Nutrient-Gene Interactions

Dietary Fat Interacts with the −514C>T Polymorphism in the Hepatic Lipase Gene Promoter on Plasma Lipid Profiles in a Multiethnic Asian Population: The 1998 Singapore National Health Survey

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ABSTRACT We have previously reported an interaction between −514C>T polymorphism at the hepatic lipase (HL) gene and dietary fat on high-density lipoprotein-cholesterol (HDL-C) metabolism in a representative sample of white subjects participating in the Framingham Heart Study. Replication of these findings in other populations will provide proof for the relevance and consistency of this marker as a tool for risk assessment and more personalized cardiovascular disease prevention. Therefore, we examined this gene-nutrient interaction in a representative sample of Singaporeans (1324 Chinese, 471 Malays and 375 Asian Indians) whose dietary fat intake was recorded by a validated questionnaire. When no stratification by fat intake was considered, the T allele was associated with higher plasma HDL-C concentrations (P = 0.001), higher triglyceride (TG) concentrations (P = 0.001) and higher HDL-C/TG ratios (P = 0.041). We found a highly significant interaction (P = 0.001) between polymorphism and fat intake in determining TG concentrations and the HDL-C/TG ratio (P = 0.001) in the overall sample even after adjustment for potential confounders. Thus, TT subjects showed higher TG concentrations only when fat intake supplied >30% of total energy. This interaction was also found when fat intake was considered as continuous (P = 0.035). Moreover, in the upper tertile of fat intake, TT subjects had 45% more TG than CC individuals (P < 0.01). For HDL-C concentration, the gene-diet interaction was significant (P = 0.015) only in subjects of Indian origin. In conclusion, our results indicate that there are differences in the association of −514C>T polymorphism with plasma lipids according to dietary intake and ethnic background. Specifically, the TT genotype is associated with a more atherogenic lipid profile when subjects consume diets with a fat content > 30%. J. Nutr. 133: 3399–3408, 2003.

KEY WORDS: • Dietary fat • hepatic lipase • gene x nutrient interaction • cardiovascular risk.

The prevalence of cardiovascular disease (CVD) is increasing in many Asian countries, especially in the most developed areas (1). In addition to type 2 diabetes mellitus (T2DM), plasma lipids are an important contributing factor in the increased risk of CVD (1,2). Although pharmaceutical agents can successfully normalize plasma lipid concentrations, dietary modification is currently the cornerstone of CVD primary prevention. Nevertheless, the success of this approach depends on the individual response to the recommended dietary changes, which is determined in great part by gene-nutrient interactions. Therefore, exposure to the same quantitative level of dietary factor(s) can decrease risk in one individual but not in another, depending on specific gene variants (3,4).

Focusing on genetic variants related to lipid metabolism, we have recently reported a strong gene-nutrient interaction between −514C>T polymorphism in the promoter of the hepatic lipase gene (LIPC) and dietary fat intake in determining high-density lipoprotein-cholesterol (HDL-C) concentration and particle size (5). Hepatic lipase (HL) is a lipolytic enzyme that catalyzes hydrolysis of triglycerides and phospholipids in all major classes of lipoproteins and plays a key role in the metabolism of HDL-C (6,7). Overall, the T allele is associated...
with decreased plasma HL activity and increased HDL-C concentrations (8–13). However, this effect varies among populations, suggesting gene-environmental interactions (14). Accordingly, in the Framingham cohort we found that the T allele was associated with greater HDL-C concentrations in subjects whose total fat intake supplied <30% of total energy (P < 0.001). Conversely, when total fat intake supplied ≥30% of energy, the predicted HDL-C concentrations were lowest among those with the TT genotype (5). Therefore, our findings in the Framingham Heart Study suggest that TT subjects may have an impaired adaptation to higher fat diets that could result in higher CVD risk. However, to identify the extent of this finding and its implications for public health actions, it is necessary to investigate the consistency of these findings in other populations. It has been reported that the −514T allele occurs more frequently in Asians than in Caucasians (15,16). This fact provides an opportunity to specifically test the hypothesis of the deleterious effect of a high fat diet in TT individuals, whose prevalence in the North-American population is <4% (17).

Singapore offers an excellent opportunity to study this gene-nutrient interaction because its Asian population comprises three ethnic groups living in a highly urbanized environment and exhibiting different incidences of CVD (18,19). Asian Indians have the highest incidence, followed by Malays and Chinese, respectively. Therefore, the aim of this study was to investigate the possible gene-nutrient interaction between −514C>T polymorphism in the HL gene promoter and dietary fat on HDL metabolism in a multiethnic population cohort in Singapore consisting of Chinese, Malays and Indians subjected to a highly Westernized lifestyle.

MATERIALS AND METHODS

Subjects and study design. The study sample consisted of 2170 individuals (1011 males and 1159 females) who participated in 1998 Singapore National Health Survey. The detailed methodology of this survey of a nationally representative household sample has been described elsewhere (20). Briefly, the survey protocol was based on the WHO-recommended model for field surveys of diabetes and other noncommunicable diseases, and the WHO MONICA protocol for population surveys. Initially, 11,200 individuals from addresses representing the house-type (a proxy for socioeconomic status) distribution of the entire Singapore housing population were selected from the National Database on Dwellings. A process of disproportionate stratified and systematic sampling was used to select individuals between 18 and 69 y from this data set, with oversampling of the minority groups to ensure that prevalence estimates for the minority groups were reliable and to allow statistical comparison between ethnic groups (Chinese, Malays and Indians). Finally, 4723 individuals participated in this survey. Data on lifestyle factors were collected using an interviewer-administered questionnaire. The classification for physical activity participation used was adapted from the American College of Sports Medicine’s classification (21). Alcohol intake was assessed using a questionnaire based on the Behavior Risk Factor Surveillance Questionnaire from the Centers for Disease Control and Prevention (22). Daily smokers were defined as those who smoked at least 1 cigarette/d.

