Brief Genetics Report

The Peroxisome Poliferator–Activated Receptor-γ2 Pro12Ala Variant

Association With Type 2 Diabetes and Trait Differences

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Recent studies have identified a common proline-to-alanine substitution (Pro12Ala) in the peroxisome proliferator–activated receptor-γ2 (PPAR-γ2), a nuclear receptor that regulates adipocyte differentiation and possibly insulin sensitivity. The Pro12Ala variant has been associated in some studies with diabetes-related traits and/or protection against type 2 diabetes. We examined this variant in 935 Finnish subjects, including 522 subjects with type 2 diabetes, 193 nondiabetic spouses, and 220 elderly nondiabetic control subjects. The frequency of the Pro12Ala variant was significantly lower in diabetic subjects than in nondiabetic subjects (0.15 vs. 0.21; $P = 0.001$). We also compared diabetes-related traits between subjects with and without the Pro12Ala variant within subgroups. Among diabetic subjects, the variant was associated with greater weight gain after age 20 years ($P = 0.023$) and lower triglyceride levels ($P = 0.033$). Diastolic blood pressure was higher in grossly obese (BMI >40 kg/m^2) diabetic subjects with the variant. In nondiabetic spouses, the variant was associated with higher fasting insulin ($P = 0.033$), systolic blood pressure ($P = 0.021$), and diastolic blood pressure ($P = 0.045$). These findings support a role for the PPAR-γ2 Pro12Ala variant in the etiology of type 2 diabetes and the insulin resistance syndrome. *Diabetes* 50:886–890, 2001

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Additional information can be found in an online appendix at www.diabetes.org/diabetes/appendix.asp.

AIR_G, acute insulin response to glucose; dBP, diastolic blood pressure; DI, disposition index; FUSION, Finland–United States Investigation of Non–Insulin-Dependent Diabetes Mellitus Genetics; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; OGTT, oral glucose tolerance test; PPAR, peroxisome proliferator–activated receptor; PROBE, primer oligo base extension reaction; sBP, systolic blood pressure; S_I, insulin sensitivity.

Peroxisome proliferator–activated receptors (PPARs) are members of the nuclear hormone receptor family of transcription factors and are involved in adipocyte differentiation and gene expression. They are also believed to play an important role in type 2 diabetes and diabetes-related traits, including insulin sensitivity and lipid and energy metabolism (1). In fact, studies have shown that ligands for PPAR-γ, including both endogenous ones and those that are synthetic (e.g., thiazolidinedione drugs), stimulate adipogenesis and increase insulin action (2). A common proline-to-alanine substitution at codon 12 (Pro12Ala) of exon B has been inconsistently associated with protection against type 2 diabetes and diabetes-related traits (3–14). These findings encouraged us to investigate the role of PPAR-γ2 in our sample from the Finnish population. The objective of our study was to examine whether the Pro12Ala variant was associated with type 2 diabetes and to examine the relationship between the Pro12Ala variant and diabetes-related traits among subgroups of diabetic and nondiabetic subjects.

The mean and standard deviation of selected trait values are given in Table 1 by subgroup. The entire sample of 935 subjects consisted of 636 Pro/Pro subjects, 271 Pro/Ala

TABLE 1
Characteristics of the subjects by clinical subgroup

	Diabetic	Spousal control	Elderly control
n	522	193	220
Sex (M:F)	288:234	62:131	106:114
Age at enrollment (years)	63.5 ± 7.5	61.4 ± 7.7	70.0 ± 0.3
Age at diagnosis (years)	50.0 ± 7.9	—	—
Diabetes duration (years)	13.6 ± 7.0	—	—
BMI (kg/m^2)	30.0 ± 4.8	28.4 ± 4.5	27.0 ± 4.0
Waist-to-hip ratio	0.94 ± 0.08	0.88 ± 0.08	0.88 ± 0.08
Fasting plasma glucose (mmol/l)	10.7 ± 3.4	5.2 ± 0.7	5.0 ± 0.5
Fasting serum insulin (pmol/l)	114.1 ± 71.4	75.6 ± 48.8	66.2 ± 34.8

Data are means ± SD.

