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Clinical genetics of Type 1 Diabetes Genetic correlates of early growth and disease progression

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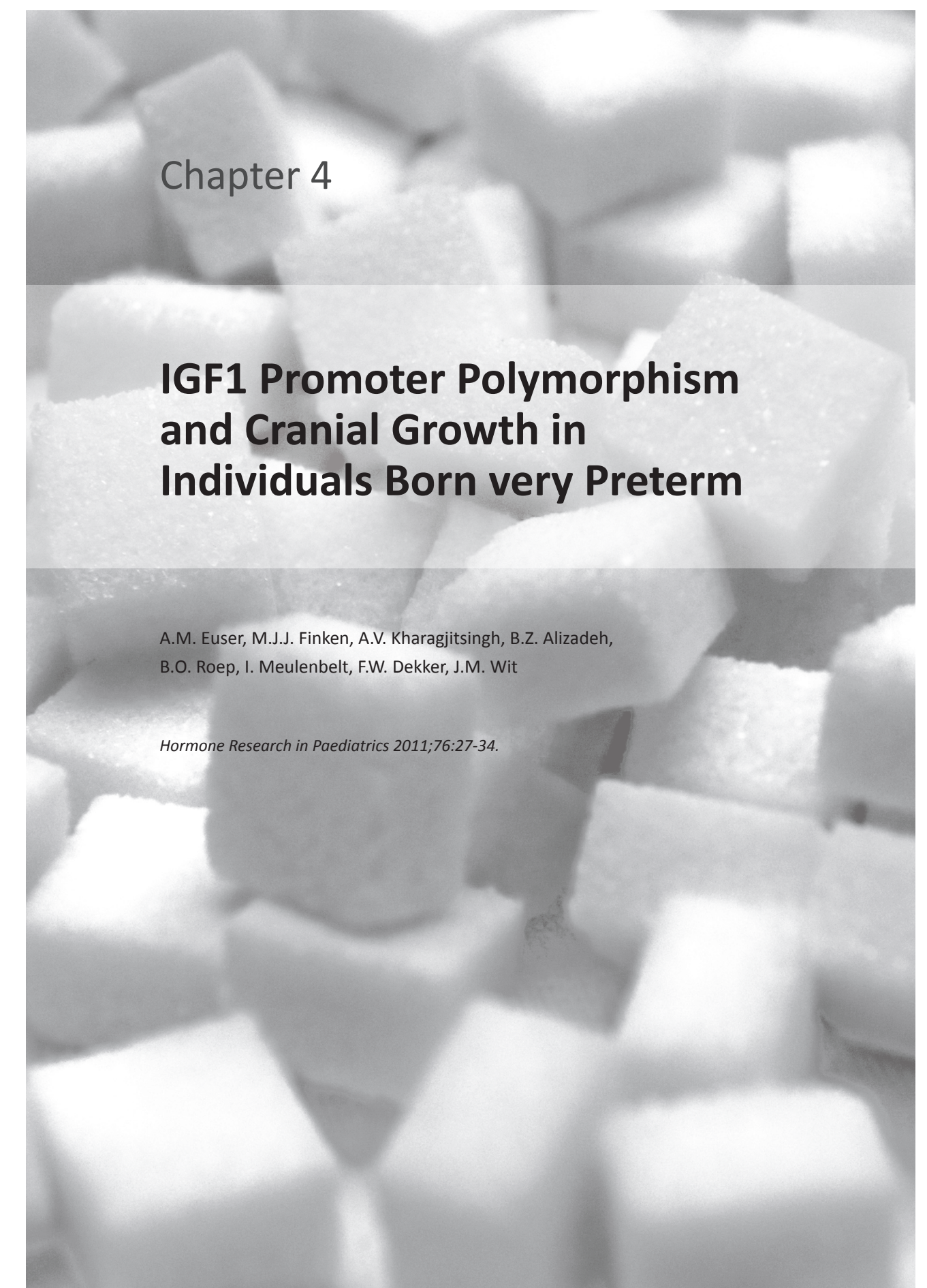
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The background of the entire page is a close-up, black and white photograph of numerous sugar cubes. The cubes are piled together, with some in sharp focus in the foreground and others blurred in the background, creating a textured, three-dimensional effect.

