ± 0.04  | 5.73 ± 0.06   | 0.597 | 5.46 ± 0.05  | 5.53 ± 0.04  | 5.47 ± 0.07   | 0.435 |    |
| 2-h glucose (mmol/l)†    | 6.64 ± 0.12  | 6.50 ± 0.10  | 6.56 ± 0.17   | 0.924 | 6.57 ± 0.11  | 6.86 ± 0.10  | 6.72 ± 0.17   | 0.213 |    |
| Fasting insulin (mU/l)   | 8.57 ± 0.78  | 7.52 ± 0.66  | 7.34 ± 1.10   | 0.400 | 7.08 ± 0.17  | 7.08 ± 0.14  | 6.88 ± 0.25   | 0.807 |    |
| 2-h insulin (mU/l)†      | 57.74 ± 2.60 | 55.15 ± 2.21 | 50.71 ± 3.63  | 0.600 | 61.38 ± 2.24 | 62.89 ± 1.90 | 57.14 ± 3.27  | 0.621 |    |
| HOMA-IR                  | 1.82 ± 0.0   | 1.91 ± 0.04  | 1.81 ± 0.07   | 0.342 | 1.71 ± 0.05  | 1.74 ± 0.04  | 1.70 ± 0.07   | 0.672 |    |
| Malay                    |              |              |               |       |              |              |               |       |    |
| n                        | 116          | 177          | 67            |       | 113          | 202          | 71            |       |    |
| Fasting glucose (mmol/l) | 6.22 ± 0.14  | 6.04 ± 0.12  | 6.14 ± 0.19   | 0.354 | 6.28 ± 0.18  | 6.22 ± 0.13  | 5.70 ± 0.22   | 0.171 |    |
| 2-h glucose (mmol/l)†    | 7.50 ± 0.29  | 7.08 ± 0.23  | 7.26 ± 0.39   | 0.818 | 7.61 ± 0.32  | 7.90 ± 0.24  | 7.05 ± 0.43   | 0.348 |    |
| Fasting insulin (mU/l)   | 7.74 ± 0.49  | 8.16 ± 0.39  | 8.60 ± 0.64   | 0.563 | 9.96 ± 0.65  | 9.55 ± 0.49  | 9.21 ± 0.83   | 0.450 |    |
| 2-h insulin (mU/l)†      | 65.18 ± 6.80 | 73.31 ± 5.38 | 64.48 ± 8.85  | 0.546 | 74.70 ± 5.53 | 76.74 ± 4.22 | 74.87 ± 7.47  | 0.777 |    |
| HOMA-IR                  | 2.18 ± 0.15) | 2.11 ± 0.12  | 2.32 ± 0.20   | 0.520 | 2.59 ± 0.20  | 2.56 ± 0.15  | 2.33 ± 0.27   | 0.429 |    |
| Indian                   |              |              |               |       |              |              |               |       |    |
| n                        | 135          | 126          | 25            |       | 128          | 154          | 30            |       |    |
| Fasting glucose (mmol/l) | 6.23 ± 0.17  | 6.68 ± 0.17  | 6.30 ± 0.39   | 0.107 | 6.11 ± 0.15  | 6.10 ± 0.13  | 6.06 ± 0.30   | 0.978 |    |
| 2-h glucose (mmol/l)†    | 7.15 ± 0.33  | 8.15 ± 0.34  | 6.22 ± 0.83   | 0.083 | 7.52 ± 0.34  | 7.88 ± 0.30  | 7.89 ± 0.70   | 0.564 |    |
| Fasting insulin (mU/l)   | 10.60 ± 0.75 | 10.86 ± 0.78 | 8.46 ± 1.77   | 0.473 | 11.40 ± 0.49 | 10.74 ± 0.45 | 11.14 ± 1.02  | 0.772 |    |
| 2-h insulin (mU/l)†      | 87.45 ± 7.87 | 91.18 ± 8.14 | 68.09 ± 19.83 | 0.470 | 92.49 ± 6.72 | 89.86 ± 6.04 | 99.33 ± 13.93 | 0.490 |    |
| HOMA-IR                  | 2.90 ± 0.27  | 3.03 ± 0.28  | 1.88 ± 0.68   | 0.282 | 2.88 ± 0.16  | 2.81 ± 0.15  | 3.12 ± 0.35   | 0.545 |    |

