LETTER TO JMG

Effect of the peroxisome proliferator activated receptor-γ gene Pro12Ala variant on body mass index: a meta-analysis

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Peroxisome proliferator activated receptor-γ (PPAR-γ) is a transcription factor abundantly expressed in adipocytes, and plays a key role in the regulation of adipocyte differentiation, lipid storage, glucose homeostasis, and blood pressure.[1] Several rare, dominant negative mutations have been detected in three families with severe insulin resistance, diabetes, and hypertension,[2] while a rare, gain of function mutation has been detected in four unrelated individuals with extreme obesity.[3] In addition, a meta-analysis based on data from over 3000 individuals has shown that a common polymorphism in the PPAR-γ gene has an influence on individual susceptibility to type 2 diabetes.[4–8] Taken together, these findings indicate that rare, severe mutations of the PPAR-γ gene may cause extreme metabolic syndrome in a small number of patients, while common, mild variants of this gene may contribute to the common, multifactorial forms of these disorders.

Systematic screening of the PPAR-γ gene for sequence variants has identified two common polymorphisms.[9–11] These are, respectively, a C→G substitution in exon B resulting in the conversion of proline to alanine at residue 12 of the PPAR-γ protein, and a synonymous C→T substitution at nucleotide position 161 in exon 6.[9–11] In this study, we examined these genetic variants in relation to body mass index (BMI) in a large cohort of white British patients with coronary artery disease (CAD), a disease closely related to obesity, dyslipidaemia, diabetes, and hypertension.

A number of previous studies have examined the Pro12Ala polymorphism in relation to BMI;[12] however, the results of these studies were not totally consistent. The disparate findings may be partly attributed to insufficient power in some studies. In addition, it has been suggested that the Pro12Ala polymorphism has an effect on BMI in individuals with marked obesity, and that this effect is not apparent in lean individuals.[13] Thus, we carried out meta-analyses using 40 datasets from 30 independent studies, to examine the effect of the Pro12Ala polymorphism on BMI in all subjects ($n = 19136$) in these studies and then separately in obese subjects ($n = 8365$) and lean subjects ($n = 10771$).

Key points

- A number of studies have shown an association between the Pro12Ala polymorphism in the peroxisome proliferator activated receptor-γ (PPAR-γ) gene and obesity, but this association has not been detected in some other studies.
- We studied this polymorphism in a large cohort ($n = 1170$) of white British patients with coronary artery disease and found that subjects homozygous for the Ala12 allele had significantly higher mean body mass index (BMI) than subjects with other genotypes ($p = 0.02$).
- We performed a meta-analysis using data from 30 independent studies with a total number of 19136 subjects. In the samples with a mean BMI value ≥ 27, Ala12 allele carriers had a significantly higher BMI than non-carriers ($p = 0.0006$). This difference was not detected in the samples with a mean BMI value < 27. A further analysis using data from the publications in which BMI for the three genotype groups were presented separately showed that the Ala12 homozygotes had significantly higher BMI than heterozygotes and Pro12 homozygotes.
- These data support the hypothesis that the Pro12Ala polymorphism is a genetic modifier of obesity and are consistent with a recessive model for the Ala12 allele.

SUBJECTS AND METHODS

Subjects

We studied a cohort of 1170 consecutive white patients with angiographically documented CAD, recruited from the Cardiothoracic Unit, Southampton General Hospital. All patients had >50% diameter stenosis in at least one major epicardial coronary artery. Anthropometric, clinical and biochemical data were recorded in a database, including age, gender, weight, height, occupation, smoking habit and number of cigarettes consumed per day by each smoker, the presence or absence of hyperlipidaemia (defined as cholesterol >5.2 mmol/l and/or triglyceride >3 mmol/l), current medications (particularly the use of lipid lowering drugs), the presence or absence of hypertension (defined as diastolic blood pressure >95 mmHg and/or systolic blood pressure >160 mmHg), the presence or absence of type 1 or type 2 diabetes, the presence or absence of previous myocardial infarction, and the presence or absence of coronary heart disease in first degree relatives under 65 years of age. Total cholesterol and triglyceride levels were measured by the clinical chemistry department of Southampton General Hospital using standard quality controlled enzymatic methods. The study was approved by the local ethics committee and all subjects gave written consent. A 10 ml blood sample was taken from each subject and DNA was extracted using a salt precipitation method.[14]

Genotyping

Genotype analysis of the samples was carried out using the Tetra-primer ARMS-PCR method, in which two pairs of

Abbreviations: BMI, body mass index; CAD, coronary artery disease; PPAR-γ, peroxisome proliferator activated receptor-γ