Chapter 4

IGF1 Promoter Polymorphism and Cranial Growth in Individuals Born very Preterm

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Abstract

Background: Major defects in the *IGF1* gene are associated with severely reduced cranial and linear growth. The association between *IGF1* promoter polymorphisms and growth is uncertain.

Aims: To test the effect of the *IGF1* 192-bp allele on cranial and linear growth and body mass index (BMI) from birth until age 5 years, and on IQ and serum IGF-1 at age 19 years.

Methods: In a birth cohort, including 285 individuals born at a gestational age <32 weeks from the Project On Preterm and Small-for-gestational age infants (POPS), cohort anthropometric measurements were analyzed. At age 19 years *IGF1* genotype, serum IGF-1 level and IQ were determined. Regression analyses were performed with mixed models.

Results: Homozygotes for the 192-bp allele had a slower cranial growth from birth until age 5 years, and a tendency towards less brain sparing and a slower linear growth compared to the other 2 genotype groups. *IGF1* genotype was not associated with IQ or BMI development. Head circumference SDS at age 5 years was positively associated with IQ at age 19 years.

Conclusion: Homozygosity for the *IGF1* 192-bp allele is associated with a slower cranial growth from birth until age 5 years in individuals born very preterm.

Introduction

Insulin-like growth factor1 (IGF-1) plays an important and pleiotropic role in human growth, both prenatally and postnatally [1]. The wild-type allele of 192 bp of a polymorphism in the promoter region of the *IGF1* gene has been associated with a greater birth weight [2], slower weight gain in infancy [3], and a greater adult height [4]. However, other studies [3,5,6] could not confirm associations of the 192-bp polymorphism with birth weight or stature.

In patients with rare mutations in the *IGF1* gene, like a deletion [7] or a missense mutation [8], microcephaly is a characteristic phenotypic feature observed in combination with other anomalies, such as deafness and severely retarded growth and psychomotor development. Heterozygous carriers of the missense mutation had a 1.5-SD smaller head circumference compared to wild-type relatives [8].

It is still unclear whether functional *IGF1* promoter polymorphisms contribute to variation in head circumference [9]. Associations between the two have been reported in children born small for gestational age [10,11] and in healthy newborns of diverse gestational ages [12]. However, these relations could not be replicated by other groups [5,6].

In preterm infants, cranial growth has been associated with subsequent neurodevelopment [13,14]. It is unknown whether common genetic variations in the *IGF1* gene contribute to these associations. The primary aim of this study was to test, in individuals born very preterm (i.e., <32 weeks of gestation), the effect of the *IGF1* 192-bp polymorphism on cranial growth and

brain sparing from birth until 5 years of age, and on intellectual performance at 19 years of age. The secondary aim was to test the effect of the 192-bp polymorphism on linear growth and BMI development.

Methods

Study Population

This genetic study was embedded in the Project On Preterm and Small-for-gestational-age infants (POPS) study. The POPS cohort comprises 94% of all liveborn very preterm (<32 weeks of gestation) and/or very low birth weight (<1,500 g) infants born in the Netherlands in 1983 [15]. For the current study, only the 1,012 subjects with a gestational age < 32 weeks were eligible for inclusion, of whom 676 were still alive at 19 years of age. Of these subjects, 419 consented to participate (62% response rate). Subjects with congenital malformations leading to changes in body proportions and/or body composition, like phocomely, i.e. chromosomal abnormalities or inborn errors of metabolism, were excluded (n = 4). Of the remaining individuals, 285 were genotyped. The derivation of the sample studied is displayed in figure 1. The study was approved by the medical ethics committee of all participating centers, and written informed consent was obtained from all participants.

