CLINICAL STUDY

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

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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.

European Journal of Endocrinology 162 49–52

Introduction

GH treatment may be a modifier of the association between polymorphisms in the peroxisome proliferator-activated receptor γ (PPAR- γ) gene and glucose homeostasis and the risk of type 2 diabetes mellitus (DM) in later life. PPAR- γ is a nuclear hormone receptor that controls genes involved in adipogenesis, and lipid and glucose metabolism (1). The Ala12 variant of the PPAR- γ gene has been associated with improved insulin sensitivity and a lower risk for type 2 DM compared with the Pro12Pro genotype (2). In contrast, in a study in overweight individuals with impaired glucose tolerance (3) and in young adults born small for gestational age (SGA) (4), the Ala12 allele was associated with an increased risk for type 2 DM.

Results about the association of the Ala12 variant with body mass index (BMI) are conflicting. The Ala12 variant has been associated with a higher BMI (5, 6) and a tendency to gain weight over time (7).

However, in subjects with a normal body weight, the Ala12Ala genotype was associated with a lower BMI and a lower increase in BMI at follow-up (8), and in a meta-analysis of subjects with a mean BMI < 27 kg/m², there was no significant difference in BMI between genotype groups (6). Size at birth also interacts with the effect of the Ala12 allele. In young adults who were born SGA, increased BMI amplified the effect of PPAR- γ polymorphism on glucose homeostasis, while in those born appropriate for gestational age there was not such an association (4). These conflicting results regarding associations of the Ala12 allele with insulin resistance and BMI demonstrate that the allele has a different effect in different environments, and that environmental factors may play a role to increase the genetic effect of PPAR- γ on metabolic risk factors.

Subjects born SGA might have an increased risk to develop type 2 DM in later life (9), particularly those with catch-up in weight (10). It is, however, unknown whether this increased risk is genetically or

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 χ^2 test. To assess longitudinally measured growth and metabolic characteristics from the start

Table 1 Patient characteristics before the start of GH treatment.

	All ($n = 238$)	Pro/Pro ($n = 168$)	Pro/Ala or Ala/Ala ($n = 70$)	P value
Height SDS	−2.98 (0.7)	−2.97 (0.7)	−3.01 (0.7)	0.72
Weight SDS _{height}	−1.14 (1.2)	−1.16 (1.3)	−1.09 (1.0)	0.68
BMI SDS _{age}	−1.25 (1.0)	−1.29 (1.0)	−1.16 (0.9)	0.36
Body fat %	12.68 (7.0)	12.83 (7.7)	12.32 (5.0)	0.77
Body fat % SDS _{age}	−0.76 (1.0)	−0.76 (1.1)	−0.76 (1.0)	1.00
Body fat % SDS _{height}	−0.77 (1.3)	−0.79 (1.4)	−0.73 (1.2)	0.87
LBM SDS _{age}	−2.49 (0.7)	−2.54 (0.8)	−2.39 (0.4)	0.38
LBM SDS _{height}	−0.87 (1.9)	−1.03 (1.9)	−0.50 (1.8)	0.25
Systolic bp SDS	1.0 (1.1)	1.0 (1.1)	1.2 (1.1)	0.24
Diastolic bp SDS	0.3 (1.0)	0.2 (1.0)	0.4 (1.1)	0.36
F glucose (mmol/l)	4.7 (0.6)	4.7 (0.6)	4.7 (0.6)	0.73
F insulin (pmol/l)	24.0 (14.0–36.0)	25.0 (14.0–40.0)	30.0 (15.0–48.0)	0.39
HOMA-IR	0.4 (0.3–0.7)	0.5 (0.3–0.8)	0.6 (0.3–0.9)	0.31
Si*10 ^{−4} /min (μU/ml)	13.4 (9.9–19.0)	12.2 (7.7–18.4)	12.1 (8.9–16.0)	0.76
AIR (mU/l)	267 (180–352)	214 (170–355)	317 (266–440)	0.11
DI (AIR*Si)	3139 (2575–4715)	2880 (2190–4600)	3115 (2671–4671)	0.32
Cholesterol (mmol/l)	4.3 (0.8)	4.3 (0.8)	4.2 (0.7)	0.69
HDL (mmol/l)	1.5 (0.4)	1.4 (0.4)	1.5 (0.4)	0.91
LDL (mmol/l)	2.4 (0.7)	2.4 (0.7)	2.4 (0.6)	0.69
Triglycerides (mmol/l)	0.8 (0.4)	0.8 (0.3)	0.8 (0.5)	0.69

Values are expressed as mean (s.d.) 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; DI, 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 DI.

