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

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Abstract

BACKGROUND: Small for gestational age children (SGA) is born on term with BW and or BL of -2.0 standard deviation score (SDS). SGA children have an increased risk of being short, developing DM, and cardiovascular and cerebrovascular disease. Often defects of *IGF1R* are the cause of SGA. Most frequently affected part of the IGF1R gene is the exon 2.

AIM: To investigate whether the exon 2 of the *IGF1R* gene is affected in the SGA children.

PATIENTS AND METHODS: A cohort of 100 SGA children born in term was evaluated for alterations in *IG1R* gene. Their anthropometric parameters, IGF1 serum concentrations and IGF1 SDS values were analysed. The molecular analysis of IGF1R gene was performed by PCR restriction-site analysis and followed by direct sequencing of conspicuous fragments.

RESULTS: Within our cohort, 64 SGA children were with short stature (height SDS -3.25 ± 0.90 SDS), and 36 were with normal height for their age and sex, (H SDS was 0.20 ± 1.1 SDS). None of these children had microcephaly (occipitofrontal circumference -0.70 ± 1.01 SDS vs 0.06 ± 0.56 SDS in SGA children with normal height) or dysmorphic features. The IGF1 serum concentrations and IGF1 SDS values of all children were within normal range. Only one child had lower normal serum IGF1 concentration. No alterations in exon 2 of *IGF1R* gene were detected.

CONCLUSIONS: The genetic analysis of the exon 2 of the *IGF1R* gene did not detect any gene defects in the analysed patients. The putative genetic defect in those children affects other parts of the *IGF1R* gene or another gene (s), or yet unidentified factors.

Introduction

SGA children comprise 3% of all births. Those are the children with birth weight (BW) and/or birth length (BL) less than 2.0 standard deviation score (SDS). Only 10% of children do not attain normal height until the age of four or five years. In fact, that 10 % have a higher risk to remain short, as well as increased prone to diabetes mellitus, cerebrovascular and cardiovascular diseases in adulthood [1].

The aetiology of SGA is heterogeneous. Genetic factors are a culprit in some of them. Defects in *IGF1R* gene are reported as a cause of SGA [2] [3] [4]. The most affected part of the *IGF1R* gene is its exon 2.

Insulin-like growth factor 1 receptor (IGF1R) is a heterotetrameric (α2β2) transmembrane glycoprotein with intrinsic kinase moiety. It contains 2 alfa and two beta subunits synthetized by one mRNA precursor (α2β2). IGF-1R and IR (insulin receptor) are parts of the same family together with IGF-2R and their ligands IGF-1 and IGF-2 and at least 6 IGF-binding proteins, so-called protein kinase superfamily or tyrosine protein kinase family and insulin receptor subfamily [5] [6] [7] [8]. GF1R and IR receptors are found in skeletal muscles, heart, kidneys, fat tissue, liver, spleen, fibroblasts and placenta.

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We investigated the exon 2 of the *IGF-1R* gene, as this gene part is the often affected by nucleotide alterations. 100 SGA children were analysed.

Patients and Methods

The cohort of 100 (M:F = 40:60) children born in term (≥ 37 GW), but small for gestational age is composed of two groups: a group of 64 (M:F = 31:33) SGA born children who did not achieve catch-up growth after the 2nd year and remained short and a group of 36 (M:F = 13:23) SGA born children (older than 4 years) with normal growth spurts for their age and sex.

Anthropometric data and IGF-1 concentrations were evaluated. IGF1-BP3 was not available. Clinical birth data include children's birth weight (BW) in kilograms, BW standard deviation score (SDS), birth length (BL) in centimetres, BL SDS and gestation week (GW). Also, the height SDS, weight SDS, occipitofrontal circumference SDS, body mass index (BMI), BMI z-score and target height (TH) SDS were also analysed.

The serum concentrations of Insulin-like growth factor 1 (IGF1) were determined by chemiluminescence immune assay method on IMMULITE 2000 Siemens, Immunoassay System apparatus.