Anthropometric and Intelligence Measurements

The POPS cohort has been studied intensely over the years, with regard to physical and psychosocial outcomes [15,16]. Weight (g), length (cm) and head circumference (cm) were measured at birth, and expressed as an SD score (SDS) using Swedish references for preterm infants [17].

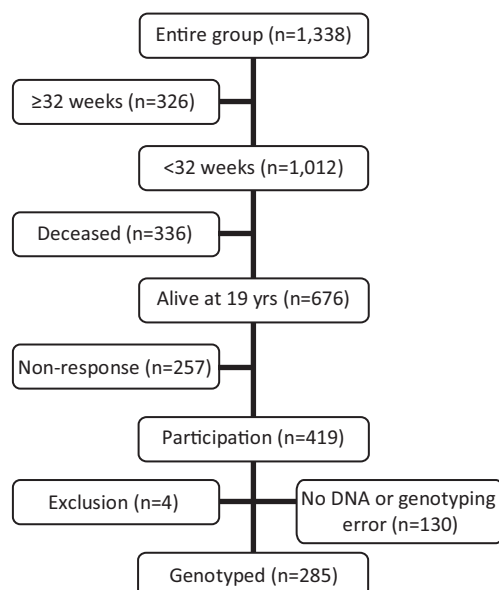


Figure 1 | Derivation of the sample studied.

During follow-up, weight, length/height and head circumference were measured at the ages of 3 and 6 months, and 1, 2 and 5 years. These measurements were expressed as SDS using Dutch reference values (corrected for prematurity until the age of 2 years) [18]. Brain sparing was defined as: head circumference SDS – length/height SDS [11].

At the age of 19 years, individuals were approached to participate in follow-up research, which included a venous blood sample for DNA studies and determination of serum IGF-1 level (Dr. Jaap van Doorn, Utrecht, the Netherlands), cognitive testing, and measurement of height and weight. Serum IGF-1 levels were converted to SDS using reference levels for age and sex in the same laboratory [19]. Intelligence was assessed with the computer version of the Multicultural

Capacity Test – Intermediate Level developed by Bleichrodt and Berg [20]. This standardized intelligence test differentiates within the lower half of the IQ spectrum and measures capacity and skills of individuals with a secondary education. It generates an IQ with a mean of 100 and SD of 15 in the Dutch norm sample.

Genotyping

Polymerase chain reaction (PCR) was performed using oligonucleotide primers designed to amplify the polymorphic cytosine-adenine (CA) repeat 1 kb upstream of the human *IGF1* gene [21]. The reaction was carried out in a final volume of 10 ml containing 50 ng of genomic DNA obtained from peripheral blood cells, 0.5 nmol/l forward primer (5' ACCACT-CTGGGAGAAGGGTA-3'), 0.5 nmol/l reverse primer (5'GCTAGCCAGCTGGTGTTATT- 3'), 0.25 mmol/l 2' -dNTP, 2.2 mmol/l MgCl₂, 0.01% W1 (Gibco BRL; Invitrogen, Carlsbad, Calif., USA) and 0.4 Taq DNA polymerase (Gibco BRL). PCR was performed in 96-well plates (94°C for 10 min; 35 PCR cycles of 30 s at 94°C, 30 s at 55°C, and 30 s at 72°C; 72°C for 10 min; 4°C hold). Forward primers were labeled with FAM, HEX or NED to determine the size of PCR products by autosequencer (ABI 3730 Applied Biosystems, Foster City, Calif., USA). The size of the PCR products was determined by comparison with an internal ROX-size standard (Perkin Elmer, Waltham, Mass., USA).

Six different alleles of the *IGF1* promoter region were identified (table 1). Alleles which occurred only once were taken together and classified as rare alleles. *IGF1* genotypes were categorized by the presence of the 192-bp allele. This resulted in 3 genotype groups: homozygotes for the 192-bp allele, heterozygotes for the 192-bp allele, and noncarriers of the 192-bp allele. Genotype frequencies were well in range with those found

Table 1 | Alle frequency and genotype distribution of the *IGF1* 192-bp polymorphism.

