Peroxisome Proliferator-Activated Receptor \( \alpha \) Gene Variants Influence Progression of Coronary Atherosclerosis and Risk of Coronary Artery Disease

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**Background**—Peroxisome proliferator-activated receptor \( \alpha \) (PPAR\( \alpha \)) regulates the expression of genes involved in lipid metabolism and inflammation, making it a candidate gene for atherosclerosis and ischemic heart disease (IHD).

**Methods and Results**—We investigated the association between the leucine 162 to valine (L162V) polymorphism and a G to C transversion in intron 7 of the PPAR\( \alpha \) gene and progression of atherosclerosis in the Lipid Coronary Angiography Trial (LOCAT), a trial examining the effect of gemfibrozil treatment on progression of atherosclerosis after bypass surgery and on risk of IHD in the second Northwick Park Heart Study (NPHS2), a prospective study of healthy middle-aged men in the United Kingdom. There was no association with plasma lipid concentrations in either study. Both polymorphisms influenced progression of atherosclerosis and risk of IHD. V162 allele carriers had less progression of diffuse atherosclerosis than did L162 allele homozygotes with a similar trend for focal atherosclerosis. Intron 7 C allele carriers had greater progression of atherosclerosis than did G allele homozygotes. The V162 allele attenuated the proatherosclerotic effect of the intron 7 C allele. Homozygotes for the intron 7 C allele had increased risk of IHD, an effect modulated by the L162V polymorphism.

**Conclusions**—The PPAR\( \alpha \) gene affects progression of atherosclerosis and risk of IHD. Absence of association with plasma lipid concentrations suggests that PPAR\( \alpha \) affects atherosclerotic progression directly in the vessel wall. (*Circulation*. 2002;105:1440-1445.)

**Key Words:** atherosclerosis ■ genetics ■ coronary disease

Atherosclerosis is a complex process influenced by numerous factors, including dyslipidemia, endothelial dysfunction, oxidative stress, and inflammation, and involves the interaction of monocyte/macrophages, endothelial cells, and smooth muscle cells. Peroxisome proliferator-activated receptor \( \alpha \) (PPAR\( \alpha \)) is a member of the nuclear hormone receptor superfamily of ligand-activated transcription factors. Ligands for PPAR\( \alpha \) include fatty acids, eicosanoids, and the fibrate class of hypolipidemic drugs. PPAR\( \alpha \) regulates the expression of genes involved in lipid metabolism and fibrate treatment causes a dramatic decrease in triglycerides, a lesser decrease in LDL cholesterol, and an increase in HDL cholesterol, producing a less atherogenic lipid phenotype. PPAR\( \alpha \) is expressed at high levels in liver, heart, skeletal muscle, and kidney, tissues that catabolize fatty acids.

PPAR\( \alpha \) also regulates proatherosclerotic processes in the vessel wall. PPAR\( \alpha \) is expressed in endothelial cells and smooth muscle cells and in monocyte/macrophages, endothelial cells, and smooth muscle cells. Peroxisome proliferator-activated receptor \( \alpha \) (PPAR\( \alpha \)) is a member of the nuclear hormone receptor superfamily of ligand-activated transcription factors. Ligands for PPAR\( \alpha \) include fatty acids, eicosanoids, and the fibrate class of hypolipidemic drugs. PPAR\( \alpha \) regulates the expression of genes involved in lipid metabolism, and fibrate treatment causes a dramatic decrease in triglycerides, a lesser decrease in LDL cholesterol, and an increase in HDL cholesterol, producing a less atherogenic lipid phenotype. PPAR\( \alpha \) is expressed at high levels in liver, heart, skeletal muscle, and kidney, tissues that catabolize fatty acids.

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mice have a greater inflammatory response. Oxidative stress also influences atherosclerosis. PPARα activators reduce oxidative stress, and PPARα knockout mice have higher levels of proinflammatory cytokines and lipid peroxides.

Fibrates reduced the progression of coronary atherosclerosis in the Bezafibrate Coronary Atherosclerosis Intermittent Therapy (BECAIT) and the Lopid Coronary Angiography Trial (LOCAT) and reduced the incidence of coronary artery disease in the Helsinki Heart Study and Veterans Administration HDL-cholesterol Intervention Trial (VA-HIT). Thus, PPARα is expressed in cell types involved in atherosclerosis, and PPARα activators have pleiotropic beneficial effects on proatherosclerotic factors. To investigate the role of PPARα in human atherosclerosis, we examined the association between two polymorphisms in the PPARα gene, the functional leucine 162 to valine (L162V) variant, and a novel polymorphism in intron 7, and progression of atherosclerosis in Finnish patients with CABG and low HDL and in the second Northwick Park Heart Study (NPHS2), a prospective study examining the risk of heart disease in healthy middle-aged men in the United Kingdom.

