

Short Stature due to SHOX Deficiency: Genotype, Phenotype, and Therapy

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Key Words

Leri-Weill syndrome · Dyschondrosteosis · Haploinsufficiency · Madelung deformity · Idiopathic short stature

Abstract

SHOX deficiency is a frequent cause of short stature. The short stature homeobox-containing gene resides in the telomeric PAR1 region on the short arm of both sex chromosomes and escapes X inactivation. For this review, abstracts of 207 publications presented by PubMed for the search term 'SHOX' were screened. Heterozygote *SHOX* mutations (80% deletions) were detected in 2–15% of individuals with formerly idiopathic short stature, in 50–90% of individuals with Leri-Weill dyschondrosteosis, and in almost 100% of girls with Turner syndrome. Mutational analysis is primarily performed by MLPA analysis followed by gene sequencing if necessary. SHOX is a nuclear protein that binds to DNA and acts as a transcriptional activator. Orthologs are present in many vertebrates but not in rodents. Gene expression starting as early as 33 days postconception in humans is predominant in the mid portion of the buds and in the first and second pharyngeal arches. In the growth plate, hypertrophic chondrocytes express *SHOX* where it seems to have antiproliferative potency. The penetrance of SHOX deficiency is high, but its clinical expression is very variable becoming more pronounced with age and being more severe in fe-

males. Growth failure starts early during the first years of life and the height deficit present at preschool age seems not to deteriorate further. The mean adult height is –2.2 SDS. Auxological analysis of the body proportions (mesomelia), the presence of minor abnormalities, and the search for subtle radiographic signs are important keys to the diagnosis which has to be confirmed by genetic analysis. The growth-promoting effect of GH therapy approved for individuals with *SHOX* mutations seems to be equal to the effect seen in Turner syndrome.

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Introduction

Statural growth and adult height are regulated by a complex system of endocrine factors including the GH-IGF-I axis as the most prominent system but also by intrinsic nonendocrine systems of the skeletal bones which guarantee the correct balance of proliferation and differentiation of chondrocytes for physiological extension in length and width. The physiology and the disorders of this bone-intrinsic regulation are less well understood than the endocrine system. Although more than 300 different monogenic disorders of the bone which are frequently associated with short stature and bone dysmorphism have been described, only a handful of disorders are so frequent that the clinician and the radiologist are

able to recognize the phenotype and make the correct diagnosis. *SHOX* deficiency is just one monogenic disorder of the bone, but collected evidence supports the notion that this highly variable osteodysplasia is likely the most frequent one.

***SHOX* Gene**

SHOX – the short stature homeobox-containing gene – was discovered by deletion mapping of the short arm of chromosome X by Rao et al. [1] and independently by Ellison et al. [2] who named the gene *PHOG* but published later. The gene is located on the very tip of the short arms of both sex chromosomes X and Y inside the telomeric part of pseudoautosomal region 1 (PAR1) which comprises around 2.6 Mb (fig. 1). This region contains genes which escape X inactivation. Because of this circumstance, *SHOX* is expressed on both sex chromosomes and as far as we know there is no difference between *SHOX* (X) and *SHOX* (Y). During the meiosis pairing of spermatogenesis, the *SHOX* region of PAR1 is a hotspot for very frequent recombination events between the X and Y chromosomes [3]. The initial discovery of the gene *SHOX* was promoted by the known association of short stature with deletions of the short arm of the X chromosome or small terminal deletions of the short arm of the Y chromosome. Independent from this research, there was a search for the disease gene involved in the dominantly inherited Leri-Weill syndrome or Leri-Weill dyschondrosteosis (OMIM 127300) which revealed linkage to microsatellite DNA markers located in PAR1 next to the discovered *SHOX* gene: *SHOX* deletions were found to be causative in Leri-Weill syndrome as well [4, 5].

The *SHOX* gene contains 2 recognized motifs: the homeobox encodes a homeodomain which enables specific DNA binding and gene transactivation of the protein [1, 2]. In addition, this protein domain is relevant for nuclear translocation [6] and for dimerization of *SHOX* [7]. The second motif encodes for the C-terminal-located OAR domain which is also relevant for the transactivational activity of *SHOX* [8]. The *SHOX* mRNA is encoded by 7 exons (1–5, 6a, and 6b) encompassing about 40 kb of genomic DNA (fig. 1). Alternative splicing results in 2 products: *SHOXa* and its shortened version *SHOXb* which lacks the information for the OAR domain and is therefore not active as a transcriptional activator. *SHOX* is a member of the paired-like type of homeobox-containing genes [1, 2]. Homeobox-containing genes play im-

portant roles during embryogenesis and development by regulating pattern formation in time and space.

