Peroxisome Proliferator-Activated Receptor-γ2 P12A Polymorphism and Risk of Coronary Heart Disease in US Men and Women

Tobias Pischon, Jennifer K. Pai, JoAnn E. Manson, Frank B. Hu, Kathryn M. Rexrode, David Hunter, Eric B. Rimm

Objective—Activation of the peroxisome proliferator-activated receptor-γ (PPARγ) improves insulin sensitivity and exerts antiatherogenic effects. A common alanine for proline substitution at codon 12 in the PPARG2 gene is related to lower receptor activity. Studies suggest that the A12 allele is associated with reduced risk of type 2 diabetes; however, data on the risk of coronary heart disease (CHD) are scarce and controversial.

Methods and Results—We examined the relationship between PPARG2 P12A and CHD risk in women (Nurses’ Health Study) and men (Health Professionals Follow-Up Study) in nested case control settings. Among participants free of cardiovascular disease at baseline, 249 women and 266 men developed nonfatal myocardial infarction (MI) or fatal CHD during 8 and 6 years of follow-up, respectively. Using risk-set sampling, controls were selected 2:1 matched on age, smoking, and date of blood draw. The relative risk (RR) of nonfatal MI or fatal CHD of carriers compared with noncarriers of the A12 allele was 1.17 (95% CI, 0.82 to 1.68) among women and 1.44 (95% CI, 1.00 to 2.07) among men (pooled RR, 1.30 [95% CI, 1.00 to 1.67]). We found a significantly increased risk associated with the A12 allele among individuals with a body mass index $\geq$25 kg/m$^2$ (women: RR, 1.88; 95% CI, 1.01 to 3.50; men: RR, 1.55; 95% CI, 0.92 to 2.60; pooled: RR, 1.68; 95% CI, 1.13 to 2.50) but not among those <25 kg/m$^2$ (pooled RR, 0.86; 95% CI, 0.37 to 1.97; $P$ heterogeneity overweight versus nonoverweight 0.16).

Conclusions—These data do not support the hypothesis that the A12 allele is associated with a decreased risk of CHD. The potential interaction between PPARG2 P12A, overweight, and increased CHD risk needs further evaluation.

Key Words: genetics ■ epidemiology ■ coronary heart disease ■ polymorphism

The peroxisome proliferator-activated receptor-γ (PPARγ) belongs to a family of transcription factors involved in the regulation of lipid and glucose metabolism, cellular proliferation and differentiation, and inflammation. Activation of PPARγ leads to adipocyte differentiation and a wide range of metabolic effects, which are generally considered to be beneficial for the cardiovascular system. Clinically, the PPARγ ligands thiazolidinediones (TZDs) are used as an established treatment of type 2 diabetes by improving insulin sensitivity; however, paradoxically, inhibition of PPARγ also improves insulin sensitivity in animal models. In humans, PPARγ exists in 2 protein isoforms. Although PPARγ1 is expressed in most tissues, PPARγ2 is almost exclusively expressed in the adipose tissue. A common alanine (A) for proline (P) substitution at codon 12 in the PPARG2 gene was identified and shown to be associated with reduced PPARγ2 activity. The initial study reported a 75% lower risk of type 2 diabetes conferred by the A12 allele. A subsequent meta-analysis of 6 studies suggested a 21% risk reduction. Individual studies yielded a range of results, with several reporting null findings and decreased or increased risk of type 2 diabetes associated with the A12 allele.

Data on this polymorphism and risk of coronary heart disease (CHD) are scarce and also inconsistent. A recent prospective study suggested a decreased risk of CHD among carriers of the A12 allele, although another cross-sectional study showed no association.

The aim of our study was to examine prospectively the association between PPARG2 P12A polymorphism and risk of CHD in women (Nurses’ Health Study [NHS]) and men (Health Professionals Follow-up Study [HPFS]) in nested case control settings. Our hypothesis was that this polymorphism is associated with a reduced risk of CHD. Recent data also suggest that the A12 allele may be related to
increased body weight and weight gain. Therefore, we aimed to examine its potential interaction with body weight.

