

## Brief Genetics Report

# Peroxisome Proliferator-Activated Receptor $\alpha$ Gene Variation Influences Age of Onset and Progression of Type 2 Diabetes

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Dysregulation of fatty acid metabolism is important in the pathogenesis of type 2 diabetes. Peroxisome proliferator-activated receptor (PPAR) $\alpha$  is a master regulator of fatty acid catabolism, and PPAR $\alpha$  activators delay the onset of type 2 diabetes. We examined association between three PPAR $\alpha$  gene polymorphisms (an A $\rightarrow$ C variant in intron 1, the L162V variant, and the intron 7 G $\rightarrow$ C variant) and age at diagnosis of type 2 diabetes in 912 Caucasian type 2 diabetic subjects. Individually, PPAR $\alpha$  gene variants did not influence age at diagnosis, but in combination, the rare alleles of both the intron 1 A $\rightarrow$ C ( $P < 0.001$ ) and intron 7 G $\rightarrow$ C ( $P = 0.025$ ) variants synergistically lowered age at diagnosis (interaction  $P < 0.001$ ). Overall, the PPAR $\alpha$  haplotype significantly influenced age at diagnosis ( $P = 0.027$ ), with the C-L-C and C-V-C haplotypes (intron 1-L162V-intron 7) accelerating onset of diabetes by 5.9 ( $P = 0.02$ ) and 10 ( $P = 0.03$ ) years, respectively, as compared with the common A-L-G haplotype, and was associated with an odds ratio for early-onset diabetes (age at diagnosis  $\leq 45$  years) of 3.75 (95% CI 1.65–8.56,  $P = 0.002$ ). Intron 1 C-allele carriers also progressed more rapidly to insulin monotherapy (AA  $9.4 \pm 1.5$  and AC + CC  $5.3 \pm 1.1$  years,  $P = 0.002$ ). These data indicate that PPAR $\alpha$  gene variation influences the onset and progression of type 2 diabetes. *Diabetes* 54:582–586, 2005

**T**he etiology of type 2 diabetes involves the progressive development of insulin resistance in skeletal muscle and culminates in pancreatic  $\beta$ -cell failure. Fatty acids contribute to the development and progression of type 2 diabetes. Increased fatty

acid concentrations reduce skeletal muscle glucose uptake and oxidation, stimulate hepatic gluconeogenesis while inhibiting insulin suppression of gluconeogenesis, and inhibit insulin production and glucose-stimulated insulin secretion in pancreatic  $\beta$ -cells (rev. in 1). Increased fasting plasma fatty acid concentrations predict the deterioration of glucose tolerance (2) and are a risk factor for the development of type 2 diabetes (3).

Peroxisome proliferator-activated receptor (PPAR) $\alpha$  is a member of the nuclear hormone receptor superfamily of ligand-regulated transcription factors for which ligands include long-chain fatty acids, eicosanoids, and the fibrate class of lipid-lowering drugs (4). PPAR $\alpha$  is expressed at high levels in tissues that catabolize fatty acids, notably liver, skeletal muscle, and heart, and at lower levels in other tissues, including pancreas (5). PPAR $\alpha$  is a master regulator of fatty acid utilization (6), and activation of PPAR $\alpha$  causes a dramatic lowering of plasma, hepatic, and intramuscular triglycerides (7). In rodent models of type 2 diabetes, administration of PPAR $\alpha$  agonists reduces adiposity, normalizes fasting plasma glucose and insulin levels, and improves insulin suppression of endogenous glucose production (8–10). Furthermore, insulin secretion and pancreatic hypertrophy and degeneration were improved by PPAR $\alpha$  activation (9,10). Recently, bezafibrate was shown to reduce the incidence and delay the onset of type 2 diabetes, indicating that such effects may also occur in humans (11).

These data indicate that PPAR $\alpha$  acts pleiotropically to ameliorate insulin resistance in the major cell types involved in the pathogenesis of type 2 diabetes. Variation in the PPAR $\alpha$  gene influences plasma lipid levels (12,13), cardiac growth (14), and risk of coronary artery disease (15). We therefore examined association between variation in the PPAR $\alpha$  gene and age at diagnosis (as a surrogate for age of onset) and progression to type 2 diabetes in Caucasian type 2 diabetic participants in the University College Diabetes and Cardiovascular Study (UDACS) (16) and Ealing Diabetic Study of Coagulation (EDSC).

