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

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.

From the 1Department of Epidemiology, Harvard School of Public Health, Boston, Massachusetts; the 2Department of Nutrition, Harvard School of Public Health, Boston, Massachusetts; the 3Channing Laboratory, Department of Medicine, Harvard Medical School and Brigham and Women’s Hospital, Boston, Massachusetts; the 4Division of Preventive Medicine, Department of Medicine, Harvard Medical School and Brigham and Women’s Hospital, Boston, Massachusetts; the 5Department of Genetics, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts; the 6Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts; and the 7Harvard School of Public Health, Center for Cancer Prevention, Boston, Massachusetts.

Address correspondence and reprint requests to Asli Memisoglu, SCD, Harvard School of Public Health, 677 Huntington Ave., Bldg. II Rm. 109, Boston, MA 02115. E-mail: amemisog@hsph.harvard.edu.

Received for publication 26 March 2003 and accepted in revised form 25 June 2003.

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

© 2003 by the American Diabetes Association.

© 2003 by the American Diabetes Association.

Original Article

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 cohort 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. Incident cases were diagnosed before 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. Incident cases were diagnosed before !996, 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. Incident cases were diagnosed before 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.
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 $PPAR\gamma$ 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 $PPAR\gamma$ 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). $PPAR\gamma$ 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 $PPAR\gamma$ 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 pmol/ml for Pro/Pro and 12Ala allele carriers, respectively, $P = 0.31$).

**CONCLUSIONS** — The data presented here support an inverse association between 12Ala $PPAR\gamma$ allele and type 2 diabetes. In contrast to case-control studies that address the role of Pro12Ala $PPAR\gamma$ 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 $PPAR\gamma$ allele and type 2 diabetes is convincing. $PPAR\gamma$ 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.

**Acknowledgments** — Supported by National Institute of Health grants CA49449, DK058845, and DK046519. A.M. was supported by a postdoctoral training grant from the National Cancer Institute (CA09001).

**References**

1. Yen CJ, Beamer BA, Negri C, Silver K, Brown KA, Yarnall DP, Burns DK, Roth J, Shuldiner AR: Molecular scanning of the human peroxisome proliferator activated receptor gamma (hPPAR gamma) gene in...