Cardiovascular Risk in Type 2 Diabetes Is Associated With Variation at the 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 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 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. (Arterioscler Thromb Vasc Biol. 2004;24:1-5.)

Key Words: type 2 diabetes ■ myocardial infarction ■ PPARG ■ polymorphism

The peroxisome proliferator-activated receptor-\( \gamma \) (PPAR\( \gamma \)) has a key role in the molecular pathophysiology of obesity and type 2 diabetes.\(^7\) It is directly involved in adipogenesis\(^8\) and liver and muscle responses to glucose,\(^9,10\) 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 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 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 Indian 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...
recorded according to a standard data set and case records regularly validated by a team of dedicated research nurses. This continuously updated data set comprises the clinical information system known as DARTS (Diabetes Audit and Research in Tayside Scotland), which has been described previously. Blood has been collected for genetic studies from a cohort of individuals in DARTS. This genetic substudy, known as Go-DARTS, therefore consists of a free-living clinical cohort selected only on the basis of having type 2 diabetes and attending a diabetes clinic in Tayside. All subjects in Go-DARTS are of white ethnicity. Rigorous compliance with National Health Service data protection and encryption standards is maintained at all times, and the study was approved by the local research ethics committee.

At the study outset, there were a total of 2016 white individuals with type 2 diabetes in Go-DARTS. Genotyping for PPARγ Pro12Ala and C1431T polymorphisms was performed using Taqman (Applied Biosystems) allelic discrimination assays as described previously. All individuals were followed up until a cardiovascular event (nonfatal myocardial infarction or revascularization) or death from any cause after recruitment or to the end of the study period. Hospitalization events for a myocardial infarction were obtained from the Scottish Morbidity Register. Myocardial infarction events that did not result in hospitalization were obtained from the DARTS database based on information derived from all general practices in the region. Hence, ascertainment of events was close to 100%. Although the date-of-death data are available rapidly on DARTS, there is a delay in obtaining adjudicated cause of death centrally from the Office of the General Registrar; therefore, these data were only available on a proportion of individuals who died early in the study, and thus we chose to consider only death by any cause.

Cox proportional hazards model was used to represent the association of genotype with time to event, with the end point being nonfatal myocardial infarction, revascularization, or all-cause death. The analysis was initially performed in the entire group, that is whether or not they had experienced a previous nonfatal event. To avoid potential problems associated with survival bias and dilution of genetic effects through the multiple events that accrue with age, we also investigated a younger group that had been enrolled into the study <70 years of age. For the same reasons, we also considered a further subgroup of individuals in whom each event after recruitment was their first by excluding all those having had a stroke, myocardial infarction, or coronary revascularization before enrollment. Age at recruitment, sex, and smoking status (never smokers or ever smokers) were included with genotype in the initial models. The Pro12Ala and C1431T variants were included in all models. It was found that for the Pro12Ala polymorphism, a dominant model produced the best fit, whereas for C1431T, a codominant model was appropriate. A subsequent multivariate analysis was performed that also included first-recorded total cholesterol, high-density lipoprotein (HDL)-cholesterol, log_{10} triglycerides, and mean arterial blood pressure (calculated as [(diastolic blood pressure×2)+systolic blood pressure]/3) together with mean log_{10} BMI and years with diabetes. Log values were used where required to normalize the distribution. STATA version 7 was used for all data manipulation and analyses.

### Table 1.

<table>
<thead>
<tr>
<th>No. Events/No. at Risk</th>
<th>Pro12Ala</th>
<th>C1431T</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>CI</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>

Results

Allele frequencies together with linkage data of the Pro12Ala and C1431T in the Go-DARTS cohort have been reported previously. 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 enrollment (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, log_{10} mean BMI, first-recorded HDL-cholesterol, total cholesterol, log_{10} 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 specificity and have confirmed a previous report that the Ala allele of Pro12Ala is associated with a reduced hazard of myocardial infarction. 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 TT homozygotes. 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. These findings corroborate previous observations that the Ala12 and the linked T1431 are associated with opposing phenotypes. 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
Indeed be true also for the Ala allele, although there is some evidence that it may have functional consequences.9

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,18 and interestingly, the Ala12 variant has been shown recently to be enriched in very elderly Italians.73 These findings, together with our findings that healthy middle-aged control populations have a higher Ala12 frequency relative to children,17 support our hypothesis that common variation at control populations have a higher Ala1 frequency relative to findings, together with our findings that healthy middle-aged 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 would thus 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.25 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,11 blood pressure,12 and lipids,26 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 approximately equal proportion of opposing Ala alleles and would thus be relatively protected.

The question whether the greater risk associated with possession of the T allele can be ameliorated with thiazolidinedione 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,28 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 |
|-----------------|-----------------|-----------------|-----------------|
| No. Events/No. at Risk | Pro12Ala HR CI P | C1431T HR CI P |
|-----------------|-----------------|-----------------|-----------------|
| All nonfatal events | 87/1898 | 0.52 0.26–1.05 0.07 | 2.65 0.88–8.00 0.08 |
| All nonfatal events <70 years | 59/1309 | 0.43 0.18–0.99 0.05 | 4.64 1.19–18.11 0.03 |
| First nonfatal event <70 years | 35/1138 | 0.22 0.08–0.72 0.01 | 8.30 1.59–43.47 0.01 |
| All events including death <70 years | 177/1309 | 0.80 0.50–1.27 0.33 | 2.08 0.90–4.79 0.09 |
| Death <70 years | 126/1309 | 1.02 0.60–1.73 0.95 | 2.11 0.80–5.62 0.13 |

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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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