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Case Report

Duplication in the *SHOX* Gene as a Rare Genetic Cause of Short Stature and/or Skeletal Abnormalities: A Clinical Report and Review of the Literature

Turan B et al. Duplication in the SHOX Gene as a Rare Genetic Cause of Short Stature and/or Skeletal Abnormalities: A Clinical Report and Review of the Literature

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What is already known on this topic?
SHOX duplications, although typically associated with tall stature, can result in short stature and/or extremity anomalies in rare cases when partial or complete duplications involving the SHOX region occur. In this case report, two patients with extremity anomalies who were found to have SHOX region duplications with two different clinical features are presented.

What this study adds?
SHOX duplications can result in short, normal, or tall stature, depending on the size, location, and transcriptional characteristics of the duplicated region, such as whether or not it contains non-coding elements. In this case presentation, we aimed to emphasize that SHOX duplications, which cannot be detected by routine short stature panels but which can be identified through advanced investigations, should be considered alongside other common genetic causes in the presence of idiopathic short stature and/or limb anomalies.

ABSTRACT
The *SHOX* (short stature homeobox containing gene) haploinsufficiency can result in phenotypes ranging from idiopathic short stature to Leri-Weill dyschondrosteosis (LWD). It has been reported to have been detected in 5-17% of children diagnosed with idiopathic short stature, and in 60-90% of children with LWD. *SHOX* duplications, although typically associated with tall stature, can result in short stature and/or extremity anomalies in rare cases when partial or complete duplications involving the *SHOX* region occur. In this case report, two patients with extremity anomalies who were found to have *SHOX* region duplications with two different clinical features are presented. The first case was an eleven-month-old male, referred to the pediatric endocrinology clinic due to short stature, and skeletal deformities. On physical examination, the patient's weight was 8.6 kilograms (-1.19 standard deviation score; SDS), and his height was 68 cm (-2.57 SDS). The systemic examination was unremarkable, but examination of the extremities revealed the absence of the right thumb and left forearm bones. Radiographic images of the bones revealed possible rudimentary bone tissue of the radius and ulna in the left upper extremity. DNA extracted from the patient's peripheral blood was subjected to multiplex ligation-dependent probe amplification (MLPA) analysis, which revealed a duplication extending from the upstream regulatory regions of the *SHOX* gene on Xp22.3/Yp11.32 to the downstream CNE8 (conserved noncoding elements) region, including all of the gene's coding regions and upstream regulatory areas. The second case involved a fourteen-month-old male, who was referred after *SHOX* duplication was detected in a microarray analysis performed due to epilepsy. On physical examination, his weight was 10.3 kg (-1.83 SDS), and his height was 79 cm (-0.11 SDS). Systemic examination was normal, but Madelung deformities were observed in the extremity examination. DNA obtained from the patient's peripheral blood was analyzed using MLPA for deletions and duplications of the *SHOX* gene and the associated regulatory regions on Xp22.3/Yp11.32. This analysis revealed a heterozygous duplication which extended from the entire *SHOX* gene and upstream CNE regions to the CNE7/8 regions downstream. *SHOX* duplications can result in short, normal, or tall stature depending on the size, location, and transcriptional characteristics (such as containing non-coding elements) of the duplicated region. This case report emphasizes that, in the presence of idiopathic short stature and/or extremity anomalies, *SHOX* duplications should be considered in addition to the other common genetic causes.
Keywords: Extremity abnormalities, microarray, MLPA, skeletal disorders

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INTRODUCTION
The *SHOX* gene (Short Stature Homeobox-containing gene), located distally on the pseudoautosomal region of the short arm of the sex chromosomes (PAR1), is a transcription factor involved in cell differentiation and organ development [1]. For the normal physiological function of the *SHOX* gene, two active copies of the gene are required. Variants in the *SHOX* gene and its regulatory regions, including changes in copy number, lead to dysfunctions of the gene [2,3]. This condition causes disruptions in the intrinsic pathways of bone and cartilage tissue, resulting in short stature and/or skeletal anomalies [1]. In cases of idiopathic short stature, where the cause of short stature cannot be determined through standard diagnostic methods, and when other systemic diseases, endocrinological disorders, and nutritional pathologies have been excluded, an evaluation for *SHOX* deficiency should be conducted [1,3]. Mutations and deletions in the *SHOX* gene are associated with short stature and extremity anomalies such as Madelung deformity, while duplications are more commonly associated with tall stature [4]. However, *SHOX* gene duplications have also been reported to be associated with short stature in some rare cases as well [4,5]. This case presentation aims to present two cases of patients who presented with limb anomalies and were found to have duplications in the *SHOX* gene through MLPA analysis.

