Prospective Study of the Association Between the Proline to Alanine Codon 12 Polymorphism in the *PPARγ* Gene and Type 2 Diabetes

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OBJECTIVE — To determine whether the Pro12Ala polymorphism in the *PPARγ* gene was associated with risk of type 2 diabetes in the Nurses' Health Study.

RESEARCH DESIGN AND METHODS — The study was a nested case-control study of 387 incident cases of type 2 diabetes and 771 matching control subjects nested within the Nurses' Health Study, a prospective cohort study. Association between *PPARγ* genotype and incident type 2 diabetes was estimated using logistic regression.

RESULTS — Carriers of the *PPARγ* variant 12Ala allele had reduced risk of type 2 diabetes compared with noncarriers. Unadjusted and adjusted odds ratios of type 2 diabetes were 0.74 (95% CI 0.55–1.00) and 0.72 (0.52–0.99), respectively.

CONCLUSIONS — The results of this study provide further support for an inverse association between the *PPARγ* variant 12Ala allele and risk of type 2 diabetes.

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One of the most promising and extensively studied genetic risk factors for type 2 diabetes is a polymorphism in the peroxisome proliferator–activated receptor *PPARγ* gene. In addition to its role in adipogenesis, PPARγ has a role in insulin signaling, insulin resistance, and development of type 2 diabetes and is the target for the thiazolidinedione class of antidiabetic drugs. The common codon 12 proline to alanine (Pro12Ala) substitution polymorphism produces PPARγ protein with lower transcriptional activity (1,2). Studies suggest that carriers of the 12Ala variant allele are at reduced risk of type 2 diabetes. The aim of the current study was to determine whether *PPARγ* Pro12Ala polymorphism was associated with reduced risk of type 2 diabetes in the Nurses' Health Study.

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Abbreviations: NDDG, National Diabetes Data Group; PPAR, peroxisome proliferator–activated receptor.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

RESEARCH DESIGN AND METHODS

— The Nurses' Health Study began in 1976 with the recruitment of 121,700 female registered nurses between the ages of 30 and 55 years (3). The participants were largely Caucasian (>95%). Samples for the present study were selected from a subcohort of 32,826 women who provided blood between 1989 and 1990 and were free from cardiovascular disease, cancer, and diabetes before giving blood. Incident cases were defined as self-reported diabetes confirmed by supplementary questionnaire and diagnosed at least 1 year after blood collection through 1996. The supplementary questionnaire obtained information on symptoms, diagnostic tests, and hypoglycemic therapy used to define type 2 diabetic cases. Diagnosis of type 2 diabetes was made using criteria consistent with those proposed by the National Diabetes Data Group (NDDG); the validity of this method has been confirmed (4,5). Although type 2 diabetes diagnosis criteria were changed in 1996, nearly all of these cases were diagnosed before 1996 and thus earlier NDDG criteria were used. Two control subjects were selected from the Nurses' Health study blood cohort and matched to each case on the following variables: age, month and year of blood draw, and fasting status at blood draw. One of the two control subjects was also matched according to BMI (±1 kg/m^2). Control subjects were free of any self-reported diabetes, cardiovascular disease, and cancer.

Genomic DNA was genotyped by Pyrosequencing using the following primers: PCR primers, 5′-BIOTIN-TTCACAAATTCTGTTACTTCA-3′ and 5′-TTGTGATATGTTTGCAGACA-3′, sequencing primer, 5′-ATCAGTGAAG GAATCGCTTTCT-3′ (Pyrosequencing AB, Uppsala, Sweden). Replicate quality control samples were included and genotyped with 100% concordance. Genotype

Table 1—*Descriptive characteristics of subjects by PPARγ genotype status*

Factor	Pro/Pro homozygotes	Ala allele carriers	*P*
n	894	264	
Age (years)	54.8 ± 7.0	54.2 ± 6.9	0.20*
BMI (kg/m^2)	29.0 ± 5.9	28.7 ± 6.0	0.42*
Alcohol			
Nondrinkers (%)	23.9	24.0	
Mean intake among drinkers (g/day)	8.1 ± 11.5	6.5 ± 8.0	0.35*
Activity (metabolic units/week)	13.4 ± 16.6	14.7 ± 17.0	0.15*
Smoking status			
Never smoked	396 (44.4)	121 (46.0)	0.70†
Former smoker	378 (42.4)	108 (41.1)	
1–14 cigarettes/day	37 (4.2)	14 (5.3)	
15–25 cigarettes/day	46 (5.2)	9 (3.4)	
>25 cigarettes/day	34 (3.8)	11 (4.2)	
Family history of diabetes			
No	611 (68.3)	192 (72.7)	0.18†
Yes	283 (31.7)	72 (27.3)	

Data are mean ± SD or *n* (%). **P* value from Wilcoxon rank-sum test; †*P* value from χ^2 test.

frequencies were in Hardy-Weinberg equilibrium ($P = 0.99$).

