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SHOX Variations in Idiopathic Short Stature in North India and a Review of Cases from Asian Countries

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What is already known on this topic?

The worldwide prevalence of *SHOX* variations in idiopathic short stature (ISS) varies from 2.5% to 15%. *SHOX* variants are one of the most common causes of ISS. *SHOX* deletions are the most common type of variation encountered worldwide.

What this study adds?

This study highlights the role and prevalence of *SHOX* variations in ISS in a North Indian population. In addition, a meta-analysis is presented which compiled findings from the last decade and provides an updated insight into the prevalence of *SHOX* variations in the whole Asian population, underscoring their potential as therapeutic targets in ISS patients.

Abstract

Objective: Short stature homeobox *(SHOX)* haploinsufficiency underlies idiopathic short stature (ISS) and Leri-Weill dyschondrosteosis. The worldwide prevalence of *SHOX* variations in ISS varies from 2.5% to 15.0%. The aim of this study was to assess the implication of *SHOX* variation in ISS in North Indians and compare this with other cases of *SHOX* variations from Asian population.

Methods: *SHOX* gene analysis was carried out by multiplex ligation-dependent probe amplification followed by Sanger sequencing in 54 patients with variable phenotypes. Comparison with other reports in a meta-analysis comprising the current study and 11 previous studies (n = 979) was performed.

Results: *SHOX* analysis resulted in 12.9% positivity (7.4% deletions and 5.5% duplications). *SHOX* association was seen significantly related to gender, with predominance in females (p = 0.047). Short arms and forearms were the only significantly associated trait seen in 51.9% of children. The overall prevalence of *SHOX* variation was 15.2% in Asians with ISS. No significant difference was found in geographical region-specific analysis.

Conclusion: This study summarises findings from the last decade and provides an updated picture of the prevalence of *SHOX* variations in Asians, emphasizing their potential as therapeutic targets in ISS patients. Further high quality, large investigations including functional validation is warranted to validate this association.

Keywords: Idiopathic short stature, *SHOX*, MLPA, Sanger sequencing, meta-analysis, prevalence

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Introduction

Idiopathic short stature (ISS) is defined as height which is more than two standard deviations (>2 SD) below the corresponding average height in a given age, sex, and population without features suggestive of specific causative disorders (1). ISS affects around 2.0% of children and is a genetically heterogeneous condition. With the advance in techniques, a battery of genes is known to be involved in longitudinal growth in human beings, but despite this, genetic causes constitute only a small proportion of cases of short stature (2). One of the genes closely associated with human short stature is the short-stature homeobox-containing gene (*SHOX*), alternatively known as pseudoautosomal homeobox-containing osteogenic gene (3). The importance of this gene is highlighted it is one of the most common genetic causes leading to short stature in children, with either isolated or familial short stature.

The location of *SHOX* is characteristic and is responsible for the variable phenotype. Being located over the tip of the short arms of both sex chromosomes X and Y inside the telomeric region of the pseudoautosomal region 1 (PAR1), named due to its presence over both these chromosomes (4). The location is also pivotal as genes in this area escape the process of lyonization. *SHOX* has been shown to function in a dose-dependent manner, that is loss-of-function variation which involves only a part of the gene, thus involving one of the *SHOX* alleles, technically known as haploinsufficiency, can lead to a wide variety of phenotypes and resulting in short stature of varying degrees (5). Furthermore, *SHOX* deletions are the most common type of variations encountered worldwide, known to account for 80.0% of all variations (6). These deletions can be of varying sizes and can involve either all or part of the gene or regions above and below it, which contain a regulatory enhancer region (7). Other types of variations reported include nonsense and missense sequence alterations. Most common variations are seen in exons 3 and 4, which are known to encode the functional part of this pivotal homeodomain (7).