The molecular analysis of *IGF1R* gene was performed on ("Biometra"-T3 Thermocycler PCR apparatus) in Laboratory for Molecular medicine in University Children's Hospital Skopje. Start DNA material was isolated from leukocytes with high concentrated 5 M NaCl solution. This genomic DNA material of particular exon 2 was amplified using the polymerase chain reaction (PCR) to perform the restriction-site analysis. We used following primers: 5'TCGACATCCGCAACGACTATC3' as *the* forward primer and the 5'CGAAGATGACCAGGGCGTAG3' as reverse primer. PCR products were digested with *Dde* I (Sigma Aldrich*)*, and the resulting fragments were characterized by 1% agarose gel horizontal electrophoresis and staining with ethidium bromide. PCR products of coding *IGF1R* exon 2 were screened by direct sequencing of conspicuous fragments in Molecular Laboratory of Faculty of Pharmacy.

The Kolmogorov-Smirnov test (KS test) is used to check whether the values have a normal (Gaussian) distribution. A confidence interval (CI) is calculated for the mean of each of the quantities. For the comparison of 2 groups, Fisher's test is used to check the equivalence of variances. The result of this test is relevant for applying the test for equivalence of means. If the 2 samples have a size greater than 30,

a z-test is used, otherwise a t-test. All tests are with a 99% significance or α = 0.01.

Results

The mean birth weight (BW) and BW standard deviation score (BW SDS), birth length (BL) and BL SDS were: in 64 short children BW 2284.37 gr ± 433 SDS and BW SDS -2.71 ± 1.05 SDS, BL 47.06 ± 2.09 SDS and BL SDS **-**1.33 ± 1.03 SDS and in 36 children with normal height BW 2502.5 gr ± 317.8 SDS and BW SDS -2.15 ± SDS and BL 46.63 cm ± 2.12 SDS and BL SDS -1.61 ± 1.12 SDS (Table 1).

Table 1: Birth parameters: Birth weight (BW), BW SDS, birth length (BL), BL SDS and Gestation Week (GW) in 2 groups of children: a group of 64 short SGA born children and group of 36 SGA born children with normal height

Groups		Short SGA Children		SGA children with Normal height		Comparison Between Two groups
Parameters		Mean	P-value From Ks test	Mean	P-value From Ks test	P-value
BW	Gr	2284.37±433 SDS	0.18	2502.5±317.8 SDS	0.97	0.0095
BW SDS	-	-2.71±1.05 SDS	0.78	-2.15± 0.56SDS	0.48	0.0009
BL	Cm	47.06±2.09 SDS	0.56	46.63±2.12 SDS	0.87	0.33
BL SDS	-	-1.33±1.03 SDS	0.34	-1.61±1.12 SDS	0.93	0.22
GW	W	39.17±0.94 SDS	0.46	39.34±0.90 SDS	0.61	0.37
Patients	M F	N=31 N=33		N=13 N=23		

Short children had measured height SDS (H SDS) (-3.25 ± 0.90 SDS), weight SDS (W SDS) (-2.72 ± 1.39 SDS), BMI z-score (0.88 ± 1.78 SDS) and occipitofrontal circumference SDS (OFC SDS) (-0.70 ± 1.01 SDS) at the time of entrance the study. Growth parameters of children with caught up growth were: H SDS (0.20 ± 1.1 SDS), W SDS (0.29 ± 1.53 SDS), BMI z-score (0.01 ± 1.68 SDS) and OFC SDS (0.06 ± 0.56 SDS) (Table 2).

Table 2: Auxology parameters: height SDS (H SDS), weight SDS (W SDS), BMI and BMI z-score, occipitofrontal circumference SDS (OFC SDS), Target Height SDS (TH SDS) in 2 groups of children: a group of 64 short SGA born children and group of 36 SGA born children with normal height

Groups		Short SGA Children		SGA children with Normal height		Comparison Between Two groups
Parameters		Mean	P-value From Ks test	Mean	P-value From Ks test	P-value
H SDS	-	-3.25±0.90 SDS	0.19	0.20±1.1 SDS	0.62	0.024
W SDS	-	-2.72±1.39 SDS	0.45	0.29±1.53 SDS	0.65	3.30e-16
BMI	Kg/m²	15.25±2.15 SDS	0.41	17.28±4.39 SDS	0.14	0.012
BMI Z-SCORE	-	-0.88±1.78 SDS	0.31	0.01±1.68 SDS	0.70	0.015
OFC SDS	-	-0.70±1.01 SDS	0.91	0.06±0.56 SDS	0.95	3.82e-06
TH SDS	-	-1.00±0.87 SDS	0.52	0.15±0.91 SDS	0.98	6.98e-9

None of our SGA born children had dysmorphic features.

