

# PPAR $\gamma$ Variant Influences Angiographic Outcome and 10-Year Cardiovascular Risk in Male Symptomatic Coronary Artery Disease Patients

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**OBJECTIVE** — Activation of peroxisome proliferator-activated receptor (PPAR)- $\gamma$  signaling influences metabolic profiles and the propensity toward inflammation. Small-molecule stimulation of PPAR $\gamma$  is investigated for secondary prevention of cardiovascular disease. The common PPAR $\gamma$  Pro12Ala variant has functional and prognostic consequences. A protective effect of the 12Ala-allele carriership on diabetes and myocardial infarction in healthy populations has been suggested. The relevance of this pathway also needs exploration in patients with manifest vascular disease. We investigated the effects of carriership of the Pro12Ala variant on angiographic and cardiovascular event outcomes in male patients with symptomatic coronary artery disease (CAD).

**RESEARCH DESIGN AND METHODS** — The Regression Growth Evaluation Statin Study (REGRESS) cohort was genotyped for the Pro12Ala variant (rs1801282). Ten-year follow-up was derived from nation-wide registries, and risks were estimated using proportional hazards. Quantitative coronary angiography measurements were obtained and relations with genotype estimated using a generalized linear model.

**RESULTS** — Genotypes ascertained ( $n = 679$ ) comprised 540 (80%) Pro/Pro, 126 (19%) Pro/Ala, and 13 (2%) Ala/Ala subjects. The 12Ala allele was associated with less extensive focal ( $P = 0.001$ ) and diffuse ( $P = 0.002$ ) atherosclerosis and lower 10-year cardiovascular risk. Hazard ratios were 0.10 (95% CI 0.01–0.70,  $P = 0.02$ ) for ischemic heart disease and 0.24 (0.08–0.74,  $P = 0.013$ ) for vascular death, per each added copy of 12Ala, respectively.

**CONCLUSIONS** — Carriers of the 12Ala allele of PPAR $\gamma$  have less widespread CAD and are considerably protected against 10-year (cardio)vascular morbidity and mortality. These long-term findings in patients with manifest CAD support an important role of PPAR $\gamma$  in determining vascular risk.

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**P**eroxisome proliferator-activated receptors (PPARs) are steroid hormone nuclear receptors that modify expression of several genes. The PPAR $\gamma$  subtype is preferentially expressed in adipocytes, vascular smooth muscle cells, endothelial cells, and macrophages (1) and modulates various aspects of metabolism (lipids and glucose) and inflammation (adhesion molecule, chemokine, and

metalloproteinase-9 expression) (2) by integrating environmental and (epi)genetic signals. Target gene transcription control by PPAR $\gamma$  arises from molecular interactions among various PPAR ligands, cofactors, dimerization with retinoid X receptor, and binding to PPAR-responsive elements on a number of target promoter regions.

The clinical relevance of the PPAR $\gamma$  pathway was established ever since its identification as a high-affinity target of the anti-diabetic drugs thiazolidinediones (TZDs) (3).

The PPAR $\gamma$  gene carries a nonsynonymous C/G polymorphism (rs 1801282) resulting in a proline to alanine substitution at codon 12 (Pro12Ala) of one of the two (4) known PPAR $\gamma$  transcripts, PPAR $\gamma$ 2. Functional consequences of this substitution are likely given its location in an insulin-sensitive activation domain (5), which is highly conserved across species, and given the fact that proline insertion generally leads to a disruption of secondary structures.

In line with the role of PPAR $\gamma$  in glucose metabolism, carriership of the alanine allele at this polymorphic site (12Ala) is consistently associated with a lower risk of developing type 2 diabetes in various populations (6–8). Moreover, the 12Ala variant has also been found to be associated with a decreased risk for myocardial infarction in the general population (9). These findings suggest a role of PPAR $\gamma$  in the development of vascular disease, either through its influence on the occurrence of diabetes or directly on atherosclerotic vascular disease.

