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Short Communication

Association of the Pro12Ala and C1431T variants of *PPARG* and their haplotypes with susceptibility to Type 2 diabetes

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Abstract

Aims/hypothesis. The Pro12Ala polymorphism of peroxisome proliferator-activated receptor (PPAR)γ has been consistently associated with Type 2 diabetes. The rare Ala12 variant is estimated to reduce the risk of developing Type 2 diabetes by 20 percent. This variant is in linkage disequilibrium with another common variant, T1431. Both have opposing associations with body weight. We therefore examined the association of specific haplotypes marked by these two variants with susceptibility to Type 2 diabetes.
Methods. We determined the *PPARG* genotype of a large Scottish cohort of Type 2 diabetic patients (n=1997) and compared allele frequencies with a cohort of local children (n=2444) and a middle-aged, population-based cohort from Scotland (n=1061).
Results. Frequency of the Ala12 allele was slightly lower in the Type 2 diabetic cohort than in the children [odds ratio (OR)=0.91, p=0.1]. In contrast, the Ala12 variant was under-represented in the Type 2 diabetic population when compared with similarly aged non-diabetic adults (OR=0.74, p=0.0006). When the Ala12 variant was on a haplotype not bearing the 1431T variant, it conferred greater protection (OR=0.66, p=0.003). However, when it was present in haplotypes containing the 1431T variant (70% of Ala12 carriers), this protection was absent (OR=0.99, p=0.94).
Conclusions/interpretation. We replicated the finding that the Ala12 variant of PPARγ affords protection from Type 2 diabetes, and suggest that this protection is modulated by additional common variation at the *PPARG* locus. [Diabetologia (2004) 47:555–558]

Keywords Type 2 diabetes · Susceptibility · Haplotype · Polymorphism · Peroxisome proliferator

The Ala12 variant of *PPARG* has been associated with protection from Type 2 diabetes [1]. It has also been associated with increased insulin sensitivity and lower body mass, and seems to modulate response to environmental cues including diet and exercise [2, 3].

Although not changing the coding sequence of *PPARG*, the C1431T polymorphism has been associated with increased body weight and is in tight allelic disequilibrium with the Ala12 variant [3]. Allelic disequilibrium can result in cross contamination of association studies where only one of the variants is considered. In this report we consider both variants in haplotypic context and dissect their specific associations with susceptibility to Type 2 diabetes.

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Abbreviations: PPAR, Peroxisome proliferator-activated receptor · DARTS, Diabetes Audit and Research in Tayside Scotland · APOSS, Aberdeen Prospective Osteoporosis Screening Study

Subjects and methods

Tayside diabetic population. In Tayside, Scotland detailed information on all individuals with diabetes mellitus is recorded on an electronic clinical information system which is known as DARTS (Diabetes Audit and Research in Tayside Scotland) and has been described elsewhere [4]. At the time of this study, about 2500 of the approximately 11,500 people registered on DARTS were enrolled in the genetic study. Of these, 1997 had Type 2 diabetes. This free-living clinical population was therefore selected solely on the basis of having Type 2 diabetes and attending diabetes clinics in Tayside. All subjects were white.

Non-diabetic children population (Tayside). Healthy school children (4–9 years old) recruited for a gene–nutrition interaction study in Tayside, Scotland were used as a control population (n=2444). This population was largely white (>97%), and being selected at random from local primary schools, was derived from the same population as the Type 2 diabetic patients.

Middle-aged control population. This consisted of 1061 white middle-aged adults from three Scottish cities, recruited without regard to diabetic status. The first group numbered 192 adults randomly selected from the Glasgow area [3]. The second group consisted of 489 adults from Tayside, recruited as control subjects for studies of breast cancer, colon cancer and systemic sclerosis [3]. The third population of 380 women was randomly selected from the Aberdeen Prospective Osteoporosis Screening Study (APOSS) [5]. These women had been genotyped for *PPARG* polymorphisms as part of a prospective study on the genetic and environmental determinants of bone mass, bone loss and osteoporotic fracture. Whereas the APOSS population comprised women only, the allele frequencies between men and women were identical in the other study populations. The allele frequencies in each individual control population reflected the final combined dataset. The individual case-control comparisons for each study will be presented elsewhere. Full ethics approval and informed consent were obtained for each of the studies referred to above.

