ELECTRONIC LETTER

Evidence of differing genotypic effects of PPAR α in women and men

Q H Khan, D E Pontefract, S Iyengar, S Ye

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Peroxisome proliferator activated receptor alpha (PPAR α), a ligand inducible transcription factor of the nuclear receptor family, plays an important part in the regulation of many genes involved in lipid metabolism and atherogenesis.¹⁻³ This nuclear receptor is the cellular target of the fibrate drugs, which can lower triglyceride levels and reduce risk of acute coronary ischaemic events.⁴⁻⁵ Gender specific differences in PPAR α expression and in response to PPAR α deficiency have been observed in many animal studies.⁶⁻¹²

There is emerging evidence suggesting that, in humans, variation in the gene encoding PPAR α contributes to interindividual variability in lipid levels,¹³⁻¹⁴ body weight,¹⁵⁻¹⁶ and risk of coronary ischaemic events.¹⁷ In this study, we examined the PPAR α gene Leu162Val and intron 7 G>C polymorphisms¹⁷⁻¹⁹ in a large cohort of Caucasian patients with coronary artery disease, and found that the genotypic effects of PPAR α on plasma triglyceride level, hypertension prevalence, and myocardial infarction differed between men and women.

SUBJECTS AND METHODS

Subjects

The subjects in this study were participants in the Southampton atherosclerosis study, all having atherosclerosis in at least one coronary artery, as described previously.²⁰⁻²¹ The demographic and clinical characteristics of the subjects are summarised in table 1. Data on fasting levels of triglycerides, total cholesterol, and HDL cholesterol were available for 1017, 1110, and 630 subjects, respectively, measured in the clinical chemistry department of the Southampton General Hospital using standard quality controlled enzymatic methods. A total of 649 patients were receiving statin treatment and 16 were receiving fibrate (PPAR α agonist) treatment. The study was approved by the local research ethics committee, and all subjects gave written consent. A 10 ml blood sample was taken from each participant, and DNA was extracted using a salt precipitation method.

Determination of genotypes

Genotypes for the Leu162Val and intron 7 (G>C) polymorphisms were determined using previously described methods¹⁷⁻¹⁸ with modifications. In brief, PCR reactions were carried out in a total volume of 20 μ l, containing 20 mM Tris-Cl (pH 8.4); 50 mM KCl, 0.05% (v/v) W1; 2.5 mM (for Leu162Val) or 2 mM (for Intron 7 G>C) MgCl₂; 0.2 mM each dNTP (dATP, dCTP, dGTP, and dTTP); 5 pmol forward primers; 5 pmol reverse primers; 1 unit *Taq* polymerase; and 21 ng of genomic template DNA. The solution was overlaid with 20 μ l of paraffin and subjected to 95°C for three minutes; and to 35 cycles of: one minute of denaturation at 95°C, one minute annealing at 72°C (decreasing by 1°C per cycle to 59°C and at 59°C for the remaining cycles for Leu162Val, and decreasing by 1°C per cycle to 54°C and at 54°C for the remaining cycles for intron 7 G>C), and

Key points

- Peroxisome proliferators activated receptor alpha (PPAR α) regulates the expression of genes involved in lipid metabolism, and the pathogenesis of atherosclerosis.
- The Leu162Val and intron 7 G>C polymorphisms in the PPAR α gene were analysed in a cohort (899 men and 279 women) of Caucasian patients with coronary artery disease.
- There was a significant gender-genotype interaction in relation to plasma levels of triglyceride in the women.
- In the men, there was an interaction between statin treatment and the Leu162Val polymorphism.
- Hypertension was significantly less prevalent in female162Val allele carriers than in female non-carriers.
- Myocardial infarction was significantly more prevalent in female patients carrying the C allele of the intron 7 polymorphism than in female non-carriers.
- These results provide evidence of gender dependent effects of PPAR α genotypes on triglyceride levels and cardiovascular events.

