Alanine for Proline Substitution in the Peroxisome Proliferator–Activated Receptor Gamma-2 (PPARG2) Gene and the Risk of Incident Myocardial Infarction

Paul M. Ridker, Nancy R. Cook, Suzanne Cheng, Henry A. Erlich, Klaus Lindpaintner, Jorge Plutzky, Robert Y.L. Zee

Objective—Recent studies have implicated the potential importance of peroxisome proliferator–activated receptors as a molecular mechanism involved in atherothrombosis. A common alanine (A) for proline (P) substitution at codon 12 in the peroxisome proliferator activated receptor gamma-2 gene (PPARG2) has been associated with reduced risk of developing type 2 diabetes mellitus. Because diabetes and atherothrombosis share common antecedents, we sought evidence that this polymorphism might also be associated with reduced risk of myocardial infarction.

Methods and Results—Using DNA samples collected at baseline in a prospective cohort of 14 916 initially healthy American men, we evaluated a P12A polymorphism in the PPARG2 among 523 individuals who subsequently developed myocardial infarction and among 2092 individuals who remained free of reported cardiovascular disease over a mean follow-up period of 13.2 years. As hypothesized, presence of the A12 allele was associated with significantly reduced risk of myocardial infarction (odds ratio in an age- and smoking-adjusted dominant model of inheritance, 0.77; 95% CI, 0.60 to 0.98; \( P = 0.034 \)). This protective effect remained statistically significant in analyses controlling for traditional cardiovascular risk factors, was present among nondiabetic study participants, was observed to be of similar magnitude in analyses assuming codominant or dominant modes of inheritance, and was seen in fully adjusted post hoc analyses in which we limited our control group to those individuals specifically matched to myocardial infarction cases (OR, 0.71; 95% CI, 0.53 to 0.96; \( P = 0.024 \)).

Conclusions—In this cohort, a common A for P substitution at codon 12 in the PPARG2 was associated with reduced incidence of myocardial infarction. If confirmed in other cohorts, these data would have implications for novel treatments of cardiovascular disease, including development of PPARG-targeted therapy. (Arterioscler Thromb Vasc Biol. 2003;23:859-863.)

Key Words: genetics ■ epidemiology ■ myocardial infarction ■ risk prediction ■ polymorphism

Recent data suggest that a common alanine for proline substitution at codon 12 (P12A) in the peroxisome proliferator–activated receptor gamma-2 gene (PPARG2) is associated with reduced incidence of type 2 diabetes.\(^1\)–\(^4\) This observation is of considerable interest because patients with diabetes have accelerated atherosclerosis and insulin resistance is now recognized as a risk factor for myocardial infarction (MI). Recent experimental work has implicated that PPARG may act directly on local vasculature in several critical aspects of atherothrombosis, including lipid metabolism, foam cell responses, and inflammation, additionally suggesting that PPARG may be an important determinant of gene expression during atherogenesis.\(^5\)–\(^6\) We therefore hypothesized that the P12A polymorphism in the PPARG2 might also be a determinant of incident myocardial infarction (MI).

Methods
Patient Selection and Clinical Investigation
We sought evidence of association between a common P12A polymorphism in the PPARG2 and risk of incident MI as part of an ongoing genetic epidemiology evaluation of incident cardiovascular events being performed within the Physicians’ Health Study.\(^7\) In brief, of 22 071 American men aged 40 to 84 years who were free of prior MI, stroke, transient ischemic attack, and cancer, 14 916 provided baseline blood samples that were available for genetic analysis. After an average time of 13.2 years since enrollment, follow-up has been complete for all deaths and for 99.4% of all reported morbid events.

