Journal Pre-proofs

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PII: S0378-1119(22)00057-9
DOI: https://doi.org/10.1016/j.gene.2022.146238
Reference: GENE 146238

To appear in: *Gene Gene*

Received Date: 3 September 2021
Revised Date: 12 January 2022
Accepted Date: 18 January 2022

Please cite this article as: Z. Spurna, P. Capkova, J. Srovnal, J. Duchoslavova, L. Punova, D. Aleksijevic, R. Vrtel, Clinical impact of variants in non-coding regions of *SHOX* - current knowledge, *Gene Gene* (2022), doi: https://doi.org/10.1016/j.gene.2022.146238

Clinical impact of variants in non-coding regions of *SHOX* - current knowledge.

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Keywords:

SHOX, ISS, LWD, CNV, SNV, non-coding region

Grants or fellowships supporting the writing of the paper:

This review was supported by Palacky University Olomouc (LF 2021_019), MH CZ – DRO (FNOL, 00098892) and European Regional Development Fund (ENOCH CZ.02.1.01/0.0/0.0/16_019/0000868, ACGT CZ.02.1.01/0.0/0.0/16_026/0008448).

Disclosure Summary

Zuzana Capkova received internal grant support from University Hospital Olomouc (FNOl, 00098892). Josef Srovnal received grant support from European Regional Development Fund (ENOCH CZ.02.1.01/0.0/0.0/16_019/0000868, ACGT CZ.02.1.01/0.0/0.0/16_026/0008448) and internal grant support from Palacky University Olomouc (LF 2021_019). Pavlina Capkova, Jana Duchoslavova, Lucia Punova, Darina Aleksijevic participate on the solution of the grant project FNOl, 00098892. Lucia Punova participates on the solution of the grant project LF 2021_019. Radek Vrtel and Marian Hajduch have nothing to declare.

Abstract:

The short stature homeobox-containing (*SHOX*) is the most frequently analysed gene in patients classified as short stature patients (ISS) or diagnosed with Leri-Weill dyschondrosteosis (LWD), Langer mesomelic dysplasia (LMD), or Madelung deformity (MD). However, clinical testing of this gene focuses primarily on single nucleotide variants (SNV) in its coding sequences and copy number variants (CNV) overlapping *SHOX* gene. This review summarizes the clinical impact of variants in noncoding regions of *SHOX*.

Recent findings: CNV extending exclusively into the regulatory elements (i.e., not interrupting the coding sequence) are found more frequently in downstream regulatory elements of *SHOX*. Further, duplications are more frequent than deletions. Interestingly, downstream duplications are more common than deletions in patients with ISS or LWD but no such differences exist for upstream CNV. Moreover, the presence of specific CNVs in the patient population suggests the involvement of additional unknown factors. Some of its intronic variants, notably NM_000451.3(SHOX):c.-9delG and c.-65C>A in the 5´UTR, have unclear clinical roles. However, these intronic SNV may increase the probability that other CNV will arise *de novo* in the *SHOX* gene based on homologous recombination or incorrect splicing of mRNA.

Summary: This review highlights the clinical impact of noncoding changes in the *SHOX* gene and the need to apply new technologies and genotype-phenotype correlation in their analysis.

Background

Short stature homeobox-containing (*SHOX*; MIM *312865) is one of the most frequently analysed genes in patients classified as short stature patients (ISS) or diagnosed with Leri-Weill dyschondrosteosis (LWD), Langer mesomelic dysplasia (LMD), or Madelung deformity (MD). Each of these disorders are caused by different aberrations of the *SHOX* gene (Costantini et al 2021, Kurnaz et al 2018, Rappold et al 2006). Recent studies have shown that aberrant *SHOX* is present in 5.0 % of ISS cases, up to 70 % of LWD cases, and in all LMD and MD cases (Capkova et al 2020, Binder et al 2004, Wit et al 2016, Shima et al 2016). Although the clinical impact of changes in the SHOX gene has been studied, such studies have focused mainly on changes occurring in its coding regions; the effects of changes in its regulatory/non-coding elements have received less attention (Hattori et al 2017, Dávid et al 2017). However, it is known that changes in non-coding regions can reduce gene expression and cause incorrect splicing of messenger RNA (mRNA) or misfolding of polypeptide strands (Kumar et al 2018, Durand et al 2011). This review summarizes the published data on changes in the non-coding regions of *SHOX* and their impact on human growth.

