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Polymorphism in the IGF-I gene: Clinical Relevance for Short Children Born Small for Gestational Age (SGA).

NICOLETTE ARENDS, LINDA JOHNSTON, ANITA HOKKEN-KOELEGA, CORNELIA VAN DUIJN, MARIA DE RIDDER, MARTIN SAVAGE, ADRIAN CLARK

Department of Pediatrics, Division of Endocrinology, Sophia Children's Hospital / Erasmus University Rotterdam, The Netherlands (NA, AH-K); Department of Endocrinology, St. Bartholomew's and the Royal London School of Medicine and Dentistry, London, United Kingdom (LJ, MS, AC); Department of Epidemiology and Biostatistics, Erasmus University Rotterdam, The Netherlands (CvD, MdeR).

ABSTRACT Low birth weight is associated with an increased risk in adult life of type 2 diabetes, hypertension and cardiovascular disease (CVD). The fetal insulin hypothesis postulates that genes involving insulin resistance could effect birth weight and disease in later life (Hattersley, 1999). Besides insulin, there is extensive evidence that insulin-like growth factor-I and –II (IGF-I, IGF-II) play an important role in fetal growth. We hypothesized that minor genetic variation in the IGF-I gene could influence pre- and postnatal growth. Three microsatellite markers located in the IGF-I gene in 124 short children (height <-1.88 SDS) who were born small for gestational age (SGA) and their parents were studied. SGA was defined as both a birth weight and birth length below –1.88 SDS for gestational age. Two polymorphic markers showed transmission disequilibrium. Allele 191 of the IGF1.PCR1 marker was transmitted more frequently from parent to child (χ^2 = 4.8 and p=0.02) and allele 198 of the 737/738 marker was transmitted less frequently from parent to child (χ^2 = 4.5 and p=0.03) . Children carrying the 191-allele had significantly lower IGF-I levels than children not carrying this allele (-1.1 SDS vs. –0.50 SDS; p=0.03). Also, head circumference SDS remained smaller in children with allele 191 compared to children without allele 191 (-2.1 SDS vs. –0.9 SDS; p=0.003). Our results show that genetically determined low IGF-I levels may lead to a reduction in birth weight, length *and* head circumference and to persistent short stature and small head circumference in later life (proportionate small). Since low IGF-I levels are associated with type 2 diabetes and CVD, we propose that the IGF-I gene may provide a link between low birth weight and such diseases in later life.

Introduction

About 10-15 % of children born small for gestational age (SGA) have an increased risk of being short as adults (1, 2). The mechanism underlying persistent short stature in these children is not fully understood. Insulin-like growth factor-I (IGF-I) plays an important role in both pre- and postnatal growth and its serum levels are regulated by both metabolic and genetic factors. In fetuses and neonates born SGA low circulating IGF-I levels have been observed suggesting a role for IGF-I in fetal growth retardation (3-5). More direct evidence for a role of IGF-I in fetal and postnatal growth comes from gene deletion studies in mice (6). Weight and length at birth were significantly reduced in IGF-I knock-out mice (birth weight about 60% of normal) (6), and postnatally they showed a further deterioration of growth resulting in adult weights of about 30 % of normal mice (7, 8). Until now only one human homozygous partial deletion of the IGF-I gene has been described in a 15-year old boy (9). This child was born SGA and showed severe postnatal growth failure, sensorineural deafness and mental retardation.
Low serum IGF-I levels have been reported in short children born SGA (10, 11). We hypothesized that minor genetic variation in the IGF-I gene might cause a change in serum IGF-I levels resulting in altered fetal and postnatal growth. We therefore investigated the IGF-I gene in a large group of short children born SGA and their parents. Three polymorphic dinucleotide repeat markers were studied and the results were analysed using the transmission disequilibrium test (TDT) (12).
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Methods

