Brief Genetics Report

Pro12Ala of the Peroxisome Proliferator–Activated Receptor-γ2 Gene Is Associated With Lower Serum Insulin Levels in Nonobese African Americans

The Atherosclerosis Risk in Communities Study

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Recent research suggests that the Pro12Ala variant in peroxisome proliferator–activated receptor-γ2 (PPAR-γ2) is associated with diabetes- and obesity-related traits, and that its effects may be modified by obesity status. We characterized this variant in a population-based sample of 1,441 middle-aged African-American individuals with respect to diabetes-, obesity-, and other cardiovascular-related traits, both cross-sectionally and prospectively. The overall frequency of Ala12 was 1.9% (95% CI 1.5–2.5%), significantly lower than in Caucasian populations. Consistent with previous findings in Caucasians, African Americans with type 2 diabetes tended to be less likely to have the Pro/Ala genotype than those without (odds ratio [OR] 0.64, 95% CI 0.34–1.20); however, this OR was not statistically significant. Among nonobese individuals, the Pro/Ala genotype was associated with significantly lower ln(insulin) (P = 0.001), lower ln(HOMA-IR) (homeostasis model assessment of insulin resistance) (P = 0.002), higher fasting glucose-to-insulin ratio (P = 0.005), and lower diastolic blood pressure (P = 0.02). Among overweight individuals (BMI 25–29.9 kg/m^2), the Pro/Ala genotype was associated with greater BMI (P = 0.02), waist-to-hip ratio (P = 0.01), and waist circumference (P = 0.04). Among obese individuals, there was no association between any of the diabetes- or obesity-related traits and the Pro12Ala PPAR-γ2 variant. We conclude that among nonobese African Americans, the Pro/Ala genotype is associated with markers of greater insulin sensitivity. ***Diabetes*** **52:1568–1572, 2003**

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Received for publication 29 August 2002 and accepted in revised form 18 February 2003.

ARIC, Atherosclerosis Risk in Communities; HOMA-IR, homeostasis model assessment of insulin resistance; PPAR-γ2, peroxisome proliferator–activated receptor-γ2.

Peroxisome proliferator–activated receptor-γ2 (PPAR-γ2) is a transcription factor that is expressed predominantly in adipose tissues and is believed to have a critical role in adipogenesis and insulin action (1,2). A nonconservative substitution at codon 12 (Pro12Ala) was identified (3), and several association studies have been conducted to examine the associations between the Pro12Ala variant and adiposity or type 2 diabetes (4–25). The results have been mixed; one of the more consistent findings is the association of the Ala12 allele and insulin sensitivity (5,10,12,24) and protection from type 2 diabetes (16,26) while several studies have raised the possibility of an interaction among the Pro12Ala PPAR-γ2 variant, obesity, and insulin action (4,6,10). Little is known about this variant in African Americans. Since ethnicity-related genetic background may affect the phenotypic expression of a given susceptibility gene variant, we characterized this variant in a population-based sample of middle-aged African-American individuals with respect to diabetes-, obesity-, and other cardiovascular-related traits.

RESULTS

A total of 1,441 African-American Atherosclerosis Risk in Communities (ARIC) participants were included in the present study. The participants age was 53.4 ± 5.8 years (means ± SD), 40% were men, 86% were from Jackson, MS, and the mean BMI was 29.0 ± 5.8 kg/m^2.

Unlike in Caucasian populations, Ala12 of PPAR-γ2 is a relatively rare allele in this population. The overall frequency of the Ala allele in our African-American subjects was 1.9% (95% CI 1.5–2.5%). The genotype distribution was in accordance with Hardy-Weinberg expectation, and no Ala/Ala homozygotes were found.

Association between Pro12Ala PPAR-γ2 and type 2 diabetes. There were 269 individuals with type 2 diabetes at baseline and 167 individuals who developed type 2 diabetes during follow-up, leaving 1,005 diabetes-free individuals. The Pro12Ala variant was not significantly associated with diabetes at the P = 0.05 level (Table 1).

