G-250A Substitution in Promoter of Hepatic Lipase Gene Is Associated With Dyslipidemia and Insulin Resistance in Healthy Control Subjects and in Members of Families With Familial Combined Hyperlipidemia

Jussi Pihlajamäki, Leena Karjalainen, Pauli Karhapää, Ilkka Vauhkonen, Marja-Riitta Taskinen, Samir S. Deeb, Markku Laakso

Abstract—Low activity of hepatic lipase (HL) has been associated with high levels of triglycerides and high density lipoproteins, but the association of the HL promoter variants with insulin sensitivity has not been investigated. Therefore, in this study, the relationship of the G-250A promoter variant of the HL gene to the rates of insulin-stimulated glucose uptake measured by the hyperinsulinemic euglycemic clamp was investigated in 110 control subjects (82 men and 28 women, aged 50.7 ± 7.6 [mean ± SD] years, body mass index 26.1 ± 3.6 kg/m²) and in 105 first-degree relatives (65 men and 40 women, aged 47.8 ± 16.0 years, body mass index 26.9 ± 5.3 kg/m²) of 34 families with familial combined hyperlipidemia (FCHL). The A-250 allele of the HL promoter was associated with low rates of insulin-stimulated whole-body nonoxidative glucose disposal in control subjects (41.1 ± 12.7 μmol · kg⁻¹ · min⁻¹ in subjects with the G-250G genotype, 36.9 ± 13.1 μmol · kg⁻¹ · min⁻¹ in subjects with the G-250A genotype, and 29.9 ± 13.5 μmol · kg⁻¹ · min⁻¹ in subjects with the A-250A genotype; P = 0.012 adjusted for age and sex) and with low rates of insulin-stimulated whole-body glucose oxidation in FCHL family members (16.7 ± 4.2 versus 15.0 ± 4.4 versus 14.1 ± 4.4 μmol · kg⁻¹ · min⁻¹, P = 0.024). In addition, the A-250 allele was associated with high levels of fasting insulin (P = 0.047), very low density lipoprotein cholesterol (P = 0.007), and total (P = 0.009) and very low density lipoprotein triglycerides (P = 0.005) triglycerides in control subjects and with high levels of low density lipoprotein triglycerides (P = 0.001) in FCHL family members (n = 340). We conclude that the G-250A promoter variant of the HL gene is associated with dyslipidemia and insulin resistance. Mechanisms via which this polymorphism could affect insulin sensitivity remain to be elucidated.

Key Words: hepatic lipase ■ insulin resistance ■ familial combined hyperlipidemia ■ hypertriglyceridemia

Impaired insulin action has been associated with diabetes, obesity, dyslipidemia, and hypertension and also with atherosclerotic vascular disease.1-2 Insulin resistance can be inherited3-5 and is a part of the most common inherited dyslipidemia, familial combined hyperlipidemia (FCHL),6-9 which suggests that genes that predispose to FCHL also regulate insulin action. However, no major genes that cause insulin resistance in FCHL or other diseases have been identified.10

Hepatic lipase (HL) catalyzes the hydrolysis of triglycerides from IDLs and LDLs and takes part in the metabolism of HDLs and the uptake of remnant lipoproteins in the liver.11,12 Therefore, it is not surprising that HL deficiency, caused by defects in the HL gene, leads to hypertriglyceridemia, high HDL levels, and coronary heart disease (CHD).13,14 Hypertriglyceridemia and CHD have also been associated with FCHL.15,16 Therefore, the HL gene is one of the candidate genes for FCHL. Although amino acid substitutions in the coding region of the HL gene are uncommon,13,14 4 common polymorphisms (G-250A, C-514T, T-710C, and A-763G) in the promoter of the HL gene have been associated with low HL activity. These polymorphisms are in complete linkage disequilibrium, forming 2 haplotypes.17,18 In several studies, the rare allele, or the rare haplotype, has been associated with low HL activity and with high triglyceride levels,19,20 high HDL cholesterol levels,20-22 buoyant LDL particles,22 and CHD.21

