

Brief Genetics Report

Evidence for Gene-Nutrient Interaction at the *PPAR* γ Locus

Jian'an Luan,¹ Paul O. Browne,² Anne-Helen Harding,¹ David J. Halsall,² Stephen O'Rahilly,² V.K. Krishna Chatterjee,² and Nicholas J. Wareham¹

The importance of the nuclear receptor peroxisome proliferator-activated receptor- γ (*PPAR* γ) in regulating insulin resistance and blood pressure has been demonstrated in families with loss of function mutations. Gain of function mutations has been associated with severe obesity. However, previous population studies of the common variant Pro12Ala have produced conflicting results. As it is likely that the natural ligands for this receptor may include fatty acids, we hypothesized that the effect of this common variant may be altered by the character of the diet, particularly the ratio of dietary polyunsaturated fat to saturated fat (P:S ratio). We studied 592 nondiabetic participants in an ongoing population-based cohort study who were genotyped for the Pro12Ala polymorphism in the *PPAR* γ 2 isoform. As the Ala homozygotes were uncommon (2.0%), all analyses were conducted comparing Pro homozygotes (79.1%) to Ala allele carriers. There was no difference in fasting insulin concentration or BMI between Ala allele carriers and Pro homozygotes. The fasting insulin concentration was negatively associated with the P:S ratio ($P = 0.0119$) after adjustment for age and sex, and a strong interaction was evident between the P:S ratio and the Pro12Ala polymorphism for both BMI ($P = 0.0038$) and fasting insulin ($P = 0.0097$). The data suggest that when the dietary P:S ratio is low, the BMI in Ala carriers is greater than that in Pro homozygotes, but when the dietary ratio is high, the opposite is seen. This gene-nutrient interaction emphasizes the difficulty of examining the effect of common polymorphisms in the absence of data on nongenetic exposures, and may explain the heterogeneity of findings in previous studies. *Diabetes* 50:686–689, 2001

From the Departments of ¹Public Health and Primary Care and ²Medicine and Clinical Biochemistry, University of Cambridge, Cambridge, U.K.

Address correspondence and reprint requests to Nicholas J. Wareham, MB, PhD, Department of Public Health and Primary Care, Institute of Public Health, University of Cambridge, Cambridge CB2 2SR, U.K. E-mail: njw1004@medschl.cam.ac.uk.

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J.L. and P.O.B. contributed equally to this work.

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A complete understanding of the etiology of common complex diseases such as diabetes, ischemic heart disease, and obesity will require exploration of the interactions between environmental factors and common genetic variants (1). To date, few examples of such interactions have been described. We present evidence for an environment-gene interaction influencing BMI and insulin sensitivity involving the nuclear receptor peroxisome proliferator-activated receptor- γ (*PPAR* γ) and the pattern of dietary fat intake.

A critical role for *PPAR* γ in the development of mammalian adipose tissue has been confirmed by the absence of adipose tissue in *PPAR* γ null murine embryos (2). Additionally, the importance of *PPAR* γ in the control of human insulin sensitivity and blood pressure has recently been established by the finding of severe insulin-resistant diabetes and early-onset hypertension in two families with loss of function mutations in this gene (3). A gain of function mutation in *PPAR* γ has also been reported in three subjects with severe obesity (4). Several studies have examined the relationship between a common variant (Pro12Ala) in the *PPAR* γ isoform (5) and metabolic variables (6–11). In terms of adiposity, studies have had variable results with some reporting increased (6), some decreased (7,8), and some neutral effects on BMI (9–11).

The natural ligands for *PPAR* γ have yet to be definitively identified. While PGd15J2 is a potent activator of *PPAR* γ (12), it is uncertain whether this prostanoid exists in appreciable concentrations in vivo. Kliewer et al. (12) and Desvergne and Wahli (13) have suggested that a single specific ligand may not exist and that *PPAR* γ may be a fatty acid sensor, with the affinity of fatty acids for the receptor varying according to their chain length and degree of desaturation. Given the potential role of *PPAR* γ as a nutrient sensor regulating adipogenesis and insulin sensitivity, we have examined the interaction between the ratio of dietary polyunsaturated fat to saturated fat (P:S ratio) and the Pro12Ala polymorphism in a large U.K. Caucasian population.

