SHOX Haploinsufficiency as a Cause of Syndromic and Nonsyndromic Short Stature

Maki Fukami^a Atsuhito Seki^b Tsutomu Ogata^{a, c}

^aDepartment of Molecular Endocrinology, National Research Institute for Child Health and Development, and ^bDepartment of Orthopedic Surgery, National Center for Child Health and Development, Tokyo, and

^cDepartment of Pediatrics, Hamamatsu University School of Medicine, Hamamatsu, Japan

Key Words

Bone · Léri-Weill syndrome · Mutation · Pseudoautosomal region · Short stature · Skeletal deformity · Turner syndrome

Abstract

SHOX in the short arm pseudoautosomal region (PAR1) of sex chromosomes is one of the major growth genes in humans. *SHOX* haploinsufficiency results in idiopathic short stature and Léri-Weill dyschondrosteosis and is associated with the short stature of patients with Turner syndrome. The *SHOX* protein likely controls chondrocyte apoptosis by regulating multiple target genes including *BNP*, *Fgfr3*, *Agc1*, and *Ctgf*. *SHOX* haploinsufficiency frequently results from deletions and duplications in PAR1 involving *SHOX* exons and/or the *cis*-acting enhancers, while exonic point mutations account for a small percentage of cases. The clinical severity of *SHOX* haploinsufficiency reflects hormonal conditions rather than mutation types. Growth hormone treatment seems to be beneficial for cases with *SHOX* haploinsufficiency, although the long-term outcomes of this therapy require confirmation. Future challenges in *SHOX* research include elucidating its precise function in the developing limbs, identifying additional *cis*-acting enhancers, and determining optimal therapeutic strategies for patients.

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In 1997, *SHOX* was reported as the causative gene for short stature in Turner syndrome [Rao et al., 1997]. Subsequently, heterozygous mutations of *SHOX* have been identified in patients with nonsyndromic short stature (idiopathic short stature, ISS) and Léri-Weill dyschondrosteosis (LWD) [Rao et al., 1997; Belin et al., 1998; Shears et al., 1998; Rappold et al., 2002]. Furthermore, *SHOX* abnormalities have been associated with various skeletal features of Turner syndrome such as scoliosis, high-arched palate, and micrognathia [Kosho et al., 1999; Binder, 2011]. Genetic defects leading to *SHOX* haploinsufficiency include intragenic mutations and deletions as well as copy number variations (CNVs) in the gene-flanking regions that possibly affect *cis*-regulatory machinery. This article provides an overview of the current understanding of *SHOX*.

The *SHOX* Gene

SHOX is located in the short arm pseudoautosomal region (PAR1) of the X and Y chromosomes (fig. 1). *SHOX* consists of exons 1–5 and alternatively spliced exons 6a and b (fig. 2) [Rao et al., 1997]. Like other genes in PAR1, *SHOX* escapes X-inactivation and therefore is

Fig. 1. Genomic structure of *SHOX* and its putative enhancer regions. *SHOX* (red box) is located in PAR1 of sex chromosomes. Previous studies identified 7 highly evolutionarily conserved noncoding DNA elements (CNEs) with *cis*-regulatory activity (blue boxes). These elements were designated as CNE-5, -3, -2, 4, 5, and 9 [Chen et al., 2009; Durand et al., 2010]; evolutionarily conserved region (ECR) 1 [Benito-Sanz et al., 2012b]; and evolutionarily conserved sequence (ECS) 4 [Fukami et al., 2006]. The horizontal line indicates the physical distance from the Xp/Yp telomere (hg 19, build 37).

Fig. 2. A, B Structural comparison among SHOXa, SHOX2a, and SHOXb. All of these proteins contain a homeodomain, while an OAR domain is present only in SHOXa and SHOX2a.

Table 1. Frequency of *SHOX* abnormalities in patients with idiopathic short stature, bilateral Madelung deformity, or LWD

Report	Methods for mutation screening	Ethnic origin	Patient	Frequency of <i>SHOX</i> abnormality
Binder et al. [2000]	SSCP and microsatellite genotyping	not described	ISS	1/68 (1.5)
Rappold et al. [2002]	SSCP	Japanese, German,	ISS	9/750 (1.2)
	FISH	Greek		3/150 (2.0)
Flanagan et al. [2002]	FISH and direct sequencing	not described	BMD	12/18 (66.7)
Schneider et al. [2005]	SNP/microsatellite genotyping and FISH	international	LWD	40/118 (33.9)
Huber et al. [2006]	SNP/microsatellite marker genotyping and direct sequencing	French	LWD	42/56 (75.0)
			ISS	12/84 (14.3)
Benito-Sanz et al. [2006]	SNP/microsatellite genotyping and MLPA	Spanish	LWD	16/26 (61.5)
Gatta et al. [2007]	MLPA and direct sequencing	not described	LWD	7/15 (46.7)
Jorge et al. [2007]	Southern blotting, microsatellite marker genotyping, FISH, and direct sequencing	not described	LWD	8/9 (88.9)
			ISS ^a	2/63 (3.2)
			DSS	2/9 (22.2)
Fukami et al. [2008]	MLPA and direct sequencing	Japanese	LWD	26/29 (89.7)
Chen et al. [2009]	SNP/microsatellite marker genotyping, FISH and MLPA	international	LWD	29/58 (50.0)
			ISS	31/735 (4.2)
Funari et al. [2010]	MLPA	Brazilian	LWD	8/8 (100)
			DSS	4/36 (11.1)
Benito-Sanz et al. [2011]	MLPA	not described	LWD	9/122 (7.4) ^b
			ISS	6/613 (0.9) ^b
Benito-Sanz et al. [2012b]	MLPA	international	LWD	19/124 (15.3) ^c
			ISS	11/576 (1.9) ^c
Rosilio et al. [2012]	SNP genotyping and direct sequencing	French	LWD	87/178 (48.9)
			ISS ^a	49/290 (16.9)
			DSS	13/69 (18.8)
Hirschfeldova et al. [2012]	MLPA and direct sequencing	not described	LWD	11/16 (68.7)
			ISS	6/51 (11.8)
			DSS	6/6 (100)
Bunyan et al. [2013]	MLPA and direct sequencing	not described	LWD	88/132 (66.7)
Sandoval et al. [2014]	MLPA	Colombian	ISS	5/62 (8.1)
Poggi et al. [2015]	MLPA	Chilean	LWD	18/27 (66.7)
			ISS	4/18 (22.2)