Thus, PPAR $\gamma$  represents a new target in the secondary prevention of recurrent atherosclerotic disease. To date, no studies have established the impact of individual variation in the PPAR $\gamma$  gene on long-term cardiovascular risk in patients with manifest vascular disease. Therefore, we investigated the impact of the gene polymorphism Pro12Ala on angiographic and 10-year cardiovascular outcomes and addressed the roles of inflammation and

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metabolic syndrome in a cohort of patients with ischemic heart disease (IHD).

## RESEARCH DESIGN AND METHODS

The study participants were derived from the Regression Growth Evaluation Statin Study (REGRESS) angiographic trial cohort, which enrolled 884 male patients with symptomatic coronary artery disease (CAD) between 1989 and 1993. The trial design and main findings have been reported previously (10,11). In brief, the primary objectives of this angiographic trial were to evaluate the effects of 24 months of 40 mg pravastatin therapy on the evolution of atherosclerotic lesions in male patients with proven CAD. All participants gave written informed consent, and the clinical trial and subsequent DNA studies were approved by all seven institutional review boards of the participating centers and by the medical ethics committees of all centers.

### Morbidity and mortality 10-year follow-up study

Within the framework of the original trial, clinical outcomes were assessed at 24 months comprising fatal and nonfatal myocardial infarction (MI), death due to CAD, repeated coronary revascularization (coronary artery bypass graft or percutaneous transluminal coronary angioplasty), stroke, and death due to noncoronary causes. To obtain 10-year follow-up data of the participants, cause-specific mortality and hospitalization data up until 1 January 2001 were extracted from nationwide registries. All diagnoses in these registers are coded according to the *International Classification of Diseases, Ninth Revision, Clinical Modification* (ICD-9 and ICD-10 for mortality and ICD-9-CM for morbidity). The research protocol was approved by the institutional review board and ethics committee of the coordinating center (University Medical Center Utrecht). The linkage process method is further described in an online appendix at <http://care.diabetesjournals.org/cgi/content/full/dc09-1819/DC1> (see METHODS section A1).

### Outcome events

In the outcome events analyses, we considered the primary causes of death and the primary clinical diagnosis recorded during hospitalization. The composite end point “death due to ischemic heart disease” consisted of all primary causes of death within the ICD-9 codes 410–414

and ICD-10 codes I20–I25. “Death due to ischemic heart disease or nonfatal myocardial infarction” additionally comprised the clinical occurrence of ICD-9-CM codes 4100–4109. The composite end point “death due to vascular disease” consisted of all primary causes of death within the ICD-9 codes 410–414, 430–438, and 440–448 and ICD-10 codes I20–I25, I60–I69, I70–I79, and F01.

### Angiographic outcomes

Patients underwent coronary arteriography upon enrollment and at 24 months of follow-up, according to a uniform previously described protocol (10). After completion of the follow-up period, all angiograms were analyzed by quantitative coronary angiography (QCA). The following angiographic outcomes were used: the average mean segment diameter per patient, reflecting the extent of diffuse atherosclerosis, and the average minimal obstruction diameter per patient, reflecting extent of focal atherosclerosis, calculated as described previously (10). Both the baseline and follow-up angiographic data were used in the current analysis.

### DNA analyses

Genomic DNA was extracted from blood collected at baseline of the study according to standard procedures and was available from 679 participants because of a stock shortage. Genotyping of the PPAR $\gamma$  Pro12Ala variant (dbSNP rs1801282) was performed using a multiplex PCR and immobilized probe-based assay (12) (Roche Molecular Systems, Alameda, CA).

### Data analyses

Differences in baseline demographic and clinical characteristics and concentrations of lipids among the three genotypes were assessed by linear regression or Pearson's  $\chi^2$  analysis, as was the assumption of Hardy-Weinberg equilibrium.

The effect of the genotype on angiographic outcomes mean segment diameter and minimal obstruction diameter were analyzed using linear regression. C-reactive protein (CRP) levels were logarithmically transformed for use as covariates, because they were not normally distributed. The absolute risk in each genotype group was estimated using the Kaplan-Meier method. To explore the presence of bias due to competing risks in the cause-specific end points, a competing risk analysis was performed according to the method of Fine and Gray (13). The

effect of genotype on outcome was analyzed using Cox proportional hazards.