The Isle of Ely Study (14,15) is a prospective population-based cohort study of the etiology and pathogenesis of type 2 diabetes and related metabolic disorders. A total of 592 nondiabetic Caucasian subjects (mean age 53.9 ± 10.9 years, 259 men and 333 women) were genotyped for the

TABLE 1
Adjusted means of BMI, fasting insulin, and P:S ratio (adjusted for age)

	Pro homozygotes	Ala allele carriers	<i>P</i>
Men			
<i>n</i>	203	56	
BMI (kg/m ²)	26.54 (26.20–26.88)	26.77 (26.10–27.43)	0.554
Fasting insulin (pmol/l)*	39.50 (37.11–42.04)	39.56 (35.03–44.69)	0.981
P:S ratio	0.55 (0.52–0.58)	0.56 (0.52–0.59)	0.986
Women			
<i>n</i>	265	68	
BMI (kg/m ²)	25.93 (25.50–26.33)	25.72 (24.88–26.57)	0.678
Fasting insulin (pmol/l)*	38.04 (36.16–40.01)	38.28 (34.59–42.36)	0.914
P:S ratio	0.55 (0.52–0.57)	0.56 (0.50–0.61)	0.823

Data are arithmetic means (95% CI) and *geometric means (95% CI).

Pro12Ala polymorphism (Pro/Pro 79.1%, Pro/Ala 18.9%, and Ala/Ala 2.0%; proportions in Hardy-Weinberg equilibrium). Because the Ala homozygotes were uncommon, all analyses were conducted comparing Pro homozygotes with Ala allele carriers. At baseline (1990–1992) and the 4.5-year follow-up (1994–1996), anthropometric measurements were taken by two trained observers with subjects in indoor clothing with the use of a rigid stadiometer and calibrated scales. Habitual diet during the previous year was assessed using a self-completion semiquantitative food frequency questionnaire (16). Reported consumption of specific foods was converted into nutrients using U.K. food tables. All blood samples were taken after a 10-h overnight fast and were immediately placed on ice and centrifuged on site. Serum samples were aliquotted, packed in ice, and transferred to the laboratory where they were stored at -70°C within 4 h. Fasting plasma insulin was measured on both occasions using two-site immunometric assays with either ^{125}I or alkaline phosphatase labels (17,18). The main outcome variables, fasting insulin and BMI, for successive time periods were analyzed with a repeated-measures design using an SAS procedure (MIXED) for unbalanced repeated data.

There were no significant associations between either BMI or fasting insulin and the Pro12Ala polymorphism stratified by sex (Table 1). Because of the critical role of fat consumption in the etiology of obesity and insulin resistance, we examined the association between dietary P:S ratio and fasting insulin, finding a significant inverse

relationship after adjustment for age and sex ($P = 0.0119$). In light of the possibility that fatty acids may be the natural ligands for PPAR γ , we investigated the interaction between the P:S ratio and the Pro12Ala polymorphism. Figure 1 shows BMI and fasting insulin in Ala carriers and Pro homozygotes stratified according to quartiles of the dietary P:S ratio. As the P:S ratio increases, there is a clear tendency for both BMI and fasting insulin to decrease in Ala carriers, but not in the Pro homozygotes. The interactions between the P:S ratio and the Pro12Ala polymorphism in determining both BMI ($P = 0.0038$) and fasting insulin ($P = 0.0097$) are both highly statistically significant (Table 2). We repeated the analyses using total fat intake as a proportion of total energy, and there was no detectable interaction with genotype on BMI or fasting insulin.

When BMI was included as a covariate, the interaction observed between genotype and the P:S ratio for fasting insulin was attenuated, suggesting that the effect is mediated through obesity. When the data were analyzed without taking the interaction with the P:S ratio into consideration, the PPAR γ genotype was not significantly associated with either BMI ($P = 0.88$) or fasting insulin ($P = 0.80$).

In summary, this study provides one of the first examples of a diet-gene interaction relevant to a common complex metabolic phenotype, as we have detected a potentially important interaction between dietary patterns of fatty acid intake and a common polymorphism in the PPAR γ gene. The data suggest that when the dietary P:S

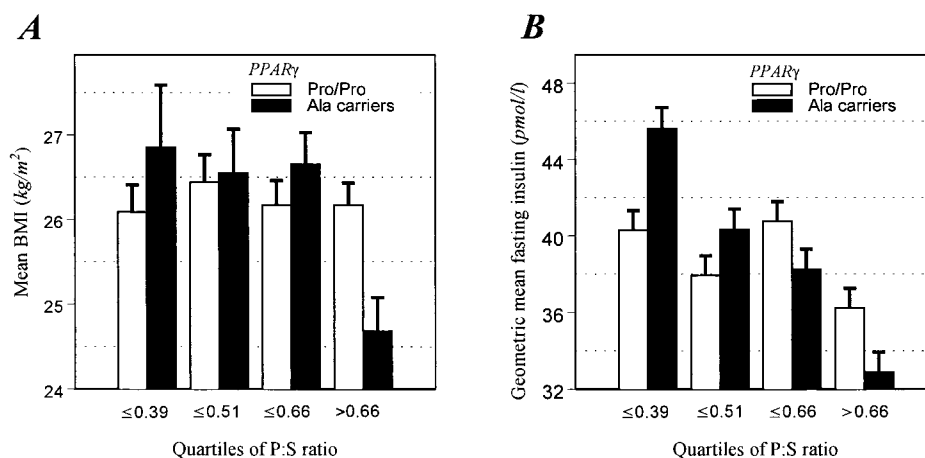


FIG. 1. Mean BMI (\pm SE) (kg/m²) (A) and geometric mean (\pm SE) fasting insulin (pmol/l) (B) by P:S ratio and PPAR γ .

