

Cytosine-Adenosine (CA)_n repeats polymorphism in IGF-I gene and early growth in infants born appropriate and small for gestational age

Jitka KYTNAROVA¹, Katerina VESELA¹, Blanka ZLATOHLAVKOVA², Alena DOHNALOVA³, Maria FEDOROVA², Michal KRSEK⁴ and Jiri ZEMAN¹

¹ Department of Paediatrics, Charles University in Prague; ² Department of Gynaecology and Obstetrics; ³ Department of Physiology; ⁴ Third Department of Medicine; First Faculty of Medicine, Prague, Czech Republic;

Correspondence to: Prof. Jiří Zeman, MD., PhD.
Department of Paediatrics First Faculty of Medicine, Charles University
Ke Karlovu 2, 120 00 Prague 2, Czech Republic
PHONE: +420 224967734; E-MAIL: jzem@lf1.cuni.cz

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Abstract

BACKGROUND: IGF-I gene polymorphisms might alter IGF-I level resulting in decreased foetal and postnatal growth and increased risk for diabetes mellitus type 2 and cardiovascular diseases in adulthood.

OBJECTIVES: We analyzed the association between Cytosine-Adenosine (CA)₁₀₋₂₄ repeats polymorphism in promoter region of the IGF-I gene and early growth in infants with birth weight appropriate for gestational age (AGA) and small for gestational age (SGA).

DESIGN AND METHODS: All neonates were born at term, 196 of them were AGA and 26 SGA. Blood for DNA analyses was obtained from placental part of umbilical vein. Genotyping was performed using fragment analyses of IGF-I gene promoter region. The data about postnatal growth in the group of AGA children were obtained at the age of 18 months, in SGA children at 12 months.

RESULTS: No differences in the frequency of wild type allele with (CA)₁₉ repeats and polymorphisms with (CA)_{<19} or (CA)_{>19} repeats were observed between AGA and SGA children. The average birth weight and length in AGA wild type (CA)₁₉ homozygotes were lower in comparison with AGA carriers of various (CA)_n polymorphisms but all observed anthropometric differences disappeared at the age of 18 months. In SGA children, no differences were found between number of (CA)_n repeats and anthropometric parameters both at birth and at the age of 12 months.

CONCLUSIONS: Although (CA)_n repeats polymorphism in IGF-I gene might affect prenatal growth in AGA children, our results have not shown any impact of variable number of (CA)_n repeats in IGF-I gene on postnatal growth.

Abbreviations :

(CA)_n repeats polymorphism – Cytosine-Adenosine repeats polymorphism
n – number of repeats
IGF-I – insulin-like growth factor I

CVD – cardiovascular disease
AGA – appropriate for gestational age children
SGA – small for gestational age children
SD – standard deviation
SDS – standard deviation score

INTRODUCTION

Insulin-like growth factor I (IGF-I) is important for foetal and postnatal growth (Orbak *et al.* 2001) although the predictive role of umbilical IGF-I level on postnatal growth remains controversial (Kajantie *et al.* 2003). Actual IGF-I level is influenced by genetic background and environmental factors including nutrition and plays an important role in the maintenance of insulin secretion and glucose homeostasis (Allen *et al.* 2003; Clemmons, 2004). Low IGF-I levels have been associated with an increased risk of cardiovascular disease (CVD) and cardiac muscle cell survival (Vaessen *et al.* 2001; Schut *et al.* 2003; Bleumink *et al.* 2004; Kawachi *et al.* 2005; Hunt *et al.* 2006).

Several minor genetic variations near the promoter region of IGF-I gene have been described including variable number of intronic Cytosine-Adenosine (CA)_n repeats from (CA)₁₀ to (CA)₂₄ [Arends *et al.* 2002; Vaessen *et al.* 2002]. In the Caucasian population the most frequent allele contains (CA)₁₉ repeats and corresponds to the 192-bp long fragment described in earlier studies (Vaessen *et al.* 2001, Vaessen *et al.* 2002). It was suggested that polymorphisms in IGF-I gene promoter may play a role in gene expression with negative impact on circulating IGF-I levels (Rietveld *et al.* 2003), IGF-I polymorphisms may affect fat accumulation in infants and adults and may result in accelerated weight gain during infancy, too (Voorhoeve *et al.* 2006; Landman *et al.* 2006). IGF-I levels in adult homozygotes for IGF-I polymorphisms with (CA)_{<19} or (CA)_{>19} alleles are lower in comparison with homozygotes for wild type allele (CA)₁₉ (Rietveld *et al.* 2004). In addition, the non-carriers of wild type (CA)₁₉ allele have reduced insulin sensitivity (Leen *et al.* 2004) and increased risk for myocardial infarction (Yazdanpanah *et al.* 2006). The IGF-I polymorphism (CA)₁₈ may also play a role in development of diabetic retinopathy (Rietveld *et al.* 2006).

