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# Association Analysis of Ten Candidate Genes in a Large Multinational Cohort of Small for Gestational Age Children and Children with Idiopathic Short Stature (NESTEGG study)

L.C.G. de Graaff[a] A.J.L. Clark[c] M. Tauber[e, f] M.B. Ranke[g] L.B. Johnston[c] J. Caliebe[g] C. Molinas[e, f] N. Amin[b] C. van Duijn[b] H. Wollmann[d] H. Wallaschofski[h] M.O. Savage[c] A.C.S. Hokken-Koelega[a] on behalf of the NESTEGG group

[a]Division of Endocrinology, Department of Paediatrics, Erasmus MC-Sophia Children's Hospital and [b]Department of Epidemiology and Biostatistics, Erasmus MC, Rotterdam, The Netherlands; [c]Department of Endocrinology, Barts and the London Queen Mary School of Medicine, John Vane Science Centre, London, and [d]Medicines Development Group, Pfizer Specialty Care, Surrey, UK; [e]Division of Endocrinology, Genetics, Gynaecology and Bone Diseases, Hôpital des Enfants, and [f]Unité INSERM, U563 (CPTP), IFR 30, Hôpital Purpan, Toulouse, France; [g]Paediatric Endocrinology Section, University Children's Hospital, Tuebingen, and [h]Institute of Clinical Chemistry and Laboratory Medicine, Metabolic Center, Ernst-Moritz-Arndt University of Greifswald, Greifswald, Germany

**Key Words**
Polymorphisms · Genotype · Phenotype · Growth hormone · Birth length · Birth weight · Gestational age

**Abstract**
***Background:*** Fetal growth failure has been associated with an increased risk of hypertension, cardiovascular disease and diabetes in adulthood. Exploring the mechanisms underlying this association should improve our understanding of these common adult diseases. ***Patients and Methods:*** We investigated 225 SNPs in 10 genes involved in growth and glucose metabolism (*GH1, GHR, IGF1, IGF1R, STAT5A, STAT5B, MAPK1, MAPK3, PPARγ* and *INS*) in 1,437 children from the multinational NESTEGG consortium: 345 patients born small for gestational age who remained short (SGA-S), 288 who showed catch-up growth (SGA-Cu), 410 idiopathic short stature (ISS) and 394 controls. We related genotype to pre- and/or postnatal growth parameters, response to growth hormone (if applicable) and blood pressure. ***Results:*** We found several clinical associations for *GH1, GHR, IGF1, IGF1R, PPARγ* and *MAPK1*. One SNP remained significant after Bonferroni's correction: *IGF1R* SNP rs4966035's minor allele A was significantly more prevalent among SGA and associated with smaller birth length (p = 0.000378) and birth weight (weaker association), independent of gestational age. ***Conclusion:*** *IGF1R* SNP rs4966035 is significantly associated with birth length, independent of gestational age. This and other associations suggest that polymorphisms in these genes might partly explain the phenotype of short children born SGA and children with ISS.


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E-Mail karger@karger.com
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Laura de Graaff, MD, PhD
Division of Endocrinology, Erasmus MC, University Medical Center
's Gravendijkwal 230
NL–3015 CE Rotterdam (The Netherlands)
E-Mail l.degraaff@erasmusmc.nl

## Introduction

Growth in early life is a complex process that is influenced by genetic, endocrine, nutritional and many other factors. Although the major endocrine determinants of growth have been intensively studied, our understanding of other aetiological mechanisms is incomplete. This is important for several reasons. Firstly, fetal growth failure is associated with long-term consequences to adult cardiometabolic health. Specifically, small size at birth has been associated with an increased risk of diabetes, hypertension and cardiovascular disease in adulthood [1–4]. Secondly, up to now therapeutic options in fetal growth failure are very limited and tend not to focus on reversal of the underlying defect. Thirdly, although improvement in linear postnatal growth of the child can often be achieved with growth hormone (GH), the underlying defect is usually not GH deficiency. Therefore, increased insight into the pathophysiology might be the first step towards an individualized therapeutic stratification.

Several large-scale epidemiological studies have shown that maternal, paternal and fetal genetic factors play a major role in determination of birth size [5–11], as reviewed by Dunger et al. [5]. However, progress in understanding precisely which genetic factors are important has been slow. As has been found with a number of complex disorders, investigation of uncommon single gene disorders provides some insight into the prevailing mechanisms. Mutations in insulin-like growth factor 1 *(IGF1)*, its receptor *(IGF1R)* and glucokinase have been recognised as having a causative role in rare cases of small for gestational age birth size [12–19]. Demonstrating a role for these and other genes in populations has been more difficult and has frequently focused on a small number of polymorphisms in the genes studied in small- or medium-sized affected cohorts in comparison to controls.

The Network of European Studies of Genes in Growth (NESTEGG) study is a large European study that aims to characterize in detail children with pre- and/or postnatal growth failure and their parents in order to study the genetic basis of growth failure [20, 21]. The study includes children born small for gestational age (SGA) who remained short later in life (SGA-S) and those who showed spontaneous catch-up growth (SGA-Cu). SGA is defined as birth weight and/or length at least 2 standard deviations (SD) below the mean for gestational age (<–2 SD) [22]. Furthermore, the study includes children with idiopathic short stature (ISS). ISS is defined auxologically by a height below –2 SD score (SDS) after exclusion of any known specific cause for short stature [23]. The NESTEGG study was established in 2001 in four major centres (Rotterdam, Toulouse, Tuebingen and London) and concentrated on rigorous phenotyping of affected trios, unaffected siblings when available, and normal controls. For all patients, birth weight, birth length, birth head circumference, systolic (SBP) and diastolic (DBP) blood pressure, and height and weight before start of GH treatment were available, as well as their response to GH treatment.