Constitution and expression of *SHOX* gene

The human genome contains two copies of *SHOX*; one each on the X and Y chromosomes in males, and one on each X chromosome in females (Rao et al 1997). *SHOX* escapes X chromosome inactivation (also known as lyonization) during embryogenesis and exhibits biallelic expression in both genders. Both copies of the gene are needed for correct development (Carrel and Willard 2005).

SHOX has seven exons encoding amino acid sequences (excluding exon 1), five introns, three untranslated regions (5´UTR, SHOXa - 3´UTR, SHOXb - 3´UTR), and eleven regulatory regions (CNE-2, CNE-3, CNE-5, CNE2, CNE3, CNE4, CNE5, CNE6, CNE7, CNE8, CNE9). All of these regions are necessary for correct expression of the gene and folding of the resulting protein (Navaro et al 2021)(Fig. 1).

The expression of any gene starts with its transcription. Transcription is the complex process of copying a segment of DNA into pre-ribonucleic acid (pre-mRNA). Deoxyribonucleic acid (DNA) and pre-mRNA differ in their primary and secondary structure. The primary structure of DNA is defined by the order in which the bases (also known nucleotides) A (adenine), T (thymine), G (guanine), and C (cytosine) appear; in pre-mRNA, T is replaced by uracil (U). The order of the nucleotides is called the *sequence* of the DNA. The secondary structure is defined by the interactions between the nucleotides; in the case of DNA, these interactions cause the formation of a double-stranded structure whereas pre-mRNA is single-stranded (Xu et al 2020, Mohd et al 2021). Transcription is initiated by the binding of RNA polymerase II to DNA. Binding occurs at the 5´UTR (5´end of untranslated region) region of the gene, which is referred to as the *promoter*. Double-stranded DNA is untangled and serves as a template for

the correct ordering of nucleotides in prepared pre-mRNA. The RNA polymerase then moves along the DNA, extending the pre-mRNA chain as it goes, until it reaches the 3´UTR (3´end of untranslated region) region of the gene, which contains a termination sequence recognized by the polymerase. This generates a pre-mRNA molecule that contains all of the gene's exons, introns, and UTRs. The pre-mRNA then undergoes a process known as splicing during which the introns and sometimes some exons are removed to form the final mRNA, which is known as the transcript. Different transcripts of the same gene may have different numbers of exons or the same exons in different orders. Although the splicing process can generate several different transcripts from a single gene, it is a highly controlled process that is governed by special sequences located at the borders between exons and introns that function as "barcodes" to guide the action of the spliceosome – the protein complex responsible for splicing (Griffiths et al 2000).

After translation, the mRNA is transported from the nucleus to the cytoplasm and translated. During translation, the mRNA's nucleotide sequence (i.e. the order in which the nucleotides appear) is used to control the synthesis of proteins; sequences of three consecutive nucleotides known as *triplets* or *codons* within the mRNA encode specific amino acids (AA), which are bound together to form the protein. Each *codon* is specific for a particular AA. While the UTR regions and some exons (such as exon 1 and part of exon 2 in the case of *SHOX*) are not translated, their presence is important for successful translation (Griffiths et al 2000)(Fig. 2).

Two major *SHOX* transcripts have been reported. Transcript variant 1 (NM_000451.3, 7934 nt, 878 amino acids) is produced preferentially and corresponds to the longer *SHOXa* form of the gene, while variant 2 encodes the *SHOXb* form (SHOX transcript variant 2, NM_006883.2). Both transcripts include exons 1-5 but they differ in the last exon: *SHOXa* lacks exon 7, while *SHOXb* lacks exon 6 (Binder et al 2005, Oliveira et al 2011) (Fig. 2). Other *SHOX* mRNAs have also been identified but are only weakly expressed in most tissues and their effects are unclear (Durand et al 2011).