CASE 1
An eleven-month-old male patient was referred to the pediatric endocrinology clinic due to short stature and an absence of the right thumb and left forearm bones. The patient's medical history indicated he was born at 32 weeks gestation to a healthy 32-year-old mother via spontaneous vaginal delivery, weighing 2,990 grams (-0.97 SDS) and measuring 49 cm (-0.58 SDS). No anomalies were reported during the

antenatal period. His neurocognitive development was appropriate for his age, and both parents were healthy with no consanguinity.
On physical examination, his weight was 8.6 kg (-1.19 SDS), his height was 68 cm (-2.57 SDS), and weight for height was %107. The systemic examination was normal. Examination of the extremities revealed the absence of the right thumb and left forearm bones (Figures 1 and 2). Hemogram, biochemical, and hormonal tests were normal. His insulin-like growth factor-1 (IGF-1) level was 35.4 ng/ml (-2.13 SDS). Bone survey imaging revealed possible rudimentary bone tissue of the radius and ulna in the left upper extremity (Figure 3). Evaluations for associated anomalies, including vision, hearing, and cardiological examinations, were normal. The differential diagnosis included Fanconi aplastic anemia, Diamond-Blackfan anemia, and Thrombocytopenia-Radius absence syndrome, which were excluded by pediatric hematology.
DNA analysis was performed using the ligation-based multiplex probe amplification (MLPA) method. A duplication was detected in the *SHOX* gene (NM_000451.3) and its regulatory regions located on Xp22.3/Yp11.32, extending from the upstream regions to the CNE8 region in the downstream area. The duplicated region included the region up to 5.4 kb downstream of CNE8 but did not extend into the CNE9 region (Figure 7).
The patient was monitored for growth velocity; during follow-up, the growth velocity was found to be normal for age, and there was an improvement in height standard deviation score. Growth hormone therapy was not considered.
SHOX MLPA analysis revealed that the mother was normal, while the father was heterozygous for the same copy gain. In patients with a copy gain in this region, growth abnormalities and musculoskeletal system anomalies are expected. Clinical findings typically include intrauterine growth restriction, macrocephaly, excessive growth, and global developmental delay.

CASE 2

A 1-year-and-2-month-old male patient was referred to the pediatric endocrinology clinic after a microarray analysis revealed a duplication in the *SHOX* region during testing for epilepsy. His history indicated he was born via cesarean section at 34 weeks gestation, weighing 2,040 grams (-0.12 SDS) and measuring 48 cm (1.38 SDS). No specific features were noted during the antenatal or postnatal periods. His neurocognitive development was appropriate for his age. The parents were healthy but had fourth degree consanguinity.
On physical examination, his weight was 10.3 kg (-0.3 SDS), his height was 79 cm (-0.11 SDS), and weight for height was %98, second case's was %98. The systemic examination was unremarkable, but examination of the extremities revealed Madelung deformities (Figures 4 and 5). Hemogram, biochemical, and hormonal tests were normal.
For the detection of deletions and duplications in the *SHOX* gene and enhancer regions within the PAR1 region, the first method to consider is chromosomal microarray. In cases where microarray analysis does not detect abnormalities, MLPA is the alternative method [12]. In our first case, both microarray analysis and *SHOX* gene MLPA analysis were performed. The microarray analysis was conducted using the AFFYMETRIX CYTOSCAN OPTIMA kit, which identified five OMIM genes within the Xp22.33 region spanning 605 kbp: [PLCXD1 (300974), GTPBP6 (300124), PPP2R3B (300339), *SHOX* (312865), *SHOX* (400020)]. Additionally, a DNA sample obtained from the patient's peripheral blood was analyzed using the MLPA method for deletions and copy number variations in the *SHOX* gene (NM_000451.3) located at Xp22.3/Yp11.32 The analysis was performed using the P018-*SHOX* MLPA probe mix and COFFALYSER analysis software.
DNA analysis of peripheral blood via the MLPA method revealed a heterozygous duplication involving the entire *SHOX* gene and a significant portion of its regulatory regions, extending to the CNE7/8 region in the downstream area (Figure 8). Segregation analysis showed that the same duplication was present in the father, who was of normal stature and showed no skeletal findings.