Plasma insulin, C-peptide, and proinsulin were determined by radioimmunoassay in the laboratory of Dr. Robert M. Cohen (University of Cincinnati, Cincinnati, OH). Proinsulin and C-peptide were determined as previously described (6), and specific insulin was determined using a radioimmunoassay (Linco Research, St. Charles, MO). Within-individual coefficients of variation among the redundant samples were 13.9, 6.9, and 7.3% for insulin, C-peptide, and proinsulin, respectively.

All statistical analyses were performed using SAS version 6.12 (SAS Institute, Cary, NC). Odds ratios (ORs) were determined using unconditional multivariate logistic regression adjusting for type 2 diabetes risk factors, as indicated.

RESULTS — The *PPARγ* Pro/Pro homozygote, Pro/Ala heterozygote, and Ala/Ala homozygote genotype frequencies were 75.5% ($n = 582$), 23.0% (177), and 1.6% (12) among control subjects and 80.6% ($n = 312$), 18.6% (72), and 0.8% (3) among incident cases. Compared with Pro/Pro homozygotes, crude ORs were 0.76 (0.56–1.03) and 0.47 (0.13–1.67) for Pro/Ala heterozygotes and Ala/Ala homozygotes, respectively (*P* for trend = 0.04). Due to the low number of Ala/Ala individuals (15) and for consistency with published reports, Pro/Ala and Ala/Ala individuals were considered one group and compared with Pro/Pro individuals in all subsequent analyses. 12Ala *PPARγ* variant allele carriers did not differ appreciably from noncarriers with regard to the following diabetes risk factors: age, BMI, alcohol consumption, physical activity, and smoking (Table 1). *PPARγ* variant allele carriers had a reduced risk of type 2 diabetes with an unadjusted OR of 0.74 (0.55–1.00) (Table 2). Adjustment for age in addition to other type 2 diabetes risk factors (alcohol consumption, menopause status, BMI, physical activity, and smoking) did not substantially change the reduced diabetes risk associated with carrying the variant 12Ala *PPARγ* allele (Table 2).

Among control subjects, no association was detected between Pro12Ala polymorphism and plasma fasting insulin (mean value 12.0 and 11.3 μU/ml for Pro/Pro and 12Ala allele carriers, respectively, $P = 0.68$), C-peptide (mean value 0.63 and 0.56 pmol/ml for Pro/Pro and 12Ala allele carriers, respectively, $P = 0.30$) or proinsulin (mean value 12.1 and 10.5 fmol/ml for Pro/Pro and 12Ala allele carriers, respectively, $P = 0.31$).

Table 2—*OR for carriers of the variant Ala allele*

	Wild-type homozygotes	Variant allele carriers	*P*
Control subjects	582	189	—
Cases	312	75	—
Unadjusted*	1.0	0.74 (0.55–1.00)	0.05
Multivariate†	1.0	0.72 (0.52–0.99)	0.05

*Unconditional logistic regression with genotype as the only predictor; †Unconditional logistic regression adjusting for age, alcohol consumption, physical activity, smoking, and BMI.

CONCLUSIONS — The data presented here support an inverse association between 12Ala *PPARγ* allele and type 2 diabetes. In contrast to case-control studies that address the role of Pro12Ala *PPARγ* polymorphism, the current study is prospective. It has been argued that case-control studies, in general, are vulnerable to bias resulting from population stratification (7,8). In the current nested case-control study design, both incident cases and control subjects were chosen from the same largely Caucasian cohort assembled prospectively before disease incidence and thus control selection is less likely to be biased. The consistency observed between the current prospective study and previous reports suggests that population stratification did not appreciably bias the previous case-control studies. Although the present study shows a marginally significant association, when data from multiple association studies are considered collectively, the inverse association between the 12Ala variant *PPARγ* allele and type 2 diabetes is convincing. *PPARγ* Pro12Ala polymorphism is the most consistent genetic predictor of type 2 diabetes to date. Given the increasing incidence of type 2 diabetes, identification of genetically susceptible individuals may be particularly important for the success of early diagnosis, prevention, and intervention.

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