Table 1 PCR primers and conditions

Polymorphism	Primer sequence	T_m	Touch-down	Annealing	Mg^{2+}	Amplicon size
Pro12Ala	Forward inner primer (C allele): 5′-GAAACTCTGGGAGATTCTCCTATTGTCC	65°C	Yes	61°C	0.5 mmol/l	221 bp (C allele)
	Reverse inner primer (G allele): 5′-GTATCAGTGAAGGAATCGCTTTCAGC	65°C				288 bp (G allele)
	Forward outer primer: 5′-AACTTTTTGTCACAGCTGGCTCCTAATA	65°C				455 bp (from two outer primers)
	Reverse outer primer: 5′-CAACGAGCTAAGCATTAAAATACTGGA	65°C				
C161T	Forward inner primer (C allele): 5′-AGACCTCAGACAGATTGTCACGGAAAAC	69°C	No	65°C	0.5 mmol/l	165 bp (C allele)
	Reverse inner primer (T allele): 5′-TCTTGATCACCTGCAGTAGCTGCCCA	71°C				202 bp (T allele)
	Forward outer primer: 5′-ATTATTCTCAGTGGAGACCGCCCAGGTT	70°C				312 bp (from two outer primers)
	Reverse outer primer: 5′-AGTGCAACTGGAAGAAGGGAAATGTTGG	70°C				

primers are used to amplify, respectively, the two different alleles of a single nucleotide polymorphism in a single PCR reaction.[15] Each PCR reaction was carried out in a total volume of 10 µl, containing 30 ng of genomic DNA, 10 × PCR buffer, 0.5 mmol/l $MgCl_2$, 0.2 mmol/l dNTP, 10 pmol of each inner primer (see table 1), 1 pmol of each outer primer (see table 1), and 1 U of *Taq* polymerase. The solution was subjected to 95°C for 3 min, followed by 30 cycles of 95°C for 1 min, 61°C or 65°C for 1 min (see table 1) and 72°C for 1 min, and a final extension at 72°C for 3 min. PCR products were subjected to electrophoresis on an 8% polyacrylamide gel and stained with Vistra green.

Statistical analysis

The HWE program was used to test whether the observed genotype distributions deviated from the Hardy–Weinberg equilibrium. Linkage disequilibrium between the Pro12Ala and C161T polymorphisms was analysed using the Associate program (both programs available from ftp://linkage.rockefeller.edu/software/utilities). The effect of PPAR-γ polymorphisms on BMI was analysed by one way analysis of variance and Student's *t* test, with both polymorphisms as factor variables, and age, gender, diabetes, and hypertension as covariates in a general linear model.

Meta-analyses

Clinical studies in which the PPAR-γ gene Pro12Ala polymorphism had been related to BMI were identified by electronic searches of PubMed. A total of 29 studies published before August 2002 were identified.[5 10 12 13 16–39] A total of 40 datasets, including 39 from the 29 previous studies and one from the present study were included in the meta-analyses. Studies that had analysed the Pro12Ala polymorphism but could not be included in this meta-analysis were those in which there were no available BMI data in different Pro12Ala genotype groups and those in which different Pro12Ala genotype groups matched for BMI were recruited for other purposes. The meta-analyses were carried out firstly in all subjects and then separately in obese subjects and lean subjects, using the StatsDirect program, which gives *g* (modified Glass statistic with pooled sample standard deviation), d^+ (pooled mean effect size estimate),[40] Q (non-combinability) statistic, and DerSimonian–Laird random effects analysis.[41] Funnel plots were used to detect evidence of possible bias resulting from selective publication of positive studies.

RESULTS

Effect of the Pro12Ala variant on body mass index in a large cohort of white British subjects

In this study, we first examined the two common polymorphisms in the PPAR-γ gene in a cohort of 1170 white British patients with CAD. In this sample, the frequency of the Ala12 allele of the Pro12Ala polymorphism was 0.13 and that of the T allele of the C161T polymorphism was 0.14, consistent with the findings in several other studies of white samples.[12 18 19 23] The genotype distributions (Pro/Pro = 817, Pro/Ala = 252, Ala/Ala = 19; and C/C = 822, C/T = 257, T/T = 25) were in agreement with Hardy–Weinberg equilibrium. The two polymorphisms were in strong linkage disequilibrium, with the Ala allele of the Pro12Ala polymorphism being linked to the T allele of the C161T polymorphism ($D' = 0.675$, $p<0.0000001$).

Mean BMI in this sample as a whole was 27.51 (SD 4.25). One way analysis of variance showed that there was a significant difference in mean BMI between Pro12Ala genotype groups ($p = 0.020$, table 2), with the Ala/Ala group having higher mean BMI than both the Pro/Pro and Pro/Ala groups ($p = 0.014$ and 0.019 respectively, table 2). The analysis indicated that the Pro12Ala polymorphism accounted for approximately 1% of the total variance of BMI in this sample ($R^2 = 0.008$). There was a similar trend towards greater BMI in individuals with the T/T genotype for the C161T polymorphism, but the differences were not statistically significant (table 2). There was no interaction between the two polymorphisms. Age, gender, plasma levels of triglyceride, and rates of hypertension did not significantly differ among the genotype groups (table 3). Plasma cholesterol levels were highest in the Ala/Ala group, inter-

Table 2 Mean (SD) BMI in different PPAR-γ genotype groups

Genotype	BMI (kg/m²)	p value*
Ala/Ala (*n* = 19)	29.65 (4.68)	0.020 for Ala/Ala *v* Ala/Pro *v* Pro/Pro
Pro/Ala (*n* = 252)	27.49 (4.11)	0.014 for Ala/Ala *v* Pro/Pro
Pro/Pro (*n* = 817)	27.42 (4.29)	0.019 for Ala/Ala *v* Pro/Ala 0.957 for Pro/Pro *v* Pro/Ala
T/T (*n* = 25)	28.74 (5.13)	0.190 for T/T *v* C/T *v* C/C
C/T (*n* = 257)	27.40 (4.35)	0.137 for T/T *v* C/C
C/C (*n* = 822)	27.47 (4.19)	0.124 for T/T *v* C/T 0.639 for C/C *v* C/T

Adjusted for age, gender, type 1 and 2 diabetes, and hypertension.

