CLINICAL STUDY

The −G1245A IGF1 polymorphism is related with small head size and less brain sparing in small for gestational age born children

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Abstract

Context: Small for gestational age (SGA) subjects experience pre- and postnatal growth restriction, which might be influenced by polymorphisms in the IGF1 gene. The well-known −841(CA)n/192 bp polymorphism has been associated with birth size, cardiovascular disease, and IGF-1 levels, and is in linkage disequilibrium with the −G1245A single nucleotide polymorphism (SNP; rs35767).

Objective: To associate the −G1245A SNP with head circumference (HC) and brain sparing (a greater head compared with height SDS) in short SGA and SGA catch-up subjects.

Design: Gene association study.

Patients: We studied 635 SGA subjects out of which 439 remained short and 196 had a postnatal height <−2.00 SDS.


Results: All SGA subjects had a postnatal head size below the population mean (−1.01 SDS, \( P<0.001 \)). Whereas SGA catch-up subjects had a head size that was in proportion with their height, short SGA subjects displayed extensive brain sparing (HC – height: SGA CU: 0.01 versus short SGA: 1.75 SDS, \( P<0.001 \)). The most severely SGA born subjects had a 0.4 SDS smaller postnatal head size and 0.6 SDS less brain sparing when carrying the −1245 A-allele in contrast to G-allele carriers (\( P=0.03 \)). The association between the −G1245A SNP and head size remained significant after correction for birth weight and postnatal height SDS (\( P=0.03 \)). Birth weight, birth length and postnatal height SDS were not related with the −G1245A SNP.

Conclusions: The −1245 A-allele of the IGF1 promoter SNP is associated with a small head size and less brain sparing in SGA born subjects and particularly those with the lowest birth weight.

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Introduction

Around 2% of all live-born neonates are born small for gestational age (SGA) and ~10% of them remain short (1,2). Since reduced size at birth may result from fetal, maternal, placental or demographic influences, SGA subjects comprise a heterogeneous group. SGA subjects without catch-up growth are known to have a smaller head circumference (HC) and a lower IQ (3) compared with SGA subjects catching up in height (4, 5). Head size preservation, or ‘brain sparing’ is defined as centralization of the fetal circulation in the brain (6) and is essential for the growth retarded fetus to prevent brain damage (7). Head size preservation might be related to SGA severity as studies have shown that short SGA children with both a birth length, birth weight, and HC at birth \( \leq −2.0 \) SDS, had the most extensive brain sparing compared with short SGA children with only a birth length \( \leq −2.0 \) SDS or a birth length and a birth weight \( \leq −2.0 \) SDS (8).

The IGF1 gene (IGF1, OMIM*147440, NM_000618, gene map locus: 12q22–q24.1) is a major candidate gene for explaining parts of the SGA phenotype as animal studies have shown that IGF1 knock-out mice had a 40% birth weight reduction and retarded postnatal growth in length and weight (9, 10). Case-reports have described patients with IGF1 gene mutations who were born SGA, had a persistent short stature and sensorineural deafness, a small HC and a low performance IQ (11–13).

The most common investigated polymorphism in the IGF1 gene is the 192 bp CA-repeat in the promoter. The non-192 bp allele is associated with a low birth weight, low adult height, low IGF-1 serum level and a higher risk...
for diabetes mellitus and myocardial infarction although conflicting results are reported (14–19). The 192 bp allele polymorphism and the −G1245A SNP (rs35767) are located in the same linkage disequilibrium block and studies have shown that the −G1245A SNP can be used as a marker for the 192-bp polymorphism (HapMap. http://www.hapmap.org) (20, WA Ester, JB van Meurs, NJ Arends, AG Uitterlinden, MA de Ridder & AC Hokken-Koelega, unpublished observations). A subgroup of the short SGA population that is described in the present study has previously shown that a haplotype, of which the −1245 A-allele was the marker, was associated with a smaller HC during spontaneous postnatal growth, but not during GH treatment in short SGA children (WA Ester, JB van Meurs, NJ Arends, AG Uitterlinden, MA de Ridder & AC Hokken-Koelega, unpublished observations).