In different studies from around the world, estimates of the presence of *SHOX* gene variation leading to ISS range from 2.5% to 15.0% (8,9,10,11). However, the prevalence of ISS in India due to *SHOX* variations is not known. In the last few decades, many studies have been done on the association between different gene variations and ISS. The prevalence of *SHOX* variation depends on ethnicity and selection of patients. Some studies from Asian populations have reported important frequencies in comparison to the worldwide populations. In the present study, individuals with ISS, with height <2 SD of population reference range height, with no identified cause were included. These selected individuals had detailed anthropometric evaluation, dysmorphism evaluation and skeletal survey by radiographs. Multiplex ligation-dependent probe amplification (MLPA) and Sanger sequencing of *SHOX* exons 2-6 were done to identify deletions and sequence variations, respectively. In addition, a meta-analysis of other cases from Asia with *SHOX* variations was performed to estimate and evaluate the relationship between *SHOX* variation and ISS in these populations.

Methods

Subjects

This was a prospective study conducted in a tertiary hospital from July 2020 to March 2022, with approval from the Ethics Committee of the Postgraduate Institute of Medical Education & Research (PGIMER) (approval no: NK/6656/MS/315, date: 01.12.2020). A detailed physical examination of children with ISS was performed to look for dysmorphic features, such as micrognathia, high-arched palate, short forearm, cubitus valgus, short hands, characteristic Madelung deformity, dislocation of ulna at the elbow, short lower leg, bowing of tibia, genu valgus, short feet, scoliosis, and/or muscular hypertrophy. Rappold scoring was done for better delineation of the clinical expression in our cohort (12). The children included in the study were measured for the following anthropometric parameters using standardized techniques and instruments, in the growth laboratory/clinic: height (cm); weight (kg); sitting height (cm); and arm span (cm). Body mass index (BMI; kg/m^2) and arm span to height ratio, as well as upper segment to lower segment ratio, was also calculated. Written informed consent was obtained from each patient or their parents or legal guardians, in the case of minors after explaining the purpose and type of all procedures used.

Molecular Analysis

Genomic DNA was extracted from peripheral blood by QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany) for subsequent molecular analysis. *SHOX* analysis was performed using MLPA technique using a SALSA P018-G2 kit (MRC-Holland, Netherlands) as per the manufacturer's instructions. The entire coding region of the *SHOX* gene (exons 2-6) and splice junctions were sequenced by Sanger sequencing using an automatic sequencer, the ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, California). Primer sequences are available upon request.

Meta-analysis

Meta-analysis of prevalence was done using the MedCalc software, version 20.111 (https://www.medcalc.org/). This study followed the PRISMA guidelines (13). To identify the relevant publications concerning *SHOX* gene variations in ISS in Asia, the databases PubMed, Scopus and Web of Science were investigated. The search string was a combination of the following keywords: "Idiopathic Short Stature", "*SHOX*", "deletions/duplications/variations", "copy number variations". All the studies published in last 10 years (2012-2022) were selected for this study. The included studies met the following eligibility criteria: region - Asia; period - last 10 years; language - English; and peer-reviewed publication. Included studies were original research articles, related to *SHOX* prevalence, variations and/or incidence, along with the number of individuals included. The authors independently assessed the risk of bias for the included studies, using the Newcastle-Ottawa Scale (NOS) tool for quality assessment (14).

Statistical Analysis

The strength of the association between *SHOX* variation and ISS in the Asian population was evaluated by calculating the odds ratio using the Mantel-Haenszel statistics method. A fixed/random-effect model was applied, along with a corresponding 95% confidence interval (CI). Fixed-effects models were used to assess the pooled prevalence of genes for results with low heterogeneity (I^2 ≤50%). Otherwise, random-effects models were applied for the analyses. MedCalc software was used for all data synthesis and statistical analyses. Chi^2 and I^2 statistics were used to calculate heterogeneity across individual studies and subgroups. Population bias was assessed by Funnel-plot analysis. In addition, subgroup analysis was performed based on ethnicity or regional variation between subgroups. All statistical analyses were performed by using IBM Statistical Package for the Social Sciences (SPSS) Statistics for Windows, version 22.0 Armonk, NY: IBM Corp.