Materials and Methods

Study Population

The NHS and HPFS are prospective cohort investigations among 121,700 female US registered nurses aged 30 to 54 years at baseline in 1976 (NHS) and 51,529 US male health professionals, aged 40 to 75 years at baseline in 1986 (HPFS). Information about anthropometric data, medical history, lifestyle choices, and incident disease is assessed biennially by self-administered questionnaires. The validity and reproducibility of the collected data have been reported in detail previously. Between 1989 and 1990, a blood sample was requested from all living participants of the NHS and was returned by 32,826 women. Similarly, between 1993 and 1995, a blood sample was requested from all living participants of the HPFS and was returned by 18,225 men. Participants who provided blood samples were similar to those who did not, albeit the men who provided samples were somewhat younger than those who did not. In the NHS, we identified 249 women with incident nonfatal myocardial infarction (MI) or fatal CHD between date of blood drawing and June 1998. In the HPFS, we identified 266 men with incident nonfatal MI or fatal CHD between date of blood draw and return of the 2000 questionnaire. As a secondary endpoint, we additionally identified 564 men who had coronary artery bypass graft surgery (CABG) or percutaneous transluminal coronary angioplasty (PTCA) during follow-up. Using risk-set sampling, controls were selected randomly 2:1 matched on age, smoking, and date of blood draw, from participants free of diagnosed cardiovascular disease (CVD) at time of case ascertainment. For the NHS, we also matched on fasting status and reported problems with blood drawing. These nested case control studies were designed primarily to examine novel and established biological predictors of CHD. The rationale for matching cases and controls on the described factors was to enhance efficiency if controlling for these potential confounders was deemed necessary. For our analysis, we chose nonfatal MI or fatal CHD as primary endpoint and considered CABG/PTCA in secondary analyses only.

MI was confirmed using World Health Organization criteria. Deaths were identified from state vital records and the National Death Index or reported by subjects’ next of kin or the postal system. Fatal CHD was confirmed by hospital records or on autopsy, or if CHD was listed as the cause of death on the death certificate, if it was the underlying and most plausible cause, and if evidence of previous CHD was available. Confirmation of CABG/PTCA was based on self-report only; hospital records obtained for a sample of 102 men confirmed the procedure for 96% of these.

The study protocol was approved by the institutional review board of the Brigham and Women’s Hospital and the Harvard School of Public Health human subjects committee review board.

Genotyping

DNA was extracted from the buffy coat fraction of centrifuged blood using the QIAmp Blood Kit (Qiagen). The primary genotyping technique was Taqman single nucleotide polymorphism allelic discrimination by means of an ABI 7900HT (Applied Biosystems). Replicate quality control samples were included and genotyped with 100% concordance. Genotype data were available for 752 men (250 cases and 502 controls) and 730 women (245 cases and 485 controls). In addition, genotype data were available for 537 men with CABG and 1077 men as their matched controls. Cases were not matched perfectly to controls because a few subjects could not be genotyped with this platform.

Biomarkers

Biomarkers were measured for nonfatal MI and fatal CHD cases and their controls only (the set of CABG/PTCA cases and controls did not have available plasma biomarkers). Details on the measurement procedures and on biomarker levels in cases and controls have been published previously.

Statistical Analyses

The 2 cohorts were analyzed separately using conditional logistic regression. We stratified the analysis by body mass index (BMI) <25 kg/m² or ≥25 kg/m² and added a cross-product term to our model to examine differences in the associations with CHD between overweight and nonoverweight individuals. In secondary analyses, we also further adjusted for BMI (<20, 20 to 24, 25 to 29, 30 to 34, and ≥35 kg/m²), parental history of CHD at <60 years of age, history of hypertension, history of diabetes, alcohol consumption (nondrinker, 0.1 to 4.9, 5.0 to 14.9, 15.0 to 29.9, or ≥30.0 g per day), and physical activity (quintiles). To pool the relative risk (RR) estimates for women and men, we used the weighted average of estimates using the DerSimonian and Laird random-effects model. We also pooled men and women stratified by body weight and tested for heterogeneity between overweight and nonoverweight subjects.