How the variants in the HL promoter could increase the risk of CHD20 is currently unknown. Low HL activity and, consequently, hypertriglyceridemia19,20 in subjects with these variants may contribute to CHD, but on the other hand, high HDL cholesterol levels20-22 and buoyant LDL particles22 in these subjects may be less atherogenic. Whether the HL promoter variants contribute to impaired insulin-stimulated glucose uptake and insulin resistance requires further investigation.
glucose uptake, which is often seen in patients with hypertriglyceridemia, has not been investigated. Therefore, in the present study, the association of the G-250A variant in the LDL receptor gene, which explained 90% of all cases of familial hypercholesterolemia (14 men and 5 women), 22 with hypertriglyceridemia (8 men and 7 women), 20 with mixed hyperlipidemia (8 men and 7 women), and 21 with normolipidemia (8 men and 7 women). All subjects participating in the present study were Finnish. The Finnish population is genetically relatively homogeneous, originating mainly from southern (European) and eastern (Asian) immigrants 2000 years ago.23,24 All controls and FCHL family members had a normal glucose tolerance according to the World Health Organization criteria,25 normal liver, kidney, and thyroid function tests, no history of excessive alcohol intake, and no severe chronic disease. In addition, control subjects did not have hypertension, symptoms or signs of CHD, or continuous drug treatment.

**Methods**

**Subjects**

All subjects participating in the present study were Finnish. The Finnish population is genetically relatively homogeneous, originating mainly from southern (European) and eastern (Asian) immigrants 2000 years ago.23,24 All controls and FCHL family members had a normal glucose tolerance according to the World Health Organization criteria,25 normal liver, kidney, and thyroid function tests, no history of excessive alcohol intake, and no severe chronic disease. In addition, control subjects did not have hypertension, symptoms or signs of CHD, or continuous drug treatment.

**FCHL Family Members**

Twenty-five of the probands with FCHL were selected from the myocardial infarction survivor family study carried out at our department.26 Selection of these subjects has been previously described in detail.9 Briefly, cutoff points for lipids were 7.7 mmol/L for total cholesterol in men and in women and 2.2 mmol/L for total triglycerides in men and 2.4 mmol/L in men. These cutoff points were based on the lipid values of the control population, which consisted of 250 persons (161 men and 89 women) of the same myocardial infarction survivor family study from which FCHL families were identified. The cutoff points for abnormal lipids were defined as the 90th percentile for total cholesterol and 90th percentile for total triglycerides. The 80th percentile for total cholesterol was used because of the high cholesterol level among subjects living in eastern Finland. After adjustment for age with linear regression analysis, the values for the median age (55 years) of this population were used as cutoff points for abnormal lipids. To meet the criteria for FCHL, each family had to have at least 3 affected members with different types of dyslipidemia and at least 1 affected family member in 2 generations. Additionally, 9 FCHL probands and their families were identified from the Coronary Angiography Register of the Kuopio University Hospital according to the same lipid criteria. None of the study subjects had tendon xanthomas or defects in the LDL receptor gene, which explained ~90% of all cases of familial hypercholesterolemia in this area.27 Altogether, 34 families with FCHL and their 340 family members met the criteria and were included in the present study (Table 1).
minutes to raise plasma insulin concentration quickly to the desired level, where it was maintained by a continuous insulin infusion of 480 pmol/m² per minute. Under these study conditions, hepatic glucose production is completely suppressed in nonobese subjects and in patients with FCHL. Blood glucose was clamped at 5.0 mmol/L for the next 180 minutes by the infusion of 20% glucose at varying rates according to the blood glucose measurements performed at 5-minute intervals. The mean rates of glucose infusion during the last hour of the clamp were used to calculate the rates of insulin-stimulated whole-body glucose uptake (WBGU).