The resulting SHOX protein has been detected in prehypertrophic and hypertrophic chondrocytes of fetal and childhood growth plates by immunohistochemistry (Beiser et al 2014). Additionally, it was identified as a growth regulator that functions as a transcriptional activator of genes including Natriuretic peptide B (*NPPB*) as well as a repressor of other genes including Fibroblast growth factor receptor 3 (*FGFR3*) (Yokokura et al 2017, Rappold et al 2012). Functional studies have shown that the SHOX protein can induce growth arrest and apoptosis, suggesting that it may regulate chondrocyte hypertrophy by inducing apoptosis (Hristov et al 2014). Furthermore, SHOX interacts with SOX5 and SOX6. This interaction regulates aggrecan, which is a key factor of chondrogenesis that plays an important role in cartilage matrix synthesis (Jee et al 2018, Aza-Carmona et al 2011).

In addition to being expressed in growth-related tissues, *SHOX* is expressed in the developing limb bud at Carnegie stage 14 (33 days post-conception). Its mRNA has also been detected in muscles, skin, and several neural tissues including the brain, spinal cord, eye, meninges, cerebellum, thalamus, and basal ganglia of embryos and foetuses. In adult tissues, *SHOX* has been detected in the bone marrow, adipose tissue, placenta and skeletal muscle as well as the thalamus, cerebellum, and frontal cortex (Durand et al 2011).

The *SHOX* gene includes approximately 35 thousand nucleotides (Stelzer et al 2016). However, clinical studies on its effects have focuses mainly on its coding regions (SHOXa = 7.9 kb, SHOXb = 1.9 kb), i.e. the regions that encode the polypeptide sequence of the protein produced by its translation (Landrum et al 2018). It is believed that approximately 80 % of genetic disorders are caused by coding abnormalities (Jackson et al 2018). Base pair changes in genes can be analysed using sequencing technique such as Sanger sequencing (SS) or next generation sequencing (NGS) methods (Lashari et al 2013, Lee et al 2021, Capkova 2020, Wit et al 2016, Shima et al 2016). However, conventional G-banding and karyotyping are used to classify the X and Y chromosomes on which SHOX is located (Yunis et al 1978).

Although it may seem at first glance that the *SHOX* gene has been studied relatively thoroughly, it is important to realise that only specific aspects of its variability have actually been studied. Additionally, some of the reported findings concerning its effects lack detailed information on the associated clinical phenotype or on other important issues (Firth et al 2009, Stelzer et al 2016).

Clinical phenotype of *SHOX* haploinsufficiency

Patients with *SHOX* deficiencies are characterized by decreased cortical volumetric bone mineral density and cortical thickness together with enlarged diaphysis (Soucek et al 2013). Histopathological testing can be used to clarify the diagnosis of such patients based on the disruption of the growth plates and their architecture and the (ir)regularity of chondrocyte stacking (Beiser et al 2014). Additionally, *SHOX* overexpression is known to cause blockage of the cell cycle, arresting proliferation and inducing apoptosis of hypertrophic chondrocytes in the epiphyseal growth plate (Binder et al 2011).

Two functional copies of *SHOX* are needed for correct development of the long bones in humans. If one copy of *SHOX* is damaged (heterozygous), Leri-Weill dyschondrosteosis (LWD, OMIM: 127300) is observed. Langer mesomelic dysplasia (LMD, MIM: 249700) occurs when both copies of *SHOX* are damaged (homozygous) (Ogushi et al 2019, Schneider et al 2005, Tung et al 2018; Benito-Sanz et al 2012, Costantini et al2021).

These genotypes have been linked to intellectual disability, autism, and language impairment in some cases, but there is little evidence indicating that these are general consequences of such deficiencies (Firth et al 2009, Tropeano et al 2016). Aberrations in *SHOX* expression are identified as the cause of idiopathic short stature (ISS; OMIM 300582) in 1.9 - 22.2 % of all cases, but the severity of the condition is highly variable; in some cases, it causes severe growth impairment while in others the individual's height remains within the normal range. Abnormal body proportions may also result, resulting in a sitting height/height ratio for age and sex (SH/H SDS) above 2 (Binder et al 2005). Other minor abnormalities may also be observed in subjects with ISS and *SHOX* deficiency such as shortening of the fourth and fifth metacarpals, a high-arched palate, increased angle of the elbow, scoliosis, and micrognathia (Faienza et al 2021, Binder et al 2005).

