Current Trends in Endocrinology

Mini-Review

The role of the SHOX gene in the development of short stature: an overview of clinical and molecular evaluation

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ABSTRACT

SHOX gene (short stature homeobox-containing gene) deficiency is related to a diversity of clinical conditions such as Leri-Weill dyschondrosteosis, Turner syndrome and idiopathic short stature, all characterized by growth failure. SHOX haploinsufficiency shows a spectrum of phenotypes whose clinical expression is variable and becomes more pronounced with age and is more severe in females. Phenotypic heterogeneity is observed in patients with haploinsufficiency of SHOX where short stature is the main clinical feature linked to the skeletal abnormalities. The SHOX gene is located in the telomeric PAR1 region in the short arm of both X and Y chromosomes and escapes X inactivation, and as a consequence its mutations and microdeletions exert a dosage effect. SHOX is expressed at its highest levels in bone morphogenic tissue, and encodes a homeodomain protein, which acts as a transcription factor known to be involved in key regulatory and development processes. SHOX defects, mutations, duplications, and most importantly deletions involving SHOX exons and/or the cis-acting enhancers, have been detected in 50-90% of patients with Leri-Weill dyschondrosteosis, in almost 100% of girls with Turner syndrome, and in 2-15% of individuals with idiopathic short stature. The growth-promoting effect of growth hormone (GH) therapy may improve final adult height, as its efficacy seems to be similar to that observed in Turner syndrome.

KEYWORDS: SHOX gene, haploinsufficiency, short stature, Leri-Weill dyschondrosteosis, Madelung deformity, Turner syndrome

INTRODUCTION

The SHOX gene (OMIM 312865) is most strongly expressed in bone marrow fibroblast, implying that SHOX plays a positive role in human skelotogenesis and thus in final height determination. SHOX haploinsufficiency results in idiopathic short stature and Léri-Weill dyschondrosteosis and is associated with the short stature of patients with Turner syndrome.

SHOX gene structure

Growth is a complex, multifactorial process that is regulated by environmental and genetic factors. A critical gene involved in growth determination is located in the major pseudoautosomal region (PAR1) of sex chromosomes (Xp22.3 and Yp11.3). Deletions in PAR1 implicating SHOX gene haploinsufficiency were first related to short stature and Léri-Weill syndrome. Also, subtle distal deletion in Xpter or Ypter was subsequently demonstrated in patients with idiopathic short stature or in patients with Turner syndrome [1]. Since X inactivation is not complete for several loci in Xp22.3, SHOX is expressed on the inactive as well as the active X and Y chromosomes. For this reason, genes on PAR1 are presented in two active copies indicating a dosage effect of the SHOX gene in cases with aberrations in sex chromosomes. Therefore, the short stature phenotypes result as a consequence of haploinsufficiency of the critical short stature gene [2].

During the early foetal life, SHOX gene expression is present in the limb regions of radius, ulna, wrist, tibia, fibula, knee and ankle. This expression

pattern is in accordance with the short stature phenotype and skeletal dysmorphisms that have been shown by patients with SHOX gene defects and Turner syndrome.

To identify the critical gene, mapping studies narrowed the short stature interval covering the furthest 700 kb portion of PAR1. Rao and colleagues narrowed this region to 270 kb, and by genotype-phenotype correlations of patients with deletion of PAR1 they identified the growth gene in a 170 kb fragment. This gene was named short stature homeobox-containing gene or SHOX [3, 4]. Other authors reported one identical gene, and they named the gene PHOG, for pseudoautosomal homeobox-containing osteogenic gene [5].

The SHOX gene shows strong conservation throughout evolution with an important role in skeletal development. SHOX encodes a homeodomain protein that has been shown to act as a transcription factor, and SHOX product is likely to be a key regulator of growth [6]. The gene SHOX consists of six exons which encode two differentially spliced mRNA, SHOXa and SHOXb. The exon 1 is non-coding, and exon 2 is the first coding exon which contains the ATG initiation codon, and resides within a CpG island. The homeobox sequence is located in exon III and exon IV. The isoforms SHOXa and SHOXb have identical N-terminal sequences but differ in the C-terminal. Sequences from exons 1-5 are similar, but there are differences in the exon 6. The SHOX protein is located in the nucleus and binds to DNA in a sequence-specific manner, and acts by its C-terminal transactivation domain as a transcriptional activator in osteogenic cell lines. Studies of the activity of truncated SHOX protein were the evidence of short stature phenotype at molecular level. SHOXb isoform is inactive as a transcriptional activator and may act as a transcription modulator of SHOXa activity.

Although the two isoforms have different patterns of expression, both are expressed at the highest levels in bone marrow fibroblasts.

SHOX gene deficiency: Phenotypic expression

Molecular defects of SHOX show a spectrum of phenotypes whose clinical severity is variable [7]. Short stature is the main clinical feature associated with skeletal abnormalities. The prevalence of SHOX mutations in individuals with Léri-Weill dyschondrosteosis (LWD) is around 50-90% [8]. The loss of both SHOX alleles causes the complete absence of SHOX and a severe phenotype of short stature and dysmorphic features in Langer mesomelic dysplasia (OMIM 249700). SHOX haploinsufficiency is also responsible for the short stature and skeletal abnormalities in almost 100% of Turner syndrome. The prevalence in children with idiopathic short stature (ISS) ranges from 2-15% [9]. In contrast, females or males with an extra sex chromosome as 47,XXX; 47,XXY; 47,XYY with three copies of the SHOX gene are taller than normal 46,XX females or 46,XY males. The gain of one or more copies of SHOX due to structural aberrations of X-chromosome can be associated with high stature [10, 11]. In light of these cases it would seem that height could be associated with the dosage of the SHOX gene.

