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Variation in the PPARα gene is associated with altered function in vitro and plasma lipid concentrations in Type II diabetic subjects

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Abstract

Aims/hypothesis. Peroxisome proliferator activated receptor alpha (PPARα) regulates genes involved in lipid metabolism, haemostasis and inflammation, in response to fatty acids and fibrates, making it a candidate gene for risk of dyslipidaemia, atherosclerosis and coronary artery disease. Plasma non-esterified fatty acids are increased in subjects with Type II (non-insulin-dependent) diabetes mellitus, suggesting that PPARα could link Type II diabetes and dyslipidaemia, and affect response to fibrates. This has been investigated in association studies in healthy and diabetic subjects and in vitro studies.
Methods. The human PPARα gene was isolated and screened for variation by single strand conformation polymorphism analysis. Genotypes were determined for 129 Type II diabetic subjects and 2508 healthy men. The association with plasma lipid concentrations was examined. The function of the V162 variant was examined in co-transfection assays.
Results. We identified two polymorphisms, one in intron 3 and a missense mutation, leucine 162 to valine, in the DNA binding domain. In Type II diabetic patients, V162 allele carriers had higher total cholesterol, HDL cholesterol and apoAI whereas intron 3 rare allele carriers had higher apoAI concentrations. By contrast, no effect was observed in healthy rare allele carriers. In vitro, the V162 variant showed greater transactivation of a reporter gene construct.
Conclusion/interpretation. Naturally occurring variation alters PPARα function, influencing plasma lipid concentrations in Type II diabetic patients but not healthy people. This demonstrates that PPARα is a link between diabetes and dyslipidaemia, and so could influence the risk of coronary artery disease, the greatest cause of morbidity and mortality in Type II diabetes. [Diabetologia (2000) 43: 673–680]

Keywords Polymorphism, Type II diabetes, nuclear receptors, PPARα, dyslipidaemia.

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Abbreviations: CAD, coronary artery disease; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; NPHS2, The Second Northwick Park Heart Study, PPARα, peroxisome proliferator-activated receptor alpha; PPRE, peroxisome proliferator response element; SENDCAP, St. Mary's, Ealing, Northwick Park Diabetes Cardiovascular Disease Prevention Study; SSCP, single strand conformational polymorphism.

Risk of coronary artery disease (CAD) is determined by a combination of genetic and environmental factors which influence plasma lipid homeostasis, haemostasis and inflammation. Peroxisome proliferator activated receptor alpha (PPARα) is a ligand-inducible transcription factor [1] which regulates the expression of genes involved in fatty acid oxidation, extracellular lipid metabolism, haemostasis [2] and inflammation [3]. Ligands for PPARα include long chain fatty acids, eicosanoids, peroxisome proliferators, non-steroidal anti-inflammatory drugs and the fibrate class of hypolipidaemic drugs [4–6]. *PPARα* is highly expressed in tissues with a high rate of fatty

acid oxidation, particularly liver, kidney, skeletal muscle and heart and has a lower expression in other tissues [7] including smooth muscle cells [8], endothelial cells [9] and macrophage [10].

Fibrates are used in the treatment of dyslipidaemia and cause a reduction in plasma triglycerides, total cholesterol and LDL-cholesterol (LDL-C) and an increase in HDL-cholesterol (HDL-C) [2]. This produces a less atherogenic lipid profile and fibrates reduce the progression of atherosclerosis [11] and the incidence of CAD in clinical trials [12,13]. Diabetic dyslipidaemia is characterised by hypertriglyceridaemia and low HDL concentrations [14], for which fibrates are an effective therapy. Type II (non-insulin-dependent) diabetes mellitus is associated with increased mortality, primarily due to CAD [15] and fibrates reduce the incidence of CAD in Type II diabetic subjects [16]. Knockout of the mouse *PPARα* gene results in abolition of the fibrate response [17], hyperlipidaemia [18] and sexually dimorphic steatosis and obesity in older animals [19]. Animals deficient in *PPARα* cannot induce expression of hepatic *CYP4A* genes in response to diabetes and starvation [20] and die of severe hypoglycaemia upon pharmacological blocking of fatty acid oxidation [21]. Therefore PPARα is a key metabolic regulator involved in the regulation of lipid and glucose homeostasis. It modulates the expression of many genes and so a minor alteration in function could have a pronounced effect, particularly in a pathophysiological situation such as Type II diabetes in which non-esterified fatty acid (NEFA) concentrations are raised [22]. The purpose of this study was to identify variation in the human *PPARα* gene and to determine the association between variants and plasma lipid concentrations in Type II diabetic and healthy subjects.