Results

A total of 54 patients were enrolled. Of these, 5 (9.3%) were in the age group 1-5 years, 20 (37.0%) were in the age group 5-10 years, 26 patients (48.0%) were 10-15 years of age and 3 patients (5.5%) were above 15 years of age. The median (interquartile range) age of children in this study was 11years (8 years, 13 years). The study included 26 females (48.1%) and 28 males (51.9%). Phenotypic characteristics of enrolled patients are given in Table 1. Short upper limbs (arms and forearms) was the most consistent feature (28/54, 51.85%) and was more common than short lower limbs (24/54, 44.4%). Short upper limbs was a significant finding in the variation positive subjects (Table 1). Mean, SD and range for the measured anthropometric parameters among the study cohort is shown in Supplementary Table 1. Most of the anthropometric parameters analyzed using SPSS software represented a moderately skewed distribution (almost towards normal distribution for practical purposes) with a skewness index ranging between +1 to -1. Detailed physical growth and pubertal characteristics of study subjects who were positive for *SHOX* variants (n=7, 13%) are depicted in Table 1. Interestingly, four out of these

Table 1. Association of phenotypic features with *SHOX* mutation

Phenotypic characters	*SHOX* mutation		p
	Yes (n=7)	No (n=47)	
	n (%)	n (%)	
Height (SDS)	-3.1+0.7	-2.7+1.2	0.316
Weight (SDS)	-2.0+0.5	-1.8+1.3	0.669
BMI (SDS)	-0.6+0.3	-0.5+1.2	0.826
Male	1 (1.9%)	27 (50.0%)	**0.047**
Female	6 (11.1%)	20 (37.1%)	
Micrognathia	2 (50.0%)	2 (50.0%)	0.077
High-arched palate	0 (0.0%)	7 (100.0%)	0.576
Short arm and forearm	5 (17.6%)	23 (82.1%)	**0.024**
Cubitus valgus	1 (33.3%)	2 (66.7%)	0.346
Madelung deformity	1 (50.0%)	1 (50.0%)	0.245
Short leg and feet	5 (20.8%)	19 (79.2%)	0.250
Genu varum	1 (0.0%)	0 (0.0%)	0.130
Muscle hypertrophy	0 (0.0%)	0 (0.0%)	-

SDS: standard deviation score, BMI: body mass index

seven cases had initially presented to the endocrinology OPD of Department of Pediatrics and were advised growth hormone therapy. However, due to financial constraints or being lost to follow-up, growth hormone was not initiated in these children, except for one case (Patient 3) in whom a marked increase in height gain was evident [from -6.4 SD score (SDS) at 8 years to -3.3 SDS at 13 years].

Molecular Analysis Results

SHOX del/dup was found in 12.9% patients (7/54) using MLPA, including heterozygous deletion of exons in 4 (7.4%), and duplications in 3 (5.5%). MLPA results of positive patients are given in Table 2 and Supplementary Figure 1. In the family of Patient 1, her mother and 17-year-old elder sister were also short. Mother was 139 cm (-3.73 SD) and elder sister was 140.5 cm (-3.46 SD) in height; they also had the same heterozygous *SHOX* exon 4 deletion. Father (171 cm) was also tested but did not have the deletion. In the family of Patient 4, in whom a heterozygous *SHOX* exon 4 deletion was detected, the mother was 128.5 cm (-5.31 SD). The family however did not consent for further testing in the mother. There was an apparently healthy younger brother with no short stature. In the other five families, two families had no other offspring and the other three families had one more offspring each with no history of short stature. From the above, it is apparent that 1 out of 7 (14%) copy number variations was familial. Sanger sequencing revealed no sequence variations in any of the MLPA negative patients. In two female patients with heterozygous exon 4 deletion on MLPA, Sanger sequencing revealed no sequence variations, thus ruling out point mutation at the ligation site as the cause.

Clinical Characteristics

Clinical parameters of the seven patients with *SHOX* variants, their Rappold scores (Supplementary Table 2) and phenotypic features are given in Table 1.