According to the overall nested case-control design of our genetic epidemiology program, each participant who provided an adequate sample of whole blood at baseline and had a confirmed MI, thromboembolic stroke, or venous thromboembolism during follow-up was matched wherever possible to 2 or 3 controls. The

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controls were study participants who had also provided a baseline blood sample and who remained free of any reported cardiovascular disease at the time the index event occurred in the case patient. Controls were selected at random from among those who met the matching criteria of age (±2 years), smoking habits (former, current, or never), and time since study entry. As described elsewhere,7 for all reported incident vascular events occurring after study enrollment, hospital records, death certificates, and autopsy reports were requested and reviewed by an end points committee using standardized diagnostic criteria. With specific regard to MI, the diagnosis was confirmed by evidence of symptoms in the presence of either diagnostic elevations of cardiac enzymes or diagnostic changes on electrocardiograms. In the case of fatal events, the diagnosis of MI was also accepted based on autopsy findings. For consistency of presentation, all controls were pooled and only those that remained incident-free through follow-up were used in the common reference group; using these methods, a total of 523 cases of incident MI and 2092 controls were available for the present analysis.

**Genotyping**

For each case and control, whole blood collected and frozen at baseline underwent DNA extraction and was genotyped for the P12A substitution in the PPARG2. Genotyping was accomplished using multiplex polymerase chain reaction and immobilized probe-based assays for markers of cardiovascular disease, immune response, and inflammation, essentially as described elsewhere, but modified to also include genotyping capability for the P12A substitution (Roche Molecular Systems).1,3,8,9 To confirm genotype assignments, the polymerase chain reaction procedure was performed in replicate on all samples, and scoring was carried out by 2 independent observers. Disagreements (<2%) were resolved by an additional joint reading and, where necessary, by repeat genotyping reaction. All results were scored blinded to case-control status.

**Statistical Analysis**

Baseline clinical characteristics of the case and control participants were computed, and the significance of any difference in means was tested by the Student’s t test, whereas the significance of any difference in proportions was tested by the χ² statistic. Genotype frequencies between case and control participants were evaluated in a series of logistic regression analyses, which were adjusted for age and smoking status and were used to compute odds ratios and 95% confidence intervals. To avoid assumptions regarding modes of inheritance, all analyses were performed using codominant, dominant, and recessive modes for each allele. We used additional multivariate models to estimate odds ratios after additional adjustment for baseline body mass index (kg/m²), history of hypertension, history of hyperlipidemia, and the presence of diabetes. On a post hoc basis, we also computed crude and fully adjusted odds ratios in analyses limited to those controls specifically matched to the myocardial infarction cases. All probability values are 2-tailed.

**Results**

Table 1 presents baseline clinical characteristics of study participants who subsequently developed MI (cases, N=523) and of those who did not (controls, N=2092). As would be expected in a prospective cohort study, participants who subsequently developed MI had a higher prevalence of conventional atherosclerotic risk factors at baseline than did the control subjects. Among the control group, there were no major differences in traditional cardiovascular risk factors among carriers of the A12 allele (N=482) compared with those with the more common P12/P12 genotype (N=1610), although P12/P12 participants were marginally older (58.6 versus 57.7 years, P=0.04) and had a somewhat more frequent history of hyperlipidemia (8.9% versus 6.2%, P=0.07). Similarly, as also shown in Table 1, there were no major differences in traditional cardiovascular risk factors among case participants when comparing carriers of the A12 allele to noncarriers.

As shown in Table 2, among the 2092 controls, frequencies for the A12/A12, A12/P12, and P12/P12 genotypes were 0.015, 0.216, and 0.770, respectively. This distribution of alleles was consistent with Hardy-Weinberg equilibrium and similar to that reported in other populations. As also shown in Table 2, case participants had significantly reduced carrier rates for the A12 allele (P=0.038). Specifically, frequencies for the A12/A12, A12/P12, and P12/P12 genotypes among the case participants were 0.012, 0.176, and 0.813, respec-

**TABLE 1. Baseline Clinical Characteristics of Initially Healthy Study Participants Who Subsequently Developed MI (Cases) and Those Who Remained Free of Cardiovascular Disease During Follow-Up (Controls) and Distributions of Clinical Characteristics According to Genotype Among the Control Participants**