Data are means ± SE. \*Test for homogeneity across genotypes. Means were adjusted for age, BMI, smoking, alcohol intake, physical activity, and diabetes status. †After the collection of the fasting sample, a 75-g oral glucose tolerance test was taken. After 2 h, a blood sample was obtained and glucose (2-h glucose) and insulin (2-h insulin) were measured. These tests were not carried out in diabetic subjects on medication (68 men: 34 Chinese, 13 Malays, and 21 Asian Indians; and 65 women: 24 Chinese, 22 Malays, and 22 Asian Indians).

bitual dietary fat and carbohydrate consumption on insulin resistance in a large sample of Asian women from the general population. The association between dietary fat or carbohydrate content and insulin resistance is currently generating a wide degree of public interest and academic debate (17–19). For several decades, medical societies have been promoting high-carbohydrate diets as part of a healthy diet aimed to prevent chronic diseases. More recently, low-carbohydrate diets have been on the rise fostered by the notion that low-carbohydrate, high-fat diets improve insulin resistance in obese patients (18–21); however, there is insufficient evidence to make general recommendations for or against the use of low-carbohydrate diets (18–20). Moreover, the vast majority of studies have not taken into account the genetic characteristics of participants. Therefore, based on the enticing evidence supporting that genetic variation has an important influence in modulating the dietary effect on phenotypic traits (22), it is reasonable to assume that the effects of dietary composition on insulin resistance measures may be different depending on the individual genotype. However, the identification of relevant polymor-

phisms in candidate genes is not an easy task, as shown by the observed inconsistencies (23,24).

In the present study, we have investigated the *PLIN* gene because of the relevant physiological role that the adipose tissue plays in insulin resistance (2). Perilipin is the predominant protein associated with lipid storage droplets in adipocytes and one of the critical regulators implicated in the lipid mobilization (4–8). In examining *PLIN* variation, we have found a statistically significant gene-diet interaction between the *PLIN* 11482G→A and the *PLIN* 14995A→T polymorphisms and dietary fat and carbohydrate intake in determining insulin resistance in women. As these polymorphisms were in strong LD, it was difficult to determine their separate effects. However, the higher statistical significance obtained with the 11482G→A polymorphism as well as the results of haplotype analysis suggested that *PLIN* 11482G→A was the most relevant marker, and we considered this variant as the tag polymorphism.

It is interesting to highlight that the gene-fat interactions were observed only for SFAs but not for MUFAs or PUFAs.

This supports a large body of research in animal models and humans suggesting that SFAs clearly worsen insulin resistance, while PUFAs and MUFAs may improve it (25). According to our results, Asian women in the highest SFA tertile had higher HOMA-IR (48% increase) than women in the lowest tertile, if they were homozygotes for the *PLIN* 11482G→A polymorphism. Conversely, *PLIN* 11482A homozygous women in the lowest carbohydrate tertile had a 24% higher HOMA-IR score than women in the highest carbohydrate tertile. When combined intakes of SFAs and carbohydrate were considered as a ratio, the differences were magnified. Our results show that the effects of SFA and carbohydrate content in the diet on insulin resistance in Asian women largely depends on the genotype and may contribute to explain the inconsistent results found in different epidemiological studies. Thus, McKeown et al. (26), in the Framingham Offspring cohort, found no association between total carbohydrate intake and HOMA-IR. Conversely, Lau et al. (27) reported that total carbohydrate were inversely related to HOMA-IR in Danish men and women. Several epidemiological

**Table 3—Interactions between PLIN 11482G→A and PLIN 14995A→T polymorphisms and intakes of total fat, SFAs, and carbohydrate in determining plasma insulin resistance–related measures in Singapore women (Chinese, Malay, and Asian Indian)**

