Intronic Polymorphism in the Fatty Acid Transport Protein 1 Gene Is Associated With Increased Plasma Triglyceride Levels in a French Population

Aline Meirhaeghe, Geneviève Martin,* Masami Nemoto,* Samir Deeb, Dominique Cottel, Johan Auwerx, Philippe Amouyel, Nicole Helbecque

Abstract—Fatty acids play important biological roles in cells. The precise mechanism whereby fatty acids cross the plasma membrane is still poorly understood. They can cross membranes because of their hydrophobic properties and/or be transported by specific proteins. Recently, a gene coding for fatty acid transport protein 1 (FATP1), an integral plasma membrane protein implicated in this process, was cloned in humans. We screened the gene by single-strand conformation polymorphism analysis and detected an A/G polymorphism in intron 8. We analyzed the potential relations of this genetic polymorphism with various obesity markers and with plasma lipid profiles in a random sample of 1144 French subjects aged 35 to 64 years. We detected statistically significant associations between this FATP1 A/G polymorphism and an increase in plasma triglyceride levels, mainly in women. These results suggest that genetic variability in the FATP1 gene may affect lipid metabolism, especially in women, and reinforce the potential implication of FATP1 in lipid homeostasis. (Arterioscler Thromb Vasc Biol. 2000;20:1330-1334.)

Key Words: fatty acid transport proteins ■ fatty acid binding proteins ■ polymorphism ■ association studies ■ fatty acids

Fatty acids (FAs) play an important role in cell metabolism. Their biological role is multiple: they are a source of energy for the cell, and most vertebrate tissues synthesize ATP by oxidation of FAs; they are also components of the cell membrane, precursors of mediators such as prostaglandins, and themselves mediators of gene expression. Free FAs are found in plasma, mainly as albumin complexes, and stored as triglycerides in lipid droplets and in cells such as adipocytes. FAs are liberated from lipoproteins by the enzyme lipoprotein lipase. In the cytoplasm, they are bound to small proteins named FA binding proteins (FABPs). The cellular uptake of FAs may be either a free diffusion phenomenon or, more probably, the result of a facilitated transport process, as suggested by the discovery of membrane lipid binding proteins. Five candidates for FA transport have been described; among these are the plasma membrane FABP, the FA translocase, and the FA transport protein (FATP1). It has been shown that FATP1 increases the long chain FA (LCFA) uptake when expressed in mammalian cells, suggesting that FATP1 could act as a transporter. FATP1 is a 63-kDa plasma membrane protein with 6 predicted membrane-spanning regions. FATP1 has been suggested to be part, perhaps in association with FA translocase, of a multimeric complex implicated in LCFA cellular uptake. FATP1 is regulated coordinately with lipoprotein lipase and acyl coenzyme A synthetase, which contains a small stretch of amino acids homologous to FATP1. Acyl coenzyme A synthetase is implicated in the activation of the FAs in acyl coenzyme A derivatives. Transcriptional regulation of the FATP1 gene is followed by functional changes in FA uptake into cells. Like lipoprotein lipase and acyl coenzyme A synthetase, FATP1 is a key target protein controlling triglycerides and FA metabolism. Five murine and 6 human FATPs have been identified and are highly conserved. In rodents, FATP1 is expressed preferentially in adipocytes, heart, brain, skeletal muscle, and testis. We cloned the gene coding for this protein in humans. The human FATP1 gene has 12 exons and extends over >13 kb of genomic DNA (G.M. et al, unpublished data, 1999), and we searched for polymorphisms in this gene by single-strand conformation polymorphism (SSCP) analysis. Scanning the whole gene (except for the 5′ end of exon 1 and the promoter, which are not yet cloned) led to the detection of 3 very close variants in introns 8 and 9, probably in linkage disequilibrium with each other. We analyzed the potential relations between the intron 8 A/G substitution and obesity markers and plasma lipid profile in a large population from northern France.

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From the Service d’Épidémiologie et de Santé Publique, INSERM U.508 (A.M., D.C., P.A., N.H.) and INSERM U.325 (G.M., J.A.), Institut Pasteur de Lille, Lille, France; Division of Medical Genetics (M.N., S.D.), University of Washington, Seattle; and Centre Hospitalier et Universitaire de Lille (P.A.), Lille, France.