Thus, the IGF1 gene is involved in determining head size, and SGA subjects have a smaller HC and lower IQ depending on the extent of postnatal catch-up growth. It is unknown whether the IGF1 192 bp gene polymorphism is related to head size in short SGA versus SGA catch-up subjects. In addition, it is unknown whether SGA severity modifies the relation between brain sparing and this genetic polymorphism.

Therefore, we investigated whether the −G1245A SNP was associated with HC and brain sparing in a large cohort of 439 short SGA and 196 SGA catch-up subjects.

Methods

Patients

All 635 subjects were born SGA (birth length and/or weight ≤−2 s.d. score or SDS for their gestational age (21)) and fulfilled the inclusion criteria of Caucasian ethnicity and an uncomplicated postnatal period. Subjects with severe chronic illness or endocrine disorders, chromosomal or genetic abnormalities, positive endomysial or transglutaminase antibodies, skeletal abnormalities, and psychosocial dwarfism were excluded from the study. The study protocol was approved by the medical ethics committee and written informed consent was obtained from the parents/guardians and subjects above 12 years.

SGA subjects were assigned to two subgroups according to their ability to attain a postnatal height in the normal range, consisting of 439 short (WA Ester, JB van Meurs, NJ Arends, AG Uitterlinden, MA de Ridder & AC Hokken-Koelega, unpublished observations) and 196 catch-up subjects. The SGA subjects who remained short (height ≤−2.00 SDS: short SGA, (22)) were investigated at the start of GH treatment and were drawn from prospective cohort trials evaluating the effect of GH treatment (23, 24). SGA subjects who had catch-up growth to a normal height (height >−2.00 SDS: SGA catch-up (22)) were randomly selected from hospitals in The Netherlands where they had been registered because of being small at birth.

Clinical and biochemical measurements

Birth data of the SGA subjects were retrieved from records of hospitals, community health services, and general practitioners. Birth weight SDS was used as a proxy for SGA severity as birth length and birth HC measurements are not always performed in case of a severely growth-retarded newborn. Anthropometrical measurements of the short SGA subjects were performed at the start of the GH trials (24). Anthropometrical measurements of the SGA catch-up group were obtained at the out-patient clinic. Delta height was calculated by subtracting birth length (SDS) from height (SDS). Body mass index (BMI) was calculated (weight in kg/height in m²) and adjusted for age and sex, expressed as SDS (25). Brain sparing was calculated by subtracting height (SDS) from HC (SDS). Both height and HC measurements were present in a subset of 597 SGA subjects. Serum IGF-1 levels were measured in the SGA subjects as previously described (26) and values were transformed in SDS by adjusting for sex and age (27).

Genotyping

Genomic DNA was extracted from samples of peripheral venous blood according to salting-out procedure (28). Genotypes were determined using the Taqman allelic discrimination assay. The Assay-by-Design service (www.appliedbio-systems.com) was used to set up a Taqman allelic discrimination assay for the −G1245A SNP (rs35767). Primer sequences were: forward: GGAATTCAAGCAGAACGTGTGGTTTGA, reverse: GGTG-GAATAACCTGGACCTTGAAT. Probe sequences were for −G1245A, forward: VIC-TTTTTTCGGCATGACTCT, reverse: FAM-TTTTTTTCCACATGACTCT. The PCR reaction mixture included 5 ng genomic DNA, 0.125 μl TaqMan assay (40*, ABI), 2.5 μl Master mix (ABI) and 2.375 μl water. PCR was performed in 384 well 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 the ABI Taqman 7900HT using the sequence detection system 2.22 software (Applied Biosystems Inc).

Statistical analysis

The total SGA population was compared with the average population, defined at 0.00 SDS, using T-test. Continuous data were compared between the short SGA and the SGA catch-up population by univariate ANOVA. The χ² test was used to analyze categorical variables.
Multiple regression analysis was performed by using HC SDS as dependent variable and gestational age, birth weight SDS, age and height SDS as independent variables. As height SDS and BMI SDS were highly interrelated, only height SDS was investigated in the regression analysis. Correlations were determined by Spearman correlation coefficient.