Subjects
This study included 124 children (66 boys and 58 girls) with short stature born SGA and their parents. All children fulfilled the same inclusion criteria: 1) birth length and birth weight standard deviation score (SDS) below – 1.88 for gestational age (13); 2) height SDS for age below –1.88 according to Dutch standards (14); 3) height velocity SDS for age below zero to exclude children with spontaneous catch-up growth; 4) an uncomplicated neonatal period, without signs of severe asphyxia (defined as Apgar score below 3 after 5 minutes), sepsis or long-term complications of respiratory ventilation such as bronchopulmonary dysplasia. Children with endocrine or metabolic disorders, chromosomal defects, syndromes and growth failure caused by other conditions (e.g. emotional deprivation, severe chronic illness, chondrodysplasia) were excluded, with the exception of Silver-Russell syndrome. The ethnicity was Caucasian for 113 families, Asian for 1 family, Indo-Mediterranean for 4 families and mixed ethnicity for 6 families. The study was approved by the Ethics Committee. Written informed consent was obtained from the parents or custodians of each child and from children older than the age of 8 years.

Clinical and biochemical measurements
In children and parents standing height was measured and expressed as SDS adjusting for sex and chronological age using Dutch standards (14). Body mass index (BMI) was

calculated as weight (in kilogram) divided by square of height (in meters) and expressed as SDS for sex and age (15). Serum IGF-I levels were measured in children using a specific RIA (16) and values were transformed into SDS adjusting for sex and age (16).

IGF-I gene

The polymerase chain reaction (PCR) was used to amplify three dinucleotide repeat markers located in the IGF-I gene (Fig. 1)(17). The three markers were 737/738, a cytosine-adenine (CA) repeat in the promoter region of the gene (18), IGF1.PCR1, an intronic cytosine-thymine (CT) repeat lying between exon 2 and 3 (19) and D12S318, a microsatellite marker (CA repeat) lying 3' to the gene (20) (21). All reactions were carried out in a volume of 10 μl in the presence of 0.1 mM dNTP, 1.5 mM $MgCl_2$ and 0.25 units Taq DNA polymerase (Sigma, Poole, UK).
For the IGF1.PCR1 and 737/738markers 100 ng of peripheral leucocyte genomic DNA was amplified using 0.5 nmol/l forward primer (IGF1.PCR1: 5'-TTGTGTCAACTGCTGATATG-3'; 737/738: 5'-GCTAGCCAGCTGGTGTTATT-3') and 0.5 nmol/l reverse primer (IGF1.PCR1: 5'-AACCAAAACATCATTCC-3'; 737/738: 5'-ACCACTCTGGGAGAAGG-3'). Amplification was for 37 cycles of 30 s at 94 °C, 30 s at 58 °C and 30 s at 72 °C. For D12S318 50 ng of genomic DNA was amplified using 0.3 nmol/l forward primer (5'-TGCTTGGGTCATCAATCTGC-3') and 0.3 nmol/l reverse primer (5'-GGTTATAGACATATAAA-3') for 35 cycles of 30 s at 94 °C, 30 s at 58 °C and 30 s at 72 °C. Forward primers were labelled with either HEX or FAM. The sizes of PCR products were determined by the ABI 377 DNA Analyser using ROX 500 as a fluorescent size marker. The results were analysed using GENESCAN and GENOTYPER software (Applied Biosystems, UK).

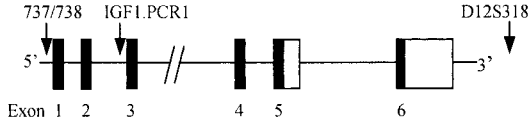

Fig. 1. Schematic organization of the IGF-I gene. Exons 1 to 6 are indicated by boxes, with coding regions in black and non-coding regions in white. Promoter regions are indicated by a dashed line. Dinucleotide markers are indicated by arrows.

Statistical analysis

The transmission disequilibrium test (TDT) was performed using the TDT/S-TDT program 1.1 of Spielman (12, 22). The TDT test is a valid test for linkage and association, even when the population under study consists of subjects of different ethnic origin. The TDT method evaluates whether the frequency of transmission of alleles from heterozygote parents to their affected children deviates from 50%, the expected Mendelian frequency when there is no linkage.

Statistical tests to analyse genotype-phenotype relationships were performed with use of SPSS package (version 10.0). Independent sample t-testing was used to analyse differences in phenotype between different genotypes. To investigate the possible influence of allele 191 on birth size and growth, a repeated measurement analysis (SAS Proc Mixed) was performed with all length and weight measurements, converted to their SD-score, from birth to the age of 4.0 years. Head circumference measurements were available from birth until the age of 1.5 years in 83 children. As random covariables were used the intercept, age and age-squared, as fixed covariables age, age-squared (if significant), presence of allele 191 and its interaction with age (unadjusted model). If significant, the model was adjusted for sex, gestational age, multiple birth and their interaction with age (adjusted model). Statistical significance was defined as $p < 0.05$.