Although both genotyping and analytical techniques have evolved substantially since the inception of this study, the resulting database and DNA collection provides a unique resource with which to investigate the genetic basis of growth failure. Furthermore, our study population of 1,437 patients might seem small in an era in which large genome-wide association studies (GWAS) dominate the literature. However, since the incidence of SGA births is low (by definition 2.5% of all births, [22]) our SGA population represents a population of about 25,000 individuals.

In the current study, we investigated 225 SNPs in 10 genes involved in pre- and/or postnatal growth and glucose metabolism (fig. 1): *GH1, GHR, IGF1, IGF1R, STAT5A, STAT5B, MAPK1, MAPK3, PPARγ* and *INS*. We hypothesised that minor genetic changes (polymorphisms) in these genes might explain part of the phenotype of children born SGA or children with ISS and their response to GH treatment.

## Patients and Methods

The NESTEGG project consists of the study of children born SGA, children with ISS and healthy controls in four different European countries according to a standard protocol. Detailed phenotypic features were recorded and blood sampling was undertaken to provide DNA [21]. Of the 1,437 children studied, 544 were recruited in the Netherlands (Sophia's Children's Hospital, Erasmus Medical Centre Rotterdam), 455 in France (University Hospital, Toulouse), 239 in Germany (University Children's Hospital, Tuebingen) and 199 in the UK (St. Bartholomew's and the Royal London Hospitals, London). Approval was obtained from the corresponding ethical committees in all countries.

SGA children were included when birth weight and/or birth length was less than –2.0 SDS, according to national growth charts for each centre.

Height at or after 3 years of age determined whether a child had experienced spontaneous catch-up growth (height SDS >–2.0 SDS: SGA catch-up or SGA-Cu) or remained short (height SDS <–2.0 SDS: short SGA or SGA-S). Subjects in the ISS group had a birth weight and/or birth length above –2.0 but less than +2.0 SDS and a height at the time of study of <–2.0 SDS without specific pathology.

Exclusion criteria were severe chronic illness or endocrine disease, positive gliadin, endomysial or reticulin antibodies, GH defi-

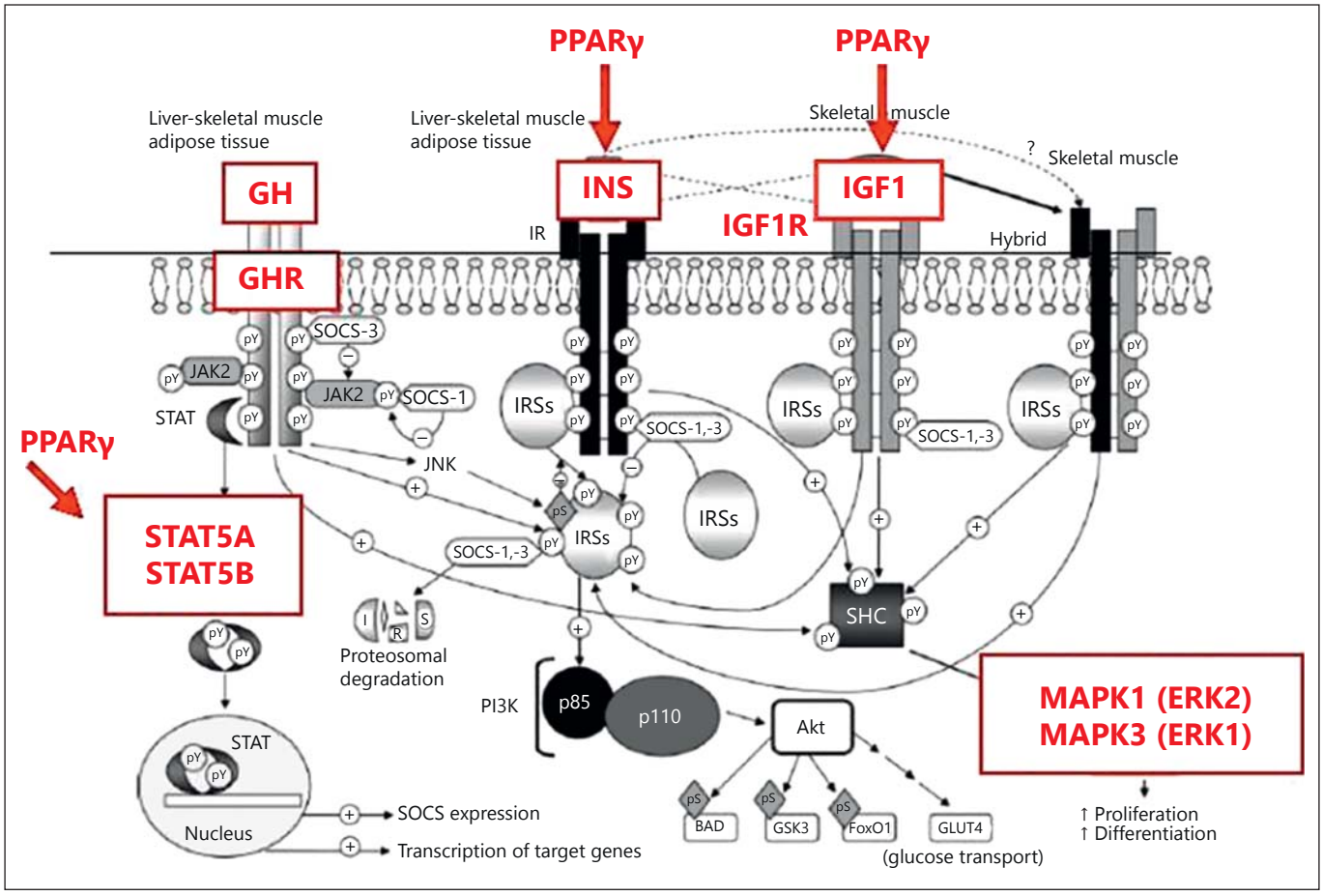


**Fig. 1.** Interrelationships between GH, *IGFI* and insulin signalling [adapted from 42].

ciency, severe disproportionate short stature, psychosocial dwarfism, chromosomal or genetic anomalies, syndromes or dysmorphic features (except Silver-Russell syndrome), any psychiatric, neurodegenerative or chronic illness in the patients or parents, adoption and/or lack of ability to give informed consent.