Indirect calorimetry was performed with a computerized flow-through canopy gas analyzer system (Deltatrac, Datex) as previously described. Gas exchange was measured for 30 minutes after a 12-hour fast and during the last 30 minutes of the euglycemic clamp. The first 10 minutes of each measurement was discarded, and the mean value of the last 20 minutes was used in calculations. The rates of glucose oxidation were calculated according to Ferrannini (determined by indirect calorimetry in the last 20 minutes of the euglycemic clamp). The rates of nonoxidative glucose disposal during the euglycemic clamp were estimated by subtracting the glucose oxidation rate from the rates of WBGU.

### Analytical Methods

Plasma glucose levels in the fasting state and after an oral glucose load and blood glucose and plasma lactate levels during the euglycemic clamp were measured by the glucose oxidase method (2300 Stat Plus, Yellow Springs Instrument Co Inc). For the determination of plasma insulin, blood was collected in EDTA-containing tubes, and after centrifugation, the plasma was stored at −20°C until the analysis. Plasma insulin concentration was determined by a commercial double-antibody solid-phase radioimmunoassay (Phadebus Insulin RIA 100, Pharmacia Diagnostics AB). Lipoprotein fractionation was performed by ultracentrifugation and selective precipitation, as previously described. Cholesterol and triglyceride levels from whole serum and lipoprotein fractions were assayed by automated enzymatic methods (Boehringer-Mannheim). ApoB and apoA-I levels were determined by a commercial immunoturbidimetric method (Kone Instruments), and serum free fatty acids (FFAs) from fresh frozen samples were determined by an enzymatic method (Wako Chemicals GmbH). Nonprotein urinary nitrogen was measured by an automated Kjeldahl method. Postheparin plasma HL activity was measured after an intravenous bolus of heparin (100 IU/kg body wt). Blood was drawn 15 minutes later into chilled heparinized tubes kept on ice. Plasma was immediately isolated by centrifugation at −20°C. HL activity was measured after inactivation of lipoprotein lipase with a substrate containing 1 mol/L NaCl, and LPL activity was measured immunochromatically by use of a specific antisera against HL raised in rabbits.

### Determination of G-250A Substitution of HL Gene

The genotype at position −250 of the HL promoter was determined by polymerase chain reaction and DraI digestion as previously described.

### Statistical Analysis

All basic calculations were performed with the SPSS/Win programs (version 7.5, SPSS Inc). The differences in the measured parameters among the control subjects with 3 different genotypes were tested by ANCOVA, with age and sex as covariates. In FCHL families, results were analyzed by using family-based association analysis with the program ASSOC (S.A.G.E., version 2.2). The ASSOC program uses linear regression analysis, allowing the quantitative trait to have familial correlation among individuals. The likelihood for the pedigree is computed with a linear regression model in which the quantitative trait is the dependent variable and the genetic variation and discrete and/or continuous covariates are independent variables. Residual variation is modeled by assuming an additive polygenic pattern of correlation among relatives. By use of this model, the likelihood for each pedigree was maximized twice, with and without the genetic variant in the model. The difference in natural logarithms of these 2 maximized likelihoods follows the χ² distribution, from which the corresponding P value is taken with 2 degrees of freedom.

(3 groups of genotypes). VLDL cholesterol, total triglycerides, all subfractions of triglycerides, insulin, and FFA levels were logarithmically transformed to obtain normal distribution before statistical analyses. A value of P < 0.05 was considered statistically significant. All data are presented as mean ± SD.