All P values presented are 2-tailed, and P values <0.05 were considered statistically significant. Analyses were performed using SAS 8.2 (SAS Institute) and STATA 8.2 (STATA Corporation).

Results

Expected associations were observed when comparing baseline characteristics of cases and controls (Table 1). The prevalence of the P12/A12 and A12/A12 genotypes among controls was 19.2% and 1.2% for women and 18.1% and 0.8% for men (no significant deviation from Hardy–Weinberg equilibrium based on χ² goodness-of-fit test; P=0.66 for men and 0.88 for women). This distribution was not significantly different from the cases (22.0% and 1.6% in women, 23.6% and 1.6% in men, P=0.11).

Overall, there were no significant differences in baseline characteristics among controls between subjects carrying and those not carrying the A12 allele (Table 2). Female carriers of the A12 allele had a slightly lower prevalence of reported hypertension, whereas BMI, reported diabetes, and parental history of CHD at <60 years of age were similar between carriers and noncarriers of the A12 allele. Similarly, there were no significant differences in biomarker levels between carriers and noncarriers of the A12 allele. There was no additional significant differences in biomarker levels between carriers and noncarriers of the A12 allele, although female carriers had slightly higher levels of low-density lipoprotein cholesterol (LDL-C). Adiponectin levels (measured among men only) were not significantly different between carriers and noncarriers of the A12 allele. We also did not find significant differences for baseline characteristics and biomarkers between carriers and noncarriers of the A12 allele when stratified by BMI (<25 kg/m² or ≥25 kg/m²; data not shown).

The RR of nonfatal MI or fatal CHD for carriers compared with noncarriers of the A12 allele was 1.17 (95% CI, 0.82 to 1.68) among women and 1.44 (95% CI, 1.00 to 2.07) among men (Table 3). When we pooled the risk estimates for women and men, carriers of the A12 allele had a 1.30-fold (95% CI, 1.00 to 1.67) increased risk of nonfatal MI or fatal CHD (Table 3). Further adjustment for BMI, parental history of CHD at <60 years of age, history of hypertension, history of diabetes, alcohol consumption, and physical activity did not substantially affect the risk estimates (data not shown). Stratification by BMI suggested a difference between normal weight and overweight participants for the association...
of the Ala allele and risk of CHD (Table 3). Thus, when we pooled men and women, overweight carriers of the A12 allele had a 1.68-fold increased risk of CHD (95% CI, 1.13 to 2.50), whereas normal weight carriers were not at increased risk (RR, 0.86; 95% CI, 0.37 to 1.97).

The RR of CABG/PTCA for male carriers compared with noncarriers of the A12 allele was 0.84 (95% CI, 0.65 to 1.09; Table 3). The RR was 0.81 (95% CI, 0.54 to 1.21) among overweight and 0.99 (95% CI, 0.55 to 1.78) among nonoverweight men.

### TABLE 1. Baseline Characteristics of Women (NHS; 8 Years of Follow-Up) and Men (HPFS; 6 Years of Follow-Up) With Incident Nonfatal MI or CHD (Cases) and Matched* Event-Free Controls