BMD = Bilateral Madelung deformity; DSS = disproportionate short stature; ISS = idiopathic short stature; SSCP = single-strand conformation polymorphism. Percentages are given in parentheses.

^a This patient cohort included both proportionate and disproportionate short stature. ^b This study focused on copy number gain of *SHOX*. ^c This study focused on *SHOX* downstream deletion.

present in 2 active forms in both males and females [Rao et al., 1997]. *SHOX* is expressed in the developing limbs and pharyngeal arches in human embryos and likely regulates differentiation and proliferation of chondrocytes [Clement-Jones et al., 2000]. Loss-of-function mutations of *SHOX* affect skeletal growth in a dose-dependent manner.

SHOX haploinsufficiency underlies the short stature of Turner syndrome patients and is associated with ISS and LWD. *SHOX* haploinsufficiency is estimated to account for 2–3% of ISS cases and ~70% of LWD cases. Although the frequency of *SHOX* mutations and deletions in previously reported ISS and LWD cases varied from 1.5 to 16.9% and from 33.9 to 100%, respectively (table 1), this may reflect the differences in methods of mutation screening and inclusion criteria of participants. *SHOX* nullizy-

gosity leads to Langer mesomelic dysplasia, an extremely rare condition characterized by severe short stature and skeletal deformity [Shears et al., 2002; Zinn et al., 2002]. Thus, *SHOX* is one of the major growth genes in humans. Although *SHOX* is located in the sex chromosomes, *SHOX* haploinsufficiency follows an autosomal dominant inheritance pattern. This phenomenon is defined as pseudoautosomal dominant inheritance [Shears et al., 2002]. Kant et al. [2011] demonstrated that heterozygous *SHOX* mutations can be transferred from the Y chromosome to the X chromosome and vice versa ('the jumping *SHOX* gene'). Overdosage of *SHOX* has been implicated in the tall stature of individuals with 47,XXY (Klinefelter syndrome) or 47,XXX karyotypes (triple-X syndrome). Furthermore, trisomy of PAR1 involving *SHOX* was observed in a female with tall stature [Ogata et al., 2001a,

2002]. However, microduplications involving only small genomic intervals around *SHOX* have been identified in a few patients with ISS and LWD [Iughetti et al., 2010; Benito-Sanz et al., 2011; Fukami et al., 2015]. The mechanism of such diverse effects of *SHOX* overdosage on stature is discussed below.

A paralog of *SHOX*, *SHOX2*, is located in chromosome 3 [Blaschke et al., 1998]. *SHOX2* and *SHOX* protein structures share significant similarity (fig. 2). Although the precise function of *SHOX2* remains to be clarified, previous studies have suggested that *SHOX2* might play a role in the development of cardiac and neurological systems [Espinoza-Lewis et al., 2009; Rosin et al., 2015]. Thus, *SHOX* and *SHOX2* likely have distinct functions, although both proteins may be involved in the skeletal growth [Blaschke and Rappold, 2006; Bobick and Cobb, 2012].

The *SHOX* Protein

The *SHOX* protein contains a homeodomain, a structure frequently seen in transcription factors involved in body patterning [Izpisua-Belmonte and Duboule, 1992]. *SHOX* likely controls chondrocyte development by transactivating multiple target genes. In vitro assays demonstrated that *SHOX* induces oxidative stress in osteosarcoma cells and causes lysosomal membrane rupture to release active cathepsin B to the cytosol [Hristov et al., 2014]. Thus, *SHOX* appears to regulate the cell death process of chondrocytes in the growth plate. Since there is no *SHOX* ortholog in rodents, a knockout mouse approach cannot be applied to study the function of *SHOX*. Previous studies have utilized in vitro analysis and in vivo assays using chick micromass culture to identify putative target genes of *SHOX* [Tiecke et al., 2006; Aza-Carmona et al., 2011; Decker et al., 2011; Durand et al., 2012]. These studies suggested that *SHOX* exerts positive and negative effects on the expression of *BNP* and *Fgfr3*, respectively [Marchini et al., 2007; Decker et al., 2011]. In addition, *SHOX* interacts with the SOX trio, i.e. SOX5, SOX6, and SOX9, which function as the major chondrogenic factors, and thereby activates the enhancer of *Agc1*, a gene encoding the major component of cartilage [Aza-Carmona et al., 2011]. Moreover, *HOXA9* has been reported as a regulator of *SHOX* [Durand et al., 2012]. Recently, Beiser et al. [2014] generated transgenic mice in which human *SHOX* is expressed under the control of the murine *Col2a1* promoter and enhancer. The transgenic mice manifested no remarkable phenotypes, possibly because

of low expression levels of *SHOX* in skeletal tissues. Nevertheless, detailed molecular analysis of the mice suggested that *SHOX* controls the expression of extracellular matrix genes including *Ctgf* in the developing limbs.