Polymorphism			
Allele (base pairs)	(CA) _n	N	(%)
188	17	8	(1.4)
190	18	38	(6.7)
192 (wild-type)	19	361	(63.3)
194	20	109	(19.1)
196	21	44	(7.7)
198	22	7	(1.2)
Other rare alleles	-	3	(0.5)
Genotypes			
Homozygous 192-bp		116	(41)
Heterozygous 192-bp		129	(45)
Non-carrier		40	(14)

(CA)_n number of cytosine-adenine repeats; wild-type allele, most frequent allele in this population. Values are number of POPS subjects (%). The allele distribution is based on 2 alleles per subject.

in earlier studies in healthy Dutch populations [6,22]. The genotype distribution was in agreement with the distribution predicted by the Hardy-Weinberg equilibrium ($p = 0.67$).

Statistical Analysis

Characteristics between the *IGF1* genotype groups were compared using ANOVA and the χ^2 test for continuous and categorical variables, respectively.

The association between the *IGF1* genotype and head growth during the first 5 years of life was assessed using repeated measures regression analyses. We first tested if interaction was present between age and the *IGF1* genotype with model (a) in which the *IGF1* genotype was included both in the intercept and as an interaction term with age:

$$\text{Head circumference SDS} = \beta_0 + (\beta_1 * \text{IGF1 genotype}) + (\beta_2 * \text{age}) + (\beta_3 * \text{IGF1 genotype} * \text{age}). \quad (\text{a})$$

If no interaction was found between the *IGF1* genotype and age, possible differences in cranial growth between the *IGF1* genotypes were estimated with the subsequent model (b) with age and *IGF1* genotype as fixed variables:

$$\text{Head circumference SDS} = \beta_0 + (\beta_1 * \text{IGF1 genotype}) + (\beta_2 * \text{age}). \quad (\text{b})$$

In both models, the covariance matrix was specified as unstructured. Repeated measures analyses were performed within a more narrowly defined subgroup in which non-Caucasian subjects and twin pregnancies were excluded.

Associations between *IGF1* genotype and IQ, and between head circumference genotype and IQ, were assessed with multiple linear regression analyses. These analyses were repeated after adjustment for the possible confounders: sex, gestational age, birth weight, maternal education and socioeconomic status [23]. All analyses were performed using the Statistical Package of Social Sciences (SPSS) version 16.

Results

In table 2, the characteristics of the participants are shown in relation to their genotype. There were no differences between the genotype groups. Head circumference and length at birth were not known for 49 and 78 persons, respectively. For each genotype group, birth weight SDS did not differ between individuals whose head circumference at birth, or birth length, was known, and those for whom it was not known (data not shown). In the entire population at 19 years of age, the serum IGF-1 level was significantly below

Table 2 | Characteristics of the participants according to their *IGF1* genotype.

	N	Homozygous 192-bp (n =116)	Heterozygous 192-bp (n =129)	Non-carrier (n =40)
General				
Sex (n, % males)	285	59 (51)	66 (51)	22 (55)
Race (n, % Caucasian)	283	104 (90)	109 (85)	37 (93)
Low maternal education (n, %)	278	61 (53)	57 (46)	16 (42)
SES (1-6)	282	3.4 (1.5)	3.5 (1.6)	3.5 (1.6)
At birth				
Singleton birth (n, %)	285	93 (80)	100 (78)	28 (70)
Gestational age (weeks)	285	30.0 (1.4)	29.7 (1.6)	29.9 (1.5)
Birth weight (g)	285	1,335 (340)	1,311 (338)	1,331 (310)
(SDS)	285	-0.2 (1.0)	-0.1 (1.0)	-0.1 (0.9)
Birth length (cm)	207	39 (3)	39 (3)	39 (4)
(SDS)	207	-0.2 (1.2)	0 (1.2)	-0.2 (1.2)
Head circumference at birth (cm)	249	27.4 (2.1)	27.6 (2.1)	27.5 (1.9)
(SDS)	249	-0.2 (1.1)	0.2 (1.1)	-0.2 (1.0)
At follow-up				
Head circumference at 5 y (cm)	279	50.9 (1.8)	51.2 (1.7)	51.4 (1.6)
(SDS)	279	-0.1 (1.0)	0.1 (1.0)	0.2 (0.9)
Brain sparing at 5 y (delta-SDS)	279	0.4 (1.0)	0.6 (1.1)	0.3 (1.0)
Height at 19 y (cm)	280	172.5 (10.0)	173.6 (9.8)	176.0 (9.7)
(SDS)	280	-0.6 (1.1)	-0.5 (1.0)	-0.2 (1.1)
BMI at 19 y (kg/m ²)	279	21.6 (2.9)	21.8 (3.8)	21.3 (2.6)
(SDS)	279	-0.2 (1.2)	-0.2 (1.3)	-0.2 (1.0)
Serum IGF-1 at 19 y (ng/ml)	284	277 (78)	271 (76)	254 (64)
(SDS)	284	-0.3 (0.8)	-0.3 (0.8)	-0.5 (0.7)
IQ at 19 y	269	101 (14)	103 (16)	101 (15)

