**IGF1R Gene Alterations in Children Born Small for Gestational Age (SGA)**

Alesksandra Janchevska¹, Marina Krstevska-Konstantinova¹, Heike Pfaffle², Marina Schlicke², Nevenka Laban¹, Velibor Tasic³, Zoran Gucev¹, Kristina Mironksa¹, Aleksandar Dimovski¹, Jürgen Kratzsch³, Jürgen Klammt², Roland Pfaffle²

¹Medical Faculty, Ss. Cyril and Methodius University in Skopje, Skopje, Republic of Macedonia; ²University of Leipzig, Leipzig, Germany; ³Macedonian Academy of Sciences and Arts, Skopje, Republic of Macedonia

**Abstract**

**BACKGROUND:** Small for gestational age (SGA)-born children are a heterogeneous group with few genetic causes reported. Genetic alterations in the IGF1 receptor (IGF1R) are found in some SGA children.

**AIM:** To investigate whether alterations in IGF1R gene are present in SGA born children.

**PATIENTS AND METHODS:** We analysed 64 children born SGA who stayed short (mean -3.25 ± 0.9 SDS) within the first 4 years of age, and 36 SGA children who caught up growth (0.20 ± 1.1 SDS). PCR products of all coding IGF1R exons were screened by dHPLC followed by direct sequencing of conspicuous fragments to identify small nucleotide variants. The presence of IGF1R gene copy number alterations was determined by Multiplex Ligation-dependent Probe Amplification (MLPA).

**RESULTS:** The cohort of short SGA born children revealed a heterozygous, synonymous variant c.3453C > T in one patient and a novel heterozygous 3 bp in-frame deletion (c.3234_3236delCAT) resulting in one amino acid deletion (p.Ile1078del) in another patient. The first patient had normal serum levels of IGF1, the second patient had unusually low IGF1 serum concentrations (-1.57 SD), which contrasts previously published data where IGF1 levels rarely are found below the age-adjusted mean.

**CONCLUSIONS:** IGF1R gene alterations were present in 2 of 64 short SGA children. The patients did not have any dysmorphic features or developmental delay. It is remarkable that one of them had significantly decreased serum concentrations of IGF1. Growth response to GH treatment in one of the patients was favorable, while the second one discontinued the treatment, but with catch-up growth.

**Introduction**

Small for gestational age children (SGA; low birth weight and/or birth length) are a heterogeneous group both regarding clinical characteristics and the aetiology (fetal, maternal, placental, and/or genetic factors). Most SGA children normalise their stature by 2 yr. of age. Nevertheless, approximately 10-15% of SGA children do not achieve normal growth and height until adolescence and adulthood and remain short [1] [2]. In addition to the short stature SGA children have a reduced lean body mass, fat mass, skin folds, and body mass index (BMI) [3] [4] [5] [6] [7], as well as a lower calorie, fat, and carbohydrate intake [1] [3]. An impaired IGF1R function may lead to disturbed glucose homeostasis [8], which may partly explain the increased risk for diabetes in SGA adults.

IGF-I, the hormone ligand that binds to the IGF1R, is fundamental for prenatal and postnatal growth and development. Intrauterine and postnatal growth retardation, deafness, microcephaly, and mental retardation have been reported in homozygous deletion or mutation in the IGF1 gene [1] [5]. The effects of IGF-I are mediated through the type 1 IGF receptor (IGF1R), which is a tyrosine kinase receptor encoded by the IGF1R gene [9]. Growth failure and microcephaly have been reported in patients with IGF1R defects.

We used dHPLC and Sanger sequencing and MLPA to detect small nucleotide variants (SNV) or copy number variants (CNV), respectively, to reveal
possible genetic alterations in the *IGF1R* gene as a cause of the observed phenotype in SGA children with or without catch-up growth.

### Patients and Methods

SGA was defined as a birth length and/or weight < 2 standard deviation scores (SDS) for the gestational age. SGA children remaining short at age 4 (height > 2.00 SDS) were included in the study. All children had an uncomplicated perinatal and postnatal period. Exclusion criteria included endocrine disorders, skeletal abnormalities, chronic diseases and chromosomal abnormalities.