Molecular Basis of *SHOX* Haploinsufficiency

Previously reported *SHOX* abnormalities included various missense and nonsense mutations as well as nucleotide insertions or deletions in the coding exons 2–6a [Niesler et al., 2007; Binder, 2011]. These nucleotide alterations are listed in the *SHOX* mutation database (http://grenada.lumc.nl/LOVD2/MR/home.php?select_db=SHOX) [Niesler et al., 2007]. Known pathogenic *SHOX* mutations are widely distributed in exons 2–6a without hotspots [Binder, 2011]. Although a few nucleotide changes in exon 6b have been submitted to the database, the clinical significance of these substitutions is unclear. Indeed, *SHOX* isoform b encoded by exons 1–6b lacks a functionally important OAR domain and is therefore likely to be a nonfunctioning protein [Rao et al., 1997]. However, it is possible that the *SHOXb* isoform affects skeletal growth by regulating mRNA levels of the major *SHOXa* isoform [Durand et al., 2011]. Alternatively, *SHOXb* may compete with *SHOXa* cofactors such as SOX5 or SOX6, as suggested for *SHOX2a* and b [Aza-Carmona et al., 2014].

SHOX haploinsufficiency is more frequently caused by CNVs than point mutations [Benito-Sanz et al., 2005, 2006, 2011, 2012a, b; Fukami et al., 2008; Chen et al., 2009; Rosilio et al., 2012]. Various submicroscopic microdeletions in PAR1 involving *SHOX* exons and/or its flanking regions have been identified in ISS and LWD patients. In particular, microdeletions in the *SHOX* downstream region have been reported as the most common genetic defects in LWD patients of Spanish origin [Benito-Sanz et al., 2006]. Microdeletions in PAR1 leading to LWD and ISS are predicted to affect exons and/or *cis*-acting enhancer elements of *SHOX*. Since monoallelic *SHOX* expression was confirmed in the skeletal tissues of a patient with a downstream deletion [Flanagan et al., 2002], elimination of *SHOX* enhancers seems to be sufficient to abolish gene expression. Although the actual positions of the upstream and downstream enhancers of *SHOX* have yet to be determined, they are likely located within highly conserved noncoding DNA elements (CNEs) around the gene because *cis*-acting enhancers are usually conserved among species [Pennacchio et al., 2006]. To date, 7 CNEs with in vitro or in vivo *cis*-regulatory activity have been

identified in PAR1: 3 in the *SHOX* upstream region and 4 in the downstream region (fig. 1) [Fukami et al., 2006; Chen et al., 2009; Durand et al., 2010; Benito-Sanz et al., 2012b]. These CNEs are predicted to contain *SHOX* enhancers. Indeed, physical interaction between the CNEs and *SHOX* has been indicated by in vitro 3C assays [Benito-Sanz et al., 2012b; Verdin et al., 2015] and by in vivo assays using zebrafish [Kenyon et al., 2011]. Furthermore, since microdeletions in the far downstream region of known CNEs have recently been identified in patients with LWD-compatible skeletal features and/or short stature [Bunyan et al., 2014; Sandoval et al., 2014; Fukami et al., 2015], there may be a hitherto unidentified *cis*-acting element(s) of *SHOX*. In vitro assays have suggested that *SHOX* transcription could be regulated by multiple *cis*-acting elements distributed in a >1-Mb region in PAR1 [Verdin et al., 2015].

Recently, a few submicroscopic PAR1 microduplications were identified in patients with ISS and LWD [Iughetti et al., 2010; Benito-Sanz et al., 2011; Fukami et al., 2015]. This finding argues against the previous notion that *SHOX* overdosage underlies tall stature in individuals with Klinefelter syndrome and 47,XXX females. These apparently conflicting results can be reconciled by assuming that relatively large duplications involving all *SHOX* exons and *cis*-acting enhancers result in *SHOX* overexpression, while small duplications encompassing only some of these components may reduce *SHOX* expression levels by disrupting the *cis*-regulatory machinery [Fukami et al., 2015]. However, since microduplications around *SHOX* have also been identified in several individuals with normal stature [Benito-Sanz et al., 2011; Fukami et al., 2015], the pathogenicity of these CNVs needs to be confirmed in future studies. It is possible that the clinical consequence of each PAR1-linked duplication is determined by its genomic position and structure.

Characterization of the breakpoints of some PAR1-linked deletions suggested that nonallelic homologous recombination and nonhomologous end-joining play a role in the development of these CNVs [Fukami et al., 2006; Benito-Sanz et al., 2012b]. Likewise, the breakpoints of one duplication have been characterized, showing that this CNV was a tandem duplication mediated by *Alu* repeats [Fukami et al., 2015]. Notably, PAR1 is enriched with *Alu* repeats [Blaschke and Rappold, 2006], which may underlie the high frequency of CNVs in this region. Moreover, the high recombination rate of PAR1 during spermatogenesis may also be associated with the high frequency of PAR1-linked CNVs. Indeed, the average of the recombination rate in PAR1 during male meiosis is ~17

times higher than the average of the genome [Hinch et al., 2014]. While there were no breakpoint hotspots, a 47.5-kb deletion in the *SHOX* downstream region was repeatedly identified in English and Scandinavian patients and ascribed to a founder effect [Bunyan et al., 2013].

SHOX abnormalities were absent in about 20% of LWD patients (table 1), and the genetic defects of these cases remained unknown until recently. Hisado-Oliva et al. [2015] demonstrated that heterozygous mutations in *NPR2*, a causative gene for Maroteaux-type acromesomelic dysplasia, result in LWD-like phenotypes. These findings provide the first indication of the locus heterogeneity for LWD. Thus, mutation analysis of *NPR2* should be considered for ISS/LWD patients without *SHOX* abnormalities.