*Genetic Techniques*

Tagging SNPs were selected from HapMap within the region 20 kb upstream and 10 kb downstream of each gene, with selection criteria being $R^2$ higher than 0.8 and mean allele frequency higher than 0.05. Tag calculation methods used were Tagger and LDSelect. We selected 225 SNPs: 3 SNPs for *GH1,* 22 SNPs for *GHR*, 2 for *STAT5A*, 5 for *STAT5B*, 8 for *MAPK1*, 3 for *MAPK3,* 38 for *PPARγ*, 13 for INS, 25 for *IGF1* and 106 SNPs for *IGF1R.*

Genotypes were determined using the TaqMan allelic discrimination assay. PCR was performed in 384-well PCR plates in an ABI 9700 PCR system (Applied Biosystems Inc., Foster City, Calif., USA) and consisted of initial denaturation for 10 min at 95° and 40 cycles with denaturation of 15 s at 92° and annealing and extension for 60 s at 60°. Results were analysed by ABI TaqMan 7900HT using the sequence detection system 2.22 software (Applied Biosystems Inc.).

*Phenotypic Data*

For all patients, birth weight, birth length, birth head circumference, SBP and DBP were collected, as well as height and weight before the start of GH treatment and response to GH treatment (if applicable). Weight, height, head circumference and blood pressure were converted to SDS for statistical analysis. Body mass index (BMI) was calculated as $kg/m^2$. Response to GH treatment was defined as increase in height SDS per year of GH treatment.

*Statistical Analysis*

We used Pearson's $\chi^2$ to test for deviations from Hardy-Weinberg equilibrium and for differences in genotype distribution between the groups. We compared the entire SGA group (SGA-S and SGA-Cu taken together) to the entire appropriate for gestational age (AGA) group (ISS and controls taken together) and we compared all short patients (SGA-S and ISS taken together) to all normal height individuals (SGA-Cu and controls taken together). We also compared the individual patient groups (SGA-Cu vs. SGA-S vs. ISS vs. controls).

For the SNPs that had a significantly different frequency between the groups, we investigated whether they were associated with any of the clinical parameters (birth weight, birth length, birth

head circumference, and height and weight before the start of GH treatment, as well as SBP and DBP) by ANOVA.

Since we tested associations with clinical parameters for multiple SNPs, we applied Bonferroni's correction for the p values obtained by ANOVA by dividing 0.05 by 225 (the number of SNPs tested). Only associations with $p < 0.0002$ (0.05/225) could be considered significant at an experiment-wide level. For Pearson's $\chi^2$ analysis, Bonferroni's correction was not necessary since multiple testing is not an issue there.

*Literature Search*

For the SNPs which were significantly associated with clinical parameters, we searched the literature by entering the refSNP (rs) number into PubMed. Furthermore, we searched GWAS Central (www.gwascentral.org) for GWAS data about *GH1, GHR, IGF1, IGF1R, STAT5A, STAT5B, MAPK1, MAPK3, PPARγ* and *INS.*

## Results

The study population consisted of 1,437 children: 345 SGA-S, 288 SGA-Cu, 410 ISS and 394 controls. Phenotypic data of the patients are shown in table 1. Genotype frequencies of all 225 SNPs are shown in online suppl. table 1, see www.karger.com/doi/10.1159/000355409. All SNPs were in Hardy-Weinberg equilibrium. We discuss the SNPs for which genotype frequencies differed between the groups with p values <0.05 and (only for those SNPs) we show associations with clinical parameters with $p < 0.05$ in table 2 (table showing all associations found with $p < 0.05$ is available on request). We discuss the results according to the clinical parameters studied and according to their clinical relevance.

*SNPs Associated with Body Size at Birth (IGF1R SNPs rs4966035, rs4966038, rs11247361, rs2715439 and rs11247380, IGF1 SNP rs12423791, and PPARγ SNP rs2920500)*

*IGF1R* SNP rs4966035's minor allele was more prevalent among SGA (12.4 vs. 8.4% in AGA, p = 0.026) and was associated with smaller birth length (p = 0.000378, entire cohort; fig. 2a) and birth weight (p = 0.008, entire cohort; fig. 2b). The difference in body size at birth was not caused by a difference in gestational age: mean gestational age was 38.7 weeks for all three genotypes (fig. 2c). *IGF1R* SNP rs4966038 is in high linkage disequilibrium with rs4966035 (r = 0.85). Therefore, like rs4966035's minor allele, the minor allele of rs4966038 CC was more prevalent among SGA and associated with smaller birth length. The association with birth length remained significant after Bonferroni's correction, but the association with birth weight did not.