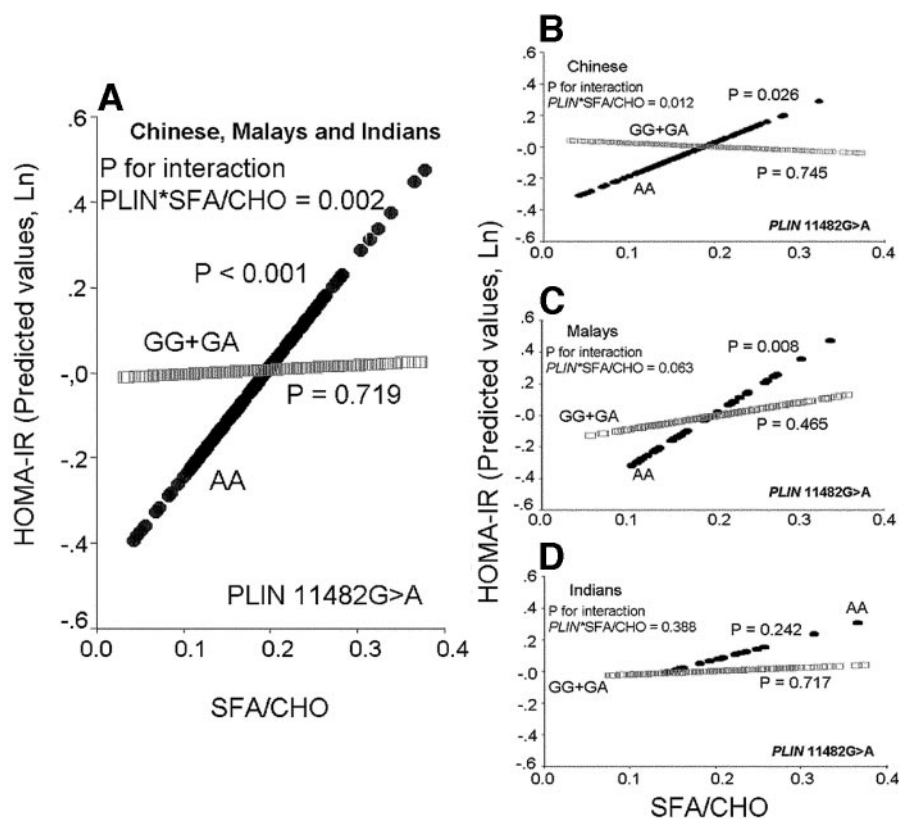
|                                   | Polymorphism | Genotypes* | Tertiles    |             |             | P trend† | P interaction‡ |
|-----------------------------------|--------------|------------|-------------|-------------|-------------|----------|----------------|
|                                   |              |            | 1 (n = 384) | 2 (n = 385) | 3 (n = 384) |          |                |
| Total fat (tertiles of intake, %) |              |            | 7.1–25.2    | 25.2–29.8   | 29.8–54.1   |          |                |
| Fasting glucose (mmol/l)          | 11482G→A     | GG + GA    | 5.62 ± 0.08 | 5.60 ± 0.07 | 5.68 ± 0.07 | 0.713    | 0.425          |
|                                   |              | AA         | 5.41 ± 0.15 | 5.56 ± 0.14 | 5.62 ± 0.13 | 0.327    |                |
| Fasting insulin (mU/l)            |              | GG + GA    | 8.62 ± 0.31 | 8.16 ± 0.29 | 8.31 ± 0.30 | 0.824    | 0.010          |
|                                   |              | AA         | 7.53 ± 0.62 | 8.10 ± 0.62 | 9.31 ± 0.59 | 0.134    |                |
| HOMA-IR                           |              | GG + GA    | 2.23 ± 0.09 | 2.10 ± 0.08 | 2.19 ± 0.08 | 0.737    | 0.007          |
|                                   |              | AA         | 1.89 ± 0.18 | 2.10 ± 0.17 | 2.41 ± 0.17 | 0.104    |                |
| Fasting glucose (mmol/l)          | 14995A→T     | AA + AT    | 5.58 ± 0.07 | 5.56 ± 0.07 | 5.69 ± 0.07 | 0.338    | 0.448          |
|                                   |              | TT         | 5.55 ± 0.14 | 5.58 ± 0.14 | 5.63 ± 0.12 | 0.574    |                |
| Fasting insulin (mU/l)            |              | AA + AT    | 8.58 ± 0.28 | 8.17 ± 0.27 | 8.24 ± 0.26 | 0.864    | 0.014          |
|                                   |              | TT         | 7.58 ± 0.88 | 7.89 ± 0.81 | 9.33 ± 0.91 | 0.333    |                |
| HOMA-IR                           |              | AA + AT    | 2.21 ± 0.08 | 2.08 ± 0.08 | 2.17 ± 0.08 | 0.726    | 0.012          |
|                                   |              | TT         | 1.93 ± 0.25 | 2.16 ± 0.23 | 2.41 ± 0.26 | 0.287    |                |
| SFA (tertiles of intake, %)       |              |            | 3.1–9.4     | 9.4–11.8    | 11.8–19.0   |          |                |
| Fasting glucose (mmol/l)          | 11482G→A     | GG + GA    | 5.62 ± 0.07 | 5.6 ± 0.07  | 5.66 ± 0.06 | 0.818    | 0.004          |
|                                   |              | AA         | 5.35 ± 0.12 | 5.55 ± 0.12 | 5.82 ± 0.12 | 0.014    |                |
| Fasting insulin (mU/l)            |              | GG + GA    | 8.49 ± 0.31 | 8.37 ± 0.33 | 8.24 ± 0.28 | 0.938    | 0.004          |
|                                   |              | AA         | 7.50 ± 0.60 | 7.52 ± 0.61 | 9.82 ± 0.60 | 0.030    |                |