TABLE 1
Allele frequency and genotype distribution of Pro12Ala, by diabetes status, in 1,441 African-American ARIC participants

	PPAR-γ2 (Pro12Ala) genotype		Frequency of Ala (%)	*P*
	Pro/Pro	Pro/Ala		
No diabetes at baseline (n = 1,172)	1,123	49	2.1	
Type 2 diabetes at baseline (n = 269)	259	10	1.8	0.73*
OR (95% CI)†	1.00 (reference)	0.89 (0.44–1.77)		
No diabetes while in the study (n = 1,005)	959	46	2.3	
Type 2 diabetes during follow-up (n = 167)	164	3	0.9	0.14‡
OR (95% CI)†	1.00 (reference)	0.38 (0.12–1.24)		
No diabetes while in the study (n = 1,005)	959	46	2.3	
All type 2 diabetes (n = 436)	423	13	1.5	0.16*
OR (95% CI)†	1.00 (reference)	0.64 (0.34–1.20)		

*P value from two-sample Z-test for binomial proportions; †adjusted for age at visit 1 and sex; ‡P value from Fisher's exact test.

However, in all analyses, diabetes tended to be less frequent in participants with the Pro/Ala genotype. After adjusting for age and sex, the odds ratio (OR) for type 2 diabetes associated with the Pro/Ala genotype was 0.64 (95% CI 0.34–1.20). No significant interaction between genotype and sex or obesity on type 2 diabetes risk was detected.

Association between Pro12Ala PPAR-γ2 and selected cardiovascular risk factors. In 1,172 nondiabetic individuals, the Pro12Ala variant was not associated with either obesity- or diabetes-related traits at baseline; however, Pro/Ala genotype was associated with lower diastolic blood pressure (Table 2). After excluding 427 individuals who were taking blood pressure–lowering medications, the age- and sex-adjusted diastolic blood pressure was 78.2 mmHg for Pro/Pro and 73.1 mmHg for Pro/Ala groups (P = 0.02). Similarly, individuals with the Pro/Ala genotype had lower systolic blood pressure than those with the Pro/Pro genotype, but this difference was not statistically significant.

Interaction between Pro12Ala PPAR-γ2, markers for obesity, and insulin sensitivity. Table 3 summarizes the association between the Pro12Ala variant and selected traits by obesity status at visit 1 of the study. Among individuals with BMI <25 kg/m^2, those with the Pro/Ala genotype had higher mean BMI, waist-to-hip ratio, and waist circumference than those with the Pro/Pro genotype, but none of the associations were significant at the P = 0.05 level. Pro/Ala genotype was associated with significantly lower insulin (P = 0.001), lower homeostasis model assessment of insulin resistance (HOMA-IR) index (P = 0.002), and higher fasting glucose-to-insulin ratio (P = 0.005), all indicating greater insulin sensitivity in those with the Pro/Ala genotype. The results remained unchanged after further adjustment for BMI within each category. Treating BMI as a continuous trait, we examined a different interaction model of insulin sensitivity, Pro12Ala genotype, and obesity by modeling log(insulin) as the dependent variable and age, sex, BMI, and Pro12Ala genotype as the independent variables. We found that the association between insulin and BMI differs depending on the Pro12Ala genotype (P = 0.0027).

Among overweight individuals (BMI 25–29.9 kg/m^2), the Pro/Ala genotype was also associated with obesity measures (Table 3). Overweight individuals with the Pro/Ala genotype had greater BMI (P = 0.02), waist-to-hip ratio (P = 0.01), and waist circumference (P = 0.004) than overweight individuals with the Pro/Pro genotype. There was no association between the Pro12Ala variant and any of the markers for insulin resistance among overweight individuals. Among obese individuals (BMI ≥30 kg/m^2), there was no association between any of the diabetes- or obesity-related traits and the Pro12Ala variant.

Longitudinal analysis of Pro12Ala PPAR-γ2 and selected cardiovascular risk factors. Since the Pro12Ala variant was associated with insulin levels among the 329 individuals with BMI <25 kg/m^2 at visit 1, we next examined this association prospectively. Of these 329 individuals, 185 were followed to visit 4 and were diabetes-free at visit 4 (n = 177 for Pro/Pro and n = 8 for Pro/Ala). There were no significant differences in the rate of change in insulin or glucose measures between Pro/Pro and Pro/Ala groups (results not shown).

