Association of the Pro12Ala Polymorphism in the PPAR-γ2 Gene With 3-Year Incidence of Type 2 Diabetes and Body Weight Change in the Finnish Diabetes Prevention Study

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The association of the Pro12Ala polymorphism of the PPAR-γ2 gene with the incidence of type 2 diabetes was investigated in 522 subjects with impaired glucose tolerance (IGT) participating in the Finnish Diabetes Prevention Study. Subjects were randomized to either an intensive diet and exercise group or a control group. By 3 years of intervention, the odds ratio of the development of type 2 diabetes for subjects with the Ala12 allele was 2.11-fold compared with that for subjects with the Pro12Pro genotype (95% CI 1.20–3.72). The risk for type 2 diabetes increased also in subjects who gained weight or belonged to the control group. In the intervention group, subjects with the Ala12Ala genotype lost more weight during the follow-up than subjects with other genotypes (Pro12Pro vs. Ala12Ala P = 0.043), and none of subjects with the Ala12Ala genotype developed type 2 diabetes in this group. In conclusion, the Ala12 allele may predispose to the development of type 2 diabetes in obese subjects with IGT. However, beneficial changes in diet, increases in physical activity, and weight loss may reverse, to some extent, the diabetogenic impact of the Ala12 allele, possibly due to an improved insulin sensitivity. *Diabetes* 51:2581–2586, 2002

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DPS, Finnish Diabetes Prevention Study; HBMI, a higher BMI group; GLM, general linear model; HOMA-IR, homeostasis model assessment for insulin resistance; IGT, impaired glucose tolerance; LBMI, a lower BMI group; OGTT, oral glucose tolerance test; PPAR-γ, peroxisome proliferator–activated receptor-γ; SSCP, single-strand conformation polymorphism.

Type 2 diabetes is preceded by impaired glucose tolerance (IGT) that can last for years before the diagnosis of diabetes. Two earlier intervention studies suggest that it is possible to reduce the incidence of type 2 diabetes among subjects with IGT by lifestyle intervention (1,2). Recently, we demonstrated that in subjects with IGT weight loss, increased physical activity and healthier diet in terms of reduced fat and increased dietary fiber intake resulted in a substantial reduction in the incidence of type 2 diabetes (3). Similarly, the efficacy of lifestyle intervention in prevention of type 2 diabetes has been confirmed in multiethnic populations in the Diabetes Prevention Program Trial (4).

Type 2 diabetes has also a strong genetic component (5). Peroxisome proliferator–activated receptor-γ (PPAR-γ), a nuclear receptor, is a promising candidate gene for type 2 diabetes and obesity, as it regulates adipocyte differentiation and lipid and glucose metabolism (6). In humans, four PPAR-γ mRNA isoforms have been identified (6,7). Several variants in the PPAR-γ gene have been reported, among them the Pro12Ala polymorphism in the PPAR-γ2 isoform-specific exon B (8,9). Recently, Altshuler et al. (10) reported that, based on cross-sectional studies of several populations, the more common Pro12 allele was associated with a 1.25-fold increased risk of type 2 diabetes. Several studies have also investigated the effect of the Pro12Ala polymorphism on body weight, but the results have been inconsistent (11–21).

The purpose of the present study was to examine whether the Pro12Ala polymorphism of the PPAR-γ2 gene is associated with the incidence of type 2 diabetes in subjects with IGT, who are at particularly high risk for the disease, followed prospectively in the Finnish Diabetes Prevention Study (DPS) (3,22). In addition, we studied whether the effect of lifestyle intervention on weight change was modified by the Pro12Ala polymorphism.

RESEARCH DESIGN AND METHODS

Study population and design. The study population, inclusion and exclusion criteria, and power calculation have been described earlier in detail (3,22). In short, the DPS is a multicenter study with five participating centers in Finland. The diagnosis of diabetes and other categories of glucose intolerance were based on the criteria adopted by the World Health Organi-

TABLE 1
Baseline characteristics of the DPS study subjects according to the Pro12Ala polymorphism of the PPAR-γ2 gene