Genotype determination. Mouthwash samples were collected from the children and blood samples were taken from the adults for DNA analysis. DNA was stored at –20°C on 96-well plates. Genotyping for *PPARG* Pro12Ala and C1431T polymorphisms was done on all populations using Taqman (Applied Biosystems, Foster City, Calif., USA.) allelic discrimination assays as described [3].

Statistical analyses. All statistical analyses were carried out using SPSS for Windows version 11 and Instat for Macintosh version 3 (Graphpad, San Diego, Calif., USA). Allele frequencies were compared between populations using Pearson's chi square. Allelic associations were analysed as described [3]. Differences in estimated haplotype frequency between populations or between phenotype were compared by log likelihood ratio using chi square. Fisher exact tests were used to determine odds ratios. A p value of less than 0.05 was considered significant.

Results and discussion

The Ala12 variant was present at a frequency of 0.111 in the 1997 individuals genotyped from the Tayside Type 2 diabetic cohort (Table 1). This is consistent with a summary frequency of 0.118 obtained from 3444 white Type 2 diabetic subjects compiled from nine studies [6, 7]. Initial analysis showed that, similarly to previous reports, the Ala12 allele was under-represented in the Type 2 diabetic cohort compared with adult population controls (Table 1) (OR=0.74, 95% CI: 0.62 to 0.88, p=0.0006). Indeed, in the adult control populations the allele frequency was highly concordant with a literature-based sample of 4065 white control subjects (0.143 vs 0.139 from the literature). The association with Type 2 diabetes was consistent with a co-dominant model, as a trend between magnitude of protection and Ala12 allele dosage was observed (chi squared test for trend = 12.8, p=0.0003). However, the Ala12 frequency was very similar in the Type 2 diabetic population and the children's cohort (OR=0.92, 95% CI: 0.79 to 1.05). The latter was even closer to the literature-based estimate of the Ala12 allele frequency in Type 2 diabetic patients (children: 0.121, diabetic: 0.118). Thus, the Ala12 variant was under-represented in the children, when compared with the adult control populations (OR=0.81, 95% CI: 0.68 to 0.95, p=0.012).

The T1431 variant was also under-represented in the Type 2 diabetic population compared to the adult population. However, this association was weaker than that observed with the Ala12 variant. The T1431 variant was in allelic association with the Ala12 variant, with about

Table 1. *PPARG* genotype frequencies in the three groups, with allele frequencies and 95% confidence intervals

Genotype	Type 2 diabetes	Adult population	Children
Pro/Pro	1575	777	1889
Pro/Ala	399	263	517
Ala/Ala	23	20	38
Ala frequency	0.111 (0.102 to 0.121)	0.143 (0.128 to 0.158)[b]	0.121 (0.112 to 0.130)
C/C	1548	725	1860
C/T	429	236	548
T/T	20	22	39
T frequency	0.117 (0.107 to 0.127)	0.142 (0.127 to 0.158)[a]	0.128 (0.119 to 0.137)

[a] p=0.0062 vs cases
[b] p=0.0003 vs cases

Table 2. *PPARG* haplotype frequencies in the three cohorts

	Type 2 diabetes	Children	Adults
Genotype			
Pro/Pro-C/C	1435	1695	640
Pro/Pro-C/T	140	178	73
Pro/Pro-T/T	0	4	3
Pro/Ala-C/C	109	149	76
Ala/Ala-C/C	4	6	2
Pro/Ala-C/T	279	345	152
Pro/Ala-T/T	11	19	11
Ala/Ala-C/T	10	17	8
Ala/Ala-T/T	9	15	8
Haplotype			
Pro-C	0.849	0.835[c]	0.810[b]
Pro-T	0.039	0.044	0.046
Ala-C	0.033	0.038	0.048[a]
Ala-T	0.078	0.083	0.094
D'	0.663	0.638	0.608

The observed combination genotypes and the statistically-derived haplotype frequencies are shown. The degree of linkage disequilibrium between the two markers is shown as D' for each population. The *p* values are shown for the comparisons of each derived-haplotype between populations

[a] p=0.0063 vs Type 2 diabetes population

[b] p=0.0001 vs Type 2 diabetes population

[c] p=0.018 vs adult non-diabetic population

Overall difference between Children and Adults by haplotype: p=0.0016

70% of individuals with Ala12 also carrying T1431 (Table 2). This raised the possibility that the protection from diabetes observed with the single polymorphisms could result from their association with each other.