## RESEARCH DESIGN AND METHODS

European type 2 diabetic subjects were selected from two studies, UDACS (16) and EDSC. UDACS and EDSC subjects were recruited consecutively from the diabetes clinics at University College London Hospital and Ealing Hospi-

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EDSC, Ealing Diabetic Study of Coagulation; PPAR, peroxisome proliferator-activated receptor; UDACS, University College Diabetes and Cardiovascular Study.

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TABLE 1  
Characteristics of the study subjects

Age at recruitment (years)	65.4 ± 12.1
Age at diagnosis (years)	54.4 ± 13.0
Duration of diabetes (years)	10.92 ± 9.1
Family history of diabetes (%)	34.0
BMI (kg/m <sup>2</sup> )	29.8 ± 5.8
Smoking status (never/former/current) (%)	45.1/38.6/16.3
Glucose (mmol/l)	11.7 ± 5.1
HbA <sub>1c</sub> (%)	8.0 ± 1.8
SBP (mmHg)	140.6 ± 20.1
DBP (mmHg)	77.3 ± 11.7
Triglycerides (mmol/l)	2.22 ± 1.57
Total cholesterol (mmol/l)	5.02 ± 1.10
LDL cholesterol (mmol/l)	2.81 ± 0.93
HDL cholesterol (mmol/l)	1.31 ± 0.41

Data are means ± SD unless otherwise indicated.

tal, respectively. Patients completed a questionnaire with details of age, ethnicity, smoking habit, fasting status, duration of diabetes, family history of diabetes, history of heart attack or stroke, current medication, and other clinical details. Blood was collected for plasma and DNA analysis. BMI, blood pressure, glucose, HbA<sub>1c</sub>, cholesterol, HDL, LDL, triglyceride, urea, creatinine, albumin-to-creatinine ratio, potassium, and proteinuria were measured. All patients had type 2 diabetes according to World Health Organization criteria (17). No subjects requiring renal dialysis were recruited. Ethical approval was obtained from University College London/University College London Hospital and Ealing Hospital ethics committees and from data protection at University College London. Middle-aged U.K. men (50–61 years, mean age 56.1 ± 3.5 years) participating in the Second Northwick Park Heart Study were used as a population-based sample for comparison of allele and haplotype frequencies (15).

**Genotyping.** The intron 1 A/C variant (refsnp 135539) was genotyped using forward primer CCAGGGGGAGGAAAGAGTGAA and reverse primer GCCACAACTAAGCAGGCAGTG to generate a product of 210 bp, which, when digested with *Hinf*I, gave products of 148 and 62 bp in the presence of the C-allele. The L162V (refsnp 1800206) and intron 7 G/C (refsnp 4253778) variants were genotyped as previously described (15).

**Statistical analysis.** Statistical analysis was performed by univariate ANOVA and linear regression using SPSS (version 12; SPSS, Chicago, IL). Factors and covariates, together with all two-way interactions, were entered into the statistical model and removed in a stepwise manner until a parsimonious model containing only factors or interactions that were statistically significant was obtained. Mean age at diagnosis, adjusted for family history of diabetes, sex, and smoking, was generated by calculating residuals and adding to the overall mean value. Haplotype frequencies and effects were determined using THESIAS (18). Smoking status and BMI at diagnosis were not available; therefore, measures at recruitment were used. BMI, triglycerides, and duration of diabetes before insulin treatment were log<sub>10</sub> transformed to normalize distribution. The three V162 allele homozygotes were combined with V162 allele carriers in all analyses. Data are shown as means ± SE, and a *P* value of 0.05 was considered statistically significant.