Materials and methods

Study samples. We screened for variation by SSCP in 48 healthy Caucasian men from the Second Northwick Park Heart Study (NPHS2) [23]. Polymorphisms were genotyped in 2508 participants in NPHS2, a prospective study of coronary artery disease in middle-aged (50–61 year-old) healthy men [23] and 129 Type II diabetic men and women (86 European, 43 Asian) from the St. Mary's, Ealing, Northwick Park Diabetes Cardiovascular Disease Prevention (SENDCAP) Study, a double-blind placebo-controlled study of the effect of bezafibrate treatment on plasma lipid concentrations and cardiovascular outcomes in Type II diabetes [16]. Exclusion criteria for NPHS2 included a history of Type II diabetes, unstable angina or previous myocardial infarction or stroke [23]. Inclusion criteria for the SENDCAP study included no history of CAD, age between 33 and 65 years and any of the following in a screening sample: total cholesterol 5.2 mmol/l or more, triglyceride 1.8 mmol/l or more, HDL 1.1 mmol/l or less and total cholesterol:HDL 4.7 or more. Exclusion criteria included total cholesterol or triglyceride more than 8 mmol/l, total cholesterol:HDL more than 7.2, severe hypertension, severe carotid stenosis and hypolipidaemic or anticoagulant therapy [16].

Table 1. Intron exon boundaries in the human *PPARα* gene

Exon	Donor	Acceptor	Exon
1	CACAG gtaaa	cacag TTCTG	2
2	AAGAG gtaca	cccag TAGCT	3
3	CACGG gtaag	cccag ACACG	4
4	GCAAG gtaga	cacag GGCTT	5
5	CAACG gtagg	cctag CGATT	6
6	ATCCA gtagg	tgtag CCTTT	7
7	TGGAG gtgag	actag ATCGT	8

Nucleotides in uppercase letters correspond to exon sequence and nucleotides in lowercase letters correspond to intronic sequence

Structure of the human PPARα gene. Intron-exon boundaries (Table 1) were obtained by sequencing a bacterial artificial chromosome (BAC) clone containing the human *PPARα* gene (kind gift of B. Wilkinson; Glaxo Wellcome, Research Triangle Park, N.C., USA).

Identification of polymorphisms. Polymerase chain reaction primer pairs (Table 2) were designed for the eight coding exons of the human *PPARα* gene (conditions available on request). The *PPARα* genomic sequence has since been released on Genbank, accession numbers Z94161, AL032818 and AL078611. Single strand conformational polymorphism (SSCP) analysis was done as described previously [24]. Samples were sequenced using the dRhodamine terminator cycle sequencing kit on an ABI PRISM 377 DNA sequencer (Perkin-Elmer, La Jolla, Calif., USA).

Genotype determination. Restriction enzyme digestion assays were designed for the polymorphisms. The intron 3 polymorphism creates an *Mae*II site but the *L162V* polymorphism does not alter a restriction enzyme site, therefore a forced-site assay was used. Polymerase chain reaction reactions were done in 20 µl, containing $1 \times NH_3$ buffer (16 mmol/l $[NH_4]_2SO_4$:67 mmol/l TRIS pH 8.4:0.01% Tween 20:0.02 mmol/l each deoxyribonucleoside triphosphate), 80 mmol/l $MgCl_2$, 8.3 pmol each primer, 0.2 units *Taq* polymerase. Polymerase chain reaction reactions were digested and analysed using the microtitre array diagonal gel electrophoresis (MADGE) system [25].

