The importance of investigation of SHOX gene deletions in etiological diagnosis of short stature; genotype-phenotype correlations

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The process of growth is determined by various environmental and genetic factors. To date, many different etiologies of short stature are known and more than two hundred genes underlying growth control have been identified.

The isolated haploinsufficiency of the *SHOX* gene (SHOX, MIM 312865) is the most common cause of short stature determined by monogenic mutations. The gene was described almost at the same time in 1997 by two different research groups, the Rappold-led German working group and the US based Ellison and co-workers, as a gene responsible for the short stature in Turner syndrome patients. Further studies have shown that it is localized in the pseudoautosomal region 1 of the sex chromosomes and encodes a transcription factor implicated in skeletal development and human growth.

The heterozygous deviation of the gene can be detected in 2-15% of patients with idiopathic short stature (ISS), in 50-90% of patients with Leri-Weill Dyschondrosteosis syndrome (LWS), and in almost 100% of patients with Turner syndrome.

The *SHOX* gene is located in the pseudoautosomal region 1 (PAR1) of the short arm of the chromosomes X and Y (Xp22.33, Yp11.32). PAR1 genes do not undergo X inactivation and 2 copies of the *SHOX* gene are expressed, one from each of the sex chromosomes. The *SHOX* gene encodes a transcription factor expressed during human embryogenesis in the pharyngeal arches and developing limbs. It plays a fundamental role in chondrocyte function in the growth plate, as a regulator of cellular proliferation and differentiation. Isolated SHOX deficiency leads to a variety of different skeletal phenotypes and clinical conditions.

The multiple ligation-dependent probe amplification (MLPA) is currently the first recommended molecular biological method used for the detection of deletions that occur in the SHOX gene and can be used to detect PAR1 deletions and duplications including SHOX and the downstream enhancer region.

The first study of the thesis showed the usefulness of the MLPA technique which was performed in the Laboratory of Endocrine Genetics of the Semmelweis University of Budapest, Hungary, using the SALSA P018 MLPA kit (MRC-Holland, Amsterdam, The Netherlands), in accordance with the manufacturer's protocol. About 100 ng of genomic DNA was used for the test. Data normalization and data analysis were performed according to the manufacturer's recommendation. All received data were aggregated in excel spreadsheets on which we carried out the block normalization and we calculated the hypothetical patients and control subjects quotient gene-specific intensities measurements in each sample. The MLPA is currently the

first recommended molecular method for the detection of deletions of *SHOX* gene, having a rate of 70-75% success. It is a simple method that permits the concomitant analysis of wide number of samples and can be applied for detection of PAR1 deletions and duplications including *SHOX* and the downstream enhancer region. It also permits the evaluation of the extension of the deletion. MLPA is more sensitive, less expensive and less time consuming than other techniques (eg. FISH technique, or microsatellite analysis).

The aim of the second study was to evaluate the frequency of *SHOX* gene haploinsufficiency in children with ISS, LWS and in patients having Turner syndrome phenotype, but normal karyotype, and to identify the dysmorphic signs characteristic for *SHOX* gene deficiency. 11 of the 144 analyzed patients showed *SHOX* gene deficiency with female dominance (9). There were no statistically significant differences between the mean age, mean height and auxological measurements (sitting height/height, arm span/height) between the SHOX-positive and SHOX-negative patients. The *SHOX* positive patients had a significantly higher BMI and presented more frequent dysmorphic signs. Madelung deformity of the upper limbs was also significantly more frequent among the *SHOX*-positive patients. Regarding the phenotype-genotype correlations, we have not found any evidence of deletions of certain exons correlated with phenotypic characteristics: we found several patients with the same type of deletion but with a variable phenotype from small stature with minimal dysmorphic signs, to phenotype with multiple DLW specific signs. We found, however, correlations between the extension of exon deletions and the associated phenotype: fewer dysmorphic signs were observed when single exon deletion was observed, compared to multiple deletions of SHOX gene exons. In the partial deletion of the SHOX gene, ogiva palatina and short forearms were more common, while in the complete deletions the Madelung deformity, the pathological sitting height/height ratio and arm span/height ratio occurred more often. The incidence of deletions was similar in the two randomly investigated populations (5,5% in Romanians and 7,93% in Hungarians). All the deletions were de novo forms.

In the third study we presented the clinical case of an 11-year-old girl diagnosed with idiopathic short stature. Multiplex Ligation-dependent Probe Amplification technique for identification of *SHOX gene haploinsufficiency* revealed a heterozygous deletion spanning exons 4-5 of SHOX gene. This case indicates the necessity of screening for SHOX deletions in patients diagnosed with idiopathic short stature, especially in children having increased sitting height-to-height ratio or decreased extremities-to-trunk ratio.

We concluded that molecular genetic examination is justified for short stature patients who present typical clinical symptoms of SHOX phenotype, or suffer of idiopathic short stature. Similarly, it is indicated in patients with Turner syndrome-like phenotype, in Leri-Weill Dyschondrosteosis syndrome, as well as in Langer mesomelic dysplasia.