Cardiovascular Risk in Type 2 Diabetes Is Associated With Variation at the \textit{PPARG} Locus

A Go-DARTS Study

Alex S.F. Doney, Bettina Fischer, Graham Leese, Andrew D. Morris, Colin N.A. Palmer

Objective—The Pro12Ala polymorphism of \textit{PPARG} modulates risk of developing type 2 diabetes. The Ala allele has also been associated with a reduced risk of cardiovascular events. We have shown previously that the linked T allele of the C1431T polymorphism influences Ala12-associated diabetes risk and that the 2 polymorphisms have opposing associations with body weight. We therefore investigated the association of these 2 variants with cardiovascular events in people with type 2 diabetes.

Methods and Results—We performed a cohort study of 2016 individuals and used Cox proportional hazards to analyze risk of myocardial infarction or death by \textit{PPARG} Pro12Ala and C1431T genotypes, adjusting for age, sex, and smoking status. In individuals enrolled <70 years of age, the hazard for a first nonfatal event associated with the Ala12 allele was 0.21 (CI, 0.06 to 0.69; \(P = 0.01\)) and the T1431 allele 9.9 (CI, 1.90 to 51.29; \(P = 0.007\)). These opposing associations remained significant after correction for other conventional risk factors. The T1431 allele was also associated with all-cause mortality.

Conclusions—This study confirms the association of the Ala12 allele with reduced risk of myocardial infarction in a type 2 diabetic population and demonstrates that the T allele independently associates with an increased risk. (\textit{Arterioscler Thromb Vasc Biol.} 2004;24:2403-2407.)

Key Words: type 2 diabetes ■ myocardial infarction ■ \textit{PPARG} ■ polymorphism

The peroxisome proliferator–activated receptor-\(\gamma\) (PPAR\(\gamma\)) has a key role in the molecular pathophysiology of obesity and type 2 diabetes.\(^1\) It is directly involved in adipogenesis\(^2\) and liver and muscle responses to glucose,\(^3,4\) as well as some aspects of pancreatic \(\beta\)-cell function.\(^5\) It is also the molecular target of the thiazolidinedione class of insulin sensitizing drugs.\(^6\) Genetic variation at the \textit{PPARG} locus may modulate individual susceptibility to type 2 diabetes mellitus and related traits associated with premature cardiovascular disease.\(^7,9\) Notably, the Ala allele of the Pro12Ala polymorphism has been associated with greater insulin sensitivity,\(^7,9\) reduced risk of type 2 diabetes,\(^10\) reduced body mass index (BMI),\(^11\) lower blood pressure,\(^12\) and reduced risk of myocardial infarction.\(^13\) Furthermore, the action of this variant may be subject to interaction with diet and exercise.\(^14,15\)

We demonstrated previously that a further silent variant in exon 6 of \textit{PPARG}, C1431T, which is in strong linkage disequilibrium (LD) with the Pro12Ala variant, has an opposing association with body mass in several populations with and without type 2 diabetes.\(^11\) We found the Ala12 variant to be associated with a reduced BMI, whereas the T allele was associated with an increased BMI. Similar findings have been reported recently in a study of polycystic ovary syndrome,\(^16\) and several reports have suggested that the T1431 allele is associated with higher leptin levels in obese women.\(^8,16\) Although the relationship between T1431, leptin, and BMI is as yet undefined. Furthermore, we have demonstrated that the strength of the association of the Ala allele with a reduced risk of type 2 diabetes may be modulated by the presence of LD with the T allele.\(^17\) Interestingly, in contrast to the balanced frequency and strong LD observed in the white population, T1431 occurs much more frequently than Ala12 in Asian and Oji-Cree populations, and reports from these populations have suggested a role of T1431 in increased risk of cardiovascular disease.\(^9,18,19\)

Because the T allele of C1431T and the Ala allele of Pro12Ala appear to be consistently associated with opposing metabolic traits, we hypothesized this may also be observed for the development of cardiovascular disease in a large group of individuals with type 2 diabetes.