Table 3 Characteristics of subjects in different genotype groups

	Age (years)	Male gender (%)	Cholesterol (mmol/L)	Triglyceride (mmol/L)	Hyper-tension (%)
Ala/Ala (*n*=19)	62.76 (9.82)	84.2	5.62 (1.04)	1.87 (1.09)	31.6
Pro/Ala (*n*=252)	63.32 (10.00)	75.0	5.02 (1.01)	1.90 (1.55)	46.8
Pro/Pro (*n*=817)	63.34 (9.87)	76.1	5.13 (1.01)	1.85 (1.12)	44.4
p value	0.968	0.654	0.047	0.889	0.405
T/T (*n*=25)	62.58 (8.90)	88.0	5.16 (1.20)	1.85 (1.02)	44.0
C/T (*n*=257)	62.75 (9.90)	72.8	5.11 (1.03)	1.84 (1.04)	49.0
C/C (*n*=822)	63.31 (10.01)	77.1	5.12 (1.00)	1.86 (1.13)	42.6
p value	0.699	0.136	0.975	0.985	0.192

Mean (SD) is shown for continuous variables, and percentage shown for categorical variables.

mediate in the Pro/Pro group, and lowest in heterozygotes, but the difference was only borderline significant (p = 0.047, table 3).

Meta-analyses of the Pro12Ala polymorphism in relation to body mass index

Because the results of previous studies of the Pro12Ala polymorphism in relation to BMI were not totally consistent, we used the meta-analysis approach to examine the overall effect of this polymorphism in these studies. A meta-analysis using 40 datasets from 30 independent studies with a total number of 19136 subjects showed that overall, BMI was 0.07 d^+ units (95% confidence interval, 0.01 to 0.12) higher in individuals ($n = 4358$) carrying the Ala12 allele than in those ($n = 14778$) who were homozygous for the Pro12 allele (p = 0.019, fig 1). However, the analysis also showed that there was significant heterogeneity among the results of the different studies (p<0.0001, fig 1). Funnel plots of effect estimates against sample size showed symmetrical distribution with intercept = 0.57 (p = 0.327), indicating that there was no apparent publication bias.

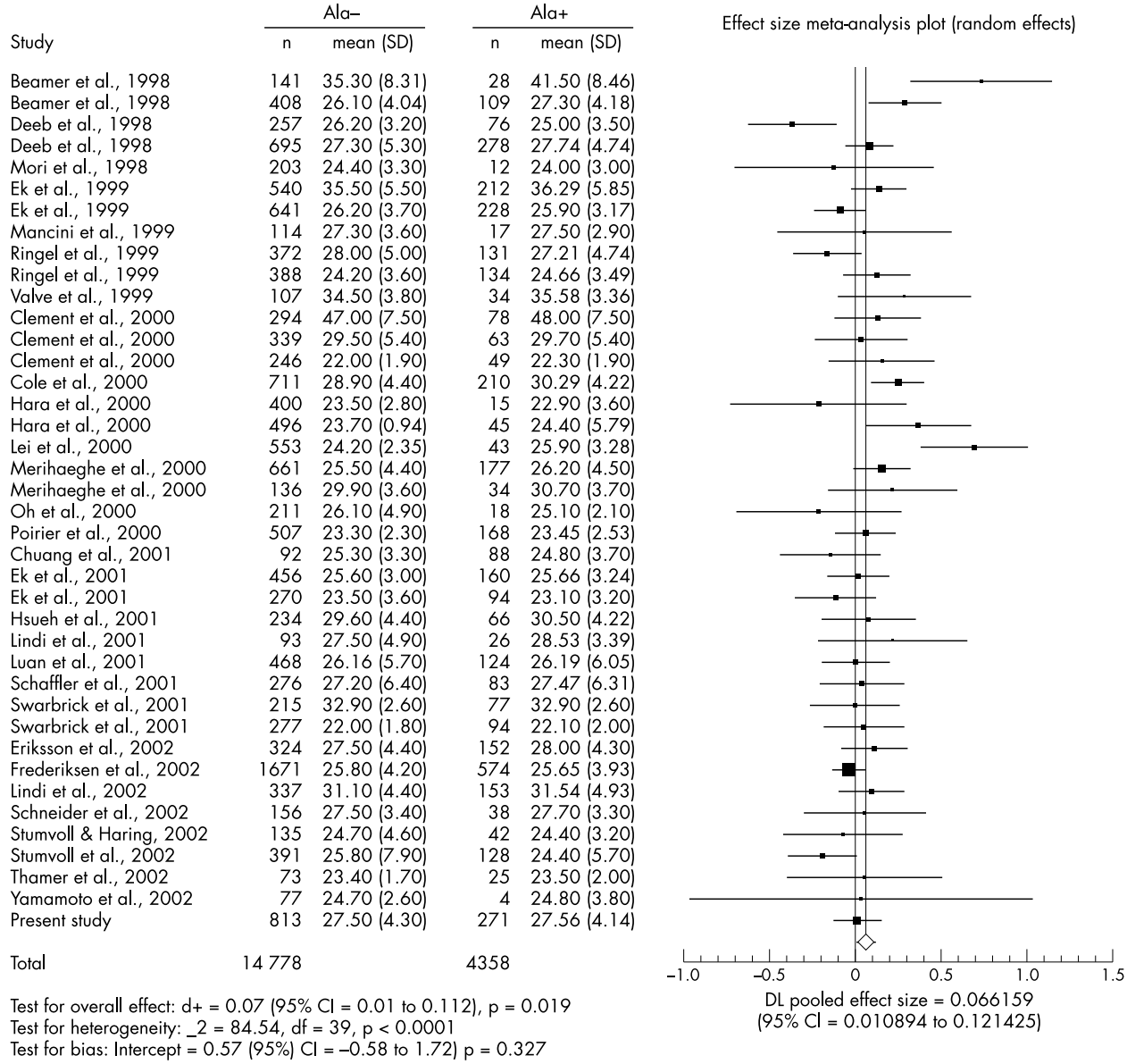
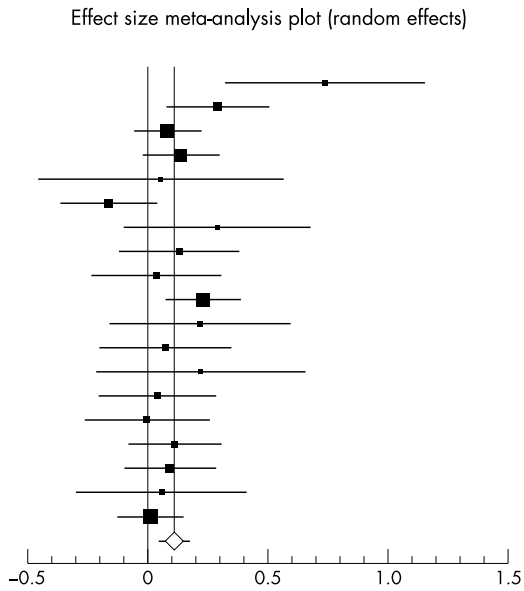