TABLE 2
Frequency of the PPAR-γ2 Pro12Ala variant by clinical subgroup

	n	Ala frequency	χ^{2}*	*P*
Diabetic subjects	522	0.15	—	—
Spousal control subjects	193	0.19	3.30	0.069
Elderly control subjects	220	0.22	11.72	<0.0007

*Compared with diabetic subjects.

subjects, and 28 Ala/Ala subjects. The allele frequency of the Pro12Ala variant in the PPAR-γ2 gene was 0.15 among diabetic subjects, 0.19 among spousal control subjects, and 0.22 among elderly control subjects (Table 2), which mirrors the continuum of diabetes susceptibility across these subgroups. The frequency of the variant was significantly lower in diabetic subjects than in elderly control subjects ($\chi^2 = 11.72$, df = 1, $P < 0.0007$) and marginally lower than in spousal control subjects ($P = 0.069$). Comparison with combined spousal and elderly control subjects gave a significant association result ($\chi^2 = 10.60$, df = 1, $P = 0.001$). A second independent sample of 263 Finnish diabetic subjects in our study subsequently confirmed the variant frequency of 0.15 in the original 522 diabetic subjects (data not shown). The observed genotype data were consistent with Hardy-Weinberg equilibrium.

Results for the quantitative traits were less compelling. Genotype-specific means for all traits for diabetic subjects, elderly control subjects, and spousal control subjects, respectively, are available in an online appendix (Tables A1–3) at www.diabetes.org/diabetes/appendix.asp. Table 3 shows the significant trait differences between subjects with and without the Pro12Ala variant by subgroup. In diabetic subjects, the presence of the variant was associated with greater weight change after 20 years of age (22.2 ± 14.0 vs. 19.5 ± 13.0 kg) and lower serum triglyceride levels (2.29 ± 1.65 vs. 2.68 ± 2.21 mmol/l). Both results were significant after adjustment for sex, age, and (for triglyceride levels) BMI ($P = 0.023$ and 0.033, respectively). There was a significant interaction ($P = 0.038$) between the variant and BMI for diastolic blood pressure (dBP); the variant was associated with higher dBP only among grossly obese diabetic subjects (Table 4). A similar trend was also observed for systolic blood pressure (sBP), although the interaction was not statistically significant ($P = 0.299$).

TABLE 4
dBP and sBP in diabetic subjects by presence/absence of the PPAR-γ2 Pro12Ala variant and BMI

	Pro/Pro	Pro/Ala or Ala/Ala
dBP* (mmHg)		
BMI ≤20	78.0 ± 7.5 (3)	82.5 ± 4.9 (2)
20 < BMI ≤ 25	82.9 ± 9.8 (39)	79.5 ± 10.7 (21)
25 < BMI ≤ 30	83.5 ± 10.5 (167)	83.8 ± 11.0 (47)
30 < BMI ≤ 35	85.5 ± 9.6 (109)	84.5 ± 10.8 (47)
35 < BMI ≤ 40	87.2 ± 10.7 (34)	90.7 ± 11.6 (16)
BMI > 40	86.6 ± 9.9 (14)	94.4 ± 7.1 (7)
sBP* (mmHg)		
BMI ≤ 20	157.3 ± 44.2 (3)	148.0 ± 18.4 (2)
20 < BMI ≤ 25	148.5 ± 23.5 (39)	145.4 ± 22.8 (21)
25 < BMI ≤ 30	150.9 ± 21.9 (167)	155.1 ± 21.7 (47)
30 < BMI ≤ 35	151.3 ± 20.6 (109)	155.8 ± 22.5 (47)
35 < BMI ≤ 40	151.0 ± 21.3 (34)	156.3 ± 24.5 (16)
BMI > 40	149.0 ± 19.8 (14)	166.9 ± 25.8 (7)

Data are means ± SD (*n*). *Mean of two measurements.

Among elderly control subjects, only maximum lifetime weight was significantly associated with the Pro12Ala variant ($P = 0.049$), and it was no longer significant after adjustment for sex ($P = 0.191$) (Table 3). Among nondiabetic spousal control subjects, the variant was significantly associated with higher sBP (151.4 ± 25.5 vs. 142.5 ± 18.9 mm Hg) ($P = 0.014$). When both sBP and dBP and fasting serum insulin were adjusted for sex, age, and BMI, differences between spousal control subjects with and without the Pro12Ala variant remained and/or became significant ($P = 0.021$, 0.045, and 0.033, respectively) (Table 3). All three traits were significantly higher among subjects with the variant.