Statistical analysis. Genotype information was analysed using the SPSS 6.1 statistical package (Chicago, Ill., USA). Allele frequencies were determined by the gene counting method and compared using the chi-squared test. Distribution of variables were checked and log transformed where necessary. For the SENDCAP study, means of baseline plasma lipid concentrations between Europeans and Asians were compared by independent samples *t* test. The effect of *PPARα* genotype on baseline and on treatment plasma lipid concentrations was examined by ANOVA using genotype as a factor and age, BMI, sex and smoking as covariates. The effect of genotype in the bezafibrate-treated group was determined by ANOVA with genotype as a factor. For the NPHS2 study, BMI and smoking were used as covariates. A value of p 0.05 or less was considered significant.

Plasmid construction. The *V162-PPARα* expression plasmid was derived from the pSG5-hPPARα vector containing a full length *PPARα* cDNA [8]. The C→G change was introduced using the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, Calif., USA) with oligonucleotide 5′-CGATTTCACAAGTGCGTTTCTGTCGGGATG-3′ and its com-

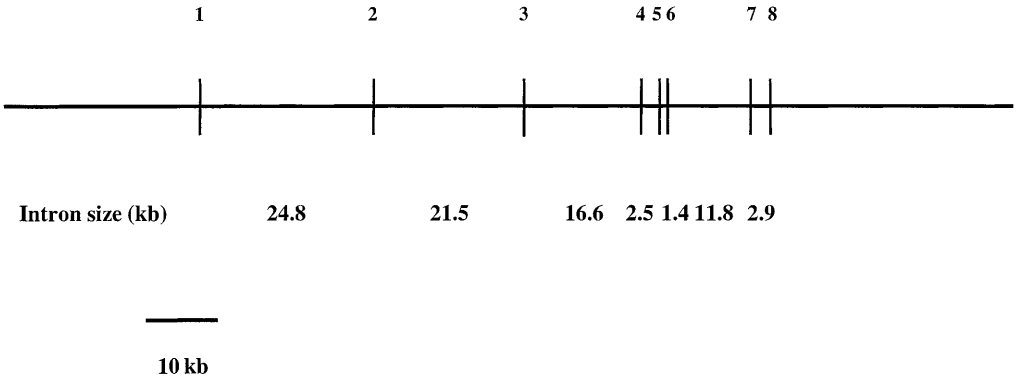

Fig. 1. Intron-exon structure of the human *PPARα* gene

plementary strand. The PPRE × 6-TKpGL3 reporter construct containing 6 copies of a peroxisome proliferator response element (PPRE) was generated by PCR using plasmid J3TKpGL3 [26] and oligonucleotides 5′-ACGTGTCGACA-CTAGTGGCTAGAGGATCTCTACCAGG-3′ and 5′-CG-ATGGTACCCTCGAGCAATGTGCTAGCGAGATCCTT-CAACCTTTACC-3′. The PCR product was digested with KpnI and SpeI and cloned into FTKpGL3 plasmid digested with KpnI and NheI. Sequence was confirmed using an ABI PRISM 377 automatic sequencer (Perkin Elmer).

Transfection assays and western blot analysis. We transfected HepG2 cells using the cationic lipid RPR 120535B (Rhone Poulenc Rorer, Vitry, France) with a mixture of plasmids containing 10 ng of the firefly luciferase reporter plasmid driven by six copies of a PPRE (PPRE × 6-TKpGL3), 30 ng of the pSG5-hPPARα expression vectors (L162- or V162-) and 2 ng of the *Renilla* luciferase reporter plasmid, pRL-SV40, as the internal control (Promega, Madison, Wis., USA). When *PPARα* plasmid was not co-transfected, pSG5 vector (Stratagene, La Jolla, Calif., USA) was added to the transfection mixture. Samples were made up to 500 ng with pBSKS plasmid (Stratagene). After 2 h cells were incubated with Wy14,643 or vehicle (dimethyl sulphoxide) in medium containing 2% Ultroser (Biosepra, Marlboro, Mass., USA). Luciferase activity was assayed 36 h later using the Dual-Luciferase reporter assay system (Promega). Results were expressed in relative light units (RLU). Cells were transfected in sextuplicate, three samples were used for the luciferase assay and three samples were analysed for hPPARα protein concentrations using a hPPARα specific antibody as described [10].