A validated food-frequency questionnaire was used to assess intakes of energy, total fat, cholesterol and specific fatty acids (23) in a random subsample of the participants. Subjects were systematically selected (1 in 2) to participate in the dietary survey. The questionnaire comprised a list of 159 individual food items, grouped into 23 main food types and 25 food subtypes. Each food group was carefully considered to ensure that foods consumed by the three ethnic groups were represented. The food composition database of the Singapore Ministry of Health was used to estimate the nutrient content. This questionnaire was previously validated in the Singaporean population against multiple 24-h recalls as well as urinary N excretion (24). In this work we present data from the random sampling of 2170 individuals (1324 Chinese, 471 Malays and 375 Indians) whose data were complete for all the variables examined (clinical, genetic, biochemical and lifestyle variables). All participants gave their informed consent, and the ethics committee of the Singapore General Hospital approved the study.

Clinical and biochemical determinations. Subjects were instructed to fast overnight for at least 10 h. A fasting blood sample was collected, and plasma lipid, glucose and insulin were determined as previously described (25). All subjects except diabetics on medication took a 75-g oral glucose tolerance test. Plasma lipid and glucose concentrations were measured using kits from Boehringer Mannheim (Boehringer Mannheim Systems, Mannheim, Germany) and read on a BM/Hirata K-747 analyser (Roche Diagnostics, Indianapolis, IN). Total cholesterol, TG and glucose were measured using enzymatic colorimetric assays. Plasma HDL-C was measured using a homogenous colorimetric assay, whereas low-density lipoprotein-cholesterol (LDL-C) was measured using a homogenous turbidimetric assay. Insulin was measured by microparticle enzyme immunoassay methods using an Abbot AxSYM (Abbot AxSYM, Chicago, IL) insulin assay. Intraassay and interassay coefficients of variation for every measure were previously reported (25).

Other parameters measured included BMI and waist-to-hip ratio. Diabetes status was determined according to American Diabetes Association recommendations (26) for the diagnosis of diabetes mellitus, and subjects were classified into two groups: diabetics and nondiabetics.

Genetic analysis. Extraction of DNA was carried out using QIAamp DNA Blood Midi kits (Qiagen, Hilden, Germany), following the manufacturer’s recommended protocol. A 255-bp fragment of the LIPC promoter encompassing the base at position −514 was amplified by polymerase chain reaction (PCR) using the following primers: 5′-TGG TCG CCT TTT CCC TAC CTG A-3′ and 5′-CCC CAG AGG GTC CAA ATT TCT-3′. The PCR amplification was carried out in a 10-μL reaction volume containing 0.1 mmol/L of each dNTP, 1.5 mmol/L of magnesium chloride, 0.4 μmol/L of each primer and 0.06 U of Qiagen HotStar Taq polymerase. The PCR cycling conditions were as previously described (5). The PCR products were incubated for 90 min at 37°C followed by 15 min at 75°C (to inactivate the enzymes) with 5 μL of each Exo I (USB, Cleveland, OH) and calf intestinal phosphatase (New England Biolabs, Beverly, MA) to remove unincorporated primers and dNTPs. Subsequently, genotyping was carried out by single nucleotide extension (27), using the ABI Prism SNaPshot multiplex system (Applied Biosystems, Foster City, CA) and the oligonucleotide probe 5′-GAC TGA CTG ACT GAC TGA CTG ACT AAA ACC CTT CAC CCC C-3′.

Statistical analyses. We examined all continuous variables for normality of distribution. The TG, HDL-C/TG ratio and insulin were skewed, and these variables were logarithmically transformed to improve normality. The transformed data for these variables were statistically analyzed. Categorical variables were compared using χ² testing. Mean differences for continuous variables among genotypes or among ethnic groups were compared using the ANOVA procedure. Once it was established that differences existed among means, Bonferroni testing was applied to determine which means differed with correction for multiple comparisons. In addition, P-values for linear trends between categories were calculated by ANOVA analysis. The influence of covariates in the comparison of means was controlled by ANCOVA analyses. Lipid concentrations were adjusted for ethnic group, gender, age, BMI, tobacco smoking, alcohol intake, exercise and diabetes status. Homogeneity of allelic effects according to ethnic group was tested by introducing the corresponding terms of interaction (in a hierarchical way) in the more parsimonious linear regression model. Multivariate linear regression analysis with dummy variables for categorical and interaction terms was used to test the null hypothesis of no interaction between HL polymorphism and fat intake in determining lipid concentrations. These regression models were fitted for the whole population and for each of the three ethnic groups, and they included controls for the potential confounding factors (age, sex, BMI, tobacco smoking, alcohol intake, physical exercise and diabetes status) as well as for energy intake.
Physical exercise, % energy PUFA, adjusted regression model and plotting these values against fat intake depicted by computing the predicted values for each individual from the continuous variable, its interaction with HL polymorphism was deemed inappropriate of these models. When fat intake was used as a categorical variable, its interaction with HL polymorphism was depicted by computing the predicted values for each individual from the adjusted regression model and plotting these values against fat intake depending on the HL genotype. All statistical tests were two-tailed, and a P-value < 0.05 was considered statistically significant. Statistical analyses were carried out using SPSS version 10.1 (SPSS, Chicago, IL).