Clinical Manifestations of Patients with *SHOX* Haploinsufficiency

Patients with *SHOX* haploinsufficiency usually present with mesomelic short stature. In most cases, head circumferences and sitting height are within the normal range, while arm span is decreased and sitting height/height ratio is increased [Rappold et al., 2007; Binder, 2011; Malaquias et al., 2013]. Although apparent mesomelia can be absent in patients with *SHOX* haploinsufficiency, axiological examinations detect body disproportion in most patients [Rappold et al., 2007; Binder, 2011]. Longitudinal follow-up studies of female patients with *SHOX* haploinsufficiency showed that body disproportion often deteriorates during puberty [Fukami et al., 2003, 2004].

Growth failure in patients with *SHOX* haploinsufficiency usually occurs from the first years of age [Binder et al., 2004]. The mean adult height of ISS patients with *SHOX* haploinsufficiency and normal karyotype is around -2.2 SD, although growth failure can be more severe in patients with LWD phenotypes [Binder, 2011]. Thus, the mean growth deficit of *SHOX* haploinsufficiency is estimated to be ~12 cm. This suggests that *SHOX* haploinsufficiency does not necessarily cause clinically discernible short stature. Consistent with this, *SHOX* haploinsufficiency has been identified in several individuals with normal stature. Since the mean adult height of females with Turner syndrome is about -3.2 SD, it appears that *SHOX* deficiency accounts for most but not all of the short stature in Turner patients.

The most characteristic clinical feature of *SHOX* haploinsufficiency is Madelung deformity (fig. 3). Madelung

Fig. 3. Madelung deformity of LWD. Bowing and shortening of the radius, prominence of the ulnar head, palmar and ulnar deviation of the carpal bones, and dorsal subluxation of the ulnar head are shown.

deformity is a combination of anatomical changes in the wrist consisting of bowing and shortening of the radius, prominence of the ulnar head, and palmar and ulnar deviation of the carpal bones (fig. 3) [Binder et al., 2001; Schmidt-Rohlfing et al., 2001]. Madelung deformity can be radiologically diagnosed by the absence or narrowing of the ulnar portion of the distal radial physis, anterior bowing of the radial shaft, and dorsal subluxation of the ulnar head (fig. 3). Histopathological analysis showed a disturbed columnar arrangement of chondrocytes in the growth plate, where tandem stacking of chondrocytes was replaced by a side-by-side arrangement [Munns et al., 2001]. Furthermore, abnormal enchondral ossification was indicated by hypertrophic osteoid in the radial metaphysis. The primary lesion of Madelung deformity appears to be the premature fusion of the distal radial epiphysis, which possibly results from an aberrant cell death process in the growth plate [Seki et al., 2014]. Furthermore, an aberrant ligament tethering the lunate to the distal portion of the radius was found in patients with Madelung deformity [Vickers and Nielsen, 1992; Harley et al., 2006; Steinman et al., 2013; Seki et al., 2014]. This ‘Vickers ligament’ likely compresses the distal epiphysis of the radius and further disturbs its linear growth. This ligament

is predicted to develop under an aberrant mechanical force due to asymmetrical growth of the radius and ulna. Notably, although Madelung deformity is a characteristic feature of LWD, it can also occur in association with other disorders such as multiple exostoses syndrome, multiple epiphyseal dysplasia, mucopolysaccharidosis, pseudohypoparathyroidism type 1b, and injury.

The severity of skeletal changes of *SHOX* haploinsufficiency is variable among patients and tends to be more severe in females than in males [Kosho et al., 1999; Binder, 2011]. While adult female patients often present with LWD, adult male patients and children usually exhibit ISS or only mild Madelung deformity. Relatively severe manifestations in adult females can be explained by the effect of gonadal estrogens. Since estrogens are known to enhance the fusion of growth plates in healthy males and females, they may accelerate premature epiphyseal fusion in individuals with *SHOX* haploinsufficiency. A relatively low frequency of LWD in Turner patients despite *SHOX* deficiency is consistent with attenuated estrogen production in these individuals.

Soucek et al. [2013] investigated bone mineral density and bone geometry in prepubertal patients with *SHOX* haploinsufficiency. They found a significantly increased total bone area, decreased relative cortical bone area, and a thin cortex. A possible interpretation of these findings is that the total bone area was increased to maintain the bone strength under the presence of mechanical loading. Similar findings were observed in prepubertal Turner patients, suggesting that *SHOX* plays a major role in bone geometrical changes of Turner syndrome.

SHOX haploinsufficiency is associated with additional clinical features [Rappold et al., 2007; Binder, 2011]. Of these, muscular hypertrophy in the lower limbs is of clinical importance because it has been reported in about one-third of *SHOX*-deficient patients [Rappold et al., 2007]. In addition, skeletal features of Turner syndrome, such as scoliosis, high-arched palate, short metacarpals, and micrognathia, were shared by a certain percentage of patients with *SHOX* haploinsufficiency and a normal karyotype [Rappold et al., 2007; Binder, 2011; Rosilio et al., 2012].