Results

The structure of the *PPARα* gene was determined by comparing the sequence of the human *PPARα* cDNA [27] and the sequence obtained from a bacterial artificial chromosome (BAC) clone containing the human *PPARα* gene. The human *PPARα* gene spans at least 88.5 kb of genomic DNA (Fig. 1). As in the mouse gene, the human gene contains at least eight exons in which exon 1, 2 and the 5′ end of exon 3 and 3′ end of exon 8 are not translated. The 3′ untranslated region could be very large as the *PPARα* mRNA is approximately 10 kb [28] and there are multiple expressed sequence tag matches to the region 3′ of exon 8. Intron positions are conserved between human and mouse and the splice acceptor and donor sites followed the gt/ag rule and were very similar to the mouse gene [29] (Table 1).

All coding exons of the human *PPARα* gene were screened by SSCP in 48 British men and variant patterns were seen in two fragments. Sequencing of the exon 2 variant showed a G to A transversion 11 bp into intron 3 (intron 3 *G/A*) at nucleotide 2176 of Genbank sequence AL032818 (sequence reversed). The exon 5 variant is a G to C transversion at the first

Table 2. PCR primers

Exon	Forward Primer	Reverse Primer	Product (bp)
2	GTCCATTCAAGCTGCTATAA	AGTGCTCGAAGGATCACCTA	230
3 5′	CCAATGGTTCCTCTTTCCTC	CTTCCAGAACTATCCTCGCC	209
3 3′	CTCGGCGGCACAACAGCA	CGGCACACTTACCCGTGATGAC	251
4	ACGGGATAGTGATGCCTGGA	AAGTAGTTGATGGTGGCGGC	258
5	AGATCCACTGTGTATTACC	GAAAATGTGGAGGGCCACCT	193
6	GCCTGTGTTTCCCCCTCCAA	AACCCAGAACAGCCGCAAAC	255
7 5′	CCTTGGTGTCCTCCTTTG	GTTTGCGAAGCCTGGGAT	207
7	ACCGTCACGGAGCTCACGGA	CTTGGGTTCCATGATATCAC	227
7 3′	TATTCGCCATGCTGTCTTCT	GTGACGTGATACCGGCAGAT	246
8 5′	AACCTCTCTCTCTTCTTTCG	ACTCCGTCTTCTTGATGAT	222
8	CTGGTGACGGAGCATGCGCA	CTACAGCTCAGACTGTCCAA	235
8 3′	CAGGAGTTCTGAAGCTGACA	CTTCCCAGTCCTGAGATTAG	204
Intron 3	Exon 2 forward	AGGAAGACACGATGCTCCTAC	189
L162V	Exon 5 forward	GTGACATCCCGACAGAAG	158

Table 3. Allele frequencies of *PPARα* polymorphisms

n	Type II diabetic Europeans (83)	Type II diabetic Asian (40)	Healthy European (2508)
Intron 3	0.064 (0.03–0.10)	0.037 (0–0.08)	0.086 (0.08–0.09)
L162V	0.066 (0.03–0.10)	0.025 (– 0.01–0.06)	0.062 (0.05–0.07)

(95 % confidence intervals)

base of codon 162 (nt 309 of reverse of Genbank sequence AL078611), creating a missense mutation which alters leucine to valine (*L162V*). Genotypes of the intron 3 and *L162V* polymorphisms were determined for 129 European and Asian Type II diabetic subjects participating in the SENDCAP study [16]. The frequency of the intron 3 *A* allele was 0.064 (95 % CI 0.03–0.10) in Europeans and 0.037 (95 % CI 0–0.08) in Asians whereas the *V162* allele frequency was 0.066 (95 % CI 0.03–0.10) in Europeans and 0.025 (95 % CI 0–0.06) in Asians (Table 2). Rare allele frequencies were not different between Type II diabetic Europeans and Asians (intron 3 $\chi^2 = 0.78$, L162V $\chi^2 = 1.82$). No homozygotes for either rare allele were detected in the SENDCAP sample and genotype distribution was not different from expected Hardy-Weinberg proportions.