Values are means (SD) unless otherwise indicated. Differences between the genotype groups were tested with one-way ANOVA for continuous and the χ^2 test for categorical variables: no statically significant differences were found.

the reference mean, with an SDS of -0.32 (95% CI -0.41 to -0.23).

Since no interaction was found between age and head circumference SDS, differences in cranial growth between the various genotypes were estimated with model (b), with age and the *IGF1* genotype as fixed variables. Figure 2 a shows the

differences in cranial growth between the genotype groups. The *IGF1* genotype had a significant effect on the cranial growth from birth until the age of 5 years ($p = 0.027$). This effect could be almost completely explained by the difference in cranial growth between the homozygotes and the other 2 genotype groups (figure 2 b). So analyses were subsequently repeated in a recessive model.

It was shown that homozygotes had a significantly slower cranial growth in the first 5 years of life ($p = 0.007$).

When analyses were repeated after exclusion of nonsingletons and non-Caucasians ($n = 64$ and 35 , respectively, and together 92 , due to overlap), results did not change. There was no statistically significant difference between genders, although the association tended to be more pronounced in males ($p = 0.05$) than in females ($p = 0.12$). In homozygous males the peak in head circumference SDS at 3 months of age was absent.

Homozygotes showed a tendency towards less brain sparing ($p = 0.11$) (figure 3). There was no indication for a stronger brain sparing effect in those with the lowest birth weights (data not shown). Head circumference SDS at 5 years of age was positively associated with total IQ at 19 years of age (beta 4.6 , 95% CI 2.1 – 7.0). This association remained significant after adjustments for sex, gestational age, birth weight, maternal education and socioeconomic status (beta 4.0 , 95% CI 1.5 – 6.4), and was similar for the various genotypes (figure 4).

The direction of association was similar for linear growth (figure 5a, b). Homozygotes had a slower linear growth than the other 2 genotype groups but the difference was not statistically significant ($p = 0.45$). The *IGF1* genotype was unrelated to BMI development.

Discussion

In this study of individuals born very preterm, we found that homozygosity for the *IGF1* promoter 192-bp allele was associated with a slower cranial growth from birth until 5 years of age but not

with IQ at 19 years of age. Head circumference at 5 years of age was positively associated with IQ at 19 years of age.

Our results may have been affected by limitations in the study design or in the data collection. Firstly, it is possible that the serum IGF-1 levels at 19 years of age may not be representative of those measured in infancy or childhood. Secondly, the size of the sample was not large enough to perform analyses for each sex separately. Thirdly, for a considerable number of individuals, head circumference and/or length at birth were not known. However, for all genotype groups the birth weight SDS of these individuals was not different from those with a known head circumference or length at birth, implying that missing values were randomly distributed and did not influence our results.

