

# A SOX5 gene variant as a possible contributor to short stature

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## Summary

SOX5 plays an important role in chondrogenesis and chondrocyte differentiation. SOX5 defects in humans (often deletions) result in a Lamb-Shaffer syndrome (LSS), presenting with speech delay, behavioral problems and minor dysmorphic features. We present a patient with idiopathic short stature (ISS) who carried a heterozygous novel variant in SOX5. The patient had no dysmorphic features, but a skeletal survey revealed minor skeletal abnormalities. Laboratory and endocrine evaluation for known causes of growth disorders was negative. The missense variant in SOX5 gene (c.1783A>G, p.K595E) was *de novo* and was predicted to be deleterious by *in silico* programs. In summary, we present a patient whose presentation may provide evidence that gene defects in SOX5 may contribute to the etiology of short stature and/or mild skeletal defects beyond LSS.

## Learning points:

- We report a girl with idiopathic short stature and mild skeletal defects presenting with a *de novo* variant in SOX5 gene, predicted *in silico* to be deleterious.
- Although SOX5 has not been previously specifically associated with short stature, several evidences support its contributing effect on dyschondrogenesis.
- Missense variants in SOX5 gene may lead to mild phenotypes, differing from typical presentation of patients with Lamb-Shaffer syndrome.

## Background

Proteins of the SOX family are transcription factors essential for the development and differentiation of chondrocytes, neuronal cells and oligodendrocytes (1, 2). Specifically in chondrocytes, SOX5, in cooperation with SOX6 and SOX9, constitute the so called 'SOX-trio' which is involved in cartilage differentiation and promotes skeletogenesis (2). The SOX-trio acts through expression of alpha-1 chain of type II collagen, and cartilage matrix proteins, such as aggrecan, an essential factor of normal skeletal development.

Animal models support the important role of SOX5 in cartilage and bone development. For example, Dy *et al.*

showed that inactivation of *Sox5* and/or *Sox6* in mice leads to destruction of synovial joints beyond the stage of the joint interzone formation (3). While double *Sox5/Sox6* knock-out mice had severe chondrodysplasia and died *in utero*, single *Sox5* knock-out mice presented with milder skeletal abnormalities (3). The chondrodysplastic phenotype in *Sox5* knock-out mice however displays a spectrum and the results have not been replicated in all studies.

With regards to human skeletogenesis, inactivation of the gene has been associated with skeletal abnormalities. Haploinsufficiency of SOX5 on chromosome 12p12.1 is described as the cause of Lamb-Shaffer syndrome (LSS)

(OMIM# 618303) (4). Chromosomal defects, such as whole gene deletions, chromosomal translocations, and more recently single nucleotide variants of the gene, have been identified in these patients (1, 4, 5, 6, 7, 8, 9, 10). Patients with LSS present with intellectual disability, speech delay, behavioral disorders, dysmorphic features (including strabismus, ear abnormalities, low nasal bridge, high forehead, down slanting palpebral fissures, thin upper lip, open mouth, pointed chin and frontal bossing), and musculoskeletal abnormalities (clinodactyly and scoliosis) (4).

We present a patient with idiopathic short stature and certain abnormal findings on skeletal survey. Her genetic evaluation revealed a heterozygous *de novo* variant of the SOX5 gene, which may have caused or contributed to her phenotype.