<table>
<thead>
<tr>
<th></th>
<th>Cases Only</th>
<th>Controls Only</th>
<th>Cases Only</th>
<th>Controls Only</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N=523)</td>
<td>(N=2092)</td>
<td>(N=482)</td>
<td>(N=1610)</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>58.3</td>
<td>58.4</td>
<td>58.6</td>
<td>57.7</td>
</tr>
<tr>
<td><strong>Smoking status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>42.9</td>
<td>43.5</td>
<td>43.4</td>
<td>43.6</td>
</tr>
<tr>
<td>Past</td>
<td>42.0</td>
<td>41.3</td>
<td>41.0</td>
<td>42.1</td>
</tr>
<tr>
<td><strong>Body mass index</strong></td>
<td>25.5</td>
<td>25.0</td>
<td>24.9</td>
<td>25.0</td>
</tr>
<tr>
<td><strong>Blood pressure</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>129.8</td>
<td>127.4</td>
<td>127.5</td>
<td>127.2</td>
</tr>
<tr>
<td>Diastolic</td>
<td>80.6</td>
<td>79.0</td>
<td>79.0</td>
<td>79.2</td>
</tr>
<tr>
<td><strong>Hyperlipidemia</strong></td>
<td>13.2</td>
<td>8.3</td>
<td>8.9</td>
<td>6.2</td>
</tr>
<tr>
<td><strong>Diabetes</strong></td>
<td>5.6</td>
<td>2.7</td>
<td>2.7</td>
<td>2.9</td>
</tr>
<tr>
<td><strong>Family history of CAD &lt;60 years</strong></td>
<td>17.9</td>
<td>11.3</td>
<td>11.0</td>
<td>12.1</td>
</tr>
</tbody>
</table>

All values given as percents except age (years) and body mass index (kg/m²).

P12/P12 indicates proline-proline; A12/P12, alanine-proline; A12/A12, alanine-alanine.
TABLE 2. Genotype Frequencies by Case and Control Status and Calculated Odds Ratios of Incident MI in Association With Carriage of the Alanine For Proline Substitution in the PPARG2 Gene

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls</th>
<th>Cases</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A12/A12</td>
<td>0.015</td>
<td>0.012</td>
<td></td>
</tr>
<tr>
<td>A12/P12</td>
<td>0.216</td>
<td>0.176</td>
<td></td>
</tr>
<tr>
<td>P12/P12</td>
<td>0.770</td>
<td>0.813</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mode of Inheritance</th>
<th>Odds Ratio*</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Codominant</td>
<td>0.79</td>
<td>0.63–0.99</td>
<td>0.037</td>
</tr>
<tr>
<td>Dominant</td>
<td>0.77</td>
<td>0.60–0.98</td>
<td>0.034</td>
</tr>
<tr>
<td>Recessive</td>
<td>0.76</td>
<td>0.32–1.85</td>
<td>0.551</td>
</tr>
</tbody>
</table>

*Adjusted for age and smoking status.

visively. Thus, in a dominant mode of inheritance, the age- and smoking-adjusted risk of future MI associated with the A for P substitution was 0.77 (95% CI, 0.60 to 0.98; P=0.034). Under a codominant mode of inheritance, the observed odds ratio was 0.79 (95% CI, 0.63 to 0.99; P=0.037).

In multivariate models, which, in addition to smoking status and age, additionally adjusted for body mass index, hypertension, hyperlipidemia, diabetes, and randomized treatment assignment, the observed difference in frequency of the A12 allele remained statistically significant in comparisons of those who subsequently developed MI and those who did not (fully adjusted odds ratio in a dominant mode of inheritance, 0.76; 95% CI, 0.59 to 0.99; P=0.041).

Because the A for P substitution at codon 12 in the PPARG2 has previously been associated with incident type 2 diabetes,1–4 a subgroup analysis was performed in which we evaluated only those participants free of this disease at study entry. In this subgroup, an almost identical overall protective effect was observed (OR, 0.78; 95% CI, 0.61 to 1.00).