In our population, we found a typical pattern of cranial growth for all 3 genotype groups. After birth, a large upward peak by the age of 3 months postterm was followed by a more gradual return to values slightly higher than the head circumference SDS at birth. This is in accordance with the growth curves of preterm infants published by Gibson et al. [24]. Knockout models have elucidated the role of IGF production and signaling in prenatal and postnatal growth. Mice lacking *igf1*, *igf2* or *igf1r* have a severely reduced body weight and length [25,26]. Also in humans, mutations in both the *IGF1* [7,8] and *IGF1R* genes [27] are associated with severely compromised prenatal and postnatal growth, while duplication of the *IGF1R* gene is associated with tall stature [28]. In animals, homozygous *igf1* or *igf1r* ablation is associated with reduced brain growth, while *igf1* overexpressing genotypes are associated with increased brain growth [29]. In humans, *IGF1* and

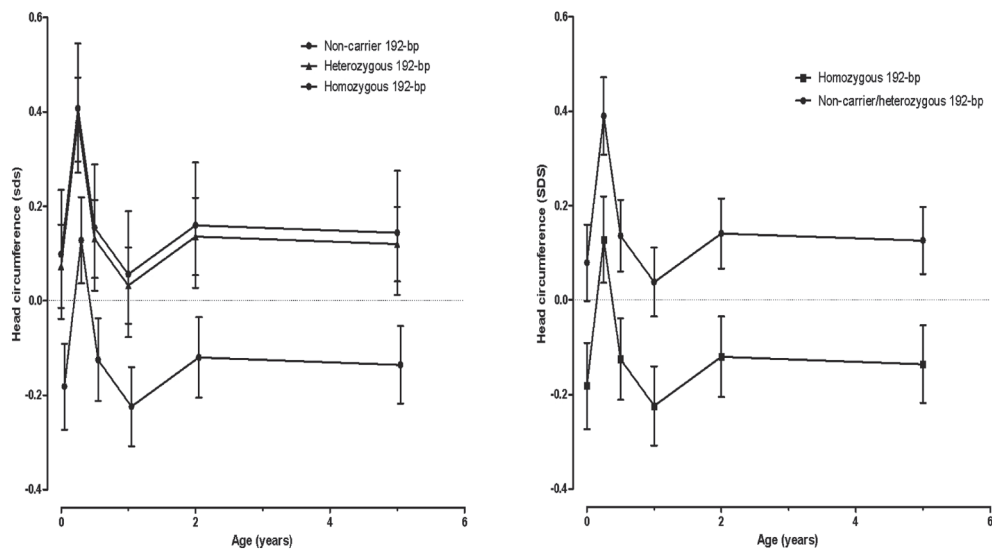


Figure 2 | Head circumference SDS (mean $\pm$ SE) from birth until the age of 5 years in homozygous 192-bp carriers, heterozygous 192-bp carriers, and non-carriers of the 192-bp allele (left panel), and in homozygous 192-bp carriers versus heterozygous 192-bp carriers and non-carriers (right panel).

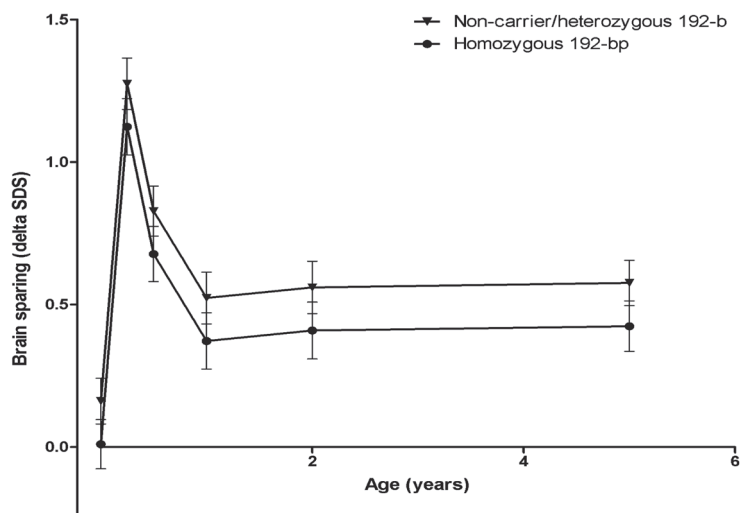


Figure 3 | Brain sparing delta-SDS (mean $\pm$ SE) from birth until the age of 5 years in homozygous 192-bp carriers, heterozygous 192-bp carriers, and non-carriers of the 192-bp allele.