### Methods

#### Study Samples

A cohort of 395 Finnish men aged 70 years old with HDL cholesterol ≤1.1 mmol/L, LDL cholesterol ≤4.5 mmol/L, and triglycerides ≤4.0 mmol/L entered the study (baseline characteristics are shown in Table 1). Current smokers and subjects with severe hypertension, obesity, history of diabetes, or fasting glucose concentration >7.8 mmol/L were excluded. Patients had undergone coronary artery bypass surgery and were treated with placebo or gemfibrozil (1200 mg/d) (Parke Davis) for 2.5 years. Coronary angiography was performed at baseline and on average at 32 months after therapy commencement. Cholesterol and triglyceride concentrations were determined in LDL and HDL subfractions. Angiographic images were analyzed with a computer-assisted quantitative method. Diffuse atherosclerosis is defined as a change in average diameter of coronary segments (ΔADS) from baseline to follow-up angiogram. Focal atherosclerosis is a change in minimum luminal diameter (ΔMLD) of stenoses. Of subjects with L162V genotype, 302 had ΔADS data and 297 subjects had ΔMLD data. For those with intron 7 genotype, 283 had ΔADS and 278 had ΔMLD data.

#### Statistical Analysis

The maximum number of genotypes available for each parameter was used in analysis. Polymorphisms were checked for deviation from Hardy-Weinberg equilibrium by means of the χ² test. Allelic association was estimated by means of the Estimate Haplotyp program. In LOCAT, the relation between baseline lipid levels, change in lipid levels on treatment, and PPARα genotype was examined by t test or ANOVA. Triglycerides were log-transformed. Multivariate ANOVA was used to control for baseline levels of ADS or MLD, time between the baseline and follow-up angiograms, and randomized therapy. Results are reported as unadjusted and adjusted least-squares mean ± SEM.

In NPHS2, statistical analysis was conducted with intercooled STATA version 6.0. Body mass index (BMI), systolic blood pressure, triglycerides, and fibrinogen were log-transformed. One-way ANOVA was used to assess the effect of genotype on baseline characteristics. Survival analysis was carried out with Cox proportional hazards model, with significance assessed by the likelihood ratio test. Adjustment was performed for age, BMI, smoking status, systolic blood pressure, fibrinogen, and cholesterol. Because of the low numbers of individuals with L162V VV genotype, genotypes LV and VV were combined.

#### Results

Genotypes for the L162V and intron 7 polymorphisms were determined for 317 and 298 participants, respectively, in the LOCAT study, and for 2620 and 2684 participants, respectively, in the Second Northwick Park Heart Study. In LOCAT, allele frequencies were 0.028 (95% CI, 0.015 to 0.041) for the V162 allele and 0.134 (95% CI, 0.107 to 0.162) for the intron 7 C allele. In NPHS2, allele frequencies were 0.063 (95% CI, 0.056 to 0.069) for the V162 allele and 0.174 (95% CI, 0.164 to 0.184) for the intron 7 C allele. Frequencies of the L162V (P = 0.0006) and intron 7 (P = 0.01) polymorphisms were significantly lower in the LOCAT study. Polymorphisms were in Hardy-Weinberg equilibrium, with the exception of the intron 7 polymorphism in NPHS2.
TABLE 2. Estimated Haplotype Frequencies in LOCAT and NPHS2

<table>
<thead>
<tr>
<th>L162V</th>
<th>Intron 7</th>
<th>LOCAT</th>
<th>NPHS2</th>
</tr>
</thead>
<tbody>
<tr>
<td>LL</td>
<td>G</td>
<td>0.860</td>
<td>0.804</td>
</tr>
<tr>
<td>LV</td>
<td>C</td>
<td>0.113</td>
<td>0.132</td>
</tr>
<tr>
<td>VV</td>
<td>G</td>
<td>0.006</td>
<td>0.021</td>
</tr>
<tr>
<td>VC</td>
<td>C</td>
<td>0.021</td>
<td>0.041</td>
</tr>
</tbody>
</table>

(\(P=0.04\)), due to regional variation in allele frequency. The V162 and the intron 7 C allele were in allelic association in LOCAT (\(\Delta=0.32\), \(P<0.01\)) and NPHS2 (\(\Delta=0.34\), \(P<0.0001\)). Estimated haplotype frequencies are shown in Table 2.

