The Pro12Ala variant of the peroxisome proliferator-activated receptor γ2 gene influences insulin sensitivity in healthy subjects participating in the RISC study

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Introduction

- The thiazolidinediones (TZDs) are insulin-sensitizing agents used in the treatment of type 2 diabetes, and mediate their effects through the nuclear transcription factor, peroxisome proliferator-activated receptor PPARγ2.
- This is encoded by the *PPARG* gene, which has been found to be a type 2 diabetes susceptibility gene.
- The Pro allele of the Pro12Ala variant was shown to confer an increased risk of type 2 diabetes.[1] The Pro12Ala variant was also shown to influence insulin sensitivity in man, with evidence suggesting that this is mediated through altered body composition.[2]
- Frederiksen et al (2002) studied a cohort of non-diabetic Caucasian subjects and found that subjects homozygous for the Ala allele had decreased levels of serum triglyceride and diastolic blood pressure.[3] However, there was no clear association with insulin sensitivity as assessed by HOMA.

Aims

The aims of this study were to investigate the relationship between the Pro12Ala variant and whole body insulin sensitivity determined by the hyperinsulinaemic clamp technique in a cohort of healthy Caucasians.

Subjects and methods

- Healthy subjects aged 30–60 years were recruited at 19 centres in 13 European countries as part of the RISC (**R**elationship between **I**nsulin **S**ensitivity and **C**ardiovascular Disease) study, to investigate the role of insulin resistance in the development of cardiovascular disease. Participating centres are shown in **Figure 1**.
- A range of data was collected, which included anthropometric, demographic and life style data.
- In addition each subject underwent oral glucose tolerance test (OGTT) and euglycaemic hyperinsulinaemic (40 mU/m^2/min) clamp.
- Here we report on 1278 subjects who completed the baseline studies and for whom DNA was extracted and available for genotyping.

Figure 1

RISC Study - Participating Centres

Pisa
London
Amsterdam
Newcastle
Lyon
Odense
Dublin
Perugia
Geneva
Frankfurt

Malmö
Rome
Glasgow
Vienna
Madrid
Athens
Milan
Belgrade
Kuopio

Participating centres indicated in black circles

Statistical analysis

- Statistical analyses were carried out using Minitab version12.
- Skewed variables were log transformed to normalise distributions. P values <0.05 were considered significant. The M value, as a measure of insulin sensitivity, was calculated and adjusted for lean body mass and free fat mass.
- The genotype-phenotype association was tested by ANOVA and adjusted for confounding factors (includes sex, age, BMI, waist and recruitment centre) using general linear model analysis.

Table 1

ANOVA comparisons of means for Pro12Ala genotypes with anthropometric and metabolic variables (Data presented as means [SE])

	Pro/Pro	Pro/Ala	Ala/Ala
Numbers	1001	264	13
Age (years)	43.6 [0.3]	44.7 [0.5]	45.6 [2.9]
BMI (kg/m^2)	25.5 [0.1]	25.6 [0.3]	26.6 [0.8]
Waist circumference (cm)	86.6 [0.4]	87.3 [0.8]	92.8 [2.7]
Fasting glucose (mmol/L)	5.1 [0.01]	5.2 [0.07]	5.2 [0.1]
Fasting Insulin (pmol/L)*	30.5 [3–118]	29.1 [8–117]	24.8 [15–40]
Fasting NEFA (mmol/L)	0.53 [0.01]	0.55 [0.01]	0.56 [0.07]
Total cholesterol (mmol/L)	4.8 [0.03]	4.9 [0.05]	4.5 [0.22]
Triglycerides (mmol/L)*	0.96 [0.3–7.4]	0.98 [0.3–5.4]	0.78 [0.4–1.7]
HDL-cholesterol (mmol/L)	1.4 [0.001]	1.4 [0.02]	1.4 [0.11]
LDL-cholesterol (mmol/L)	2.9 [0.03]	3.0 [0.05]	2.7 [0.15]
Systolic BP (mmHg)	117.4 [0.4]	117.6 [0.8]	117.4 [2.8]
Diastolic BP (mmHg)	74.3 [0.3]	75.0 [0.5]	74.1 [1.8]
M value (µmol/min/kg$_{ffm}$)	56.3 [0.7]	56.6 [1.4]	64 [6.3]