The mode of inheritance exerting the effects of this gene variant remains unknown. We compared recessive, dominant, and additive models and selected the optimal model to describe the data based on the lowest Akaike information criterion. The additive model appeared to meet this criteria for all cardiovascular outcomes. Hence, the number of rare allele (alanine) copies was entered as a linear covariate into the model. The proportional hazards assumption was assessed for all end points through testing the regression coefficient of the interaction between the covariate(s) and time and was verified visually by a log-log plot. In all instances, the assumption was satisfied.

For both the cardiovascular outcomes as the angiographic outcomes, the role of possible intermediate factors or possible bias from confounding factors was explored in multivariate analyses. These included the components of the metabolic syndrome and baseline CRP levels. To take into account the randomized nature of the first 2 years of the follow-up study, we verified if adjustment for randomization group changed the effects found. Throughout, a two-tailed *P* value of 0.05 was interpreted as indicating a statistically significant difference. All statistical analyses were carried out by two of the authors (J.J.R. and A.H.Z.) using SPSS for Windows, release 15.0 (SPSS, Chicago, IL). All authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the article as written.

## RESULTS

### Distribution of allele frequencies

The study population (*n* = 679) comprised 540 (80%) Pro/Pro, 126 (19%) Pro/Ala, and 13 (2%) Ala/Ala subjects, respectively, meeting the Hardy-Weinberg equilibrium assumption (*P* = 0.094) and closely resembling distributions reported in other Caucasian populations (9). Baseline characteristics according to genotype are shown in Table 1.

Of note, the 12Ala carriers had a lower systolic blood pressure, which reached borderline significance for diastolic blood pressure. Otherwise, no significant differences were observed among the genotypes in baseline coronary heart disease risk factors including lipoprotein profile or lifestyle parameters.

**Table 1—Baseline demographic and CAD characteristics according to the PPAR $\gamma$  Pro12Ala genotype**

	Pro/Pro	Pro/Ala	Ala/Ala	P
Age (years) (SEM)	55.7 $\pm$ 0.34	55.7 $\pm$ 0.74	57.0 $\pm$ 1.7	0.82*
BMI (kg/m <sup>2</sup> )	25.9 $\pm$ 0.11	26.3 $\pm$ 0.26	25.8 $\pm$ 0.60	0.24*
Systolic blood pressure (mmHg)	135.8 $\pm$ 0.78	134.1 $\pm$ 1.74	126.2 $\pm$ 4.1	0.07*
Diastolic blood pressure (mmHg)	81.8 $\pm$ 0.43	81.0 $\pm$ 0.91	74.6 $\pm$ 3.3	0.05*
Current smoking (%)	153 (28%)	34 (27%)	0 (0%)	0.08†
Ejection fraction	70.2 $\pm$ 0.55	70.2 $\pm$ 1.13	73.5 $\pm$ 3.7	0.66*
NYHA functional class				0.17†
I	63 (12%)	8 (6%)	3 (23%)	
II	262 (49%)	64 (50%)	8 (62%)	
III	178 (33%)	39 (39%)	1 (8%)	
IV	33 (6%)	6 (5%)	1 (8%)	
History of hypertension (%)	160 (30%)	35 (28%)	3 (23%)	0.80†
Previous MI (%)	265 (49%)	57 (45%)	4 (31%)	0.32†
Number of vessels diseased				
1 (%)	229 (43%)	55 (44%)	5 (38%)	
2 (%)	185 (34%)	40 (32%)	7 (54%)	
3 (%)	124 (23%)	31 (25%)	1 (8%)	0.52†
Fasting glucose (mmol/l)	5.28 $\pm$ 0.05	5.37 $\pm$ 0.11	5.22 $\pm$ 0.23	0.62*
CRP (mg/l)	5.42 $\pm$ 0.54	6.37 $\pm$ 1.15	2.71 $\pm$ 0.85	0.99*§
$\beta$ -Fibrinogen (g/l)	3.39 $\pm$ 0.07	3.21 $\pm$ 0.17	3.15 $\pm$ 0.32	0.18*§
Total cholesterol (mmol/l)	6.05 $\pm$ 0.04	6.04 $\pm$ 0.08	5.86 $\pm$ 0.25	0.75*
HDL (mmol/l)	0.93 $\pm$ 0.01	0.90 $\pm$ 0.02	1.01 $\pm$ 0.07	0.12*
Triglycerides (mmol/l)	1.78 $\pm$ 0.03	1.87 $\pm$ 0.07	1.62 $\pm$ 0.21	0.42*§
LDL (mmol/l)	4.31 $\pm$ 0.03	4.03 $\pm$ 0.07	4.07 $\pm$ 0.20	0.6*