The association between the PPAR\(\alpha\) gene polymorphisms and plasma lipid concentrations at baseline and change in plasma lipid concentrations after gemfibrozil treatment was examined. There were no significant differences in plasma lipid concentrations in either study (Table 3) or in the change in plasma lipids in response to gemfibrozil in LOCAT (not shown) by PPAR\(\alpha\) genotype.

Association between PPAR\(\alpha\) polymorphisms and progression of atherosclerosis was examined. Compared with L162 homozygotes, V162 allele carriers had significantly reduced progression of diffuse atherosclerosis in native coronary arteries (LL, 0.026±0.006 mm, \(n=284\); LV, 0.032±0.025 mm, \(n=18\); \(P=0.022\)) (Figure 1). In multivariate ANOVA, adjusting for baseline ADS, time between angiograms, and treatment, the effect remained significant (\(P=0.049\)). The effect was similar in the placebo and treated groups, with no evidence of interaction between genotype and treatment. Moreover, the LV genotype showed a protective influence against progression of focal atherosclerosis in coronary arteries (\(\Delta\)MLD) (LL, 0.073±0.010 mm, \(n=279\); versus LV, 0.006±0.041 mm, \(n=18\); \(P=0.064\)) (Figure 1A), which was again independent of gemfibrozil treatment. Thus, carriers of the V162 allele were protected from progression of atherosclerosis.

The intron 7 polymorphism also influenced the rate of progression of atherosclerosis. C allele homozygotes (\(n=7\)) were combined with C allele carriers. Carriers of the C allele showed a nonsignificant trend toward greater progression of diffuse atherosclerosis (\(\Delta\)ADS) than G allele homozygotes (GG, −0.017±0.007 mm, \(n=213\); GC + CC, −0.041±0.015 mm, \(n=70\); \(P=0.095\)) (Figure 1B). Intron 7 genotype had similar effects in both placebo and treated groups. Intron 7 genotype significantly influenced progression of focal atherosclerosis (\(\Delta\)MLD). Carriers of the C allele had a significantly greater decrease in MLD than G allele homozygotes (GG, −0.041±0.015 mm, \(n=70\); GC + CC, −0.017±0.007 mm, \(n=213\); \(P=0.022\)). In multivariate ANOVA, adjusting for baseline ADS, time between angiograms, and treatment, the effect remained significant (\(P=0.007\)).

Figure 1. Effect of PPAR\(\alpha\) genotype on progression of atherosclerosis in LOCAT. A, L162V genotype. B, Intron 7 genotype. Carriers and homozygotes for the intron 7 C allele are combined. Values are mean±SEM. Values in brackets indicate number of subjects. C, Regression model for PPAR\(\alpha\) L162V and intron 7 variants. Values are \(\beta\)-coefficients.

TABLE 3. Association Between PPAR\(\alpha\) Polymorphisms and Baseline Plasma Lipid Concentrations