Regulatory regions of *SHOX* (CNE)

Regulatory elements of genes may be either enhancers or repressors, and they can be located at some distance from the gene itself (Chatterjee et al 2017). These elements are essential for controlling gene expression and for allowing genes to be expressed at different levels in different tissues (Chen et al 2009). Many of them are highly evolutionarily conserved, occurring in distantly related species such as chicken and zebrafish, and are therefore termed conserved non-coding elements or CNEs (Durand et al 2010, Kenyon et al 2011).

The existence of regulatory elements that are widely separated from the *SHOX* gene has been suggested but their number and location remain to be elucidated. Moreover, the effects of such CNEs on clinical phenotype are unclear (Durand et al 2010).

Three upstream regulatory regions of *SHOX* have been identified (CNE-2, CNE-3, CNE-5) along with eight conserved non-coding DNA elements (CNE2, CNE3, CNE4, CNE5, CNE6, CNE7, CNE8, CNE9) (Fukami et al 2006, Chen et al 2009, Durand et al 2010, Verdin et al 2015, Homma et al 2018)(Tab. 1).

CNVs that disrupt CNEs could potentially have phenotypic effects similar to that of changes in the gene itself. Recent studies have shown that the frequency of CNVs is somewhat higher in the downstream elements of *SHOX* than in its upstream elements, and that duplications are more frequent than deletions (Shima H et al 2018, Fukami et al 2006). However, other studies found that deletions of downstream elements are rarer than duplications in patients with ISS or LWD (deletions = 27, duplications = 12) but that no such difference existed among CNVs in upstream elements Capkova et al 2020, Shima H et al 2018, Chen J et al 2009 Sandoval GT et al 2014, Benito-Sanz S et al 2006)(Tab. 2). Other studies have found that the frequency of CNVs in CNEs is 10 – 24 times higher in LWD patients than in ISS patients (ISS = 1.28 % - 1.61 %, LWD = 12.5% - 38.46 %) (Shima H et al 2018, Chen J et al 2009, Sandoval GT et al 2014, Benito-Sanz S et al 2006, Bunyan et al 2013)(Tab.2). ISS, MD, LWD,

microcephalus and disproportionate growth have been reported in patients with CNV in downstream CNE. However, these variants are also detected in the healthy population (Chen J et al 2009, Fukami et al 2006, Capkova et al 2020).

Untranslated regions (UTR)

The untranslated regions (UTRs) are the sequences on either side of a gene's coding sequence. They are transcribed to pre-mRNA despite not encoding amino acids (Hinnebusch et al 2016). The UTR on the 5' side of the gene is called the 5'UTR or leader sequence and is critical for binding to the ribosome and recognition of the start coding. Accordingly, it strongly affects translation efficiency and helps shape the cellular proteome (Hinnebusch et al 2016). The UTR on the 3' side is called the 3'UTR or trailer sequence, and is a powerful regulatory element that determines the rate at which protein translation proceeds (Schwerk et al 2015).

The 5'UTR region is the major determinant controlling the initial steps of protein expression (Hinnebusch et al 2016). The *SHOXa* and *SHOXb* mRNAs both include the 5'UTR located upstream of the gene, which consists of the non-coding exon 1 and part of exon 2. It is 694 nucleotides in length and has a GC content of 63 %. Interestingly, it contains 7 AUG triplets, each of which is associated with different open reading frames (ORFs). In addition, its Gibbs free energy of folding is high (ΔG = -282 kcal/mol), indicating that it has a highly stable secondary structure, suggesting that the 5'UTR plays a key regulatory role (Blaschke et al 2003).