RESULTS

Significant ethnic differences for BMI, fasting glucose, fasting insulin and plasma lipid profiles were observed in both men and women (Table 1). The HDL-C concentrations were higher in Chinese and lower in Indians; the opposite was true for TG (P < 0.05 for every comparison after the Bonferroni correction). Likewise, the three ethnic groups exhibited differences in lifestyle variables such as tobacco smoking, alcohol consumption and dietary intake (Table 1). In general, Chinese had lower total fat intake compared with Malays and Indians. Overall, a large proportion of the population consumes a diet that meets the criteria of supplying <30% of energy from fat. This percentage varies from 72.6% in Chinese to 64.3% and 64.1% in Indians and Malays, respectively. However, 54.5% of Chinese, 66.9% of Indians and 71.5% of Malays had an estimated daily intake of saturated fatty acids (SFA) ≥ 10% of energy. The global prevalence of diabetic subjects in this population was 7.7%. It was higher in Indians (11.5%), followed by Malays (10.8%) and Chinese (5.6%); P < 0.001.

For all the ethnic groups the distribution of genotypes for −514C>T polymorphism at the LIPC locus (Table 2) did not deviate from the Hardy-Weinberg expectations (28) in any gender. Asians presented the lowest allele frequency for the T variant (0.273), followed by Chinese (0.377) and Malays (0.437); P < 0.001. Differences by gender across the −514C>T genotypes were nonsignificant (P = 0.879).

To examine the association between −514C>T polymorphism and plasma lipid concentrations by ethnic group, men and women were pooled in the analyses after verifying that there was no heterogeneity of genotype effects by gender (Table 3). This polymorphism was statistically associated with total cholesterol, HDL-C, LDL-C and TG in Chinese, with women showing a trend for TG deviating from the Hardy-Weinberg expectations (28) in any gender. Asians presented the lowest allele frequency for the T variant (0.273), followed by Chinese (0.377) and Malays (0.437); P < 0.001. Differences by gender across the −514C>T genotypes were nonsignificant (P = 0.879).

TABLE 1
Demographic, biochemical, clinical and lifestyle characteristics of the study subjects stratified by ethnic group and gender

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Chinese (n = 604)</th>
<th>Malays (n = 226)</th>
<th>Indians (n = 181)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>38.9 ± 12.7</td>
<td>38.5 ± 12.4</td>
<td>40.7 ± 11.0</td>
<td>0.171</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>23.6 ± 3.7</td>
<td>24.9 ± 3.9</td>
<td>24.1 ± 3.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>5.6 ± 0.8</td>
<td>5.8 ± 1.5</td>
<td>5.9 ± 1.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasting insulin, pmol/L</td>
<td>55.2 ± 36.6</td>
<td>58.8 ± 37.3</td>
<td>70.3 ± 65.9</td>
<td>0.003</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>3.5 ± 0.9</td>
<td>3.9 ± 1.0</td>
<td>3.8 ± 1.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.3 ± 0.3</td>
<td>1.2 ± 0.3</td>
<td>1.0 ± 0.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>1.7 ± 1.9</td>
<td>2.0 ± 1.7</td>
<td>1.9 ± 1.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-C/TG</td>
<td>1.1 ± 0.7</td>
<td>0.9 ± 0.6</td>
<td>0.8 ± 0.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Protein intake, g/d</td>
<td>77.9 ± 26.8</td>
<td>75.0 ± 37.5</td>
<td>71.5 ± 25.3</td>
<td>0.027</td>
</tr>
<tr>
<td>Total carbohydrate intake, g/d</td>
<td>348.7 ± 113.8</td>
<td>364.3 ± 142.3</td>
<td>360.1 ± 110.6</td>
<td>0.163</td>
</tr>
<tr>
<td>Total fat intake, g/d</td>
<td>70.6 ± 31.4</td>
<td>80.7 ± 50.1</td>
<td>73.5 ± 31.2</td>
<td>0.002</td>
</tr>
<tr>
<td>Total fat, % energy</td>
<td>26.6 ± 5.3</td>
<td>28.0 ± 6.9</td>
<td>27.3 ± 5.5</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>SFA, % energy</td>
<td>10.4 ± 2.5</td>
<td>12.0 ± 3.6</td>
<td>11.4 ± 2.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MUFA, % energy</td>
<td>9.3 ± 2.2</td>
<td>9.2 ± 2.7</td>
<td>8.7 ± 2.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PUFA, % energy</td>
<td>4.9 ± 1.7</td>
<td>4.6 ± 1.9</td>
<td>5.5 ± 2.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Daily smokers, %</td>
<td>21.4 ± 36.3</td>
<td>30.4 ± 30.4</td>
<td>30.4 ± 30.4</td>
<td>0.001</td>
</tr>
<tr>
<td>Nondrinkers, %</td>
<td>43.2 ± 88.5</td>
<td>48.6 ± 48.6</td>
<td>48.6 ± 48.6</td>
<td>0.001</td>
</tr>
<tr>
<td>Physical exercise, %</td>
<td>46.6 ± 43.4</td>
<td>43.6 ± 46.3</td>
<td>46.6 ± 43.6</td>
<td>0.161</td>
</tr>
<tr>
<td>No exercise</td>
<td>22.7 ± 20.7</td>
<td>20.8 ± 29.3</td>
<td>20.8 ± 29.3</td>
<td>0.180</td>
</tr>
</tbody>
</table>

1 Values are means ± SD. Proportions are given as percentages. 2 P-value obtained in the comparison among ethnic groups (ANOVA test for means and χ² test for percentages). 3 HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; SFA, saturated fatty acids; TG, triglycerides.