The study protocol was approved by the Medical Ethics Committee of the Medical Faculty Skopje, Macedonia.

Birth and growth data before the start of treatment were retrieved from records of nurseries, and general practitioners. Height and head circumference were expressed as SD scores [10]. Body mass index was calculated (weight in kg/height in meters²) and expressed as SD scores for age and sex. Bone age was determined according to Greulich and Pyle [11]. The dysmorphological examination was performed by an experienced clinical geneticist.

GH pituitary reserve was assessed by L-dopa and clonidine GH tests. Serum samples were analysed for IGF-1 and IGFBP-3 by either chemiluminescent immunoassays (Mediagnost, Reutlingen, Germany), or by colourimetric ELISA (Mediagnost, Reutlingen, Germany).

IGF-1 inter- and intra-assay variation coefficients were 6.8 and 6.7%, respectively; IGFBP-3 inter- and intra-assay variation coefficients 6.30 and 4.51%, respectively. Serum GH was measured by a solid-phase, two-site, chemiluminescent immunoassay (ARUP, Salt Lake City, Utah, USA). Cortisol, testosterone and estrogens were measured by colourimetric ELISA (Diagnostic Products Corporation, Los Angeles, Calif., USA).

NA was extracted from peripheral blood samples using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). PCR products of all coding exons and adjacent intronic sequences of the *IGF1R* gene were generated and subjected to denaturing HPLC (dHPLC) pre-screening (WAVE System; Transgenomic, Glasgow, UK). PCR products with conspicuous chromatograms were further analysed by Sanger dyeoex-sequencing (ABI PRISM 310 Genetic Analyzer; Thermo Fisher Scientific, Waltham, MA). Primer sequences can be obtained upon request. Sequences were compared to the human reference genome (UCSC, version 19 (GRCh37)) and annotated according to the GenBank reference coding sequence of the *IGF1R* NM_000875 and UniProtKB protein reference P08069. Multiplex Ligation-dependent Probe Amplification (MLPA) to detect copy-number variants (CNV) in the *IGF1R* gene was performed according to the manufacturer’s recommendations (SALSA MLPA P217; MRC Holland, Amsterdam, The Netherlands).

### Results

All 64 short SGA children were investigated, and mutations in two patients were identified by dHPLC and direct sequencing.

Patient A is a boy who was born spontaneously after 37 weeks of gestation and after uneventful pregnancy and delivery. He is the second child of young, non-consanguineous parents. His brother had a normal birth size, and postnatal growth. The proband’s birth weight was 1700 g (-3.36 SDS score) and birth length 41 cm (-3.66 SDS score). The parents’ height was: father 166cm (-0.76 SDS score) and of his mother 154.5 cm (-1.9 SDS score), with a target height of 166.2 cm (-1.27 SDS score). His psychomotor development, sight and hearing were normal.

At 6.3 yr. of age, his height was 101.4 cm (-3.90 SDS score), weight 13.8 kg (-3.24 SDS weight for height), and head circumference was not available. L-dopa and clonidine stimulation tests were performed at age 5 years with a maximal GH response of 6.31 ng/ml and 10.8 ng/ml, respectively. At this age, his bone age was 4 yr. His IGF-I level was 52.3 ng/ml (-1.57 SDS score) and IGFBP-3 level 1.17 mg/liter (-1.63 SDS score). Ultrasound of the heart and kidneys were uneventful, antibodies for gliadin negative. MRI of the hypothalamic and pituitary region revealed normal size pituitary and no anomalies. Morphologic examination showed no anomalies. He attends to a regular primary school. His IQ score was 89. Since the age of 11.1 years, the GH treatment (37 ìg/kg/day) was given for 18 months. The treatment resulted in catch-up growth, and he reached 144.6 cm (-0.21 SDS) at 12.7 years when the parents interrupted the treatment.