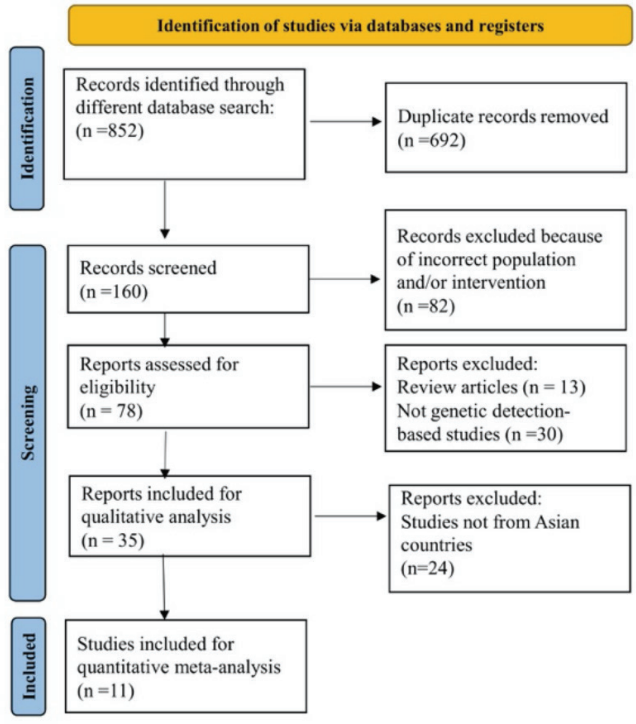

Figure 1. Flow chart for selection of studies (PRISMA 2020)

Table 2. Clinical and molecular features of the children with *SHOX* deficiency

	Age at enrolment and gender	Height	Weight	BMI	MPH	Father's height	Mother's height	Bone age	MLPA
Patient 1	14 years, female	137.1 cm (-2.7 SDS)	34.4 kg (-1.6 SDS)	18.3 (-0.3 SDS)	148.5 cm	171.0 cm	139.0 cm (mother was short)	Advanced	Heterozygous deletion of exon 4 of *SHOX* gene on Xp22
Patient 2	12 years, female	119.4 cm (-4.0 SDS)	25.0 kg (-2.3 SDS)	17.6 (-0.2 SDS)	154.5 cm	166.0 cm	156.0 cm	Normal	Heterozygous deletion of exon 5
Patient 3 (on GH therapy)	13 years, male	128.5 cm (-3.3 SDS)	28.0 kg (-2.0 SDS)	17.1 (-0.4 SDS)	164.2 cm	163.4 cm	152.0 cm	Delayed	Heterozygous deletion of exon 5, 6 of *SHOX* gene on Xp22
Patient 4	14 years, female	128.0 cm (-4.1 SDS)	27.0 kg (-2.8 SDS)	16.4 (-1.0 SDS)	138.0 cm	160.5 cm	128.5 cm (mother was short)	Normal	Heterozygous deletion of exon 4 of *SHOX* gene on Xp22
Patient 5	15 years, female	140 cm (-2.6 SDS)	35.5 kg (-1.7 SDS)	17.8 (-0.6 SDS)	157.5 cm	167.0 cm	161.0 cm	Delayed	Complete duplication of *SHOX* gene
Patient 6	12 years, female	128.5 cm (-2.9 SDS)	25.9 kg (-2.2 SDS)	15.8 (-0.9 SDS)	150.7 cm	162.0 cm	152.3 cm	Normal	Duplication of exons 1, 2, 3 including upstream region
Patient 7	8 years, female	110.6 cm (-2.4 SDS)	18.9 kg (-1.4 SDS)	15.6 (0.1 SDS)	152.6 cm	164.0 cm	154.1 cm	Normal	Duplication of downstream region of *SHOX* gene on Xp22

SDS: standard deviation score, GH: growth hormone, BMI: body mass index, MLPA: multiplex ligation-dependent probe amplification, *SHOX: Short-stature homeobox-containing gene*

Among the 54 cases, *SHOX* gene variation was present in 3.5% of boys and 23.1% of girls enrolled. This was a significant relationship between *SHOX* gene variations and female sex ($p = 0.047$) (Table 1).