Variation in properties of the SHOX protein could explain the phenotypic heterogeneity observed in patients with haploinsufficiency of SHOX**.** One explanation for the variability could be the presence of modifier genes, intragenic mutations that have not been identified, or cases of epigenetic effects.

Moreover, it was suggested that oestrogens exert a maturation effect on skeletal tissues that are susceptible to premature fusion of growth plates facilitating the development of skeletal lesions in females with LWD [12]. It is known that one function of SHOX is to act as a repressor of growth plate fusion and skeletal maturation in the distal limbs, counteracting the maturation effect of oestrogens on skeletal tissue.

Diagnosis of patients with SHOX deficiency

Patients with isolated short stature and patients with any skeletal dysmorphisms such as Madelung deformity, mesomelia, cubitus valgus, genu valgum, and in addition, short fourth metacarpals and other related skeletal features should be investigated by molecular analysis for the presence of SHOX mutations [13]. The main indicators of a SHOX gene defect are short stature, family history of Léri-Weill syndrome or Langer syndrome, Madelung deformity, disproportionate limbs such as short forearms, short legs, and dysmorphic signs like

distal limbs, high arched palate, and radiological abnormalities.

To determine the nature of the short stature, auxological and radiological observations are collected, followed by biochemical analysis, including karyotype study in lymphocytes cultures. By high resolution and G-bands by trypsin using Giemsa (GTG) banding it is possible to demonstrate an important SHOX deletion. Fluorescence *in situ* hybridization (FISH) analysis can be used to demonstrate SHOX microdeletion [14].

Molecular basis of SHOX haploinsufficiency

Various clinically significant missense and nonsense mutations as well as nucleotide deletions or insertions have been identified within exons 2,3,4,5 and 6a of the SHOX gene [12].

Deletions are the most frequent defects found because PAR1 region is rich in repetitive DNA sequences with a high frequency of recombination at meiosis I. However, it was seen that the size of the deletion does not correlate with the severity of the phenotype. The most frequent mutations are nonsense mutations which are found throughout the gene. R195X isolated in both ISS and LWD accounts for 20% of all nonsense mutations identified. This mutation leads to the expression of a non-functional truncated protein where the highly conserved C-terminal region is missing. In addition missence mutations have also been identified in the highly conserved homeobox [15].

Comparative genomic analysis has identified evolutionarily conserved non-coding DNA elements (CNEs) defined as enhancers, which can act upstream and downstream of the SHOX gene [16, 17]. To date, 7 CNEs with cis-regulatory activity have been identified in PAR1. A few microduplications, and particularly microdeletions in PAR1 involving SHOX exons and/or cis-acting enhancer elements, most in downstream flanking regions of SHOX, have been identified in LWD and ISS patients [18, 19].

Genetic testing of SHOX mutations

Several techniques have been used to identify abnormalities of SHOX gene. The single strand conformation polymorphism (SSCP) analysis has been applied to identify many of the mutations, but it is not as sensitive as other methods. To evaluate large deletions, the analysis of the intragenic SHOX microsatellites such as the CA repeated marker located in the 5´ UTR in exon 1 of the SHOX gene, and telomere proximal markers (DXYS233, DXYS234 among others), have proved to be useful for the detection of SHOX haploinsufficiency. Samples are evaluated for heterozygosity or homozygosity of these polymorphic markers which flank the gene.

Laboratory diagnosis involves many steps. At first chromosome analysis is performed to search for a deletion of the PAR1region. Fluorescence *in situ* hybridation (FISH) can be used to identify a SHOX deffect in patients and families with clinical signs of classical LWD. The MLPA (multiplex ligation dependent probe amplification) is a high sensitive method which enables the detection of small deletions of the SHOX gene. In cases in which the deletion is not found the entire gene should be sequenced [20].

Treatment of short stature

The molecular diagnosis of SHOX gene in children with ISS or LWD has therapeutic implications [21]. Growth hormone treatment of short stature due to Turner syndrome is well established, and considering the common etiology of short stature in patients with SHOX deficiency, this treatment is also suggested for patients with ISS and LWD [22, 23]. The growth-promoting effect associated with recombinant human growth hormone (rhGH) therapy for individuals with mutations or deletions of SHOX seems to be similar to the effect achieved in Turner syndrome. Although the treatment improves the growth of these children and their final adult height, management protocols have not been established. Recently, it has been demonstrated that the effect of 24 months of rhGH therapy is effective for the disproportionate short stature of patients with SHOX haploinsufficiency [24]. Growth hormone treatment seems to be beneficial for cases with SHOX haploinsufficiency, although the long-term outcomes of this therapy require a proper control.

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CONFLICT OF INTEREST STATEMENT

The author has nothing to disclose.

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