TABLE 2
Genotype distribution and allele frequency of −514C>T polymorphism by ethnic group in the Singaporean population

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Chinese</th>
<th>Malays</th>
<th>Indians</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>501</td>
<td>37.8</td>
<td>195</td>
</tr>
<tr>
<td>CT</td>
<td>655</td>
<td>49.5</td>
<td>155</td>
</tr>
<tr>
<td>TT</td>
<td>168</td>
<td>12.7</td>
<td>26</td>
</tr>
<tr>
<td>T allele2</td>
<td>0.374ab</td>
<td>0.437b</td>
<td>0.273c</td>
</tr>
</tbody>
</table>

1 Values are number and percentage. P < 0.001. 2 Values are mean allele frequency and 95% confidence interval; a = different from Malays (P < 0.01); b = different from Indians (P < 0.01); c = different from Chinese (P < 0.01).
The possible effect of interaction between −514C>T polymorphism and dietary fat intake on plasma lipid concentrations was further examined. First, the population as a whole was analyzed, and the heterogeneity of the interaction effect by ethnic group was tested in a hierarchical multivariate regression model. In this model, dietary fat intake was considered as categorical (<30 and ≥30% of energy from total fat, respectively), based on previous results from the Framingham Heart Study. This regression model was also adjusted for age, gender, BMI, tobacco smoking, alcohol consumption, physical exercise, diabetes status and total energy intake. The statistical significance of the second-order interactions among HL polymorphism, total fat intake (dichotomous) and ethnic group was \( P = 0.050 \) and \( P = 0.755 \) for HDL-C and TG concentrations, respectively. This indicates homogeneity of the interactive effect of −514C>T polymorphism and total fat intake on TG across the three ethnic groups, and borderline heterogeneity of the fat-HL interaction effect on HDL-C.

We found that the interaction of HL polymorphism and total fat intake (dichotomous) strongly affected fasting TG in the whole Singaporean population \( (P = 0.001; \text{Fig. 1}) \). A diet supplying ≥30% of energy from total fat was associated with higher plasma TG (−37%) in TT subjects \( (P = 0.01) \), compared with a diet that supplied <30% of energy from total fat. This interaction remained significant \( (P = 0.009) \) even after additional adjustment for daily intake of carbohydrates and proteins. The significance of the interaction term was maintained \( (P = 0.010) \) when dietary fat was dichotomized according to the mean intake of the Singaporean population \( (<\simeq 27.5\% \text{ of energy}) \). Moreover, categorizing dietary fat by the tertiles of fat intake in the Singaporean population showed a dose-response trend in the modification of the effect (Table 5). The increase in total fat intake (from tertile 1 to tertile 3) correlated with an increase in TG concentrations only in TT subjects \( (P = 0.047) \). Consequently, major differences in TG were detected across the HL genotypes in the highest tertile of fat intake \( (P < 0.001) \), with TT subjects having 45% more TG than CC homozygotes in the same category of fat intake. Analysis of the interactive effect of −514C>T polymorphism and total fat intake on TG concentration by ethnic group consistently showed modification of the effect of high fat intake on TG concentrations in TT subjects in Chinese, Malays and Indians (Fig. 2A, B and C, respectively). How-

\[
\begin{array}{ccccccccc}
\text{Age, y} & \text{BMI, kg/m}^2 & \text{HDL-C, mmol/L} & \text{LDL-C, mmol/L} & \text{TG, mmol/L} & \text{HDL-C/TG} & \text{Chinese} & \text{Malays} & \text{Indians} \\
\text{CC} (n = 501) & \text{CT} (n = 655) & \text{TT} (n = 168) & \text{CC} (n = 137) & \text{CT} (n = 256) & \text{TT} (n = 78) & \text{CC} (n = 195) & \text{CT} (n = 155) & \text{TT} (n = 25) \\
37.9 \pm 11.7 & 39.1 \pm 12.9 & 38.9 \pm 12.5 & 0.258 & 37.7 \pm 12.2 & 37.5 \pm 12.3 & 40.1 \pm 10.4 & 0.194 & 39.5 \pm 11.2 & 40.7 \pm 11.1 & 36.7 \pm 10.1 & 0.206 \\
22.70 \pm 5.30 & 22.70 \pm 3.90 & 23.10 \pm 3.80 & 0.428 & 25.60 \pm 4.80 & 25.10 \pm 4.40 & 26.60 \pm 4.40 & 0.031 & 24.70 \pm 4.20 & 24.80 \pm 4.40 & 25.05 \pm 3.69 & 0.952 \\
5.28 \pm 1.02 & 5.50 \pm 1.10 & 5.58 \pm 1.02 & 0.001 & 5.71 \pm 1.16 & 5.73 \pm 1.15 & 6.01 \pm 1.13 & 0.143 & 5.43 \pm 1.00 & 5.57 \pm 1.06 & 5.47 \pm 0.99 & 0.483 \\
3.31 \pm 0.93 & 3.41 \pm 0.98 & 3.51 \pm 0.91 & 0.032 & 3.79 \pm 1.14 & 3.75 \pm 1.05 & 3.92 \pm 1.12 & 0.468 & 3.59 \pm 0.94 & 3.71 \pm 1.00 & 3.72 \pm 0.85 & 0.545 \\
1.29 \pm 0.81 & 1.42 \pm 1.32 & 1.77 \pm 2.79 & 0.003 & 1.52 \pm 0.82 & 1.51 \pm 1.61 & 1.89 \pm 2.20 & 0.058 & 1.52 \pm 0.89 & 1.68 \pm 1.16 & 1.70 \pm 0.75 & 0.148 \\
1.52 \pm 0.99 & 1.49 \pm 0.95 & 1.42 \pm 0.95 & 0.128 & 1.14 \pm 0.73 & 1.12 \pm 0.78 & 1.04 \pm 0.73 & 0.133 & 1.02 \pm 0.72 & 1.09 \pm 0.70 & 0.90 \pm 0.56 & 0.677 \\
\end{array}
\]

**Table 3**

Association between −514C>T polymorphism and plasma lipid concentration by ethnic group in the Singaporean population