Figure 1 Meta-analysis of the effect Pro12Ala on BMI in all samples.

Study	Ala– n	Ala– mean (SD)	Ala+ n	Ala+ mean (SD)
Beamer et al., 1998	141	35.30 (8.31)	28	41.50 (8.46)
Beamer et al., 1998	408	26.10 (4.04)	109	27.30 (4.18)
Deeb et al., 1998	695	27.30 (5.30)	278	27.74 (4.74)
Ek et al., 1999	540	35.50 (5.50)	212	36.29 (5.85)
Mancini et al., 1999	114	27.30 (3.60)	17	27.50 (2.90)
Ringel et al., 1999	372	28.00 (5.00)	131	27.21 (4.74)
Valve et al., 1999	107	34.50 (3.80)	34	35.58 (3.36)
Clement et al., 2000	294	47.00 (7.50)	78	48.00 (7.50)
Clement et al., 2000	339	29.50 (5.40)	63	29.70 (5.40)
Cole et al., 2000	711	28.90 (4.40)	210	30.29 (4.22)
Merihaeghe et al., 2000	136	29.90 (3.60)	34	30.70 (3.70)
Hsueh et al., 2001	234	29.60 (4.40)	66	30.05 (4.22)
Lindi et al., 2001	93	27.50 (4.90)	26	28.53 (3.39)
Schaffler et al., 2001	276	27.20 (6.40)	83	27.47 (6.31)
Swarbrick et al., 2001	215	32.90 (2.60)	77	32.90 (2.60)
Eriksson et al., 2002	324	27.50 (4.40)	152	28.00 (4.30)
Lindi et al., 2002	337	31.10 (4.40)	153	31.54 (4.93)
Schneider et al., 2002	156	27.50 (3.40)	38	27.70 (3.30)
Present study	813	27.50 (4.30)	271	27.56 (4.14)
Total	6305		2060	

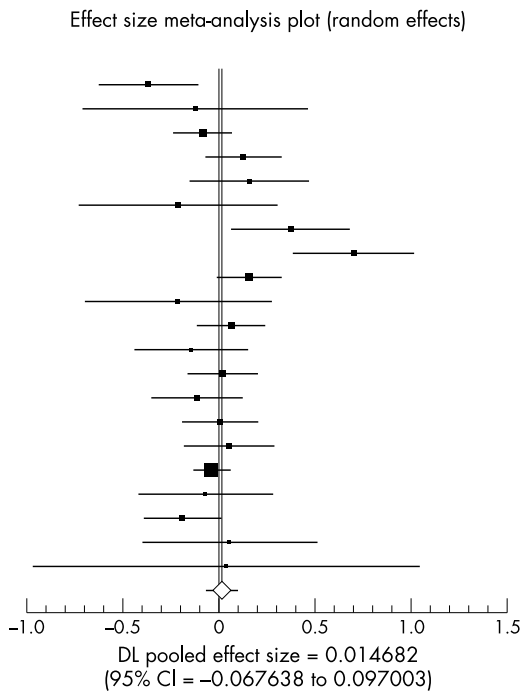


Test for overall effect: d+ = 0.11 (95% CI = 0.05 to 0.18), p = 0.0006
Test for heterogeneity: _2 = 26.32, df = 18, p = 0.093
Test for bias: Intercept = 0.90 (95%) CI = –0.73 to 2.54) p = 0.261

Figure 2 Meta-analysis of Pro12Ala in samples with mean BMI ⩾27 kg/m^2.