Since the Pro12Ala variant was associated with measures of obesity among the 451 overweight individuals at Visit 1, we also examined the association between Pro12Ala PPAR-γ2 and obesity measurements from follow-up visits in these individuals (Table 4). The Pro/Ala genotype was associated with increased BMI, waist-to-hip ratio, and waist circumference during the follow-up visits.

TABLE 2
Adjusted mean in 1,172 nondiabetic African-American ARIC participants by Pro12Ala genotype

Characteristic	PPAR-γ2 genotype		*P*
	Pro/Pro	Pro/Ala	
n	1123	49	
BMI (kg/m^2)	28.6	28.5	0.90
Waist-to-hip ratio	0.906	0.918	0.26
Waist circumference (cm)	96.3	97.3	0.64
Subscapular skinfold (mm)	29.7	29.5	0.91
Triceps skinfold (mm)	25.8	25.8	0.98
Glucose (mmol/l)	5.47	5.35	0.13
ln(insulin) (pmol/l)	4.30	4.27	0.73
Glucose/insulin (mg/dl ÷ uU/ml)	11.9	13.1	0.45
ln(HOMA-IR)	0.91	0.86	0.59
HDL cholesterol (mmol/l)	1.48	1.40	0.24
LDL cholesterol (mmol/l)	3.52	3.59	0.69
ln(triglycerides) (mmol/l)	0.02	0.12	0.09
Systolic blood pressure (mmHg)	127.0	122.9	0.16
Diastolic blood pressure (mmHg)	79.9	74.9	0.004

All analyses adjusted for age at visit 1 and sex.

TABLE 3
Associations between selected quantitative traits (mean) and Pro12Ala genotype in 1,172 nondiabetic African-American ARIC participants by obesity status

	BMI <25 kg/m^2			BMI 25–30 kg/m^2			BMI ≥30 kg/m^2		
	Pro/Pro	Pro/Ala	*P*	Pro/Pro	Pro/Ala	*P*	Pro/Pro	Pro/Ala	*P*
n	316	13		429	22		378	14	
BMI (kg/m^2)	22.5	23.2	0.18	27.3	28.0	0.02	35.0	34.2	0.50
Waist-to-hip ratio	0.867	0.874	0.70	0.906	0.940	0.01	0.939	0.925	0.42
Waist circumference (cm)	82.6	85.0	0.20	93.9	98.1	0.004	110.5	107.5	0.40
Glucose (mmol/l)*	5.29	5.20	0.57	5.49	5.27	0.06	5.61	5.50	0.49
ln(insulin)/(pmol/l)*	3.85	3.34	0.001	4.30	4.50	0.08	4.69	4.59	0.49
Glucose/insulin (mg/dl ÷ uU/ml)*	17.4	28.6	0.005	11.4	8.6	0.16	7.7	8.0	0.86
ln(HOMA-IR)*	0.42	−0.10	0.002	0.91	1.07	0.19	1.33	1.21	0.45
ln(triglycerides)/(mmol/l)*	−0.12	−0.10	0.87	0.03	0.22	0.05	0.11	0.14	0.81
DBP (mmHg)*	78.1	69.8	0.01	80.2	76.6	0.19	80.9	77.8	0.32

All analysis adjusted for age and sex. *Further adjusted for BMI at visit 1. DBP, diastolic blood pressure.

For any given visit of the study, Pro/Ala genotype was associated with higher BMI ($P = 0.03$), waist-to-hip ratio ($P = 0.01$), and waist circumference ($P = 0.006$). Although the mean values of these traits in the follow-up visits differed between genotypes, the rate of change (e.g., change in BMI per 3 years) in these obesity-related traits over follow-up did not differ significantly between genotypes.

DISCUSSION

The data lead to two main conclusions about the Pro12Ala PPAR-γ2 variant in African Americans. First, the Ala12 allele was associated with markers of greater insulin sensitivity in nonobese individuals. Second, the Ala12 allele tended to be associated with a lower prevalence of type 2 diabetes. Although the magnitude of this association was similar to those described by others (5,12,16, 16,26–28), it was not statistically significant due to the low frequency of the Ala allele in this population.