<table>
<thead>
<tr>
<th>LOCAT</th>
<th>LL ((n=284))</th>
<th>LV ((n=18))</th>
<th>VW ((n=0))</th>
<th>P</th>
<th>GG ((n=213))</th>
<th>GC ((n=63))</th>
<th>CC ((n=7))</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides</td>
<td>1.62±0.73</td>
<td>1.52±0.71</td>
<td>...</td>
<td>0.55</td>
<td>1.61±0.74</td>
<td>1.72±0.69</td>
<td>1.17±0.27</td>
<td>0.12</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>5.17±0.71</td>
<td>5.17±0.74</td>
<td>...</td>
<td>0.99</td>
<td>5.18±0.74</td>
<td>5.19±0.60</td>
<td>5.40±0.82</td>
<td>0.74</td>
</tr>
<tr>
<td>LDL</td>
<td>3.42±0.60</td>
<td>3.41±0.54</td>
<td>...</td>
<td>0.94</td>
<td>3.44±0.61</td>
<td>3.38±0.49</td>
<td>3.71±0.16</td>
<td>0.34</td>
</tr>
<tr>
<td>HDL total</td>
<td>1.02±0.17</td>
<td>1.05±0.17</td>
<td>...</td>
<td>0.55</td>
<td>1.03±0.18</td>
<td>1.01±0.16</td>
<td>1.11±0.17</td>
<td>0.31</td>
</tr>
<tr>
<td>NPHS2</td>
<td>((n=2302))</td>
<td>((n=307))</td>
<td>((n=11))</td>
<td>((n=1847))</td>
<td>((n=740))</td>
<td>((n=97))</td>
<td>((n=740))</td>
<td>((n=97))</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.80±0.96</td>
<td>1.79±0.91</td>
<td>1.80±0.79</td>
<td>0.95</td>
<td>1.78±0.93</td>
<td>1.84±0.98</td>
<td>1.74±0.85</td>
<td>0.35</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>5.74±1.02</td>
<td>5.71±1.02</td>
<td>6.01±1.34</td>
<td>0.61</td>
<td>5.74±1.01</td>
<td>5.73±1.02</td>
<td>5.69±1.17</td>
<td>0.89</td>
</tr>
<tr>
<td>ApoB</td>
<td>0.88±0.26</td>
<td>0.84±0.26</td>
<td>0.94±0.32</td>
<td>0.11</td>
<td>0.88±0.26</td>
<td>0.88±0.26</td>
<td>0.87±0.29</td>
<td>0.77</td>
</tr>
<tr>
<td>HDL</td>
<td>0.80±0.24</td>
<td>0.80±0.25</td>
<td>0.69±0.14</td>
<td>0.35</td>
<td>0.80±0.25</td>
<td>0.79±0.24</td>
<td>0.82±0.22</td>
<td>0.48</td>
</tr>
<tr>
<td>ApoAI</td>
<td>1.61±0.34</td>
<td>1.60±0.34</td>
<td>1.56±0.24</td>
<td>0.88</td>
<td>1.62±0.34</td>
<td>1.59±0.34</td>
<td>1.61±0.34</td>
<td>0.29</td>
</tr>
</tbody>
</table>

Values are mmol/L, mean±SD.
The L162V and intron 7 polymorphisms are in positive allelic association yet have opposing effects on progression of atherosclerosis. The estimated frequency of the V162-intron 7 C haplotype in LOCAT was 0.021, whereas the haplotype frequency of the V162-G haplotype was 0.006; that is, 78% of V162 alleles are on the same haplotype as the intron 7 C allele (Table 2). In a regression model, both polymorphisms showed significant effects on progression of diffuse (ΔADS; constant, −0.018±0.077 mm, P=0.014; V162 allele, 0.082±0.029 mm, P=0.005; intron 7 C allele, −0.038±0.015 mm, P=0.013) and focal (ΔMLD; constant, −0.054±0.011 mm, P<0.001; V162 allele, 0.132±0.045 mm, P=0.003; intron 7 C allele, −0.086±0.024 mm, P=0.001) atherosclerosis (Figure 1C). In a multivariate regression model including baseline measures, time between angiogram, both PPARα genotypes and examining for interaction between the PPARα variants, a significant interaction was observed between PPARα genotypes for diffuse atherosclerosis (ΔADS; P=0.05), with a similar trend observed for focal atherosclerosis (ΔMLD P=0.07).

We investigated whether PPARα variants influence risk of IHD in the prospective second Northwick Park Heart Study. L162V genotype did not influence risk of events in a Cox proportional hazards model adjusted for age, BMI, cholesterol, fibrinogen, smoking, and systolic blood pressure. Carriers and homozygotes for the V162 allele combined had a hazard ratio of 0.75 (95% CI, 0.45 to 1.26, P=0.28). Introns 7 C allele homozygotes showed a nonsignificant trend for greater risk of IHD, with a hazard ratio of 1.83 (95% CI, 0.96 to 3.51, P=0.07), whereas carriers of the intron 7 C allele had a hazard ratio of 1.17 (95% CI, 0.84 to 1.63, P=0.34). When intron 7 and L162V genotype combination was examined, the V162 allele again attenuated the effect of the intron 7 C allele. Individuals homozygous for the L162 allele who were carriers of the intron 7 C allele had a 26% greater hazard ratio (1.26; 95% CI, 0.88 to 1.80, P=0.21), whereas those who were also C allele homozygotes had a 2.6-fold increased hazard ratio (2.61; 95% CI, 1.27 to 5.36, P=0.009) (Table 4). Intron 7 C allele homozygotes had a 3.2-fold greater risk of IHD if they were homozygous for the L162 allele than if they were V162 allele carriers. Haplotype frequencies were significantly different between cases and healthy individuals in NPHS2 (P=0.05), with the L-C haplotype being overrepresented in cases (0.178) compared with control subjects (0.129). Conversely, the V162 allele–containing haplotypes had a lower frequency in cases than did control subjects (V-G 0.014 in cases, 0.021 in control subjects; V-C 0.032 in cases, 0.043 in control subjects) (Table 2). Kaplan-Meier survival curves are shown in Figure 2, with homozygotes for the L-C haplotype showing a significantly greater risk of IHD than combined carriers of the V162 allele, L-G haplotype homozygotes, or L-G/L-C heterozygotes. There was no interaction between PPARα genotype and smoking, BMI, or systolic blood pressure on risk of ischemic events.