<table>
<thead>
<tr>
<th></th>
<th>Women Cases</th>
<th>Controls</th>
<th>P†</th>
<th>Men Cases</th>
<th>Controls</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>245</td>
<td>485</td>
<td>–</td>
<td>250</td>
<td>502</td>
<td>–</td>
</tr>
<tr>
<td>Age, years, mean±SE</td>
<td>60.4±0.4</td>
<td>60.3±0.3</td>
<td>–</td>
<td>65.2±0.5</td>
<td>65.1±0.4</td>
<td>–</td>
</tr>
<tr>
<td>Current smoker, %</td>
<td>32.2</td>
<td>31.6</td>
<td>–</td>
<td>12.4</td>
<td>12.3</td>
<td>–</td>
</tr>
<tr>
<td>BMI, kg/m², mean±SE</td>
<td>26.8±0.4</td>
<td>25.4±0.2</td>
<td>0.001</td>
<td>26.2±0.2</td>
<td>25.7±0.2</td>
<td>0.06</td>
</tr>
<tr>
<td>Parental history of CHD at &lt;60 years of age, %</td>
<td>21.6</td>
<td>12.4</td>
<td>0.001</td>
<td>16.0</td>
<td>11.0</td>
<td>0.05</td>
</tr>
<tr>
<td>Postmenopausal, %</td>
<td>90.1</td>
<td>87.7</td>
<td>0.34</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>HRT use among postmenopausal women, %</td>
<td>31.9</td>
<td>39.7</td>
<td>0.06</td>
<td>39.8</td>
<td>34.7</td>
<td>0.17</td>
</tr>
<tr>
<td>Aspirin use, %</td>
<td>14.7</td>
<td>20.4</td>
<td>0.06</td>
<td>39.8</td>
<td>34.7</td>
<td>0.17</td>
</tr>
<tr>
<td>History of hypertension, %</td>
<td>57.1</td>
<td>29.5</td>
<td>0.001</td>
<td>41.6</td>
<td>29.9</td>
<td>0.001</td>
</tr>
<tr>
<td>History of diabetes, %</td>
<td>19.6</td>
<td>6.6</td>
<td>0.001</td>
<td>9.2</td>
<td>4.4</td>
<td>0.009</td>
</tr>
<tr>
<td>Alcohol consumption, g/d, median (IQR)</td>
<td>0.9 (0–3.7)</td>
<td>1.8 (0–8.5)</td>
<td>&lt;0.001</td>
<td>5.9 (0.9–15.9)</td>
<td>7.0 (0.9–18.5)</td>
<td>0.15</td>
</tr>
<tr>
<td>Physical activity, MET-hrs/wk, median (IQR)</td>
<td>11.0 (3.9–22.9)</td>
<td>11.5 (4.8–23.3)</td>
<td>0.42</td>
<td>22.8 (8.3–44.7)</td>
<td>27.3 (11.9–48.9)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

IQR indicates interquartile range; HRT, hormone replacement therapy; MET-hrs, metabolic equivalent hours.

*Matching criteria were age, smoking status, and date of blood drawing. In women, matching factors also include fasting status and problems with blood drawing.

†Variables were compared between cases and controls using Student unpaired t-test, Wilcoxon unpaired rank-sum test, or the χ² test.

### TABLE 2. Baseline Characteristics and Biomarker Levels* Among Women (NHS) and Men (HPFS) Selected as Controls to Subjects With Incident Nonfatal MI or Fatal CHD According to PPARG Genotype