To address the possibility that our a priori choice to include all available controls might have led to a spurious false-positive result, we performed an additional post hoc analysis in which we limited our control group to those individuals specifically matched to the MI cases. In this analysis, odds ratios in both crude (OR, 0.73; 95% CI, 0.57 to 0.95; P=0.021) and fully adjusted analyses (OR, 0.71; 95% CI, 0.53 to 0.96; P=0.024) were almost identical in magnitude to that found using the total control population as a whole.

Discussion

PPARG, a member of the nuclear hormone receptor family, is a ligand-activated transcription factor. Initially recognized to play an important part in adipogenesis, PPARG was subsequently identified as having roles in glucose homeostasis, an effect underscored by the serendipitous discovery that insulin-sensitizing thiazolidinediones (TZDs) such as pioglitazone and rosiglitazone are synthetic PPARG ligands. More recent work implicated PPARG in several vascular processes. PPARG activation results in decreased monocyte/macrophage and T lymphocyte cytokine production as well as reduced endothelial cell adhesion molecule, chemokine, and MMP expression.5,6,10 Indeed, synthetic PPARG agonists limit atherosclerosis in a variety of mouse models.

Given this background, PPARG polymorphisms have been investigated as potential genetic determinants of diabetes. In particular, the finding that 2 rare loss-of-function mutations in PPARG were associated with severe insulin resistance11 led to the observation that a common P12A polymorphism was associated with reduced risk of diabetes.1–4 Recent reports have shown that rare loss-of-function mutations in PPARG cause partial lipodystrophy with associated insulin resistance and hypertension,12–14 additionally suggesting rare PPARG mutations on mechanisms contributing to atherogenesis. Interestingly, the P12A polymorphism is found only in 1 of 2 known PPARG splice variants, namely PPARG2, which is expressed primarily in adipocytes. Despite increasing recognition of inflammatory links between diabetes and atherothrombosis,15 relatively little data exist regarding PPARG variants and risk for atherosclerosis. Wang et al16 found a significant association between a 161C>T PPARG polymorphism (a closely linked polymorphism to P12A) and coronary artery disease (CAD) in an Australian white population. In that study, the T161 allele carriers had a significantly reduced risk of CAD, which appeared independent of diabetes, body mass index, waist-to-hip ratio, and lipid profiles. This effect of a PPARG variant on atherosclerosis, which seemed to be independent of metabolic changes, suggests possible direct PPARG effects on the arterial wall.

Indeed, the present study provides additional evidence of a possible alternative mechanism of PPARG2 biologic effects, in this case through P12A polymorphism, which may occur independent of adipocyte differentiation, fat storage, and glucose homeostasis. To the best of our knowledge, this is the first study evaluating the P12A polymorphism in the PPARG2 as a determinant of acute coronary occlusion.

Despite the intriguing nature of these data, limitations of our study require consideration. First, because the Physician’s Health Study cohort is almost exclusively comprised of white middle-aged men, these hypothesis-generating data cannot be generalized to women or other groups, such as African Americans, for whom rates of both atherothrombosis and diabetes are known to differ. In addition, although the observed effect of the A for P substitution at codon 12 in the PPARG2 was statistically significant in both crude and fully adjusted models, the upper bound of the calculated confidence interval in our data are close to 1.0. As we have pointed out previously,17 the possibility of false-positive findings in any genetic association study must be considered, and thus these data require direct confirmation in other cohorts. Nonetheless, there are considerable strengths of our study design that balance these concerns. Most importantly, we used a closed prospective cohort in which the determination of case status was based solely on the subsequent development of disease rather than on any arbitrary selection criteria designed by the investigators. Furthermore, our sample size is large and we found no evidence of any change in risk estimates after adjustment for other vascular risk factors in analyses excluding subjects with diabetes or in analyses limited to controls specifically matched to the myocardial infarction cases. As also outlined in Table 1, there were no significant differences in frequency of traditional cardiovascular risk factors in comparisons between carriers of the A12
allele and noncarriers, either among controls or cases. We thus believe inadvertent epidemiologic bias or uncontrolled confounding are unlikely to account for these observations. Finally, the fact that the observed allele frequencies in our control population are similar to prior reports and that both the magnitude and direction of effect in our data for incident MI mirrors that previously reported for incident diabetes additionally supports the robustness of these data.