| HOMA-IR                           |              | GG + GA    | 2.19 ± 0.09 | 2.16 ± 0.10 | 2.17 ± 0.08 | 0.978    | 0.003          |
|                                   |              | AA         | 1.79 ± 0.17 | 1.90 ± 0.17 | 2.65 ± 0.17 | 0.006    |                |
| Fasting glucose (mmol/l)          | 14995A→T     | AA + AT    | 5.63 ± 0.07 | 5.57 ± 0.07 | 5.64 ± 0.06 | 0.816    | 0.009          |
|                                   |              | TT         | 5.38 ± 0.14 | 5.69 ± 0.15 | 5.91 ± 0.14 | 0.006    |                |
| Fasting insulin (mU/l)            |              | AA + AT    | 8.29 ± 0.25 | 8.18 ± 0.24 | 7.89 ± 0.30 | 0.963    | 0.014          |
|                                   |              | TT         | 7.59 ± 0.85 | 7.70 ± 0.90 | 9.20 ± 0.87 | 0.332    |                |
| HOMA-IR                           |              | AA + AT    | 2.16 ± 0.08 | 2.15 ± 0.09 | 2.15 ± 0.07 | 0.983    | 0.005          |
|                                   |              | TT         | 1.86 ± 0.24 | 2.00 ± 0.26 | 2.57 ± 0.25 | 0.095    |                |
| CHO (tertiles of intake, %)       |              |            | 34.5–56.3   | 56.3–61.7   | 61.7–84.5   |          |                |
| Fasting glucose (mmol/l)          | 11482G→A     | GG + GA    | 5.52 ± 0.07 | 5.59 ± 0.07 | 5.72 ± 0.06 | 0.050    | 0.145          |
|                                   |              | AA         | 5.72 ± 0.13 | 5.65 ± 0.12 | 5.39 ± 0.12 | 0.121    |                |
| Fasting insulin (mU/l)            |              | GG + GA    | 8.07 ± 0.30 | 8.43 ± 0.30 | 8.55 ± 0.30 | 0.244    | 0.007          |
|                                   |              | AA         | 9.10 ± 0.61 | 8.54 ± 0.57 | 7.32 ± 0.64 | 0.042    |                |
| HOMA-IR                           |              | GG + GA    | 2.12 ± 0.08 | 2.06 ± 0.09 | 2.18 ± 0.09 | 0.338    | 0.004          |
|                                   |              | AA         | 2.35 ± 0.17 | 2.16 ± 0.16 | 1.89 ± 0.18 | 0.046    |                |
| Fasting glucose (mmol/l)          | 14995A→T     | AA + AT    | 5.63 ± 0.07 | 5.50 ± 0.07 | 5.68 ± 0.07 | 0.726    | 0.293          |
|                                   |              | TT         | 5.74 ± 0.12 | 5.53 ± 0.13 | 5.42 ± 0.12 | 0.213    |                |
| Fasting insulin (mU/l)            |              | AA + AT    | 7.97 ± 0.26 | 8.52 ± 0.27 | 8.50 ± 0.27 | 0.162    | 0.008          |
|                                   |              | TT         | 9.22 ± 0.96 | 8.28 ± 0.78 | 7.32 ± 0.89 | 0.127    |                |
| HOMA-IR                           |              | AA + AT    | 2.10 ± 0.08 | 2.15 ± 0.08 | 2.21 ± 0.08 | 0.282    | 0.012          |
|                                   |              | TT         | 2.39 ± 0.28 | 2.20 ± 0.22 | 1.92 ± 0.26 | 0.143    |                |