	Genotype			
	Pro12Pro	Pro12Ala	Ala12Ala	*P**
n	337	140	13	
M/F	114/223	44/96	3/10	0.658
Age (years)	55 ± 7	56 ± 7	53 ± 8	0.307
Weight (kg)	85.8 ± 13.9	86.8 ± 15.1	89.9 ± 18.0	0.822
BMI (kg/m^2)	31.1 ± 4.4	31.4 ± 4.8	33.0 ± 6.3	0.959
Waist circumference (cm)†	101.0 ± 10.6	102.0 ± 11.4	103.0 ± 14.3	0.793
Hip circumference (cm)	109.9 ± 9.5	110.2 ± 10.7	117.1 ± 17.0	0.836
Waist-to-hip ratio†	0.92 ± 0.08	0.93 ± 0.07	0.88 ± 0.06	0.174
Fasting plasma glucose (mmol/l)	6.1 ± 0.8	6.2 ± 0.7	6.4 ± 0.7	0.076
2-h plasma glucose (mmol/l)	8.8 ± 1.5	9.0 ± 1.6	8.9 ± 1.2	0.861
Fasting serum insulin (pmol/l)‡	89.5 ± 46.8	90.5 ± 40.6	72.5 ± 27.5	0.349
2-h serum insulin (pmol/l)§	572.9 ± 360.0	582.0 ± 471.2	472.0 ± 205.6	0.955
HOMA-IR (mol · μU · l^{-2})‡	4.13 ± 2.38	4.25 ± 2.16	3.48 ± 1.47	0.353

Data are means ± SD. **P* values for the anthropometric measurements and plasma glucose, serum insulin, and HOMA-IR values were adjusted for age and sex. In addition, *P* values for plasma glucose and serum insulin concentrations were adjusted for BMI; †number of subjects: Pro12Pro = 335, Pro12Ala = 140, Ala12Ala = 13; ‡number of subjects: Pro12Pro = 308, Pro12Ala = 126, Ala12Ala = 12; §number of subjects: Pro12Pro = 306, Pro12Ala = 126, Ala12Ala = 12.

zation in 1985 (23). IGT was defined as fasting plasma glucose <7.8 mmol/l and a 2-h plasma glucose 7.8–11.0 mmol/l (oral glucose tolerance test [OGTT] 75 g). Altogether 522 overweight subjects (BMI 31.1 ± 4.6 kg/m^2) aged 40–68 years and with IGT were randomly allocated to one of the two treatment modalities, the intensive diet and exercise intervention group or the control group. The screening for the Pro12Ala polymorphism of the PPAR-γ2 gene was performed in 490 (161 men and 329 women) subjects.

The intervention program has been described previously (3,22). Briefly, subjects in the intervention group were given individually tailored dietary advice aimed at reducing weight and the intake of total and saturated fat and increasing the intake of dietary fiber. Furthermore, subjects in the intervention group were individually guided to increase their level of physical activity. The control group received general information on the benefits of weight reduction, physical activity, and healthy diet.

The study protocol was approved by the ethics committee of the National Public Health Institute in Helsinki, Finland. All subjects gave written informed consent.

Methods. A medical history and a physical examination were done on a yearly basis. In this report, measurements at baseline and at the 3-year examination were used. Waist circumference was measured midway between the lowest rib and iliac crest and hip circumference over the great trochanters, with 0.5-cm precision with the subjects in a standing position. BMI was calculated as weight (kg) divided by height (m) squared (2). The relative weight change during the 3-year follow-up was calculated as follows: $([\text{weight}_{\text{3-year follow-up}} - \text{weight}_{\text{baseline}}]/\text{weight}_{\text{baseline}}) \times 100$. In a 2-h OGTT, samples for glucose and insulin were taken before (0 min) and 120 min after a glucose load (75 g). Plasma glucose was measured at each center by standard methods, and the measurements were standardized by the central laboratory in Helsinki (3). Serum insulin was determined with a radioimmunoassay (Pharmacia, Uppsala, Sweden). The homeostasis model assessment for insulin resistance (HOMA-IR) was calculated using the following formula: fasting plasma glucose (mol/l) × fasting serum insulin (μU/l)/22.5.

The Pro12Ala polymorphism of the PPAR-γ2 gene was determined by the PCR-SSCP (single-strand conformation polymorphism) method as previously reported (17).