Data are means  $\pm$  SE unless otherwise indicated. P values were determined by \*linear regression, † $\chi^2$  for cross-tabs. §P value applies to log-transformed triglycerides, CRP, and  $\beta$ -fibrinogen levels due to non-normality. Pro, proline; Ala, alanine at codon 12 of PPAR $\gamma$ .

### Genotype and the occurrence of outcome events

Among the 679 patients included in the survival analysis, 111 died during follow-up; 53 patients died from vascular disease, and 43 deaths were caused by IHD. The composite of nonfatal MI or death from IHD occurred in 81 subjects. After 10 years of follow-up, carriers of the 12Ala allele had a considerably reduced risk. For instance, risk of vascular death was 10.0% (SE 1.4%) in Pro12/Pro12, whereas it was 2.5% (SE 1.4%) in Pro12/12Ala and 0% in 12Ala/12Ala carriers, as displayed also in Table 3. The 12Ala allele thus conferred both a reduced risk of vascular death (hazard ratio [HR] 0.24 [95% CI 0.08–0.74]), death from IHD (0.10 [0.01–0.70]), and nonfatal MI or IHD (0.42 [0.21–0.84]). Table 3 displays these respective HRs per each added allele copy of 12Ala. HRs adjusted for components of the metabolic syndrome and randomization group (first 2 years of the follow-up) or baseline CRP level and randomization group did not yield substantial changes in the effects found. In all instances, the competing risk analy-

sis (data not displayed) did not change the effect found.

### Genotype and angiographic extent of CAD

The possession of a 12Ala allele was associated with a moderate increase in coronary arterial diameters (Table 2) in both the average mean segment diameter and the average minimal obstruction diameter and in both the baseline and the 24-month follow-up angiograms in the study.

**Table 2—Effect of genotype (Pro12Ala) on angiographic extent of CAD: baseline and 24-month follow-up angiographic recordings**

	Pro/Pro	Pro/Ala	Ala/Ala	B*	P
Angiographic end point					
Baseline MOD (mm)	1.84 (0–3.78)	1.93 (0–3.75)	2.29 (1.0–3.85)	0.162	0.002
Follow-up MOD (mm)	1.76 (0–4.28)	1.90 (0–3.87)	2.15 (1.17–3.2)	0.137	0.011
Baseline MSD (mm)	2.77 $\pm$ 0.02	2.94 $\pm$ 0.06	2.96 $\pm$ 0.26	0.145	0.001
Follow-up MSD (mm)	2.70 $\pm$ 0.02	2.84 $\pm$ 0.06	2.80 $\pm$ 0.18	0.110	0.014

Data are means  $\pm$  SE or medians (minimum to maximum). MOD, median minimal obstruction diameter, representing focal atherosclerotic changes. Medians are presented here to illustrate genotype effects because of the skewed distribution of MOD. MSD, mean average mean segment diameter, representing diffuse atherosclerotic changes. \*Regression coefficient from general linear model.

The coefficients from multivariate models are displayed in online appendix Table A1. Notably, these multivariate analyses revealed that the relations were found both on cardiovascular and angiographic end points generally continued to be significant in models 1 and 2. In these models, relations between established risk factors and outcomes were generally not significant, presumably due to lack of statistical power in this cohort.

**CONCLUSIONS**— This long-term follow-up study in 679 CAD patients demonstrates the significance on both angiographic outcome and cardiovascular mortality of a frequent variant in PPAR $\gamma$ 2. The 12Ala isotype in this group of patients was associated with less extensive coronary atherosclerosis and better 10-year prognosis. Although the mechanism underlying these observations remains unexplained, this supports the hypothesis that PPAR $\gamma$ 2, expressed predominantly in adipose tissue (14), plays an important role in the prognosis of patients with symptomatic CAD.