Limitations of our study include limited characterization of insulin resistance, possibility of false positive associations due to type 1 error, small sample size for analyses of qualitative traits given the low allele frequency of the Ala12 allele, and the lack of observations regarding homozygosity for this allele. Furthermore, the Pro12Ala variant may be in linkage disequilibrium with a nearby variant in PPAR-γ2 or a nearby gene. Nonetheless, the uniqueness of this understudied study population and the collection of longitudinal data add strength to this study.

TABLE 4
Analysis of selected quantitative traits from follow-up visits by PPAR-γ2 genotypes in 451 overweight African-American ARIC participants

	Adjusted difference (β) between Pro/Ala and Pro/Pro	*P*
BMI (kg/m^2)		
Mean	0.89	0.03
Change per visit	0.12	0.52
Waist-to-hip ratio		
Mean	0.030	0.01
Change per visit	−0.004	0.31
Waist circumference (cm)		
Mean	4.11	0.006
Change per visit	−0.05	0.94
ln(triglycerides)/(mmol/l)*		
Mean	0.17	0.06
Change per visit	−0.04	0.24

All analyses adjusted for age at visit 1 and sex. Mean represents the adjusted difference in a trait between Pro/Ala and Pro/Pro at any given visit of the study. Change per visit represents the adjusted difference in the rate of change (i.e., change in BMI per 3-year period) between Pro/Ala and Pro/Pro. *Limited to fasting individuals.

One of the most consistent associations of the Pro12Ala variant among studies is that of the Ala12 allele and increased insulin sensitivity. This has been shown in several nondiabetic populations (5,10,12,17,19,20,29). In addition, the Pro12Ala polymorphism is associated with increased insulin sensitivity of glucose disposal and suppression of lipolysis (30). In our study, the Pro/Ala genotype was associated with insulin sensitivity only among the nonobese African-American individuals. The lack of association between the Pro/Ala genotype and insulin sensitivity in obese individuals could be attributed to increased environmental influences, additional unknown genetic influences in this group, or the confounding influences of obesity itself (25).

While some studies have observed either no significant association between type 2 diabetes and this variant (7,8,11) or increased diabetes risk with the Ala12 allele (21), we observed a decrease in the prevalence of diabetes among African Americans with the Pro/Ala genotype. The magnitude of this association was similar to those found in Japanese (5,12,27), Scandinavian (16,28), U.S., and Polish populations (26). In addition, a study that pooled previous smaller studies of this association showed that the Pro/Ala genotype is associated with decreased diabetes prevalence (16). Protection from diabetes is consistent with our findings and those of others showing increased insulin sensitivity in subjects with the Pro/Ala genotype.

Positive (4,11,14,20), null (8,9,12,13,15), and negative associations (5) between the Pro/Ala genotype and obesity measures have also been reported in various populations. A possible explanation for the inconsistencies was suggested by the study of Ek et al. (6), in which the Ala12 allele was associated with lower BMI in nonobese subjects and higher BMI in obese subjects, and by Luan et al. (31), in which interaction between the Ala12 allele and dietary fat intake on insulin sensitivity and BMI was observed.

These interactions are not unlikely in light of the highly codependent relationship between PPAR-γ2 and adipocytes, as well as the fact that some dietary fats are ligands for PPAR-γ2.

In summary, results from our study indicate significant association between the Pro/Ala genotype of PPAR-γ2 and markers of insulin sensitivity and perhaps also protection from the development of type 2 diabetes among nonobese African-American individuals. It is clear that the physiologic roles of PPAR-γ2 are numerous and that complex mechanisms exist for the associations between PPAR-γ2 and increased adiposity and between PPAR-γ2 and increased insulin sensitivity. Further studies are warranted to investigate the cellular and molecular mechanisms underlying these associations.