## RESULTS

The clinical characteristics of type 2 diabetic subjects are shown in Table 1. The intron 1 A→C variant is situated ~12.5 kb 3' of the site of transcriptional initiation of the PPARα gene, 55 kb 5' of L162V, and 71 kb 5' of the intron 7 G→C variant. Rare allele frequencies were intron 1 A→C 0.404 (95% CI 0.381–0.427), L162V 0.064 (0.053–0.076), and intron 7 G→C 0.187 (0.169–0.205). The intron 1 A→C variant was selected due to its rare allele frequency and close proximity to the 5' end of the PPARα gene, whereas the L162V and intron 7 G→C variants have previously shown positive results in association studies (12–15). No other PPARα variants were examined. Genotype distributions were in Hardy-Weinberg equilibrium and were not different between the two study centers. Allele frequencies were not significantly different between type 2

TABLE 2  
Effect of individual PPARα genotypes on age at diagnosis of type 2 diabetes

Intron 1 A/C	
AA	53.1 ± 0.7 (315)
AC	53.2 ± 0.6 (408)
CC	52.0 ± 1.1 (148)
<i>P</i>	0.60
L162V	
LL	52.7 ± 0.5 (762)
LV + VV	54.7 ± 1.2 (109)
<i>P</i>	0.13
Intron 7 G/C	
GG	52.9 ± 0.6 (574)
GC	53.1 ± 0.7 (268)
CC	51.8 ± 2.7 (29)
<i>P</i>	0.87

Data are means ± SE (no. per genotype group). *P* values were determined by ANOVA.

diabetic subjects and those previously reported in healthy U.K. middle-aged men (15). All three variants were in linkage disequilibrium (intron 1–L162V *D'* = 0.57, *P* = 0.0001; intron 1–intron 7 *D'* = 0.46, *P* = 2.6 × 10<sup>-8</sup>; L162V–intron 7 *D'* = 0.58, *P* = 2.7 × 10<sup>-23</sup>).

Individually, PPARα gene variants did not influence age at diagnosis when examined by ANOVA (Table 2), but when examined in combination, both the intron 1 A→C (*P* < 0.001) and intron 7 G→C (*P* = 0.025) variants significantly influenced age at diagnosis and showed a significant interaction (intron 1 × intron 7 interaction, *P* < 0.001), acting synergistically to lower age at diagnosis (Table 3). In multivariate linear regression, sex (*P* = 0.001), currently smoking (*P* = 0.026), family history of diabetes (*P* < 0.001), intron 1 genotype (*P* = 0.001), intron 7 genotype (*P* = 0.045), and the intron 1 × intron 7 interaction (*P* = 0.003) were independent predictors of age at diagnosis. The effects of PPARα genotype were the same by study center, which did not independently influence age at diagnosis. Haplotype analysis revealed that PPARα haplotype significantly influenced age at diagnosis (*P* = 0.037) (Table 4). Haplotypes 6 and 8, comprising the intron 1 C- and intron 7 C-alleles with the L162V L162 allele (haplotype 6, C-L-C) or the V162 allele (haplotype 8, C-V-C), were associated with an age at diagnosis of 5.86 ± 2.57 (*P* = 0.02) and 9.98 ± 4.65 (*P* = 0.03) years, respectively, earlier than the common haplotype.

Given the dramatic haplotype effect on age at diagnosis, the role of PPARα in early-onset type 2 diabetes was examined. PPARα haplotype frequency was calculated in subjects stratified by age of diagnosis of ≤45 or >45 years, based on the previous use of this cutoff age in a linkage

TABLE 3  
Combined effect of intron 1 A→C and intron 7 G→C variants on age at diagnosis of type 2 diabetes

Intron 1 A→C	Intron 7 G>C		
	GG	GC	CC
AA	54.2 ± 1.0 (199)	54.8 ± 1.0 (117)	58.8 ± 2.7 (18)
AC	54.7 ± 0.8 (269)	55.1 ± 1.0 (148)	45.2 ± 6.4 (6)
CC	54.4 ± 1.2 (130)	49.3 ± 2.9 (20)	39.0 ± 6.9 (5)

Data are means ± SE (no. per genotype group).