As it had been previously suggested that the Pro12Ala polymorphism might have an effect on BMI only in individuals with marked obesity but not in lean individuals,[13] we carried out meta-analyses separately in samples ($n = 8365$) with a mean BMI value ⩾27 kg/m^2 (overall mean BMI value 30.18) and in those ($n = 10771$) with a mean BMI value <27 kg/m^2 (overall mean BMI value 24.90). In the samples with a mean BMI value, Ala12 allele carriers ($n = 2060$) had BMI values of 0.11 d$^+$ units (95% confidence interval 0.05 to 0.18) higher than did non-carriers ($n = 6305$, p = 0.0006), without significant heterogeneity among the data from different individual studies (fig 2). Excluding the present study in the meta-analysis did not markedly change the result (d$^+ = 0.12$, p<0.0001). In contrast, in the

Study	Ala– n	Ala– mean (SD)	Ala+ n	Ala+ mean (SD)
Deeb et al., 1998	257	26.20 (3.20)	76	25.00 (3.50)
Mori et al., 1998	203	24.40 (3.30)	12	24.00 (3.00)
Ek et al., 1999	641	26.20 (3.70)	228	25.90 (3.17)
Ringel et al., 1999	388	24.20 (3.60)	134	24.66 (3.49)
Clement et al., 2000	246	22.00 (1.90)	49	22.30 (1.90)
Hara et al., 2000	400	23.50 (2.80)	15	22.90 (3.60)
Hara et al., 2000	496	23.70 (0.94)	45	24.40 (5.79)
Lei et al., 2000	553	24.20 (2.35)	43	25.90 (3.28)
Merihaeghe et al., 2000	661	25.50 (4.40)	177	26.20 (4.50)
Oh et al., 2000	211	26.10 (4.90)	18	25.10 (2.10)
Poirier et al., 2000	507	23.30 (2.30)	168	23.45 (2.53)
Chuang et al., 2001	92	25.30 (3.30)	88	24.80 (3.70)
Ek et al., 2001	456	25.60 (3.00)	160	25.66 (3.24)
Ek et al., 2001	270	23.50 (3.60)	94	23.10 (3.20)
Luan et al., 2001	468	26.16 (5.70)	124	26.19 (6.05)
Swarbrick et al., 2001	277	22.00 (1.80)	94	22.10 (2.00)
Frederiksen et al., 2002	1671	25.80 (4.20)	574	25.65 (3.93)
Stumvoll & Haring, 2002	135	24.70 (4.60)	42	24.40 (3.20)
Stumvoll et al., 2002	391	25.80 (7.90)	128	24.40 (5.70)
Thamer et al., 2002	73	23.40 (1.70)	25	23.50 (2.00)
Yamamoto et al., 2002	77	24.70 (2.60)	4	24.80 (3.80)
Total	8473		2298	



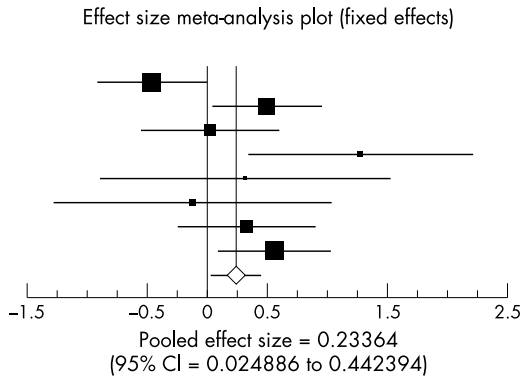
Test for overall effect: d+ = 0.01 (95% CI = –0.07 to 0.11), p = 0.727
Test for heterogeneity: _2 = 48.24, df = 20, p = 0.0004
Test for bias: Intercept = 0.28 (95%) CI = –1.33 to 1.90) p = 0.718

Figure 3 Meta-analysis of Pro12Ala in samples with mean BMI <27 kg/m^2.

A Pro/Pro versus Ala/Ala

Study	Pro/Pro n	Pro/Pro mean (SD)	Ala/Ala n	Ala/Ala mean (SD)
Deeb et al., 1998	695	27.30 (5.27)	20	25.70 (4.02)
Ek et al., 1999	540	35.50 (5.50)	21	38.90 (5.40)
Ringel et al., 1999	372	28.00 (5.00)	13	27.30 (5.10)
Valve et al., 1999	107	34.50 (3.80)	6	39.20 (4.60)
Lindi et al., 2001	93	27.50 (4.90)	3	29.50 (3.30)
Schaffler et al., 2001	276	27.20 (6.40)	3	26.70 (6.60)
Lindi et al., 2002	337	31.10 (4.40)	13	33.00 (6.30)
This study, 2002	813	27.50 (4.30)	19	29.70 (4.70)