*Both authors contributed equally to this study.

Correspondence to Prof Philippe Amouyel, Service d’Épidémiologie et de Santé Publique, INSERM U.508, Institut Pasteur de Lille, 1 rue du Professeur Calmette, BP 245, 59019 Lille Cedex, France. E-mail Philippe.Amouyel@pasteur-lille.fr

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Methods

Subjects

The population was recruited from 1995 to 1997 in the course of the Multinational Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) project of the World Health Organization (WHO),8,9 which has been exhaustively collecting cases of myocardial infarction and coronary deaths in 38 populations from 21 countries in 4 continents for 10 years. A sample of 1195 individuals (601 men and 594 women) living in the Urban Community of Lille (northern France), aged 35 to 64 years, was recruited. The present study was randomly sampled from the electoral rolls and equally distributed according to 10-year age groups and sex. The study was approved by the Ethics Committee of the Centre Hospitalier et Universitaire de Lille. Each individual signed an informed consent. A set of questionnaires was completed; questions included details of personal history, drug intake, cigarette smoking, and alcohol consumption. Body mass index (BMI), waist-to-hip ratio, and blood pressure were measured.

Laboratory Methods

Glucose was measured by the glucose oxidase method (DuPont Dimension). Plasma insulin was measured by radioimmunoassay (BiInsuline, ERIA Pasteur). Plasma total cholesterol and triglyceride levels were measured enzymatically (DuPont Dimension).

SSCP Analysis

SSCP analysis was performed with the primers listed in Table 1. The 582-bp fragment comprising exons 8 to 10 was digested by Sau3AI into 3 fragments before SSCP analysis. Briefly, polymerase chain reactions (PCRs) contained 100 ng genomic DNA, 62.5 μmol/L of each dNTP, 10 pmol of each primer, 2.5 mmol/L MgCl₂, 0.25 U Taq polymerase, and 0.1 μL α-[32P]dCTP (3000 Ci/mmol, 10 mCi/mL) in a volume of 10 μL. Each sample was electrophoresed at 2 different gel temperatures: 4°C or room temperature. After electrophoresis, gels were transferred to Whatman 3MM paper and dried. Autoradiography with Kodak BIO-MAX MS film was performed at 70°C. PCR products that yielded aberrantly migrating band patterns were subjected to automated dideoxy sequence analysis.

Genetic Analysis

Genomic DNA was prepared from white blood cells by a “salting out” procedure.10 DNA amplification was performed by use of PCR.11 The primers that were used to amplify the mutated intronic sequence were derived from the genomic sequence of the FATP1 locus and are listed in Table 1. The PCR conditions were as follows: 94°C for 1 minute, 56°C for 1 minute, and 72°C for 30 cycles and a final extension at 72°C for 10 minutes with 1.5 mmol/L MgCl₂ and 5% dimethyl sulfoxide. The intron 8 A/G polymorphism was detected by using allele-specific oligonucleotide hybridization with the following primers: 5'-TCCCCACACCCTGCCT-3' for the A allele and 5'-TCCCCACGCGCCTGCGT-3' for the G allele (polymorphism underlined). Membranes were hybridized at 48°C for 1 hour, washed twice in 1× SSC and 10% SDS buffer for 5 minutes, then washed in 0.5× SSC and 10% SDS buffer for 5 minutes, and finally washed in 0.5× SSC and 10% SDS buffer at 49°C for 3 minutes.

![SSCP analysis of intron 8 of the human FATP1 gene. The arrow shows the A/G polymorphism, resulting in a mobility shift.](image-url)
Complete results were obtained for 1144 subjects. The data were analyzed by use of SAS statistical software (release 6.12, SAS Institute Inc). We considered the statistical significance to be at the 0.05 level. Because of skewness, triglycerides, glucose, and insulin data were logarithmically transformed to achieve normal distributions. Statistical tests were carried out on the transformed values. The Pearson $\chi^2$ test was used to compare genotypic distributions and allelic frequencies between groups. When necessary, the Fisher test of exact probability ($2 \times 2$ table) was used instead of the $\chi^2$ test.