The −G1245A SNP was determined in the total SGA group of 635 subjects. The Hardy-Weinberg equilibrium (HWE) was calculated by computing the \( \chi^2 \) test for deviations in HWE. Allele frequencies were calculated and tested by \( \chi^2 \). The polymorphism was grouped according to a dominant model into the [GG] genotype versus the [GA] + [AA] genotype. The genotypic groups were compared for all anthropometric parameters by univariate ANOVA. As head size preservation is related to SGA severity (8), we also analyzed the associations between the −G1245A SNP and head size according to birth weight quartiles of the study groups. \( P \) value ≤0.05 was considered significant. Statistical tests were performed using SPSS 15.0 (SPSS Inc., Chicago, IL, USA).

**Results**

**Clinical data**

The total SGA group had a mean birth length of −3.00 SDS and a birth weight of −2.34 SDS (Table 1). Postnatally, they had a significantly lower BMI and smaller HC than the average population (\( P < 0.001 \)), but compared with their height SDS, they had a large head (HC − height, 1.18 SDS). Their IGF-1 level was significantly lower than the average population (−0.93 SDS), but relatively high compared with their height of −2.24 SDS.

Short SGA subjects were born with a lower birth length SDS and tended to have a shorter gestational age than SGA catch-up subjects (Table 1). Short SGA subjects were younger at examination and by definition, shorter and had less catch-up growth in height from birth onwards compared with SGA catch-up subjects. Short SGA subjects also had a lower BMI, smaller head size and lower IGF-1 levels than SGA catch-up subjects. Whereas, there was no brain sparing within SGA catch-up subjects (0.01 SDS), short SGA subjects had a 1.75 SDS larger head than height SDS. Although SGA catch-up subjects had caught up to a normal height and HC, these measurements and their IGF-1 level remained below the population mean of 0 SDS (\( P \) value: <0.001, <0.001 and 0.005 respectively).

**Factors associated with HC SDS**

The −1245 A-allele was associated with a 0.3 SDS smaller HC in the total SGA population, although explaining a minor proportion of the variation in head size (\( P = 0.006, 1.3\% \), Table 2). Birth weight was positively associated with postnatal HC, each SDS in birth weight resulting in a 0.2 SDS increases in postnatal HC. The −G1245A SNP remained a significant explanatory factor, even after inserting birth weight and postnatal height into the model (\( P = 0.03, R^2 13.1\% \)).

**−G1245A polymorphism**

The total SGA population consisted of 71.7% [GG], 24.6% [GA] and 2.2% [AA] carriers. The short SGA and SGA catch-up group had comparable genotype frequencies (\( P = 0.40 \)). Genotypes were in HWE (\( P = 0.88 \)). The association between head size and the −G1245A SNP was examined in each birth weight quartile in the total SGA population. Subjects who were most severe SGA at birth, being in the lowest birth weight quartile,