In our analysis, we found a significantly lower frequency of the Pro12Ala variant of the PPAR-γ2 gene in diabetic subjects than in nondiabetic subjects. The directionality of these highly significant ($P = 0.001$) findings is consistent with results from the studies of Deeb et al. (3), Mancini et al. (4), and Altshuler et al. (14), although the difference in allele frequencies in the second study failed to reach statistical significance. Coupled with these studies and the biological importance of PPARγ, these findings suggest a

TABLE 3
Significant results by clinical subgroup and presence/absence of the PPAR-γ2 Pro12Ala variant

	Pro/Pro	Pro/Ala or Ala/Ala	*P*	
			Unadjusted analysis	Adjusted analysis
Diabetic subjects				
Weight change* (kg)	19.5 ± 13.0 (362)	22.2 ± 14.0 (143)	0.052	0.023
Triglycerides (mmol/l)	2.68 ± 2.21 (373)	2.29 ± 1.65 (143)	0.047	0.033
Elderly control subjects				
Maximum lifetime weight (kg)	76.8 ± 11.6 (134)	80.7 ± 15.7 (84)	0.049	0.191
Spousal control subjects				
Fasting serum insulin (pmol/l)	70.6 ± 35.6 (123)	84.6 ± 18.9 (68)	0.083	0.033
sBP† (mmHg)	142.5 ± 18.9 (122)	151.4 ± 25.5 (65)	0.014	0.021
dBP† (mmHg)	84.0 ± 10.0 (122)	86.9 ± 11.9 (65)	0.090	0.045

Data are means ± SD (*n*) unless otherwise indicated. Adjusted analysis *P* value includes adjustment for sex, age, and (except for weight-related traits). BMI; *P* values are not adjusted for multiple comparisons. *Current weight minus weight at age 20 years; †mean of two measurements.

link between the Pro12Ala variant of the PPAR-γ2 gene and the pathogenesis of type 2 diabetes. The increased frequency of the variant in nondiabetic subjects would seem to suggest that the Pro12Ala variant confers some protective effect against diabetes.

Despite the increased frequency of the Pro12Ala variant among elderly control subjects, we failed to find any significant trait associations within this subgroup. Instead, we observed weak but significant associations between the Pro12Ala variant and traits characteristic of the insulin resistance syndrome in both diabetic and nondiabetic subjects. For example, greater weight gain was associated with the Pro12Ala variant in diabetic subjects, whereas higher fasting insulin, sBP, and dBP were associated with the variant in nondiabetic spouses. It should be emphasized that, in contrast to the spousal control subjects, the elderly control subjects represent a quite distinct subgroup of nondiabetic subjects who are unlikely to ever develop type 2 diabetes. As such, they are unlikely to carry the cluster of susceptibility genes that may interact with variants in PPAR-γ2 to result in the insulin resistance syndrome phenotype. The spousal control subjects are somewhat younger and remain at risk for developing type 2 diabetes during their lifetime.

Alterations in functional characteristics of the PPAR-γ2 gene induced by the Ala isoform may be partly responsible for the manifestation of some characteristics of the insulin resistance syndrome. Deeb et al. (3) identified lowered transactivation capacity and reduced stimulation of PPAR-γ target genes as a potential molecular mechanism underlying the association of the Pro12Ala variant with lower BMI and increased insulin sensitivity, a hypothesis consistent with their observations in Finnish subjects. Although this hypothesis may appear to be at odds with (or at least not supported by) our trait findings, several points should be clarified. First, the middle-aged subjects in the study by Deeb et al. (3) were much younger and leaner than our nondiabetic spouses and elderly control subjects. Second, although the elderly subjects from both studies were better matched, we could not parallel their genotype-based analysis because of insufficient numbers of Pro12Ala homozygotes. If the Pro/Ala and Ala/Ala subjects from their study had been pooled, it is unlikely that they would have observed significant trait differences because trends within their elderly subjects were inconsistent (e.g., fasting insulin was highest for Pro/Ala heterozygotes).

Consistent with at least one report of a differential effect of the PPAR-γ2 Pro12Ala variant in the lean and obese states (12), we also found an interaction between BMI and the variant for dBP in the diabetic subjects. Among severely obese subjects, those with the Pro12Ala variant had substantially higher blood pressure. Higher values for sBP and dBP were also associated with the variant among nondiabetic spousal control subjects, though there was no evidence for an interaction between BMI and the Pro12Ala variant. These associations are of interest, given the recent report by Barroso et al. (13) of three type 2 diabetic subjects with early-onset hypertension and polymorphisms in the PPAR-γ2 gene, suggesting that this receptor is important in both blood pressure and glucose homeostasis.

In summary, we found that the Pro12Ala variant of the PPAR-γ2 gene was associated with protection against type 2 diabetes in Finnish subjects, a finding consistent with several reports in the literature (3,4,14). Because we only screened for this particular variant, we cannot exclude the role of other PPAR-γ2 variants or variants in nearby genes, possibly in linkage disequilibrium with the Pro12Ala variant. Further studies, including functional analyses, will be required to fully understand the role of this gene in type 2 diabetes. Our data suggest that the PPAR-γ2 Pro12Ala variant has variable effects among subgroups of individuals with different levels of diabetes risk.