We believe the current data have several implications. First, our novel finding that carriers of the A12 allele seem to be at reduced risk of MI (OR, 0.77; 95% CI, 0.60 to 0.98) is of clinical interest given prior findings linking the P12A polymorphism to reduced risk of type 2 diabetes mellitus.1–4 Given the complex and multifactorial nature of both diabetes and atherosclerosis, such associations may occur independent of the presence of diabetes itself.

Second, and perhaps of greater immediate importance, these data also have implications for the development of PPARG-targeted pharmacological therapy as potential modalities in the treatment of atherosclerotic disease. In particular, the present genetic data are likely to spur accelerated interest in the use of PPARG ligands, including the TZD insulin-sensitizing agents, for several interrelated atherothrombotic conditions. In this regard, preliminary reports suggest that TZD use may limit in-stent restenosis, slow carotid intimal thickening, and reduce plasma levels of several inflammatory biomarkers, including CRP.5,6 Recent studies have also demonstrated that PPARG activators limit proatherosclerotic targets in vascular and atheroma-associated cells, including inhibition of adhesion molecules, chemokines, and matrix metalloproteinase expression as well as monocyte/macrophage homing to atherosclerotic plaques.18 PPARG agonists also decrease atherosclerosis in a variety of murine mouse models.

Of note, the P12A polymorphism has been suggested to be a loss of PPARG function mutation, seemingly at odds with the data that PPARG agonists limit atherosclerotic responses in vascular and inflammatory cells. Several relevant points can be raised in this regard. First, P12A as a loss of function variant in vivo in humans remains to be established. Despite the consistency between the two carefully done in vitro reports on P12A published to date,1,19 those studies used common in vitro approaches with known limitations. In fact, using such approaches, a modest diminution of response is seen more than an overt loss of function. The significance of this is unclear. In the only functional data available,19 transfection of the P12A variant into an adipocyte cell line decreased adipogenesis only at 0.5 μmol/L and not 5 μmol/L of the PPARG agonist. Thus, the biologic plausibility of the P12A variant as limiting atherosclerosis remains to be determined, just as the effect of PPARG agonists on atherosclerosis continues to be pursued.

Independent of the nature of the P12A variant, fundamental differences may exist between synthetic PPAR agonists and endogenous PPAR ligands. Thus, it may not be possible to compare the effects predicted through the use of synthetic PPAR agonists, ie, decreased atherosclerosis, and the cardiovascular phenotypes associated with genetic variants in vivo, in which altered signaling by endogenous PPAR ligands occurs through activation of a mutant receptor. Finally, although most of the data suggest PPARG activation should limit atherosclerosis, other known vascular PPARG-induced targets would be predicted to promote atherosclerosis, for example the oxidized LDL receptor CD36. Thus, in certain tissues and under certain conditions, the P12A variant, even as a loss of PPARG function variant, could be atheroprotective by limiting the induction of some known PPARG-induced targets.5,6,10

Clearly the published associations between the functional changes of the P12A polymorphism with various traits will need careful investigation, because the putative antidiabetic and antiatherosclerotic effects of TZDs are believed to derive from PPARG activation.1,5,13 The possibility also exists that the observed association in these data were not the direct result of the functional consequence of P12A polymorphism but rather of a linkage disequilibrium with a yet-to-be-identified functional polymorphism at or nearby the PPARG locus. As mentioned previously, a closely linked 161C>T single nucleotide polymorphism has been shown to be associated with CAD.16 The possible importance of linkage disequilibrium with this genetic polymorphism merits additional investigation.

Taken together, the present data provide important genetic-epidemiologic evidence of association between polymorphism in the PPARG2 and acute coronary occlusion. At the same time, these observations underscore the need for substantial work to fully understand how PPARG, its variants, and its agonists might influence atherosclerosis.

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References


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