one minute of extension at 72°C. For the Leu162Val polymorphism, the forward and reverse primers were 5'GACTCAAGCTGGTGTATGACAAGT and 5'CGTTGTGTGACATCCCGACAGAAT, respectively, and the PCR products were incubated with the restriction enzyme *Hinf*I (recognition site 5'GANTC3') which cleaved the amplicon (117 bp) from the 162Val allele into two fragments, 93 bp and 24 bp in length.¹⁸ For the intron 7 G>C polymorphism, the forward and reverse primers were 5'ACAATCACTCCTTAAATATGG and 5'AAGTAGGGACAGACAGGACCAGTA, respectively, and the PCR products were incubated with the restriction enzyme *Taq*I (recognition site 5'TCGA3') which cleaved the amplicon (266 bp) from the C allele into two fragments, 216 bp and 50 bp in length.¹⁷ The products of digestion were then separated using gel electrophoresis, and detected by Vistra Green staining.

Statistical analysis

The HWE programme (<ftp://linkage.rockefeller.edu/software/utilities>) was employed to assess whether the genotype distribution of the polymorphisms was in Hardy-Weinberg equilibrium; *t* tests and χ^2 tests, respectively, examined differences in continuous and categorical variables between genotype groups. The distribution of triglyceride levels was found to be skewed, and therefore log transformed triglyceride values were used in the analyses. Interactions between genotype and other variables were tested using a univariate

general linear model if the dependent variable was continuous, or logistic regression analysis if the dependent variable was categorical. Odds ratios were calculated using logistic regression analysis. Linkage disequilibrium between the polymorphisms and the association metric D were analysed with the use of the ASSOCIATE program (<ftp://linkage.rockefeller.edu/software/utilities>). Haplotype frequencies were estimated using the Haplotyper program which employs a Bayesian algorithm.²² Stepwise linear regression analysis and stepwise logistic regression analysis with all genotypes and haplotypes, input as independent variables, were performed to investigate which polymorphism(s) and/or haplotype(s) accounted for the association of continuous and categorical variables with the PPAR α gene.

RESULTS

A total of 1108 subjects was successfully genotyped for the Leu162Val polymorphism, and 1088 subjects were genotyped for the intron 7 G>C polymorphism. The frequencies of the Leu/Leu, Leu/Val, and Val/Val genotypes were 84.1% (n = 991), 9.6% (n = 113), and 0.3% (n = 4), respectively, and those for the G/G, G/C, and C/C genotypes were 63.7% (n = 750), 25.9% (n = 305), and 2.8% (n = 33), respectively. These genotype distributions were in concordance with Hardy–Weinberg equilibrium. The 162Val allele had a frequency of 0.055 (95% CI from 0.046 to 0.065) and the C allele had a frequency of 0.170 (95% CI from 0.155 to 0.187), both similar to those reported in other Caucasian samples.^{13–17,19} Mean age, gender ratio, smoking status, and family history of coronary artery disease did not significantly differ between genotype groups (tables 2 and 3). The percentages of patients on fibrate treatment were higher among the women than the men (p = 0.011, table 1). Since fibrate treatment might influence the genotypic effects of PPAR α , the 16 patients (eight men and eight women) receiving fibrate treatment were excluded in all following analyses. The percentages of patients receiving statin treatment were similar among men and women (p = 0.104, table 1).

Genetic effects of PPAR α on plasma lipid levels

A univariate analysis of variance using a general linear model revealed an interaction between gender and the Leu162Val polymorphism in relation to plasma triglyceride levels (p = 0.001). Analyses of the genotypic effect in male patients and female patients separately showed that, among the women, 162Val carriers had 30% lower mean triglyceride levels than non-carriers (p = 0.007, table 2) whereas, among the men, there was no significant difference in triglyceride level between the genotype groups (table 2).