Only in one boy with short stature (-2.63 SDS) had low normal IGF1 serum concentration (52.3

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ng/ml, N = 50-286) and IGF1 SDS (-1.57 SDS) for his age and sex. The PCR restriction-site analysis in all SGA born children did not show any genetic alteration in exon 2 of *IGF1R* gene. The screened PCR products coding *IGF1R* exon 2 by direct sequencing of conspicuous fragments were uneventful.

Discussion

Several alterations (point mutations and deletions) in the *IGF1* and insulin-like growth factor 1 receptor (*IGF-1R*) genes have been demonstrated last decade. Alterations have been identified that affect IGF1R biosynthesis, signal reception and receptor kinase activity [9] [10] [11] [12] [13] [14] [15] [16] [17] [18] [19].

Homozygous defects in the *IGF1* gene have been found in patients with major developmental impairments and severe intrauterine and postnatal growth retardation. On the other hand, mutations in *IGF1R* gene that have been predominantly heterozygous are 3 found in patients with mild phenotype and clinically heterogeneous presentation [20].

Since 2003y Abuzzahab et al., [10] described 2 point mutations in exon 2 of *IGF1R* gene: the first was compound heterozygosity with the exchange of arginine by glutamine at amino acid 108 (p. R108Q) and exchange for lysine by asparagine in the amino acid 115 (p. K115N) in SGA born girl with delayed motor development, psychiatric problems and growth retardation. The authors also described another heterozygous mutation in the éxon 2 (p. R89X) in an IUGR born boy with microcephaly, short stature and delayed motor and speech development.

Kawashima et al., [11] in 2005 described missense mutation in heterozygosity at 11 éxon the *IGF1R* (p. R739Q) in a SGA born patient with significant mental retardation and postnatal growth. In SGA born 35 y old patient with microcephaly and elevated IGF1 values, Walenkamp et al., [12] 2006y found a mutation in the éxon 16 (p. E1050K). Inagaki et al., [13] 2007y found heterozygous point mutation resulting in R481Q in a girl with short stature and elevated IGF1 values. Kruis et al., 2010y [14] in 7 members of the same family with low BW, microcephaly and normal mental development, reported similar mutation which resulted in G1125A protein.

Wallborn et al., [15] found a mutation of *IGF1R* gene p. V599E in SGA born patient with microcephaly, mental retardation and elevated IGF1 levels. Fang et al., 2009 [16] reported novel heterozygous 19 nucleotides duplication within 18 éxon of *IGF1R* gene and consequently to

haploinsufficiency of IGF1R protein in 4 short statured family members with normal IGF1 levels. Mohn et al., 2011 [17] described 4 SGA family members with short stature and impaired glucose metabolism with a novel mutation (p.Tyr387X).

Labarta et al., 2013 [21] described novel heterozygous *IGF1R* missense mutation in exon 7 (c.A1549T, p.Y487F) in 3 IUGR born females from the same family with short stature and microcephaly. Juanes et al., [22] 2015 identified three novel heterozygous missense mutations in 3 patients with microcephaly and growth retardation, de novo p.Arg1256Ser, de novo p.Asn359Tyr and p.Tyr865Cys.

We investigated 100 SGA born children, 64 children were with short stature -H SDS (-3.25 ± 0.90 SDS) and 36 children with normal height -H SDS (0.20 ± 1.1 SDS). They were born with low BW SDS (-2.71 ± 1.05 SDS) vs (-2.15 ± 0.56 SDS) and/or BL SDS (-1.33 ± 1.03 SDS) vs (-1.61 ± 1.12 SDS). No alterations in exon 2 of the IGF1R gene were found.

The IGF1 serum concentration only in one patient of our cohort was in the lower normal range for his age and sex at the time of diagnosis. The defects of IGF1R usually result in elevated IGF1 serum concentrations [2] [12] [13] [15].

In conclusion, within a cohort of 100 SGA born children without microcephaly or dysmorphic features we did not find alterations in the exon 2 of the *IGF1R* gene. Exon 2 of the *IGF1R* gene might not be a hotspot for alterations. Investigating most or the whole of the *IGF1R* gene together with other genes implicated in SGA might yield an answer on the SGA cause of a particular child.

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