Brief Genetics Report

Peroxisome Proliferator–Activated Receptor α Gene Variation Influences Age of Onset and Progression of Type 2 Diabetes

David M. Flavell,[1] Helen Ireland,[1] Jeffrey W. Stephens,[1] Emma Hawe,[1] Jay Acharya,[1] Hugh Mather,[2] Steven J. Hurel,[3] and Steve E. Humphries[1]

Dysregulation of fatty acid metabolism is important in the pathogenesis of type 2 diabetes. Peroxisome proliferator–activated receptor (PPAR)α is a master regulator of fatty acid catabolism, and PPARα activators delay the onset of type 2 diabetes. We examined association between three PPARα gene polymorphisms (an A→C variant in intron 1, the L162V variant, and the intron 7 G→C variant) and age at diagnosis of type 2 diabetes in 912 Caucasian type 2 diabetic subjects. Individually, PPARα gene variants did not influence age at diagnosis, but in combination, the rare alleles of both the intron 1 A→C ($P < 0.001$) and intron 7 G→C ($P = 0.025$) variants synergistically lowered age at diagnosis (interaction $P < 0.001$). Overall, the PPARα haplotype signficantly 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 ≤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 ± 1.5 and AC + CC 5.3 ± 1.1 years, $P = 0.002$). These data indicate that PPARα gene variation influences the onset and progression of type 2 diabetes. *Diabetes* 54:582–586, 2005

The etiology of type 2 diabetes involves the progressive development of insulin resistance in skeletal muscle and culminates in pancreatic β-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 β-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)α 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). PPARα 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). PPARα is a master regulator of fatty acid utilization (6), and activation of PPARα causes a dramatic lowering of plasma, hepatic, and intramuscular triglycerides (7). In rodent models of type 2 diabetes, administration of PPARα agonists reduces adiposity, normalizes fasting plasma glucose and insulin levels, and improves insulin suppresson of endogeneous glucose production (8–10). Furthermore, insulin secretion and pancreatic hypertrophy and degeneration were improved by PPARα 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 PPARα acts pleiotropically to ameliorate insulin resistance in the major cell types involved in the pathogenesis of type 2 diabetes. Variation in the PPARα 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 PPARα 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-

From the [1]Centre for Cardiovascular Genetics, Department of Medicine, Royal Free and University College Medical School, London, U.K.; the [2]Department of Medicine, Ealing Hospital, London, U.K.; and the [3]Department of Diabetes & Endocrinology, University College London Hospital, London, U.K.

Address correspondence and reprint requests to Dr. David M. Flavell, Centre for Cardiovascular Genetics, The Rayne Building, 5 University St., London WC1E 6JF, U.K. E-mail: d.flavell@ucl.ac.uk.

Received for publication 2 June 2004 and accepted in revised form 27 October 2004.

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

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^2)	29.8 ± 5.8
Smoking status (never/former/current) (%)	45.1/38.6/16.3
Glucose (mmol/l)	11.7 ± 5.1
HbA_{1c} (%)	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_{1c}, 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 GCCAC AACTAAGCAGGCAGTG to generate a product of 210 bp, which, when digested with *Hin*fI, 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 $\log_{10}$ 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 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, $P = 2.6 \times 10^{-8}$; L162V–intron 7 D′ = 0.58, $P = 2.7 \times 10^{-23}$).

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)
P	0.60
L162V	
LL	52.7 ± 0.5 (762)
LV + VV	54.7 ± 1.2 (109)
P	0.13
Intron 7 G/C	
GG	52.9 ± 0.6 (574)
GC	53.1 ± 0.7 (268)
CC	51.8 ± 2.7 (29)
P	0.87

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

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
PPARα 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 ± 0.40	—	0.453	0.418	1	—	0.412
2	A-L-C	0.114	1.54 ± 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 ± 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 ± 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 ± 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 ± 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 ± 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 ± 4.65	0.03	0.017	0.006	2.42 (0.70–8.29)	0.16	0.010

Data are means ± 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, PPARα 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 PPARα 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 ± 1.5 and AC + CC 5.3 ± 1.1 years, $P = 0.002$). The L162V and intron 7 G→C variants did not influence duration before insulin therapy. PPARα 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 PPARα activators beneficially alters plasma lipid profile, and we previously demonstrated that variation in the PPARα gene influences plasma lipid levels in dyslipidemic type 2 diabetic subjects (12). We therefore examined whether PPARα 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 PPARα 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 ≤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 PPARα predisposes to early-onset type 2 diabetes. PPARα 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 PPARα gene.