Obesity markers and plasma lipid, lipoprotein, or apolipoprotein levels were compared between genotypes by an ANCOVA that used a general linear model (GLM procedure, type III test).

### Results

To identify polymorphisms in the FATP1 human gene, the entire coding region (except for exon 1 because the 5' end is not yet known) and intron-exon junctions were screened by SSCP analysis. Ten pairs of primers were designed to screen the FATP1 gene. The sequences of these primers are given in Table 1. For larger exons (ie, exons 2 and 7), 2 pairs of primers were used to create overlapping products of smaller size for SSCP analysis. After screening of 20 DNA samples by SSCP, 3 abnormal conformers were detected. They correspond to an A/G substitution in intron 8 and 2 substitutions 28 bp apart (G/A and G/T) in intron 9. These polymorphisms were very close and probably in linkage disequilibrium with each other. The Figure is an autoradiograph of the SSCP gel showing the A/G intron 8 polymorphism. We analyzed the potential relations between the A/G intron 8 polymorphism and obesity markers and plasma lipid profile in a large population from northern France. The studied population (n=1144) was a representative sample of the general population living in the Urban Community of Lille (northern France), equally distributed according to 10-year age groups and sex. Forty-seven individuals suffered from non–insulin-dependent diabetes mellitus (NIDDM), and 306 (26.7%) were treated for hyperlipidemia, diabetes (all types), or hypertension. To avoid any interference between treatment and biological variables, treated subjects were excluded in the following analyses. All subjects were genotyped for the FATP1 A/G intron 8 polymorphism. The genotype distributions (Table 2) were in Hardy-Weinberg equilibrium. Allelic frequencies were 0.60 for the A (wild-type) allele and 0.40 for the G allele. There was no difference in genotypic and allelic distributions between the entire population and the untreated group or between the whole population and the untreated group. Values in parentheses are ranges. NS indicates not significant.

**TABLE 2. Genotypic and Allelic Distributions of FATP1 A/G Intron 8 Polymorphism**

<table>
<thead>
<tr>
<th>Genotypic Frequencies, n (%)</th>
<th>Allelic Frequencies, n (frequency)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n AA AG GG</td>
<td>A G</td>
</tr>
<tr>
<td>All subjects  1144</td>
<td>411 (35.9)</td>
</tr>
<tr>
<td>Untreated subjects 838</td>
<td>303 (36.2)</td>
</tr>
<tr>
<td>Men 421</td>
<td>165 (39.2)</td>
</tr>
<tr>
<td>Women 417</td>
<td>138 (33.1)</td>
</tr>
<tr>
<td>NIDDM subjects 47</td>
<td>14 (29.8)</td>
</tr>
</tbody>
</table>

**TABLE 3. Impact of FATP1 A/G Polymorphism in Untreated Subjects**

<table>
<thead>
<tr>
<th>Anthropometric variables</th>
<th>All</th>
<th>Men</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>301</td>
<td>164</td>
</tr>
<tr>
<td>Age, y</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>BMI, kg/m$^2$</td>
<td>25.8±4.5</td>
<td>25.9±4.1</td>
</tr>
<tr>
<td>Plasma lipid variables</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>290</td>
<td>155</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.80±0.99</td>
<td>5.77±1.00</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.07</td>
<td>1.23</td>
</tr>
<tr>
<td>Insulin, $\mu$U/mL</td>
<td>12.55</td>
<td>12.68</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.26</td>
<td>5.42</td>
</tr>
</tbody>
</table>

Values in parentheses are ranges. NS indicates not significant.

*Test for linear trend.
**P value was adjusted for age, sex, BMI, alcohol consumption, and smoking status.
†P value was adjusted for age, BMI, alcohol consumption, and smoking status.
sample and NIDDM subjects (allele A 0.58, allele G 0.42). Similarly, there was no statistically significant difference in genotypic and allelic distributions between men (n=421, allele A 0.62) and women (n=417, allele A 0.57).