Materials and Methods

All individuals with diabetes mellitus in the population of Tayside, Scotland, have been identified previously through record linkage techniques with a sensitivity of 97%.\(^20\) Clinically relevant data are

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From the Institute for Cardiovascular Research (B.F., C.N.A.P.), Biomedical Research Centre, and the Department of Medicine and Therapeutics (A.S.F.D., G.L., A.D.M.), Ninewells Hospital and Medical School, Dundee, Scotland.
Correspondence to Dr Colin N.A. Palmer, Biomedical Research Centre, Ninewells Hospital and Medical School, University of Dundee, Dundee, Scotland, DD1 9SY. E-mail nuclear-receptor@dundee.ac.uk

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2403
TABLE 1. Ala Allele of Pro12Ala Is Associated With a Reduced Cardiovascular Risk and the T Allele of C1431T With Increased Risk

<table>
<thead>
<tr>
<th></th>
<th>Pro12Ala</th>
<th>C1431T</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. Events/No. at Risk</td>
<td>HR</td>
</tr>
<tr>
<td>All nonfatal events</td>
<td>91/2016</td>
<td>0.54</td>
</tr>
<tr>
<td>All nonfatal events &lt;70 years</td>
<td>59/1349</td>
<td>0.43</td>
</tr>
<tr>
<td>First nonfatal event &lt;70 years</td>
<td>35/1176</td>
<td>0.21</td>
</tr>
<tr>
<td>All events including death &lt;70 years</td>
<td>184/1349</td>
<td>0.68</td>
</tr>
<tr>
<td>Death &lt;70 years</td>
<td>133/1349</td>
<td>0.82</td>
</tr>
</tbody>
</table>

Corrected for sex age and smoking status.

Results

Allele frequencies together with linkage data of the Pro12Ala and C1431T in the Go-DARTS cohort have been reported previously.11,21 The mean age of the population at enrollment was 64.4 years (SD 11.6) and the mean duration of diabetes 7.9 years (SD 6.8). The mean follow-up time for all events was 36.6 months (SD 15.0). We found no evidence of an association of either polymorphism with age of diagnosis with diabetes or diabetes duration before enrolment (data not shown).

When we included either the Pro12Ala polymorphism or the C1431T polymorphism separately in the Cox model, we found only a weak, nonsignificant association of the Ala12 allele with reduced risk of nonfatal cardiovascular events and an even weaker nonsignificant association of the T allele with increased events (data not shown). However, including both variants in the model resulted in a stronger and opposite association of each variant with events. These data are shown in Table 1, which also gives the number of individuals at risk and the number of events occurring for each group of patients considered. In the initial model, which considered all nonfatal events in the entire population, we found a borderline nonsignificant association of the Ala allele with reduced risk of a nonfatal myocardial infarction or revascularization (hazard ratio [HR], 0.54; CI, 0.27 to 1.08), whereas the T allele was associated with a nonsignificant increased risk (HR, 2.34; CI, 0.77 to 7.11). However, when we considered the younger population enrolled before 70 years of age, we found the strength of the associations increased and became more significant with the HR associated with the Ala allele being lower (HR, 0.43; CI, 0.18 to 0.99) and the hazard associated with the T allele higher (HR, 4.75; CI, 1.24 to 18.25). When we analyzed time to first event in each individual in this younger cohort, excluding individuals who had experienced stroke, myocardial infarction, or revascularization before enrollment, we found a further strengthening of the observed associations (HR for the Ala allele, 0.21; CI, 0.06 to 0.69; HR T allele, 9.90; CI, 1.9 to 51.29). We then considered the combined end point of nonfatal events and all-cause death in this subgroup, and it was found that the T allele was again associated with a significantly increased risk (HR, 2.55; CI, 1.13 to 5.75), whereas the Ala allele was associated with a reduced risk that was only of borderline significance (HR, 0.68; CI, 0.43 to 1.1). The interaction of the T1431 and Ala12 alleles in this group are illustrated as Kaplan–Meier plots in the Figure. In A, time to event is compared between...
individuals with and without the Ala12 allele while adjusting for the presence of the T1431 allele. In B, time to event is compared between individuals with and without a T1431 allele while adjusting for the presence of the Ala12 allele. In C, the combined genotypes are plotted, demonstrating that individuals possessing an Ala12 allele in the absence of a T1431 allele are relatively protected from events, whereas conversely, individuals possessing a T1431 allele in the absence of an Ala12 allele are at a relatively greater risk. When an individual possesses both alleles, his/her opposing risks cancel each other, resulting in an intermediate risk similar to possession of neither allele.

