The PPAR-γ Pro12Ala polymorphism associates with weight gain during GH-treatment in short children born small for gestational age

Sandra W K de Kort and Anita C S Hokken-Koelega
Division of Endocrinology, Department of Pediatrics, Erasmus University Medical Center, Sophia Children's Hospital, Room number SB-2603, Dr Molewaterplein 60, 3015 GJ Rotterdam, The Netherlands
(Correspondence should be addressed to S W K de Kort; Email: s.dekort@erasmusmc.nl)

Abstract

Context: Short children born small for gestational age (SGA) have a lean phenotype with lower insulin sensitivity and higher blood pressure. GH treatment results in weight gain, and a decrease in blood pressure and insulin sensitivity. However, not all children respond in the same way. The Pro12Ala polymorphism of the peroxisome proliferator-activated receptor (PPAR-γ) gene is inversely associated with body mass index (BMI), changes in BMI and the risk to develop type 2 diabetes mellitus.

Objective: To analyze the contribution of the PPAR-γ Pro12Ala polymorphism to GH induced changes in determinants of metabolic and cardiovascular disease in short SGA children.

Methods: PPAR-γ was genotyped in 238 Caucasian short SGA children (mean age 7.5 years). Height, weight, blood pressure, and serum lipids were measured before start and during 4 years of GH treatment. In addition, glucose homeostasis by homeostasis model assessment insulin resistance ratio (HOMA-IR) (n=148) and by frequently sampled i.v. glucose tolerance test (n=51), and body composition by dual energy X-ray absorptiometry (n=79) were measured.

Results: At baseline, the Ala12 allele was not associated with any determinant of metabolic and cardiovascular disease. After 4 years of GH treatment, the increase in weight for height SDS and BMI SDS was significantly greater in carriers of an Ala12 allele than in noncarriers. The change in all other parameters was not associated with Pro12Ala genotype.

Conclusion: The Ala12 variant of the PPAR-γ gene is associated with higher weight gain during GH treatment but not with changes in determinants of metabolic and cardiovascular diseases in Caucasian subjects born SGA.
environmentally determined or by a combination of both. Most children with short stature who were born SGA are nowadays treated with GH. GH treatment generally results in weight gain, a decrease in blood pressure, serum lipid levels, and insulin sensitivity (11, 12). Also, there is a compensatory increase in insulin secretion (11). However, not all children respond in the same way. Since the PPAR-γ gene was associated with BMI, glucose homeostasis, and atherosclerosis (1), genotyping might indicate which children are less capable to compensate for the effects of GH treatment on metabolic and cardiovascular risk factors and are thus more at risk to develop adult diseases.

The present study aimed to investigate whether PPAR-γ polymorphisms correlate with changes in the metabolic and cardiovascular profile during GH treatment. To investigate this question, we performed genotyping of the PPAR-γ gene, measurements of anthropometry, blood pressure, serum lipids, frequently sampled i.v. glucose tolerance tests (FSIGTs), and calculations of HOMA-IR in 238 short children who were born SGA and treated with GH.

**Subjects and methods**

**Subjects and study design**

The study group comprised 238 children (124 boys) with short stature (height SDS for age and gender < −2 (13)) who were born SGA (birth length and/or birth weight SDS < −2.0 for gestational age (14)) and treated with biosynthetic GH at a dose of 1 mg/m² body surface area per day. Inclusion criteria have previously been described (15, 16). Height, weight, blood pressure, and serum lipids were measured before the start and during 4 years of GH treatment as previously described (17). In addition, glucose homeostasis by HOMA-IR (n = 148) and by FSIGT (n = 51) (11, 18), and body composition by dual energy X-ray absorptiometry scans (DEXA, type Lunar DPX-L, GE Healthcare, Madison, WI, USA) (17) (n = 79) were measured. The Medical Ethics Committees of Erasmus Medical Center, Rotterdam, and the other participating centers approved all studies and written informed consent was obtained from all participants and their parents.

**Genotyping**

Genomic DNA was extracted from samples of peripheral venous blood according to salting out procedure (19). Genotyping of the PPAR-γ gene Pro12Ala polymorphism (rs1801282) was performed using the Taqman allelic discrimination assay. PCR was performed in 384 wells PCR plates in an ABI 9700 PCR system (Applied Biosystems Inc., Foster City, CA, USA) and consisted of initial denaturation for 10 min at 95 °C and 40 cycles with denaturation of 15 s at 92 °C and annealing and extension for 60 s at 60 °C. Results were analyzed by ABI Taqman 7900HT using the sequence detection system 2.22 software (Applied Biosystems Inc).