Genotypes were determined for 2508 healthy middle-aged men participating in the NPHSII study. Genotype distributions were in Hardy-Weinberg proportions. The allele frequency for the intron 3 A allele was 0.086 (95 % CI 0.08–0.09) and for the *V162* allele 0.062 (95 % CI 0.05–0.07) (Table 3). The intron 3 and *L162V* polymorphisms were not in linkage disequilibrium (Delta = 0.01, $p = 1.0$). Allele frequencies were not statistically significantly different between the NPHS2 and SENDCAP studies.

Association between the PPARα variants and baseline plasma lipid concentration in European and Asian Type II diabetic subjects was examined (Table 4). Duration of diabetes, treatment (hypertension and oral glycaemic) and race did not affect the statistical significance levels of the results. Mean baseline plasma lipid concentrations were not different between Europeans and Asians and the PPARα genotypes showed effects of similar magnitude in both ethnic groups and so these ethnic groups were combined. Intron 3 *A* allele carriers had 11 % higher HDL-C ($p = 0.06$), 32 % higher HDL_2 cholesterol ($p = 0.08$) and 16 % higher apoAI ($p = 0.002$) concentrations whereas total cholesterol:HDL-C ratio was 13 % lower in *A* allele carriers than *G* allele homozygotes ($p = 0.03$). Carriers of the *V162* allele had 9 % higher plasma total cholesterol ($p = 0.04$) and non-HDL-C ($p = 0.07$) than *L162* allele homozygotes. Concentrations of HDL-C were 10 % higher ($p = 0.05$), HDL_2 cholesterol 31 % higher

Table 4. Effect of *PPARα* polymorphisms on baseline plasma lipid concentrations in the SENDCAP study

Trait (mmol/l)	Intron 3			*L162V*		
	G/G n = 113	*G/A n* = 14	*p*	*L/L n* = 109	*L/V n* = 13	*p*
Triglyceride	2.59 (2.37–2.81)	2.36 (1.88–2.84)	0.55	2.61 (2.38–2.83)	2.26 (1.70–2.83)	0.32
TC	5.85 (5.68–6.01)	5.76 (5.15–6.37)	0.61	5.77 (5.59–5.94)	6.31 (5.98–6.63)	0.04
Non-HDL-C	4.86 (4.70–5.01)	4.66 (4.13–5.18)	0.33	4.78 (4.62–4.94)	5.22 (4.85–5.59)	0.07
ApoB (g/l)	1.36 (1.29–1.43)	1.35 (1.10–1.60)	0.91	1.34 (1.27–1.42)	1.48 (1.33–1.63)	0.19
HDL-C	0.99 (0.95–1.02)	1.10 (0.96–1.25)	0.06	0.99 (0.95–1.03)	1.09 (0.95–1.23)	0.05
HDL_2	0.19 (0.17–0.21)	0.25 (0.16–0.34)	0.08	0.19 (0.17–0.21)	0.25 (0.17–0.32)	0.05
HDL_3	0.76 (0.73–0.80)	0.80 (0.70–0.90)	0.45	0.77 (0.73–0.80)	0.82 (0.71–0.93)	0.24
ApoAI (g/l)	1.35 (1.31–1.40)	1.57 (1.39–1.76)	0.002	1.36 (1.32–1.41)	1.56 (1.39–1.74)	0.003
TC:HDL-C	6.14 (5.90–6.36)	5.36 (4.88–5.84)	0.03	6.02 (5.79–6.23)	6.11 (5.18–7.03)	0.88
HbA_1 (%)	9.68 (9.30–10.06)	9.90 (8.82–10.98)	0.69	9.74 (9.36–10.14)	9.75 (8.68–10.82)	0.94
Glucose	10.61 (9.94–11.28)	10.23 (8.59–11.87)	0.72	10.65 (9.98–11.32)	10.07 (8.22–11.91)	0.55

Values are means (95 % CI) of individual median pretreatment measures. All values are mmol/l except where indicated. TC = total cholesterol

Table 5. Effect of *PPARα* polymorphisms on plasmid lipid measures in healthy middle-aged men