In men but not in women, there was an interaction between statin treatment and the Leu162Val polymorphism in relation to triglyceride levels (p = 0.022 in male patients and p = 0.122 in female patients). Among men who were not receiving statin treatment, the 162Val carriers had a 26% higher mean triglyceride level than the non-carriers (1.85 ± 1.13 mmol/l in carriers, and 2.33 ± 1.30 mmol/l in non-carriers; p = 0.027) (fig 1); whereas among men treated with statins, the 162Val carriers had a 13% lower mean triglyceride level than the non-carriers (1.97 ± 1.15 mmol/l in carriers, and 1.72 ± 1.27 mmol/l in non-carriers; p = 0.021) (fig 1). Among the women, in contrast, triglyceride levels were about 30% lower in 162Val carriers than in non-carriers, regardless of whether or not they were receiving statin treatment (fig 1).

Among the women, there was a non-significant trend towards lower total cholesterol levels and higher HDL levels in 162Val allele carriers (table 2). Among the men, the levels were similar in different Leu162Val genotype groups. In both

Table 1 Characteristics of subjects

Characteristic	All (n = 1178)	Men (n = 899)	Women (n = 279)	Comparison*
Age (years)	63.3 (10.0)	62.5 (10.1)	65.7 (9.2)	p<0.001
Current/former smokers	74.6% (874)	81.0% (725)	54.0% (149)	p<0.001
Body mass index (kg/m ²)	27.5 (4.2)	27.5 (3.7)	27.5 (5.0)	p = 0.925
Triglyceride (mmol/l)	1.86 (1.22)	1.92 (1.17)	1.68 (1.34)	p = 0.008
Cholesterol (mmol/l)	5.11 (1.02)	5.05 (0.97)	5.31 (1.14)	p<0.001
HDL cholesterol (mmol/l)	1.28 (0.46)	1.24 (0.47)	1.45 (0.36)	p<0.001
Statin treatment	55.9% (649)	54.5% (486)	60.1% (163)	p = 0.104
Fibrate treatment	16 (3.0%)	8 (1.9%)	8 (6.9%)	p = 0.011
Hypertension	45% (530)	42.3% (899)	53.8% (150)	P = 0.001
Diabetes mellitus	13.3% (156)	12.3% (110)	16.6% (46)	p = 0.064
Myocardial infarction	48.4% (504)	51.2% (412)	38.7% (92)	p = 0.001
Family history of CAD	48.5% (571)	46.8% (421)	53.8% (150)	p = 0.043

Data shown are mean (SD) for continuous variables and percentage (n) for categorical variables.

*Comparison between male and female patients. n, number; CAD, coronary artery disease.

men and women, no significant interaction between statin treatment and Leu162Val genotype on total cholesterol or HDL levels was detected, nor was there an association between the intron 7 G>C polymorphism and triglyceride, total cholesterol, or HDL-cholesterol levels (table 2).

PPAR α genotypes in relation to hypertension, diabetes, and body mass index

Among the female subjects, the prevalence of hypertension was significantly lower in 162Val carriers than in non-carriers (p = 0.007, table 3). Among the male subjects, hypertension was slightly less prevalent in 162Val carriers than in non-carriers, but the difference was not statistically significant (table 3). There was no interaction between genotype and statin treatment in relation to hypertension, in men or women.

There was no significant association between the intron 7 G>C polymorphism and prevalence of hypertension (table 3). Neither the Leu162Val polymorphism nor the intron 7 G>C polymorphism was associated with body mass index or prevalence of diabetes mellitus (tables 2 and 3).

PPAR α genotypes in relation to severity of atherosclerosis and risk of myocardial infarction

Among the women, the prevalence of myocardial infarction was higher in those carrying the C allele of the intron 7 polymorphism than in non-carriers (p = 0.019, table 3). In addition, the frequencies of C allele carriers were highest among those who had a history of both myocardial infarction and unstable angina, intermediate among those who had either myocardial infarction or unstable angina, and lowest among those had neither of these phenotypes (46.8%, 35.2%, and 29.0% respectively, p = 0.044). Among the men, myocardial infarction was slightly more prevalent in C allele carriers than in non-carriers, but the difference was not statistically significant (table 3).