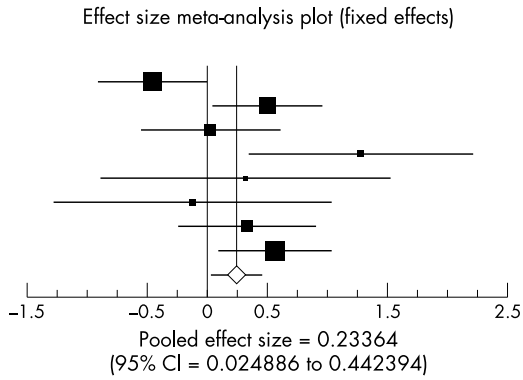
Test for overall effect: d+ = 0.29 (95% CI = 0.08 to 0.49), p = 0.005
Test for heterogeneity: _2 = 17.60, df = 7, p = 0.013
Test for bias: Intercept = 0.68 (95%) CI = –3.89 to 5.26) p = 0.727



B Pro/Ala versus Ala/Ala

Study	Pro/Ala n	Pro/Ala mean (SD)	Ala/Ala n	Ala/Ala mean (SD)
Deeb et al., 1998	258	27.90 (4.82)	20	25.70 (4.02)
Ek et al., 1999	191	36.00 (5.90)	21	38.90 (5.40)
Ringel et al., 1999	118	27.20 (4.70)	13	27.30 (5.10)
Valve et al., 1999	28	34.80 (3.10)	6	39.20 (4.60)
Lindi et al., 2001	23	28.40 (3.40)	3	29.50 (3.30)
Schaffler et al., 2001	80	27.50 (6.26)	3	26.70 (6.60)
Lindi et al., 2002	140	31.40 (4.80)	13	33.00 (6.30)
This study, 2002	252	27.40 (4.10)	19	29.70 (4.70)

Test for overall effect: d+ = 0.23 (95% CI = 0.02 to 0.44), p = 0.028
Test for heterogeneity: _2 = 17.71, df = 7, p = 0.013
Test for bias: Intercept = 0.89 (95%) CI = –3.56 to 5.34) p = 0.642



C Pro/Pro versus Pro/Ala

Study	Pro/Pro n	Pro/Pro mean (SD)	Pro/Ala n	Pro/Ala mean (SD)
Deeb et al., 1998	695	27.30 (5.27)	258	27.90 (4.82)
Ek et al., 1999	540	35.50 (5.50)	191	36.00 (5.90)
Ringel et al., 1999	372	28.00 (5.00)	118	27.20 (4.70)
Valve et al., 1999	107	34.50 (3.80)	28	34.80 (3.10)
Lindi et al., 2001	93	27.50 (4.90)	23	28.40 (3.40)
Schaffler et al., 2001	276	27.20 (6.40)	80	27.50 (6.29)
Lindi et al., 2002	337	31.10 (4.40)	140	31.40 (4.80)
This study, 2002	813	27.50 (4.30)	252	27.40 (4.10)

Test for overall effect: d+ = 0.04 (95% CI = –0.03 to 0.11), p = 0.289
Test foir heterogeneity: _2 = 6.39, df = 7, p = 0.495
Test for bias: Intercept = 0.23 (95%) CI = –2.37 to 2.82) p = 0.838

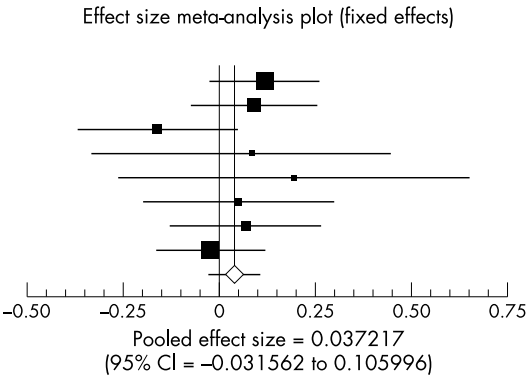


Figure 4 Pairwise comparisons between genotypes in samples with mean BMI ⩾27 kg/m^2.

meta-analysis of samples with a mean BMI <27 kg/m^2, there was no significant difference in BMI between the genotype groups (p = 0.727, fig 3).

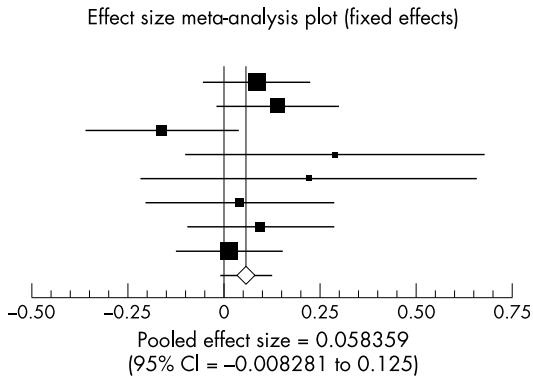
In agreement with the finding of an association between the Ala12 allele and increased BMI, the frequency of Ala12 allele carriers was higher in the samples with a mean BMI value ⩾27 kg/m^2 than in the samples with a mean BMI value <27 kg/m^2 (25% (2060 of 8365 subjects) in the former group and 21% (2298 of 10771 subjects) in the latter group, p<0.0001). Furthermore, the frequency of Ala12 allele carriers was higher in the samples with a mean BMI value ⩾30 kg/m^2 than in those with a mean BMI value between 27 and 30 kg/m^2 (26% (582 of 2216 subjects) in those with a mean BMI value ⩾30 kg/m^2 compared with 24% (1478 of 6149 subjects) in those with a mean BMI value between 27 and 30 kg/m^2; p = 0.0008).