Trait	Intron 3			*L162V*		
	G/G + G/A (*n* = 2487)	*A/A* (*n* = 16)	*p*	*L/L + L/V* (*n* = 2500)	*V/V* (*n* = 8)	*p*
Triglyceride	1.80 (1.76–1.84)	1.39 (1.08–1.77)	0.04	1.80 (1.77–1.97)	1.61 (1.14–2.29)	0.54
Cholesterol	5.74 (5.70–5.78)	5.64 (5.15–6.13)	0.70	5.73 (5.69–5.77)	5.79 (5.09–6.48)	0.88
	(*n* = 2126)	(*n* = 14)		(*n* = 2134)	(*n* = 257)	
apoB	0.87 (0.86–0.88)	0.78 (0.67–0.91)	0.15	0.87 (0.86–0.88)	0.95 (0.75–1.21)	0.46
apoAI	1.62 (1.60–1.53)	1.73 (1.56–1.91)	0.19	1.62 (1.60–1.63)	1.56 (1.29–1.83)	0.69

Values are means (95% CI) (geometric mean for triglyceride). ApoAI and apoB concentrations were only measured in a subset of the sample

Table 6. Effect of *PPARα* polymorphisms on change in plasma lipids concentrations in bezafibrate-treated participants in the SENDCAP study

Trait (mmol/l)	Intron 3			*L162V*		
	G/G n = 53	*G/A n* = 9	*p*	*L/L n* = 50	*L/V n* = 8	*p*
TC	– 0.42 ± 0.08	– 0.71 ± 0.29	0.28	– 0.42 ± 0.09	– 0.90 ± 0.22	0.04
Non-HDL-C	– 0.51 ± 0.09	– 0.79 ± 0.31	0.36	– 0.50 ± 0.09	– 1.01 ± 0.24	0.04
ApoB (g/l)	+ 0.09 ± 0.05	– 0.07 ± 0.14	0.29	+ 0.09 ± 0.05	– 0.17 ± 0.11	0.15
HDL-C	+ 0.09 ± 0.02	+ 0.11 ± 0.03	0.92	+ 0.07 ± 0.02	+ 0.12 ± 0.06	0.39
ApoAI (g/l)	– 0.02 ± 0.03	– 0.16 ± 0.11	0.08	– 0.03 ± 0.03	– 0.15 ± 0.07	0.18
TC:HDL-C	– 0.75 ± 0.15	– 0.96 ± 0.35	0.87	– 0.72 ± 0.15	– 1.29 ± 0.31	0.19

Values are means ± SEM of individual pretreatment median values subtracted from individual post-treatment median values. TC = total cholesterol

($p = 0.05$) and apoAI 15% higher ($p = 0.003$) in *V162* carriers compared with *L162* homozygotes. No effects on fasting glucose or glycated haemoglobin (HbA_1) were observed with either polymorphism (Table 4).

We also examined the association between the *PPARα* polymorphisms and plasma total cholesterol, triglycerides, apoB and apoAI concentrations in the 2508 healthy NPHSII men. There were no statistically significant differences in plasma lipid concentrations between common allele homozygotes and heterozygotes. Homozygotes for the rare intron 3 *A* allele had 26% lower plasma triglycerides compared with common *G* allele homozygotes and *G/A* heterozygotes. No statistically significant differences in plasma lipid concentrations were observed for *V162* homozygotes (Table 5).

We also examined the association between the *PPARα* gene variants and response of plasma lipid traits to bezafibrate treatment in the SENDCAP study (Table 6). In the SENDCAP study, bezafibrate treatment caused a reduction in total cholesterol of 7.4%, non-HDL-C of 12.3% and total cholesterol:HDL-C ratios of 12% and an increase in HDL-C of 6.4% over 3 years [16]. Bezafibrate treated *V162* allele carriers showed a twofold greater lowering of total cholesterol (-0.90 vs –0.42 mmol/l, $p = 0.04$) and non-HDL-C (–1.01 vs –0.50 mmol/l, $p = 0.04$) than treated *L162* allele homozygotes. There was no statistically significant effect of intron 3 genotype on change in plasma lipid concentrations on bezafibrate treatment.

Transient transfection assays in the HepG2 human hepatoma cell line were done to compare L162-hPPARα to V162-hPPARα transcriptional activity. The V162-hPPARα variant showed similar unstimulated (vehicle) basal transcriptional activity compared with L162-PPARα on a synthetic promoter driven by multimerized copies of a PPRE. In the presence of the PPARα ligand Wy14,643, V162-PPARα showed enhanced transactivation activity compared with L162-PPARα (Fig. 2). This difference was not due to enhanced protein production by the V162-hPPARα expression vector because protein concentrations of both variants were similar after transient transfection as assessed by western blot analysis (not shown).