*IGF1R* SNP rs11247361's minor allele GG was significantly more frequent in those with normal birth weight: 16.4% in AGA versus 10.9% in SGA (p = 0.006). It was associated with greater birth length (p = 0.004) and birth weight in the entire cohort (p = 0.0036) and with lower SBP SDS (p = 0.025) in the SGA-Cu cohort. Likewise, *IGF1R* SNP rs2715439's A allele was more frequent among those born AGA (56.5 vs. 53.3%, p = 0.010) and associated with greater birth length and higher DBP (p = 0.024, all SGA). For the *IGF1R* SNP rs11247380, homozygosity for the A allele was slightly more frequent among SGA (32.4 vs. 31.4%, p = 0.048) and it was clearly associated with smaller birth length (p = 0.001, entire cohort).

In *IGF1*, SNP rs12423791's C allele was more frequent among those born AGA (2.1 vs. 1.0%, p = 0.027) and was associated with greater birth length and lower DBP SDS. No one was homozygous for this minor allele.

In *PPARγ,* SNP rs2920500's minor allele GG was more prevalent in AGA than in SGA (28.9 vs. 22.3%, p = 0.021) and was associated with greater birth weight (entire cohort, p = 0.017) and greater birth length SDS (entire cohort, p = 0.029) as well as greater height (p = 0.01 in the entire cohort and 0.001 in controls). Furthermore, the heterozygous state was associated with higher SBP SDS (SGA-Cu, p = 0.017) and higher DBP SDS (SGA-Cu, p = 0.008)

*SNPs Associated with Body Size Later in Life (IGF1R SNPs rs939626 and rs4965438, MAPK1 SNP rs5999842)*

*IGF1R* SNP rs939626's minor allele CC was more prevalent among those who were short later in life (SGA-S and ISS together 24.8 vs. 17.9% in SGA-Cu and controls taken together, p = 0.002) and was associated with higher weight SDS and BMI SDS in SGA-Cu (p = 0.007 and 0.041, respectively). It was also associated with DBP SDS in SGA (p = 0.031) and ISS (p = 0.039). Likewise, *IGF1R* SNP rs4965438's minor allele GG was more prevalent in homozygosity among short individuals (6.8 vs. 4.3% in individuals of normal adult height, p = 0.024) and was associated with higher SBP SDS in SGA-S (p = 0.044) and all SGA (p = 0.03).

In *MAPK1,* SNP rs5999842's major allele GG was more prevalent among those who were short later in life (SGA-S and ISS together 72.9 vs. 69.1% in SGA-Cu and controls taken together, p = 0.044) and was associated with lower height SDS (entire cohort, p = 0.025).

There was 1 SNP which was associated with body size, although its genotype frequency did not differ between

**Table 1.** Phenotypic data of the patients participating in the study

| | | n | Mean | SD | Min | Max |
|---|---|---|---|---|---|---|
| Birth length SDS | controls | 282 | –0.23 | 0.95 | –1.87 | 3.84 |
| | SGA-Cu | 233 | –2.47 | 1.11 | –8.22 | 0.35 |
| | ISS | 360 | –0.57 | 0.83 | –1.88 | 3.88 |
| | SGA-S | 320 | –2.74 | 1.12 | –7.42 | 0.35 |
| Birth weight SDS | controls | 394 | –0.29 | 1.02 | –1.88 | 4.42 |
| | SGA-Cu | 286 | –2.28 | 0.88 | –4.81 | 3.34 |
| | ISS | 409 | –0.46 | 0.86 | –1.85 | 6.75 |
| | SGA-S | 345 | –2.12 | 0.97 | –4.74 | 0.51 |
| Birth head circumference SDS | controls | 83 | –0.20 | 1.24 | –3.03 | 3.59 |
| | SGA-Cu | 123 | –1.18 | 1.24 | –3.91 | 4.09 |
| | ISS | 225 | –0.17 | 1.06 | –3.90 | 3.33 |
| | SGA-S | 160 | –1.35 | 1.15 | –4.20 | 2.40 |
| Gestational age, weeks | Controls | 394 | 39.3 | 2.0 | 30 | 43 |
| | SGA-Cu | 286 | 37.8 | 3.0 | 30 | 43 |
| | ISS | 410 | 39.0 | 2.2 | 30 | 42 |
| | SGA-S | 345 | 38.4 | 2.7 | 30 | 43 |
| Age at follow-up, years | controls | 394 | 24.2 | 13.6 | 2.03 | 59.7 |
| | SGA-Cu | 286 | 15.3 | 9.36 | 1.95 | 59.8 |
| | ISS | 410 | 29.8 | 6.23 | 1.91 | 40.6 |
| | SGA-S | 345 | 8.23 | 5.83 | 1.98 | 27.7 |
| BMI SDS | controls | 391 | 0.10 | 1.10 | –3.94 | 3.68 |
| | SGA-Cu | 282 | –0.08 | 1.22 | –3.25 | 4.54 |
| | ISS | 395 | –0.35 | 1.05 | –4.86 | 2.92 |
| | SGA-S | 339 | –0.45 | 1.08 | –4.92 | 3.58 |
| Height SDS[1] | controls | 394 | –0.02 | 1.07 | –1.88 | 3.27 |
| | SGA-Cu | 286 | –0.55 | 0.92 | –1.88 | 4.54 |
| | ISS | 410 | –2.48 | 0.53 | –4.86 | 1.88 |
| | SGA-S | 345 | –2.78 | 0.69 | –5.18 | –1.88 |
| Response to GH, ΔhtSDS/year | ISS | 105 | 0.03 | 0.44 | –1.73 | 1.23 |
| | SGA-S | 214 | 0.16 | 0.64 | –1.79 | 6.36 |
| DBP SDS | controls | 101 | 0.43 | 0.95 | –2.09 | 2.50 |
| | SGA-Cu | 122 | 0.64 | 0.91 | –1.93 | 3.65 |
| | ISS | 316 | 0.59 | 0.95 | –2.01 | 4.80 |
| | SGA-S | 275 | 0.80 | 0.94 | –2.01 | 3.60 |
| SBP SDS | controls | 101 | 0.62 | 0.78 | –1.55 | 2.29 |
| | SGA-Cu | 122 | 0.63 | 0.87 | –1.97 | 4.08 |
| | ISS | 316 | 0.58 | 0.72 | –1.45 | 3.07 |
| | SGA-S | 275 | 0.66 | 0.88 | –2.66 | 3.37 |

ΔhtSDS = Increase in height SDS.