In our study we have analyzed the frequency of variable number of Cytosine-Adenosine (CA)_n repeats in the promoter of IGF-I gene in 196 neonates with birth weight appropriate for gestational age (AGA) and 26 neonates small for gestational age (SGA) with respect to early postnatal growth. Although (CA)_n repeat polymorphism in IGF-I gene might affect prenatal growth in AGA children, our results have not shown any impact of variable number of (CA)_n repeats in IGF-I gene on early postnatal growth.

METHODS

Subjects

All children prospectively involved in the study were born between January 2006 and April 2007. Exclusion criteria were: multiple pregnancies, chromosomal aberrations, genetic syndromes, long-lasting corticosteroid therapy, severe neurological impairment and other serious diseases that may have affected growth and weight gain.

Appropriate for gestational age (AGA) children

200 newborns born at term (> 37 weeks, <42 weeks) with appropriate birth length and weight for gestational age (AGA), that means birth weight and length between ± 2 SDS (standard deviation score) according to Czech standards (Kobzová *et al.* 2004) were enrolled to the study. In four children genotyping from blood sample failed, so they had to be excluded from the study.

In 196 children (104 boys and 92 girls) the birth weight and birth length were measured at the delivery room using standard medical devices. The length, weight and head circumference were obtained at the age of 18 months as a part of obligatory well-child (preventive) visit in 164 children (83%).

Small for gestational age (SGA) children

26 children born small for gestational age (SGA), that means with birth length and/or weight below 2 SDS (Lee *et al.* 2003) were involved in the study and were followed up in regular intervals for twelve months (6 months, 12 months). 17 (65%) of them had intrauterine growth retardation documented at least by two prenatal ultrasound examinations. Length and weight were measured in a standardised way.

Genotyping methods

Blood for genomic DNA was obtained after the birth from placental part of umbilical vein. Cytosine-Adenosine (CA)_n repeats polymorphisms in IGF1 gene were analyzed (OMIM: 147440; GeneBank: NT_019546, complement (26271866-26356514). The (CA)_n sequence is at the 5' end of non-coding promoter region and ends by the 967th bp ahead of the start codon of transcripts variants NM_000618, NM_001111283 and NM_001111285 (NM_000618:c.1-967(CA)11-22). The fragment containing polymorphism was amplified by PCR using 50 ng of DNA, primers (forward: 5' ctggcacacagactcctct 3', reverse: 5' 6-FAMaccactctgggagaaggga 3') and Plain Combi PPP Master Mix (by Top-Bio s.r.o.). Reverse primer was marked by 6-FAM fluorescent dye. Mixture containing 1 μ l of PCR product, 0.5 μ l of size standard (GeneScan-500ROX; Applied Biosystems) and 8.5 μ l of formamide (Hi-Di formamide – Applied Biosystems) was analyzed on gel capillary electrophoresis system (ABI 3100 Avant; Applied Biosystems). Results were analyzed by GeneMapper Software version 3.5 (Applied Biosystems).

Ethics

The Medical Ethics Committee of 1st Medical Faculty of Charles University, Prague, approved the study. Written informed consent was obtained from parents of all participating infants.

Statistical analysis

The association between Cytosine-Adenosine (CA)₁₁₋₂₃ repeats polymorphism in IGF-I gene and anthropometric parameters including weight, length and head cir-

cumference expressed as a mean \pm SD at birth and at the age of 18 and 12 months respectively was analyzed by the SPSS software version 13.0. Statistical significance for continuous variables was evaluated using analysis of variance (ANOVA) and t-test for two independent samplings. Pearson χ^2 test or Fisher exact test were used for analysis of categorical variables.

RESULTS

In our study, the variable number of Cytosine-Adenosine (CA)_n repeats in IGF-I gene ranged in size from (CA)₁₁ to (CA)₂₃. Polymorphisms (CA)₁₀ and (CA)₂₄ were not found. The distribution of IGF-I polymorphisms in AGA and SGA children is shown in Table 1. No significant differences in genotype distribution were found between SGA and AGA children.

