SNP+45 of the *(APM1)* is associated with Insulin Resistance in Healthy Subjects participating in the RISC Study

Ibrahim Ibrahim, Sheila Patel, Muthu Jayapaul, Mark Walker and the RISC Investigators

Diabetes Research Group, University of Newcastle, UK

Introduction

- The adipose most abundant gene transcript 1*(APM1)* that encodes adiponectin has been mapped to chromosome 3q27[1]
- *(APM1)* gene has been shown to increase susceptibility for type 2 diabetes (T2DM), the metabolic syndrome and abdominal obesity[2]
- In both animal and human studies an inverse relationship has been observed between circulating adiponectin levels and the degree of insulin resistance[3,4]
- Investigation of the adiponectin gene has revealed several SNPs associated with T2DM and insulin resistance
- In a Japanese cohort, the SNP +45 G allele in exon 2 of the *(APM1)* gene was associated with an increased risk of T2DM,[5] although this was not confirmed in a study of Caucasian T2DM patients[6]

Aims

The aim of this study was to investigate the relationship between SNP +45 of the *(APM1)* gene 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**
- Subjects underwent an oral glucose tolerance test (OGTT) and euglycaemic hyperinsulinaemic (40 mU/m^2/min) clamp
- SNP +45 of the *(APM1)* was genotyped using the Sequenom Mass ARRAY (San Diego, California, USA)
- Here we report on 1278 subjects who completed the baseline studies and for whom DNA was extracted and available for genotyping
- The genotype-phenotype association was tested by ANOVA and ANCOVA adjusting for confounding factors (sex, age, BMI, and recruitment centre)

RISC Study - Recruiting Centres

Pisa
London
Amsterdam
Newcastle
Lyon
Odense
Dublin
Perugia
Geneva
Frankfurt

Malmö
Rome
Glasgow
Wien
Madrid
Athens
Milan
Belgrade
Kuopio

Figure 1: map of Europe with RISC participating centers indicated in black dots

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 of SNP +45T/G were 0.89 and 0.11 for the T and G alleles respectively, and they were in Hardy-Weinberg equilibrium
- **Table 1** summarizes the metabolic and anthropometric data for the 3 genotypes of the SNP +45 of the *APM1* gene. There were no significant differences between the 3 groups when analyzed by ANOVA
- General linear model analysis revealed significant differences between the 3 genotype groups for the M value (T/T vs. T/G vs. G/G: 56.3 ± 0.7 vs. 56.9 ±1.4 vs. 48.8 ± 5.4 [mean ± SE] µmol/min/kg$_{ffm}$; p=0.04) and Fasting NEFA levels (T/T vs. T/G vs. G/G: 0.54(0.01) ± vs. 0.53(0.01) ± vs. 0.68(0.1) ± [geometric mean (SE)] mmol/l; p=0.03) after correction for age, sex, BMI, and recruitment centre
- It is evident from these data that the key differences are between the G/G carriers and the other genotype groups. For this reason, we then compared subjects homozygous for the G allele to the T allele carriers (T/T + T/G) as shown in **Table 2**
- Subjects homozygous for the G allele had a lower M value (44.7[0.04] vs. 54.5[0.6] µmol/min/kg$_{ffm}$; p=0.04, higher waist circumference (90[1.5] vs. 87[0.2] cm; p=0.02) and higher fasting NEFA levels (0.70 [0.05] vs. 0.53[0.01] mmol/l; p=0.004) after correction for the same factors

Table 1: ANOVA comparisons of means for SNP +45 genotypes with anthropometric and metabolic variables

	T/T	T/G	G/G
Numbers	1003	258	17
Age (years)	44 [0.3]	44 [0.5]	45 [2.2]
BMI (kg/m^2)	25.6 [0.1]	25.5 [0.3]	24.8 [0.8]
Waist circumference (cm)	87 [0.4]	87 [0.8]	88 [2.8]
Fasting Glucose (mmol/l)	5.1 [0.03]	5.0 [0.04]	5.0 [0.15]
*Fasting Insulin (pmol/l)	28.8 [3–118]	26.8[8–117]	26.3 [15–40]
Fasting NEFA (mmol/l)	0.54 [0.01]	0.53 [0.01]	0.68 [0.1]
*Triglycerides (mmol/l)	0.95 [0.3–7.4]	1.02 [0.3–5.4]	0.89 [0.4–1.7]
HDL–cholesterol (mmol/l)	1.4 [0.01]	1.4 [0.02]	1.4 [0.09]
LDL–cholesterol (mmol/l)	2.9 [0.03]	2.9 [0.05]	2.9 [0.19]
Systolic BP (mmHg)	118 [0.4]	117 [0.8]	118 [2.8]
Diastolic BP(mmHg)	75 [0.3]	74 [0.5]	75 [1.2]
M value (µmol/min/kg$_{ffm}$)	56.3 [0.7]	56.9 [1.4]	48.8 [4.2]

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

Table 2: Analysis of Covariance of SNP +45 of the *(APM1)* gene adjusted for age, sex, BMI, and recruitment centre