DISCUSSION

This case report aims to present two cases with skeletal abnormalities in which a duplication in the *SHOX* gene was identified. An 11-month-old male patient presenting with short stature and extremity anomalies, along with a 2-year-and-2-month-old male patient exhibiting Madelung deformities but without short stature, are reported.
The *SHOX* gene plays a significant role in growth and bone development. The function of the *SHOX* gene is dose-dependent, and point mutations and more frequently deletions typically lead to short stature, while duplications usually result in tall stature [6].
SHOX gene mutations can cause a wide range of phenotypes. Individuals with the same mutations/deletions may present with normal stature, while others may show some features. In cases where mutations occur in the gene itself, disproportionate growth is more commonly observed [7-9].
In addition to these, duplications within the gene itself or in the enhancer regions can also lead to *SHOX* deficiency. It has been shown that, depending on the characteristics of the duplicated region, the gene expression can decrease as a result of structural disruptions in the gene [3,4]. A limited number of cases in the literature have reported short stature following *SHOX* gene duplication [5]. It is believed that partial or full duplications of the *SHOX* gene result in reduced or lost expression of the gene on the allele where the duplication occurs, thereby impairing the gene's function. There are three hypotheses related to this. The first one suggests that the duplicated region may be non-functional because it does not contain necessary regulatory sequences. The second possible reason is that the duplicated region could affect the regulatory sequences of the *SHOX* gene. The third possibility is that the duplication increases the distance between the gene and its regulatory elements, leading to disruption in gene function [11].
For the detection of deletions and duplications in the *SHOX* gene and enhancer regions within the PAR1 region, the first method to consider is chromosomal microarray. In our first case, a heterozygous copy gain was detected covering the entire coding region of the *SHOX* gene, including the regulatory regions extending to the CNE8 region, but excluding the CNE9 region. In patients with a copy gain in this region, growth abnormalities and musculoskeletal system anomalies are expected. Clinical findings typically include intrauterine growth restriction, macrocephaly, excessive growth, and global developmental delay.
In our second case, the *SHOX* region was investigated using the MLPA method, and a heterozygous duplication was detected, covering the entire *SHOX* gene and a significant portion of its regulatory regions, extending to the CNE7/8 region in the downstream area. In contrast to the first case, this patient did not present with short stature, but exhibited Madelung deformity. Although the same duplication was detected in the father, no clinical manifestations were observed.
A review of the literature showed a study by Benito Sanz et al. [5] involving 122 cases of Leri-Weill Dyschondrosteosis (LWD) and 613 cases of idiopathic short stature. In their study, 4 complete and 10 partial *SHOX* duplications were identified. The cases with partial duplications were found to have more frequent skeletal dysplasia features compared to those with complete duplications. These features were predominantly ulna shortening, cubitus valgus, and expansions at the hand joints [5] (Table 1). In our first case, in addition to short stature, skeletal findings were noted, including the absence of the thumb and forearm bones, which were attributable to the *SHOX* duplication.
In a series of 9 cases by David Bunjan et al., 5 cases were diagnosed with idiopathic short stature, while 4 cases were evaluated as having normal stature. The duplication regions in all cases encompassed the entire *SHOX* gene and were considered complete duplications. The duplication region in our second case showed similarities to the P3 case in the series by David Bunjan et al., but with a significantly different clinical presentation [13]. In the case from Bunjan et al., craniosynostosis and intellectual disability were present, whereas in our case, only Madelung deformity was observed. Additionally, the father of our second case also had the same duplication in the same region. His height was normal, and no skeletal findings were present. Our case's father also have the same duplication with their children without any skeletal finding or short stature.
A review of the literature clearly shows that similar duplications are not always characterized by the same phenotype. The size of the duplication, whether or not it includes regulatory regions, and its proximity to the *SHOX* gene are considered to be the factors responsible for

this variability. Additionally, duplications are often detected incidentally and are most frequently inherited from phenotypically normal parents. This leads to a high degree of phenotypic variability between individuals, which can complicate diagnosis and genetic counseling [15].

CONCLUSION

SHOX duplications can result in short, normal, or tall stature, depending on the size, location, and transcriptional characteristics of the duplicated region, such as whether or not it contains non-coding elements. In this case presentation, we aimed to emphasize that *SHOX* duplications, which cannot be detected by routine short stature panels but which can be identified through advanced investigations, should be considered alongside other common genetic causes in the presence of idiopathic short stature and/or limb anomalies.

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Table 1. The assessment of the individuals with *SHOX* gene duplications in the literature.