Although in the group of AGA children, the homozygotes for wild type allele with (CA)₁₉ repeats in IGF-I gene had birth weight and length lower in comparison with the carriers of (CA)_{<19} or (CA)_{>19} polymorphisms on one or both alleles (Figures 1 and 2), these differences disappeared during infancy and were no more present at the age of 18 months. No differences were also found in the group of SGA children both at birth and at the age of 12 months.

DISCUSSION

Children born small for gestational age (SGA) or with IUGR are at increased risk for development of insulin resistance, diabetes mellitus and/or cardiovascular disease (CVD) in adulthood. It seems that most endangered are children without postnatal catch-up of growth and children with rapid weight gain after birth. It may be explained by genetic background and altered IGF-I level and insulin sensitivity (Leen *et al.* 2004).

Animal study with knock-out mice demonstrated the role of IGF-I in pre- and postnatal growth (Liu *et al.* 1993). In human, the homozygous deletion of IGF-I gene was identified in a 15 years old boy with intrauterine growth retardation (IUGR) and postnatal growth failure (Woods *et al.* 1996). Also, the second described patient homozygous for mutation in the IGF-I gene had IUGR and postnatal growth failure (Bonapace *et al.* 2003). In addition, both children had mental retardation and deafness.

IGF-I polymorphism with variable number of Cytosine-Adenosine (CA)_n repeats in the promoter region of the gene was also recognized as the link to weight pattern in infancy (Landman *et al.* 2006). Most studies of the impact of polymorphisms in IGF-I gene on anthropometric parameters at birth were done in adulthood and the data about birth weight and length were obtained retrospectively (Frayling *et al.* 2002; Vaessen *et al.* 2002; Velde *et al.* 2005). The impact of (CA)_n repeats polymorphism on linear growth in pre- and early postnatal period is still unclear. The results of several previous studies have remained controversial (Vaessen *et al.* 2002; Velde *et al.* 2005; Landman *et al.* 2006; Ester & Hokken-Koelega, 2008). For example in the group of Dutch adults, the IGF-I genotype was significantly associated with birth size and the non-carriers of wild type allele had lower birth weight (Vaessen *et al.* 2002; Velde *et al.* 2005). In a recent study described by Geelhoed *et al.* (2008), early foetal growth of homozygotes for wild type allele with (CA)₁₉ repeats in the IGF-I gene promoter is delayed in the middle of gestation in comparison to fetuses with (CA)_n repeats polymorphisms, but these differences consequently vanished with subsequent foetal and postnatal growth.

On the contrary, in two studies realized in the United Kingdom (Frayling *et al.* 2002) and Germany (Landman *et al.* 2006) the association between (CA)_n repeats poly-

Table 1. The frequency of Cytosine-Adenosine (CA)₁₁₋₂₃ repeats polymorphism in the promoter of IGF-I gene in 196 children with birth weight appropriate (AGA) and 26 children born small (SGA) for gestational age.

Group	Cytosine-Adenosine (CA) ₁₁₋₂₃ repeats polymorphism in IGF-I gene						
	(CA) ₁₉ / (CA) ₁₉		(CA) ₁₉ / (CA) _{<19} or (CA) _{>19}		no (CA) ₁₉ allele		Total
	n	%	n	%	n	%	
AGA children	89	45.4	86	43.9	21	10.7	196
SGA children	15	57.7	9	34.6	2	7.7	26
Total	104	46.8	95	42.8	23	10.4	222

(CA)₁₉ / (CA)₁₉ homozygotes for wild type allele with (CA)₁₉ repeats in the promoter of IGF-I gene

(CA)₁₉ / (CA)₁₁₋₁₈ or (CA)₁₉ / (CA)₂₀₋₂₃ heterozygotes for one wild type allele with (CA)₁₉ repeats in the promoter of IGF-I gene and one (CA)_n repeats polymorphism on the other allele

no (CA)₁₉ allele non-carriers of wild type allele (CA)₁₉ (children with (CA)₁₁₋₁₈ or (CA)₂₀₋₂₃ repeats polymorphisms on both alleles in the promoter of IGF-I gene)