Genotype-Phenotype Correlation

The phenotypic severity of individuals with *SHOX* haploinsufficiency does not reflect the mutation types [Binder et al., 2004]. In fact, identical *SHOX* abnormalities have been detected in patients with ISS and LWD and

in individuals with normal stature. Furthermore, no apparent phenotypic differences were reported between patients with missense mutations and those with nonsense or frameshift mutations [Binder et al., 2004]. Since the clinical manifestation of *SHOX* haploinsufficiency is usually more severe in adult female patients than in adult male and prepubertal patients, hormonal conditions rather than mutation types seem to determine the clinical consequences of *SHOX* haploinsufficiency. On the other hand, Rosilio et al. [2012] suggested that CNVs involving only the downstream enhancer regions lead to slightly milder phenotypes than mutations or deletions in the exons. *SHOX* downstream deletions may be associated with a broad clinical spectrum because Chen et al. [2009] documented a prominent phenotype in patients with such deletions. Furthermore, Donze et al. [2015] reported that patients with enhancer deletions were equally short as those with *SHOX* intragenic defects but were less disproportionate and showed better responses to growth hormone (GH) therapy. Benito-Sanz et al. [2011] suggested that *SHOX* duplications are often associated with relatively mild phenotypes. These findings suggest that there may be some phenotypic difference between *SHOX* exonic mutations/deletions and enhancer abnormalities.

Diagnosis of *SHOX* Abnormalities

SHOX haploinsufficiency can be diagnosed by the presence of Madelung deformity and mesomelic short stature, although these features are shared by only a certain percentage of the patients. A family history of autosomal dominant short stature supports the diagnosis of *SHOX* haploinsufficiency. Malaquias et al. [2013] demonstrated that body disproportion is a useful indicator of *SHOX* haploinsufficiency. Rappold et al. [2007] developed a scoring system for identification of the appropriate subjects for *SHOX* genetic testing. Score items of the system included arm span/height ratio, sitting height/height ratio, body mass index, cubitus valgus, short forearm, bowing of the forearm, muscular hypertrophy, and dislocation of the ulna.

Molecular analysis is useful to confirm the diagnosis of *SHOX* haploinsufficiency. Since *SHOX* haploinsufficiency is more frequently caused by submicroscopic CNVs than exonic point mutations, copy number analysis should be the first approach for molecular diagnosis. Multiplex ligation-dependent probe amplification (MLPA; MRC Holland, Amsterdam, The Netherlands) is frequently used for the initial screening of *SHOX* abnormalities (table 1) because it allows detection of copy num-

ber gains and losses of *SHOX* exons and the CNEs in a single assay. Array CGH is frequently used to confirm and characterize the CNVs identified by MLPA. Mutation analysis of the *SHOX*-coding region should be performed for patients without pathogenic CNVs. The *SHOX* mutation database is useful to assess the pathogenicity of missense mutations.

Treatment of Patients with *SHOX* Abnormalities

To date, management protocols for patients with *SHOX* haploinsufficiency have not been fully established. GH treatment has successfully improved growth velocity in several patients [Blum et al., 2013; Wit and Oostdijk, 2015]. The effects of GH on stature growth were comparable between patients with *SHOX* haploinsufficiency and those with Turner syndrome [Blum et al., 2013]. However, long-term outcomes of GH treatment need to be evaluated in future studies. Since Donze et al. [2015] reported that GH treatment was more effective in patients with enhancer deletions than in those with intragenic abnormalities, patients should be classified according to their mutation types. In addition, while Ogata et al. [2001b] suggested that gonadal suppression therapy may be useful to prevent the development of Madelung deformity in female patients, the outcome of this therapy remains to be investigated.

Surgical interventions have been carried out to reduce pain or improve wrist function in a few cases with severe Madelung deformity. In addition, previous studies suggested that surgical removal of the Vickers ligament in combination with dome osteotomy may benefit patients with Madelung deformity [Vickers and Nielsen, 1992; Harley et al., 2006; Steinman et al., 2013; Seki et al., 2014]. However, an optimal surgical procedure for Madelung deformity has yet to be determined.

Conclusions

SHOX is one of the major growth genes in humans, and its haploinsufficiency underlies syndromic and non-syndromic short stature. *SHOX* haploinsufficiency represents a unique pseudoautosomal dominant disorder that mainly results from submicroscopic CNVs in PAR1. Future challenges in *SHOX* research include elucidation of its precise role in the developing limbs, identification of further *cis*-acting enhancers, and the development of optimal therapeutic strategies for patients.

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Statement of Ethics

The authors have no ethical conflicts to disclose.