Statistical analysis. The significance of differences in allele frequencies was analyzed using two-tailed Fisher's exact test in the StatXact-4 program version 4.0.1 (Cytel Software Corporation, Cambridge, MA). Otherwise, the data were analyzed using the SPSS/WIN program version 9.0 (SPSS, Chicago). χ^2 analysis was applied to test for the significance of differences in sex distribution among genotypes. Normal distribution of variables was checked with the Kolmogorov-Smirnov (Lilliefors) test, and logarithmic transformation was used for those not normally distributed. The differences among the genotypes were evaluated by the univariate ANOVA (general linear model [GLM]) with adjustment for age, sex, and BMI, when appropriate. The differences in the 3-year weight change among genotypes were assessed by GLM with glucose tolerance status at the 3-year examination (IGT versus type 2 diabetes) as a covariate. Logistic regression analysis was used to assess whether the Ala12 allele predicted the development of type 2 diabetes. In the logistic regression analysis, genotypes were encoded as 0 = Pro12Pro and 1 = Pro12Ala or Ala12Ala and study groups were encoded as 1 = intervention group and 2 = control group. Weight change used in the logistic regression analysis was calculated as $\text{weight (kg)}_{\text{3 year}} - \text{weight (kg)}_{\text{baseline}}$. In subjects whose diabetes was diagnosed before the 3-year examination, the baseline weight was subtracted from the weight measured at the visit when the diabetes diagnosis was done. In addition, age and sex were considered in the logistic regression model together with genotype, study group, and weight change, but they were not significantly related to the risk of type 2 diabetes (data not shown), and therefore they were omitted from the final logistic regression model. A *P* value <0.05 was considered statistically significant. Data are presented as means ± SD, unless otherwise indicated.

RESULTS

In the intervention group, the Pro12Pro genotype was found in 163 subjects, the Pro12Ala genotype was found in 79 subjects, and 6 subjects were homozygous for the Ala12 allele. In the control group, 174 subjects had the Pro12Pro genotype, 61 had the Pro12Ala genotype, and 7 had the Ala12Ala genotype. Observed genotype frequencies of the polymorphism were in agreement with Hardy-Weinberg expectations. The frequency of the Ala12 allele did not differ between the intervention group and the control group (0.183 vs. 0.155, respectively, $P = 0.268$).

At baseline, the Pro12Ala polymorphism was not associated with body weight, BMI, waist and hip circumferences, waist-to-hip ratio, HOMA-IR, 2-h plasma glucose, or fasting and 2-h serum insulin concentrations (Table 1). However, subjects with the Ala12Ala genotype tended to have higher fasting plasma glucose concentrations than those with other genotypes.

By 3 years of intervention, 19 subjects in the intervention group and 50 subjects in the control group had developed type 2 diabetes (Fig. 1*B*). When the association between the Pro12Ala polymorphism and the incidence of type 2 diabetes was considered in the logistic regression model, the odds of the development of type 2 diabetes for those with the Ala12 allele was over twofold compared with the odds of the development of type 2 diabetes for subjects with the Pro12Pro genotype (Table 2). Similarly, the odds of the development of type 2 diabetes was over twofold for subjects in the control group compared with