### Case presentation

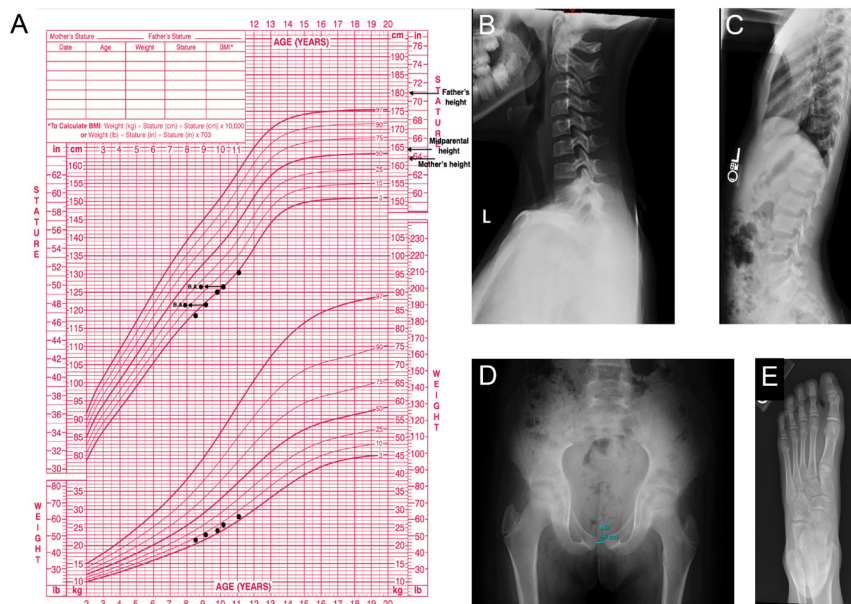
The patient presented at the pediatric endocrine clinic at the age of 8.5 years for evaluation of short stature. She was born at 40 weeks of gestational age with birth weight of 3.5 kg (0 standard deviation score (SDS)) and birth length of 50 cm (0 SDS). Although review of growth charts from birth to 4 years old was not available (patient was living abroad until that age), growth charts from 4 to 8.5 years revealed that at the age of 4 years old, her height was at -1.65 SDS based on 2000 CDC growth charts (shown in Fig. 1A). Her weight had been tracking at -1.65 SDS, with normal BMI. On review of her presentation, she reported normal diet, and denied any gastrointestinal symptom,

headache, visual problem, polyuria or other complain. She was developmentally appropriate and has met all developmental milestones on time. Her past medical history was noncontributory, without any hospitalization or chronic medical condition. Family history did not reveal any significant medical conditions, nor any family member with significant short stature. Her mid-parental target height was 164.5 cm ( $\pm 8.5$  cm) corresponding to 0.18 SDS.

On physical examination her height was 118.6 cm (-2.1 standard deviation score (SDS)), weight 21.7 kg (-1.5 SDS) and BMI was 15.4 kg/m<sup>2</sup> (-0.34 SDS). Sitting height to height ratio was between 75th and 85th percentile based on NHANES standards of growth and between +1 and +2 SDS based on Fredriks *et al.* standard of growth for the Dutch population (11). Arm span was 4 cm shorter than standing height. She did not have any apparent dysmorphic features, including no clinodactyly, scoliosis, frontal bossing, abnormally shaped or positioned ears, nor any significant eye or teeth findings. She was Tanner 1 for breast development and pubic hair.

### Investigation

Her initial laboratory evaluation, including CBC, chemistry, thyroid function tests, and did not reveal any abnormality, and a karyotype was reported as 46,XX (>50 cells counted). A bone age performed at a chronologic age of 8 years old was consistent with 6 years 10 months old based on Greulich and Pyle method, giving her an estimated predicted adult height of 154 cm, lower than



**Figure 1**  
(A) Growth chart of the patient. BA: bone age. (B, C, D and E) Select images from skeletal survey: (B) Lateral radiograph of cervical spinal, showing slight loss of physiologic cervical lordosis and possible subtle beaking at C3 and C4 vertebral bodies, (C) lateral radiograph of spine showing mild anterior beaking of multiple thoracolumbar vertebral bodies, more prominent at the lumbar spine and slight increased concavity at the endplates of L4 and L5 vertebral bodies, (D) pelvic radiograph with slightly increased pubic symphyseal width at 0.9 cm, (E) foot radiograph showing mild clawing fourth and fifth toes.



her genetic potential. Given an IGF-1 level on the lower half of the normal range (-1.3 SDS) and despite a growth velocity within normal limits (4.4 cm/year), sex-hormone-primed (3 days of oral estradiol) GH stimulation test (with sequential use of arginine and clonidine during the same day) was performed and showed an adequate GH secretion (peak level: 17.6 ng/mL after clonidine administration). Due to lack of evidence for other endocrine causes of short stature (TSH: 4.87 mIU/mL (normal limits: 0.48–4.81) and free thyroxine: 1.2 ng/dL (normal limits: 0.9–1.7)), further genetic, and imaging studies were pursued.