[1] Before start of GH treatment (in cases where GH treatment had already started).

the groups: *GH1* SNP rs11079515. For this SNP, GG genotype was associated with lower birth length (entire cohort, p = 0.007) and lower SBP SDS (SGA-S, p = 0.008). However, this association lost statistical significance after Bonferroni's correction.

*SNPs Associated with Head Circumference (PPARγ SNP rs13070963)*

*PPARγ* SNP rs13070963's minor allele CC was more prevalent in SGA (p = 0.023) and associated with smaller birth head circumference in controls (p = 0.000178).

**Table 2.** Clinical associations of SNPs in *GH1, GHR, IGF1, IGF1R, MAPK1, PPARγ* and *INS* with $p < 0.01$ or $p < 0.05$ and genotype frequencies significantly differing between the groups at $p < 0.05$

| Gene | SNP | Parameter | Genotype | n | Mean | SD | Min | Max | p | Cohort |
|---|---|---|---|---|---|---|---|---|---|---|
| *GH1* | rs11079515 | birth length SDS | CC | 419 | –1.44 | 1.50 | –7.42 | 3.19 | 0.007 | entire cohort |
| | | | CG | 577 | –1.36 | 1.45 | –8.22 | 3.88 | | |
| | | | GG | 183 | –1.76 | 1.55 | –6.98 | 1.49 | | |
| | | SBP SDS | CC | 105 | 0.61 | 0.82 | –1.54 | 2.58 | 0.008 | SGA-S |
| | | | CG | 115 | 0.82 | 0.83 | –0.76 | 3.37 | | |
| | | | GG | 52 | 0.38 | 1.01 | –2.66 | 2.39 | | |
| *GHR* | rs6898743 | GH response, ΔhtSDS/year | CC | 14 | 0.87 | 0.85 | 0.16 | 3.49 | 0.01 | all SGA |
| | | | CG | 84 | 0.75 | 0.45 | –0.46 | 2.03 | | |
| | | | GG | 114 | 0.98 | 0.51 | –0.13 | 2.89 | | |
| | | GH response, ΔhtSDS/year | CC | 22 | 0.71 | 0.73 | –0.25 | 3.49 | 0.006 | all short |
| | | | CG | 123 | 0.70 | 0.46 | –0.46 | 2.03 | | |
| | | | GG | 166 | 0.89 | 0.51 | –0.13 | 2.89 | | |
| | rs719756 | SBP SDS | AA | 61 | 0.70 | 0.91 | –1.54 | 2.82 | 0.035 | SGA-S |
| | | | AT | 142 | 0.73 | 0.83 | –2.12 | 3.37 | | |
| | | | TT | 66 | 0.40 | 0.90 | –2.66 | 2.58 | | |
| | | DBP SDS | AA | 61 | 0.74 | 0.91 | –1.22 | 2.99 | 0.042 | SGA-S |
| | | | AT | 142 | 0.91 | 0.90 | –1.09 | 3.60 | | |
| | | | TT | 66 | 0.57 | 1.02 | –2.01 | 30.07 | | |
| *IGF1* | rs12423791 | birth length SDS | CG | 33 | –0.88 | 1.27 | –2.74 | 3.19 | 0.025 | entire cohort |
| | | | GG | 1,143 | –1.46 | 1.49 | –8.22 | 3.88 | | |
| | | DBP SDS | CG | 9 | 0.13 | 0.90 | –1.41 | 1.24 | 0.038 | all SGA |
| | | | GG | 381 | 0.77 | 0.92 | –1.93 | 3.65 | | |
| *IGF1R* | rs12901358 | SBP SDS | CC | 56 | 0.70 | 0.94 | –1.44 | 4.08 | 0.025 | all SGA |
| | | | CT | 184 | 0.76 | 0.87 | –2.12 | 3.37 | | |
| | | | TT | 152 | 0.50 | 0.85 | –2.66 | 2.39 | | |
| | | SBP SDS | CC | 40 | 0.67 | 0.91 | –1.44 | 2.82 | 0.039 | SGA-S |
| | | | CT | 140 | 0.77 | 0.87 | –2.12 | 3.37 | | |
| | | | TT | 91 | 0.47 | 0.87 | –2.66 | 2.39 | | |
| | rs11247361 | birth length SDS | CC | 487 | –1.48 | 1.52 | –8.22 | 3.88 | 0.005 | entire cohort |
| | | | CG | 531 | –1.50 | 1.53 | –7.19 | 3.84 | | |
| | | | GG | 153 | –1.07 | 1.24 | –3.95 | 2.67 | | |
| | | birth weight SDS | CC | 576 | –1.14 | 1.32 | –4.81 | 6.75 | 0.036 | entire cohort |
| | | | CG | 631 | –1.26 | 1.32 | –4.74 | 4.42 | | |
| | | | GG | 195 | –1.00 | 1.22 | –4.36 | 3.23 | | |
| | | SBP SDS | CC | 52 | 0.66 | 0.99 | –1.81 | 4.08 | 0.025 | SGA-Cu |
| | | | CG | 64 | 0.69 | 0.75 | –1.97 | 2.12 | | |
| | | | GG | 3 | –0.71 | 0.36 | –1.03 | –0.32 | | |
| | rs4966035[1] | birth length SDS | AA | 123 | –1.88 | 1.46 | –6.98 | 1.22 | 0.000378 | entire cohort |
| | | | AG | 508 | –1.30 | 1.48 | –7.42 | 3.84 | | |
| | | | GG | 556 | –1.47 | 1.48 | –8.22 | 3.88 | | |
| | | birth weight SDS | AA | 144 | –1.49 | 1.24 | –4.59 | 1.48 | 0.008 | entire cohort |
| | | | AG | 606 | –1.11 | 1.30 | –4.74 | 6.75 | | |
| | | | GG | 671 | –1.17 | 1.30 | –4.81 | 3.74 | | |
| | rs11247380 | birth length SDS | AA | 123 | –1.86 | 1.47 | –6.98 | 1.22 | 0.001 | entire cohort |
| | | | AG | 515 | –1.30 | 1.47 | –7.42 | 3.84 | | |
| | | | GG | 550 | –1.48 | 1.48 | –8.22 | 3.88 | | |