First, we found that carriership of the PPAR $\gamma$ 2 12Ala isotype, as can be found in 21% of Caucasians (9,15), is associated with a decreased 10-year cardiovascular mortality compared with the Pro12 isotype. An allele-dose effect seemed present, notably for IHD and vascular disease–caused deaths and incident myocardial infarction. To our best knowledge, these are the first prospective data on the prognostic impact of the Pro12Ala variant in patients with manifest coronary or other vascular disease. However, this relation has been addressed in the general population. A matched nested case-control study (9) of incident MI among 2,615 healthy men followed for 13.2 years in the Physicians' Health Study observed a protective effect of the 12Ala al-

Table 3—Effect of PPAR $\gamma$  genotype on outcome

Clinical end point	Genotype			HR perAla allele copy		
	Pro/Pro (n = 540)	Pro/Ala (n = 126)	Ala/Ala (n = 13)	Univariate model	Multivariate model 1	Multivariate model 2
Death from IHD	8.2 (1.2)	0.8 (0.8)	0	0.10 (0.01–0.70)	0.10 (0.14–0.72)	—
n	42	1	0			
P				0.02	0.02	
Death from IHD or non-fatal MI	17.6 (2.3)	7.3 (2.5)	0	0.42 (0.21–0.84)	0.46 (0.23–0.92)	0.28 (0.09–0.85)
n	73	8	0			
P				0.01	0.03	0.03
Death from vascular disease	10.0 (1.4)	2.5 (1.4)	0	0.24 (0.08–0.74)	0.17 (0.04–0.67)	0.13 (0.018–0.92)
n	50	3	0			
P				0.01	0.01	0.04
All-cause mortality	18.9 (1.9)	12.6 (2.9)	0	0.66 (0.41–1.07)	0.57 (0.34–0.97)	0.63 (0.34–1.18)
n	94	17	0			
P				0.09	0.04	0.02

Data are % (SE %) or hazard ratio (95% CI) unless otherwise indicated. Ten-year absolute risk (% and SE) of composite outcome events (Kaplan-Meier estimate) and absolute number of cases (uncensored data) are displayed per genotype on the left. Hazard ratios (with corresponding 95% CI and P value) per each additional Alanine allele copy are displayed on the right. Multivariate model 1 controls for randomization group, BMI, fasting blood glucose, triglyceride and HDL cholesterol levels, systolic and diastolic blood pressure, self-reported hypertension, antihypertensive drug use, and antidiabetic drug use. Multivariate model 2 controls for randomization group and ln(CRP). (—), Unable to estimate due to empty cell.

allele (odds ratio [OR] 0.79, 95% CI 0.63–0.99). The finding was not replicated in a later report of incident MI or CHD death in two matched case-control samples of shorter follow-up durations in 730 men and 752 women nested within the Health Professionals Follow-up Study and Nurses' Health Study, respectively (15). In the latter report, the pooled effect of both samples suggested an elevated risk in presence of the 12Ala allele, reaching borderline significance (OR 1.30, 95% CI 1.00–1.67). Further, a case-control report in 844 Han Chinese described a significantly increased risk of MI in 12Ala allele carriers (unadjusted OR 1.83, 95% CI 1.06–3.14). One potential explanation for opposing findings may be found in the existence of gene-environment interactions. Notably, the data of Pischon et al. (15) suggest that the prognostic effect was modified by the presence of obesity.

Second, we observed a lower extent of focal and diffuse atherosclerosis (respectively higher minimal obstruction diameter and mean segment diameter) associated with the presence of the 12Ala allele, and an allele-dose effect seemed present. However, no effect was detected on the rate of progression of atherosclerosis during the 24-month angiographic follow-up. Although possibly due to low statistical power, in this regard, it is tempting to speculate that vascular processes apart from atherogenesis might additionally explain the 10-year prognostic effect of the PPAR $\gamma$  variant.