For both genders, the genotypic effect on myocardial infarction was more pronounced among those who were not receiving statin treatment. Among untreated women, the

Table 2 Continuous variables in different genotype groups according to gender

Variable	Leu/Leu		Leu/Val + Val/Val		p Value	G/G		G/C + C/C		p Value
	Mean (SD)	n	Mean (SD)	n		Mean (SD)	n	Mean (SD)	n	
Women*										
Triglyceride (mmol/l)	1.68 (0.87)	199	1.18 (0.40)	22	0.007	1.61 (0.84)	140	1.67 (0.89)	75	0.799
Cholesterol (mmol/l)	5.37 (1.16)	215	5.07 (1.21)	24	0.242	5.34 (1.11)	151	5.39 (1.27)	82	0.799
HDL (mmol/l)	1.43 (0.36)	94	1.59 (0.35)	12	0.162	1.44 (0.39)	68	1.42 (0.30)	38	0.792
BMI (kg/m ²)	27.54 (4.97)	223	27.67 (5.28)	26	0.896	27.59 (4.85)	155	27.56 (5.21)	88	0.965
Age (years)	65.89 (8.95)	225	62.94 (11.02)	26	0.122	65.51 (9.11)	157	65.66 (9.66)	88	0.904
Men*										
Triglyceride (mmol/l)	1.91 (1.15)	644	1.96 (1.31)	77	0.770	1.89 (1.15)	496	1.98 (1.15)	216	0.224
Cholesterol (mmol/l)	5.07 (0.97)	697	5.00 (1.07)	84	0.538	5.08 (0.99)	536	5.05 (0.92)	235	0.556
HDL (mmol/l)	1.24 (0.49)	434	1.24 (0.26)	45	0.988	1.26 (0.54)	327	1.19 (0.26)	139	0.129
BMI (kg/m ²)	27.52 (3.89)	740	27.39 (3.49)	90	0.775	27.54 (3.93)	572	27.59 (4.15)	245	0.859
Age (years)	62.77 (9.99)	752	61.70 (11.08)	90	0.345	62.78 (9.90)	580	62.19 (10.49)	258	0.443

*Eight men and eight women receiving fibrate treatment were excluded. Emboldened value is statistically significant. SD, standard deviation; n, number; HDL, high density cholesterol; BMI, body mass index.

odds ratio for myocardial infarction was 2.27 (95% CI from 1.01 to 5.11) for C allele carriers compared with non-carriers, whereas among women receiving statin treatment, the odds ratio was 1.67 (95% CI from 0.82 to 3.40). Among untreated and treated men, the odds ratios were 1.56 (95% CI from 0.99 to 2.48) and 0.86 (95% CI from 0.57 to 1.28), respectively.

There was no significant difference in the number of coronary arteries with >50% stenosis between genotype groups of the Leu162Val or intron 7 G>C polymorphism (table 3).

Haplotype analysis

The Leu162Val and intron 7 G>C polymorphisms were in strong linkage disequilibrium, with the 162Val allele being linked with the C allele ($D = 0.0248$; $p < 10^{-6}$). The frequencies of the Leu-G, Leu-C, Val-G, and Val-C haplotypes were 0.776, 0.160, 0.028, and 0.036, respectively. Among the women, the mean triglyceride level was lower in carriers of the Val-C haplotype compared with non-carriers ($p = 0.033$, table 4). The other phenotypes studied did not significantly differ between carriers and non-carriers (tables 4 and 5). Stepwise regression analyses showed that none of the haplotypes had a more significant association with triglyceride level or hypertension than the 162Val allele, and that none of the haplotypes had a more significant association with myocardial infarction than the C allele of the intron 7 polymorphism.