Data are means ± SE. \*The distribution of PLIN genotypes in these 1,153 women were 424 GG, 552 GA, and 177 AA for the 11482G→A and 378 AA, 567 AT, and 208 TT for the 14995A→T. †P for lineal trend was estimated by comparing adjusted means of insulin resistance variables across tertiles of macronutrient intake depending on the genotype group (expressed as percent of energy). Means were adjusted for ethnicity, age, BMI, cigarette smoking, alcohol consumption, physical activity, diabetes status, and energy intake. Further adjustment of means for fat or carbohydrate did not modify the statistical significance of results. ‡P value for the interaction term between the PLIN polymorphisms and tertiles of the corresponding macronutrient in the hierarchical multivariate regression model. CHO, carbohydrate.

studies (28–30) have found that total carbohydrate intake is unrelated to fasting insulin and diabetes risk. Some (31) have suggested that carbohydrate intake is not a sensitive enough measure, and the glycemic index has been proposed as a better measure to classify carbohydrate-containing foods. In the present study, we have not determined the glycemic index, precluding us from examining its interac-

tion with *PLIN* variation. However, although a high dietary glycemic index has been positively associated with HOMA-IR in some studies, uncertainties regarding its estimation reduce the glycemic index validity in observational studies (32). On the other hand, despite the widespread belief that SFA intake worsens cardiovascular disease risk–related variables (33), inconsistency also exists when the associ-

ation between SFAs and insulin resistance is examined in epidemiological studies (19,25). In the present study, SFAs were not associated with insulin resistance in the population as a whole. However, high-SFA and low-carbohydrate intakes were strongly associated with higher HOMA-IR in the subgroup of women who were homozygotes for the *PLIN* 11482G→A polymorphism. Interest-



**Figure 1**—Predicted values of HOMA-IR in women from the whole Singapore population (A), Chinese (B), Malays (C), and Indians (D) according to the *PLIN* 11482G→A polymorphism (carriers of the 11482G allele: n = 976 in A, n = 598 in B, n = 196 in C, and n = 182 in D; 11482A homozygotes: n = 177 in A; n = 116 in B, n = 43 in C, and n = 18 in D) plotted against the SFA-to-carbohydrate (CHO) ratio. Predicted values (Ln) were calculated from the regression model after adjustment for ethnic group, age, BMI, cigarette smoking, alcohol consumption, physical activity, diabetes status, and energy intake. P values indicate the statistical significance of the interaction term in the adjusted regression model, the statistical significance of the adjusted regression coefficient in the regression line corresponding to *PLIN* 11482A homozygotes, and the statistical significance of the adjusted regression coefficient in the regression line corresponding to carriers of the *PLIN* 11482G allele. When Malay and Indian women were analyzed together in the same multivariate adjusted model, the interaction term between SFA/carbohydrate and the *PLIN* 11482G→A polymorphism was statistically significant ( $P = 0.018$ ). The P values for the regression coefficients were  $P = 0.005$  in *PLIN* 11482A homozygotes and  $P = 0.442$  in carriers of the *PLIN* 11482G allele (results not shown).

ingly, this gene-diet interaction was consistently found among Chinese, Malay, and Indian women, despite their different susceptibilities to insulin resistance, increasing the level of causality of this statistical association. In contrast, we did not find statistically significant interactions in men. This finding is not surprising. Previous studies (9–11) analyzing *PLIN* genetic variation have revealed sex-specific associations in women. Moreover, Mitterdorfer (34) examined the evidence regarding sex differences in insulin resistance and concluded that women are intrinsically more insulin resistant than men, possibly because of specific sex-linked gene expression and the resulting differences in metabolic control elements.

The biological mechanism underlying this gene-diet interaction remains to be determined; however, some experimental evidence supports our results. Thus, *PLIN* knockout mice that were resistant to obesity induced by a high-fat diet developed more insulin resistance than control mice (7). In addition, when studying the metabolic adaptation of *PLIN*-null mice (35), increased  $\beta$ -oxidation and increased insulin resistance with age was observed by another independent group. Although the *PLIN* 11482G→A polymorphism is located in an intron, Mottagui-Tabar et al. (8) reported that individuals carrying the 11482A allele had significantly reduced perilipin expression in their adipocytes, linking this polymorphism to a metabolic

situation similar to that reported in *PLIN*-null mice.