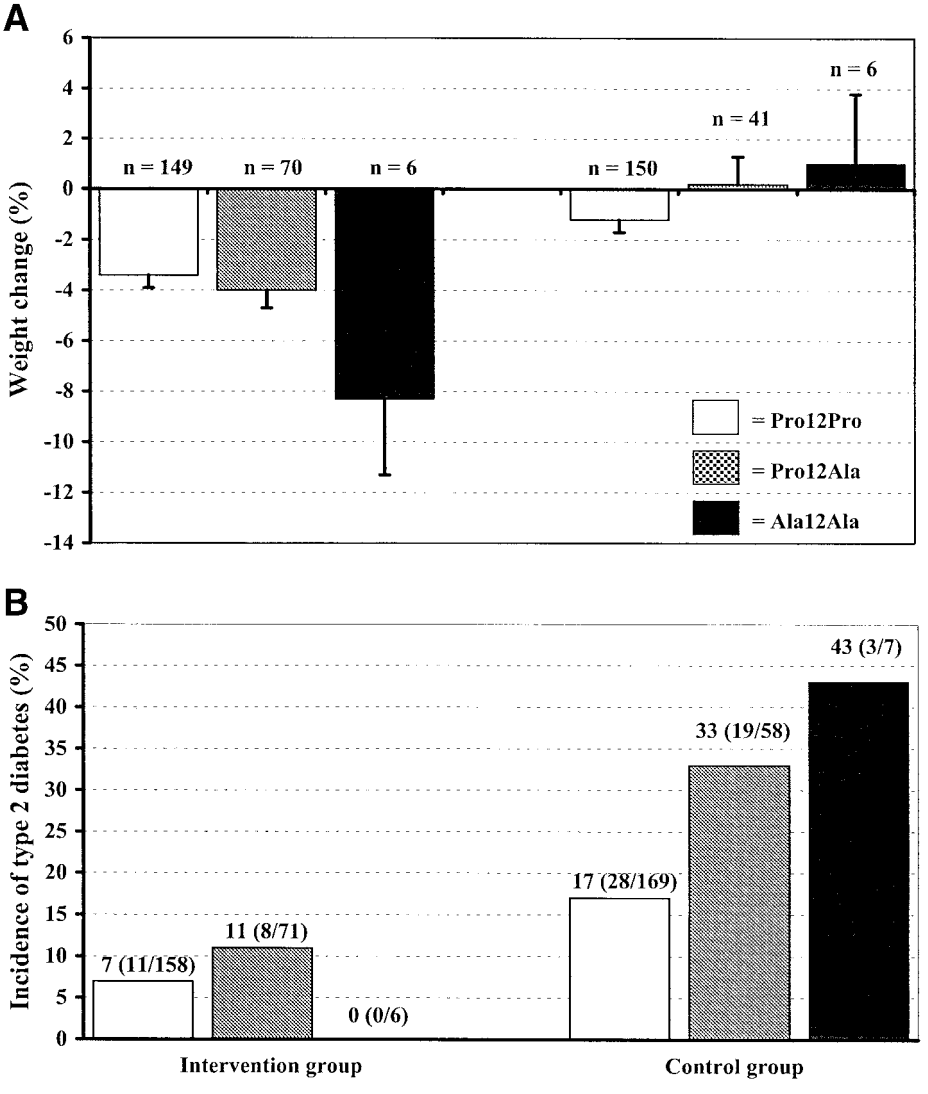

FIG. 1. ***A*****: Three-year weight change (%) in the intervention group and the control group by the Pro12Ala polymorphism of the PPAR-γ2 gene (mean and SE). In the intervention group, $P = 0.191$ for comparison among all three genotypes and $P = 0.043$ for difference between the Pro12Pro and the Ala12Ala genotypes. In the control group, $P = 0.733$ for comparison among all three genotypes.** ***B*****: Three-year incidence of type 2 diabetes in the intervention group and the control group by the Pro12Ala polymorphism of the PPAR-γ2 gene [% (number of subjects who developed type 2 diabetes/ total number of subjects)].**

subjects in the intervention group. A higher weight at the baseline examination as well as weight gain during the follow-up were also significant predictors for the development of type 2 diabetes (Table 2, Model 3).

The logistic regression analysis was also carried out separately in the intervention group and in the control group. When the weight change during the follow-up was included in the model, in the control group the odds of the development of type 2 diabetes for those with the Ala12 allele was 2.36-fold compared with the odds of the development of type 2 diabetes for subjects with the Pro12Pro genotype (95% CI 1.21–4.60). In the intervention group, the Ala12 allele was not significantly associated with an increased risk of type 2 diabetes, even though the risk was

TABLE 2
Logistic regression analysis for the development of type 2 diabetes

	Regression coefficient	*P*	Odds ratio	95% CI
Model 1				
Genotype*	0.68	0.011	1.98	1.17–3.34
Model 2				
Genotype	0.78	0.005	2.19	1.27–3.75
Study group†	1.19	<0.001	3.29	1.86–5.83
Model 3				
Genotype	0.75	0.010	2.11	1.20–3.72
Study group	0.90	0.004	2.46	1.35–4.51
Weight at baseline‡	0.03	<0.001	1.03	1.01–1.05
Weight change§	0.12	<0.001	1.13	1.06–1.19

Age and sex were not predictors of development of type 2 diabetes and were therefore omitted from the models. *Genotypes were encoded as 0 = Pro12Pro and 1 = Pro12Ala or Ala12Ala; †study groups were encoded as 1 = intervention group and 2 = control group; ‡weight at baseline is in kilograms; §weight change was calculated as weight $(kg)_{3\ year}$ − weight $(kg)_{baseline}$.