DISCUSSION

The main finding of this study is that in persons with coronary artery disease and without fibrate or statin treatment, there is a gender dependent genotypic effect of PPAR α on triglyceride levels, such that the 162Val allele is associated with a lower triglyceride level in female patients but with a higher triglyceride level in male patients. Gender specific differences in PPAR α expression and in PPAR α genotypic effect on lipid metabolism have been demonstrated in a number of animal studies.⁶⁻¹² For example—it has been shown that in rodents, PPAR α expression levels in the liver are higher in males than in females, and that inactivating the PPAR α gene results in an increased hepatic triglyceride secretion rate in females but does not affect this rate in males.^{7,8} It has also been shown that gonadectomy abolishes the differences in hepatic PPAR α expression level between male and female rats, suggesting an influence of sex hormones on PPAR α expression.⁸ The results of the present study provide evidence of a gender dependent genotypic effect of PPAR α in humans. To our knowledge, there has been no reported study of PPAR α gene polymorphisms in coronary heart disease patients with parallel analyses in men and women. A previous study of male patients with coronary heart disease who were not receiving lipid lowering agents showed that 162Val carriers had higher plasma triglyceride levels than non-carriers,²³ which is consistent with the findings in the male patients in the present study. Several reported studies of PPAR α polymorphisms have been

Table 3 Categorical variables in different genotype groups according to gender

Variable	Leu/Leu	Leu/Val + Val/Val	p Value	G/G	G/C + C/C	p Value
Women*	n = 225	n = 26		n = 157	n = 88	
Hypertension	54.7%	26.9%	0.007	54.8%	50.0%	0.472
Diabetes	13.8%	19.2%	0.459	14.2%	17.0%	0.552
Smoking	53.8%	50.0%	0.954	50.6%	56.8%	0.336
Family CAD history	53.8%	46.2%	0.461	54.8%	51.1%	0.584
Diseased vessels (n)†	46.7/34.7/18.7%	53.8/34.6/11.5%	0.633	45.9/36.9/17.2%	50.0/31.8/18.2%	0.720
Unstable angina	28.9%	38.5%	0.313	28.0%	34.1%	0.321
Myocardial infarction	46.9%	53.8%	0.500	42.3%	58.0%	0.019
Men*	n = 752	n = 90		n = 580	n = 248	
Hypertension	43.0%	36.7%	0.254	42.8%	40.3%	0.515
Diabetes	12.4%	9.0%	0.352	11.4%	14.2%	0.269
Smokers	81.3%	76.1%	0.242	81.2%	79.7%	0.617
Family CAD history	46.8%	51.1%	0.440	54.8%	51.1%	0.584
Diseased vessels (n)†	39.2/33.2/27.5%	37.8/30.0/32.2%	0.627	39.7/34.0/26.4%	36.7/30.2/33.1%	0.145
Unstable angina	27.9%	32.2%	0.393	28.8%	26.2%	0.448
Myocardial infarction	56.3%	52.2%	0.468	54.8%	58.5%	0.334

*Eight men and eight women receiving fibrate treatment were excluded. †Percentages of patients with one, two, or three coronary arteries with >50% stenosis. Emboldened p value is statistically significant. n, number; CAD, coronary artery disease.

Figure 1 Plasma triglyceride levels in different genotype groups stratified by statin treatment. Patients (eight men and eight women) receiving fibrate treatment were excluded. Data shown are mean $\pm$ standard error of mean.

conducted in healthy subjects, general population subjects, and diabetic patients. A study in healthy Japanese individuals showed that a PPAR α gene polymorphism—that is, Val227Ala, which is present in Japanese persons but not in Caucasians—is associated with triglyceride levels among women but not among men.²⁴ However, a study of the Leu162Val polymorphism in a population sample of Caucasians did not show significant differences in triglyceride levels between the different genotype groups in men or women.¹³ Given that lipid levels are influenced by many genetic and environment factors, it is possible that the genotypic effects of PPAR α can vary, depending on the combinations of other factors.