Finally, considering only all-cause death in the younger group, it was found again that the T allele was associated with an increased risk of earlier death (HR, 2.61; CI, 1.02 to 6.65); however, association of the Ala allele was considerably weakened (HR, 0.82; CI, 0.49 to 1.39). We also examined cardiovascular death using this model and observed hazards of a similar magnitude; however, this was underpowered compared with the all-cause death data because of the shorter follow-up period available for the cardiovascular death diagnosis, as detailed in the methods (data not shown). Because the majority of deaths in type 2 diabetes are of cardiovascular origin, our study was underpowered to specifically determine the role of the variants in noncardiovascular causes of death.

We then determined the extent to which the observed associations were dependent on other conventional risk factors and so repeated the analysis including smoking status, log10 mean BMI, first-recorded HDL-cholesterol, total cholesterol, log10 triglycerides, and mean arterial blood pressure. We found that with inclusion of these conventional risk factors in the model, the observed associations of genotype with outcome was modestly attenuated but remained significant (Table 2).

Discussion

We have investigated the association of the Pro12Ala and C1431T polymorphisms of PPARG on cardiovascular events in a large population of patients with type 2 diabetes. We have exploited advanced record-linkage technology developed through DARTS to enable all individuals in the cohort to be prospectively followed with a high degree of sensitivity and specificity22 and have confirmed a previous report that the Ala allele of Pro12Ala is associated with a reduced hazard of myocardial infarction.13 Furthermore, we have demonstrated that the T allele of C1431T is associated with an increased hazard and that its coexistence influences the hazard associated with the Ala allele. The potential importance of the T1431 variant as a marker for cardiovascular risk is supported by a recent case control study from Taiwan that considered this variant in isolation and demonstrated a significantly increased risk of premature myocardial infarction as well as an increased level of atherogenic oxidized low-density lipoprotein-cholesterol associated with T1431 homozygotes.18 Furthermore, a recent study in the Oji-Cree considered the C1431T and Pro12Ala polymorphisms and demonstrated an association of the Ala12 allele with reduced carotid intima media thickness, whereas the T1431 allele was associated with an increase in total atherosclerotic plaque volume in the carotid artery.19 These findings corroborate previous observations that the Ala12 and the linked T1431 are associated with opposing phenotypes.11,16,17 Because the T allele is silent, it is likely that these observed associations are attributable to its LD with a further common variant with functional consequences at the PPARG locus. This may
We found that the opposing hazards associated with the PPARG variants were more apparent in a younger population enrolled in the study <70 years of age, probably reflecting survival biases operating in the very elderly, together with the fact that at that at an older age, the influence of genotype on events will be attenuated. For example, the Taiwanese study was able to detect the increased myocardial infarction risk associated with the T1431 allele in individuals <50 years old, and interestingly, the Ala12 variant has been shown recently to be enriched in very elderly Italians. These findings, together with our findings that healthy middle-aged control populations have a higher Ala12 frequency relative to conventional risk factors. This is supported by recent evidence that common variation at PPARG has pleiotropic consequences for general morbidity and mortality. It is likely that the stronger association observed in the group with no previous cardiovascular events before enrollment reflects the effects of an increased number of cardiovascular protective drugs prescribed to individuals with previous cardiovascular events.

Because the Ala12 and T1431 alleles are in strong LD, they often occur together in the same individual and, as we have demonstrated previously, when they occur together, they effectively cancel out their associated phenotype. This is illustrated in the Figure (C), in which the survival curves are superimposed when both alleles are absent or both present. It is for this reason that considering either allele in the model alone results in only a weak and nonsignificant association with cardiovascular risk compared with including both variants in the model, in which case the presence of the opposing allele is being accounted for statistically. Another consideration when interpreting reports of these variants is that the LD between the variants differs markedly by ethnicity. In white populations, the D’ is 0.6 and the allele frequencies are almost identical, giving a similar R² and D’ value. It would appear that the frequency of the T1431 variant in Asian populations may be 10-fold higher than Ala12 and 3-fold higher in Oji Cree, thus providing many more individuals who contain the T1431 variant without the Ala12 variant than would be present in the white samples, which represent the bulk of the literature. Therefore, the “cross-contamination” of association by LD will be less in these samples.