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

multiple levels. In rodents, PPARα 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 β-cell function. Genetically determined reduced PPARα levels may influence any of the abovementioned factors. We did not observe association between PPARα 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 PPARα gene variants on levels of intramuscular and intrahepatic triglycerides, whole-body glucose uptake, and β-cell function will elucidate the mechanisms underlying the observed effects.

The PPARα 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 ± 0.5 years, presence 56.5 ± 0.7 years, $P = 0.001$) and did not influence the effect of PPARα 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 PPARα 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 PPARα 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 PPARα 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, PPARα activators delay onset of type 2 diabetes in humans (11) and rats (10), indicating that increased PPARα activity protects against the onset of type 2 diabetes.

PPARα interacts with several genes that influence type 2 diabetes. Hepatic PPARα gene expression is regulated by hepatocyte nuclear factor 4α (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 PPARγ coactivator-1, which also coactivates PPARα, influences the insulin secretory response in Pima Indians (24). The retinoid X receptor α, with which PPARα forms heterodimers, also influences insulin resistance (25). It will be interesting to examine interaction between PPARα and hepatocyte nuclear factor 4α, PPARγ coactivator-1, and retinoid X receptor α gene variants in subsequent studies.

In summary, we demonstrate that PPARα 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.

ACKNOWLEDGMENTS

D.M.F. and S.E.H. are supported by grants from the British Heart Foundation (FS 2001/075 to D.M.F. and RG2000015 to S.E.H.). J.W.S. is supported by a clinical training fellowship from Diabetes U.K. (BDA: RD01/0001357). Recruitment of patients in EDSC was supported by The Coronary Thrombosis Trust.

The authors thank the medical staff and patients who contributed to the UDACS and EDSC.

REFERENCES

1. McGarry JD: Banting Lecture 2001: Dysregulation of fatty acid metabolism in the etiology of type 2 diabetes. *Diabetes* 51:7–18, 2002
2. Charles MA, Eschwege E, Thibult N, Claude JR, Warnet JM, Rosselin GE, Girard J, Balkau B: The role of non-esterified fatty acids in the deterioration of glucose tolerance in Caucasian subjects: results of the Paris Prospective Study. *Diabetologia* 40:1101–1106, 1997
3. Paolisso G, Tataranni PA, Foley JE, Bogardus C, Howard BV, Ravussin E: A high concentration of fasting plasma non-esterified fatty acids is a risk factor for the development of NIDDM. *Diabetologia* 38:1213–1217, 1995
4. Krey G, Braissant O, L'Horset F, Kalkhoven E, Perroud M, Parker MG, Wahli W: Fatty acids, eicosanoids, and hypolipidemic agents identified as ligands of peroxisome proliferator-activated receptors by coactivator-dependent receptor ligand assay. *Mol Endocrinol* 11:779–791, 1997
5. Braissant O, Foufelle F, Scotto C, Dauca M, Wahli W: Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-alpha, -beta, and -gamma in the adult rat. *Endocrinology* 137:354–366, 1996
6. Kersten S, Desvergne B, Wahli W: Roles of PPARs in health and disease. *Nature* 405:421–424, 2000
7. Ye JM, Doyle PJ, Iglesias MA, Watson DG, Cooney GJ, Kraegen EW: Peroxisome proliferator–activated receptor (PPAR)-α activation lowers muscle lipids and improves insulin sensitivity in high fat–fed rats: comparison with PPAR-γ activation. *Diabetes* 50:411–417, 2001
8. Guerre-Millo M, Gervois P, Raspe E, Madsen L, Poulain P, Derudas B, Herbert J-M, Winegar DA, Willson TM, Fruchart J-C, Berge RK, Staels B: PPARα activators improve insulin sensitivity and reduce adiposity. *J Biol Chem* 275: 2000
9. Kim H, Haluzik M, Asghar Z, Yau D, Joseph JW, Fernandez AM, Reitman ML, Yakar S, Stannard B, Heron-Milhavet L, Wheeler MB, LeRoith D: Peroxisome proliferator–activated receptor-α agonist treatment in a transgenic model of type 2 diabetes reverses the lipotoxic state and improves glucose homeostasis. *Diabetes* 52:1770–1778, 2003
10. Koh EH, Kim MS, Park JY, Kim HS, Youn JY, Park HS, Youn JH, Lee KU: Peroxisome proliferator–activated receptor (PPAR)-α activation prevents diabetes in OLETF rats: comparison with PPAR-γ activation. *Diabetes* 52:2331–2337, 2003
11. Tenenbaum A, Motro M, Fisman EZ, Schwammenthal E, Adler Y, Goldenberg I, Leor J, Boyko V, Mandelzweig L, Behar S: Peroxisome proliferator-activated receptor ligand bezafibrate for prevention of type 2 diabetes mellitus in patients with coronary artery disease. *Circulation* 109:2197–2202, 2004
12. Flavell DM, Pineda Torra I, Jamshidi Y, Evans D, Diamond JR, Elkeles RS, Bujac SR, Miller G, Talmud PJ, Staels B, Humphries SE: Variation in the PPARα gene is associated with altered function *in vitro* and plasma lipid concentrations in type II diabetic subjects. *Diabetologia* 43:673–680, 2000
13. Vohl M-C, Lepage P, Gaudet D, Brewer CG, Betard C, Perron P, Houde G, Cellier C, Faith JM, Despres J-C, Morgan K, Hudson TJ: Molecular scanning of the human PPARα gene: association of the L162V mutation with hyperapobetalipoproteinemia. *J Lipid Res* 41:945–952, 2000
14. Jamshidi Y, Montgomery HE, Hense HW, Myerson SG, Torra IP, Staels B, World MJ, Doering A, Erdmann J, Hengstenberg C, Humphries SE, Schunkert H, Flavell DM: Peroxisome proliferator–activated receptor alpha gene regulates left ventricular growth in response to exercise and hypertension. *Circulation* 105:950–955, 2002
15. Flavell DM, Jamshidi Y, Hawe E, Pineda Torra I, Taskinen M-R, Frick MH, Nieminen MS, Kesäniemi A, Pasternack A, Staels B, Miller G, Humphries SE, Talmud PJ, Syvanne M: Peroxisome proliferator-activated receptor α gene variants influence progression of coronary atherosclerosis and risk of coronary artery disease. *Circulation* 105:1440–1445, 2002
16. Stephens JW, Hurel SJ, Acharya J, Humphries SE: An interaction between the interleukin-6–174G>C gene variant and urinary protein excretion influences plasma oxidative stress in subjects with type 2 diabetes. *Cardiovasc Diabetol* 3: 2, 2004
17. Alberti KG, Zimmet PZ: New diagnostic criteria and classification of diabetes–again? *Diabet Med* 15:535–536, 1998
18. Tregouet DA, Escolano S, Tiret L, Mallet A, Golmard JL: A new algorithm for haplotype-based association analysis: the Stochastic-EM algorithm. *Ann Intern Med* 68:165–177, 2004