### Results

#### Clinical Characteristics

Characteristics of the study groups are shown in Table 1. The subgroup of FCHL family members (n = 105) who participated in the hyperinsulinemic clamp did not differ from the group of all FCHL family members (n = 340). Compared with the control group, both FCHL groups included more women and had higher fasting levels of insulin, total cholesterol, LDL cholesterol, VLDL cholesterol, total triglycerides, HDL triglycerides, VLDL triglycerides, and apoB (all P < 0.05, Table 1). In addition, the subgroup of clamped FCHL family members had higher levels of LDL triglycerides than did the control subjects, and the mean age of the group of all FCHL family members (n = 340) was lower than the mean age of the controls (all P < 0.01, Table 1). During the hyperinsulinemic clamp, FCHL family members, compared with control subjects, had higher levels of FFAs (P < 0.001), higher rates of lipid oxidation (P < 0.001), and lower rates of insulin-stimulated glucose uptake (P < 0.001), glucose oxidation (P < 0.001), and nonoxidative glucose disposal (P < 0.008, data not shown).

#### Role of the G-250A Substitution of the HL Gene in the Fasting State

The frequencies of the rare A-250 allele of the HL gene were similar in control subjects and in 34 probands with FCHL (0.25 versus 0.30), and the genotypes were in Hardy-Weinberg equilibrium. In control subjects, this allele was associated with high levels of fasting insulin (P = 0.047, adjusted for age and sex), VLDL cholesterol (P = 0.007), total triglycerides (P = 0.009), and VLDL triglycerides (P = 0.005, Table 2). In FCHL family members, the A-250 allele was associated with high levels of LDL triglycerides (P = 0.001) and also with low activity of postheparin HL (P = 0.010). No associations were observed between this polymorphism and body mass index (BMI), waist-to-hip ratio, fasting glucose, HDL or LDL cholesterol, apoA-I, and apoB in either of the study groups (Table 2). When we analyzed the data in FCHL families separately in FCHL patients and their relatives without dyslipidemia, no significant associations were seen (all P > 0.100).

#### Effect of the G-250A Substitution of the HL Gene on Insulin Action

In control subjects, no association was observed between the G-250A polymorphism of the HL promoter and the fasting levels of FFAs or the fasting rates of lipid and glucose oxidation or between the G-250A polymorphism and the levels of FFAs, rates of lipid oxidation, or rates of insulin-stimulated WBGU during the clamp (Table 3). However, the A-250 allele of the HL promoter was associated with low rates of insulin-stimulated nonoxidative glucose disposal (41.1 ± 12.7 μmol·kg⁻¹·min⁻¹ in subjects with the G-250G genotype, 36.9 ± 13.1 μmol·kg⁻¹·min⁻¹ in subjects with the G-250A genotype, and 29.9 ± 13.5 μmol·kg⁻¹·min⁻¹ in subjects with the A-250A genotype; P = 0.012 adjusted for
TABLE 2. Fasting Values According to G-250A Polymorphism of the HL Gene