TABLE 3
Logistic regression analysis for the development of type 2 diabetes in the lower BMI group and in the higher BMI group.

	Regression coefficient	*P*	Odds ratio	95% CI
Lower BMI group				
Model 1				
Genotype*	0.99	0.018	2.68	1.19–6.07
Model 2				
Genotype	1.07	0.012	2.92	1.26–6.75
Study group†	1.31	0.005	3.70	1.48–9.26
Model 3				
Genotype	1.00	0.021	2.73	1.16–6.40
Study group	1.09	0.024	2.97	1.16–7.64
Weight change‡	0.10	0.059	1.10	1.00–1.22
Higher BMI group				
Model 1				
Genotype*	0.44	0.215	1.55	0.78–3.08
Model 2				
Genotype	0.55	0.128	1.74	0.85–3.53
Study group†	1.10	0.004	3.00	1.44–6.27
Model 3				
Genotype	0.54	0.150	1.72	0.82–3.59
Study group	0.69	0.081	2.00	0.92–4.35
Weight change‡	0.13	<0.001	1.14	1.06–1.23

*Genotypes were encoded as 0 = Pro12Pro and 1 = Pro12Ala or Ala12Ala; †study groups were encoded as 1 = intervention group and 2 = control group; ‡weight change was calculated as weight $(kg)_{3\ year}$ − weight $(kg)_{baseline}$.

almost twice as high as in those with the Pro12Pro genotype (odds ratio = 1.90, 95% CI 0.70–5.18).

Next we studied whether the association of the Ala12 allele with type 2 diabetes risk was modified by the degree of obesity. Therefore, DPS study subjects were divided into the two groups according to median of the baseline BMI. The first group, a lower BMI group (LBMI, mean BMI 27.7 ± 1.6 kg/m^2, n = 244), consisted of subjects whose baseline BMI was below or equal to the median of the whole study group baseline BMI. The second group, a higher BMI group (HBMI, mean BMI 34.7 ± 4.0 kg/m^2, n = 246), consisted of subjects whose baseline BMI was over the median of the whole study group baseline BMI. The Ala12 allele was a significant predictor of the development of type 2 diabetes in the LBMI group only (Table 3).

Finally, the influence of the Pro12Ala polymorphism on 3-year weight change was examined. In the intervention group, subjects with the Ala12Ala genotype lost more weight than subjects with the Pro12Pro genotype (−8.3 ± 7.3% vs. −3.4 ± 5.7%, P = 0.043) and the weight loss in subjects with the Pro12Ala genotype (−4.0 ± 6.0%) was close to that in subjects with the Pro12Pro genotype (Fig. 1*A*). In contrast, in the control group, 3-year weight changes were relatively small and the Pro12Ala polymorphism was not associated with the weight changes (−1.2 ± 6.1% for the Pro12Pro genotype, 0.2 ± 6.9% for the Pro12Ala genotype, and 1.0 ± 6.9% for the Ala12Ala genotype, P = 0.733) (Fig. 1*A*).

DISCUSSION

The present study is the first to examine the association of the Pro12Ala polymorphism of the PPAR-γ2 gene with the incidence of type 2 diabetes in a high-risk IGT population in a longitudinal study design. Interestingly, we found that in the IGT subjects, the Ala12 allele may predispose to the development of type 2 diabetes. In line with our study, the Ala12 allele has been reported to associate with type 2 diabetes in Canadian Oji-Cree women (24). Similarly, Evans et al. (25) found an association between the Ala12 allele and type 2 diabetes. Hasstedt et al. (26) studied members of familial type 2 diabetic kindreds and reported that the proportion of individuals with type 2 diabetes increased with the number of Ala12 alleles, although the result was not statistically significant.

The Ala12 allele has been associated with high insulin sensitivity (12,27,28), but there are also studies in which this association has not been found (13,26,29). The PPAR-γ gene is also expressed in pancreatic β-cells (30), and therefore the Ala12 allele could interfere with β-cell function. Indeed, type 2 diabetic patients with the Ala12 allele might have a reduced capacity for insulin secretion (31). In addition, Stefan et al. (29) reported that in healthy normal weight carriers of the Ala12 allele, second-phase insulin secretion after the experimental elevation of serum free fatty acids was decreased compared with that in subjects with the Pro12Pro genotype. In the present study, the Ala12 allele was associated with the type 2 diabetes risk particularly in less obese IGT subjects, in whom the reason for IGT could be related to disturbances in the insulin secretion rather than impaired insulin action. Thus, it is tempting to speculate that the Ala12 allele may predispose to insulin deficiency in the high-risk IGT population and therefore contribute to the development of type 2 diabetes.