So far, no other QCA data have been reported in relation with the Pro12Ala polymorphism. However, a study in 267 Korean subjects undergoing coronary angiography reported no effect of the Pro12Ala polymorphism on the number of vessels diseased, possibly due to low statistical power or precision, as the authors stated (16). However, presence of the Alanine12 isotype has been consistently related to decreased intima-media thickness (IMT) in 622 German individuals bearing risk factors for diabetes (17), 154 Japanese diabetes patients (18), and 161 healthy Canadian aboriginals (19). This agrees with the current QCA findings and supports effects of PPAR $\gamma$  on atherogenesis.

### Mechanism

The mechanism mediating the protective effects of the 12Ala allele as a determinant of angiographic and clinical outcomes observed here remains unresolved. To disclose intermediate factors, we performed several multivariate analyses.

First, given involvement of PPAR $\gamma$ 2 in adipogenesis (14), and Pro12Ala in particular as a genetic determinant of type 2 diabetes (7,20), BMI (17,21,22), and blood pressure (23), we elected to incorporate components of the metabolic syndrome as covariates. In the current analysis, carrier-ship of the 12Ala allele continued to be significantly associated with a decrease of 10-year cardiovascular outcome. This is supported by data obtained in the general population (9), which did not reveal any indication of diabetes as an intermediate

factor. Hence, the metabolic syndrome or its components appear not to account for the relation between the Pro12Ala polymorphism and cardiovascular risk.

Second, in REGRESS, effects of the Pro12Ala variant on cardiovascular risk were independent of baseline CRP levels in multivariate analyses. Carriers of 12Ala appeared to exhibit slightly lower mean baseline levels of CRP and  $\beta$ -fibrinogen, and this trend had also been observed in other reports (15) but was not statistically significant. Thus, reduced inflammation most likely is not the mechanism explaining the effects observed.

Interestingly, it has been previously reported that the Alanine allele determines lower lipoprotein lipase activity with a high triglyceride–low HDL cholesterol profile, in two independent populations ( $n = 294$ ), both diabetic and nondiabetic (24). However, the prognostic effect of lower lipoprotein lipase activity remains unknown. Our current data from REGRESS do not reveal such an effect of the Pro12Ala polymorphism on lipoprotein profile. Accordingly, lipoprotein lipase activity is not likely an explanation for the effects of Pro12Ala on angiographic and 10-year clinical outcomes.

### Study strengths and limitations

To appreciate these findings, some aspects of our study merit consideration. First, the longitudinal REGRESS study constitutes a cohort of CAD patients with 10 years of follow-up. The QCA

data obtained within the study enable us to observe effects on the extent of atherosclerosis. A second consideration is that the data originate from a randomized trial in which assessment of the benefits of pravastatin treatment was the primary objective. Because the study medication taken during 2 years of the follow-up time was allocated at random, i.e., irrespective of genotype, this will not have affected our findings. Third, our follow-up dataset was not complete for all patients: 3 and 16% of patients could not be uniquely identified in the mortality and hospital registries, respectively. Because these patients were right-censored at lost-to-follow-up time, again it seems unlikely that this would have affected the primary outcome of the current study. We elected to calculate actuarial survival across genotypes, in contrast to the matched (9,15) or unmatched (25) case-control design. Survival analysis enabled us to efficiently study all available information, including that of censored participants. Fourth, with the current data, we did not identify the underlying mechanisms (putatively, the metabolic syndrome and inflammatory state). The lack of information on the occurrence of the metabolic syndrome or inflammatory parameters over time limits definitive conclusions in this respect. Finally, an important issue is that the results in this study were obtained in a cohort of male patients with established CAD, and further research is needed to demonstrate whether the results of our study also apply to women. Nevertheless, the current findings from REGRESS are the first long-term data on hard clinical end points collected prospectively and add to the understanding on the role of PPAR $\gamma$  in cardiovascular disease.

In conclusion, among symptomatic CAD patients, carriership of the 12Ala variant of PPAR $\gamma$  is associated with less extensive atherosclerotic disease and appears to protect against recurrent vascular disease and vascular mortality, as observed in a long-term follow-up study. These long-term findings in patients with manifest CAD support an important role of PPAR $\gamma$  in determining vascular risk.

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