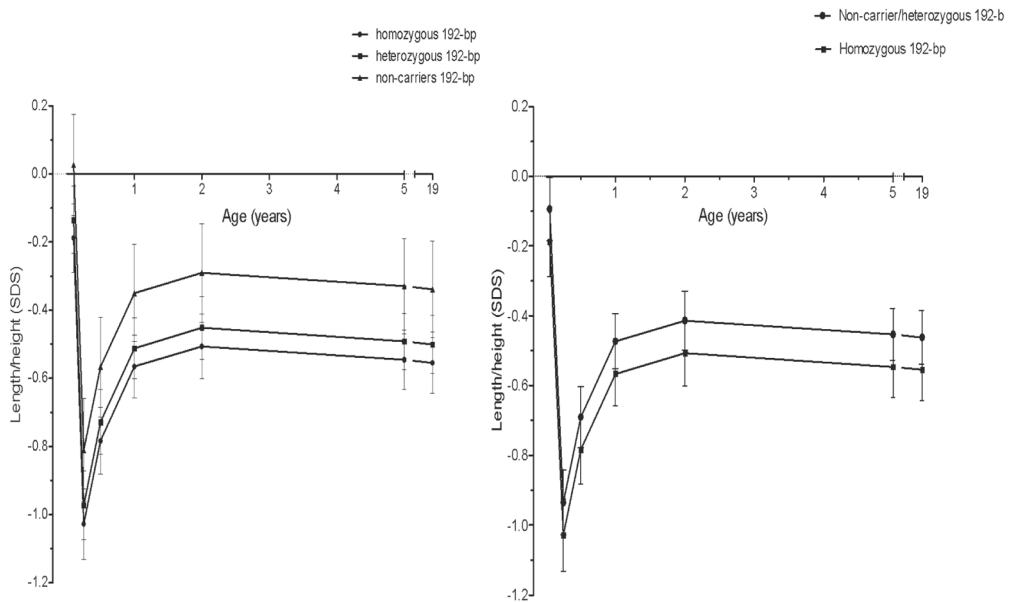


Figure 4 | Length/height SDS (mean +/- SE) from birth until the age of 19 years in homozygous 192-bp carriers, heterozygous 192-bp carriers, and non-carriers of the 192-bp allele (left panel), and in homozygous 192-bp carriers versus heterozygous 192-bp carriers and non-carriers (right panel).

IGF1R mutations are associated with a reduced head circumference [7,8,27], while duplication of the *IGF1R* gene is associated with a larger head circumference [28].

We found that homozygosity for the 192-bp allele was associated with a slower cranial growth but no relations of the 192-bp allele with the other growth parameters were found. Previous studies have found associations between *IGF1* promoter polymorphisms and birth weight, head circumference, stature, and IGF-1 level [2,3,5,6,9,11,12,30,31]. However, the direction of the reported associations varied considerably between these studies. In our study, the lack of association with stature may be explained by an overrepresentation of conditions acting in perinatal life and exerting a larger effect on linear

growth [32] than *IGF1* gene variation. In our study, smaller head circumference SDS in childhood was significantly associated with a lower total IQ at young adult age, which is in agreement with other findings for infants [33,34] and adults [13] with a low birth weight and/or born prematurely. The lack of association between the 192-bp allele and IQ at age 19 years is probably a matter of sample size, since in our study, associations were found between the 192-bp allele and cranial growth, and between cranial growth and IQ.

In conclusion, in this study of individuals born very preterm, we found that homozygosity for the *IGF1* promoter 192-bp allele was associated with a slower cranial growth from birth until 5 years of age but not with IQ at 19 years of age. Head circumference at 5 years of age was positively associated with IQ at 19 years of age.

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