
Brief Genetics Report

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

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The allele frequencies for a Pro¹²→Ala substitution in peroxisome proliferator-activated receptor- γ differ among ethnic groups, and its relationship with diabetes and associated diseases is controversial. The prevalence of this polymorphism and its effects on clinical characteristics have now been evaluated with a large number of Japanese individuals with type 2 diabetes ($n = 2,201$) and normal control subjects ($n = 1,212$) recruited by 10 institutions located in seven different cities in Japan. The allele frequency for the Ala¹² variant was significantly lower in the type 2 diabetic group than in the control group (2.39 vs. 4.13%, $P = 0.000054$). However, compared with subjects without the Ala¹² variant, the

diabetic subjects with this variant exhibited a significantly higher serum concentration of total cholesterol ($P = 0.001$), manifested a reduced capacity for insulin secretion as evaluated by homeostasis model assessment ($P = 0.007$), and tended to possess a higher level of HbA_{1c}. These data suggest that the Ala¹² variant is associated with a reduced risk for the development of diabetes in the general population, but that it may be also a risk factor for insulin deficiency and disease severity in individuals with type 2 diabetes. *Diabetes* 50:891–894, 2001

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ANCOVA, analysis of covariance; f-IRI, fasting plasma immunoreactive insulin; FPG, fasting plasma glucose; HOMA- β , homeostasis model assessment for β -cell function; HOMA-IR, HOMA for insulin resistance; OR, odds ratio; PCR, polymerase chain reaction; PPAR- γ , peroxisome proliferator-activated receptor- γ ; RFLP, restriction fragment-length polymorphism.

Two loss-of-function mutations of peroxisome proliferator-activated receptor- γ (PPAR- γ) were shown to cause type 2 diabetes with severe insulin resistance, indicating that PPAR- γ is essential for insulin action and glucose homeostasis (1). A more common Pro¹²→Ala substitution in PPAR- γ was detected in several ethnic groups (2) and was shown to be associated with lower BMI and higher insulin sensitivity in Finns (3). In two Japanese populations, the frequency of the Ala¹² allele was shown to be significantly lower in individuals with type 2 diabetes than in normal subjects (3,4). Altshuler et al. (5) also showed a significant decrease in diabetes risk associated with the Ala¹² allele in a Caucasian population; however, they could not find significant alterations in BMI or metabolic phenotypes.

Other studies of the Pro¹²→Ala polymorphism of PPAR- γ have yielded apparently discrepant results (6–10). The small numbers of subjects enrolled in these various investigations and the apparently conflicting data obtained have highlighted the need for much larger studies. We organized a multi-institutional study involving 10 institutions located in seven different cities in Japan and enrolled a large number of subjects with and without glucose intolerance.

The characteristics of the type 2 diabetic and normal

TABLE 1
Characteristics of the study subjects

Characteristic	Type 2 diabetic subjects	Normal control subjects
<i>n</i>	2,201	1,212
M/F	995/1,206	517/695
Age (years)	60.9 \pm 11.7 (2,201)	71.3 \pm 8.0 (1,212)
Age at onset of diabetes (years)	47.1 \pm 12.2 (2,081)	
BMI (kg/m ²)	23.2 \pm 3.6 (2,144)	22.4 \pm 3.3 (1,126)
Maximum BMI (kg/m ²)	26.8 \pm 4.2 (1,425)	
Waist-to-hip ratio	0.89 \pm 0.08 (241)	
HbA _{1c} (%)	7.8 \pm 1.8 (2,114)	5.0 \pm 0.3 (1,029)
FPG (mg/dl)	157 \pm 57 (1,598)	94 \pm 11 (989)
f-IRI (μ U/ml)	6.78 \pm 4.73 (577)	
HOMA-R (mol \cdot μ U \cdot l ⁻²)	2.54 \pm 1.97 (577)	
HOMA- β (%)	36.2 \pm 34.1 (577)	
Serum total cholesterol (mg/dl)	203 \pm 40 (1,586)	
Serum triglycerides (mg/dl)	142 \pm 133 (1,444)	
Hypertension prevalence	45.5 (1,829)	
Retinopathy prevalence	43.3 (1,626)	
Nephropathy prevalence	37.3 (1,632)	
Neuropathy prevalence	40.7 (1,546)	
Treatment for diabetes	2,176	
Diet	475	
Oral hypoglycemic agents	985	
Insulin	716	