### Table 1 Clinical data of the total, short small for gestational age (SGA), and SGA catch-up group.

<table>
<thead>
<tr>
<th>Child</th>
<th>Total SGA group</th>
<th>SGA subgroups</th>
<th>SGA catch-up</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Short</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>SGA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth:</td>
<td>635</td>
<td>439</td>
<td>196</td>
<td>0.01</td>
</tr>
<tr>
<td>Sex (boys/girls)</td>
<td>302/333</td>
<td>223/216</td>
<td>79/117</td>
<td>0.10</td>
</tr>
<tr>
<td>GA (weeks)</td>
<td>36.7 (3.6)</td>
<td>36.6 (3.8)</td>
<td>37.1 (3.0)</td>
<td>0.10</td>
</tr>
<tr>
<td>Length (SDS)</td>
<td>−3.00 (1.49)*</td>
<td>−3.14 (1.49)*</td>
<td>−2.70 (1.41)*</td>
<td>0.001</td>
</tr>
<tr>
<td>Weight (SDS)</td>
<td>−2.34 (1.01)*</td>
<td>−2.28 (1.03)*</td>
<td>−2.47 (0.81)*</td>
<td>0.03</td>
</tr>
<tr>
<td>At measurement:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>12.7 (7.2)</td>
<td>8.9 (5.1)</td>
<td>21.1 (2.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Height (SDS)</td>
<td>−2.24 (1.27)*</td>
<td>−2.95 (0.63)*</td>
<td>−0.64 (0.84)*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Height (SDS) − birth length (SDS)</td>
<td>0.81 (1.81)*</td>
<td>0.20 (1.60)</td>
<td>2.04 (1.57)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (SDS)</td>
<td>−0.73 (1.34)*</td>
<td>−1.13 (1.16)*</td>
<td>0.15 (1.31)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HC (SDS)</td>
<td>−1.01 (1.00)*</td>
<td>−1.19 (0.95)*</td>
<td>−0.64 (0.99)*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HC (SDS) − height (SDS)</td>
<td>1.18 (1.38)</td>
<td>1.75 (1.06)</td>
<td>0.01 (1.22)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IGF-1 level (SDS)</td>
<td>−0.93 (1.32)*</td>
<td>−0.99 (1.33)*</td>
<td>−0.45 (1.10)*</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Values are means (s.d.) unless indicated otherwise. When compared with 0.00 SDS; \( *P \) value < 0.001, \( ^*P \) value < 0.01, \( ^{1}P \) value < 0.05. The overall \( P \) value is presented between the short SGA and the SGA catch-up group by ANOVA for continuous and by \( \chi^2 \) test for categorical variables. GA, gestational age; SDS, s.d. score; HC, head circumference.
had a significantly 0.4 SDS smaller head size when carrying the −1245 A-allele (Table 3, Fig. 1). SGA subjects with the −1245 A-allele also had 0.6 SDS less brain sparing than [GG] carriers (Fig. 1). Short SGA and SGA catch-up groups showed the same difference, although it did not reach statistical significance. No associations were found with birth length, birth weight, and height SDS.

### Discussion

The aim of our study was to investigate whether the −G1245A SNP was associated with HC and brain sparing in a large cohort of 439 short SGA and 196 SGA catch-up subjects. The −1245 A-allele of the IGF1 promoter SNP was associated with a small head size and less brain sparing in SGA born subjects and particularly those with the lowest birth weight. Birth weight, birth length, and postnatal height SDS were not associated with the −G1245A SNP.

This study demonstrated that the relation between the −G1245A SNP, head size, and brain sparing was present in SGA born subjects and particularly those with the smallest size at birth. The head sparing adaptation of these severely SGA born neonates was reduced when carrying the −G1245 A-allele. As the −1245 A-allele is known to be in close linkage with the non-192 bp-allele in the IGF1 gene promoter, our study has shown another feature of carriers of this allele. Our study also showed that the association between the −G1245A SNP, head size, and brain sparing, was present in the total SGA population which is suggestive for a general feature of SGA born children.

Several studies have shown the important role of the IGF1 gene in brain growth (29, 30). Lee et al. (29) demonstrated that transgenic mice overexpressing IGF1 could ameliorate brain growth retardation caused by undernutrition, which resulted in a comparable brain growth as the well-fed control mice. They also demonstrated that IGF1 overexpression resulted in postnatal brain growth, which was localized in cerebral cortex, hippocampus and diencephalon. Also Simmons et al. (30) suggested a tissue-specific effect of IGF1, showing that glucose transport in the brain was preserved and unaffected by treatment with IGF-1 or insulin. To test, whether neuronal IGF-1 serves as an anabolic role, glucose utilization in IGF1-targeted gene

### Table 3 The total, short small for gestational age (SGA) and SGA catch-up group associations between the −G1245A SNP and head size according to birth weight quartiles.

