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RESEARCH LETTER

The Pro12Ala polymorphism in *PPARG2* increases the effectiveness of primary prevention of cardiovascular disease by a lifestyle intervention

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To the Editor: Genetic factors interact with modifiable risk factors to influence the risk of cardiovascular disease [1]. They also modulate the effectiveness of interventions for the primary prevention of cardiovascular disease [2]; thus, the identification of the genes involved may help to more efficiently prevent cardiovascular disease [1, 2]. With regard to glucose metabolism, gene–environment and gene–nutrient interactions have previously been demonstrated for the polymorphism in the peroxisome proliferator–activator receptor γ2 gene (*PPARG2*) that results in the substitution of Pro for Ala at codon 12 [3]. In addition, carriers of the Ala-encoding allele have an improved response to aerobic exercise in terms of glucose metabolism [3]. Because *PPARG2* is also considered to play a role in the process of atherosclerosis [3], we tested whether *PPARG2* interacts with the effects of lifestyle intervention on cardiovascular traits. Specifically, we studied the effect of this common polymorphism on the increase in flow-mediated vasodilation (FMD) and the decrease in serum levels of C-reactive protein (CRP) during a 9 month lifestyle intervention in 166 non-diabetic subjects (58 men, 108 women; age 46±11 years).

The population was at increased risk of type 2 diabetes and cardiovascular disease because of one or more of the following risk factors: being overweight (BMI >27 kg/m^2), being a first-degree relative of a patient with type 2 diabetes, or having IGT or a history of gestational diabetes. After the baseline visit, which included an OGTT and FMD, all subjects started an exercise and dietary lifestyle intervention (Tubingen Lifestyle Intervention Program [TULIP]). The study was approved by the local ethics committee. All subjects gave their written informed consent. This programme includes the goals of the Diabetes Prevention Study [4]: a reduction of body weight by >5%, a reduction of dietary fat intake to <30% of total energy intake, a reduction of saturated fatty acid intake to <10% of total energy intake, an increase in the daily amount of ingested fibre to >15 g per 4,187 kJ, and an increase in the amount of weekly exercise to >3 h/week. The TULIP study design includes a baseline and two follow-up visits, one after 9 months and the other after 24 months.

In this analysis, data from 166 subjects who completed the baseline visit and the first follow-up visit are reported (mean follow-up time [±SD] 264±56 days, range 151–465 days). At the time of data analysis, less than 50% of these subjects had completed the second follow-up visit, and so these data are not presented. At baseline and at follow-up, FMD of the brachial artery was measured with high-resolution ultrasound (13 MHz) [5]. Subjects with serum levels of CRP of >10 mg/l at baseline or at follow-up were excluded from the analysis. Genomic DNA was extracted from peripheral blood lymphocytes. Genetic analyses were performed by PCR with subsequent restriction analysis with *Bst*XI, as described previously [6]. The statistical software package JMP (SAS Institute, Cary, NC, USA) was used. Of the 166 subjects, 131 had the Pro/Pro genotype and 35 had an X/Ala genotype. These two genotype groups were not different in terms of baseline FMD (7.2±0.3 vs 7.3±0.6%, respectively; p=0.69) or CRP (1.6±0.1 vs 1.6±0.3 mg/l, respectively; p=0.43). During lifestyle intervention, BMI was reduced (from 29.3±0.4 to

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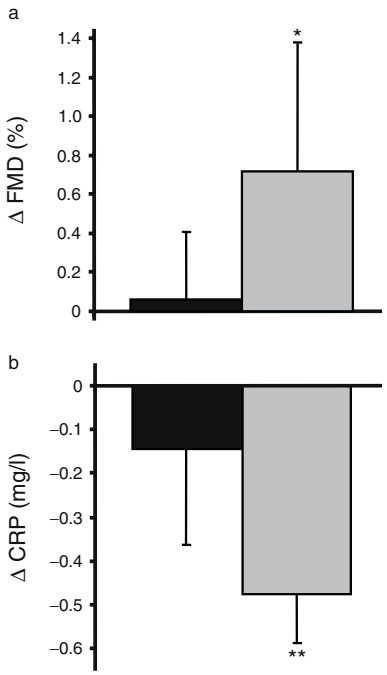

Fig. 1 Changes in FMD (**a**) and CRP (**b**) during lifestyle intervention in individuals with the Pro/Pro (*black bars*) and X/Ala (*grey bars*) genotype. Data are presented as means±SEM. *p 0.025, **p<0.01 vs Pro/Pro after adjustment for sex, age, baseline BMI and relative change in BMI, plus baseline FMD in **a** and baseline CRP in **b**

28.6±0.4 kg/m^2; p<0.0001) and 2 h glucose during the OGTT was improved (from 6.9±0.1 to 6.4±0.1 mmol/l; p<0.001). Weight was significantly decreased in both the Pro/Pro (from 29.3±0.4 to 28.6±0.5 kg/m^2; p<0.0001) and X/Ala groups (29.6±0.8 to 28.8±0.8 kg/m^2; p<0.001), but the difference in weight loss between the groups was not significant (p=0.55). In the group as a whole, there were no statistically significant changes in FMD (change from 7.2± 0.3 to 7.4±0.3%; p=0.36) or CRP (change from 1.6±0.1 to 1.4±0.01 mg/l; p=0.11). There was an increase in FMD with lifestyle intervention in carriers of the Ala-encoding allele (from 7.3±0.6 to 8.0±0.6%; p=0.04), while no statistically significant change was observed in subjects homozygous for the Pro-encoding allele (change from 7.2± 0.3 to 7.3±0.04%; p=0.67). The difference in FMD between the genotypes, adjusted for sex, age, baseline BMI, relative change in BMI and baseline FMD was statistically significant (p=0.025) (Fig. 1a). In addition, the reduction in serum CRP levels with lifestyle intervention was greater in carriers of the Ala-encoding allele (from 1.6± 0.3 to 1.2±0.2 mg/l; p<0.01) than in subjects homozygous for the Pro-encoding allele (from 1.6±0.1 to 1.5±0.1 mg/l; p=0.50). Again, the difference between the genotype groups adjusted for sex, age, baseline BMI, relative change in BMI and baseline CRP was statistically significant (p< 0.01) (Fig. 1b). The associations of the Pro12Ala genotype with the changes in FMD and CRP remained statistically significant after adjusting for multiple comparisons for these two traits at the new α-level of 0.0253.

Lifestyle intervention has been shown to be effective in the primary prevention of cardiovascular disease [7]. During lifestyle intervention the change in CRP is correlated to the weight loss achieved [8]. The relatively small weight loss achieved in our study did not result in improvements in CRP levels or FMD across the group as a whole. These two early markers of atherosclerotic risk were only improved in carriers of the Ala-encoding alleles in *PPARG2*. This result is consistent with the observation that the Ala-encoding allele in *PPARG2* is associated with anti-atherogenic and anti-inflammatory effects [9]. These effects are possibly mediated via changes in systemic inflammation and macrophage activation [3, 9]. Recent evidence suggests that, in non-diabetic Europid populations the Pro12Ala genotype is associated with obesity itself and with glucose metabolism in the presence of obesity [10]. However, in the present study, the weight loss achieved did not depend on the genotype of this polymorphism. We conclude that lifestyle intervention is more effective with regard to primary cardiovascular prevention in carriers of the Ala-encoding allele in *PPARG2* than in those with the Pro/Pro genotype.

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