Data are *n*, means \pm SD (*n*), or % (*n*). Criteria for diagnosis of hypertension and diabetic complications are described in RESEARCH DESIGN AND METHODS.

control subjects are summarized in Table 1. Polymerase chain reaction (PCR) and restriction fragment-length polymorphism (RFLP) analysis revealed that the allele frequency for the Ala variant of the Pro¹²→Ala polymorphism of PPAR- γ was significantly lower in the type 2 diabetes group (2.39%) than in the control group (4.13%) (Table 2), indicating that the Ala¹² variant is associated with reduced risk for the development of diabetes (odds ratio [OR] 0.57, 95% CI 0.43–0.75). This association is still observed after adjustment for age, sex, and BMI (OR 0.57, 95% CI 0.41–0.77). The total OR lies in the CI of OR at each institution (data not shown). A test of heterogeneity in ORs using the Breslow-Day technique showed no significant evidence against homogeneity (*P* = 0.80). Therefore,

TABLE 2
Prevalence of the Pro¹²→Ala polymorphism of PPAR- γ in type 2 diabetic individuals and control subjects

	Type 2 diabetic subjects	Normal control subjects
<i>n</i>	2,201	1,212
Genotype		
Pro/Pro	2,097	1,114
Pro/Ala	103	96
Ala/Ala	1	2
Ala allele frequency	2.39* (1.93–2.84)	4.13 (3.33–4.92)

Data are *n* or % (95% CI). *OR for association of the Ala allele with type 2 diabetes is 0.57 (95% CI 0.43–0.75). $\chi^2 = 16.3$; *P* = 0.000054 vs. the normal control group.

TABLE 3
Comparison of clinical profiles between those normal control subjects with and without the Ala¹² allele of the Pro¹²→Ala polymorphism of PPAR- γ

Characteristic	Pro/Pro	Pro/Ala + Ala/Ala	<i>P</i> *
<i>n</i>	1,114	98	
M/F	474/640	43/55	0.998
Age (years)	71.4 \pm 8.0 (1,114)	71.2 \pm 8.2 (98)	0.750
BMI (kg/m ²)	22.4 \pm 3.3 (1,034)	22.4 \pm 3.4 (92)	0.881
HbA _{1c} (%)	5.0 \pm 0.3 (944)	5.0 \pm 0.3 (85)	0.676
FPG (mg/dl)	94 \pm 11 (913)	94 \pm 10 (76)	0.595

Data are *n* or means \pm SD (*n*). **P* values were obtained by the two-tailed Student's *t* test.

it is unlikely that there was any interaction of institution in conjunction with this polymorphism.