TABLE 4  
PPAR $\alpha$  haplotype frequencies, effect on adjusted age at diagnosis, and frequency in young- and later-onset subjects

Haplotype	Intron 1-L162V-intron 7 genotype	Frequency in type 2 diabetic subjects (n = 912)	Haplotype effect on age at diagnosis (years)	P*	Frequency in young-onset subjects (n = 238)	Frequency in later-onset subjects (n = 674)	Odds ratio (95% CI) for early age at diagnosis	P*	Frequency in U.K. middle-aged men without overt type 2 diabetes (n = 2,630)
1	A-L-G	0.428	26.17 $\pm$ 0.40	—	0.453	0.418	1	—	0.412
2	A-L-C	0.114	1.54 $\pm$ 1.19	0.19	0.083	0.126	0.66 (0.42–1.05)	0.08	0.102
3	A-V-G	0.021	0.94 $\pm$ 2.64	0.72	0.018	0.021	0.82 (0.33–2.05)	0.67	0.019
4	A-V-C	0.035	2.96 $\pm$ 2.12	0.16	0.028	0.038	0.73 (0.36–1.48)	0.38	0.034
5	C-L-G	0.362	0.52 $\pm$ 0.67	0.44	0.325	0.376	0.85 (0.66–1.09)	0.20	0.391
6	C-L-C	0.030	–5.86 $\pm$ 2.57	0.02	0.071	0.014	3.75 (1.65–8.56)	0.002	0.030
7	C-V-G	0.001	–5.44 $\pm$ 84.7	0.95	0.004	0	1.73 (0.00–9.99)	0.94	0.001
8	C-V-C	0.009	–9.98 $\pm$ 4.65	0.03	0.017	0.006	2.42 (0.70–8.29)	0.16	0.010

Data are means  $\pm$  SE unless otherwise indicated. Age at diagnosis was adjusted for family history of diabetes, sex, and smoking. \*P-values vs. reference haplotype 1.

scan for age at diagnosis (19). Overall, PPAR $\alpha$  haplotype frequency was highly significantly different between young-onset and later-onset subjects ( $P = 1.4 \times 10^{-7}$ ), with haplotype 6 increasing risk of early-onset type 2 diabetes (odds ratio 3.75, 95% CI 1.65–8.56,  $P = 0.002$ ). Haplotype frequencies were not different between the entire type 2 diabetes sample and middle-aged U.K. men without overt type 2 diabetes ( $P = 0.50$ ) but were significantly different between middle-aged U.K. men and early-onset subjects ( $P = 0.024$ ) (Table 4).

We investigated whether PPAR $\alpha$  variants influence the progression of type 2 diabetes, as estimated by the duration of diabetes before commencing insulin monotherapy. In UDACS, carriers and homozygotes of the rare C-allele progressed to insulin treatment significantly earlier than A allele homozygotes (AA  $9.4 \pm 1.5$  and AC + CC  $5.3 \pm 1.1$  years,  $P = 0.002$ ). The L162V and intron 7 G $\rightarrow$ C variants did not influence duration before insulin therapy. PPAR $\alpha$  genotype did not influence whether subjects were on an oral hypoglycemic agent, insulin, or both an oral hypoglycemic agent and insulin therapy (not shown). Measures of progression were not available in EDSC.

Treatment with PPAR $\alpha$  activators beneficially alters plasma lipid profile, and we previously demonstrated that variation in the PPAR $\alpha$  gene influences plasma lipid levels in dyslipidemic type 2 diabetic subjects (12). We therefore examined whether PPAR $\alpha$  gene variation influences plasma lipid levels in type 2 diabetic subjects, but observed no significant association with plasma triglycerides or LDL, HDL, or total cholesterol concentrations (not shown).

## DISCUSSION

This study demonstrates that variation in the PPAR $\alpha$  gene influences the onset and progression of type 2 diabetes, in concurrence with the recent observation that bezafibrate reduces the incidence and delays the onset of type 2 diabetes (11). Allele and haplotype frequencies were not different between healthy middle-aged U.K. men and the type 2 diabetes sample, but haplotype frequency was different between subjects with age at diagnosis  $\leq 45$  years and both those with later age at diagnosis and with healthy middle-aged U.K. men without overt type 2 diabetes, indicating that PPAR $\alpha$  predisposes to early-onset type 2 diabetes. PPAR $\alpha$  gene variants influenced age at diagnosis in combination and in haplotype analysis. Carriers of the intron 1 and 7 rare C-alleles had a dramatically younger age at diagnosis, an effect confirmed in haplotype analysis, in which haplotypes 6 and 8, comprising the intron 1 C- and intron 7 C-alleles with the L162 or V162 alleles, respectively, were associated with an age at diagnosis 5.8 and 10.0 years earlier than the common haplotype. Genome scans have identified several loci linked to age at diagnosis (19,20), one of which maps in a subset of families with age at diagnosis  $< 45$  years to chromosome 22q11 (19), 28 Mb centromeric to the PPAR $\alpha$  gene.