SHOX gene function is dosage dependent: the loss-of-function mutation of one *SHOX* allele (haploinsufficiency) results in the disorder of *SHOX* deficiency which causes growth failure. The most frequent *SHOX* mutations found are gene deletions of different size which encompass the *SHOX* gene itself [9] or a regulatory enhancer region which is located 50–250 kb downstream of the coding region [10]. These deletions account for around 80% of all mutations. The other gene defects found are missense and nonsense mutations which are spread all over the gene, but most of them are located within exons 3 and 4 which encode the functional important homeodomain (fig. 1). These mutations are predicted to cause inactivation of the protein or to block nuclear translocation or dimerization of *SHOX* [7]. Very recently, partial and complete *SHOX* duplications with a size range from 13 to 346 kb were reported in patients with short stature and the *SHOX* deficiency phenotype [11, 12]. Such chromosome rearrangements are thought to cause a disruption of the interaction between the enhancer regions and the promoter of *SHOX* resulting in a decreased transactivation of gene expression [11, 12].

The prevalence of *SHOX* mutations in children with so-far-unexplained short stature (idiopathic short stature) has been estimated according to *SHOX* mutation screening studies. Estimates differed from cohort to cohort and increased with the surge of more sensitive genetic methods for the detection of small deletions and with the knowledge of the functional importance of the enhancer region downstream of the *SHOX* gene. Estimates for prevalence in short children ranked from 2 to 15% [13–15]. The prevalence of *SHOX* mutations in individuals with Leri-Weill syndrome (see below) is around 50–90% [16, 17]. Turner syndrome is almost always associated with the loss of one *SHOX* gene because of the numerical or structural aberration of the X chromosome associated with this syndrome.

The loss of both *SHOX* alleles causes the complete lack of *SHOX* and an extreme phenotype of osteodysplasia called Langer syndrome [18]. This syndrome (MIM 249700) is relatively rare in comparison to what could be expected from the estimated prevalence of *SHOX* haploinsufficiency in short children. The gain of 1 or 2 additional copies of *SHOX* due to structural aberrations of the X chromosome can be associated with tall stature [19, 20].

The rational genetic approach for detecting *SHOX* mutations starts with the search for deletions because these