All P values >0.05, *geometric means (range)

Results

- The study cohort consists of 1278 subjects (579 men and 699 women) aged 43.8 ± 8.4 yrs (mean ±SD), with a mean BMI of 25.6 ± 4.0 kg/m^2.
- The allele frequencies were 0.89 and 0.11 for the Pro and Ala alleles, respectively, and they were in Hardy-Weinberg equilibrium.
- **Table 1** summarises the metabolic and anthropometric data for the 3 genotypes of the Pro12 Ala variant. There were no significant differences between the 3 groups when analysed by ANOVA.
- General linear model analysis revealed significant differences between the 3 genotype groups for the M value (Pro/Pro vs. Pro/Ala vs. Ala/Ala, mean ± SE: 54.3 ± 0.7 vs. 53.7 ±1.2 vs. 67.4 ± 5.4 µmol/min/kg$_{ffm}$, P=0.04) and serum triglycerides levels (geometric mean (range)) 0.95(0.3–7.4) vs. 0.93(0.3-5.4) vs. 0.69(0.4–1.7) mmol/L, P=0.03) after correction for age, sex, BMI, waist circumference and recruitment centre.
- It is evident from these data that the key differences are between the Ala/Ala carriers and the other genotype groups. For this reason, we then compared subjects homozygous for the Ala allele to the Pro allele carriers (Pro/Pro + Pro/Ala) as shown in **Table 2**.
- General linear model analysis showed that the Ala/Ala carriers had a higher M value (66.7[5.4] vs. 54.3[0.6] µmol/min/kg$_{ffm}$; p=0.02) and lower serum triglyceride levels (0.70 (change from 0.69 [0.4-1.7] vs. 0.95[0.3-7.4] mmol/L; p=0.01) after correction for age, sex, BMI, waist circumference and recruitment centre. However, this did not explain the greater insulin sensitivity which remained after including triglyceride levels as a covariate.

Table 2

Analysis of Covariance *PPARG* (Pro12Ala) (age, sex, BMI, waist and centre adjusted (Data presented as means [SE])

	P/P +P/A	Ala/Ala	P Value
Numbers	1265	13	
Fasting glucose (mmol/L)	5.1 [0.02]	5.0 [0.2]	NS
Fasting Insulin (pmol/L)*	29.9 [3–118]	22.6 [15–40]	0.02
Fasting NEFA (mmol/L)	0.53 [0.01]	0.56 [0.06]	NS
Total cholesterol (mmol/L)	4.8 [0.03]	4.4 [0.23]	0.07
Triglycerides (mmol/L)*	0.95 [0.3–7.4]	0.70 [0.4–1.7]	0.01
HDL-cholesterol (mmol/L)	1.4 [0.01]	1.5 [0.08]	NS
LDL-cholesterol (mmol/L)	2.9 [0.02]	2.6 [0.2]	NS
Systolic BP (mmHg)	117.6 [0.3]	114.4 [3.0]	NS
Diastolic BP (mmHg)	74.6 [0.3]	72.7 [2.0]	NS
M value (µmol/min/kg$_{ffm}$)	54.3 [0.6]	66.7 [5.4]	0.02

NS=not significant, *geometric means (range)

Conclusions

We confirm that the Pro12Ala *PPARG* variant influences insulin sensitivity in the healthy population. Specifically, subjects homozygous for the Ala allele are more insulin sensitive compared to the rest of the population, and this appears in part to be independent of differences in circulating triglyceride levels and measures of adiposity.

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Further information on the RISC project and participating centres can be found on www.egir.org.

References

1. **Altshuler D, Hirschhorn JN, Lander ES, et al.** ***Nat Genet*** **2000;26(1):76–80.**
2. **Deeb S, Fajais L, Auwerx J, et al.** ***Nat Genet*** **1998;20(3):284–287.**
3. **Frederiksen L, Brødbaek K, Urhammer S, et al.** ***J Clin Endocrinol Metab*** **2002;87(8):3989–3992.**

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