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects</th>
<th></th>
<th>FCHL Families</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G/G</td>
<td>G/A</td>
<td>A/A</td>
<td>G/G</td>
</tr>
<tr>
<td>Without FCHL/FCHL, n/h</td>
<td>62/0</td>
<td>40/0</td>
<td>8/0</td>
<td>92/52</td>
</tr>
<tr>
<td>Sex (men/women), n/h</td>
<td>48/14</td>
<td>26/14</td>
<td>8/0</td>
<td>70/74</td>
</tr>
<tr>
<td>Age, y</td>
<td>51.6±6.7</td>
<td>48.1±8.1</td>
<td>57.5±5.2</td>
<td>48.4±15.7</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.1±3.3</td>
<td>25.8±4.0</td>
<td>27.9±3.5</td>
<td>26.5±4.7</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.94±0.07</td>
<td>0.92±0.09</td>
<td>0.99±0.04</td>
<td>0.92±0.12</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>133±14</td>
<td>132±13</td>
<td>131±13</td>
<td>134±19</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>84±8</td>
<td>84±7</td>
<td>83±6</td>
<td>80±11</td>
</tr>
<tr>
<td>Fasting plasma glucose, mmol/L</td>
<td>5.5±0.6</td>
<td>5.5±0.5</td>
<td>5.6±0.6</td>
<td>5.5±0.8</td>
</tr>
<tr>
<td>Fasting plasma insulin, mU/L</td>
<td>53.4±31.2</td>
<td>54.0±25.2</td>
<td>85.2±63.0†</td>
<td>70.2±44.4</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.81±1.15</td>
<td>5.52±0.96</td>
<td>6.65±1.38</td>
<td>6.47±1.44</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.28±0.29</td>
<td>1.38±0.29</td>
<td>1.43±0.30</td>
<td>1.28±0.30</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>3.96±0.99</td>
<td>3.57±0.86</td>
<td>3.91±1.53</td>
<td>4.43±1.22</td>
</tr>
<tr>
<td>VLDL cholesterol, mmol/L</td>
<td>0.59±0.31</td>
<td>0.56±0.25</td>
<td>1.32±1.97‡</td>
<td>0.75±0.51</td>
</tr>
<tr>
<td>Total triglycerides, mmol/L</td>
<td>1.24±0.48</td>
<td>1.35±0.53</td>
<td>2.54±3.14‡</td>
<td>1.77±1.05</td>
</tr>
<tr>
<td>HDL triglycerides, mmol/L</td>
<td>0.19±0.11</td>
<td>0.21±0.10</td>
<td>0.25±0.10</td>
<td>0.21±0.07</td>
</tr>
<tr>
<td>LDL triglycerides, mmol/L</td>
<td>0.35±0.13</td>
<td>0.33±0.12</td>
<td>0.45±0.17</td>
<td>0.35±0.15</td>
</tr>
<tr>
<td>VLDL triglycerides, mmol/L</td>
<td>0.71±0.40</td>
<td>0.81±0.41</td>
<td>1.84±2.94‡</td>
<td>1.21±0.94</td>
</tr>
<tr>
<td>Apo A-I, g/L</td>
<td>1.56±0.26</td>
<td>1.62±0.27</td>
<td>1.38±0.45</td>
<td>1.42±0.26</td>
</tr>
<tr>
<td>Apo B, g/L</td>
<td>1.03±0.26</td>
<td>0.91±0.25</td>
<td>1.09±0.30</td>
<td>1.14±0.31</td>
</tr>
<tr>
<td>Postheparin HL activity, mU/mL*</td>
<td>264±118</td>
<td>193±90</td>
<td>144±25‡</td>
<td>264±118</td>
</tr>
</tbody>
</table>

Values are mean±SD. *P<0.05 and †P<0.01 by ANCOVA in control subjects or by ASSOC in FCHL families over the 3 genotypes, with age and sex as covariates.

Correlations between fasting insulin levels, insulin action, and postheparin HL activity were determined in 69 FCHL family members who had undergone the hyperinsulinemic clamp and the heparin test. Fasting insulin levels and insulin-stimulated WBGU were highly negatively correlated (P<0.001). In the present study, no correlation was observed between WBGU and HL activity (r=-0.072, P=0.565). Fasting insulin levels correlated positively with HL activity in subjects with the G-250G genotype (n=28, r=0.419, and P=0.027) but not in the pooled group of subjects with the G-250A (n=39) and A-250A (n=2) genotypes (r=0.133, P=0.408).

Discussion

The novel findings of the present study were the associations of the A-250 allele in the promoter of the HL gene with low rates of nonoxidative glucose disposal in control subjects and with low rates of insulin-stimulated glucose oxidation in

TABLE 3. Results of Hyperinsulinemic Euglycemic Clamp According to G-250A Polymorphism of the HL Gene