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 = $\beta_0 + \beta_1 \text{age} + \beta_2 \text{gender} + \beta_3 \text{puberty} + \beta_4 \text{study duration} + \beta_5 \text{genotype} + \beta_6 \text{genotype} \times \text{study duration}$.

Statistical significance was defined as $P < 0.05$. Analyses were performed using the statistical package 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 -2.27 (1.1), and birth length SDS -3.15 (1.4). No significant differences in age, gender distribution, size at birth, anthropometry, body composition, blood pressure, glucose homeostasis, and serum lipid levels were observed between the Ala12 carriers and non-carriers (Table 1). Twenty-three children (9.7%) were in puberty at the start of study. The proportion of pubertal subjects was similar in both genotype groups ($P = 0.91$), and all results were similar when pubertal subjects were excluded.

During 4 years of GH treatment, the increase in weight SDS_{height} (0.9 vs 0.6 SDS increase, $P = 0.02$) and in BMI SDS (0.6 vs 0.4 SDS increase, $P = 0.03$) was greater in carriers of Ala12 than in noncarriers. The change in all other evaluated parameters was not significantly different between carriers and noncarriers of the Ala12 allele. The genotype distribution of the Pro12Ala polymorphism was not significantly different for children with a change in weight SDS_{height} ($n = 186$) or BMI SDS ($n = 172$) > 0 , and children with a change in weight SDS_{height} ($\chi^2 = 2.23$, $P = 0.14$) or BMI SDS ($\chi^2 = 1.79$, $P = 0.18$) of ≤ 0 . In the subgroup of 79 children with a DEXA scan, the Pro12Ala polymorphism was not associated with the change in body fat % SDS or the change in lean body mass SDS. However, in this much smaller group, weight SDS_{height} and BMI SDS were also not associated with the Pro12Ala polymorphism, which might indicate a power problem in the relatively small DEXA group.

Discussion

In this study, we demonstrated that the PPAR- γ Pro12Ala polymorphism was associated with a greater increase in weight SDS and BMI SDS during GH treatment of short SGA children. An explanation for

this observation might be that the Ala12 allele facilitates fat accumulation. In a smaller subgroup with DEXA scans, we found no support for this hypothesis. In SGA children who caught up to a normal height and subjects with type 2 DM or obesity (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- γ 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- γ Pro12Ala genotype and glucose homeostasis or blood pressure.

In conclusion, our data demonstrate the possible involvement of the PPAR- γ 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 Huibregtse-Schouten, Mrs J van Houten, Mrs J van Nieuwkastele, 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 Ridder for her help with the statistical analysis, and Dasha Gorbenko Del Blanco 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 Odink 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; C Noordam, Radboud University Medical Center Nijmegen, Nijmegen, The Netherlands; C Westerlaken, Canisius Wilhelmina Hospital, Nijmegen, The Netherlands; N J T Arends, E M Bannink, V H Boonstra, M Van Dijk, Y K van Pareren, T C J Sas, Erasmus Medical Center Sophia, Rotterdam, The Netherlands; H M Reeser and E C A M Houdijk, Juliana Children's Hospital, The Hague, The Netherlands; E J Schroor, Isala clinics, Zwolle, The Netherlands; J P C M van der Hulst, Zaans Medical Centre, Zaandam, The Netherlands; E J Sulkers, Walcheren Hospital, Vlissingen, The Netherlands; and M Jansen, Wilhelmina Children's Hospital, Utrecht, The Netherlands.

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Received 9 September 2009

Accepted 1 October 2009