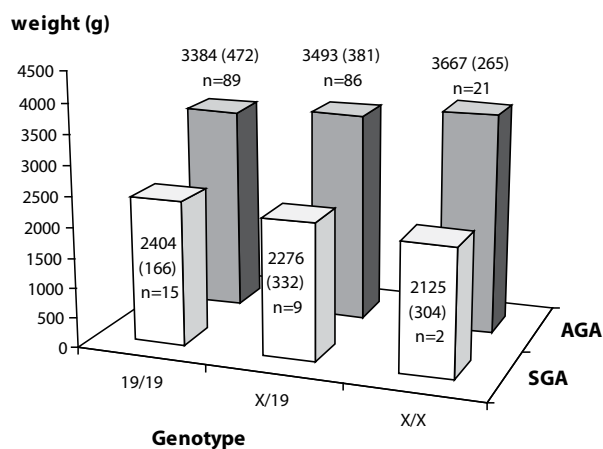


Figure 1. Birth weight of children appropriate for gestational age (AGA) and small for gestational age (SGA) in the relation to the Cytosine-Adenosine (CA)₁₁₋₂₃ repeats polymorphism in the promoter of IGF-I gene
 19/19 children homozygous for wild-type allele with (CA)₁₉ repeats in the promoter of IGF-I gene
 19/X compound heterozygous children with one wild-type allele with (CA)₁₉ repeats in the promoter of IGF-I gene and one (CA)_{<19} or (CA)_{>19} repeats polymorphism on the other allele
 X/X children with (CA)_{<19} or (CA)_{>19} repeats polymorphisms on both alleles in the promoter of IGF-I gene: non-carriers of wild type allele (CA)₁₉
 All variables are expressed as means ± standard deviation (SD).

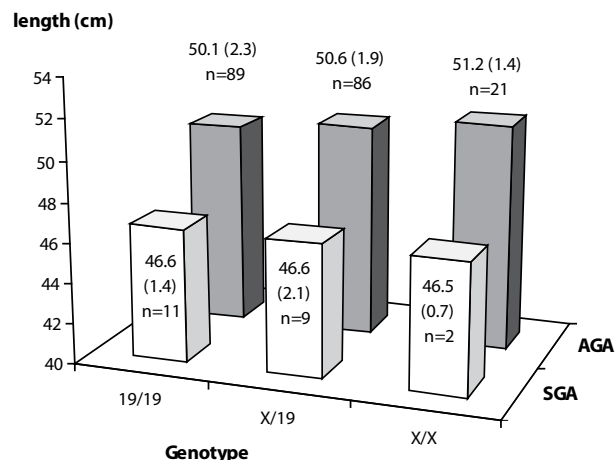


Figure 2. Birth length of children appropriate for gestational age (AGA) and small for gestational age (SGA) in the relation to the Cytosine-Adenosine (CA)₁₁₋₂₃ repeats polymorphism in the promoter of IGF-I gene
 19/19 children homozygous for wild-type allele with (CA)₁₉ repeats in the promoter of IGF-I gene
 19/X compound heterozygous children with one wild-type allele with (CA)₁₉ repeats in the promoter of IGF-I gene and one (CA)_{<19} or (CA)_{>19} repeats polymorphism on the other allele
 X/X children with (CA)_{<19} or (CA)_{>19} repeats polymorphisms on both alleles in the promoter of IGF-I gene: non-carriers of wild type allele (CA)₁₉
 All variables are expressed as means ± standard deviation (SD).

morphism and birth weight and length was not found. The published studies in children and adults differ significantly in the decade of birth of investigated subject. Described differences in the results between studies may be at least partly explained by gradual change of the lifestyle and nutritional habits during last 50 years in various populations with the impact on IGF-I levels. Epigenetic factors may from a great extent affect or even override the role of heredity. In our group of AGA children of Slavonic origine, the IGF-I homozygotes for wild type allele with (CA)₁₉ repeats had birth weight and length lower in comparison with the carriers of IGF-I polymorphisms, but these differences were no more present at the age of 18 months. In addition, we also did not found any significant differences in the group of SGA children both at birth and at the age of 12 months.

The level of IGF-I may be suboptimal both in heterozygotes for increased and for decreased number of Cytosine-Adenosine (CA)_n repeats in IGF-I gene (Rietveld *et al.* 2004) probably regardless of different number of missing (CA)_{<19} repeats or additional (CA)_{>19} repeats.

In conclusion, it seems that (CA)_n repeat polymorphism in IGF-I gene might affect prenatal growth in AGA children, absence of significant differences in SGA children in the present study may indicate strong impact of environmental factors especially on postnatal

growth. Further studies will be necessary to extend the number of investigated SGA children, but of particular importance will be the question concerning the potential role of various combined polymorphisms in IGF-I gene on the foetal and postnatal growth and metabolic consequences.

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