*Brief Genetics Report*

# The Effects of the Pro12Ala Polymorphism of the Peroxisome Proliferator–Activated Receptor-γ2 Gene on Insulin Sensitivity and Insulin Metabolism Interact With Size at Birth

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**Type 2 diabetes is known to be associated with a small body size at birth. Body size at birth is an indicator of the intrauterine environment. There is also a well-established association between the peroxisome proliferator–activated receptor (PPAR)-γ2 gene and type 2 diabetes. We therefore assessed whether the effects of the Pro12Ala polymorphism of the PPAR-γ2 gene on insulin sensitivity and insulin concentrations in adult life are modified by size at birth. We found that the effects of the Pro12Pro and Pro12Ala polymorphisms of the PPAR-γ2 gene in elderly people depended on their body size at birth. The well-known association between small body size at birth and insulin resistance was seen only in individuals with the high-risk Pro12Pro allele. In those who had low birth weight, the Pro12Pro polymorphism of the PPAR-γ2 gene was associated with increased insulin resistance ($P < 0.002$) and elevated insulin concentrations ($P < 0.003$). These interactions between the effects of the Pro12Ala polymorphisms of the PPAR-γ2 gene on adult traits and the effects of birth weight link two previously unknown associations together within the context of type 2 diabetes. We suggest that these findings reflect gene-environment interaction. *Diabetes* 51:2321–2324, 2002**

In humans, birth weight serves as a marker of the intrauterine environment. Low birth weight has been shown to be linked to higher rates of coronary heart disease and type 2 diabetes later in life (1–3). This has led to the "fetal origins" hypothesis, which proposes that these diseases originate through an adverse environment during development (4). This environment, it is argued, changes gene expression and leads to physiological or morphological phenotypes associated with disease. On this basis, one would predict that genes associated with these diseases will have different effects in people who had different birth weights. This phenomenon has already been described in relation to the effects of the vitamin D receptor gene on bone mineral density (5).

The peroxisome proliferator–activated receptor (PPAR)-γ is a nuclear hormone receptor that mediates the effects of synthetic and endogenous ligands to modulate the expression of numerous target genes (6,7). The expression changes under the influence of metabolic and hormonal factors, e.g., degree of obesity is of importance (8–10). The human PPAR-γ gene is composed of nine exons spanning >100 kb of genomic DNA on chromosome 3p25. The PPAR-γs are therapeutic targets for common disorders, including type 2 diabetes, obesity, and coronary heart disease, since they are regulators for adipocyte differentiation as well as glucose and lipid metabolism (6). Obviously, they are strong candidates for genes that influence insulin sensitivity and insulin metabolism.

Several variants in the PPAR-γ gene have been identified, among them the Pro12Ala polymorphism in the PPAR-γ2 isoform-specific exon B (6,11,12). This polymorphism reduces the transcriptional activity of PPAR-γ. The Ala12 allele has been suggested to protect against type 2 diabetes (13). However, the impact of the Pro12Ala polymorphism on the risk of type 2 diabetes is not uniform and may be dependent on lifestyle and other unrecognized factors (14,15). This polymorphism has also been found to be associated with altered insulin sensitivity (8,10).

The purpose of this study was to examine whether the effects of the Pro12Ala polymorphism of the PPAR-γ2 gene on insulin sensitivity in adult life are modified by size at birth.

A total of 152 (32%) of the 476 subjects examined had either the Pro12Ala (29%) or the Ala12Ala (3%) genotypes. The frequency of the Ala12 allele was therefore 0.173 in the study population. The observed genotype frequencies of the polymorphism were in agreement with Hardy-Weinberg expectations ($P = 0.92$).

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HOMA-IR, homeostasis model assessment for insulin resistance; PPAR, peroxisome proliferator–activated receptor.