**Statistical analysis**

Genotype distributions for significant departure from the Hardy–Weinberg equilibrium were calculated using the χ² test. To assess longitudinally measured growth and metabolic characteristics from the start

**Table 1 Patient characteristics before the start of GH treatment.**

<table>
<thead>
<tr>
<th></th>
<th>All (n=238)</th>
<th>Pro/Pro (n=168)</th>
<th>Pro/Ala or Ala/Ala (n=70)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height SDS</td>
<td>−2.98 (0.7)</td>
<td>−2.97 (0.7)</td>
<td>−3.01 (0.7)</td>
<td>0.72</td>
</tr>
<tr>
<td>Weight SDS</td>
<td>−1.14 (1.2)</td>
<td>−1.16 (1.3)</td>
<td>−1.09 (1.0)</td>
<td>0.68</td>
</tr>
<tr>
<td>BMI SDS</td>
<td>−1.25 (1.0)</td>
<td>−1.29 (1.0)</td>
<td>−1.16 (0.9)</td>
<td>0.36</td>
</tr>
<tr>
<td>Body fat %</td>
<td>12.98 (7.0)</td>
<td>12.83 (7.7)</td>
<td>12.32 (5.0)</td>
<td>0.77</td>
</tr>
<tr>
<td>Body fat % SDSage</td>
<td>−0.76 (1.0)</td>
<td>−0.70 (1.1)</td>
<td>−0.76 (1.0)</td>
<td>1.00</td>
</tr>
<tr>
<td>Body fat % SDSheight</td>
<td>−0.77 (1.3)</td>
<td>−0.79 (1.4)</td>
<td>−0.73 (1.2)</td>
<td>0.87</td>
</tr>
<tr>
<td>LBM SDSage</td>
<td>−2.49 (0.7)</td>
<td>−2.54 (0.8)</td>
<td>−2.39 (0.4)</td>
<td>0.38</td>
</tr>
<tr>
<td>LBM SDSheight</td>
<td>−0.87 (1.9)</td>
<td>−1.03 (1.9)</td>
<td>−0.50 (1.8)</td>
<td>0.25</td>
</tr>
<tr>
<td>Systolic bp SDS</td>
<td>1.0 (1.1)</td>
<td>1.0 (1.1)</td>
<td>1.2 (1.1)</td>
<td>0.24</td>
</tr>
<tr>
<td>Diastolic bp SDS</td>
<td>0.3 (1.0)</td>
<td>0.2 (1.0)</td>
<td>0.4 (1.1)</td>
<td>0.36</td>
</tr>
<tr>
<td>F glucose (mmol/l)</td>
<td>4.7 (0.6)</td>
<td>4.7 (0.6)</td>
<td>4.7 (0.6)</td>
<td>0.73</td>
</tr>
<tr>
<td>F insulin (pmol/l)</td>
<td>24.0 (14.0–36.0)</td>
<td>25.0 (14.0–40.0)</td>
<td>30.0 (15.0–48.0)</td>
<td>0.39</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.4 (0.3–0.7)</td>
<td>0.5 (0.3–0.8)</td>
<td>0.6 (0.3–0.9)</td>
<td>0.31</td>
</tr>
<tr>
<td>Si”10⁻³/min (µU/ml)</td>
<td>13.4 (9.9–19.0)</td>
<td>12.2 (7.7–18.4)</td>
<td>12.1 (8.9–16.0)</td>
<td>0.76</td>
</tr>
<tr>
<td>AIR (mU/l)</td>
<td>267 (180–352)</td>
<td>214 (170–355)</td>
<td>317 (266–440)</td>
<td>0.11</td>
</tr>
<tr>
<td>DL (AIR*Si)</td>
<td>3139 (2575–4715)</td>
<td>2880 (2190–4600)</td>
<td>3115 (2671–4671)</td>
<td>0.32</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>4.3 (0.8)</td>
<td>4.3 (0.8)</td>
<td>4.2 (0.7)</td>
<td>0.69</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>1.5 (0.4)</td>
<td>1.4 (0.4)</td>
<td>1.5 (0.4)</td>
<td>0.91</td>
</tr>
<tr>
<td>LDL (mmol/l)</td>
<td>2.4 (0.7)</td>
<td>2.4 (0.7)</td>
<td>2.4 (0.6)</td>
<td>0.69</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>0.8 (0.4)</td>
<td>0.8 (0.3)</td>
<td>0.8 (0.5)</td>
<td>0.69</td>
</tr>
</tbody>
</table>