In patient A a heterozygous synonymous nucleotide transition, c.3453C > T (p.I1151 =), was found. Position 3435 is located 5 nucleotides upstream of the last nucleotide of exon 18. In silico analysis using MutationTaster (http://www.mutationtaster.org/; accessed April 2018) predicts pathogenicity due to a potential splice site change. The additional computational analysis suggests the introduction of a new exonic splicing silencer site while a potentially existing splicing enhancer site is broken by the nucleotide substitution (Human Splicing Finder 3.1;
http://www.umd.be/HSF3/; accessed April 2018). Aberrant splicing at the intron 18 splice donor site would presumably result in a severely disturbed IGF1R function because the affected amino acid residue(s) are part of the tyrosine kinase domain.

![Figure 1: Genetic analysis of IGF 1R; a novel heterozygous variant (synonymous); c.3453C>T; a possible impact on splicing has to be verified](image)

The 8.9 old boys was referred for pediatric endocrine evaluation because of short stature. He was born at 40 wk gestation, after uneventful pregnancy and delivery. The parents are young and non-consanguineous. His brother and sister had normal birth size and normal postnatal growth. His birth weight was 2300 g (-2.76 SDS), his birth length 46 cm (-2.14 SDS), the head circumference at birth was not available. The parents' height was: father 158 cm (-2.0 SDS score) and of his mother 158.7 cm (-1.26 SDS score), with a target height of 165.0 cm (-1.38 SDS score). His psychomotor development, sight and hearing were normal. At referral, the boy had a height of 114.6 cm (-3.08 SDS) and a weight of 22.2 kg (-1.2 SDS). His head circumference was 49.8 cm (normal). The Greulich and Pyle male standards bone age was 10 years. His sight, hearing and development were normal. He had average grades in the primary and secondary school. L- Dopa and clonidine tests of pituitary GH reserve were 16.4 ng/ml and 17.7 ng/ml respectively. Initially, IGF-1 was 468 ng/ml (reference 237-996) and IGFBP-3 levels were not available, but under GH treatment IGF-I was 205 ng/ml (reference 226–903), while IGF binding protein-3 (IGFBP)-3 were not available. At the age of 15.41 years, his height was 138.2 cm (-4.11 SDS), his weight 30.3 kg (-2.71 SDS), head circumference normal. T4, TSH, cortisol, renal function, hepatic analysis were normal. Since the age of 16.25 years (-3.77 SDS) the GH treatment (37 µg/kg/day) was initiated and lasted 3 years. This resulted in a final adult height of 160.5 cm (-2.5 SDS) which was within the parental target height range.

In patient B a 3-bp in-frame deletion, c.3234_3236delCAT, was identified. The deletion probably leads to removal of isoleucine at protein position 1078 (p.Ile1078del) and is predicted to be disease-causing (MutationTaster). The affected position maps to the tyrosine kinase domain of the IGF1R. Isoleucine 1078 is highly conserved among species and paralogues (insulin receptor, insulin receptor-related receptor) with only isoleucine or valine found at this position. Disturbance of the receptor’s kinase activity can be assumed but has to be shown experimentally.

![Figure 2: Genetic analysis of IGF 1R of Patient B; a novel heterozygous 3 bp deletion (c.3234_3236delCAT) resulting in one amino acid deletion (p.Ile1078del)](image)

**Discussion**

Mutations in the IGF1R gene resulting in IGF1 resistance underlie some cases of prenatal and postnatal growth failure [4]. Interestingly, there were three types of phenotypes reported. Some SGA children with IGF1R genetic alterations had microcephaly and short stature, others had only short stature without microcephaly, while some reports describe short stature and/or microcephaly and impaired glucose tolerance. Also, most reports describe elevated circulating levels of IGF1, consistent with the expectations when there is a receptor defect. It is of note that some patients were described with low normal levels of IGF1, as was the case in one of our patients with the novel heterozygous 3 bp deletion (c.3234_3236delCAT) resulting in one amino acid deletion (p.Ile1078del). There is one report that describes hypoglycemia found in a patient with a heterozygous mutation (c.94+1G > A, p.D1105E) affecting the splicing site of the IGF1R mRNA [12].