Although hundreds of variants in the 5´UTR region have been recorded in genetic databases (TOPMED 2021, Karczewski et al 2020, Kopanos et al 2019), only 14 have been associated with ISS (Landrum et al 2018, Alharthi et al 2017, Solc et al 2014, Babu et al 2021)(Tab. 3). The pathogenicity of these variants was estimated using different prediction tools and functional studies on animal models (McLaren et al 2016, Babu et al 2021, Kopanos et al 2019, TOPMed 2021). Three of them (NM_000451.3(SHOX):c.-646_-645insTGT, c.-51G>A, and c.-19G>A) are considered to be likely pathogenic/pathogenic (Landrum et al 2018). This conclusion is supported by their frequency in the population and the 0 % frequency of homozygotes in the population (Kopanos et al 2019, TOPMed 2021). Additionally, the Ensembl Transcript Support Level tool (TSL) suggests that the mRNA sequences containing these SNV have secondary structures with low stability. A link between these SNVs and decreased *SHOX* expression was confirmed by a functional study on the c.-51G>A and c.-19G>A mutations (Kopanos et al 2019, Babu et al 2021). In contrast, the likely benign/benign variants NM_000451.3(SHOX):c.-58G>A, c.-55C>T, and c.-19G>C are frequent in the population, exist in homozygous form, and the stability of the corresponding transcripts is supported by TSL (Kopanos et al 2019, TOPMed 2021). In addition, a

functional study confirmed that these variants have no effect on *SHOX* expression (Babu et al 2021). Similarly, the variants NM_000451.3(SHOX): c.-644T>G, c.-512C>A, c.-507G>C, c.-372G>A, and c.-112G>A are likely benign/benign because it was shown that their frequency in ISS patients is not significantly greater than in the general population (Solc et al 2014).

However, two variants have an as-yet unknown role in ISS patients. NM_000451.3(SHOX):c.-9delG was confirmed to reduce *SHOX* expression in an animal model (Babu et al 2021) but it was considered as likely benign by Landrum et al. (2018) with a frequency 0.0437 % (Kopanos et al 2019). Homozygosity is not observed for the second variant in this group, c.-65C>A, and its frequency in the population is low, ranging from 0,000378 to 0.00319 % (Kopanos et al 2019, TOPMed 2021).

SHOXa and *SHOXb* have different 3´UTRs (3´UTR-a and 3´UTR-b, respectively). 3´UTR-a derives from exon 6 (3´UTR-a, length: 2187 nt) and does not include exon 7, which encodes 3´UTR-b. Conversely, 3´UTR-b derives from exon 7 (3´UTR-b, length: 582 nt) and excludes exon 6 (Uhlen et al 2017). The number of variants in the 3´UTRs is lower than in the 5´UTR and their frequency in the population is between 0.000378% - 56 % (TOPMed 2021). To date, 3´UTRs variants have not been associated with ISS patients in databases or the literature (Landrum et al., 2018).

Introns

Introns are non-coding sequences within genes located between the exons; they are typically excised from the pre-mRNA after transcription during the splicing process. Splicing allows the creation of multiple forms of the *SHOX* mRNA, and its is possible that intronic variants could adversely affect the splicing process, resulting in the production of incorrect mRNA or interchromosomal rearrangement (Garrido-Martín et al 2021). The *SHOX* gene contains 5 introns, with intron 5 being interrupted by the alternative terminal exon 6a (Navarro et al 2021).

Repetitive parts (*Alu* elements belonging to the interspersed repeat sub-family of endogenous retrovirus group K (ERVK)) have been identified as the cause of deletions/duplications of *SHOX* during non-homologous/homologous recombination. *Alu* elements were identified mainly in introns 3 (comprising 13.76 % of the intron sequence) and 5 (12.90 % of the intron sequence). These introns exhibit 78 % homology, which could lead to deletions/duplications of exons 4 and 5 or downstream regions by homologous recombination. Additionally, there is a non-homologous downstream locus containing an interspersed repeat belonging to the ERVK sub-family that causes the exclusion of exon 6a. Approximately half of the detected partial deletions/duplications in *SHOX* are due to hot spots in intron 3. The deletions have been associated with LWD and ISS, while duplications are linked to Mayer-

Rokitansky-Kuster-Hauser Syndrome and autism spectrum disorders (Benito-Sanz S et al 2017, Alexandrou et al 2016).