<table>
<thead>
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<th>FCHL Families</th>
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</tr>
<tr>
<td>Without FCHL/FCHL, n/h</td>
<td>62/0</td>
<td>40/0</td>
<td>8/0</td>
<td>21/22</td>
</tr>
<tr>
<td>Sex (men/women), n/h</td>
<td>48/14</td>
<td>26/14</td>
<td>8/0</td>
<td>30/14</td>
</tr>
<tr>
<td>FFAs, mmol/L</td>
<td>0.05±0.06</td>
<td>0.06±0.04</td>
<td>0.07±0.08</td>
<td>0.18±0.11</td>
</tr>
<tr>
<td>Lipid oxidation, mg · kg⁻¹ · min⁻¹</td>
<td>0.01±0.24</td>
<td>0.01±0.21</td>
<td>-0.04±0.27</td>
<td>0.19±0.18</td>
</tr>
<tr>
<td>WBGU, μmol · kg⁻¹ · min⁻¹</td>
<td>60.3±14.9</td>
<td>56.7±14.8</td>
<td>48.8±13.9</td>
<td>50.0±16.4</td>
</tr>
</tbody>
</table>

Values are mean±SD. No significant differences were observed by ANCOVA in control subjects or by ASSOC in FCHL families over the 3 genotypes, with age and sex as covariates.
by the findings of Jansen et al, who reported a positive insulin-mediated stimulation of the HL promoter is supported with HL activity in noncarriers of the A-250 allele but not in carriers. Therefore, variants in the HL promoter may abolishing the ability of insulin to stimulate HL activity.

The observation of Jansen et al that the rare −514T allele of the HL promoter was associated with CAD in Dutch patients has so far been without a good explanation. Subjects with this rare allele, or the respective haplotype, have low HL activity, high HDL cholesterol levels, and buoyant LDL particles, which have all been associated low risk of CHD. On the other hand, the rare haplotype has been associated with hypertriglyceridemia and, according to the present study, also with insulin resistance. Therefore, a hypothesis can be proposed that the increased risk of CHD in subjects with the promoter substitutions of the HL gene may be caused by hypertriglyceridemia, a consequence of low HL activity, and by insulin resistance.

The HL gene is one of the candidate genes for FCHL. With the use of linkage analysis, a study of Finnish FCHL families proposed that the HL gene is not a major gene for FCHL. However, because positive associations could be observed...
between the G-250A polymorphism and LDL triglyceride levels in the FCHL family members in the present study, the HL gene may have a modifying role in FCHL.

It is currently unknown which of the polymorphisms in the HL promoter (G-250A, C-514T, T-710C, and A-763G) are functionally important. Because these variants are in complete linkage disequilibrium,17,18 association studies in populations do not reveal the importance of individual variants. Therefore, each variant has to be expressed separately, and all the response elements for insulin and other regulatory hormones in the HL promoter have to be identified. First results indicated that the deletion of the region −483 to +129 in the HL gene leads to a 60% drop in promoter activity, suggesting the presence of important stimulatory elements in this region.54 In addition, the disruption of the upstream stimulatory factor binding site, which mediates insulin stimulation of protein expression, by the C-514T substitution (change of E-box motif CACGTG to CATGTG) may be of importance.55

We conclude that the A-250 allele of the HL promoter is associated with insulin resistance and high triglyceride levels in healthy controls and in FCHL family members. This result implies that the HL gene may, in addition to serum lipids and lipoproteins, also be associated with insulin-mediated glucose disposal. Mechanisms through which variants in the HL gene could affect insulin action are currently unknown, but insulin resistance could be one of the possible contributors to atherosclerosis that have been observed in patients with the variants in the HL promoter.21

Acknowledgments

This study was supported by grants from the Medical Research Council of the Academy of Finland, the Finnish Foundation for Cardiovascular Disease, the Aarne and Aili Turunen Foundation, and National Institutes of Health grant HL-64322 to S. Deeb. The results of the present study were partly obtained with the S.A.G.E. Statistical Analysis for Genetic Epidemiology (Release 2.2, 1994) computer program package available from the Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, Ohio. This program is supported by a US Public Health Service grant (1P41RR03655) from the National Center for Research Resources.

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*Arterioscler Thromb Vasc Biol.* 2000;20:1789-1795
doi: 10.1161/01.ATV.20.7.1789

*Arteriosclerosis, Thrombosis, and Vascular Biology* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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