**Discussion**

The present study demonstrates that variation in the PPARα gene is associated with progression of atherosclerosis and risk of IHD. Furthermore, this effect is not mediated through plasma lipid levels, suggesting that PPARα may influence the atherosclerotic process through mechanisms involving inflammation and/or oxidative stress. This further illustrates the importance of non–lipid-mediated mechanisms in atherosclerosis and demonstrates that PPARα influences such mechanisms in humans.

In LOCAT, both PPARα polymorphisms were associated with progression of atherosclerosis. The V162 allele was associated with reduced progression of atherosclerosis, whereas the intron 7 C allele was associated with increased progression of atherosclerosis. The V162 allele and intron 7 C allele are in strong allelic association, such that 78% of V162 alleles are found in combination with the intron 7 C allele, and the atheroprotective V162 allele strongly attenuated the

![Figure 2. Survival curves of IHD events by PPARα genotype in NPHS2. Adjustment was performed for age, BMI, smoking status, systolic blood pressure, fibrinogen, and cholesterol. Genotype information is presented as L162V/intron 7 genotype.](http://circ.ahajournals.org/doi/fig/10.1161/CIRCULATIONAHA.114.014902)
proatherosclerotic effect of the intron 7 C allele. The PPARα intron 7 polymorphism was also associated with risk of IHD in NPHS2, and although there was no significant association with the L162V polymorphism and risk, the V162 allele again reduced the effect of the intron 7 polymorphism. The risk-associated L-C haplotype is overrepresented in subjects with IHD, whereas the V-G and V-C haplotypes are found at a lower frequency in cases than in control subjects.

Surprisingly, given the well-documented effects of fibrate treatment on plasma lipid levels, the PPARα gene polymorphisms had no effect on plasma lipid concentrations in either study, confirming our previous findings. It has been reported that apoB and LDL cholesterol concentrations are higher in carriers of the V162 allele, though we did not observe such a finding, possibly because of differences in the subject selection criteria. In the LOCAT study, a similar effect of PPARα genotype on progression of atherosclerosis was observed in both placebo and treated groups, suggesting that these variants in the PPARα gene do not dramatically influence response to fibrate treatment, as previously observed.

The absence of an association between PPARα polymorphisms and plasma lipid levels suggests that the effect of the PPARα polymorphisms on IHD is not plasma lipid-mediated unless subtle alterations occur in plasma lipid profiles. Additionally, the risk of IHD was unchanged when plasma cholesterol levels were included in the Cox proportional hazards model.

PPARα is also expressed in vessel wall cell types and regulates the expression of genes that influence proatherosclerotic processes in these cells. Inflammation plays a major role in both the initiation and progression of atherosclerosis, and PPARα activation has anti-inflammatory actions. PPARα also influences oxidative stress. Thus, PPARα polymorphisms may affect progression of atherosclerosis through inflammatory and/or oxidative stress mechanisms.

The mechanism by which the intron 7 variant influences the atherosclerotic process remains unclear. PPARα-V162 has higher transcriptional activation in vitro. No other common missense variants have been identified in the PPARα gene coding region. Given the opposing effects of the V162 allele and the intron 7 C allele and the beneficial effects of stimulation of PPARα activity with fibrates, we hypothesize that the intron 7 C allele is associated with lower levels of PPARα. It is unlikely that the intron 7 variant is itself functional, due to its position in an intron. However, we suggest that it is in allelic association with a functional variant in a promoter or enhancer element of the PPARα gene that results in reduced PPARα gene expression. Surprisingly, PPARα knockout mice crossed with the apoE knockout mouse on an atherogenic diet show reduced aortic atherosclerosis compared with apoE knockout control mice.

Thus, PPARα affects factors that contribute to the atherosclerotic process, and common variation in the human PPARα gene influences these factors. These data demonstrate the important role of PPARα in human atherogenesis and provide genetic evidence that PPARα has antiatherosclerotic effects in humans in vivo and so influences risk of IHD.

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References


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