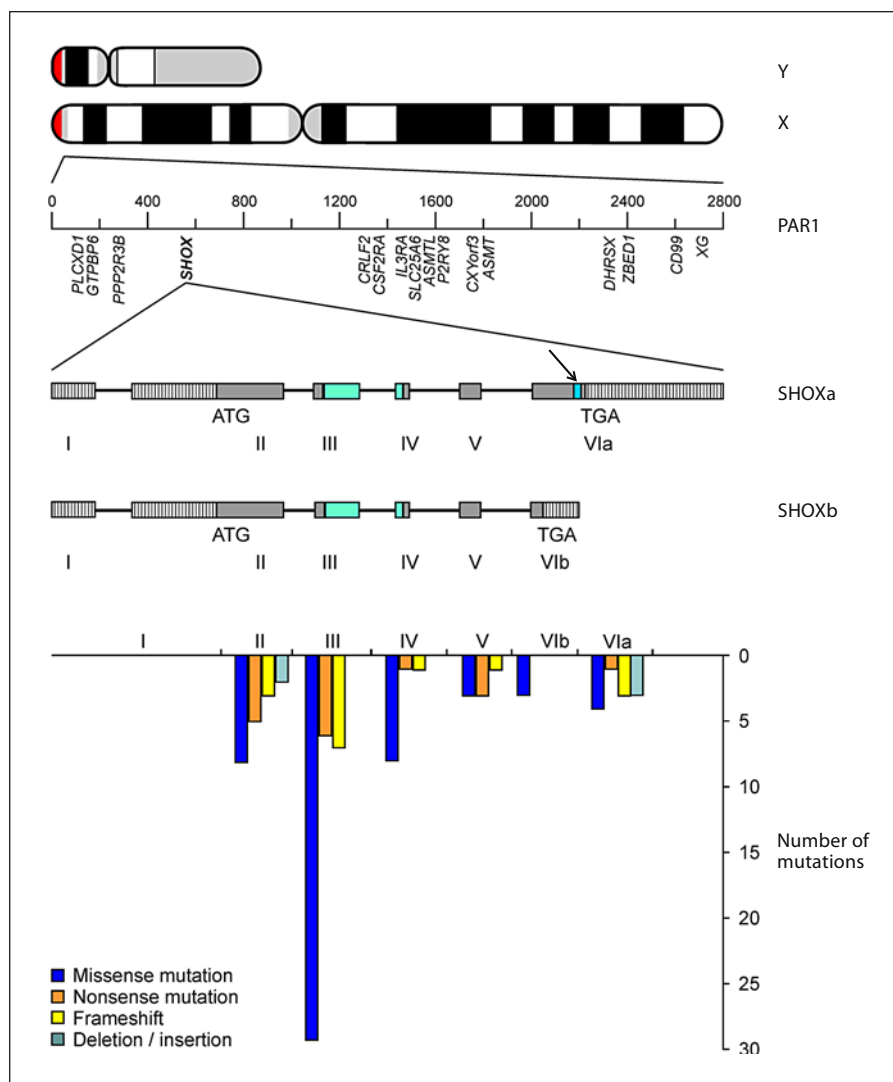


Fig. 1. The schematic overview shows the chromosomal location of PAR1, the gene content of PAR1 including SHOX, and the 2 different SHOX mRNAs produced by alternative splicing, which contain exons I to VIa and I to VIb, respectively (intron lengths are not scaled). Homeobox is indicated in turquoise (white in the print version) and the OAR, which is only present in SHOXa, is indicated by the arrow. The frequency and location of point mutations and small deletions/insertions of SHOX are shown at the bottom. The graphic is a compilation of resources taken from publications by Mangs et al. [43] and Blaschke et al. [44].

are the most frequent SHOX gene mutations found. For this purpose, the commercially available multiplex ligation probe-dependent amplification (MLPA) analysis has the highest available sensitivity [21]. This method enables the detection and quantification of small deletions in many screening samples relatively fast and is superior to formerly used methods like fluorescence in situ hybridization (FISH) or microsatellite analysis. In the absence of a deletion, direct sequencing of exons and flanking regions is necessary to detect the less frequent missense or nonsense mutations. Until now, 224 unique allelic variants have been reported to the SHOX database (<http://www.hd-lovd.uni-hd.de>). The mutational analysis is covered by a patent which is currently held by Bioscientia in Europe and by Esoterix in the USA.

SHOX Protein

In the case of SHOX, the gene and the protein have the same name. Studies on the biological meaning of the SHOX protein are still scarce and focus on SHOXa which contains 292 amino acids. The main problem is the lack of a rodent ortholog. Therefore, a knockout mouse or rat model could not be established. So far, functional analyses have been performed in chick embryos where an ortholog of SHOX exists [10, 22]. Like other homeodomain-containing proteins, SHOX is a nuclear protein and acts as a transcriptional activator [8]. SHOX specifically binds to the palindromic DNA sequences 5'-TAAT(N)ATTA-3' [8]. SHOX is phosphorylated, with serine 106 being the major SHOX phosphorylation site [23].

Analysis using zoo blots revealed that SHOX is present in vertebrates including fish, but it is absent in nonvertebrates [24]. In situ hybridizations in human embryos revealed a very early start of SHOX expression in the developing limb bud at Carnegie stage 14 which is 33 days post-conception [24]. The predominant location found was in the mid portion of the developing limb buds and, in addition, in the first and second pharyngeal arches [24]. This expression pattern resembles the locations of the anatomic structures which are affected when SHOX is deficient: the forearm and lower legs as well as maxilla, mandible, and the external ear tract.

Analysis of human growth plates revealed hypertrophic chondrocytes as the main producer of SHOX [25, 26]. In osteogenic cell line U2OS as well as in primary oral fibroblasts and primary chondrocytes, SHOX overexpression causes blockage of the cell cycle with proliferation arrest and apoptosis which may indicate that the main effect of SHOX is to promote differentiation and to stop proliferation of the hypertrophic chondrocytes in the epiphyseal growth plate [25]. A search for downstream targets has so far detected brain natriuretic peptide (BNP) as a gene whose expression is stimulated by SHOX [27]. So far, the biological meaning of this finding is unclear.