<table>
<thead>
<tr>
<th>Birth weight quartiles</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bright weight range [SDS]:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[−3.0; −2.3]</td>
<td>[−3.0; −2.3]</td>
<td>[−3.0; −2.3]</td>
<td>[−3.0; −2.3]</td>
<td>[−3.0; −2.3]</td>
</tr>
<tr>
<td>[GG]</td>
<td>[GA + AA]</td>
<td>[GG]</td>
<td>[GA + AA]</td>
<td>[GG]</td>
</tr>
<tr>
<td>Total SGA group</td>
<td>−1.11 (1.16)</td>
<td>−1.54 (1.02)</td>
<td>−0.96 (0.99)</td>
<td>−1.16 (1.04)</td>
</tr>
<tr>
<td>Head circumference SDS</td>
<td>1.27 (1.43)</td>
<td>0.69 (1.43)</td>
<td>0.75 (1.55)</td>
<td>0.77 (1.05)</td>
</tr>
<tr>
<td>Short SGA group</td>
<td>−1.29 (1.09)</td>
<td>−1.70 (1.09)</td>
<td>−1.20 (0.83)</td>
<td>−1.57 (0.88)</td>
</tr>
<tr>
<td>Head circumference SDS</td>
<td>1.61 (1.20)</td>
<td>1.32 (1.14)</td>
<td>1.77 (1.06)</td>
<td>1.38 (0.81)</td>
</tr>
<tr>
<td>SGA catch-up group</td>
<td>−0.53 (1.21)</td>
<td>−1.19 (0.75)</td>
<td>−0.73 (1.08)</td>
<td>−0.65 (1.03)</td>
</tr>
<tr>
<td>Head circumference SDS</td>
<td>0.21 (1.59)</td>
<td>−0.71 (0.92)</td>
<td>−0.21 (1.32)</td>
<td>0.03 (0.83)</td>
</tr>
</tbody>
</table>

Values are means (s.d.). P values between the [GG] and [GA + AA] genotype are: * = 0.03, † = 0.08, ‡ = 0.26, ‡ = 0.08, § = 0.06.
deletion mice was investigated by Cheng et al. (31). Both IGF1 knock-out mice who were injected with 2-deoxy-D-[1-14C]glucose, and Sprague-Dawley rats who were injected with recombinant IGF-1 and 2-deoxy-D-[1-14C]glucose, were compared. Cheng showed that IGF-1 functions in an insulin-like manner to augment brain glucose utilization during brain development (31).

A reduced IGF1 gene expression has also been associated with a reduced brain size in mice, whereas IGF1 overexpression gave rise to increased brain growth, although the IGF-1 level in brain tissue was among the lowest one measured in all body tissues (32, 33). This might also be applicable to short SGA subjects having the −1245 A-allele which might result in less local IGF-1 expression in the brain and a smaller head size. All together, these observations indicate that short SGA subjects might experience a differential IGF1 gene expression which influenced their head size.

Several studies have demonstrated that short stature and a small head size are important predictors for subnormal intellectual performance (4, 34, 35). Our study showed that a small birth size in combination with the −1245 A-allele, was associated with the smallest HC in SGA subjects, and especially in short SGA subjects (A-allele carriers: −1.70 SDS). Unfortunately, we do not have data on intellectual performance in these subjects, but it would be interesting to study the influence of the A-allele on intellectual performance in future studies.

This study did not show associations between the −G1245A SNP and birth size, postnatal height and IGF-1 level in the short SGA and SGA catch-up group. Although animal studies and case reports of subjects with IGF1 gene mutations have shown that the IGF1 gene is important in pre- and postnatal growth, the short SGA subjects only had an association with head size. The absence of associations with pre- and postnatal growth in these SGA populations confirm previous genome wide and large association studies in which IGF1 was not one of the genes that could explain variations in height (36–41).

Additionally, animal models in which hepatic IGF-1 levels were eliminated, have shown that mice kept growing with 75% reduced serum circulating IGF-1 levels (42). These observations underline that the processes determining height is multifactorial in nature and that the IGF1 gene is not a main contributor in determining adult height.

Owing to the ascertainment, short SGA subjects were investigated at 9 years of age and SGA catch-up subjects were investigated in early adulthood at 21 years of age. Several studies have demonstrated that short SGA subjects remain short for their entire life when they did not reach a normal height at the age of 5 years (1, 43). In addition, both SGA groups had similar inclusion criteria at birth and during childhood, except of a difference in stature. Thus, although we can not exclude some bias due to the difference in age at investigation, we consider this unlikely to explain the observed associations.

We observed that the most severely SGA born subjects had a 0.4 SDS smaller head size and 0.6 SDS less brain sparing when carrying the −1245 A-allele in contrast to G-allele carriers, which might be of clinical relevance. Our study has shown for the first time that the −G1245A SNP is related to postnatal head size in SGA born subjects and particularly those with the lowest birth weight.

**Declaration of interest**

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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