Single nucleotide variants (SNVs) located near exon-intron boundaries can also adversely affect pre-mRNA splicing. Of the known splicing variants of *SHOX* (TOPMED 2021, Landrum et al 2018), only c.544+1G>A and c.278-1G>C have been associated with ISS (Kumar et al 2020, Landrum et al 2018). Two variants, c.634-3C>T (3 tools, NM_000451.3) and c.486+3A>G, c.634-7C>T (2 tools, NM_000451.3), were identified as the most probable sources of splicing variants by the PredictSNP2 predictor which uses five tools (CADD, DANN, FATHMM, FunSeq2, GWAVA) (Bendl et al 2016).

Effect of variants in the *SHOX* gene on the treatment of patients with LMD, LWD, and ISS

According to the clinical guidelines, prepuberal children with short stature and haploinsufficiency of *SHOX* gene could be treated with growth hormone replacement therapy (Grimberg A., 2016). The *SHOX* gene haploinsufficiency could be caused by pathogenic or likely pathogenic variants (CNV, SNV, indel) in coding sequence as well as in non-coding regions. Up to date, there were only few pathogenic variants in non-coding regions published (Babu D., 2021). Patients with these variants and ISS could have clinical benefit from the growth hormone therapy. However, further research is needed to reveal the genotype-phenotype correlation of variants in non-coding regions of *SHOX* gene.

Conclusions

This review summarises recent findings concerning molecular defects of the *SHOX* gene in patients with LMD, LWD, and ISS and the phenotype-genotype spectrum of *SHOX* deficiency. Most recently reported studies have focused on CNVs that overlap in whole or in part with the gene itself or critical CNEs and SNV in coding exons (Gürsoy et al 2020). This review complements the existing literature by focusing on genomic changes that do not interrupt the coding sequences of *the SHOX* gene.

The pathogenic impact of CNV overlapping with regulatory elements together with coding sequences of *SHOX* can be explained by disruption of the gene's coding exons (Durand et al 2010, Fukami et al 2006, Chen et al 2009). However, the impact of CNV that overlap only with regulatory elements remains unclear, with the exception of deletions of upstream CNEs (Benito-Sanz et al 2016). In accordance with expectation, CNVs are more frequent in downstream regions than upstream regions. However, surprisingly, deletions are more common than duplications in the downstream region (Benito-Sanz et al 2016). This may reflect some bias in the literature; deletions are more frequently discussed and their clinical impact is often more serious than that of duplications. Although duplications are often considered to cause only mild or non-clinical phenotypes, some studies have provided information on them. For example, whereas deletions were associated with ISS and LWD,

duplications were described in patients with global developmental delay, cognitive impairment, language impairment, and autism (Hirschfeldova et al 2017, Solc et al 2014). This correlates with the assumption of higher expression of the *SHOXb* form in the nervous tissues during embryonal development (Durand et al 2011). Numbers of these variants are inherited and genomic studies have shown that they also occur in the healthy population. However, the possible impact of variable expression and reduced penetrance should be considered as well as additional unknown factors in the genome (Fukami et al 2015, Chen et al 2009, Capkova et al 2020).

Whereas CNVs are rarely found in upstream regions, SNVs in the 5´UTR, exon 2, and part of exon 1 of *SHOX* are frequently associated with ISS. Conflicting interpretations have been reported for c.-9delG, whose effect is currently unknown. However, a recent study indicates that its presence reduces *SHOX* expression (Babu et al 2021). The effects of c.-512C>A and c.-507G>C are also the subject of debate (Babu et al 2021, Kopanos et al 2019, Landrum et al 2018, Karczewski et al 2020). Both variants appeared together in one patient, suggesting that their co-occurrence may cause ISS (Landrum et al 2018).

The *SHOX* gene contains *Alu* repetitive sequences with the potential to cause deletions or duplications of the gene (in whole or in part) in introns 3 and 5. A combined partial deletion of *SHOX* and frameshift variant in exon 6a was reported in a patient with LWD (Benito-Sanz et al 2017). Additionally, splice variants are most frequently located in introns 3 and 5 (NM_000451.3: c.486+3A>G, 634-3C>T, and c.634-7C>T). Similar SNVs have also been observed in intron 4 and intron 1 (c.544+1G>A and c.278-1G>C, respectively) (Solc et al 2014, Alharthi et al 2017, Babu et al 2021, Kopanos et al 2019).