From among all the traits which were noted among the enrolled patients, short arms and forearms were significant and related to those with ISS due to *SHOX* variations, whereas the other phenotypic characteristics were not found to be linked with variation. The phenotypic characters and their respective association are summarized in Table 1. No significant association was found for height, mid-parental height, weight, BMI, upper segment to lower segment ratio, or arm span to linear height ratio (data not shown). Radiological survey was done using X-ray of wrist, arms and forearm, legs, thoracolumbar spine and chest X-ray. However, no significant association was established between abnormal radiological findings and *SHOX* gene variation.

Meta-analysis

A total of 852 studies were retrieved. After the removal of duplicates (692 studies), 160 studies including the present study were considered potentially eligible for evaluation, but 149 did not meet the inclusion criteria, leaving 11 studies for analysis (Figure 1). This meta-analysis comprised 10 previous studies and the present study with a total of 979 participants. Detailed characteristics of the studies are provided in Table 3 with NOS scoring. Studies were conducted in six different Asian countries, which were further subcategorised into South, West and Eastern Asia. Most of the studies used MLPA as a major method, followed by Sanger sequencing, chromosomal microarray (CMA) and fluorescent *in situ* hybridisation analysis.

Variations in *SHOX* were identified in 83 of 979 patients. Using random effect model, the mean prevalence of *SHOX* variations was 14.4% (95% CI, 1.0-3.0, $p < 0.001$, $I^2 = 95.1\%$) (Figure 2A, Table 4). The Funnel-plot was asymmetric, suggesting the possibility of publication bias Figure 2B. Further prevalence and association were calculated on the basis of the different regions of Asia, divided into South Asia (patients n = 134), West Asia (n = 226) and East Asia (n = 619). No significant differences were observed in these rates in the different regions of the Asia. The mean frequency of *SHOX* variations in South Asia was 10.4%, with this rate being higher than the rates in other regions; East Asia (8.4%) and West Asia (7.5%) (Figure 3, Table 5).

Discussion

SHOX gene deficiency is one of the single gene disorders of bone metabolism resulting in highly variable osteodysplasia and so affecting the overall height of affected children and adults. However, as understanding of the gene and allelic alterations in the gene have evolved over time, it is now known that isolated deficiency of either the *SHOX* gene or its modifiers up and down the PAR region are responsible for a spectrum of disorders, ranging from simple ISS to more severe disorders, like Leri-Weill dyschondrosteosis (LWD, MIM 127300) and Langer Mesomelic dysplasia (LMD, MIM 249700)". In the present study, *SHOX* variants were found in 12.9% of patients using MLPA and Sanger sequencing, further sub-divided into heterozygous deletion of exons (n = 4), and duplications (n = 3).

In previous studies conducted earlier the prevalence of *SHOX* variations in children with ISS ranges from 2.0-15.0% (8,9,10,11). In a study by Hirschfeldova et al. (10), MLPA analysis detected *SHOX* gene anomalies in 13.7% (n = 7),