Among control subjects, there were no significant differences in age, sex, BMI, the fasting plasma glucose (FPG) concentration, or HbA_{1c} levels between the two subgroups with and without the Ala¹² variant (Table 3). The clinical features of the type 2 diabetic subjects classified according to PPAR- γ genotype were shown in Table 4. To evaluate insulin resistance and β -cell function, we used homeostasis model assessment for β -cell function (HOMA- β) and for insulin resistance (HOMA-IR), as described in RESEARCH DESIGN AND METHODS (11). The values obtained by this approach correlate well with those obtained with the glucose clamp technique (12). We decided that in the multiple testing of clinical data (a total of 18 characteristics) shown in Table 4, *P* values should be adjusted with Bonferroni's correction. We calculated that if a *P* value <0.05 is considered to be nominally significant, this value should be divided by 18 (0.05/18 = 0.0028); accordingly, *P* values <0.0028 were considered to be truly significant. Of the clinical parameters related to obesity or insulin resistance, we found that BMI, maximum BMI, waist-to-hip ratio, and HOMA-IR did not differ significantly between the two subgroups of diabetic subjects. However, the serum concentration of total cholesterol was significantly higher in the type 2 diabetic subjects with at least one Ala¹² allele (*P* = 0.001 by analysis of covariance [ANCOVA]), as shown in Table 4. HOMA- β values were available for 577 type 2 diabetic subjects who were not receiving insulin therapy; the mean values of HOMA- β for those individuals with the Ala¹² variant tended to be lower than that for those without the variant (23.0 vs. 36.8%, *P* = 0.007 by ANCOVA), as shown in Table 4. The HbA_{1c} level was higher in diabetic subjects with the Ala¹² allele than in those without the allele (Table 4), although this difference did not achieve statistical significance (*P* = 0.058). To eliminate the effect of medication, we investigated the HbA_{1c} level of the type 2 diabetic subjects receiving diet therapy alone. Among them, the Ala variant was also associated with a higher level of HbA_{1c} (6.8 \pm 1.5 vs. 7.5 \pm 2.1%, *P* = 0.041 by ANCOVA). These results suggest that the Ala¹² variant of PPAR- γ may be associated with a risk factor for insulin deficiency and disease severity in diabetic individuals, although these associations were revealed to be less strong when the *P* values obtained were adjusted with Bonferroni's correction for multiple testing.

The result that the Ala¹² variant of PPAR- γ is associated

TABLE 4

Comparison of clinical characteristics between those type 2 diabetic subjects with and without the Ala¹² variant of the Pro¹²→Ala polymorphism of PPAR- γ

Characteristic	Pro/Pro	Pro/Ala + Ala/Ala	<i>P</i> *	<i>P</i> †
<i>n</i>	2,097	104	—	—
M/F	940/1,157	55/49	0.107	—
Age (years)	60.8 ± 11.7 (2,097)	61.8 ± 12.1 (104)	0.407	—
Age at onset of diabetes (years)	46.9 ± 12.1 (1,979)	48.0 ± 12.1 (102)	0.279	—
BMI (kg/m ²)	23.2 ± 3.6 (2,041)	23.3 ± 4.1 (103)	0.946	—
Maximum BMI (kg/m ²)	26.8 ± 4.0 (1,357)	26.3 ± 4.1 (68)	0.337	—
Waist-to-hip ratio	0.89 ± 0.08 (231)	0.93 ± 0.09 (10)	0.114	—
HbA _{1c} (%)	7.8 ± 1.8 (2,017)	8.1 ± 1.8 (97)	0.058	—
FPG (mg/dl)	157 ± 57 (1,523)	163 ± 56 (75)	0.100	—
f-IRI (μ U/ml)	6.88 ± 4.79 (553)	5.42 ± 3.49 (24)	0.222	—
HOMA-IR mol · μ U · l ⁻²	2.55 ± 1.98 (553)	2.29 ± 1.74 (24)	0.460	—
HOMA- β (%)‡	36.8 ± 34.5 (553)	23.0 ± 18.6 (24)	0.013	0.007
Serum total cholesterol (mg/dl)	203 ± 39 (1,517)	219 ± 46 (69)	0.004	0.001
Serum triglyceride (mg/dl)‡	142 ± 135 (1,380)	133 ± 83 (64)	0.605	—
Hypertension prevalence	45.1 (1,750)	53.2 (79)	0.161	—
Retinopathy prevalence	43.2 (1,555)	46.5 (71)	0.580	—
Nephropathy prevalence	37.1 (1,562)	40.0 (70)	0.628	—
Neuropathy prevalence	40.4 (1,477)	47.8 (69)	0.217	—
Therapy	2,073	103		
Diet	453	22	} 0.598	—
Oral hypoglycemic agents	943	42		
Insulin	677	39		