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Chinese</th>
<th>Malays</th>
<th>Indians</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC (n = 501)</td>
<td>37.9 ± 11.7</td>
<td>39.1 ± 12.9</td>
<td>38.9 ± 12.5</td>
</tr>
<tr>
<td>CT (n = 655)</td>
<td>37.7 ± 12.2</td>
<td>37.5 ± 12.3</td>
<td>40.1 ± 10.4</td>
</tr>
<tr>
<td>TT (n = 168)</td>
<td>39.5 ± 11.2</td>
<td>40.7 ± 11.1</td>
<td>36.7 ± 10.1</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.70 ± 5.30</td>
<td>22.70 ± 3.90</td>
<td>23.10 ± 3.80</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>5.28 ± 1.02</td>
<td>5.50 ± 1.10</td>
<td>5.58 ± 1.02</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>3.31 ± 0.93</td>
<td>3.41 ± 0.98</td>
<td>3.51 ± 0.91</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>1.29 ± 0.81</td>
<td>1.42 ± 1.32</td>
<td>1.77 ± 2.79</td>
</tr>
<tr>
<td>HDL-C/TG</td>
<td>1.52 ± 0.99</td>
<td>1.49 ± 0.95</td>
<td>1.42 ± 0.95</td>
</tr>
</tbody>
</table>

**Table 4**

Pooled analysis of the effect of −514C>T polymorphism on plasma lipid concentration in the Singaporean population, stratified by genotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Chinese (n = 833)</th>
<th>Malays (n = 1066)</th>
<th>Indians (n = 271)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.46 ± 0.05</td>
<td>5.56 ± 0.05</td>
<td>5.64 ± 0.09</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.26 ± 0.02</td>
<td>1.32 ± 0.02</td>
<td>1.33 ± 0.03</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>3.54 ± 0.05</td>
<td>3.58 ± 0.04</td>
<td>3.65 ± 0.07</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>1.49 ± 0.07</td>
<td>1.62 ± 0.06</td>
<td>1.82 ± 0.11</td>
</tr>
<tr>
<td>HDL-C/TG</td>
<td>0.94 ± 0.04</td>
<td>0.93 ± 0.04</td>
<td>0.86 ± 0.06</td>
</tr>
</tbody>
</table>

**Table 5**

Association between −514C>T polymorphism and plasma lipid concentration in the Singaporean population, stratified by genotypes

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<thead>
<tr>
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</tr>
<tr>
<td>LDL-C, mmol/L</td>
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<td>3.58 ± 0.04</td>
<td>3.65 ± 0.07</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>1.49 ± 0.07</td>
<td>1.62 ± 0.06</td>
<td>1.82 ± 0.11</td>
</tr>
<tr>
<td>HDL-C/TG</td>
<td>0.94 ± 0.04</td>
<td>0.93 ± 0.04</td>
<td>0.86 ± 0.06</td>
</tr>
</tbody>
</table>
ever, the magnitude of the effect was more prominent in Indians and Malays, as compared with Chinese.

The effect of this interaction on HDL-C concentrations in the whole population was further explored. Although higher fat intake correlated with higher HDL-C concentrations in CC subjects and lower HDL-C concentrations in TT subjects, the interaction of HL polymorphism and fat was not significant (P = 0.190). Further adjustment for daily intake of carbohydrates and proteins did not change the significance of these results (P = 0.189). Bearing in mind the heterogeneity of the effect among ethnic groups for this particular interaction, this effect was explored separately in Chinese, Malay and Indian subjects. There was no interaction of HL polymorphism and total fat in Chinese and Malays. However, there was an interactive effect in Indians (P = 0.015). In Indians consuming <30% of energy from fat (Fig. 3), mean HDL-C concentrations were different (P = 0.045) across HL genotypes, with TT subjects displaying the highest concentrations (1.27 ± 0.08 mmol/L, vs. 1.09 ± 0.04 mmol/L in CC subjects; P = 0.029) after adjustment for potential confounders. Conversely, when fat intake was ≥30% of energy, HDL-C was lower in TT subjects (1.04 ± 0.09 mmol/L). The latter represents a decrease of 21% (P = 0.049) in HDL-C concentrations in TT subjects consuming a high fat diet. The significance of this diet-fat interaction persisted when dietary fat was dichotomized according to the mean intake of the Singaporean population (27.5% of energy), as well as when additional controls for carbohydrates and proteins were applied (P = 0.015).

To avoid the problem of selecting cutoff points, the modification of the effect of total fat as a continuous variable on TG, HDL-C and HDL-C/TG ratio (Fig. 4A, B and C, respectively) in the Singaporean population was examined after adjustment for age, gender, ethnic group, BMI, tobacco smoking, alcohol consumption, physical exercise, diabetes and total energy intake. In agreement with the data obtained using dietary fat as a qualitative variable, modification of the effect of HL polymorphism by total fat intake on TG concentrations seemed to be linear (P = 0.035). Consequently, the slope in TT individuals was statistically different from those found in CT and CC subjects (P = 0.021 and P = 0.012, respectively). Although HDL-C concentrations in TT subjects also decreased as dietary total fat increased (Fig. 4B), this gene-nutrient interaction was not significant in the entire Singaporean population (P = 0.760), and there was heterogeneity among ethnic groups. Examination of the linear effect by ethnic group (results not shown) showed an interaction term between total fat intake (continuous) and LIPC polymorphism for HDL-C concentrations in Indians (P = 0.048). This interaction effect was particularly noted for TT subjects. In these subjects HDL-C concentrations decreased as total fat intake increased (P = 0.033). Likewise, when the HDL-C/TG ratio

**FIGURE 1** Fasting triglycerides (TG) in the Singaporean population plotted against −514C>T polymorphism and two levels of fat intake (<30% of energy) after adjustment for gender, age, ethnic group, BMI, tobacco smoking, alcohol consumption, physical exercise, diabetes and total energy intake. P-value for the interaction was obtained in the regression model. Values are adjusted means ± SEM, n = 2170. Values marked with a are different from other means (P < 0.05).