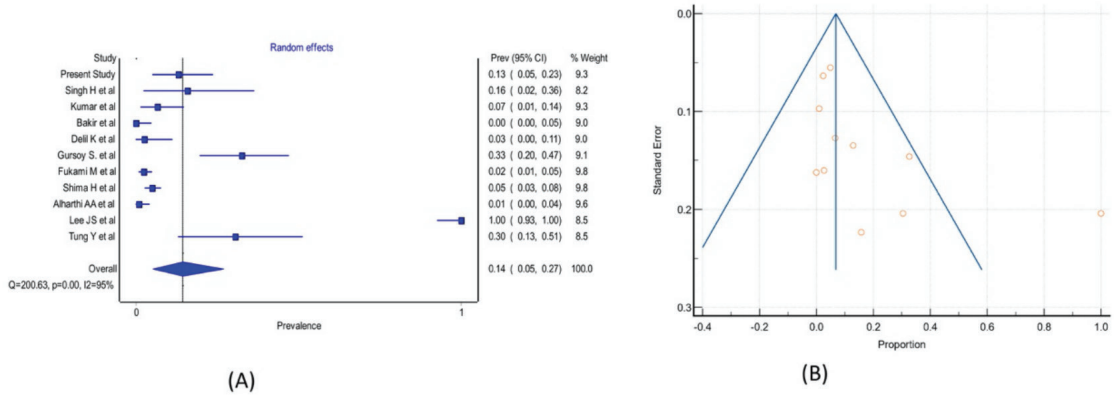

Figure 2. (A) The prevalence of *SHOX* variations is 14% (95% CI: 5.03-27.0, $p < 0.01$, $I^2 = 95\%$). (B) Funnel-plot for SHOX variation studies to analyse risk bias

CI: confidence interval

including 4 deletions, 1 duplication with an "ambiguous effect" and 2 *SHOX* gene point variations. In the same study, single isolated enhancer duplication was also observed in the LWD group. In the present study, three duplications were detected, with no apparent enhancer effect. In the present study, *SHOX* gene association was significantly ($p = 0.047$) related to gender, with predominance in female patients but there was no significant age correlation. Short arms and forearms were the only trait seen in 51.9% of the children and this was significantly ($p = 0.024$) related to *SHOX* gene variation, with the remaining phenotypic traits were not being significantly associated. The seven children with *SHOX* variants were investigated for phenotypic-genotypic correlation. Copy number changes involving the conserved non-coding DNA element (CNE) both upstream and downstream have been described in ISS (15). Duplications are very rare. In the present study, there were three heterozygous duplications, one involving the whole gene, one the first three exons and upstream and the third, downstream. All duplications gave rise to ISS. The transcriptional regulation of *SHOX* is highly complex. Genomic studies have identified multiple CNEs downstream of SHOX with there being a few upstream CNEs. CNEs have been reported to not always be highly conserved (5).

For better delineation of the clinical expression in our cohort, Rappold scoring was performed. Interestingly, the

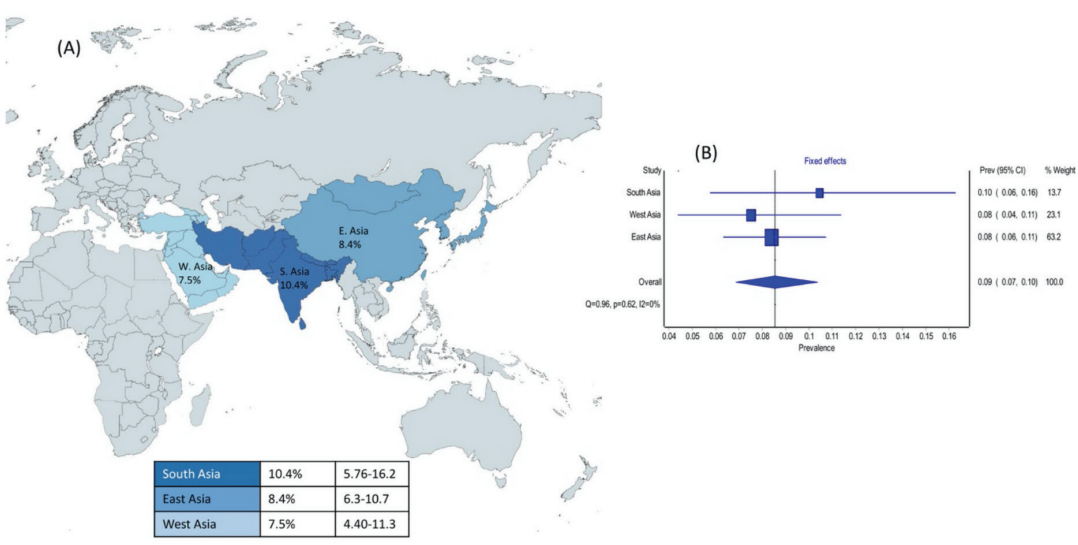

Figure 3. Prevalence of *SHOX* variations in ISS. (A) All studies were grouped according to the geographical origins of the patients. On the map of the world are shown the frequencies (%) of *SHOX* variations in South Asia, East Asia, and West Asia. (B) Dotted lines correspond to the overall prevalence and the 95% CI