Results

Baseline characteristics are shown in Table 1. Birth length SDS , birth weight SDS, head circumference SDS and height SDS were all significantly lower compared to –1.88 SDS. BMI SDS, IGF-I SDS, fathers height SDS and mothers height SDS were all significantly lower compared to zero, i.e. compared to the median for normals.

Gestational age (weeks)[1]	37.0 (32.8 – 39)
Birth length SDS[1]	-3.3 (-4.7 - -2.5)*
Birth weight SDS	-3.0 (0.8)*
Birth head circumference SDS	-2.5 (1.1)*
Age (years)	6.7 (2.4)
Height SDS	-3.0 (0.7)*
BMI SDS	-1.4 (1.3)†
IGF-I SDS	-0.6 (1.1)†
Fathers height SDS	-1.1 (1.1)†
Mothers height SDS	-1.3 (1.0)†

Table 1. Baseline characteristics of 124 short children born SGA; Values expressed as mean (SD) unless stated otherwise; BMI SDS = body mass index SDS
[1] = Median (interquartile range)
* = significantly lower compared to –1.88 ($p<0.001$)
† = significantly lower compared to zero ($p<0.001$)

TDT results and allele frequencies of the 3 markers are shown in Table 2. Significant evidence of transmission disequilibrium was found in the IGF1.PCR1 and the 737/738 markers.
The wildtype allele of the IGF1.PCR1 marker was allele 189. Significant transmission disequilibrium was found with allele 191 ($\chi^2 = 4.8$ and $p=0.02$). This allele was transmitted more frequently from parents to children. Interestingly, the mean serum IGF-I level was significantly lower in children carrying allele 191 compared to children without allele 191 (-1.1 SDS vs. –0.50 SDS; $p=0.03$) suggesting a functional relationship between this

Allele[1]	Freq. (%)	Transm.	Non-transm.	p-value
IGF1.PCR1				
185	0.8	2	0	
187	0.4	1	1	
189	*65.3*	*61*	*61*	
191	**8.5**	**21**	**9**	**0.02**
193	0.8	2	6	
195	2.4	6	10	
197	4.0	10	14	
199	4.0	8	7	
201	6.5	14	20	
203	1.6	4	3	
205	1.6	3	1	
207	3.6	9	9	
209	0.4	1	0	
211	-	0	1	
737/738				
176	0.4	1	1	
186	-	0	1	
188	1.2	3	1	
190	4.0	11	7	
192	*71.8*	*62*	*54*	
194	16.5	39	39	
196	5.6	12	19	
198	**0.4**	**1**	**7**	**0.03**
D12S318				
239	-	-	-	
241	-	0	1	
243	-	0	1	
247	6.0	16	11	
249	10.1	24	22	
251	6.5	17	16	
253	*52.0*	*63*	*60*	
255	8.5	18	25	
257	6.9	15	15	
259	5.2	14	8	
261	4.0	10	17	
263	0.8	2	1	
265	-	0	2	

Table 2. Allele frequencies of 124 short children born SGA
[1] = allele in number of base pairs; Italics = wildtype allele; Bold = allele with significant transmission disequilibrium

	Carriers allele 191	Non-carriers allele 191	p-value
HC SDS			
- at birth	-2.0 (0.3)	-2.3 (0.2)	Ns
- after 1.3 yrs	-2.1 (0.4)	-0.9 (0.2)	0.003
IGF-I SDS	-1.1 (0.2)	-0.5 (0.1)	0.03