RESEARCH DESIGN AND METHODS

The Finland–United States Investigation of Non–Insulin-Dependent Diabetes Mellitus Genetics (FUSION) Study is an international collaborative effort to map and clone genes predisposing to type 2 diabetes and related traits in Finnish subjects. The FUSION study design and family material have been described previously (15). For the present investigation, our sample included 522 unrelated subjects with type 2 diabetes, 193 nondiabetic spouses of a diabetic subject or his/her affected sibling, and 220 unrelated elderly nondiabetic control subjects. Diabetes was diagnosed by World Health Organization (16) criteria. Spouses had a single normal oral glucose tolerance test (OGTT). Elderly control subjects had normal glucose tolerance at ages 65 and 70 years.

A total of 14 traits were analyzed on all subjects: BMI, waist circumference, waist-to-hip ratio, current weight, maximum lifetime weight, fasting plasma glucose, fasting serum insulin, total cholesterol, HDL cholesterol, HDL ratio (HDL cholesterol/total cholesterol), LDL cholesterol, triglycerides, sBP, and dBP. Values for sBP and dBP were each determined as the mean of two measurements. Seven additional traits were ascertained on diabetic subjects: weight at 20 years of age, change in weight after 20 years of age, maximum lifetime weight change after 20 years of age, age at diagnosis of diabetes, diabetes duration, age at which insulin treatment started (if applicable), and fasting plasma C-peptide concentrations. In addition, glucose and insulin concentrations 2 h after OGTT were analyzed in nondiabetic subjects, whereas the insulin sensitivity index (S_I), the glucose effectiveness index, the acute insulin response to glucose (AIR_G), and the disposition index (DI) were analyzed ($DI = S_I \times AIR_G$) only in the nondiabetic spouses; the latter analyses used tolbutamide-modified frequently sampled intravenous glucose tolerance tests and minimal model analysis (17). Glucose, insulin, C-peptide, and lipid concentrations were assayed using standard methods (15).

Genotyping by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. The PPAR-γ2 Pro12Ala variant was analyzed by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. A 69-bp fragment containing the Pro12Ala variant site was amplified by polymerase chain reaction from 20 ng genomic DNA using 25 pmol forward primer 5′-GCTGTTATGGGTGAAACTCTG, 2 pmol of a universal sequence-tailed reverse primer 5′-AGCGGATAACAATTTCACACAGGCAGTGTATCAGTGAAGGAATCG, and 10 pmol of a biotinylated universal primer 5′-biotin-AGCGGATAACAATTTCACACAGG under standard reaction conditions (Fig. 1*A*). After 15 min of denaturation at 95°C, 55 cycles (5 s at 95°C, 20 s at 53°C, and 30 s at 72°C) were performed. To recover the single-stranded DNA template, the product was immobilized on streptavidin-coated magnetic beads (Dynal, Great Neck, NY), washed with 10 mmol/l Tris-HCl at pH 8.0, denatured in 50 μl 0.1 mol/l NaOH, and washed again with 10 mmol/l Tris-HCl.

The primer oligo base extension reaction (PROBE) was performed by the addition of 20 pmol extension primer 5′-TCTGGGAGATTCTCCTATTGAC under conditions similar to those previously described (18). The extension reaction products were applied to a SpectroChip (Sequenom, San Diego, CA) prespotted with a matrix of 3-hydroxypicolinic acid using a Spectrojet piezoelectric nanoliter dispensing system (19). A modified Bruker Biflex III MALDI-TOF mass spectrometer (DNA MassArray; Sequenom) was used to determine genotypes by the appearance of peaks corresponding to the expected extension product masses (Fig. 1*B*).

Statistical analyses. Associations of the Pro12Ala variant of the PPAR-γ2 gene between diabetic subjects and both nondiabetic spouses and elderly control subjects were examined by χ^2 tests of independence. Trait differences within diabetic, elderly control, or spousal control subgroups were examined by analysis of variance. Initially, we tested whether trait means differed significantly among subjects with the Pro/Pro, Pro/Ala, and Ala/Ala genotypes. Due to the small number of individuals with the Ala/Ala genotype, we subsequently tested whether the trait means differed between subjects with and without the Pro12Ala variant (Pro/Ala and Ala/Ala versus Pro/Pro). All