Histological examination of the epiphyseal plate of the distal radius in patients with Leri-Weill syndrome and SHOX haploinsufficiency revealed a disorganization of the hypertrophic chondrocytes which built cell nests instead of columns, given a plausible explanation for insufficient growth in length and bowing of the radius in SHOX deficiency [28].

SHOX Deficiency Phenotype

SHOX deficiency causes short stature with a highly variable phenotype which is frequently nonspecific in preschool children because the main characteristics of mesomelic disproportion of the limbs and Madelung deformity of the forearm develop over time and appear during the second decade of life or never [29–31]. Therefore, the clinical spectrum observed resembles different phenotypes: short stature with no specific findings other than short stature (so-called idiopathic short stature), short stature with subtle auxological and radiological findings, and the full picture of Leri-Weill syndrome (osteochondrosteosis). Characteristic signs are more frequent and more severe in girls, a finding which may be explained by the presence of higher estrogen levels in females [32].

Interestingly, in contrast to many other growth disorders like growth hormone deficiency or even idiopathic short stature, in SHOX deficiency females outnumber males. This phenomenon has been explained by the more severe phenotype in females which may cause ascertainment bias [30]. However, female predominance was also observed in SHOX mutation screening studies where the only phenotype targeted was short stature [29]. Therefore, ascertainment bias alone is unlikely to explain the gender dichotomy. Another explanation for the female preponderance in SHOX deficiency may be a genetic one: SHOX (X) deletions have been more frequently reported than SHOX (Y) deletions, which may indicate that the SHOX on the X is more prone to getting deleted than the SHOX on the Y.

In many children with SHOX deficiency below 6 years of age and in some boys above 6 years of age, there may be no characteristic sign of SHOX deficiency detectable in spite of a careful clinical evaluation. In this situation, the phenotype resembles the typical presentation of idiopathic short stature. However, the diagnosis made should not be idiopathic short stature but rather short stature due to SHOX deficiency [33].

Madelung Deformity and Other Clinical Signs

The wrist deformity associated with SHOX deficiency was first described by the German surgeon Dr. Otto Wilhelm Madelung in the year 1878 [34]. He described a 'spontaneous subluxation of the distal ulna forward' which gives the hand and forearm the appearance of a dinner fork. In minor, subtle, or immature forms of Madelung deformity, one can frequently observe a rotation deficit of the forearm with a reduction of the ability to supinate and to pronate. Shortening and broadening of the forearm may also appear before the full picture of Madelung deformity develops. There is a similar effect on the lower legs with bowing of the tibia and genu valgum. Because of the dominant inheritance, the examination of the wrists of the parents can give valuable hints in familial cases.

In addition to wrist deformity, there are several less-specific signs in SHOX deficiency which are known from Turner syndrome. They are shortening of the fourth and fifth metacarpals, high arched palate, increased carrying angle of the elbow, scoliosis, and micrognathia [35]. In addition, muscular hypertrophy of the calves is found in one third of affected individuals [35]. Importantly, the absence of any of these signs including Madelung deformity does not exclude SHOX haploinsufficiency.

Growth and Auxology of SHOX Deficiency

Most of the available information on the mean adult height in SHOX deficiency is biased by ascertainment. The least biased study screened all members of families with at least 1 index patient having Leri-Weill syndrome and calculated the mean adult height to be -2.2 SDS according to North American references [30]. Birth length in SHOX deficiency is only mildly reduced, but the height deficit is already present at preschool age suggesting that early childhood is the phase of growth failure [29, 30]. Longitudinal data suggest the absence of catch-up growth at any time. Kosho et al. [36] reported an additional loss of height due to diminished pubertal growth in a small cohort of children; this second phase of growth failure, however, has not been confirmed by us and others [29, 30].

In SHOX deficiency, short stature is accompanied and to an important extent caused by (mesomelic) shortening of the extremities [29–31]. Therefore, clinical judgment requires measurement of the arm span and sitting height as well as calculation of the subsischial leg length besides measurement of the standing height. In comparison to age-related reference standards, the arm span is significantly reduced in comparison to the standing height, and the leg length is significantly shorter than expected from the sitting height. In children less than 6 years of age, skeletal disproportions may be absent. A helpful parameter which integrates these 3 parameters is the extremities-trunk ratio which was proposed by us (fig. 2). The ratio has the sum of the leg length and arm span in its numerator and the height of the trunk in its denominator. In children with SHOX deficiency, this ratio is significantly lower than in controls [29]. According to our data, a normal ratio in a school child excludes SHOX deficiency with a high negative predictive value at almost 100% [29].