This review summarizes current knowledge about genomic changes of the *SHOX* gene that do not interrupt coding regions and highlights their important roles in ISS or LWD. There is considerable uncertainty about the impact of several of these changes, indicating that further functional and clinical studies in this area are needed.

Acknowledgment

This review was supported by Palacky University Olomouc (LF 2021_019), MH CZ – DRO (FNOL, 00098892) and European Regional Development Fund (ENOCH CZ.02.1.01/0.0/0.0/16_019/0000868, ACGT CZ.02.1.01/0.0/0.0/16_026/0008448).

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Legends for Figures and Tables:

Table 1: Coordinates of CNEs of *SHOX* gene.

Table 2: Descripted deletion/duplication of non-coding regions of SHOX.

Table 3: Variants in 5´UTR associated with clinical phenotype.

Figure 1: Structure of *SHOX* gene and it´s non-coding variants in 5´UTR and introns resulted as pathogenic (red) and unknown/possible pathogenic (brown).

Figure 2: Description of transcription, splicing and translation of *SHOX* gene.

Tables:

Table 1:

		CNEs	NCBI36/hg18	GRCh37/hg19	GRCh38/hg38
Chromosome X	Telomere				
	Upstream	CNE-5	318 357-318 950	398 357-398 950	437 622-438 215
		CNE-3	380 279-380 664	460 279-460 664	499 544-499 929
		CNE-2	436 610-437 229	516 610-517 229	555 875-556 494
	Gene	SHOX	505 079-527 558	585 079-607 558	624 344-646 823
	Downstream	CNE2	588 305-588 743	668 305-668 743	707 570-708 008
		CNE3	614 440-615 062	694 440-695 062	733 705-734 327
		CNE4	634 084-634 753	714 084-714 753	753 349-754 018
		CNE5	670 824-671 850	750 824-751 850	790 089-791 115
		CNE6	685 864-686 360	765 864-766 360	805 129-805 625
		CNE7	700 700-701 220	780 700-781 220	819 965-820 485
		CNE8	731 550-732 300	811 550-812 300	850 815-851 565
		CNE9	754 740-755 572	834 740-835 572	874 005-874 837
		"down"	770 581-949 698	850 581-1 029 698	889 846-1 068 963
	Centromere				

Table 2:

References	Structure of cohort	Large of cohort	Upstream of *SHOX*		Downstream of *SHOX*	
			Deletion	Duplication	Deletion	Duplication
Capkova et al 2020	ISS/LWD	174	0	1	1	7
Hirschfeldova et al 2017*	ISS/LWD	352	0	1	0	5
Bunyan et al 2016*	ISS/LWD	1200	0	2	0	10
Fukami et al 2015	ISS/LWD	245	0	0	0	1
Shima H et al 2018	ISS	312	1	0	1	2
	LWD	16	0	0	1	1
Chen J et al 2009	ISS	735	0	0	10	0
	LWD	58	0	0	14	0
Sandoval GT et al 2014	ISS	62	0	0	0	1
Benito-Sanz S et al 2006	LWD	26	0	0	10	0
Total		3154	1	4	27	27

** deletion excluded, ISS-idiopatic short stature, LWD-Leri-Weill dyschondrosteosis*

Table 3:

Pozition	Identification	Pathogenity in ClinVar	BRAVO	Frequency in Varsome	Homozygotes in Varsome	Citations
Exon 1	NM_000451.3(SHOX):c.-649C>G	Likely benign	0.00264%	N/A	N/A	Landrum et al 201 8#
	NM_000451.3(SHOX):c.-646_-645insTGT/TTG	Pathogenic	0.00038%	N/A	N/A	Alharthi et al 2017
	NM_000451.3(SHOX):c.-644T>G	N/A	0.00189%	N/A	N/A	Solc et al 2014
	NM_000451.3(SHOX):c.-512C>A	Pathogenic/bpn	0.04870%	>0,05 %	yes	Alharthi et al 2017, Solc et al 2014
	NM_000451.3(SHOX):c.-507G>C	Pathogenic/bpn	>0,05 %	>0,05 %	yes	Alharthi et al 2017, Solc et al 2014
Exon 2	NM_000451.3(SHOX):c.-372G>A	Uncertain significance	>0,05 %	>0,05 %	yes	Alharthi et al 2017, Solc et al 2014
	NM_000451.3(SHOX):c.-112G>A	N/A	N/A	N/A	N/A	Solc et al 2014
	NM_000451.3(SHOX):c.-65C>A	Uncertain significance	0.00038%	0.00319 %	no	Landrum et al 2018 #
	NM_000451.3(SHOX):c.-58G>A	Likely benign	0.00076%	N/A	N/A	Babu et al 2021
	NM_000451.3(SHOX):c.-55C>T	Likely benign	0.00038%	>0,05 %	no	Babu et al 2021
	NM_000451.3(SHOX):c.-51G>A	Likely pathogenic	N/A	0.000404 %	no	Babu et al 2021
	NM_000451.3(SHOX):c.-19G>A	Likely pathogenic	0.00038%	0.000803 %	no	Babu et al 2021
	NM_000451.3(SHOX):c.-19G>C	Benign	>0,05 %	>0,05 %	yes	Landrum et al 2018 # #
	NM_000451.3(SHOX):c.-9delG	Likely benign	N/A	0.0437 %*	no	Babu et al 2021

** frequency in Finish european population 0.00462 %, N/A not available, Origin of variants in ClinVar were: # Bioscientia Institut fuer Medizinische Diagnostik GmbH,Sonic Healthcare, 2020; ## Athena Diagnostics Inc, 2017*

Clinical impact of variants in non-coding regions of SHOX - current knowledge.

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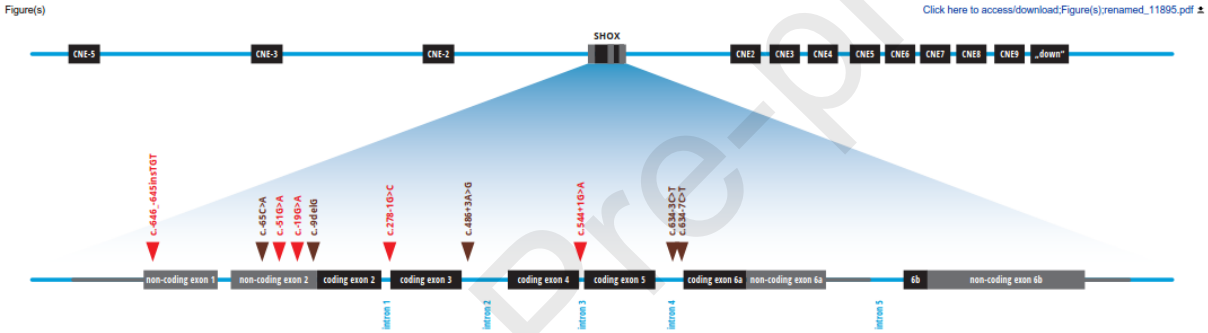
Abbreviations list

CNEs	conserved non-coding elements
CNVs	copy number variants
DNA	deoxyribonucleic acid
ERVK	endogenous retrovirus group K
FGFR3	Fibroblast growth factor receptor 3
ISS	idiopathic short stature
LMD	Langer mesomelic dysplasia
LWD	Leri-Weill dyschondrosteosis
MD	Madelung deformity
mRNA	messenger RNA
NPPB	Natriuretic peptide B
RNA	ribonucleic acid
SHOX	short stature homeobox-containing
SNVs	single nucleotide variants
UTRs	Untranslated regions

Clinical impact of variants in non-coding regions of *SHOX* - current knowledge.

- Noncoding variants of *SHOX* have a similar clinical impact as coding variants
- CNVs of the regulatory elements are more frequently downstream of the *SHOX* gene
- Duplications of *SHOX*s´ downstream are more frequent than deletions
- Intronic variants may cause *de novo* CNV of *SHOX*



Figure(s)

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