We studied the effect of the A/G polymorphism on different anthropometric and plasma lipid variables (Table 3). No association was observed either with anthropometric variables, such as body weight, BMI, and waist-to-hip ratio, or with plasma insulin or glucose levels. However, a significant association was observed between the intron 8 A/G polymorphism and plasma triglyceride levels, which were increased in the presence of the mutated allele, in an allelic dose-dependent manner. Because our population was recruited to have the same number subjects of each sex, we stratified the population according to sex. The effect of the G/A polymorphism on plasma triglyceride levels was observed mainly in women (11% in GG subjects compared with AG subjects) and was not statistically significant in men. No association was detected in the obese subjects (BMI ≥30 kg/m²) or in NIDDM subjects (data not shown).

The association between the intron 8 G/A polymorphism and plasma lipid levels was further investigated by using multiple regression analysis in women (data not shown). The regression model was examined for plasma triglyceride and total cholesterol levels as dependent variables and for age, BMI, alcohol consumption, smoking status, and the polymorphism as independent variables. The model showed that BMI and alcohol consumption were the most significant determinants of triglyceride levels (P<0.001), with the G/A polymorphism contributing significantly to this model (P=0.03). Age, BMI, alcohol consumption, smoking status, and the intron 8 G/A polymorphism accounted for 29% of the plasma triglyceride level variance in women.

**Discussion**

In this population study from northern France, we found a significant association between a frequent intronic A/G polymorphism in the *FATP1* gene and increased plasma triglyceride levels, mainly in women. Conversely, no association could be detected with any markers of obesity (BMI and waist-to-hip ratio) or with the insulin resistance syndrome.

FATP1 is a 63-kDa plasma membrane protein mainly expressed in heart, testis, brain, and adipose tissue. Cell lines expressing FATP1 demonstrate a marked increase in the uptake of LCFA; this effect was much smaller for medium chain FAs (<C8), and no effect was observed for butyric acid (C4) for instance. It has been suggested that FATP1 could be a part, in association with other proteins such as FA translocase, of a multimeric complex that facilitates LCFA cellular uptake. Because this protein participates in LCFA cellular uptake, any defect in FATP1 affecting either the formation of the hypothetical complex mentioned above or FATP1 membrane anchoring or affecting the LCFA binding domain can inhibit this uptake, leading to an increase in plasma FA concentration.

It has been shown that an increase in plasma free FA levels stimulates VLDL production in humans. Free FAs are rapidly delivered to the liver, where they are processed to avoid accumulation of these substances. Processing includes β-oxidation and reesterification, storage as triglycerides, and, ultimately, VLDL secretion. Such a mechanism could explain the observed association between the intronic A/G polymorphism in the *FATP1* gene and an increase in plasma triglyceride levels (most triglycerides in plasma are carried in VLDLs). Although the triglyceride values still fell within the normal range, the observed association suggests an involvement of FATP1 in triglyceride metabolism. Because the *FATP1* A/G polymorphism is located in the middle of intron 8, it is unlikely to be functional. This strongly suggests that the intronic A/G polymorphism may be in linkage disequilibrium, with an active mutation located, for instance, in the regulatory region of the *FATP1* gene.

The association between the *FATP1* A/G polymorphism and plasma triglyceride levels was mainly observed in women. This observation suggests that the regulation of plasma free FA levels and/or metabolism differs according to sex. This hypothesis has been confirmed by experiments in rodents: an increase of triacylglycerol synthesis was observed in isolated liver cells from female rats compared with male rats; concomitantly, FA oxidation was higher in male cells. Studies were also conducted in human lymphocytes and fibroblasts, in which highly significant differences were observed in FA esterification, oxidation, and FA composition of lipoproteins between male and female cells.

In conclusion, the present study showed an association between an intronic polymorphism in the human *FATP1* gene and an increase in plasma triglyceride levels, mainly in women. An increase of the same variable was observed when studying the Ala54Thr polymorphism of *FABP2*, an intestinal cytosolic FABP, in obese Finns. This last polymorphism has also been associated in nondiabetic Pima Indians with insulin resistance syndrome, whereas the *FATP1* intron 8 polymorphism in the present study was not. We conclude from these results that FATP1 may be implicated in lipid metabolism, especially in women.

**Acknowledgments**

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References

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