**TABLE 5**

*Modification of the effect of −514C>T polymorphism on plasma triglyceride (TG) concentration depending on the total fat intake (tertiles) in the Singaporean population*

<table>
<thead>
<tr>
<th>−514C&gt;T polymorphism</th>
<th>Tertile 1 (6.8–23.5%)</th>
<th>Tertile 2 (23.6–29.7%)</th>
<th>Tertile 3 (29.8%–54.1%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TG (P = 0.305)² mmol/L</td>
<td>TG (P = 0.222)² mmol/L</td>
<td>TG (P &lt; 0.001)² mmol/L</td>
</tr>
<tr>
<td>CC</td>
<td>1.80 ± 0.12 (n = 252)</td>
<td>1.81 ± 0.10 (n = 279)</td>
<td>1.75 ± 0.11a (n = 303)</td>
</tr>
<tr>
<td>CT</td>
<td>1.93 ± 0.11 (n = 367)</td>
<td>1.87 ± 0.09 (n = 362)</td>
<td>1.94 ± 0.11b (n = 836)</td>
</tr>
<tr>
<td>TT</td>
<td>1.83 ± 0.15 (n = 99)</td>
<td>2.22 ± 0.15 (n = 87)</td>
<td>2.54 ± 0.16cd (n = 85)</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM; tertile 1, n = 718; tertile 2, n = 728; tertile 3, n = 724. Means were adjusted for ethnic group, gender, age, body mass index, tobacco smoking, alcohol consumption, physical exercise, diabetes, energy, carbohydrates and proteins. P-value for total fat tertile–polymorphism interaction = 0.005. Values marked with letters differ from other means: a = different from TT individuals in tertile 3 (P < 0.001); b = different from CC individuals in tertile 3 (P < 0.05); c = different from CT individuals in tertile 3; d = different from CC individuals in tertile 1.

2 P-value obtained by comparison of adjusted means across the hepatic lipase genotypes.

3 P-value obtained by comparison of adjusted means across the tertiles of fat intake.
was considered as the outcome variable in order to control one variable for another (Fig. 4C), the interaction terms for the Singaporean population as a whole were significant ($P = 0.048$).

To determine the homogeneity of the different sources of fat, we examined the effect of the specific fatty acids (SFA, MUFA and PUFA) on these interactions. There was no heterogeneity of the effect depending on the type of fat. Thus, the significance of the interaction terms between these types of fat and HL polymorphism for the lipid concentrations were borderline for SFA, MUFA and PUFA separately (results not shown), indicating a greater effect for total fat intake as compared with specific fatty acids.

Finally, considering that enhanced HL activity has been related to T2DM and obesity, the associations between this polymorphism and insulin resistance-related variables and the effect of these variables on the reported interaction were investigated. There was no association between $-514C>T$ polymorphism and diabetes in any ethnic group, and no differences in the percentage of diabetic subjects by genotype (9.0, 6.9 and 7.0% for CC, CT and TT individuals, respectively; $P = 0.222$) when the population as a whole was considered. For fasting glucose levels, there was no interaction between total fat intake and HL polymorphism, and we obtained a marginally significant term in determining fasting insulin in the whole population ($P$ for interaction HL $\times$ fat $= 0.060$). The interaction effect was greater in TT individuals. Thus, the adjusted mean of fasting insulin in TT subjects consuming $<30\%$ of energy from fat was $56.6 \pm 3.6$ pmol/L versus $71.7 \pm 5.0$ pmol/L in TT subjects consuming $>30\%$ fat ($P = 0.046$).

Further, subjects were considered obese ($\text{BMI} \geq 27\text{kg/m}^2$) and nonobese ($\text{BMI} < 27\text{kg/m}^2$) according to the recommendations for this population, and when the first-order interaction term between obesity and HL polymorphism was tested in the whole population, it was significant for HDL-C and TG ($P = 0.003$ and $P = 0.041$, respectively). Overall, in obese.

FIGURE 2 Fasting triglycerides (TG) in (A) Chinese, (B) Malays and (C) Indians plotted against $-514C>T$ polymorphism and two levels of fat intake ($</>=30\%$ of energy) after adjustment for gender, age, ethnic group, BMI, tobacco smoking, alcohol consumption, physical exercise, diabetes and total energy intake. $P$-values for the interaction terms were obtained in the corresponding regression model. Values are adjusted means $\pm$ SEM; $n = 1324$ Chinese (A), $n = 471$ Malays (B), $n = 375$ Indians (C). Values marked with letters are different from other means ($P < 0.05$): $a =$ different from $b$; $c =$ different from $b$.

FIGURE 3 High-density lipoprotein-cholesterol (HDL-C) concentrations in Indians plotted against $-514C>T$ polymorphism and two levels of fat intake ($</>=30\%$ of energy) after adjustment for gender, age, ethnic group, BMI, tobacco smoking, alcohol consumption, physical exercise, diabetes and total energy intake. $P$-value for the interaction of fat intake and hepatic lipase (HL) polymorphism was obtained in the regression model. Values are adjusted means $\pm$ SEM, $n = 375$. Values marked with letters are different from other means ($P < 0.05$): $a =$ different from $b$; $b =$ different from $d$; $c =$ different from $d$. 

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subjects, the T allele correlated with lower HDL-C and higher TG concentrations as compared with CC homozygotes. In the analysis by ethnic group, the three-way interaction term among HL, fat and obesity was marginally significant in Chinese for HDL-C ($P = 0.081$) and TG ($P = 0.090$) and nonsignificant in Malays and Indians (both $P > 0.05$). After fully multivariate adjustment, TT Chinese subjects consuming >30% of energy from fat showed lower concentrations of HDL-C only if they were obese (1.40 ± 0.04 and 1.49 ± 0.06 mmol/L in nonobese, $P = 0.147$ vs. 1.28 ± 0.09 and 0.87 ± 0.10 mmol/L in obese, $P = 0.05$, when consuming <30 and >30% fat, respectively). Accordingly, TT Chinese subjects consuming >30% of fat showed higher concentrations of TG only if they were obese (2.13 ± 0.17 and 1.96 ± 0.25 mmol/L in nonobese, $P = 0.301$ vs. 2.24 ± 0.36 and 5.20 ± 0.40 mmol/L in obese, $P = 0.049$, when consuming <30 and >30% fat, respectively). Conversely, in Asian Indians and Malays, the interaction term between HL polymorphism and fat remained statistically independent of the additional effect of obesity.