The PPAR $\alpha$  intron 1 genotype was also associated with a more rapid progression to insulin monotherapy, suggesting that PPAR $\alpha$  influences the rate of development of insulin resistance and/or progression to  $\beta$ -cell failure. PPAR $\alpha$  is expressed in liver, skeletal muscle, and pancreas (5) and could therefore affect the disease process at

multiple levels. In rodents, PPAR $\alpha$  activators lowered fasting plasma triglycerides, and free fatty acids, as well as lowered visceral adiposity and muscle and hepatic steatosis, resulting in improved hepatic and muscle insulin sensitivity and improved pancreatic  $\beta$ -cell function. Genetically determined reduced PPAR $\alpha$  levels may influence any of the above-mentioned factors. We did not observe association between PPAR $\alpha$  variation and plasma triglyceride levels (fasting triglyceride levels were not available for all subjects), thus decreasing the power to observe an effect. Studies that directly assess the impact of PPAR $\alpha$  gene variants on levels of intramuscular and intrahepatic triglycerides, whole-body glucose uptake, and  $\beta$ -cell function will elucidate the mechanisms underlying the observed effects.

The PPAR $\alpha$  genotype influences the risk of coronary artery disease (15), and it is conceivable that it could increase the likelihood of subjects presenting with heart disease and subsequently being diagnosed with type 2 diabetes, thus confounding the current study. The presence of cardiovascular disease was in fact associated with later age at diagnosis (absence  $53.4 \pm 0.5$  years, presence  $56.5 \pm 0.7$  years,  $P = 0.001$ ) and did not influence the effect of PPAR $\alpha$  genotype on age at diagnosis, indicating that these data are not confounded by cardiovascular disease. The differences between UDACS and EDSC in BMI, measures of glycemic control, blood pressure, and plasma lipids did not confound the effect of PPAR $\alpha$  haplotype on age at diagnosis.

Mechanistically, several lines of evidence lead us to speculate that the intron 1 C- and intron 7 C-alleles mark a haplotype with reduced PPAR $\alpha$  gene expression. First, in previous studies, the intron 7 C-allele shows opposing effects to the V162 allele (14,15), which encodes a more active PPAR $\alpha$  in vitro (12). Furthermore, the intron 7 C-allele is associated with reduced response to fenofibrate in the DAIS (Diabetes Atherosclerosis Intervention Study) (21). Finally, PPAR $\alpha$  activators delay onset of type 2 diabetes in humans (11) and rats (10), indicating that increased PPAR $\alpha$  activity protects against the onset of type 2 diabetes.

PPAR $\alpha$  interacts with several genes that influence type 2 diabetes. Hepatic PPAR $\alpha$  gene expression is regulated by hepatocyte nuclear factor 4 $\alpha$  (22), a closely related member of the nuclear hormone receptor superfamily that is associated with risk of type 2 diabetes (23). Additionally, genetic variation in PPAR $\gamma$  coactivator-1, which also co-activates PPAR $\alpha$ , influences the insulin secretory response in Pima Indians (24). The retinoid X receptor  $\alpha$ , with which PPAR $\alpha$  forms heterodimers, also influences insulin resistance (25). It will be interesting to examine interaction between PPAR $\alpha$  and hepatocyte nuclear factor 4 $\alpha$ , PPAR $\gamma$  coactivator-1, and retinoid X receptor  $\alpha$  gene variants in subsequent studies.

In summary, we demonstrate that PPAR $\alpha$  gene variation influences the onset and progression of type 2 diabetes in human subjects. The mechanisms underlying these effects remain unclear and warrant future investigation.

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