RESEARCH DESIGN AND METHODS

Setting and study participants. Subjects of the present analyses were selected from the 4,266 African-American participants of ARIC study. The ARIC study is a prospective epidemiologic study that examines clinical and subclinical atherosclerotic disease in a cohort of 15,792 persons, aged 45–64 years at baseline examination, selected by probability sampling from four U.S. communities: Forsyth County, NC (12% African American); Jackson, MS (100% African American); the northwest suburbs of Minneapolis, MN (<1% African American); and Washington County, MD (<1% African American). The sampling procedure and methods used in ARIC have been described in detail elsewhere (32). The present analyses included four clinic visits that were scheduled 3 years apart (visit 1–4). Classification of ethnicity was based on self-report. African-American individuals were excluded from the sampling frame of the present analyses if they had missing demographic, clinical, dietary, or laboratory data at baseline (n = 998), resulting in a sampling frame of 3,268 individuals (2,006 women and 1,262 men). Using sex-stratified simple random sampling, 1,441 individuals were selected for the present analyses (sampling fraction = 44%).

Diabetes was defined as the presence of any one of the following at visits 1–4 of the study: *1*) fasting glucose ≥7.0 mmol/l (126 mg/dl), *2*) nonfasting glucose ≥11.1 mmol/l (200 mg/dl), *3*) current use of diabetic medication, or *4*) a positive response to the question "Has a doctor ever told you that you had diabetes (sugar in the blood)?"

Exposure assessment. Baseline examination included home interviews, clinic examinations, and clinic questionnaires. Information on age, sex, race, family history of diabetes, and education were obtained from the home and clinic interviews conducted at the baseline visit. Anthropometry was performed at the baseline clinic visit, and each of the three subsequent visits, with the participants wearing light-weight, nonconstricting underwear and no shoes. Waist and hip girths were measured using an anthropometric tape applied at the level of umbilicus and of the maximal protrusion of the gluteal muscles, respectively. The reliability of girth measurements were high. Physical activity during leisure time was assessed by an index (1 = least active and 5 = most active) derived from a modified interviewer-administered version of the questionnaire developed by Baecke et al. (33) measuring usual activities. Dietary intake was also assessed by an interviewer-administered, modified version of the 61-item food frequency questionnaire developed by Willett et al. (34) from which total energy intake was calculated.

Serum glucose was assessed by a modified hexokinase/glucose-6-phosphate dehydrogenase procedure, a Centers for Disease Control and Prevention national glucose reference method. Standard radioimmunoassay was used to determine serum insulin (^{125}I Insulin [732] kit; Cambridge Medical Diagnostics, Billerica, MA). The formula for the HOMA model was as follow: HOMA-IR = [fasting insulin (uU/ml) × fasting glucose(mmol/l)/22.5].

PCR amplification of a 270-bp fragment containing the Pro12Ala variant of PPAR-γ2 was performed using standard PCR protocol and Ready-To-Go PCR bead (Pharmacia Biotech, Uppsala, Sweden) with 1.5 mmol/l magnesium. The PCR product was resolved on a 2% Nusieve:1% agarose gel with ethidium bromide in Tris-borate EDTA buffer (88 mmol/l Tris, 88 mmol/l boric acid, and 2 mmol/l EDTA).

A subset of 75 duplicate samples was genotyped for quality control of the assays, and the κ statistic was 1.0 for each gene variant. Interreader reliability of the scoring of the samples was assessed by having another member of the lab, who was blinded to the phenotypes being studied, independently score a random sample for both gene variants. The interreader κ statistic was 1.0 for the Pro12Ala PPAR-γ2 variant (n = 96).

Statistical analysis. Means ± SD and frequencies of baseline characteristics were calculated. Since fasting serum insulin and triglyceride levels and HOMA-IR indices were not normally distributed, they were *ln*-transformed for analysis, but geometric means were presented in the results tables. Allele frequencies were calculated, and two-sample test for binomial proportions was used to assess differences in allele frequencies between prevalent diabetic and nondiabetic individuals and between all diabetic and nondiabetic individuals. Fisher's exact test was used to assess difference in allele frequencies between incident diabetic and nondiabetic individuals due to the small number of diabetic individuals with Pro/Ala genotype. χ^2 goodness-of-fit test was used to assess deviation from Hardy-Weinberg equilibrium of the genotypic frequency by calculating expected frequencies of genotypes. Multiple logistic regression was used to adjust for age, sex, and other potential confounders, and ORs (with 95% Cis) for diabetes were reported. Individuals were classified as nonobese, overweight, or obese according to their BMI at baseline using the following cutoff values: BMI <25 kg/m^2 was nonobese, BMI 25–30 was overweight, and BMI ≥30 was obese. Gene-by-environment interactions among obesity-related traits, environmental exposures (physical activity, total fat intake, total polyunsaturated fatty acid, or total energy intake), and Pro12Ala genotypes were assessed by regressing the obesity-related trait (e.g., BMI) on the environmental exposure (e.g., physical activity index, Pro12Ala genotypes, and an interaction term that allowed for genotype-specific slope for the association between the environmental variable and the obesity-related outcome).