**DISCUSSION**

In this study of a representative sample of the Singaporean population, we found that a highly prominent gene-diet interaction between −514 C>T polymorphism in the HL gene promoter and dietary fat affects plasma lipid concentrations. These results are in agreement with our recent findings in the Framingham Heart Study showing that dietary fat modifies the effect of −514C>T polymorphism on HDL metabolism (5). Furthermore, the current work is the first to report an interaction between this polymorphism in the HL gene promoter and total fat intake affecting plasma TG, as well as the first to suggest an additional modulation by obesity. One factor contributing to the detection of the interaction with TG was the higher prevalence of the T allele in Asians compared with Caucasians (14,29–31). In fact, the allele frequency of this polymorphism differs widely among ethnic groups, ranging from 0.15 to 0.26 in Caucasians (8,10,13,14,17,32,33) and from 0.45 to 0.52 in African Americans (34,35) and Japanese (13,36,37). Our study confirms the higher prevalence of the T allele in Asians and shows differences at this locus between ethnic groups in Singapore. Compared with the U.S. population, the Singaporean population offers the possibility of obtaining a large number of homozygotes for association studies with a similar sample size. Thus, although the sample size in the current study (1011 men and 1159 women) was very close to the number of participants in our previous Framingham report (1020 men and 1110 women), the number of TT homozygotes was 3 times higher (271 vs. 73 TT subjects), increasing the power to detect associations showing a similar effect (5).

In Singapore rapid urbanization has been accompanied by an increase in the incidence of CVD to levels that exceed those seen in Western populations (1,18). However, the effects of urbanization have not affected all three ethnic groups equally. Indians have the highest rate of CVD (19,38), as well as the lowest HDL-C concentrations (39,40). Although differences in lifestyle factors are evident among these ethnic groups, they are not sufficient to explain the ethnic differences in lipid profiles (23), making the examination of loci related to HDL metabolism very important in this population. The first evidence that −514C>T polymorphism at the HL gene locus influences HDL-C concentrations was provided by Cohen et al. (41). In addition, they observed that this polymorphism was in complete linkage disequilibrium with three other pro-

**FIGURE 4** Predicted values of (A) triglycerides (TG), (B) high-density lipoprotein-cholesterol (HDL-C) and the (C) HDL-C/TG ratio in the Singaporean population by HL genotype (CC, $n = 833$; CT, $n = 1066$; TT, $n = 271$) plotted against amount of fat consumed (as continuous variable). Predicted values were calculated from the regression models containing total fat intake, −514C>T polymorphism and their interaction term, after adjustment for gender, age, ethnic group, body mass index (BMI), tobacco smoking, alcohol consumption, physical exercise, diabetes and total energy intake.
moter polymorphisms (34). Although the association between the T allele and plasma lipids differs among populations (14,42), numerous studies report higher HDL-C concentrations in carriers of the T variant as compared with CC homozygotes (8,10,17,36,41,43). However, only a few studies have reported an association between this polymorphism and plasma TG (15,43,44). In one study, carried out by Jansen et al. (43) in the European Atherosclerosis Research Study II, the T allele was associated with higher concentrations of plasma TG, HDL-C, apolipoprotein A-I and apolipoprotein B. However, the authors concluded that the reason for the effect of the T allele on TG concentrations was unclear.

In the Singaporean population, when no stratification for dietary fat intake is considered, the T allele is associated with higher plasma HDL-C as well as TG concentrations, with a clearer dose effect for TG in every ethnic group. There are several facts that may explain this association. One may be related to the much greater statistical power of the present study than in other populations, particularly considering that the SD of TG is higher than that of HDL-C. For example, in the Framingham Heart Study (5), mean plasma TG concentrations according to LIPC genotype were: 1.60 ± 1.10, 1.63 ± 1.19 and 1.86 ± 1.13 mmol/L for CC, CT and TT subjects, respectively. The increase in TG associated with the T allele was very close to the results obtained in the Singaporean population; however, in Framingham mean differences were not statistically significant. Another fact that may contribute to the association with TG is the hypertriglyceridermia that characterizes Asian populations because of their low fat diets as compared with those of Caucasians (1,23,38,45). This effect was probably not seen in the studies on Caucasian groups because their higher fat diets do not induce hypertriglyceridermia.

An additional reason may be the specificity of the association. The T allele is related to decreased HL activity (9,12–14). Specifically, HL promotes the conversion of large, buoyant HDL2 to small, dense HDL3 by modulating the phospholipid content of these particles. Thus, a more favorable lipid profile has been described in carriers of the T allele. This profile is characterized by increased plasma concentrations of TG-rich HDL2 and large buoyant LDL particles (6,7,13). Presumably, the changes in HDL-C are a reflection of changes in HDL3-C, and some studies show that only HDL2-C and not total HDL-C concentrations appear to be strongly associated with HL polymorphism (13,35). In the present report, HDL2-C concentrations were not determined, and we cannot test the relevance of this association. However, bearing in mind that TG have a higher negative correlation with HDL-C concentrations and a positive correlation with small LDL particles (46,47), our results may suggest that TG concentrations in this population represent a more sensitive marker than HDL-C for testing the effect of HL polymorphisms on HDL metabolism. Alternatively, a more likely explanation is that plasma TG concentrations reflect changes in HL activity per se. It has been reported that HL plays a secondary role in the clearance of chylomicron remnants by the liver (7). Low HL activity has been associated with high levels of TG and HDL-C in subjects with HL deficiency as well as in rat models (48). Therefore, reduced HL activity is also related to delayed clearance of TG-rich particles, and plasma TG concentrations may be increased in response to this impairment. Specifically, HL has been involved in the hydrolysis of phospholipids and TG of chylomicron remnants, and may influence remnant removal by the hydrolysis of chylomicron phospholipids, unmasking apolipoprotein E and thereby enhancing binding to the LDL receptor-related protein (49).