<table>
<thead>
<tr>
<th>PPARG2 Genotype</th>
<th>Women</th>
<th>Men</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>P12/P12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>386</td>
<td>407</td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m², mean±SE</td>
<td>25.5±0.2</td>
<td>25.6±0.2</td>
<td>0.64</td>
</tr>
<tr>
<td>Parental history of CHD, age &lt;60, %</td>
<td>11.4</td>
<td>11.1</td>
<td>0.85</td>
</tr>
<tr>
<td>History of hypertension, %</td>
<td>31.6</td>
<td>30.4</td>
<td>0.62</td>
</tr>
<tr>
<td>History of diabetes, %</td>
<td>6.7</td>
<td>4.1</td>
<td>0.61</td>
</tr>
<tr>
<td>sTNF-R1, pg/mL, mean±SE</td>
<td>1270±17</td>
<td>1496±23</td>
<td>0.52</td>
</tr>
<tr>
<td>sTNF-R2, pg/mL, mean±SE</td>
<td>2479±33</td>
<td>2943±39</td>
<td>0.92</td>
</tr>
<tr>
<td>IL-6, pg/mL, geometric mean (95% CI)</td>
<td>1.78 (1.67–1.90)</td>
<td>1.87 (1.57–2.25)</td>
<td>0.67</td>
</tr>
<tr>
<td>CRP, mg/L, geometric mean (95% CI)</td>
<td>2.20 (1.98–2.46)</td>
<td>0.97 (0.78–1.22)</td>
<td>0.12</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL, mean±SE</td>
<td>225±2</td>
<td>204±2</td>
<td>0.18</td>
</tr>
<tr>
<td>LDL-C, mg/dL, mean±SE</td>
<td>131±2</td>
<td>126±2</td>
<td>0.10</td>
</tr>
<tr>
<td>HDL-C, mg/dL, mean±SE</td>
<td>60±1</td>
<td>46±1</td>
<td>0.61</td>
</tr>
<tr>
<td>Triglycerides, mg/dL, mean±SE</td>
<td>126±4</td>
<td>154±6</td>
<td>0.82</td>
</tr>
<tr>
<td>Adiponectin, mg/L, mean±SE</td>
<td>n/a</td>
<td>17.7±0.4</td>
<td>0.37</td>
</tr>
</tbody>
</table>

sTNF-R indicates soluble tumor necrosis factor-α receptors; IL-6, interleukin 6; CRP, C-reactive protein; HDL-C, high-density lipoprotein cholesterol.

*All variables (except No. of subjects) are adjusted for age of the participants. Biomarkers were additionally adjusted for fasting status and date of blood draw. In women, biomarkers were additionally adjusted for reported problems with blood drawing. Results were obtained from linear regression with robust variance.29
TABLE 3. RR of CHD in Carriers Compared With Noncarriers (Reference) of the PPARG2 A12 Allele Among Women (NHS; 8 Years of Follow-Up) and Men (HPFS; 6 Years of Follow-Up) Stratified by CHD End Point* in the Total Data Sets and Stratified by Body Weight

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Total Nonoverweight</th>
<th>Overweight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RR (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>Women† Nonfatal MI or fatal CHD</td>
<td>1.17 (0.82–1.68)</td>
<td>0.39</td>
</tr>
<tr>
<td>Men† Nonfatal MI or fatal CHD</td>
<td>1.44 (1.00–2.07)</td>
<td>0.05</td>
</tr>
<tr>
<td>CABG/PTCA</td>
<td>0.84 (0.65–1.09)</td>
<td>0.18</td>
</tr>
<tr>
<td>Any CHD</td>
<td>1.00 (0.81–1.24)</td>
<td>0.99</td>
</tr>
<tr>
<td>Women+ Men‡ Nonfatal MI or fatal CHD</td>
<td>1.30 (1.00–1.67)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

*CHD end points in women include nonfatal MI or fatal CHD. CHD end point in men include nonfatal MI or fatal CHD and CABG/PTCA.
†Results obtained from conditional logistic regression. Cases and controls were matched on age, smoking status, and month of blood drawing. Women were additionally matched on fasting status and reported problems with blood drawing.
‡Results obtained from pooling the RR estimates for women and men using the weighted average of estimates using the DerSimonian and Laird random-effects model.
§P=0.03 compared with overweight women; ¶P=0.44 compared with overweight men; ||P=0.24 compared with overweight men; **P=0.62 compared with overweight men; ††P=0.16 compared with overweight subjects.

When we combined CHD end points among men, the RR of nonfatal MI, fatal CHD, or CABG/PTCA was 1.00 (95% CI, 0.81 to 1.24; Table 3).