Several reports describe SGA with growth failure: a compound heterozygote for point mutations in exon 2 of the IGF1R gene bearing a p.R108Q mutation in 1 allele and a p.K115N mutation in the second [4]. Short stature and intrauterine growth retardation (IUGR) were found to be caused by a heterozygous mutation in the IGF1R gene, Arg709 to Gin (p.R709Q) [13], while the p.R481Q mutation was described in two family members with increased serum IGF-I levels and intrauterine and postnatal growth retardation [14]. Normal IGF-I, but short stature was found in a boy and several family members with a 19-nucleotide duplication within exon 18 of the IGF1R gene and consequently haploinsufficiency of IGF1R protein [15]. One of our patients had unusually low IGF1 serum concentrations (-1.5 SD), which contrasts with previous reports.

Prenatal and postnatal growth failure was found in patients with p.Y387X mutation [16] where the proband had high IGF-I levels, while he and his two paternal aunts had impaired glucose tolerance. SGA was also reported in a novel IGF1R mutation (p.Alal 40fsX20) [17], and intrauterine and postnatal growth retardation was found in a missense mutation...
Heterozygous nonsense mutations affecting the C-terminal region (p.Q1250X, p.W1249X) of IGF1R were described in two out of 55 analysed Japanese patients with SGA and growth failure [19].

A heterozygous mutation (p.C1248Y) in the IGF1R gene was found in two brothers with prenatal and postnatal growth retardation and their father [8]. It is of note that OGTT showed progressive impaired glucose tolerance, while the father was already treated for type 2 diabetes mellitus. In a child with a deletion on 15q26.2 intrauterine growth retardation, postnatal growth failure, and recurrent hypoglycemia there was only a single copy of the IGF1R gene [20].

Microcephaly is frequently associated with IGF1R genetic alterations. SGA, microcephaly, persistent postnatal growth retardation, and elevated IGF-1 levels were reported in a 15-year-old girl with heterozygous deletion of 15q26.2-qter which included the IGF1R gene [21]. The p.R59X mutation was reported in two half-brothers with primary microcephaly, mild mental retardation, and intrauterine as well as postnatal growth deficits [4] [22]. Microcephaly, pre- and postnatal growth retardation were found in patients with heterozygous missense mutations in three unrelated patients, de novo p.R1256S, de novo p.N395Y and p.Y865C [23]. Also the c.1549A > T, the p.Y487F mutation was reported in a patient with microcephaly and prenatal and postnatal growth impairment [24]. GH treatment of a patient with short stature, microcephaly, dysmorphic features, developmental delay and a terminal deletion of 15q26.2q26.3 containing the IGF1R gene in addition to a terminal duplication of the 4q35.1q35.2 region resulted in a strong growth response [25]. It is of note that the GH treatment in our patients had mixed effects. The first patient did achieve height within the parental target range, while the second one discontinued the treatment without having a catch-up growth.

The compound heterozygous mutation p.E121K/E234K was reported as the cause of intrauterine growth retardation and severe postnatal growth failure [26]. The homozygous c.119G > T (p.R10L) was shown to be associated with dysmorphic features, severe IUGR, and insulin resistance [27].

In conclusion, IGF1R gene alterations are an important and relatively frequent cause for SGA. Microcephaly with prenatal or postnatal growth failure should alert the physician on a possible IGF1R defect. Increased IGF-1 levels are also a major sign of IGF1R defects. It is of note that low normal serum IGF-1 levels have also been reported, and therefore are not an argument not to test the IGF1R gene. A precise phenotype-genotype correlation is still lacking. The GH treatment in one of the patients did result in a height gain into the parental range. However, the second patient interrupted the treatment but induced a catch-up growth.

**Statement**

Written informed consent has been obtained from each patient or subject after full explanation of the purpose and nature of all procedures used.

**References**