**Table 2.** (continued)

| Gene | SNP | Parameter | Genotype | n | Mean | SD | Min | Max | p | Cohort |
|---|---|---|---|---|---|---|---|---|---|---|
| | rs4966038 | birth length SDS | CC | 122 | –1.74 | 1.49 | –6.98 | 1.22 | 0.044 | entire cohort |
| | | | CG | 499 | –1.37 | 1.50 | –7.42 | 3.84 | | |
| | | | GG | 552 | –1.42 | 1.46 | –8.22 | 3.88 | | |
| | rs4965438 | SBP SDS | GG | 24 | 1.01 | 0.70 | –0.68 | 2.08 | 0.033 | all SGA |
| | | | GT | 130 | 0.53 | 0.96 | –2.12 | 4.08 | | |
| | | | TT | 239 | 0.68 | 0.83 | –2.66 | 3.37 | | |
| | | SBP SDS | AA | 171 | 0.66 | 0.88 | –2.66 | 3.37 | 0.041 | SGA-S |
| | | | AG | 81 | 0.54 | 0.86 | –2.12 | 2.37 | | |
| | | | GG | 16 | 1.14 | 0.65 | –0.21 | 2.08 | | |
| | rs2715439 | birth length SDS | AA | 328 | –1.27 | 1.50 | –7.18 | 3.88 | 0.033 | entire cohort |
| | | | AG | 619 | –1.51 | 1.48 | –8.22 | 3.19 | | |
| | | | GG | 230 | –1.53 | 1.37 | –5.56 | 1.89 | | |
| | | DBP SDS | AA | 102 | 0.86 | 0.93 | –1.04 | 3.65 | 0.024 | all SGA |
| | | | AG | 209 | 0.79 | 0.93 | –2.01 | 3.60 | | |
| | | | GG | 78 | 0.50 | 0.94 | –1.53 | 2.87 | | |
| *MAPK1* | rs5755694 | GH response, ΔhtSDS/year | CC | 94 | 0.67 | 0.48 | –0.46 | 2.89 | 0.007 | all short |
| | | | CT | 159 | 0.84 | 0.54 | –0.35 | 3.49 | | |
| | | | TT | 64 | 0.90 | 0.48 | 0.08 | 2.41 | | |
| | | GH response, ΔhtSDS/year | CC | 61 | 0.74 | 0.52 | –0.46 | 2.89 | 0.048 | all SGA |
| | | | CT | 109 | 0.92 | 0.55 | –0.16 | 3.49 | | |
| | | | TT | 46 | 0.95 | 0.45 | 0.19 | 2.08 | | |
| | | SBP SDS | CC | 242 | 0.58 | 0.81 | –2.12 | 3.07 | 0.035 | entire cohort |
| | | | CT | 400 | 0.59 | 0.77 | –2.66 | 2.37 | | |
| | | | TT | 162 | 0.77 | 0.85 | –1.77 | 4.08 | | |
| | | SBP SDS | CC | 96 | 0.47 | 0.73 | –0.93 | 3.07 | 0.022 | ISS |
| | | | CT | 152 | 0.57 | 0.69 | –1.45 | 2.12 | | |
| | | | TT | 63 | 0.79 | 0.76 | –1.05 | 2.49 | | |
| | rs5999842 | height SDS | AA | 33 | –1.27 | 1.23 | –3.38 | 1.47 | 0.025 | entire cohort |
| | | | AG | 376 | –1.35 | 1.48 | –4.86 | 3.96 | | |
| | | | GG | 1,007 | –1.57 | 1.45 | –5.18 | 4.54 | | |
| | | BMI SDS | AA | 5 | –0.29 | 0.69 | –1.08 | 0.57 | 0.039 | ISS |
| | | | AG | 108 | –0.13 | 0.98 | –2.30 | 2.79 | | |
| | | | GG | 279 | –0.43 | 1.07 | –4.86 | 2.92 | | |
| | | SBP SDS | AA | 5 | 1.03 | 0.58 | 0.20 | 1.71 | 0.036 | SGA-Cu |
| | | | AG | 36 | 0.33 | 0.91 | –1.97 | 2.04 | | |
| | | | GG | 80 | 0.75 | 0.85 | –1.77 | 4.08 | | |
| *PPARγ* | rs2920500 | birth length SDS | AA | 334 | –1.61 | 1.53 | –6.71 | 3.88 | 0.010 | entire cohort |
| | | | AG | 540 | –1.44 | 1.49 | –8.22 | 3.84 | | |
| | | | GG | 302 | –1.26 | 1.40 | –5.44 | 2.03 | | |
| | | birth weight SDS | AA | 392 | –1.34 | 1.27 | –4.72 | 3.21 | 0.017 | entire cohort |
| | | | AG | 652 | –1.15 | 1.29 | –4.81 | 4.42 | | |
| | | | GG | 366 | –1.08 | 1.32 | –4.74 | 6.75 | | |
| | | height SDS | AA | 392 | –1.68 | 1.42 | –5.18 | 4.54 | 0.010 | entire cohort |
| | | | AG | 653 | –1.42 | 1.44 | –5.18 | 2.66 | | |
| | | | GG | 366 | –1.44 | 1.50 | –4.86 | 3.96 | | |