Data are *n*, means ± SD (*n*), or % (*n*). **P* values were obtained by the two-tailed Student's *t* test or the Mann-Whitney nonparametric test, as appropriate; †*P* values were obtained by ANCOVA using age and sex as covariates; ‡parameters analyzed after log-transformation as described in RESEARCH DESIGN AND METHODS. In the multiple testing, a *P* value <0.0028 calculated with Bonferroni's adjustment was considered to be statistically significant.

with a reduced risk for diabetes is consistent with previous studies of two Japanese populations (3,4) and of Scandinavian parent-offspring trios (5). As an ethnic group, the Japanese are considerably more homogeneous than Caucasians. It is likely that the influence of population stratification in our study and in two previous studies of Japanese populations (3,4) was smaller than that in studies of Caucasians (8,9). On the other hand, Altshuler et al. (5) showed the same results as in our study by using transmission disequilibrium testing, which could eliminate population stratification.

Two previous studies suggested that the nondiabetic individuals with the Ala¹² allele may possess increased insulin sensitivity compared with those without the allele (3,4); this could explain our results showing that the allele frequency of the Pro¹²→Ala variant is higher in the normal control group than in the diabetic group. However, among type 2 diabetic individuals in the present study, we could not detect the association between the Ala¹² allele and clinical parameters related to insulin resistance. On the other hand, the serum concentration of total cholesterol was higher in subjects with the Ala¹² allele than in those without the allele (Table 4). The Ala¹² variant of PPAR- γ has previously been associated with an increased concentration of HDL cholesterol and a reduced concentration of triglycerides (3), as well as with increased concentrations of total cholesterol and LDL cholesterol (13). Our data are thus consistent with those in the latter study.

Among type 2 diabetic subjects, the values for HOMA- β were lower in those with the Ala¹² allele than in those without the allele (Table 4). When assessed by an oral glucose tolerance test, both heterozygous PPAR- γ -deficient mice and wild-type mice showed hyperglycemia

under a high-fat diet, although the heterozygous PPAR- γ -deficient mice showed lower insulin secretion than the wild-type mice (14). If the heterozygous PPAR- γ -deficient mice possessed a capacity to secrete insulin that was similar to that of wild-type mice, then they might have been expected to show normal glucose tolerance. It is therefore possible that the reduced expression of PPAR- γ in the heterozygous mice may be responsible not only for protection from the development of insulin resistance but also for a reduced capacity for insulin secretion. Because the Ala¹² variant of PPAR- γ exhibits a reduced ability to activate transcription and adipogenesis compared with that of the Pro¹² variant (3,15), this observation is consistent with our finding that the Ala¹² variant of PPAR- γ is associated with impaired insulin secretion in diabetic subjects. The diabetic subjects with the Ala¹² allele also tended to exhibit higher HbA_{1c} levels than those without this allele, although individuals with the Ala¹² allele are associated with a reduced risk for the development of type 2 diabetes. This paradoxical observation could be explained by a reduced capacity to secrete insulin in these individuals.

In conclusion, the Ala variant of PPAR- γ is associated with a reduced risk for the development of diabetes. However, among individuals with type 2 diabetes, this variant may be associated with a lower level of insulin secretion, a higher serum concentration of total cholesterol, and a tendency toward increased levels of HbA_{1c}. Diabetic individuals with the Ala¹² variant are thus likely to experience a more severe form of the disease.

RESEARCH DESIGN AND METHODS

A total of 3,413 Japanese subjects were enrolled by 10 institutions for the study. They included 2,201 subjects with type 2 diabetes and 1,212 normal

control subjects. The diagnosis of diabetes was based on World Health Organization criteria (16). The normal control subjects were selected according to the following criteria: no past history of urinary glucose or glucose intolerance, an HbA_{1c} level <5.6% or a normal oral glucose (75 g) tolerance test, age >60 years, and no family history of diabetes. All study subjects were unrelated, and they gave their written consent to participate in the study after being informed of its nature. The study protocol was reviewed by the appropriate institutional review committee of each institution, and the investigation was performed in accordance with the guidelines expressed in the Declaration of Helsinki.