We therefore examined the distribution of Ala12 variant with and without the T1431 variant in the two cohorts. This was done by statistically estimating the frequency of specific haplotypes from the combined observed genotypes (Table 2). When the Ala12 variant was examined with regard to the chromosome not bearing the T1431 variant (Pro-C vs Ala-C), it was more substantially under-represented in the Type 2 diabetic population (OR=0.66, p=0.003) than in the adult control populations. However, when the Ala12 variant was examined in chromosomes bearing the T1431 variant (70% of all Ala12 carriers; Pro-T vs Ala-T), no association was seen (OR=0.99, p=0.94).

Whereas these data further support the hypothesis that Ala12 variant confers protection against Type 2 diabetes, the protective signal in our data was totally derived from a specific minority haplotype. Moreover, the protective effect attributable to the Ala12 variant was absent in cases where the Ala12 variant was on the same chromosome as the T1431 variant. This would suggest that only a minority subgroup of Ala12 carrier individuals are protected from Type 2 diabetes. These data also support our previous studies, where we observed that the association of the Ala12 allele with reduced body weight is modulated by its chromosomal context relative to the silent T1431 allele. This modulation could be due to an independent association of the T1431 variant with increased body weight [3]. As body weight is a major determinant of Type 2 diabetes susceptibility, the observed effects of these variants on body weight could well be related to differential susceptibility to Type 2 diabetes.

The use of children from the same region as the population with diabetes to estimate the local birth frequency of these variants showed that the Ala12 frequency had mainly changed in the relatively healthy middle-aged population-based controls, rather than in the diseased cohort. This is consistent with the emerging view that variation at the *PPARG* locus is likely to have pleiotropic effects, modulating susceptibility to hypertension and myocardial infarction [8, 9, 10]. The Ala12 variant could therefore provide protection not only from developing Type 2 diabetes, but also from more general metabolic morbidity. This could result in the selective recruitment of elderly Ala12 carriers in control populations.

In conclusion, we have confirmed that the Ala12 variant of *PPARG* modulates susceptibility to Type 2 diabetes in a large population-based cohort of Type 2 diabetics and have shown that this could be modulated by additional common variation at the *PPARG* locus.

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References

1. Deeb SS, Fajas L, Nemoto M, et al. (1998) A Pro12Ala substitution in PPARgamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. Nat Genet 20:284–287
2. Luan J, Browne PO, Harding AH et al. (2001) Evidence for gene-nutrient interaction at the PPARgamma locus. Diabetes 50:686–689
3. Doney A, Fischer B, Frew D et al. (2002) Haplotype analysis of the PPARgamma Pro12Ala and C1431T variants reveals opposing associations with body weight. BMC Genet 3:21

4. Morris AD, Boyle DI, MacAlpine R et al. (1997) The diabetes audit and research in Tayside Scotland (DARTS) study: electronic record linkage to create a diabetes register. DARTS/MEMO Collaboration. BMJ 315:524–528
5. McGuigan FE, Armbrecht G, Smith R, Felsenberg D, Reid DM, Ralston SH (2001) Prediction of osteoporotic fractures by bone densitometry and COLIA1 genotyping: a prospective, population-based study in men and women. Osteoporos Int 12:91–96
6. Ek J, Andersen G, Urhammer SA et al. (2001) Studies of the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor-gamma2 (PPAR-gamma2) gene in relation to insulin sensitivity among glucose tolerant caucasians. Diabetologia 44:1170–1176
7. Ardlie KG, Lunetta KL, Seielstad M (2002) Testing for population subdivision and association in four case-control studies. Am J Hum Genet 71:304–311
8. Ridker PM, Cook NR, Cheng S et al. (2003) Alanine for proline substitution in the peroxisome proliferator-activated receptor gamma-2 (PPARG2) gene and the risk of incident myocardial infarction. Arterioscler Thromb Vasc Biol 23:859–863
9. Ostgren CJ, Lindblad U, Melander O, Melander A, Groop L, Rastam L (2003) Peroxisome proliferator-activated receptor-gammaPro12Ala polymorphism and the association with blood pressure in type 2 diabetes: Skaraborg Hypertension and Diabetes Project. J Hypertens 21:1657–1662
10. Rodriguez-Esparragon FJ, Rodriguez-Perez JC, Macias-Reyes A, Alamo-Santana F (2003) Peroxisome proliferator-activated receptor-gamma2-Pro12Ala and endothelial nitric oxide synthase-4a/bgene polymorphisms are associated with essential hypertension. J Hypertens 21:1649–1655