In 1 study, the T allele has also been indirectly associated with a reduced risk of angiographically defined coronary artery disease. Interestingly, this study did not consider the Ala allele and found that the protective advantage was mainly among the C1431T heterozygotes, with C1431 and TT homozygous individuals each having evidence of increased disease. Based on our findings, we would predict that individuals homozygous for the T allele would have increased disease because of an increased ratio of T to Ala alleles, whereas CT heterozygotes, in contrast, would be expected to have an approximately equal proportion of opposing Ala alleles and thus would be relatively protected.

To the best of our knowledge, only 1 other study has considered both of these variants on coronary artery disease in a diabetic population and found no association of these or other variants with atherosclerotic vascular disease. However, this was a small retrospective case control and therefore subject to bias and lack of power.

Genetic variation at PPARG has also been associated with conventional risk factors for cardiovascular disease such as body weight, blood pressure, and lipids, suggesting the possibility that the observed association with cardiovascular risk also may be through these mechanisms. However, the inclusion of a full range of conventional risk factors including BMI in the model resulted in only a modest reduction in the impact of the alleles, suggesting that the observed genetic association was largely independent of these. However, it should be pointed out that the patients in this population were all undergoing active management of their diabetes and cardiovascular risk factors over a mean duration of ~8 years because diagnosis with diabetes and this would tend to obscure differences in these parameters because of genotype. Furthermore, measures of a particular risk factor taken at only a single point in an individual’s life span are unlikely to reflect the cumulative effect of subtle modulations in PPARγ activity throughout that individual’s life and the influence this may have in turn on development of cardiovascular disease. It is also likely that PPARγ has a diverse role in the pathophysiology of cardiovascular disease, possibly unrelated to conventional risk factors. This is supported by recent evidence that PPARγ is involved in the regulation of inflammation and endothelial function.

In this study, we have not addressed the influence of pharmacotherapy on the association of genotype and outcome. It has been postulated that thiazolidinediones, which are PPARγ activators, and increased insulin sensitivity may reduce the risk of cardiovascular risk events, which raises the question whether the greater risk associated with possession of the T allele can be ameliorated with thiazolidinedione

### Table 2. Fully Corrected Model Including First-Recorded HDL, Cholesterol Log₁₀ Triglycerides, and Mean Arterial Pressure Log₁₀ Mean BMI and Log₁₀ Years With Diabetes

<table>
<thead>
<tr>
<th>No. Events/No. at Risk</th>
<th>Pro12Ala HR (CI)</th>
<th>P</th>
<th>C1431T HR (CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All nonfatal events</td>
<td>87/1898 0.52 (0.26–1.05)</td>
<td>0.07</td>
<td>2.65 0.88–8.00</td>
<td>0.08</td>
</tr>
<tr>
<td>All nonfatal events &lt;70 years</td>
<td>59/1309 0.43 (0.18–0.99)</td>
<td>0.05</td>
<td>4.64 1.19–18.11</td>
<td>0.03</td>
</tr>
<tr>
<td>First nonfatal event &lt;70 years</td>
<td>35/1138 0.22 (0.08–0.72)</td>
<td>0.01</td>
<td>8.30 1.59–43.47</td>
<td>0.01</td>
</tr>
<tr>
<td>All events including death &lt;70 years</td>
<td>177/1309 0.80 (0.50–1.27)</td>
<td>0.33</td>
<td>2.08 0.90–4.79</td>
<td>0.09</td>
</tr>
<tr>
<td>Death &lt;70 years</td>
<td>126/1309 1.02 (0.60–1.73)</td>
<td>0.95</td>
<td>2.11 0.80–5.62</td>
<td>0.13</td>
</tr>
</tbody>
</table>
therapy or indeed any of the other pharmacological therapies commonly prescribed to individuals with type 2 diabetes. These interesting, although complex, pharmacogenetic questions are the subject of ongoing research.

Finally, although we have not been able in this study to define the mechanism whereby variation at PPARG is associated with cardiovascular events, our observation that the association of T1431 variant with coronary events can be followed through to all-cause death, of which as many as two thirds are vascular in origin in patients with type 2 diabetes, further underpins the potential clinical importance of this pleiotropic locus. This study therefore indicates the possible clinical importance of including genotype in discriminating between individuals with differing risk profiles for premature events to inform appropriate therapeutic intervention.

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References
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