Clinical WES (performed in GEnEDx, Inc, Gaithersburg, Maryland) revealed a *de novo* (after paternity confirmation) heterozygous single nucleotide variant of uncertain significance (VUS) in *SOX5* (c.1783A>G, p.K595E, NM\_006940.5). Chromosome microarray analysis excluded copy number variations. The variant is located in the high-mobility-group (HMG) domain of the gene and has not been reported in a large database of general population genetic data (gnomAD, accessed 3/1/2020). *In silico* programs predicted this variant to be probably damaging (PolyPhen-2 score: 1.000), deleterious (PROVEAN score: -3.816), or not tolerated (SIFT). The amino acid is highly conserved among several species (shown in Fig. 2). No other variant potentially pathogenic has been identified in genes known to be associated with short stature (such as *SHOX*, *NPR2*, *FGFR3*, *ACAN* and others), although evaluation for small deletions/duplications of *SHOX* with multiplex ligation-dependent probe amplification (MLPA) or similar techniques has not been completed and is included in future plans. The patient was also found to harbor a heterozygous variant in *CYP11A1* gene (c.940 G>A, p.E314K), inherited from her father.

A skeletal survey was performed to investigate potential additional evidence for skeletal dysplasia (shown in Fig. 1B, C, D and E). Slight loss of physiologic cervical lordosis with possible subtle beaking at C3 and C4 vertebral bodies were identified. No significant scoliosis was noted. There was mild anterior beaking of multiple thoracolumbar vertebral bodies; the defects were more prominent at the

lumbar spine, along with slight increased concavity at the endplates of L4 and L5 vertebral bodies. Additionally, subtle prominence of terminal phalangeal tufts of the second through fourth fingers was noted. Possibly slightly increased pubic symphyseal width at 0.9 cm was noted while bilateral obturator foramina appeared smaller due to increased angulation. There was mild clawing of the fourth and fifth toes bilaterally.

## Discussion

We present a patient who was referred for evaluation of possible idiopathic short stature (ISS). On examination she was found to have subtle skeletal abnormalities; she had no endocrine defects, except for biochemical evidence of mild subclinical hypothyroidism which could not explain her presentation and later resolved without treatment. The finding of a gene variant in a gene (*SOX5*) involved in cartilage and chondrocyte differentiation suggested that *SOX5* dysfunction may have been the cause or at least contributed to her presentation.

Review of the literature shows that patients with *de novo* or inherited gene defects of the *SOX5* gene presented often with skeletal abnormalities, along with other dysmorphic characteristics (Table 1). Some of the reported patients also presented with short stature (<-2 SDS in certain cases), although this finding was not consistent in all patients (Table 1). Skeletal features previously reported included narrow chest, high or narrow palate, micrognathia, brachycephaly, abnormally rotated ears, rhizomelia, clinodactyly, arachnodactyly or short fingers and metacarpals, flat or high arched feet (1, 4, 5, 6). Similarly, our patient's presentation involved mild skeletal abnormalities along with abnormal growth.

Our patient however does not fit the complete diagnosis of LSS since she does not have developmental delay (although thorough assessment of language abilities has not been performed), nor any of the previously mentioned characteristic dysmorphic features. She also does not present with any major skeletal deformities (like cubitus valgus, Madelung deformity, bowing of the forearm, dislocation of ulna at elbow, genu valgum, short hand or feet) except for the mild findings as mentioned above (Fig. 2E). Recently, 16 patients with missense variants were reported expanding the spectrum of the syndrome (8). Indeed, although developmental delay was a consistent finding in this cohort, a spectrum of presentations was reported. Of note, missense variants located in the HMG domain of the gene, were consistently associated with impaired DNA binding.

Homo sapiens	547	RGRGSNEPHIKRPMNAFMVWAKDERRKILQAFPMHNSNISKILGSRWKA	596
Macaca mulatta	547	RGRGSNEPHIKRPMNAFMVWAKDERRKILQAFPMHNSNISKILGSRWKA	596
Canis lupus familiaris	583	RGRGSNEPHIKRPMNAFMVWAKDERRKILQAFPMHNSNISKILGSRWKA	632
Bos taurus	512	RGRGSNEPHIKRPMNAFMVWAKDERRKILQAFPMHNSNISKILGSRWKA	561
Mus musculus	547	RGRGSNEPHIKRPMNAFMVWAKDERRKILQAFPMHNSNISKILGSRWKA	596
Rattus norvegicus	547	RGRGSNEPHIKRPMNAFMVWAKDERRKILQAFPMHNSNISKILGSRWKA	596
Gallus gallus	521	RGRGSNEPHIKRPMNAFMVWAKDERRKILQAFPMHNSNISKILGSRWKA	570
Danio rerio	549	RGRGSSEPHIKRPVNAFMVWAKDERRKILQAFPMHNSNISKILGSRWKA	598
Xenopus tropicalis	548	RGRGSNEPHIKRPMNAFMVWAKDERRKILQAFPMHNSNISKILGSRWKA	597

**Figure 2**

Conservation analysis of amino acid K595 (marked in red box) shows significant conservation among several species.