Auxology is also the core part of another scoring system for the diagnosis of SHOX deficiency proposed by Rappold et al. [35]. This score with 8 items was based on the data of 1,608 short individuals including 68 individuals with SHOX deficiency. The first 2 characteristics of this scoring system, namely the arm-span-to-height ratio and the sitting-height-to-height ratio, have the same targets as the above mentioned extremities-trunk ratio. Importantly, the limits given in percentage by this score are only valid in school children [37].

Radiology of SHOX Deficiency

The radiology of SHOX deficiency was described by Langer [38] when defining the radiological signs present in Leri-Weill syndrome. The main 3 signs are shown in

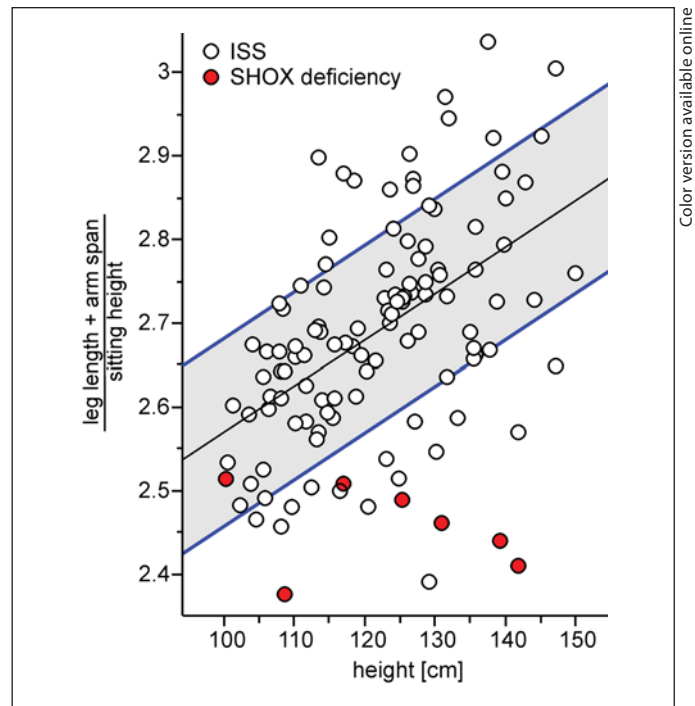


Fig. 2. Skeletal disproportions due to SHOX deficiency can be detected and quantified by the extremities-trunk ratio which is plotted against height in this figure. The mean ratio and 1 SD are indicated by lines. Each open circle indicates the ratio of a child with idiopathic short stature (ISS), and red circles (gray in the print version) are ratios from children with SHOX deficiency. With a ratio more than 1 SD below the mean at school age, SHOX deficiency is likely. A ratio above this limit in a school child (height >110 cm) excludes SHOX deficiency with a high negative predictive value at almost 100% [29].

figure 3 and are as follows: (i) the form of the distal radial epiphysis remains triangular instead of forming a trapezium at a bone age >10 years in females and >11 years in boys. This triangularization of the distal radial epiphysis is also present in girls with Turner syndrome, but not to the extent it is in Leri-Weill syndrome [39]; (ii) the carpal row becomes wedge shaped with the os lunatum building the tip of the pyramid which seems to invade the articular space (so-called pyramidalization of the carpal row), and (iii) the distal radius is radiographically lucent on the ulnar side where bone appears less dense than on the other side of the radius. This lucency can be a very early sign of SHOX deficiency because calcification of this part of the radius is present at birth [29]. Radiology can be regarded as more sensitive than clinical evaluation of the wrist in detecting signs of subtle Madelung deformity.