Since obesity-related quantitative traits were measured at each of the four visits of the study, a random effects model was fitted to assess the association between genotype and changes in obesity-related quantitative traits from visit 1 to 4 of the study. Analyses of longitudinal data were performed using Stata statistical package (College Station, TX), and all other statistical analyses were performed using the SAS statistical package (Cary, NC).

ACKNOWLEDGMENTS

This research was supported by National Institutes of Health (NIH) grant 1R01DK53959-01. F.L.B. was supported by a Established Investigator Grant from the American Heart Association, Dallas, TX, and by NIH grant 1K24-DK6222-01. W.H.L.K. was supported by NIH training grant T32HL07024-23. A.R.S. was supported by NIH grant K24DK02673 and the Baltimore Veterans Administration Geriatrics Research and Education Center. The ARIC Study is carried out as a collaborative study supported by contracts N01-HC-55015, N01-HC-55016, N01-HC-55018, N01-HC-55019, N01-HC-55020, N01-HC-55021, and N01-HC-55022 from the National Heart, Lung, and Blood Institute.

The authors thank the staff and participants in the ARIC study for their important contributions.

REFERENCES

1. Beamer BA, Negri C, Yen CJ, Gavrilova O, Rumberger JM, Durcan MJ, Yarnall DP, Hawkins AL, Griffin CA, Burns DK, Roth J, Reitman M, Shuldiner AR: Chromosomal localization and partial genomic structure of the human peroxisome proliferator activated receptor-gamma (hPPAR gamma) gene. *Biochem Biophys Res Commun* 233:756–759, 1997
2. Spiegelman BM, Hu E, Kim JB, Brun R: PPAR gamma and the control of adipogenesis. *Biochimie* 79:111–112, 1997
3. 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 diabetic Caucasians: identification of a Pro12Ala PPAR gamma 2 missense mutation. *Biochem Biophys Res Commun* 241:270–274, 1997
4. Beamer BA, Yen CJ, Andersen RE, Muller D, Elahi D, Cheskin LJ, Andres R, Roth J, Shuldiner AR: Association of the Pro12Ala variant in the peroxisome proliferator–activated receptor-γ2 gene with obesity in two Caucasian populations. *Diabetes* 47:1806–1808, 1998
5. Deeb SS, Fajas L, Nemoto M, Pihlajamaki J, Mykkanen L, Kuusisto J, Laakso M, Fujimoto W, Auwerx J: A Pro12Ala substitution in PPAR-gamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. *Nat Genet* 20:284–287, 1998
6. Ek J, Urhammer SA, Sorensen TI, Andersen T, Auwerx J, Pedersen O: Homozygosity of the Pro12Ala variant of the peroxisome proliferation-activated receptor-gamma2 (PPAR-gamma2): divergent modulating effects