Discussion

The coding region and intron-exon boundaries of the *PPARα* gene were screened for variation and a missense mutation (*L162V*) and an intronic mutation (in-

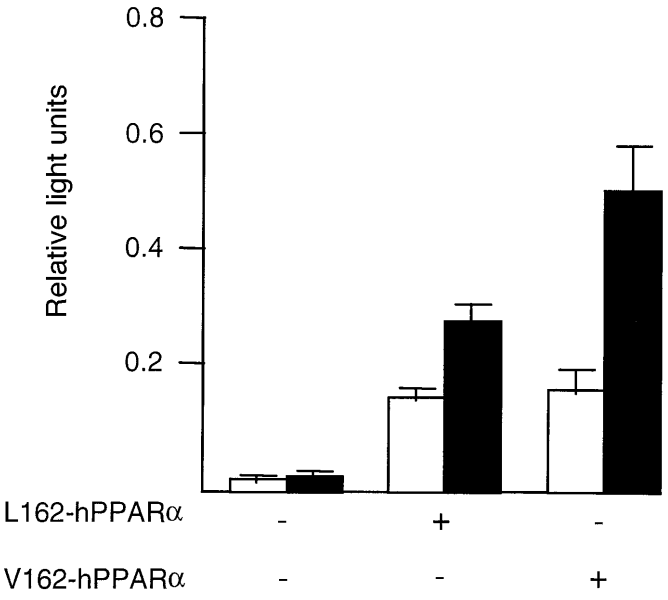

Fig. 2. The V162-PPARα isoform has increased PPRE-dependent transcriptional activity compared with the L162-PPARα isoform. HepG2 cells, transfected with a PPRE × 6-TKpGL3 reporter and either the L162-hPPARα or the V162-hPPARα expression plasmids, were incubated in the presence of Wy14,643 (50 μmol/l, ■) or vehicle (dimethyl sulphoxide, □). Values (means ± SD) represent firefly luciferase activity normalised relative to a *Renilla* luciferase transfection internal control. Three independent experiments were done with similar results

tron 3) were identified. Allele frequencies were not statistically significantly different between healthy Caucasians and Type II diabetic subjects, suggesting that they do not have a major causal role in Type II diabetes, although the SENDCAP study has limited power to detect modest effects.

In Type II diabetic subjects, the *PPARα* polymorphisms exerted a dominant effect on apoAI concentrations in rare allele carriers. By contrast, the *PPARα* polymorphisms have little effect on plasma lipids in healthy men, with no difference observed between common allele homozygotes and rare allele carriers and only intron 3 rare allele homozygotes showing an effect on plasma triglycerides. These data imply a homeostatic role for PPARα in diabetic dyslipidaemia. We hypothesise that in the healthy state, when plasma lipids and NEFA are in the normal range, PPARα activity is not limiting and so the polymorphisms, which presumably have slightly altered function, do not show an effect. In Type II diabetes, high plasma NEFA could activate PPARα, enhancing functional differences and explaining the 'dominant' effect observed. *PPARα* gene expression [30] and transcriptional activity [31] are regulated by insulin. This study confirms that PPARα is involved in diabetic dyslipidaemia.

The PPARα alters plasma lipids concentrations by regulating the expression of genes involved in fatty acid oxidation and extracellular lipid metabolism [3]. In healthy men, intron 3 rare allele homozygotes had lower plasma triglyceride than common allele homozygotes and heterozygotes. In Type II diabetic subjects carriers of either rare allele showed a trend for lower triglyceride concentrations, which might not have reached significance due to high individual variability in plasma triglyceride concentration and low sample number. Fibrates lower plasma triglyceride by PPARα-mediated down regulation of *apoCIII* gene expression [32] and the *PPARα* polymorphisms could alter regulation of the *apoCIII* gene, though plasma apoCIII concentrations were not determined in either study. In Type II diabetic subjects, carriers of either rare allele had significantly higher apoAI concentrations. The PPARα regulates expression of the human *apoAI* [33–36] and *apoAII* genes [26] and so higher HDL measures could reflect up regulation of these genes. Additionally, *V162* allele carriers had higher baseline total cholesterol and non-HDL-C, with a trend of similar magnitude for higher apoB concentrations. Fibrates lower plasma total cholesterol concentrations by lowering VLDL synthesis and increasing clearance of triglyceride-rich lipoproteins [2], although LDL-C rose in Type II diabetic subjects treated with gemfibrozil [37] and bezafibrate [38].