**Table 1** Review of the available literature for patients with *SOX5* gene defects, including anthropometric data (where available) and skeletal features.

Reference	Number of patients	<i>SOX5</i> gene defect	Height SDS range at latest evaluation	Weight SDS range at latest evaluation	Skeletal features
Schanze <i>et al.</i> (1)	3	Deletion	-1.8 to 0	-0.4 to 0	High forehead, broad nasal bridge, upturned nose with bulbous tip, narrow chest, flaring of anterior ribs, 5th finger clinodactyly, short 3rd phalanges, brachydactyly in toes, flat feet
Lamb <i>et al.</i> (6)	16	Deletion	<-2 to 1.3	<-2 to 2.1	Frontal bossing, brachycephaly, flat occiput, board and low nasal bridge, upturned nose, crowded teeth, high and narrow palate, micrognathia, arachnodactyly, short fingers and metacarpals, prominent fingertip pads, clinodactyly of toes, flat feet, high arched feet, short toes, rhizomelia
Fukushi <i>et al.</i> (7)	1	Balanced reciprocal translocation	-0.9	-1.0	Depressed nasal bridge, clinodactyly of fingers and toes
Lee <i>et al.</i> (8)	2	Deletion	-1.6 to -0.9	-1.3 to -0.1	Frontal bossing, bulbous tip of nose, depressed nasal bridge, mild right facial hypoplasia
Quintela <i>et al.</i> (9)	1	Deletion	Reported as normal	Reported as normal	Triangular face, short and narrow forehead, mild upturned and bulbous nasal tip, high palate
Zawerton <i>et al.</i> (10)	41	Deletion, Single nucleotide variant	-2.6 to +2.7	-2.7 to >+5	Microcephaly, fused vertebrae, scoliosis, thoracic kyphosis, toe syndactyly, joint hyperlaxity, hip dysplasia
Nesbitt <i>et al.</i> (11)	1	Single nucleotide variant	0.65	-1.45	Prominent maxilla, narrow palate, overcrowded teeth, retrognathia, marfanoid appearance of hands and feet, thoracic kyphoscoliosis, lumbar lordosis, pectus carinatum, toe syndactyly
Zech <i>et al.</i> (12)	2	Single nucleotide variant	NR	NR	No abnormal skeletal features reported on physical examination

NR: not reported, SDS: standard deviation score.

Several lines of evidence support a contributing involvement of *SOX5* gene in the skeletal defects of our patient. Data from previous cell and animal studies support an important role of *SOX5* for the differentiation of fibroblasts, osteoblasts and chondrocytes (2). Along with the other transcription factors participating in the SOX-trio, *SOX5* is essential for the expression of chondrogenic genes, like *Col2al*, which when mutated also causes abnormal growth of chondrocytes and growth plate malformation (2). Furthermore, the reported variant in our patient was *de novo*, predicted to be pathogenic by all our *in silico* analyses, and has not been reported in the general population. Last, it is located in the HMG domain, previously reported as highly predictive of impaired DNA binding (8).

In summary, we present the case of a patient with short stature and a *de novo* variant in the *SOX5* gene, that was predicted to be deleterious. Since *SOX5* is involved in cartilage and growth plate differentiation, pathogenic variants of this gene could represent a novel cause of mild skeletal dysplasia leading to decreased height. Whether

*SOX5* contributes significantly in the pathogenesis of short stature in the general population, remains unknown, and larger cohorts of children with familial or sporadic idiopathic short stature would need to be screened for *SOX5* mutations.

#### Declaration of interest

Dr Stratakis holds patents on technologies involving PRKAR1A, PDE11A, GPR101 and their function; his laboratory has already received research funding support by Pfizer Inc. for unrelated to this subject investigation; F R F holds patent on the *GPR101* gene and/or its function.

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#### Patient consent

Written informed consent for participation in the protocol and for all research and genetic studies was obtained by parents, along with assent from the patient.





#### Author contribution statement

A G drafted the manuscript and performed the literature review. C T and C A S provided clinical care for the patient, reviewed and interpreted the genetic studies and corrected the manuscript. B F, F R F, and C A S performed and interpreted the genetic studies.

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