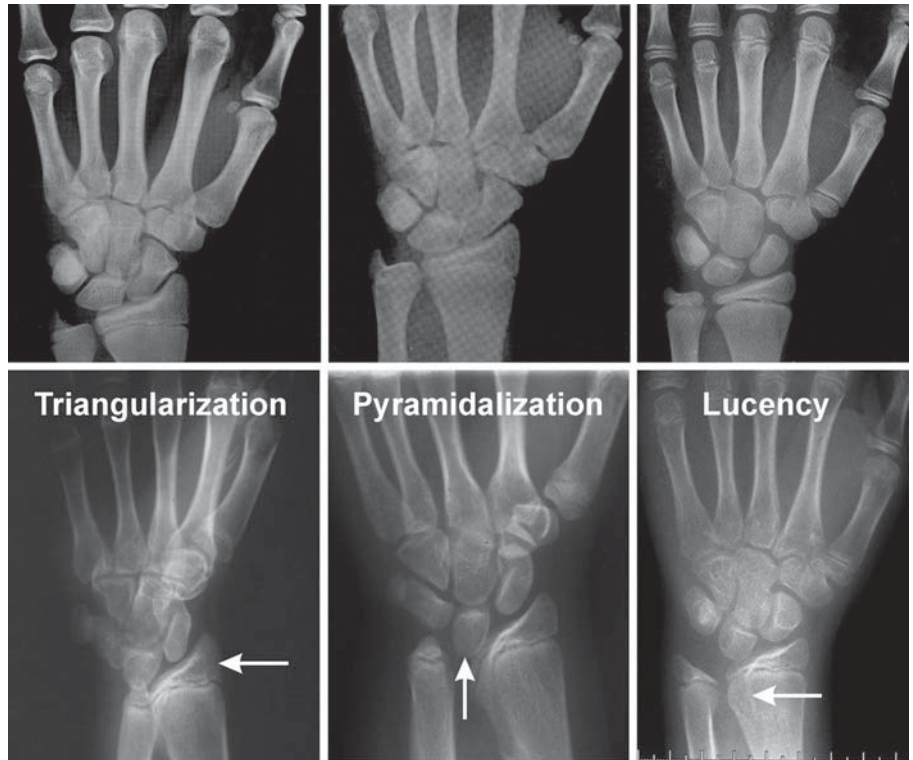


Fig. 3. The main 3 radiological signs of SHOX deficiency and Leri-Weill syndrome are shown in comparison to normal hand X-rays in the top of the figure: triangularization of the distal radial epiphysis (bone age 13 years, female); pyramidalization of the carpal row which becomes wedge-shaped with the os lunatum building the tip of a pyramid (bone age 14 years, female), and radiographic lucency: the distal radius is radiographically lucent at the ulnar side where bone appears less dense than on the other side of the radius (bone age 10 years, female).