The effect of *PPARα* polymorphisms on response to bezafibrate in the SENDCAP study was also examined. Bezafibrate-treated *V162* allele carriers showed a greater lowering of total cholesterol and non-HDL-C than bezafibrate-treated *L162* allele homozygotes. The weak effect of the variants on response could be partly explained by the observation that bezafibrate is not PPARα specific and also interacts with PPARβ/δ and PPARγ [6].

Cardiovascular disease is the major cause of morbidity and mortality in Type II diabetic subjects in western society [15] and dyslipidaemia is predictive of CAD events [39, 40] and mortality [41] in this condition. The Multiple Risk Factor Intervention Trial showed that risk attributable to increasing cholesterol was higher in diabetic than non-diabetic subjects [15] and the 4S study suggested a greater benefit of cholesterol lowering in Type II diabetic patients [42]. Both polymorphisms are associated with higher HDL-C, and intron 3 A allele carriers also have a lower total cholesterol:HDL-C ratio, both of which are strong predictors of cardiovascular mortality in Type II diabetic subjects [39, 40]. No frequency differences were observed for ischaemic events with either polymorphism, although the SENDCAP study does not have the power to detect modest effects.

The mechanisms underlying the effect of the *L162V* variant have been investigated. In co-transfection experiments, V162-PPARα showed similar basal transactivation of a PPRE-driven reporter gene compared with L162-PPARα. When treated with the PPARα ligand Wy14,643, V162-PPARα showed,

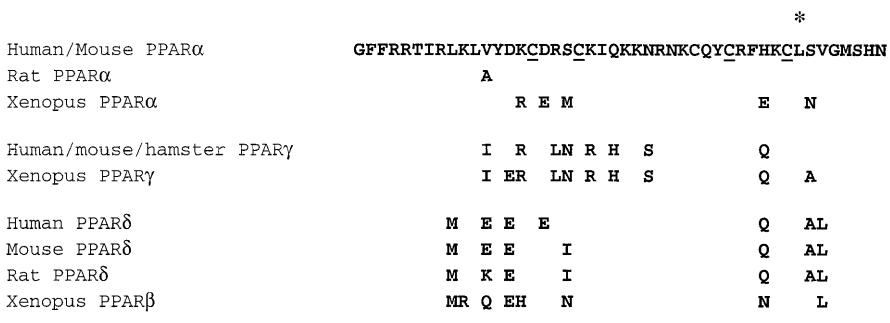

Fig. 3. Alignment of sequences encoded by exon 5 of the *PPAR* gene subfamily. Leucine 162 of PPARα is indicated by an asterisk. Cysteine residues coordinating the zinc atom of the second zinc finger are indicated by **C**

however, approximately twofold greater transactivation than L162-PPARα. Exon 5 encodes the second zinc finger of the DNA binding domain and although leucine to valine is a conservative change, L162V is situated adjacent to a cysteine which coordinates the Zn^{2+} atom of the second zinc finger (Fig. 3) and immediately upstream of a region determining the specificity and polarity of PPARα binding to different PPREs [43]. The L162V polymorphism could only differentially affect the PPREs in a subset of PPARα-regulated genes, as PPRE sequences vary in different genes [44]. The intron 3 polymorphism is situated 10 bp into intron 3, outside the consensus splice donor sequence, suggesting that it is not functional but is in allelic association with a variant in a regulatory region affecting *PPARα* gene expression and hence PPARα protein concentrations. This could explain the effects observed on HDL measures with both polymorphisms, whereas the effect on total cholesterol concentrations is only seen with the L162V polymorphism.

In conclusion, variation in the *PPARα* gene affects dyslipidaemia in Type II diabetic subjects and so could alter their risk of CAD.

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