Study	Sex	Age (year)	Phenotype	Duplication Type (Complete/Partial)	Height (SDS)	Madelung deformity	Other clinical findings
Iughetti et al.. (2000)	Male	13.9	ISS (idiopathic short stature)	Complete	-3.25 SDS	None	-
Benito sanz et al.(2011)	Female	13	ISS	Complete	-2.57 SDS	None	Short neck
	Female	adult	ISS	Complete	-2.19 SDS	None	-

	Female	13.8	ISS	Complete	-2.29 SDS	None	
	Female	6.7	ISS	Partial	-2.01 SDS	None	Atopic dermatitis
	Male	13	LWD	Partial	-2.25 SDS	Present	-
	Male	10.7	LWD	Partial	-1.8 SDS	Present	-
	Male	15.5	LWD	Partial	-3.88 SDS	Present	-
	Female	5	LWD	Partial	-4.13 SDS	Present	-
	Female	13	LWD	Partial	-2.24 SDS	Present	Pyloric stenosis
	Male	10	LWD	Partial	-2.06 SDS	Present	-
	Female	13.5	LWD	Partial	-3.95 SDS	Present	-
	Female	7.5	LWD	Partial	-1.88 SDS	None	Precocious puberty
	Female	14	ISS	Partial	-2.40 SDS	None	Small for Gestational Age
	Female	Adult	ISS	Complete	-2.10 SDS	None	Mental retardation
Davia Bowan et al. (2023)	Female	5	ISS	Complete	<-2 SDS	None	-
	Male	16	ISS	Complete	<-2SDS	None	Brachydactyly
	Male	10	ISS	Complete	<-2SDS	None	Craniosynostosis, mental retardation

	Male	5	-	Complete	<-2SDS	None	Neonatal edema
	Female	0.1	ISS	Complete	<-2SDS	None	-
	Male	4	ISS	Complete	-	None	Epilepsy
	Male	3	-	Complete	0 SDS	None	-
	Female	2	-	Complete	-	None	Heart anomaly, imperforate anus
	Female	1	ISS	Complete	<-2SDS	None	Short [illegible]
Presented case-1	Male	0.9	LWD	Complete	-2.57 SDS	None	Age[illegible]esis of thum[illegible] and [illegible]rea[illegible] bones
Presented case-2	Male	1.1	ISS	Complete	-0.11 SDS	[illegible]	Epilepsy

Figure 1-2. The clinical chara[illegible]eristics of the patient 1.

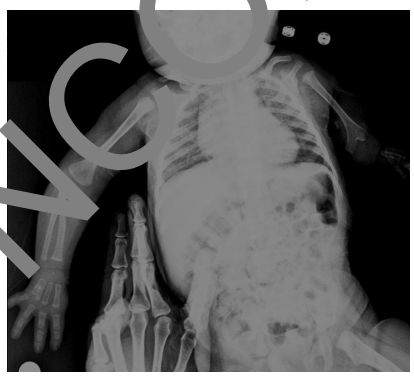

Figure 3. The radiological image of the patient 1.

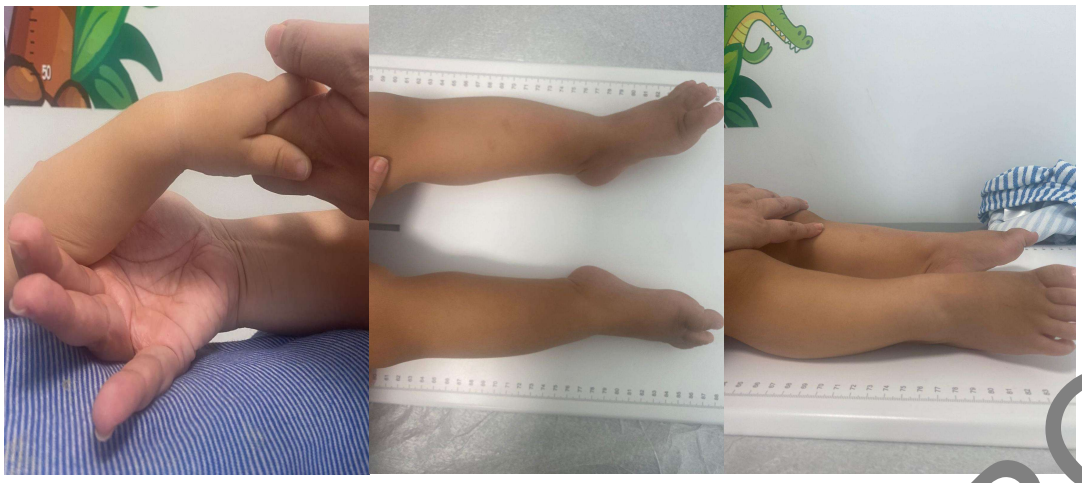

Figure 4-5-6 . The clinical characteristics of the patient 2.