Disclosure Statement

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References

- Aza-Carmona M, Shears DJ, Yuste-Checa P, Barca-Tierno V, Hisado-Oliva A, et al: SHOX interacts with the chondrogenic transcription factors SOX5 and SOX6 to activate the aggrecan enhancer. *Hum Mol Genet* 20:1547–1559 (2011).
- Aza-Carmona M, Barca-Tierno V, Hisado-Oliva A, Belinchón A, Gorbenko-del Blanco D, et al: *NPPB* and *ACAN*, two novel SHOX2 transcription targets implicated in skeletal development. *PLoS One* 9:e83104 (2014).
- Beiser KU, Glaser A, Kleinschmidt K, Scholl I, Röth R, et al: Identification of novel SHOX target genes in the developing limb using a transgenic mouse model. *PLoS One* 9:e98543 (2014).
- Belin V, Cusin V, Viot G, Girlich D, Toutain A, et al: SHOX mutations in dyschondrosteosis (Léri-Weill syndrome). *Nat Genet* 19:67–69 (1998).
- Benito-Sanz S, Thomas NS, Huber C, Gorbenko del Blanco D, Aza-Carmona M, et al: A novel class of pseudoautosomal region 1 deletions downstream of SHOX is associated with Léri-Weill dyschondrosteosis. *Am J Hum Genet* 77:533–544 (2005).
- Benito-Sanz S, del Blanco DG, Aza-Carmona M, Magano LF, Lapunzina P, et al: PAR1 deletions downstream of SHOX are the most frequent defect in a Spanish cohort of Léri-Weill dyschondrosteosis (LWD) probands. *Hum Mutat* 27:1062 (2006).
- Benito-Sanz S, Barroso E, Heine-Suñer D, Hisado-Oliva A, Romanelli V, et al: Clinical and molecular evaluation of SHOX/PAR1 duplications in Léri-Weill dyschondrosteosis (LWD) and idiopathic short stature (ISS). *J Clin Endocrinol Metab* 96:E404–E412 (2011).
- Benito-Sanz S, Aza-Carmona M, Rodríguez-Estévez A, Rica-Etxebarria I, Gracia R, et al: Identification of the first PAR1 deletion encompassing upstream SHOX enhancers in a family with idiopathic short stature. *Eur J Hum Genet* 20:125–127 (2012a).
- Benito-Sanz S, Royo JL, Barroso E, Paumard-Hernández B, Barrera-Bonis AC, et al: Identification of the first recurrent PAR1 deletion in Léri-Weill dyschondrosteosis and idiopathic short stature reveals the presence of a novel SHOX enhancer. *J Med Genet* 49:442–450 (2012b).
- Binder G: Short stature due to SHOX deficiency: genotype, phenotype, and therapy. *Horm Res Paediatr* 75:81–89 (2011).
- Binder G, Schwarze CP, Ranke MB: Identification of short stature caused by SHOX defects and therapeutic effect of recombinant human growth hormone. *J Clin Endocrinol Metab* 85:245–249 (2000).
- Binder G, Fritsch H, Schweizer R, Ranke MB: Radiological signs of Léri-Weill dyschondrosteosis in Turner syndrome. *Horm Res* 55:71–76 (2001).
- Binder G, Renz A, Martínez A, Keselman A, Hesse V, et al: SHOX haploinsufficiency and Léri-Weill dyschondrosteosis: prevalence and growth failure in relation to mutation, sex, and degree of wrist deformity. *J Clin Endocrinol Metab* 89:4403–4408 (2004).
- Blaschke RJ, Rappold G: The pseudoautosomal regions, SHOX and disease. *Curr Opin Genet Dev* 16:233–239 (2006).
- Blaschke RJ, Monaghan AP, Schiller S, Schechinger B, Rao E, et al: SHOT, a SHOX-related homeobox gene, is implicated in craniofacial, brain, heart, and limb development. *Proc Natl Acad Sci USA* 95:2406–2411 (1998).
- Blum WF, Ross JL, Zimmermann AG, Quigley CA, Child CJ, et al: GH treatment to final height produces similar height gains in patients with SHOX deficiency and Turner syndrome: results of a multicenter trial. *J Clin Endocrinol Metab* 98:E1383–E1392 (2013).
- Bobick BE, Cobb J: *Shox2* regulates progression through chondrogenesis in the mouse proximal limb. *J Cell Sci* 125:6071–6083 (2012).
- Bunyan DJ, Baker KR, Harvey JF, Thomas NS: Diagnostic screening identifies a wide range of mutations involving the SHOX gene, including a common 47.5 kb deletion 160 kb downstream with a variable phenotypic effect. *Am J Med Genet A* 161A:1329–1338 (2013).
- Bunyan DJ, Taylor EJ, Maloney VK, Blyth M: Homozygosity for a novel deletion downstream of the SHOX gene provides evidence for an additional long range regulatory region with a mild phenotypic effect. *Am J Med Genet A* 164A:2764–2768 (2014).
- Chen J, Wildhardt G, Zhong Z, Röth R, Weiss B, et al: Enhancer deletions of the SHOX gene as a frequent cause of short stature: the essential role of a 250 kb downstream regulatory domain. *J Med Genet* 46:834–839 (2009).
- Clement-Jones M, Schiller S, Rao E, Blaschke RJ, Zuniga A, et al: The short stature homeobox gene SHOX is involved in skeletal abnormalities in Turner syndrome. *Hum Mol Genet* 9:695–702 (2000).
- Decker E, Durand C, Bender S, Rödelberger C, Glaser A, et al: *FGFR3* is a target of the homeobox transcription factor SHOX in limb development. *Hum Mol Genet* 20:1524–1535 (2011).
- Donze SH, Meijer CR, Kant SG, Zandwijken GR, van der Hout AH, et al: The growth response to GH treatment is greater in patients with SHOX enhancer deletions compared to SHOX defects. *Eur J Endocrinol* 173:611–621 (2015).
- Durand C, Bangs F, Signolet J, Decker E, Tickle C, Rappold G: Enhancer elements upstream of the SHOX gene are active in the developing limb. *Eur J Hum Genet* 18:527–532 (2010).
- Durand C, Roeth R, Dweep H, Vlatkovic I, Decker E, et al: Alternative splicing and nonsense-mediated RNA decay contribute to the regulation of SHOX expression. *PLoS One* 6:e18115 (2011).
- Durand C, Decker E, Roeth R, Schneider KU, Rappold G: The homeobox transcription factor HOXA9 is a regulator of SHOX in U2OS cells and chicken micromass cultures. *PLoS One* 7:e45369 (2012).
- Espinoza-Lewis RA, Yu L, He F, Liu H, Tang R, et al: *Shox2* is essential for the differentiation of cardiac pacemaker cells by repressing *Nkx2-5*. *Dev Biol* 327:376–385 (2009).
- Flanagan SF, Munns CF, Hayes M, Williams B, Berry M, et al: Prevalence of mutations in the short stature homeobox containing gene (SHOX) in Madelung deformity of childhood. *J Med Genet* 39:758–763 (2002).
- Fukami M, Matsuo N, Hasegawa T, Sato S, Ogata T: Longitudinal auxological study in a female with SHOX (short stature homeobox containing gene) haploinsufficiency and normal ovarian function. *Eur J Endocrinol* 149:337–341 (2003).
- Fukami M, Nishi Y, Hasegawa Y, Miyoshi Y, Okabe T, et al: Statural growth in 31 Japanese patients with SHOX haploinsufficiency: support for a disadvantageous effect of gonadal estrogens. *Endocr J* 51:197–200 (2004).
- Fukami M, Kato F, Tajima T, Yokoya S, Ogata T: Transactivation function of an approximately 800-bp evolutionarily conserved sequence at the SHOX 3' region: implication for the downstream enhancer. *Am J Hum Genet* 78:167–170 (2006).
- Fukami M, Dateki S, Kato F, Hasegawa Y, Mo-chizuki H, et al: Identification and characterization of cryptic SHOX intragenic deletions in three Japanese patients with Léri-Weill dyschondrosteosis. *J Hum Genet* 53:454–459 (2008).