In the above meta-analysis, the Pro/Pro genotype was compared with the Pro/Ala and Ala/Ala genotypes combined, because in the majority of publications BMI values were presented for Pro/Ala and Ala/Ala combined rather than separately, probably due to the low frequency of the Ala/Ala genotype. As it had been suggested previously that the effect of the Ala12 allele was recessive,[13] and the data of the present study also suggested a recessive model, we performed

A Dominant model

Study	Pro/Pro n	Pro/Pro mean (SD)	Pro/Ala + Ala/Ala n	Pro/Ala + Ala/Ala mean (SD)
Deeb et al., 1998	695	27.30 (5.30)	278	27.74 (4.74)
Ek et al., 1999	540	35.50 (5.50)	212	36.29 (5.85)
Ringel et al., 1999	372	28.00 (5.00)	131	27.21 (4.74)
Valve et al., 1999	107	34.50 (3.80)	34	35.58 (3.36)
Lindi et al., 2001	93	27.50 (4.90)	26	28.53 (3.39)
Schaffler et al., 2001	276	27.20 (6.40)	83	27.47 (6.31)
Lindi et al., 2002	337	31.10 (4.40)	153	31.54 (4.93)
This study, 2002	813	27.50 (4.30)	271	27.56 (4.14)

Test for overall effect: d+ = 0.06 (95% CI = –0.01 to 0.13), p = 0.086
Test for heterogeneity: _2 = 8.26, df = 7, p = 0.310
Test for bias: Intercept = 0.86 (95%) CI = –2.02 to 3.74) p = 0.491



B Recessive model

Study	Pro/Pro + Pro/Ala n	Pro/Pro + Pro/Ala mean (SD)	Ala/Ala n	Ala/Ala mean (SD)
Deeb et al., 1998	953	27.46 (5.15)	20	25.70 (4.00)
Ek et al., 1999	731	35.63 (5.60)	21	38.90 (5.40)
Ringel et al., 1999	490	27.81 (4.93)	13	27.30 (5.10)
Valve et al., 1999	135	34.56 (3.65)	6	39.20 (4.60)
Lindi et al., 2001	116	27.68 (4.60)	3	29.50 (3.30)
Schaffler et al., 2001	356	27.27 (6.37)	3	26.70 (6.60)
Lindi et al., 2002	477	31.19 (4.52)	13	33.00 (6.30)
This study, 2002	1065	27.48 (4.25)	19	29.70 (4.70)

Test for overall effect: d+ = 0.28 (95% CI = 0.08 to 0.48), p = 0.007
Test for heterogeneity: _2 = 18.25, df = 7, p = 0.01
Test for bias: Intercept = 0.76 (95%) CI = –3.88 to 5.41) p = 0.701

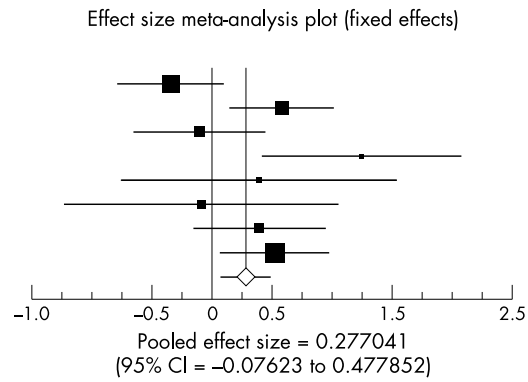


Figure 5 Dominant and recessive models in samples with mean BMI ⩾27 kg/m^2.

pairwise comparisons between the three genotypes and tested the effect of the Ala12 allele under a dominant model and then under a recessive model, using the data from those publications in which BMI values for the three genotype groups were presented separately.[10 13 18 19 25 27 29 31 34 42] In those samples with a mean BMI value <27 kg/m^2 (n = 5390),[18 25 27 42] no significant difference in BMI was found in the pairwise comparisons nor in the analysis under the dominant or recessive models. In contrast, in those samples with a mean BMI value ⩾27 kg/m^2,[10 13 18 19 29 31 34] the pairwise comparisons showed that BMI was higher in the Ala/Ala group than the Pro/Pro group (p = 0.005) as well as the Pro/Ala group (p = 0.028), but did not significantly differ between the Pro/Pro and Pro/Ala groups (p = 0.289) (fig 4). Under a dominant model (Pro/Pro was compared with Pro/Ala and Ala/Ala combined), mean BMI was slightly higher in Ala12 carriers than in non-carriers and the difference approached significance (effect size = 0.06, p = 0.086, fig 5). Under a recessive model (Pro/Pro and Pro/Ala combined were compared with Ala/Ala), mean BMI was higher in Ala12 homozygotes than in subjects with other genotypes and the difference was significant (effect size = 0.28 and p = 0.007, fig 5). Excluding the present study in this analysis reduced the effect size to 0.22 (p = 0.05).