Discussion

In these 2 nested case control studies of women and men, we did not find a decreased risk of CHD among carriers of the PPARG2 A12 allele. In addition, we did not find significant differences in the phenotype between individuals carrying and those not carrying the A12 allele, including plasma levels of total cholesterol, LDL-C, high-density lipoprotein cholesterol, C-reactive protein, interleukin-6, soluble tumor necrosis factor receptors, and adiponectin. These data do not support the hypothesis that the presence of the A12 allele is associated with a decreased risk of CHD.

Data on the PPARG2 P12A polymorphism and risk of CHD are scarce and inconsistent.12,13 An analysis in the male Physicians’ Health Study, which includes participants similar to our cohorts with respect to demography, ethnicity, and socioeconomic status, reported that carriers of the A12 allele had a 23% lower risk of MI compared with controls over a mean follow-up period of ~13 years (P=0.03).12 Notably, the prevalence of diabetes was not significantly different between carriers and noncarriers of the A12 allele in that study.12 Two more recent cross-sectional studies suggested that the PPARG2 P12A variant may be related to less atherosclerosis, as measured by intima media thickness.23,24 In contrast, a cross-sectional study by Blüher et al13 found that diabetic carriers of the A12 allele were not at reduced risk of CHD.

Because PPARγ is involved in a wide range of metabolic pathways, its activation or inhibition is likely to have complex results. Thus, treatment with TZDs improves insulin sensitivity but concomitantly promotes weight gain, especially under excess caloric intake, which may offset their beneficial long-term effects.25 Recent studies suggest that the PPARG2 P12A polymorphism, which supposedly improves insulin sensitivity, may be associated with increased body weight and weight gain.14,15,26 Thus, one may speculate whether the reduced CHD risk from improved insulin sensitivity is offset by long-term weight gain. In this respect, our finding of a significantly increased risk of CHD among overweight (but not normal weight) carriers of the A12 allele is potentially relevant. Clearly, further confirmation of these findings is necessary.

We found modest differences in risk estimates for nonfatal MI and fatal CHD among those of CABG/PTCA. Thus, male carriers of the A12 allele were at increased risk of nonfatal MI and fatal CHD but at decreased (albeit nonsignificant) risk of CABG/PTCA. It is unclear whether these results represent true differences for the relationship between the PPARG2 P12A polymorphism and these disease end points or rather they reflect random variation; however, because we did not have information on genotyping for incident CABG/PTCA among women, and because results for nonfatal MI and fatal CHD were similar between men and women, we analyzed the CABG/PTCA end points separately.

Our cohorts include relatively healthy subjects, and the prevalence of type 2 diabetes is lower than in the general US population ≥60 years.27 If type 2 diabetes is the only intermediary mechanism between PPARγ and CHD development, then our power to detect an association between the P12A variant and risk of CHD was limited. However, PPARγ is involved in a wide range of metabolic pathways beyond diabetes, and its activation or inhibition is likely to have complex results on the cardiovascular system.1 Multiple testing in association studies may produce spurious results when large numbers of polymorphisms are examined, especially without adequate a priori hypotheses. However, the role of PPARγ in the pathogenesis of CVD has long been established, and the polymorphism examined in our study has been well studied with regard to type 2 diabetes. Nevertheless, our study does not rule out that other variants in the PPARG2 gene may be related to CHD risk.28 Also, our study sample may have had undetected population stratification, although control for ethnicity did not appreciably alter our results (data not shown).

In summary, we did not find a decreased risk of CHD among carriers of the PPARG2 A12 allele in 2 nested case control studies of women and men. We also did not find significant differences in various cardiovascular risk factors between individuals carrying and those not carrying the A12 allele.
allele. Furthermore, we found that overweight (but not normal weight) carriers of the A12 allele were at increased risk of CHD, although these results require confirmation from future studies. Together, our study does not support the hypothesis that the presence of the A12 allele is associated with a decreased risk of CHD.

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References

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