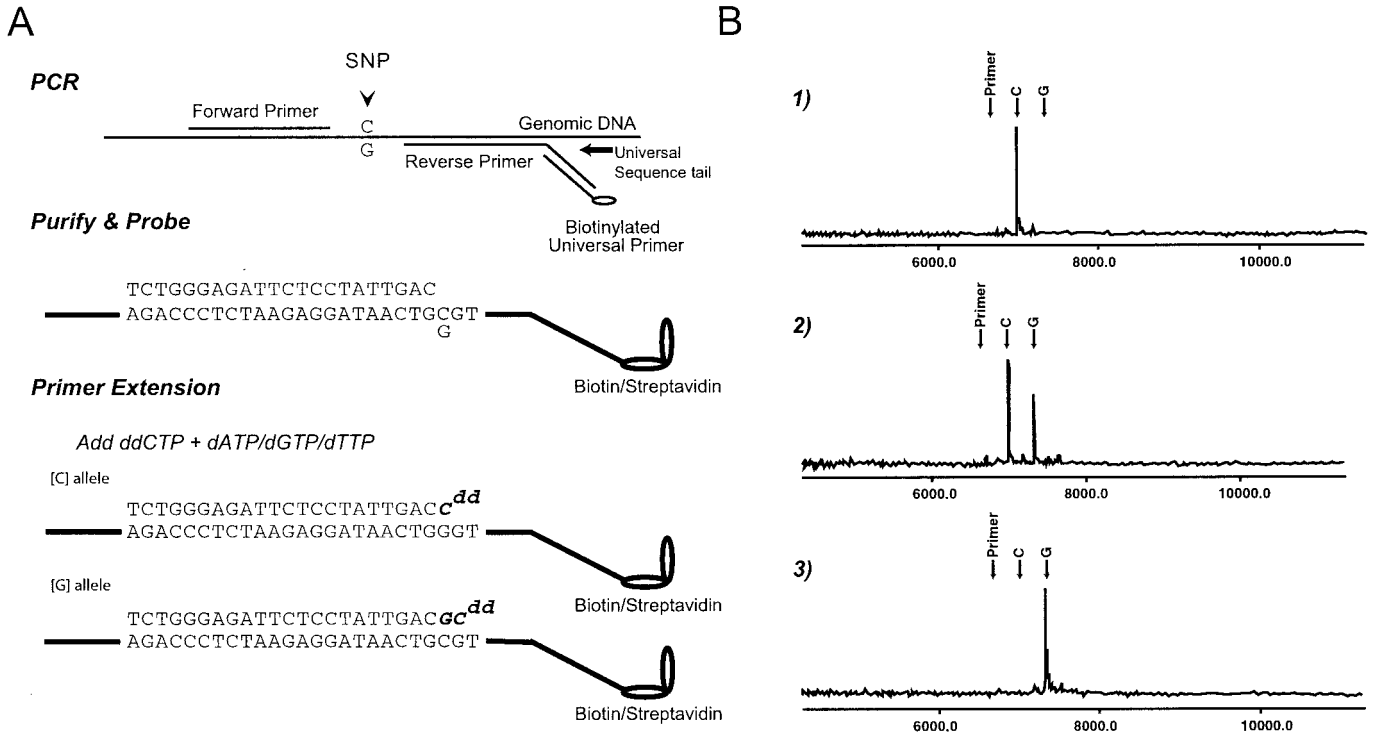

FIG. 1. Genotype analysis by MALDI-TOF spectrometry. *A*: PROBE reaction. The region containing the PPARγ2 Pro12Ala (CCA->GCA) variant is amplified with a biotinylated primer to enable purification of the single-stranded template. Next, the PROBE primer anneals to the template and is extended. When the single nucleotide polymorphism (SNP) is C, the probe is extended by one nucleotide, dideoxy-CTP. When the SNP is G, the probe is extended by two nucleotides, deoxy-GTP, and dideoxy-CTP. *B*: Mass spectrometry profiles of primer extension products. Peaks at 6,989.6 and 7,318.8 Da correspond to the mass of the probe primer extended by one or two nucleotides, respectively. Genotypes of the spectra are *1*) CC, *2*) CG, and *3*) GG. The mass of the unextended PROBE primer is indicated at 6,716.4 Da, but in these examples, none is detected.

analyses were performed with and without adjustment for covariates, including sex, age, and BMI. Preselected interactions between the variant and sex or BMI were also tested. Standard regression diagnostics were computed to examine the adequacy of model assumptions, and traits were transformed to approximate normality when necessary. P values <0.05 were considered statistically significant. No adjustments for multiple comparisons were made. We excluded from the analyses any subject who, on the day of their examinations, took medications that could influence the trait of interest. We also excluded subjects whose diabetic status was uncertain and those with a first-degree relative with type 1 diabetes.

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