TABLE 1
Clinical measurements according to PPAR-γ2 gene polymorphism

| | Pro12Pro | Pro12Ala/Ala12Ala | *P* |
|---|---|---|---|
| *N* | 324 | 152 | |
| M/F | 112/212 | 59/93 | |
| Age (years) | 69.5 ± 2.8 | 69.8 ± 2.9 | 0.27 |
| BMI (kg/m$^2$) | 27.5 ± 4.4 | 28.0 ± 4.3 | 0.30 |
| Glucose 0 min (mmol/l) | 5.68 ± 1.21* | 5.47 ± 1.24* | 0.06 |
| Glucose 120 min (mmol/l) | 7.94 ± 1.45* | 7.80 ± 1.43* | 0.64 |
| Insulin 0 min (pmol/l) | 71 ± 1.72* | 61 ± 1.75* | 0.01 |
| Insulin 120 min (pmol/l) | 487 ± 1.98* | 506 ± 1.89* | 0.58 |
| Proinsulin (pmol/l) | 3.21 ± 2.06* | 2.82 ± 2.15* | 0.07 |
| 32-33 split proinsulin (pmol/l) | 8.14 ± 2.08* | 7.83 ± 1.97* | 0.58 |
| HOMA-IR index | 18.0 ± 1.83 | 14.9 ± 1.80 | 0.05 |
| Cholesterol (mmol/l) | 5.9 ± 1.1 | 6.1 ± 1.1 | 0.12 |
| HDL cholesterol (mmol/l) | 1.43 ± 1.32* | 1.46 ± 1.32* | 0.41 |
| LDL cholesterol (mmol/l) | 3.8 ± 0.9 | 3.9 ± 1.0 | 0.25 |
| Triglycerides (mmol/l) | 1.29 ± 1.50* | 1.31 ± 1.57* | 0.73 |

Data are means ± SD. *Geometric means ± SD. *P* values were adjusted for age, sex, and adult BMI.

Birth weight was inversely related to four indexes of insulin resistance and insulin metabolism: 2-h plasma insulin ($P = 0.02$), fasting proinsulin ($P < 0.001$), 32-33 split proinsulin ($P = 0.01$) concentrations, and homeostasis model assessment for insulin resistance (HOMA-IR) index ($P = 0.05$). It was similarly inversely, though not significantly, related to fasting plasma insulin concentration ($P = 0.10$). It was positively related to HDL cholesterol concentration ($P = 0.04$). It was not related to plasma glucose or serum total or LDL cholesterol or triglyceride concentrations.

Subjects with the Pro12Ala or the Ala12Ala genotype had lower fasting insulin and proinsulin concentrations and a lower HOMA-IR index (Table 1) compared with subjects with the Pro12Pro genotype. There were no differences between the groups in serum lipid concentrations and no differences in birth weight ($P = 0.53$) or length ($P = 0.73$).

We found that the effects of the Pro12Ala polymorphism on fasting insulin and HOMA-IR index depended on birth weight (*P* values for interaction 0.03 and 0.05). The interactions with birth weight were similar in men and women. Insulin levels were only raised in subjects who had low birth weight and the Pro12Pro genotype. There were no interactions between the effects of the Pro12Ala polymorphisms on insulin sensitivity and body size at 7 years of age. Likewise, no gene/adult body mass interactions were found.

We have shown that the Pro12Pro genotype of the PPAR-γ2 gene was associated with two markers of glucose and insulin metabolism: higher fasting insulin concentrations and insulin resistance, as measured using the HOMA-IR index. However, this association was observed only among men and women whose birth weight was <3,500 g (Table 2).

The associations between the Pro12Ala polymorphisms of the PPAR-γ gene and insulin sensitivity and insulin concentrations are consistent with previous studies (8,10), as are the associations we found between birth weight and these parameters (1–4).

There are limitations of our study, which was carried out on a sample of elderly people belonging to an epidemiological cohort. The frequency of the Ala12 allele in our study population was 0.173, consistent with previous studies in Finland (9). The allele frequency was also constant across birth weight groups ($P = 0.63$ for trend). Furthermore, we have previously shown that the associations between size at birth and metabolic outcome are not affected by elderly age (16). Therefore, we believe that the strong interaction between the Pro12Ala polymorphism and birth weight on those important metabolic factors is not a cause of confounding factors.

TABLE 2
Mean fasting insulin concentration and HOMA-IR index according to PPAR-γ gene polymorphism and birth weight

| | Birth weight (g) | | | |
|---|---|---|---|---|
| | −3,000 | −3,500 | >3,500 | *P** |
| Fasting insulin (pmol/l) | | | | |
| Pro12Pro (*n*) | 84 (56) | 71 (161) | 65 (107) | 0.003 |
| Pro12Ala/Ala12Ala (*n*) | 60 (37) | 60 (67) | 65 (48) | 0.31 |
| *P*† | 0.008 | 0.02 | 0.99 | |
| HOMA-IR index | | | | |
| Pro12Pro | 21.6 | 17.9 | 16.5 | 0.002 |
| Pro12Ala/Ala12Ala | 14.6 | 15.0 | 15.2 | 0.47 |
| *P* | 0.005 | 0.03 | 0.47 | |

Numbers of subjects in each cell are shown within parentheses. *For the difference among birth weight groups; †for the difference between the Pro12Pro and Pro12Ala/Ala12Ala genotypes.