Another key finding of this study was that in men with coronary heart disease, there was an interaction between PPAR α genotype and statin treatment on triglyceride levels. Thus, in the male patients who were not receiving lipid lowering treatment, triglyceride levels were higher in 162Val carriers than in non-carriers; however, among male patients receiving statin treatment, 162Val carriers had a lower mean triglyceride level than non-carriers (fig 1). This could be interpreted as a substantial triglyceride lowering effect of statins in male 162Val carriers but not in male non-carriers (fig 2). It has been shown that PPAR α agonists (fibrates)

Table 4 Continuous variables in carriers and non-carriers of the Val-C haplotype

Variable	Non-carriers		Carriers		p Value
	Mean (SD)	n	Mean (SD)	n	
Women*					
Triglyceride (mmol/l)	1.71 (1.39)	223	1.15 (0.37)	15	0.033
Cholesterol (mmol/l)	5.32 (1.15)	241	5.21 (1.34)	16	0.713
HDL (mmol/l)	1.44 (0.37)	109	1.60 (0.36)	7	0.257
BMI (kg/m ²)	27.44 (4.87)	250	28.46 (5.58)	18	0.397
Age (years)	65.75 (8.95)	253	63.17 (12.22)	18	0.253
Men*					
Triglyceride (mmol/l)	1.91 (1.15)	709	1.92 (1.24)	56	0.754
Cholesterol (mmol/l)	5.06 (0.97)	767	4.93 (0.99)	62	0.320
HDL (mmol/l)	1.24 (0.48)	471	1.23 (0.26)	35	0.867
BMI (kg/m ²)	27.50 (3.99)	815	27.65 (3.65)	64	0.770
Age (years)	62.75 (10.02)	827	60.54 (10.80)	64	0.091

*Eight men and eight women receiving fibrate treatment were excluded. Emboldened p value is statistically significant. SD, standard deviation; n, number; HDL, high density cholesterol; BMI, body mass index.

Figure 2 Plasma triglyceride levels in patients treated and untreated with statins stratified by genotypes. Eight women and eight men receiving fibrate treatment were excluded. Data shown are mean $\pm$ standard error of mean.

have greater effects in lowering plasma triglyceride level and raising HDL cholesterol levels in 162Val carriers than in non-carriers.^{25–26} In our patient cohort, the number of subjects who were receiving PPAR α agonist treatment (eight women and eight men) was too small to analyse whether these drugs had different effects in different genotype groups. However, as described above, we found that in the male subjects there was an interaction between Leu162Val genotypes and statin (3-hydroxy-3-methylglutaryl coenzyme A reductase) treatment in determining plasma triglyceride levels. This genotype-statin interaction was not observed in the female subjects. The mechanism for the interaction between PPAR α genotype and statin treatment in the men is unclear. Laboratory experiments have shown that statins can increase the expression and activity of PPAR α , and that some of the effects of statins on lipid levels might be mediated by a PPAR α dependent pathway.^{27–29} It is possible that clinically these effects are influenced by gender and PPAR α genotype.

At the blood vessel wall, PPAR α regulates the expression of a number of genes involved in the pathogenesis of atherosclerosis and ischaemic clinical events.³⁰ Clinical trials have shown that fibrate drugs which are PPAR α agonists can

Table 5 Categorical variables in carriers and non-carriers of the Val-C haplotype

Variable	Carriers	Non-carriers	p Value
Women*			
Hypertension	54.5%	33.3%	0.081
Diabetes	15.5%	22.2%	0.455
Smokers	54.4%	50.0%	0.717
Family history of CAD	53.8%	44.4%	0.444
No. of diseased vessels†	47.0/34.8/18.2%	55.6/33.3/11.1%	0.690
Unstable angina	28.9%	44.4%	0.163
Myocardial infarction	47.2%	55.6%	0.494
Men*			
Hypertension	42.3%	37.5%	0.451
Diabetes	12.1%	11.1%	0.812
Smokers	81.0%	79.0%	0.709
Family history of CAD	46.6%	50.0%	0.595
No. of diseased vessels†	39.3/33.1/27.6%	34.4/32.8/32.8%	0.620
Unstable angina	27.7%	34.4%	0.252
Myocardial infarction	56.1%	56.3%	0.982

*Eight men and eight women receiving fibrate treatment were excluded. †Percentages of patients with one, two, or three coronary arteries with >50% stenosis. n, number; CAD, coronary artery disease.

reduce the rates of acute coronary ischaemic events in individuals with dyslipidaemia by over 20%.^{4,5} Recently, Flavell *et al* showed an association between the C allele of the PPAR α gene intron 7 polymorphism and increased risk of ischaemic heart disease in a prospective study.¹⁷ The results of our study provide further evidence of this association and indicate that this genotypic effect is greater in women than in men.