	T/T + T/G	G/G	P Value
Numbers	1261	17	
Waist circumference (cm)	87.0 [0.2]	90.0 [1.7]	0.02
Fasting Glucose (mmol/l)	5.1 [0.02]	5.0 [0.2]	NS
*Fasting Insulin (pmol/l)	28.6 [3–118]	29.9 [15–40]	NS
Fasting NEFA (mmol/l)	0.53 [0.01]	0.70 [0.05]	0.004
*Triglycerides(mmol/l)	0.95 [0.3–7.4]	0.93 [0.4–1.7]	NS
HDL–cholesterol (mmol/l)	1.4 [0.01]	1.4 [0.08]	NS
LDL–cholesterol (mmol/l)	2.9 [0.02]	2.6 [0.2]	NS
Systolic BP (mmHg)	118.0 [0.3]	119.0 [2.6]	NS
Diastolic BP(mmHg)	75.0 [0.2]	76.0 [1.8]	NS
M value (µmol/min/kg$_{ffm}$)	54.5 [0.6]	44.7 [0.04]	0.04

NS = not significant, * geometric means (interquartile range).

Conclusions

We confirm that SNP +45T/G of the *APM1* gene influences insulin sensitivity in the healthy population. Specifically, subjects homozygous for the G allele are less insulin sensitive compared to the rest of the population and have a higher waist circumference and fasting NEFAs.

RISC is supported by the European Union (QLG1-CT-2001-01252) and by AstraZeneca.

Acknowledgements

EGIR-RISC Study Group
Project Management Board:
B Balkau (Villejuif, France); SW Coppack (London, England); JM Dekker (Amsterdam, The Netherlands); E Ferrannini (Pisa, Italy); A Mari (Padova, Italy); A Natali (Pisa, Italy); M Walker (Newcastle, England).
RISC Recruitment Centres:
Amsterdam, The Netherlands: R.J. Heine, J Dekker, G Nijpels, W Boorsma; **Athens Greece:** A Mitrakou, S Tournis, K Kyriakopoulou; **Belgrade, Serbia and Montenegro:** N Lalic, K Lalic, A Jotic, L Lukic, M Civcic; **Dublin, Ireland:** J Nolan, TP Yeow, M Murphy, C DeLong, G Neary, MP Colgan; **Frankfurt, Germany:** T Konrad, H Böhles, S Fuellert, F Baer, H Zuchhold; **Geneva, Switzerland:** A Golay, V. Barthassat, V. Makoundou, TNO Lehmann, E. Harsch Bobbioni, T Merminod; **Glasgow, Scotland:** J Petrie, C Perry, F Neary, C MacDougall, K Shields, L Malcolm; **Kuopio, Finland:** M Laakso, U Salmenniemi, A Aura, R Raisanen, U Ruotsalainen, T Sistonen, M Laitinen; **London, England:** SW Coppack, N McIntosh, P Khadobaksh; **Lyon, France:** M Laville, F. Bonnet, A Brac de la Perriere, C Louche-Pelissier, C Maitrepierre, J Peyrat, A Serusclat; **Madrid, Spain:** R. Gabriel, EM Sánchez, R. Carraro, A Friera, B. Novella; **Malmö, Sweden (1):** P Nilsson, M Persson, G Östling, (2): O Melander, P Burri; **Milan, Italy:** PM Piatti, LD Monti, E Setola, F Minicucci, A Colleluori; **Newcastle-upon-Tyne, England:** M Walker, IM Ibrahim, M Jayapaul, D Carman, Y McGrady, D Richardson; **Odense, Denmark:** H Beck-Nielsen, P Staehr, K Hojlund, V Jensen, C Olsen; **Perugia, Italy:** GB Bolli, F Porcellati, C Fanelli, M Romolini, F Calcinaro, A Saturni; **Pisa, Italy:** E Ferrannini, A Natali, E Muscelli, S Pinnola, M Kozakova, L Landucci; **Rome, Italy:** G Mingrone, P Di Rocco, C Guidone, A Favuzzi; **Vienna, Austria:** W Waldhäusl, M Roden, C Anderwald, A Hofer.

Core Laboratories and Reading Centres:
Lipids – Dublin, Ireland: P Gaffney, J Nolan, G Boran. **Hormones –** Odense, Denmark: C Olsen, L Hansen; H Beck-Nielsen. **Urine Albumin:creatinine –** Amsterdam, The Netherlands: A Kok, J Dekker; **Genetics –** Newcastle-upon-Tyne, England: S Patel, M Walker. **Stable isotope analysis –** Pisa, Italy: A Gastaldelli; D Ciociaro. **Ultrasound reading centre –** Pisa, Italy: M Kozakova, E Ferrannini. **Data Management –** Villejuif, France; B Balkau, L Mhamdi. **Mathematical modelling and website management –** Padova, Italy: A Mari, G Pacini,C Cavaggion. **Coordinating office –** Pisa, Italy: SA Hills, L Mota, L Landucci.

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

References

[1]Kissebah AH, *et al. Proc Nat Acad Sci USA* 2000;**97**(26):14478–83
[2]Vionnet N, *et al. Am J Hum Genet* 2000;**67**(6):1470–1480
[3]Yamauchi T, *et al. Nat Med* 2001;**7**(8):941–46
[4]Yang WS, *et al. Diabetes Care* 2002;**25**(25):376–80
[5]Hara K, Kadowaki T, *et al. Diabetes* 2002;**51**(2):536–40
[6]Vasseur F, Froguel P, *et al. Human Molecular Genet* 2002;**11**(21):2607–14

Presented at the ADA, Washington DC, 9-13 June 2006