**Table 2.** (continued)

| Gene | SNP | Parameter | Genotype | n | Mean | SD | Min | Max | p | Cohort |
|---|---|---|---|---|---|---|---|---|---|---|
| | | DBP SDS | AA | 34 | 0.47 | 0.89 | –1.93 | 1.93 | 0.008 | SGA-Cu |
| | | | AG | 54 | 0.93 | 0.87 | –0.66 | 3.65 | | |
| | | | GG | 33 | 0.39 | 0.85 | –1.34 | 2.11 | | |
| | | SBP SDS | AA | 34 | 0.61 | 0.77 | –1.81 | 2.12 | 0.017 | SGA-Cu |
| | | | AG | 54 | 0.87 | 0.87 | –0.77 | 4.08 | | |
| | | | GG | 33 | 0.35 | 0.80 | –1.77 | 1.71 | | |
| | rs13070963 | birth head circumference SDS | CC | 3 | –2.14 | 0.85 | –2.95 | –1.25 | 0.000178 | controls |
| | | | CT | 37 | 0.30 | 1.25 | –1.65 | 3.59 | | |
| | | | TT | 43 | –0.50 | 1.02 | –3.03 | 2.16 | | |

All SGA = SGA-Cu and SGA-S; all short = SGA-S and ISS; ΔhtSDS = increase in height SDS.
[1] Association remained significant after Bonferroni's correction.

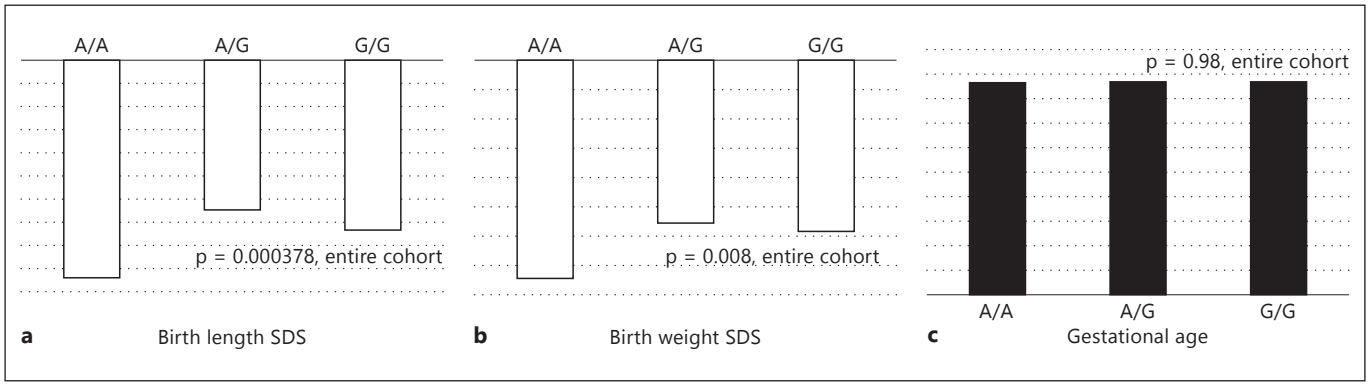


**Fig. 2.** Birth length SDS (**a**), birth weight SDS (**b**) and gestational age at birth (**c**) according to *IGF1R* SNP rs4966035 genotype.

*SNPs Associated with Response to GH Treatment (GHR SNP rs6898743 and MAPK1 SNP rs5755694)*

We found 2 SNPs associated with increase in height SDS during GH treatment with empirical p values below 0.01. *GHR* SNP rs6898743's GG genotype was equally prevalent among all groups, but it was associated with better response to GH (all short, p = 0.006 and all SGA, p = 0.01).

*MAPK1* SNP rs5755694's CC genotype was equally prevalent among all groups, but it was associated with poorer response to GH (entire cohort, p = 0.015, all SGA, p = 0.048 and all short, p = 0.007). However, these associations lost statistical significance after Bonferroni's correction.

*SNPs Associated with Blood Pressure (GHR SNP rs719756, IGF1R SNPs rs12901358 and rs11635251)*

The SNPs which were more frequent among those who were short later in life were all associated with higher blood pressure.

For *GHR* SNP rs719756, the A allele was more frequent among those who remain short (50 vs. 45% in individuals with normal adult height, p = 0.009) and was associated with higher SBP SDS (SGA-S, p = 0.035) and higher DBP SDS (SGA-S, p = 0.042).

For *IGF1R* SNP rs12901358, the C allele was more frequent among those who remain short (41 vs. 37%, p = 0.009) and was associated with higher SBP SDS (all SGA, p = 0.025 and SGA-S, p = 0.039). *IGF1R* SNP rs11635251's minor allele GG was more prevalent among short (6.4 vs. 3.9% in non-short, p = 0.035) and was associated with higher SBP SDS in the entire cohort (p = 0.021), with higher DBP SDS and SBP SDS in all SGA (p = 0.011 and 0.006, respectively) and with SBP SDS in SGA-S (p = 0.041).

For *STAT5A, STAT5B, MAPK3* and *INS*, we did not find any significant associations with body size, response to GH treatment or blood pressure.