DISCUSSION

Systematic screening in several previous studies[10] has identified two common polymorphisms in the PPAR-γ gene. In this study, we showed in a large cohort of white British subjects with a mean BMI of 27.51 kg/m^2 that the Pro12Ala polymorphism but not the C161 polymorphism was associated with BMI. Pairwise comparisons showed that the Ala/Ala genotype group had significantly higher mean BMI than both the Ala/Pro and Pro/Pro groups, indicating a recessive model for the Ala12 allele. In addition, a meta-analysis using 19 independent samples in which mean BMI was ⩾27 kg/m^2 showed a significant association between the Pro12Ala polymorphism and BMI, whereas a meta-analysis using 21 independent samples in which mean BMI was <27 kg/m^2 did not show such an association. These results support the notion that the Pro12Ala polymorphism has an apparent effect on BMI only in markedly obese individuals.[13] Further analysis using data from the publications in which BMI for the three genotype groups were presented separately showed that the Ala12 homozygotes had significantly higher BMI than heterozygotes and Pro12 homozygotes, which supports a recessive model for the Ala12 allele. Thus, both the present study and the meta-analysis indicate that the Ala12 allele is associated with increased BMI in overweight individuals under a recessive model.

The pathogenesis of obesity probably involves a large number of genetic and environmental factors. The disparate effects of the PPAR-γ gene Pro12Ala polymorphism on BMI in obese and lean individuals suggest that the impact of this genetic variant can be modified by other environmental and/or genetic factors. In general, PPAR-γ expression is increased in the adipose tissue of obese subjects, but a low calorie diet can down-regulate its expression.[43] It has been shown that when the dietary polyunsaturated fat to saturated fat ratio is low, the BMI in Ala12 carriers is greater than that in Pro12 homozygotes, but when the dietary ratio is high, the opposite is seen.[30] This gene–nutrient interaction may, in part, explain

the disparate effects of the Ala12 variant on BMI in obese and lean subjects.

Several lines of evidence suggest that Pro12Ala is a functional polymorphism. Pro12 is present in both normal human and mouse PPAR-γ sequences, and is within the domain of PPAR-γ that enhances ligand independent activation.[44] It has been shown that the non-conservative substitution of alanine for proline results in a decrease in PPAR-γ activity, (a decrease in binding affinity of PPAR-γ to the PPAR response element in its target genes), and thus a reduction in its ability to regulate the expression of these genes.[10]

The Pro12Ala polymorphism has also been associated with diabetes susceptibility. However, the effect of the polymorphism on diabetes susceptibility is only modest, and to detect this modest effect will require a large sample size to provide sufficient power. A meta-analysis of the Pro12Ala polymorphism on diabetes by Altshuler *et al*[4] has shown a highly significant association of the Pro12Ala polymorphism with diabetes susceptibility, although the effect of the polymorphism in some of the individual studies did not reach statistical significance. Thus, it has been suggested that the sample size and hence study power were not sufficient in those studies.[4] Similarly, the effect of the Pro12Ala polymorphism on BMI is only modest, accounting for only approximately 1% of the total variance of BMI in the white British cohort genotyped in this study. In a number of previous studies, the differences in BMI between Pro12Ala genotype groups did not reach statistical significance, which could be due to insufficiency in study power, a problem that can be overcome by the meta-analysis approach.

In summary, rare mutations in the PPAR-γ gene have previously been identified in patients with extreme obesity[3] and in families with severe insulin resistance, diabetes mellitus, and hypertension.[2] The results from the present study of a large cohort of white British subjects and from meta-analyses of 30 independent studies indicate that the common Pro12Ala polymorphism of the PPAR-γ gene may be a genetic modifier of obesity. These findings together provide strong genetic evidence of an important role of PPAR-γ in the regulation of body weight, insulin sensitivity, glucose homeostasis, and blood pressure.

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ECHO

Genetic influences in gastro-oesophageal reflux disease: a twin study

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Please visit the *Journal of Medical Genetics* website [www.jmedgenet.com] for a link to the full text of this article.

Background: A number of families have been described which include multiple members with symptomatic, endoscopic, or complicated gastro-oesophageal reflux disease (GORD). First degree relatives of patients with GORD are more likely to suffer with GORD symptoms. These observations raise the possibility of a genetic contribution to the aetiology of GORD.
Aims: To determine the relative contribution of genetic factors to GORD by evaluating GORD symptoms in monozygotic (MZ) and dizygotic (DZ) twins.
Methods: A total of 4480 unselected twin pairs, identified from a national volunteer twin register, were asked to complete a validated symptom questionnaire. GORD was defined as symptoms of heartburn or acid regurgitation at least weekly during the past year.
Results: Replies were obtained from 5032 subjects (56% response rate). A total of 1960 twin pairs were evaluable: 928 MZ pairs (86 male pairs, mean (SD) age 52 (13) (range 19–81) years) and 1032 DZ pairs (71 male pairs, mean age 52 (13) (20–82) years). The prevalence of GORD among both groups of twins was 18%. Casewise concordance rates were significantly higher for MZ than DZ twins (42% *v* 26%; $p<0.001$). Multifactorial liability threshold modelling suggests that additive genetic effects combined with unique environmental factors provide the best model for GORD. Heritability estimates suggest that 43% (95% confidence interval 32–55%) of the variance in liability to GORD is due to additive genetic factors.
Conclusions: There is a substantial genetic contribution to the aetiology of GORD.

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