TABLE 2

Results from the mixed-model multivariate analysis with BMI or fasting insulin concentration as the outcome variable

	Outcome variable				
	BMI	BMI	Fasting insulin (log _e)	Fasting insulin (log _e)	Fasting insulin (log _e)
Age (years)	0.032 (0.014)	0.050 (0.0002)	0.005 (0.006)	0.007 (0.0005)	0.003 (0.05)
Sex	0.525 (0.037)	0.629 (0.015)	0.032 (0.35)	0.027 (0.45)	-0.016 (0.61)
<i>PPAR</i> γ	-0.046 (0.88)	-2.412 (0.008)	-0.011 (0.80)	-0.309 (0.015)	-0.143 (0.20)
P:S ratio		-4.320 (0.005)		-0.658 (0.0003)	-0.361 (0.011)
P:S ratio × <i>PPAR</i> γ		4.366 (0.004)		0.548 (0.0097)	0.248 (0.18)
BMI (kg/m ²)					0.069 (0.0001)

Data are regression coefficients (significance). Sex coded: 0 = male, 1 = female; *PPAR*γ coded: 0 = Ala allele carrier, 1 = Pro homozygotes.

ratio is low, the mean BMI in Ala carriers is greater than that in Pro homozygotes. When the dietary ratio is high, the opposite is true and Ala carriers are leaner. The absence of similar demonstrable relationships with total fat intake expressed as a proportion of total energy intake may not necessarily indicate that these interactions do not exist, but may be a reflection of the difficulty of quantifying absolute intake with a food frequency questionnaire.

This interaction may explain the conflicting results of previous studies of the Pro12Ala *PPAR*γ variant; the results of such studies would presumably be highly dependent on the prevalent diets in those particular populations. Ideally, one would investigate this possibility using dietary information from the study populations in which genotyping has been undertaken. This information is not currently available and would require a multicohort international study with standardized dietary measurement to be examined in any detail. However, nutritional studies do indicate the magnitude of the between-country differences in the P:S ratio. In a review of such studies, the mean P:S ratio is reported to vary from 0.11 in Hungary, 0.41 in Australia, and 0.48 in the U.S. to 1.10 in Japan and 1.2 in Portugal (19). Even within this limited range of countries, the P:S ratio has thus varied by more than 10-fold, a variation that far exceeds that seen within the U.K. population.

The biological plausibility of the observed association is high. Previous functional studies suggest that the Ala variant is associated with moderately reduced transcriptional activity (7). Thus, if some derivatives of polyunsaturated fatty acids are ligands for *PPAR*γ, then they might be expected to be more effective stimulators of adipogenesis in Pro homozygotes rather than Ala carriers. This interpretation would be consistent with the decreasing adiposity that we observe in Ala carriers with increasing P:S ratio. However, overly simplistic interpretations should be avoided since the biological effects of *PPAR*γ are likely to be complex and highly dependent upon the particular ligand involved, a phenomenon demonstrated recently in relation to pharmacological *PPAR*γ ligands that antagonize thiazolidinedione-stimulated adipogenesis, yet continue to stimulate glucose uptake (20).

RESEARCH DESIGN AND METHODS

Polymerase chain reaction and restriction digestion. To detect the C34G variant in subjects from the Isle of Ely Study, two primers, *PPAR* forward (5'-GCCAATTCAGCCAGTG-3') and the mutagenic *PPAR* reverse (5'-GATATGTTTGCAGACAGTGTATCAGTGAAGGAATCGCTTTCGG-3') (the mutagenic position is underlined), were used in a polymerase chain reaction (PCR) to amplify the fragment containing the C34G substitution from genomic DNA isolated from whole blood using a QIAamp blood kit (Qiagen, London, U.K.)

and were amplified 50-fold using the *Nrich* kit (Genpak, Brighton, U.K.). PCR was performed using *Bio-Taq* (Bioline, London, U.K.) and carried out under standard conditions. A total of 35 cycles (30 s at 96°C, 30 s at 55°C, and 60 s at 72°C) were performed using a GeneAmp 2400 PCR System (Perkin-Elmer, Beaconsfield, U.K.). The mutagenic PCR introduced a *Bst*UI restriction site when the C34G substitution was present. Restriction digestion was performed using *Bst*UI (New England Biolabs, Beverly, MA) and carried out under standard conditions for 120 min at 60°C. Gel electrophoresis was performed using 2% (wt/vol) agarose gels (Gibco BRL, Paisley, U.K.), and the restriction digestion products were subsequently detected with ethidium bromide under ultraviolet illumination.

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