Clinical assessment. All clinical assessments described below were performed using uniform standards throughout the 10 institutions that participated in this study. The sex and present age were ascertained from medical records. The current BMI and waist-to-hip ratio of each individual was directly measured when we collected blood samples for DNA analysis. The age at onset of diabetes and maximum BMI were obtained from direct interviews by trained interviewers. The FPG level, concentration of serum lipids, fasting plasma immunoreactive insulin (f-IRI) concentration, and HbA_{1c} level were determined in each subject by standard laboratory techniques calibrated by the uniform standards. Based on the assumption that normal subjects aged <35 years who are of normal weight exhibit 100% β -cell function and an insulin resistance of 1, the corresponding values for individuals with diabetes can be assessed from FPG and f-IRI concentrations using the following formulas: 1) HOMA- β (expressed as a percentage) = f-IRI \times 20/(FPG - 3.5) and 2) HOMA-IR (expressed in mol \cdot μ M \cdot l⁻²) = FPG \times f-IRI/22.5 (11). The diagnosis of retinopathy was based on the medical records of the department of ophthalmology at each institution. The diagnosis of nephropathy was based on repeated evidence of urinary albumin excretion of >10 μ g/ml. The diagnosis of neuropathy was based on the subjective symptoms of each individual as revealed by the questionnaire or medical records. Blood pressure was measured on the right arm of each seated subject after >10 min of rest. The diagnosis of hypertension was based on treatment with antihypertensive drugs or a blood pressure of >140/90 mmHg. The treatment of diabetes was classified into three categories: dietary therapy (no medication), administration of oral hypoglycemic agents, and insulin therapy.

Genotype analysis of the Pro¹²→Ala polymorphism of PPAR- γ . Genomic DNA was isolated from the peripheral blood leukocytes of all study participants and subjected to PCR-RFLP analysis for determination of the genotype with regard to the Pro¹²→Ala polymorphism of human PPAR- γ . Fragments of the human PPAR- γ gene encompassing the polymorphic site were amplified by PCR with the sense primer 5'-TCTGGGAGATTCTCCTATTGGC-3' and the antisense primer 5'-CTGGAAGACAACACTACAAGAG-3'; the sense primer contains an additional single-base mutation (indicated in bold) located 2 bp upstream of the site of the substitution responsible for the Pro¹² (CCA)→Ala (GCA) polymorphism. This mismatched base results in the generation of a new *HhaI* restriction site in the PCR product obtained from subjects with the Ala¹² allele. The 154-bp PCR products of the human PPAR- γ gene were therefore digested with *HhaI*, subjected to electrophoresis on a 10% polyacrylamide gel, and visualized by staining with ethidium bromide. To confirm that detection of this C→G nucleotide substitution by PCR-RFLP analysis is reproducible, we also performed PCR-based direct sequencing analysis. The genotype of each study subject was determined blindly, without knowledge of clinical status.

Statistical analysis. The statistical difference in allele frequencies between the type 2 diabetic group and the normal control group was assessed by χ^2 test. The ORs and 95% CIs (nonadjusted or adjusted for age, sex, and BMI) were calculated by logistic regression analysis. All clinical data are expressed as either means \pm SD or proportions. Before statistical analysis, the normal distribution and homogeneity of the variables were tested. Parameters that did not fulfill these tests (HOMA- β and triglycerides) were transformed to natural logarithms to remove skewness. Comparison of variables between groups of genotypes was performed using the two-tailed Student's *t* test or the Mann-Whitney nonparametric test, as appropriate. The effect of genotype on the clinical parameters was also estimated by ANCOVA using genotype as a factor and age and sex as covariates. To evaluate the statistical significance with regard to multiple testing of a total of 18 clinical data (Table 4), the *P* values were corrected with Bonferroni's correction. Because a *P* value <0.05 was considered to be nominally significant, that value was divided by 18 (0.05/18 = 0.0028) to reflect the clinical data; therefore, *P* values <0.0028 were considered to be truly significant. Statistical analysis was performed with StatView Version 5.0 software (SAS Institute, Cary, NC).

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