Table 3. Head circumference SDS (HC SDS) and serum IGF-I SDS in carriers versus non-carriers of allele 191; Values are expressed as mean (SE)

polymorphism and the IGF-I gene (Table 3). No significant difference in height SDS was found between children with and without allele 191. Both in the unadjusted and adjusted model, no significant relation was found between the changes in height and weight SDS from birth until 4.0 years and allele 191. However, changes in head circumference SDS during the first 1.5 years were significantly associated with allele 191. At the age of 1.5 years children carrying allele 191 had a significant smaller head circumference SDS than children without allele 191 ($p=0.003$) (Table 3). Head circumference SDS of the 191-carriers did not change during the first 1.5 years of life whereas the non-carriers showed a significant increase in head circumference SDS during that period ($p<0.001$). BMI SDS and parental height SDS did not differ significantly between 191-carriers and 191-non-carriers. Allele 192 of the 737/738 marker was the most common allele and therefore likely to be the wildtype allele. Transmission disequilibrium was found with allele 198 ($\chi^2 = 4.5$ and $p=0.03$). This allele was less likely to be transmitted from parents to children. Since only one child was carrier of the 198 allele it was not possible to analyse phenotypic differences between the 198- carriers and non-carriers. None of the alleles of the D12S318 marker was in transmission disequilibrium.

Discussion

Our study provides evidence for an important role of the IGF-I gene in short children born SGA. Allele 191 of the IGF1.PCR1 marker was transmitted more frequently from parent to child suggesting this is a 'causal allele'. The association between head circumference and allele 191 indicates this allele might play a role in SGA children with persistent short stature and small head circumference (proportionate small) . It was to be expected that we could not detect significant differences in changes in height between different genotypes since our study population is a very homogenous group where all children are short (height <-1.88 SDS). Also, children with allele 191 had significantly lower serum IGF-I levels than those without allele 191 suggesting a functional relationship.

Our results are suggestive of the existence of a functional variant of the IGF-I gene located between the promoter region and exon 3 which results in significantly lower serum IGF-I levels. Further research is needed to unravel the exact location and function of this mutation.

The 198 allele of the 737/738 marker was less frequently transmitted from parent to child. Since the frequency of this allele was very low it is difficult to draw conclusions regarding its relation to serum IGF-I levels. An unexpected finding is that parents carrying the 198 allele (n = 8) were significantly shorter than parents without this allele (n = 240) (-2.0 SDS vs. –1.1 SDS; $p=0.03$). This suggests the allele itself is associated with short stature. Since this allele is less likely to be transmitted to offspring it is unlikely that a causal relation exists between this polymorphism and SGA.

Johnston et al. could not find an association between the IGF-I gene and the SGA phenotype in a cohort of French term singleton SGA subjects and a cohort of adults born appropriate for gestational age (AGA)(23). Although the mean adult height of the SGA cohort was significantly lower compared to the AGA cohort, no distinction was made between the SGA adults who had attained normal

height and those who remained short. This might explain why no significant association was found.
Insulin resistance has been reported in short children born SGA (24) and impaired glucose tolerance was described in 8% of our study population (25). Severe insulin resistance has been reported in a child with a homozygous IGF-I gene defect (26). Besides being an important contributor to fetal growth, IGF-I has a stimulatory effect on growth and development of pancreatic beta-cells. A lifetime exposure to low-normal serum IGF-I levels has been reported as a risk factor for developing cardiovascular disease and type 2 diabetes (27-29). Thus, polymorphisms in the IGF-I gene resulting in low serum IGF-I levels may increase the risk of cardiovascular disease and type 2 diabetes. We found an association between a polymorphism of the IGF-I gene and low serum IGF-I levels in a specific group of short children born SGA who remained proportionate small. Since low IGF-I levels increase the risk of developing adult disease we suggest that this IGF-I polymorphism may be a link between a low birth weight and an increased risk of adult disease. This would be consistent with the 'fetal insulin hypothesis' (30). Besides the glucokinase gene (31), the IGF-I gene might not only be involved in fetal growth but also in the pathogenesis of diabetes and cardiovascular disease. Further research is needed to confirm our findings and to explore if other genetic and environmental factors may also contribute to this phenotype.

This is the first study showing an association between a polymorphism of the IGF-I gene and low serum IGF-I levels in a group of short children born SGA. Our results suggest that genetically determined low serum IGF-I levels may lead not only to a reduction in birth length, weight *and* head circumference but also to persistent short stature and small head circumference during childhood and adulthood (proportionate small). As low serum IGF-I levels are associated with adult disease, the IGF-I gene may provide a link between the association of low birth weight and disease in later life in this specific group of small SGA children.

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