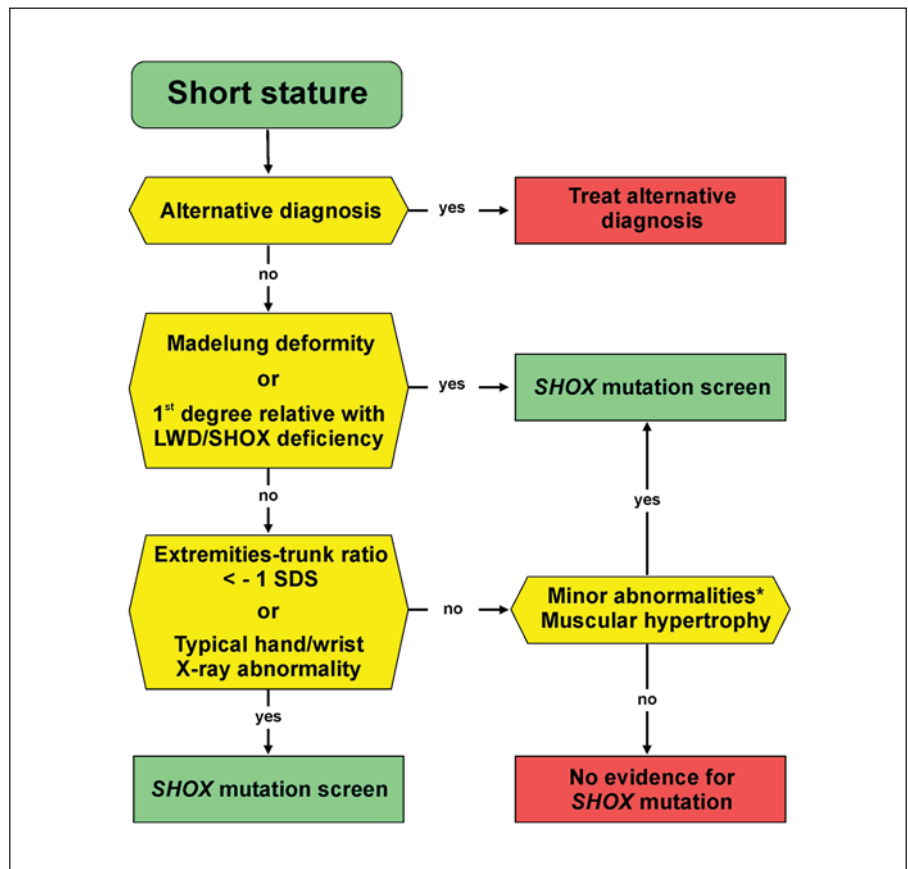


Fig. 4. This schema provides an algorithm approach to SHOX mutation screening in a short child. Minor abnormalities (*) in SHOX deficiency are shortening of the fourth and fifth metacarpals, high arched palate, increased carrying angle of the elbow, scoliosis, and micrognathia.

Differential Diagnoses for SHOX Deficiency

An algorithm approach to *SHOX* mutation screening in a short child is shown in figure 4. The vast majority of cases with idiopathic short stature (85–98%) are not caused by *SHOX* deficiency [13–15]. In around 10–50% of cases, Leri-Weill syndrome is not caused by *SHOX* deficiency [16, 17]. In these instances, there is no approval for GH therapy in Europe.

Acquired Madelung deformity due to trauma or infection should be excluded by evaluation of the history. In males, deletions of *SHOX* can be associated with deletions of other genes on the X chromosome causing a contiguous gene deletion syndrome which can not be compensated for by the Y chromosome [40]. These boys may have ichthyosis, learning difficulties, Kallmann syndrome, and chondrodysplasia punctata. In females, Turner syndrome should be excluded by karyotyping.

Therapy of Short Stature due to SHOX Deficiency

After the initial discovery of *SHOX* mutations in 2 siblings from our clinic, recombinant GH was discussed as a potential drug for growth promotion. If *SHOX* deficiency is the main cause of short stature in girls with Turner syndrome, where GH therapy is efficient and safe, it seemed appropriate to perform a trial with GH in these short children [13]. This trial was efficient in the short term and in the long term [13, 41].

In the meantime, GH therapy has been approved by the FDA and EMEA based on the data of a randomized controlled trial. This 2-year prospective open-label parallel study randomized 2 cohorts of *SHOX*-deficient patients into a treatment group and a control group [42]. In parallel, a cohort of girls with Turner syndrome was treated with GH. The mean age at the start of therapy was 7.5 years, and the GH dose was 50 $\mu\text{g}/\text{kg}\cdot\text{d}$.

The primary outcome variable was height velocity during the first year of therapy. The untreated cohort grew with a normal height velocity and their height SDS stayed unchanged. The GH-treated cohort grew faster and as fast as the girls with Turner syndrome treated in parallel. The treated group gained 3.5 cm in the first year and 1.9 cm in the second year in comparison to the untreated group [42].

In a retrospective study, final heights were evaluated from a group of children with *SHOX* deficiency who had been on GH treatment for more than 2 years. Although most of these children were treated with a low GH dose (mean initial dose 36 $\mu\text{g}/\text{kg}\cdot\text{d}$), the calculated overall gain in height (7 cm) was not different to the mean gain in height in treated Turner girls [41].

Conclusions

SHOX deficiency is frequently found (2–15%) in individuals with formerly idiopathic short stature, causes Leri-Weill syndrome (dyschondrosteosis) in the majority of cases, and is involved in the short stature phenotype of Turner syndrome. The actual mutational analysis includes MLPA analysis followed by gene sequencing. Human expression is found in the early human embryo as well as in the child whose hypertrophic chondrocytes of the growth plate express *SHOX*. In vitro experiments suggest an antiproliferative potency of *SHOX*. The penetrance of *SHOX* deficiency is high but its clinical expression is very variable, becoming more pronounced with age and being more severe in females. Growth failure starts early during the first years of life, and the mean height reached is around 2 SD below the mean. Auxological analysis of the body proportions (mesomelia), the presence of some minor abnormalities, and the search for subtle radiographic signs are important keys to the diagnosis which has to be confirmed by genetic analysis. The growth-promoting effect of GH therapy which has been approved for growth promotion in individuals with *SHOX* mutations by FDA and EMEA seems to be equal to the effect reached in Turner syndrome. In the future, the elucidation of upstream and downstream partners of *SHOX* will help to understand the high clinical variability of *SHOX* deficiency and may provide new drug targets for growth promotion.

Acknowledgements

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