In summary, we found that Asian women with a moderate degree of insulin resistance and who were homozygotes for *PLIN* 11482A allele, have increased insulin resistance when consuming a high-SFA, low-carbohydrate diet. This gene-diet interaction was homogeneous among Chinese, Malay, and Indian women, adding consistency to this genetic marker and supporting the notion that dietary recommendations to improve insulin resistance should move forward to more personalized guidelines including relevant genetic information.

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**References**

1. Zimmet P, Alberti KG, Shaw J: Global and societal implications of the diabetes epidemic. *Nature* 414:782–787, 2001
2. Eckel RH, Grundy SM, Zimmet PZ: The metabolic syndrome. *Lancet* 365:1415–1428, 2005
3. Stern MP: Strategies and prospects for finding insulin resistance genes. *J Clin Invest* 106:323–327, 2000
4. Londos C, Gruia-Gray J, Brasaemle DL, Rondinone CM, Takeda T, Dwyer NK, Barber T, Kimmel AR, Blanchette-Mackie EJ: Perilipin: possible roles in structure and metabolism of intracellular neutral lipids in adipocytes and steroidogenic cells. *Int J Obes Relat Metab Disord* 20 (Suppl.)3:97–101, 1996
5. Martinez-Botas J, Anderson JB, Tessier D, Lapillonne A, Chang BH, Quast MJ, Gorenstein D, Chen KH, Chan L: Absence of perilipin results in leanness and reverses obesity in *Lepr* (db/db) mice. *Nat Genet* 26:474–479, 2000
6. Sztalryd C, Xu G, Dorward H, Contreras JA, Kimmel AR, Londos C: Perilipin A is essential for the translocation of hormone-sensitive lipase during lipolytic activation. *J Cell Biol* 161:1093–1103, 2003
7. Tansey JT, Sztalryd C, Gruia-Gray J, Roush DL, Zee JV, Gavrilova O, Reitman ML, Deng CX, Li C, Kimmel AR, Londos C: *PLIN* ablation results in a lean mouse with aberrant adipocyte lipolysis, enhanced leptin production, and resistance to diet-induced obesity. *Proc Natl Acad Sci U S A* 98:6494–6499, 2001
8. Mottagui-Tabar S, Ryden M, Lofgren P, Faulds G, Hoffstedt J, Brookes AJ, Anders-