The PPAR-γ is a nuclear hormone receptor that plays a crucial role in several aspects of metabolism, translating nutritional, pharmacological, and metabolic stimuli into changes in gene expression (6,7). PPAR-γ expression is relatively restricted, and adipose tissue expresses the highest levels of PPAR-γ mRNA and protein. PPAR-γ is also expressed in normal human pancreatic islets at the mRNA and protein levels (17). Therefore, the PPAR-γ gene could be involved in the regulation of pancreatic β-cell function and potentially even in pancreatic β-cell development (18). Recent data suggest that the Pro12Ala polymorphism of the PPAR-γ2 gene is involved in insulin secretion in response to free fatty acids (19). Likewise, there are suggestions that the Ala12 allele, although associated with lower fasting insulin concentrations in impaired glucose–tolerant subjects, increases the risk for type 2 diabetes (20). These findings support the idea that the Ala12 allele may predispose to insulin deficiency in a high-risk group. These findings also fit the well-known heterogeneity of type 2 diabetes.

The Ala12 allele has previously been associated with higher insulin sensitivity, although the findings are inconsistent (8,18). In the present study, we have shown that the Ala12 allele seems to protect against the insulin resistance that is associated with a small body size at birth. The interaction observed between body size at birth and markers of insulin sensitivity and insulin metabolism requires further consideration. Intrauterine growth failure leading to a small birth weight is known to affect the number of pancreatic β-cells as well as the function of the β-cells (21). An individual with a low birth weight and the Pro12Pro genotype might have a compensatory β-cell hyperplasia and consequently hyperinsulinemia causing insulin resistance and predisposing to type 2 diabetes.

It has previously been proposed that the PPAR-γ2 gene is one mediator of gene-environment interactions, because the effect of the Ala12 allele on BMI and type 2 diabetes has been shown to be modified by dietary intake, degree of obesity, and level of physical activity (20,22). We have now shown gene-environment interactions during development as well as in adult life. Size at birth is primarily an indicator of the early environment because the effects of the intrauterine environment on fetal growth dominate those of the fetal genome.

Variants in the PPAR-γ2 gene could affect transcriptional activation of several genes in adipose tissue. Changes in adipose tissue metabolism could cause insulin resistance. The PPAR-γ2 gene is an obvious candidate gene for the metabolic syndrome, in which insulin resistance is a major determinant. Also, associations between gene polymorphism and birth weight have been reported, for example, in the insulin gene (23). Interactions between the effects of genes and those of birth weight could therefore reflect gene-gene interactions. However, in the present study, we found no difference in size at birth between those with and without the Ala12 allele. We therefore suggest that our findings reflect gene-environment interactions. This finding helps link two previously unknown associations, birth weight and the PPAR-γ2 gene, within the context of type 2 diabetes.

## RESEARCH DESIGN AND METHODS

The original epidemiological study of 7,086 men and women, born as singletons at Helsinki University Central Hospital during 1924–1933, has been described (24). They attended school in Helsinki and were still resident in Finland in 1971. Details of their birth and school health records have previously been described. Their birth records included birth weight and length. Their school health records included a mean of 10 (SD 4) measurements of height and weight between 6 and 16 years of age.

A total of 674 subjects from the original study cohort of 7,086 men and women, who were known to be living in the greater Helsinki area, were invited to attend a clinical study in the morning after an overnight fast. Of the 500 (74%) patients who attended the clinic, 476 had DNA samples drawn. The mean age at the time of investigation was 70 ± 3 years. An oral glucose tolerance test (75 g glucose) was performed. Plasma glucose and insulin concentrations were measured at 0, 30, and 120 min. Plasma glucose was measured by the hexokinase method. Plasma insulin, proinsulin, and 32-33 split proinsulin concentrations were determined by two-site immunometric assay (25). Serum total and HDL cholesterol and triglyceride concentrations were measured using standard enzymatic methods. LDL cholesterol concentration was derived by the Freidewald-Frederickson formula. Insulin resistance was measured by HOMA-IR as the product of fasting insulin and glucose concentrations divided by 22.5.

The Pro12Ala polymorphism of the PPAR-γ2 gene was determined by the PCR-SSCP (single-strand conformation polymorphism) method, and the genotypes were encoded as 0 = Pro12Pro and 1 = Pro12Ala or Ala12Ala.

**Statistical methods.** Data were analyzed by tabulation of means and multiple linear regression. Levels of significance refer to analyses of continuous variables. Plasma glucose, insulin, proinsulin, HDL cholesterol, and triglyceride concentrations had skewed distributions and were log transformed for analysis. We adjusted values for age, sex, and current BMI. Regresssions were done within birth weight categories and within genotype, when birth weight in grams was used.

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