- Fukami M, Naiki Y, Muroya K, Hamajima T, Soneda S, et al: Rare pseudoautosomal copy-number variations involving SHOX and/or its flanking regions in individuals with and without short stature. *J Hum Genet* 60:553–556 (2015).
- Funari MF, Jorge AA, Souza SC, Billerbeck AE, Arnhold IJ, et al: Usefulness of MLPA in the detection of SHOX deletions. *Eur J Med Genet* 53:234–238 (2010).
- Gatta V, Antonucci I, Morizio E, Palka C, Fischetto R, et al: Identification and characterization of different SHOX gene deletions in patients with Leri-Weill dyschondrosteosis by MLPA assay. *J Hum Genet* 52:21–27 (2007).
- Harley BJ, Brown C, Cummings K, Carter PR, Ezaki M: Volar ligament release and distal radius dome osteotomy for correction of Madelung's deformity. *J Hand Surg Am* 31:1499–1506 (2006).
- Hinch AG, Altemose N, Noor N, Donnelly P, Myers SR: Recombination in the human pseudoautosomal region PAR1. *PLoS Genet* 10:e1004503 (2014).
- Hirschfeldova K, Solc R, Baxova A, Zapletalova J, Kebrdlova V, et al: SHOX gene defects and selected dysmorphic signs in patients of idiopathic short stature and Léri-Weill dyschondrosteosis. *Gene* 491:123–127 (2012).
- Hisado-Oliva A, Garre-Vázquez AI, Santaolalla-Caballero F, Belinchón A, Barreda-Bonis AC, et al: Heterozygous NPR2 mutations cause disproportionate short stature, similar to Léri-Weill dyschondrosteosis. *J Clin Endocrinol Metab* 100:E1133–1142 (2015).
- Hristov G, Marttila T, Durand C, Niesler B, Rappold GA, Marchini A: SHOX triggers the lysosomal pathway of apoptosis via oxidative stress. *Hum Mol Genet* 23:1619–1630 (2014).
- Huber C, Rosilio M, Munnich A, Cormier-Daire V, French SHOX GeNeSIS Module: High incidence of SHOX anomalies in individuals with short stature. *J Med Genet* 43:735–739 (2006).
- Iughetti L, Capone L, Elsedfy H, Bertorelli R, Predieri B, et al: Unexpected phenotype in a boy with trisomy of the SHOX gene. *J Pediatr Endocrinol Metab* 23:159–169 (2010).
- Izpisua-Belmonte JC, Duboule D: Homeobox genes and pattern formation in the vertebrate limb. *Dev Biol* 152:26–36 (1992).
- Jorge AA, Souza SC, Nishi MY, Billerbeck AE, Libório DC, et al: SHOX mutations in idiopathic short stature and Leri-Weill dyschondrosteosis: frequency and phenotypic variability. *Clin Endocrinol (Oxf)* 66:130–135 (2007).
- Kant SG, van der Kamp HJ, Kriek M, Bakker E, Bakker B, et al: The jumping SHOX gene – crossover in the pseudoautosomal region resulting in unusual inheritance of Leri-Weill dyschondrosteosis. *J Clin Endocrinol Metab* 96:E356–E359 (2011).
- Kenyon EJ, McEwen GK, Callaway H, Elgar G: Functional analysis of conserved non-coding regions around the short stature hox gene (*shox*) in whole zebrafish embryos. *PLoS One* 6:e21498 (2011).
- Kosho T, Muroya K, Nagai T, Fujimoto M, Yokoya S, et al: Skeletal features and growth patterns in 14 patients with haploinsufficiency of SHOX: implications for the development of Turner syndrome. *J Clin Endocrinol Metab* 84:4613–4621 (1999).
- Malaquias AC, Scalco RC, Fontenele EG, Costalonga EF, Baldin AD, et al: The sitting height/height ratio for age in healthy and short individuals and its potential role in selecting short children for SHOX analysis. *Horm Res Paediatr* 80:449–456 (2013).
- Marchini A, Häcker B, Marttila T, Hesse V, Emons J, et al: BNP is a transcriptional target of the short stature homeobox gene SHOX. *Hum Mol Genet* 16:3081–3087 (2007).
- Munns CF, Glass IA, LaBrom R, Hayes M, Flanagan S, et al: Histopathological analysis of Leri-Weill dyschondrosteosis: disordered growth plate. *Hand Surg* 6:13–23 (2001).
- Niesler B, Röth R, Wilke S, Fujimura F, Fischer C, Rappold G: The novel human SHOX allelic variant database. *Hum Mutat* 28:933–938 (2007).
- Ogata T, Matsuo N, Nishimura G: SHOX haploinsufficiency and overdosage: impact of gonadal function status. *J Med Genet* 38:1–6 (2001a).
- Ogata T, Onigata K, Hotsubo T, Matsuo N, Rappold G: Growth hormone and gonadotropin-releasing hormone analog therapy in haploinsufficiency of SHOX. *Endocr J* 48:317–322 (2001b).