on body mass index in obese and lean Caucasian men. *Diabetologia* 42:892–895, 1999
7. Ringel J, Engeli S, Distler A, Sharma AM: Pro12Ala missense mutation of the peroxisome proliferator activated receptor gamma and diabetes mellitus. *Biochem Biophys Res Commun* 254:450–453, 1999
8. Mancini FP, Vaccaro O, Sabatino L, Tufano A, Rivellese AA, Riccardi G, Colantuoni V: Pro12Ala substitution in the peroxisome proliferator–activated receptor-γ2 is not associated with type 2 diabetes. *Diabetes* 48:1466–1468, 1999
9. Mori Y, Kim-Motoyama H, Katakura T, Yasuda K, Kadowaki H, Beamer BA, Shuldiner AR, Akanuma Y, Yazaki Y, Kadowaki T: Effect of the Pro12Ala variant of the human peroxisome proliferator-activated receptor gamma 2 gene on adiposity, fat distribution, and insulin sensitivity in Japanese men *Biochem Biophys Res Commun* 251:195–198, 1998
10. Koch M, Rett K, Maerker E, Volk A, Haist K, Deninger M, Renn W, Haring HU: The PPARgamma2 amino acid polymorphism Pro 12 Ala is prevalent in offspring of type II diabetic patients and is associated to increased insulin sensitivity in a subgroup of obese subjects. *Diabetologia* 42:758–762, 1999
11. Meirhaeghe A, Fąjas L, Helbecque N, Cottel D, Auwerx J, Deeb SS, Amouyel P: Impact of the peroxisome proliferator activated receptor gamma2 Pro12Ala polymorphism on adiposity, lipids and non-insulin-dependent diabetes mellitus. *Int J Obes Relat Metab Disord* 24:195–199, 2000
12. Hara K, Okada T, Tobe K, Yasuda K, Mori Y, Kadowaki H, Hagura R, Akanuma Y, Kimura S, Ito C, Kadowaki T: The Pro12Ala polymorphism in PPAR gamma2 may confer resistance to type 2 diabetes. *Biochem Biophys Res Commun* 271:212–216, 2000
13. Clement K, Hercberg S, Passinge B, Galan P, Varroud-Vial M, Shuldiner AR, Beamer BA, Charpentier G, Guy-Grand B, Froguel P, Vaisse C: The Pro115Gln and Pro12Ala PPAR gamma gene mutations in obesity and type 2 diabetes. *Int J Obes Relat Metab Disord* 24:391–393, 2000
14. Cole SA, Mitchell BD, Hsueh WC, Pineda P, Beamer BA, Shuldiner AR, Comuzzie AG, Blangero J, Hixson JE: The Pro12Ala variant of peroxisome proliferator-activated receptor-gamma2 (PPAR-gamma2) is associated with measures of obesity in Mexican Americans. *Int J Obes Relat Metab Disord* 24:522–524, 2000
15. Evans D, Mann WA, Heer J, Michel U, Wendt D, Kortner B, Wolf A, Beisiegel U: Variation in the gene for human peroxisome proliferator activated receptor gamma (PPARgamma) does not play a major role in the development of morbid obesity. *Int J Obes Relat Metab Disord* 24:647–651, 2000
16. Altshuler D, Hirschhorn JN, Klannemark M, Lindgren CM, Vohl MC, Nemesh J, Lane CR, Schaffner SF, Bolk S, Brewer C, Tuomi T, Gaudet D, Hudson TJ, Daly M, Groop L, Lander ES: The common PPARgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. *Nat Genet* 26:76–80, 2000
17. Eriksson JG, Lindi V, Uusitupa M, Forsen TJ, Laakso M, Osmond C, Barker DJ: The effects of the Pro12Ala polymorphism of the peroxisome proliferator–activated receptor-γ2 gene on insulin sensitivity and insulin metabolism interact with size at birth. *Diabetes* 51:2321–2324, 2002
18. Chuang LM, Hsiung CA, Chen YD, Ho LT, Sheu WH, Pei D, Nakatsuka CH, Cox D, Pratt RE, Lei HH, Tai TY: Sibling-based association study of the PPARgamma2 Pro12Ala polymorphism and metabolic variables in Chinese and Japanese hypertension families: a SAPPHIRe study: Stanford Asian-Pacific Program in Hypertension and Insulin Resistance. *J Mol Med* 79:656–664, 2001
19. Ek J, Andersen G, Urhammer SA, Hansen L, Carstensen B, Borch-Johnsen K, Drivsholm T, Berglund L, Hansen T, Lithell H, Pedersen O: Studies of the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor-gamma2 (PPAR-gamma2) gene in relation to insulin sensitivity among glucose tolerant caucasians. *Diabetologia* 44:1170–1176, 2001