However, gene-diet interactions such as those reported in the Framingham Heart Study (5) must be considered when explaining the possible heterogeneity of these associations (14). We demonstrated that the T allele was associated with greater HDL-C, HDL2-C, large HDL subtraction and HDL particle size only in subjects that consumed <30% of energy from total fat. In addition, TT subjects consuming a high fat diet presented higher TG concentrations than their counterparts when consuming a low fat diet. However, as discussed above, in the Framingham Heart Study the interaction did not affect TG.

The results of the present report showing that dietary fat modifies the effect of −514C>T polymorphism on plasma lipid concentrations in Singaporeans agree with our previous findings; specifically, the consistency of the harmful effects of a high fat diet on plasma lipids in TT individuals is notable. This is the first gene-diet interaction study to offer such homogeneity among populations. Despite these consistencies, there is still a need for more interplay between the US and the Singaporean populations that should be discussed.

First, in Singapore TG was the main lipid parameter affected by this gene-diet interaction, whereas in Framingham, HDL-C and HDL particle size were the main parameters. Some of the reasons mentioned above regarding the importance of TG concentrations in Asian subjects might contribute to this difference.

Second, the interaction between total fat intake and HL polymorphism affected HDL-C levels only in Indians and not in Chinese and Malays, suggesting a greater similarity between Indian Singaporeans and Framingham participants for some characteristics, for example, BMI, which was higher in the United States than in Singapore. Interestingly, the modulating effect of BMI and obesity-related parameters may consistently explain this fact, considering that we have reported that the interactive effect among HL polymorphism, fat intake and obesity on HDL-C in Chinese shows borderline significance. Thus, when analyzed only in obese subjects, the interaction of HL polymorphism and fat intake on HDL-C in Chinese subjects paralleled the effect found in the Framingham Heart Study. Some studies demonstrate that obesity, mainly intraabdominal fat, increases HL activity and attenuates the effect of −514C>T polymorphism on HDL-C (50,51). Our results agree with this last observation and are the first to report an additional interaction of dietary fat in modulating the effect of obesity.

Third, when the type of fatty acid was examined in the Framingham Heart Study, only SFA and MUFA were effect modifiers; PUFA showed no interaction. Conversely, the Singapore study showed no heterogeneity in the effect of the specific fatty acids. One of the main factors that would contribute to this fact is the specific source of dietary fat. In Framingham, the main sources of SFA and MUFA were animal fats (meat and dairy products), whereas the main sources of PUFA were vegetable oils (5). However, the Singapore study detected a marked difference in types of vegetable fat; there were more coconut, palm and mixed vegetable oils, which are especially rich in SFA (23,24).

This interaction may help to explain conflicting data regarding whether this polymorphism modulates the CVD risk (16,52). Despite the antiatherogenic effects of the T allele on increased HDL2-C and on LDL size (large, buoyant particles), it appears to be associated with more subclinical atherosclerosis and even with increased risk of diagnosed CVD (53,54). According to our results from the present study and the Framingham report (5), −514C>T polymorphism may be a proatherogenic or an antiatherogenic factor depending on
dietary fat intake. When total fat intake is <30% of energy, the T allele was associated with a more favorable plasma lipid profile characterized by higher HDL-C concentrations and particle size. However, in a high fat diet, the T allele, mainly in TT subjects, was associated with a detrimental lipid profile characterized by higher TG and lower HDL-C concentrations than in CC subjects. This effect could be further modulated by obesity, increasing the proatherogenic role of the T allele in a high fat diet.

Several authors report a different interindividual response to dietary fat intake, although they did not investigate the effect of −514C>T polymorphism. This is the case with Deon et al. (55), who reported that baseline LDL subclass patterns (modulated by HL activity) strongly influence the response to low fat versus high fat diets, results that may be partially explained by this specific interaction. We hypothesize a defect in the remnant lipoprotein metabolism in TT subjects that consumed a high fat diet as a mechanism that may explain the higher TG concentration observed in this population. In TT subjects, TG concentrations in postprandial states are highly responsive to the environmental stimuli of a high fat load, contributing to the atherogenic lipid profile. The action of fat intake on HL activity may directly mediate another mechanism. However, the relationship of dietary fat to HL activity is still controversial (56–58). In addition, recent evidence suggests that HL polymorphism may also interfere with components of glucose homeostasis, abolishing the ability of insulin to stimulate HL activity (16). Although increased HL activity has been reported in T2DM (5), Dugi et al. (54) described an overrepresentation of patients with diabetes mellitus in the lowest HL activity quartile. In the Singaporean population, there was no association between this polymorphism and diabetes. These results were in agreement with the Framingham Heart Study (5). However, considering the potential effects of the HL-fat interaction and the small number of diabetic subjects, a more powerful prospective design is needed to specifically test the hypothesis of an increased risk of T2DM in TT subjects that consume a high-fat diet.

In agreement with the Framingham Heart Study, we conclude that dietary fat modifies the effect of −514C>T polymorphism in the HL gene on plasma lipid profile. It is necessary to take this interaction into account when explaining the effects of this polymorphism on cardiovascular risk, especially in Asian Indians. Interestingly, TT subjects that had an antiatherogenic lipid profile when consuming a low fat diet were the most susceptible to a high fat diet. Furthermore, obesity and diabetes may be important factors modulating the effect of this interaction by increasing the deleterious effect of a high fat diet in TT subjects.

LITERATURE CITED