Values are expressed as mean (±SD) or as median (interquartile range). Data with a skewed distribution were log transformed before analysis with t-tests. LBM, lean body mass; bp, blood pressure; F, fasting; HOMA-IR, insulin resistance index; Si, insulin sensitivity; AIR, acute insulin response; DL, disposition index. n = 79 for body fat % and LBM, n = 225 for blood pressure, n = 148 for F glucose, F insulin, and HOMA-IR; n = 136 for lipid levels; n = 51 for Si, AIR, and DL.
of GH treatment to 4 years after the start of treatment, we performed repeated measures regression analysis as previously described (20, 21). The models can be written as: outcome variable = \( \beta_0 + \beta_1 \text{age} + \beta_2 \text{gender} + \beta_3 \text{puberty} + \beta_4 \text{study duration} + \beta_5 \text{genotype} + \beta_6 \text{genotype*study duration} \).

Statistical significance was defined as \( P<0.05 \). Analyses were performed using the statistical packages SPSS (version 15.0; SPSS Inc., Chicago, IL, USA) for Windows. SAS 8.2 (SAS Institute Inc., Cary, NC, USA) was used for repeated measures of variance analyses.

**Results**

The genotype distribution was Pro12Pro 70.6%, Pro12Ala 26.9%, and Ala12Ala 2.5% and was not different from the Hardy–Weinberg expectations (\( \chi^2 = 0.001, P = 0.97 \)). The frequency of the Ala12 allele was 16%, as reported in literature (3, 22). Mean (S.D.) age was 7.5 (2.9) years, birth weight SDS for children with a change in weight SDS height (Pro12Ala polymorphism was not significantly different between carriers and noncarriers (4, 22–26), results about the association between Pro12Ala and estimates of glucose homeostasis and blood pressure have been conflicting. A population of short SGA children had not been studied yet. Among the risk factors that potentiate the insulin resistance associated with low birth weight, obesity is very important (10) but short SGA subjects are usually lean. As the association of PPAR-\(\gamma\) Pro12Ala with insulin sensitivity might be mediated through body weight, it is important to study short SGA subjects separately. We found no direct association between PPAR-\(\gamma\) Pro12Ala genotype and glucose homeostasis or blood pressure.

In conclusion, our data demonstrate the possible involvement of the PPAR-\(\gamma\) Pro12Ala polymorphism in weight gain during GH treatment in short SGA children. The polymorphism was not associated with determinants of metabolic and cardiovascular disease, either at baseline or during GH treatment.

**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

**Funding**

This was an investigator-initiated study, which received independent research grants from Novo Nordisk Farma B.V. and Pfizer B.V., The Netherlands.

**Acknowledgements**

We greatly acknowledge Mrs C Bruinings-Vroombout, Mrs M Huijbregts-Schouten, Mrs J van Houten, Mrs J van Nieuwkaastele, Mrs J Dunk and Mrs E Lems, research nurses, for their assistance and help with data collection; Mrs J P Sluimer for performing and analyzing the DEXA measurements, Dr W H Hackeng for his glucose and insulin assays, M A J de Bidder for her help with the statistical analysis, and Dashan Gorbenko Del Bianco for her help with the genetics. We also greatly acknowledge the participating physicians: E G A H van Mil, Free University Hospital Amsterdam, The Netherlands; J C Mulder, Rijnstate Hospital, Arnhem, The Netherlands; R J H Oudink and J J J Waelkens, Catharina Hospital, Eindhoven, The Netherlands; W M Bakker-van Waarde, University Medical Center Groningen, Groningen, The Netherlands; W H Stokvis, B Bakker, Leiden University Medical Center, Leiden, The Netherlands; N J T Arends, E M Bannink, V H Noordam, Radboud University Medical Center Nijmegen, Nijmegen, The Netherlands; C Noordam, Radboud University Medical Center Nijmegen, Nijmegen, The Netherlands; C Westerlaken, Canisius Wilhelmina Hospital, Nijmegen, The Netherlands; C Noordam, Radboud University Medical Center Nijmegen, Nijmegen, The Netherlands; C Westerlaken, Canisius Wilhelmina Hospital, Nijmegen, The Netherlands; C Westerlaken, Canisius Wilhelmina Hospital, Nijmegen, The Netherlands; C Westerlaken, Canisius Wilhelmina Hospital, Nijmegen, The Netherlands; C Westerlaken, Canisius Wilhelmina Hospital, Nijmegen, The Netherlands; C Westerlaken, Canisius Wilhelmina Hospital, Nijmegen, The Netherlands.
References

1 Auwerx J. PPARγ, the ultimate thrifty gene. Diabetologia 1999 42 1033–1049.

Received 9 September 2009
Accepted 1 October 2009