## Discussion

We investigated 225 SNPs in 10 genes reported to be involved in pre- and/or postnatal growth and glucose metabolism: *GH1, GHR, IGF1, IGF1R, STAT5A, STAT5B, MAPK1, MAPK3, PPARγ* and *INS*. We found several clinical associations for *GH1, GHR, IGF1, IGF1R, PPARγ* and *MAPK1*. For *STAT5A, STAT5B, MAPK3* and *INS*, we did not find any significant associations with body size, response to GH treatment, SBP or DBP.

We mainly focused on SNPs for which the genotype frequency significantly differed between the groups (SGA-Cu vs. SGA-S vs. ISS vs. controls, short vs. normal height, SGA vs. non-SGA). However, we also found 3 SNPs which also raised our interest (although they did not meet this criterion): *GH1* SNP rs1109515, *GHR* SNP rs6898743 and *MAPK1* SNP rs5755694. Although their associations did not remain significant after Bonferroni's correction, we chose to show these associations since the purpose of this study is exploratory. In this case, applying Bonferroni's correction too strictly can cause false-negative results [24]. For all associations found, we suggest they be further investigated and replicated in other cohorts.

One SNP showed statistically significant genotype differences between the patient groups and showed associations with clinical parameters which remained significant after Bonferroni's correction: *IGF1R* SNP rs 4966035. The minor (A–) allele of this SNP was more prevalent among SGA (12.4 vs. 8.4% in AGA, p = 0.026). rs4966035's minor allele A was associated with smaller birth length: birth length SDS was –1.88 in AA versus –1.30 and –1.47 SDS in AG and GG, respectively (p = 0.000378, entire cohort). rs4966035's minor allele A was also associated with birth weight (p = 0.008, entire cohort). An interesting literature finding was that Haataja et al. [25] related rs4966038 (in high linkage disequilibrium with rs4966035) to spontaneous preterm birth susceptibility. However, the association with body size at birth which we found was not caused by a difference in gestational age: mean gestational age was 38.7 weeks for all 3 rs4966035 genotypes. rs4966035 was also related to insulin secretion index by Naj et al. [26]. Montasser et al. [27] found that rs4966035 was associated with DBP using generalized estimation equation regression methods in non-smokers but not in smokers. However, using bayesian quantitative trait nucleotide method which they also used, this association was non-significant.

For all SNPs associated with size at birth, we searched the literature for previously found associations. *IGF1R* SNP rs11247361 was also studied by Haataja et al. [25] in relation to spontaneous preterm birth. They did not find any association. For *IGF1R* SNP rs11247380, we did not find any publications in relation to clinical parameters. *IGF1R* SNP rs2715439 was described in relation to insulin levels and insulin sensitivity index [26], but not to size at birth or blood pressure.

In the literature, *IGF1* SNP rs12423791 was reported in relation to myopia [28, 29], but not to growth or blood pressure.

*PPARγ* SNP rs2920500's minor allele GG was less prevalent in SGA than in AGA and was associated with greater birth weight, birth length and height SDS. Furthermore, the heterozygous state was associated with higher SBP SDS and higher DBP SDS. The finding that genetic polymorphisms in the heterozygous state can be associated with a certain phenotype, which can differ from the two homozygous states, has been previously reported and has been the subject of research and discussion [30–39]. This might be a reflection of trans-regulation, i.e. the interaction of two alleles in trans, which might explain advantages or disadvantages of heterozygosity as found in several studies and for rs2920500 in our study. However, it might also reflect pure coincidence. We did not find any articles about rs2920500 in the literature.

*IGF1R* SNP rs939626's minor allele CC was more prevalent among short individuals and was associated with higher weight SDS and with BMI SDS in SGA-Cu. It was also associated with DBP SDS in SGA and ISS. However, the direction of the association was opposite in SGA vs. ISS, which renders it less likely that this is a 'real' association. We did not find any publications about this SNP in relation to clinical parameters.

We did not find any publications about *IGF1R* SNP rs4965438 and *MAPK1* SNP rs5999842 in relation to clinical parameters.

We checked whether the relevant SNPs from important GWAS, like the large height GWAS of Lango Allen et al. [40], were also significant in our cohort. rs2871865 showed an association with height (height SDS decreased according to genotype from CC –1.46 to CG –1.58 and GG –1.84 SDS), but this was not significant (p = 0.20). This discrepancy is probably due to size and phenotype differences between the populations studied.

*PPARγ* SNP rs13070963's minor allele CC was more prevalent in SGA and associated with smaller birth head circumference in controls. However, since the minor allele was present in only 3 patients, it is hard to draw any conclusions from this. We did not find any articles about this SNP in the literature.

The SNPs which were more frequent among those who were short later in life were all associated with higher blood pressure.

*GHR* SNP rs719756 has been studied in relation to longevity, where it did not reach genome-wide statistical significance [41], but it was never described in relation to blood pressure. For *IGF1R* SNPs rs12901358 and rs11635251, we did not find any articles about this SNP in the literature.

Finally, we studied the combinations of different associations and we did not find any interaction between the associations.

In conclusion, we found many clinical associations during our extensive exploratory SNP analysis of 10 candidate genes involved in pre- and/or postnatal growth and glucose metabolism which have not been reported before. The most interesting SNP was *IGF1R* SNP rs4966035, of which the minor allele A was associated with birth length and birth weight, independent of gestational age. Although the nature of our study was exploratory and the results should be validated in an independent cohort, our findings suggest that polymorphisms in *IGF1R,* as well as *GH1, GHR, IGF1, MAPK1 and PPARγ,* might explain part of the phenotype of children born SGA and children with ISS.

## Disclosure Statement

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H.W. is employed by Pfizer Inc. All other authors have nothing to declare.

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