20. Lindi V, Sivenius K, Niskanen L, Laakso M, Uusitupa MI: Effect of the Pro12Ala polymorphism of the PPAR-gamma2 gene on long-term weight change in Finnish non-diabetic subjects. *Diabetologia* 44:925–926, 2001
21. Lindi VI, Uusitupa MI, Lindstrom J, Louheranta A, Eriksson JG, Valle TT, Hamalainen H, Ilanne-Parikka P, Keinanen-Kiukaanniemi S, Laakso M, Tuomilehto J: Association of the Pro12Ala polymorphism in the PPAR-γ2 gene with 3-year incidence of type 2 diabetes and body weight change in the Finnish Diabetes Prevention Study. *Diabetes* 51:2581–2586, 2002
22. Stumvoll M, Haring H: The peroxisome proliferator–activated receptor-γ2 Pro12Ala polymorphism. *Diabetes* 51:2341–2347, 2002
23. Celi FS, Shuldiner AR: The role of peroxisome proliferator–activated receptor gamma in diabetes and obesity. *Curr Diab Rep* 2:179–185, 2002
24. Stumvoll M, Wahl HG, Loblein K, Hauer B, Grauer P, Becker R, Rett K, Machicao F, Jacob S, Haring H: The Pro12Ala polymorphism in the peroxisome proliferator activated receptor γ2 gene is associated with increased antilipolytic insulin sensitivity in lean, healthy subjects (Abstract). *Diabetes* 49 (Suppl. 1):A7, 2000
25. Nicklas BJ, van Rossum EF, Berman DM, Ryan AS, Dennis KE, Shuldiner AR: Genetic variation in the peroxisome proliferator–activated receptor-γ2 gene (Pro12Ala) affects metabolic responses to weight loss and subsequent weight regain. *Diabetes* 50:2172–2176, 2001
26. Ardlie KG, Lunetta KL, Seielstad M: Testing for population subdivision and association in four case-control studies. *Am J Hum Genet* 71:304–311, 2002
27. Mori H, Ikegami H, Kawaguchi Y, Seino S, Yokoi N, Takeda J, Inoue I, Seino Y, Yasuda K, Hanafusa T, Yamagata K, Awata T, Kadowaki T, Hara K, Yamada N, Gotoda T, Iwasaki N, Iwamoto Y, Sanke T, Nanjo K, Oka Y, Matsutani A, Maeda E, Kasuga M: The Pro12→Ala substitution in PPAR-γ is associated with resistance to development of diabetes in the general population: possible involvement in impairment of insulin secretion in individuals with type 2 diabetes. *Diabetes* 50:891–894, 2001
28. Douglas JA, Erdos MR, Watanabe RM, Braun A, Johnston CL, Oeth P, Mohlke KL, Valle TT, Ehnholm C, Buchanan TA, Bergman RN, Collins FS, Boehnke M, Tuomilehto J: The peroxisome proliferator–activated receptor-γ2 Pro12A1a variant: association with type 2 diabetes and trait differences. *Diabetes* 50:886–890, 2001
29. Ceriello A, Falleti E, Motz E, Taboga C, Tonutti L, Ezsol Z, Gonano F, Bartoli E: Hyperglycemia-induced circulating ICAM-1 increase in diabetes mellitus: the possible role of oxidative stress. *Horm Metab Res* 30:146–149, 1998
30. Stumvoll M, Wahl HG, Loblein K, Becker R, Machicao F, Jacob S, Haring H: Pro12Ala polymorphism in the peroxisome proliferator–activated receptor-γ2 gene is associated with increased antilipolytic insulin sensitivity. *Diabetes* 50:876–881, 2001
31. Luan J, Browne PO, Harding AH, Halsall DJ, O'Rahilly S, Chatterjee VK, Wareham NJ: Evidence for gene-nutrient interaction at the PPARγ locus. *Diabetes* 50:686–689, 2001
32. ARIC Investigators: The Atherosclerosis Risk in Communities (ARIC) Study: design and objectives. *Am J Epidemiol* 129:687–702, 1989
33. Baecke JA, Burema J, Frijters JE: A short questionnaire for the measurement of habitual physical activity in epidemiologial studies. *Am J Clin Nutr* 36:936–942, 1982
34. Willett WC, Stampfer MJ, Rosher B, Bain C, Witchi J, Hennekens CH, Speizer FE: Reproducibility and validity of a semiquantitative food frequency questionnaire. *Am J Epidemiol* 122:51–65, 1985