- son I, Arner P: Evidence for an important role of perilipin in the regulation of human adipocyte lipolysis. *Diabetologia* 46: 789–797, 2003
9. Qi L, Corella D, Sorli JV, Portoles O, Shen H, Coltell O, Godoy D, Greenberg AS, Ordovas JM: Genetic variation at the PLIN locus is associated with obesity-related phenotypes in white women. *Clin Genet* 66:299–310, 2004
  10. Qi L, Shen H, Larson I, Barnard JR, Schaefer EJ, Ordovas JM: Gender-specific association of a perilipin gene haplotype with obesity risk in a white population. *Obes Res* 12:1758–1765, 2004
  11. Qi L, Shen H, Tai ES, Shen H, Chew SK, Greenberg AS, Corella D, Ordovas JM: Intra-genic linkage disequilibrium structure of the human perilipin gene (*PLIN*) and haplotype association with increased obesity risk in a multi-ethnic Asian population. *J Mol Med* 83:448–456, 2005
  12. Corella D, Qi L, Sorli JV, Godoy D, Portoles O, Coltell O, Greenberg AS, Ordovas JM: Obese subjects carrying the 11482G→A polymorphism at the perilipin (*PLIN*) locus are resistant to weight loss following dietary energy restriction. *J Clin Endocrinol Metab* 90:5121–5126, 2005
  13. Cutter J, Tan BY, Chew SK: Levels of cardiovascular disease risk factors in Singapore following a national intervention programme. *Bull World Health Organ* 79: 908–915, 2001
  14. Deurenberg-Yap M, Li T, Tan WL, Tan WL, van Staveren WA, Chew SK, Deurenberg P: Can dietary factors explain differences in serum cholesterol profiles among different ethnic groups (Chinese, Malays and Indians) in Singapore? *Asia Pac J Clin Nutr* 10:39–45, 2001
  15. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28:412–419, 1985
  16. Alberti KG, Zimmet PZ: Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1. Diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med* 15:539–553, 1998
  17. Astrup A, Meinert Larsen T, Harper A: Atkins and other low-carbohydrate diets: hoax or an effective tool for weight loss? *Lancet* 364:897–899, 2004
  18. Bravata DM, Sanders L, Huang J, Krumholz HM, Olkin I, Gardner CD, Bravata DM: Efficacy and safety of low-carbohydrate diets: a systematic review. *JAMA* 289:1837–1850, 2003
  19. Schwenke DC: Insulin resistance, low-fat diets, and low-carbohydrate diets: time to test new menus. *Curr Opin Lipidol* 16:55–60, 2005
  20. Crowe TC: Safety of low-carbohydrate diets. *Obesity Rev* 6:235–245, 2005
  21. Boden G, Sargrad K, Homko C, Mozzoli M, Stein TP: Effect of a low-carbohydrate diet on appetite, blood glucose levels, and insulin resistance in obese patients with type 2 diabetes. *Ann Intern Med* 142:403–411, 2005
  22. Ordovas JM, Corella D: Nutritional genomics. *Annu Rev Genomics Hum Genet* 5:71–118, 2004
  23. Luan J, Browne PO, Harding AH, Halsall DJ, O’Rahilly S, Chatterjee VK, Wareham NJ: Evidence for gene-nutrient interaction at the PPAR $\gamma$  locus. *Diabetes* 50:686–689, 2001
  24. Robitaille J, Despres JP, Perusse L, Vohl MC: The PPAR-gamma P12A polymorphism modulates the relationship between dietary fat intake and components of the metabolic syndrome: results from the Quebec Family Study. *Clin Genet* 63: 109–116, 2003
  25. Riccardi G, Giacco R, Rivellese AA: Dietary fat, insulin sensitivity and the metabolic syndrome. *Clin Nutr* 23:447–456, 2004
  26. McKeown NM, Meigs JB, Liu S, Saltzman E, Wilson PW, Jacques PF: Carbohydrate nutrition, insulin resistance, and the prevalence of the metabolic syndrome in the Framingham Offspring Cohort. *Diabetes Care* 27:538–546, 2004
  27. Lau C, Faerch K, Glumer C, Tetens I, Pedersen O, Carstensen B, Jorgensen T, Borch-Johnsen K, the Inter99 study: Dietary glycemic index, glycemic load, fiber, simple sugars, and insulin resistance: the Inter99 study. *Diabetes Care* 28:1397–1403, 2005
  28. Ludwig DS, Pereira MA, Kroenke CH, Hilner JE, Van Horn L, Slattery ML, Jacobs DR Jr: Dietary fiber, weight gain, and cardiovascular disease risk factors in young adults. *JAMA* 282:1539–1546, 1999
  29. Salmeron J, Manson JE, Stampfer MJ, Colditz GA, Wing AL, Willett WC: Dietary fiber, glycemic load, and risk of non-insulin-dependent diabetes mellitus in women. *JAMA* 277:472–477, 1997
  30. Meyer KA, Kushi LH, Jacobs DR Jr, Slavin J, Sellers TA, Folsom AR: Carbohydrates, dietary fiber, and incident type 2 diabetes in older women. *Am J Clin Nutr* 71:921–930, 2000
  31. Jenkins DJ, Kendall CW, Augustin LS: Glycemic index: overview of implications in health and disease. *Am J Clin Nutr* 76: 266S–73S, 2002
  32. Pi-Sunyer FX: Glycemic index and disease. *Am J Clin Nutr* 76:290S–298S, 2002
  33. German JB, Dillard CJ: Saturated fats: what dietary intake? *Am J Clin Nutr* 80: 550–559, 2004
  34. Mittendorfer B: Insulin resistance: sex matters. *Curr Opin Clin Nutr Metab Care* 8:367–372, 2005
  35. Saha PK, Kojima H, Martinez-Botas J, Sunehag AL, Chan L: Metabolic adaptations in the absence of perilipin: increased beta-oxidation and decreased hepatic glucose production associated with peripheral insulin resistance but normal glucose tolerance in perilipin-null mice. *J Biol Chem* 279:35150–35158, 2004