An association between the Leu/Leu genotype and higher prevalence of hypertension, particularly in women, was also observed in this study. It has been shown in animal experiments that PPAR α agonist fenofibrate can improve endothelium and also nitric oxide mediated vasodilation.³¹ Since the 162Leu isoform has a lower transcriptional activity than the 162Val isoform,¹⁹ it is possible that there is a decrease in vasodilation in persons with the Leu/Leu genotype, which may explain the association between the Leu/Leu genotype and hypertension.

In a recent study of healthy individuals, carriers of the 162Val allele were found to have lower body mass index values than 162Leu homozygotes, and this difference was more pronounced among women.¹⁵ In another study, the 162Val allele was associated with lower body mass in persons with type 2 diabetes but not in healthy individuals, patients attending lipid clinics, or morbidly obese patients who underwent gastric banding surgery.¹⁶ In the present study, no significant difference in body mass index was found between the genotype groups. The differing findings of these studies could be related to differences in the characteristics of the participants. Since coronary artery disease is caused by interactions between multiple environmental and genetic factors, it is likely that the genetic backgrounds and the exposures to environmental risk factors, such as high fat diets, of the subjects with coronary artery disease of our study were different from those of the subjects of the other two studies mentioned above.^{15,16} These genetic and environmental factors might influence the genotypic effects of PPAR α on adiposity.

The Leu162Val and intron 7 G>C polymorphisms are in strong linkage disequilibrium, with the Val allele linked with the C allele. These two polymorphisms define four haplotypes, namely Leu-G, Leu-C, Val-G, and Val-C. None of these haplotypes was found to be more significantly associated with plasma triglyceride level or prevalence of hypertension than the Leu162Val polymorphism, and none of the haplotypes had a more significant association with myocardial infarction than the intron 7 G>C polymorphism, suggesting that the Leu162Val and intron 7 G>C polymorphisms do not have an additive effect on these traits and do not mark the effects of another genetic variant through linkage disequilibrium.

In summary, in this study of a large cohort of patients with coronary artery disease, we found that the PPAR α gene Leu162Val polymorphism was associated with plasma levels of triglyceride in a gender dependent manner and that, in the men, there was an interaction between this genetic polymorphism and statin treatment in determining triglyceride levels. We also detected an association between the Leu162Val polymorphism and prevalence of hypertension, and an association between the intron 7 G>C polymorphism and myocardial infarction, although the latter was not highly significant statistically. The subjects of this study were recruited from patients with coronary artery disease consecutively undergoing coronary angiography. In a manner consistent with the different susceptibilities to coronary artery disease of the two genders, men and women differed in some characteristics such as age, percentage of smokers, and family history of coronary artery disease. Thus, it is possible that men and women might be influenced by

differing environmental and genetic factors. Nevertheless, the findings of this study are in agreement with the pleiotropic effects of PPAR α on lipid metabolism and the pathophysiology of the cardiovascular system,³ and indicate that the genotypic effects of PPAR α on men with coronary artery disease and women with coronary artery disease do differ. Gender specific effects of another gene involved in lipid metabolism, the apolipoprotein E gene, have been reported previously.³² The gender dependent effects of these genes might be a mechanism contributing to the differences in lipid levels and incidence of cardiovascular diseases between men and women.

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Authors' affiliations

Q H Khan, Shu Ye, Human Genetics Division, School of Medicine, University of Southampton, UK

D E Pontefract, S lyengar, Wessex Cardiac Unit, Southampton General Hospital, UK

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Correspondence to: Dr S Ye, Human Genetics Division, Duthie Building (mp808), Southampton General Hospital, Southampton SO16 6YD, UK; Shu.Ye@soton.ac.uk

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