In the present study, the Ala12 allele was not significantly associated with the incidence of type 2 diabetes in the intervention group, but a small number of cases might explain this. It has also been proposed that PPAR-γ2 is one of the mediators of the gene-environment interactions (32) and, likewise, it could be possible that the beneficial changes in diet, increased physical activity, and concomitant weight loss, as well as some gene-nutrient and gene-exercise interactions, protected subjects with the Ala12 allele in the intervention group from the development of

type 2 diabetes in the present study. For example, subjects in the intervention group reduced the intake of total and saturated fat and increased the level of physical activity (3), the factors known to improve insulin sensitivity (33,34). Therefore, we suggest that weight reduction together with other beneficial lifestyle changes improved insulin sensitivity and reduced the β-cell stress and, consequently, alleviated the deleterious effect of the Ala12 allele on the development of type 2 diabetes in the intervention group. In accordance with this presumption, there is evidence from the Troglitazone in Prevention of Diabetes Study that an induced β-cell rest may protect from the development of type 2 diabetes in high-risk individuals (35). In addition, Nicklas et al. (36) found that postmenopausal women with the Ala12 allele had a greater increase in insulin sensitivity and fasting carbohydrate oxidation after a 6-month hypocaloric diet compared with women with the Pro12Pro genotype in spite of similar amounts of weight loss. However, women with the Ala12 allele had a greater decrease in fasting lipid oxidation, and they regained more weight during 1-year follow-up compared with women with the Pro12Pro genotype (36). This may imply that although subjects with the Ala12 allele may in some circumstances lose weight more easily during active weight reduction program, they may regain the weight more easily after treatment as well.

In contrast to our results, the Ala12 allele has also been supposed to be slightly protective against type 2 diabetes (10,12,21,27,31). The reason for the discrepancy between these studies and the present study may partly be related to differences in study designs. All earlier studies have been cross-sectional, and the frequency of the Ala12 allele has been compared between the groups of type 2 diabetic patients and subjects with normal glucose tolerance. In the present study, the incidence of type 2 diabetes was evaluated after the 3-year follow-up in an IGT study population known to be at an increased risk for type 2 diabetes. Thus, our study population does not represent the general population, and our results can be generalized only to those subjects with the IGT. Several of the studies investigating the relationship between the Pro12Ala polymorphism and type 2 diabetes have been carried out in Japanese Americans (12) or in Japanese subjects (27,31). In the Japanese population, the frequency of the Ala12 allele is over four times lower than in the Finnish population (12,17,21), and this complicates the comparisons of the results obtained in these two populations. Moreover, as discussed above, the degree of obesity, the composition of diet, and the level of physical activity may modify the effect of the Ala12 allele on the risk of type 2 diabetes, and differences in these lifestyle factors between the present study and previous studies may also partly explain the divergent results.

Although the size of the DPS population was sufficient for the original intervention study (3), the number of subjects with the Ala12Ala genotype was only moderate for the association study described here. Thus, carriers of the Ala12Ala genotype were combined with subjects with the Pro12Ala genotype in the logistic regression analysis in the present study. In other words, the logistic regression model utilized in this study assumed dominant effect of the Ala12 allele over the Pro12 allele. However, it would be interesting to investigate the association between the Ala12 allele and the risk of type 2 diabetes in other large prospective studies using a statistical model supposing also a recessive mode of inheritance for the Ala12 allele.

In summary, in the present study, the Ala allele in codon 12 of the PPAR-γ2 gene was associated with the development of type 2 diabetes in the high-risk IGT population. However, among those who followed an intensive diet and exercise program, subjects with the Ala12Ala genotype lost more weight than subjects having the Pro12Pro genotype, and none of subjects with the Ala12Ala genotype developed type 2 diabetes in this group. Therefore, the weight loss, together with other beneficial lifestyle changes, might reverse to some extent the diabetogenic impact of the Ala12 allele. Because our study is the first longitudinal study investigating the association between the Ala12 allele and the risk of type 2 diabetes, there is a need to confirm the present findings in other prospective studies.

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