- Ogata T, Inokuchi M, Ogawa M: Growth pattern and body proportion in a female with short stature homeobox-containing gene overdosage and gonadal estrogen deficiency. *Eur J Endocrinol* 147:249–254 (2002).
- Pennacchio LA, Ahituv N, Moses AM, Prabhakar S, Nobrega MA, et al: In vivo enhancer analysis of human conserved non-coding sequences. *Nature* 444:499–502 (2006).
- Poggi H, Vera A, Avalos C, Lagos M, Mellado C, et al: A deletion of more than 800 kb is the most recurrent mutation in Chilean patients with SHOX gene defects. *Horm Res Paediatr* 84:254–257 (2015).
- Rao E, Weiss B, Fukami M, Rump A, Niesler B, et al: Pseudoautosomal deletions encompassing a novel homeobox gene cause growth failure idiopathic short stature and Turner syndrome. *Nat Genet* 16:54–63 (1997).
- Rappold GA, Fukami M, Niesler B, Schiller S, Zunkeller W, et al: Deletions of the homeobox gene SHOX (short stature homeobox) are an important cause of growth failure in children with short stature. *J Clin Endocrinol Metab* 87:1402–1406 (2002).
- Rappold G, Blum WF, Shavrikova EP, Crowe BJ, Roeth R, et al: Genotypes and phenotypes in children with short stature: clinical indicators of SHOX haploinsufficiency. *J Med Genet* 44:306–313 (2007).
- Rosilio M, Huber-Lequesne C, Sapin H, Carel JC, Blum WF, Cormier-Daire V: Genotypes and phenotypes of children with SHOX deficiency in France. *J Clin Endocrinol Metab* 97:E1257–E1265 (2012).
- Rosin JM, McAllister BB, Dyck RH, Percival CJ, Kurrasch DM, Cobb J: Mice lacking the transcription factor SHOX2 display impaired cerebellar development and deficits in motor coordination. *Dev Biol* 399:54–67 (2015).
- Sandoval GT, Jaimes GC, Barrios MC, Cespedes C, Velasco HM: SHOX gene and conserved non-coding element deletions/duplications in Colombian patients with idiopathic short stature. *Mol Genet Genomic Med* 2:95–102 (2014).
- Schmidt-Rohlfing B, Schwöbel B, Pauschert R, Niethard FU: Madelung deformity: clinical features, therapy and results. *J Pediatr Orthop B* 10:344–348 (2001).
- Schneider KU, Sabherwal N, Jantz K, Röth R, Muncke N, et al: Identification of a major recombination hotspot in patients with short stature and SHOX deficiency. *Am J Hum Genet* 77:89–96 (2005).
- Seki A, Jinno T, Suzuki E, Takayama S, Ogata T, Fukami M: Skeletal deformity associated with SHOX deficiency. *Clin Pediatr Endocrinol* 23:65–72 (2014).
- Shears DJ, Vassal HJ, Goodman FR, Palmer RW, Reardon W, et al: Mutation and deletion of the pseudoautosomal gene SHOX cause Leri-Weill dyschondrosteosis. *Nat Genet* 19:70–73 (1998).
- Shears DJ, Guillen-Navarro E, Sempere-Mirallès M, Domingo-Jimenez R, Scambler PJ, Winter RM: Pseudodominant inheritance of Langer mesomelic dysplasia caused by a SHOX homeobox missense mutation. *Am J Med Genet* 110:153–157 (2002).
- Soucek O, Zapletalova J, Zemkova D, Snajderova M, Novotna D, et al: Prepubertal girls with Turner syndrome and children with isolated SHOX deficiency have similar bone geometry at the radius. *J Clin Endocrinol Metab* 98:E1241–E1247 (2013).
- Steinman S, Oishi S, Mills J, Bush P, Wheeler L, Ezaki M: Volar ligament release and distal radial dome osteotomy for the correction of Madelung deformity: long-term follow-up. *J Bone Joint Surg Am* 95:1198–1204 (2013).
- Tiecke E, Bangs F, Blaschke R, Farrell ER, Rappold G, Tickle C: Expression of the short stature homeobox gene *Shox* is restricted by proximal and distal signals in chick limb buds and affects the length of skeletal elements. *Dev Biol* 298:585–596 (2006).
- Verdin H, Fernández-Miñán A, Benito-Sanz S, Janssens S, Callewaert B, et al: Profiling of conserved non-coding elements upstream of SHOX and functional characterisation of the SHOX cis-regulatory landscape. *Sci Rep* 5:17667 (2015).
- Vickers D, Nielsen G: Madelung deformity: surgical prophylaxis (physiolysis) during the late growth period by resection of the dyschondrosteosis lesion. *J Hand Surg Br* 17:401–407 (1992).
- Wit JM, Oostdijk W: Novel approaches to short stature therapy. *Best Pract Res Clin Endocrinol Metab* 29:353–366 (2015).
- Zinn AR, Wei F, Zhang L, Elder FF, Scott CI Jr, et al: Complete SHOX deficiency causes Langer mesomelic dysplasia. *Am J Med Genet* 110:158–163 (2002).