19. Frayling TM, Wiltshire S, Hitman GA, Walker M, Levy JC, Sampson M, Groves CJ, Menzel S, McCarthy MI, Hattersley AT: Young-onset type 2 diabetes families are the major contributors to genetic loci in the Diabetes U.K. Warren 2 genome scan and identify putative novel loci on chromosomes 8q21, 21q22, and 22q11. *Diabetes* 52:1857–1863, 2003
20. Wiltshire S, Frayling TM, Groves CJ, Levy JC, Hitman GA, Sampson M, Walker M, Menzel S, Hattersley AT, Cardon LR, McCarthy MI: Evidence from a large U.K. family collection that genes influencing age of onset of type 2 diabetes map to chromosome 12p and to the MODY3/NIDDM2 locus on 12q24. *Diabetes* 53:855–860, 2004
21. Foucher C, Rattier S, Flavell DM, Talmud PJ, Humphries SE, Kastelein JJ, Ayyobi A, Pimstone S, Frohlich J, Ansquer JC, Steiner G, the DAIS Investigators: Response to micronised fenofibrate treatment is associated with the peroxisome-proliferator-activated receptors alpha G/C intron 7 polymorphism in subjects with type 2 diabetes. *Pharmacogenetics* 14:823–829, 2004
22. Pineda Torra I, Jamshidi Y, Flavell DM, Fruchart JC, Staels B: Characterization of the human PPARalpha promoter: identification of a functional nuclear receptor response element. *Mol Endocrinol* 16:1013–1028, 2002
23. Love-Gregory LD, Wasson J, Ma J, Jin CH, Glaser B, Suarez BK, Permutt MA: A common polymorphism in the upstream promoter region of the hepatocyte nuclear factor-4 α gene on chromosome 20q is associated with type 2 diabetes and appears to contribute to the evidence for linkage in an Ashkenazi Jewish population. *Diabetes* 53:1134–1140, 2004
24. Muller YL, Bogardus C, Pedersen O, Baier L: A Gly482Ser missense mutation in the peroxisome proliferator–activated receptor γ coactivator-1 is associated with altered lipid oxidation and early insulin secretion in Pima Indians. *Diabetes* 52:895–898, 2003
25. Faul MM, Grese TA: Selective RXR modulators for the treatment of type II diabetes. *Curr Opin Drug Discov Devel* 5:974–985, 2002