ISS: idiopathic short stature, CI: confidence interval, I^2: inconsistency index

Table 3. Characteristics of studies included for analysis

Population	Country	Study	Positive cases/total cases/%	Methodology	NOS
South Asia	India	Present study, 2022	7/54/13%	MLPA and Sanger sequencing	7
		Singh et al. (19) 2018	3/19/15.8%	CMA	8
		Kumar et al. (18) 2020	4/61/6.6%	MLPA and Sanger sequencing	7
West Asia	Turkey	Delil et al. (20) 2016	1/38/2.6%	FISH	7
		Bakır et al. (21) 2018	0/37/0%	FISH and Sanger sequencing	7
		Gürsoy et al. (23) 2020	15/46/32.6%	MLPA and Sanger sequencing	7
	Saudi Arabia	Alharthi et al. (22) 2017	1/105/0.95%	Sanger sequencing	7
East Asia	Japan	Fukami et al. (5) 2015	6/245/2.45%	MLPA and CMA	7
		Shima et al. (26) 2016	16/328/4.9%	MLPA and CMA	7
	South Korea	Lee et al. (24) 2021	23/23/100%	MLPA, CMA and Sanger sequencing	7
	Taiwan	Tung et al. (25) 2018	7/23/30.4%	MLPA and Sanger sequencing	8

FISH: fluorescent *in situ* hybridisation, NOS: Newcastle-Ottawa Scale, MLPA: multiplex ligation-dependent probe amplification, CMA: chromosomal microarray

scores were very variable with a median (range) of 3 (0-10). Short forearm was the most commonly observed feature (62.5%), while cubitus valgus was observed in two patients. All reported BMIs were less than 50th percentile. Unexpectedly, abnormal arm span/height ratio was uncommon (28.5%), whereas bowing of forearm and tibia was present in only one patient. Sitting height/height ratio (>55.5%), dislocation of ulna at the elbow and muscular hypertrophy were absent in our cohort.

In different studies conducted in different populations, the prevalence of *SHOX* variations in ISS children again varies widely. Stuppia et al. (16) reported around 7.0% of patients with ISS having *SHOX* gene deletions, while

Table 4. Prevalence of *SHOX* mutation in Asian population along with test for heterogeneity and publication bias

Study	Prevalence (%)	LCI 95%	HCI 95%	Random effect weight (%)
Present study	12.9	0.1	0.2	9.3
Singh et al. (19) 2018	15.8	0.0	0.4	8.2
Kumar et al. (18) 2020	6.6	0.0	0.1	9.3
Bakır et al. (21) 2018	0.0	0	0.0	8.9
Delil et al. (20) 2016	2.6	0	0.1	8.9
Gürsoy et al. (23) 2020	3.3	0.2	0.5	9.1
Fukami et al. (5) 2015	2.4	0.0	0.0	9.8
Shima et al. (26) 2016	4.9	0.0	0.1	9.8
Alharthi et al. (22) 2017	1.0	0.0	0.0	9.6
Lee et al. (24) 2021	100.0	0.9	1.0	8.5
Tung et al. (25) 2018	3.4	0.1	0.5	8.5
Pooled	14.4	0.1	0.3	100.0
Test for heterogeneity				
Q	204.2			
DF	10			
Significance level	$p < 0.001$			
I^2 (inconsistency)	95.1%			
95% CI for I^2	92.9 to 96.6			
Publication bias				
Egger's test				
Intercept	5.4			
95% CI	-0.5 to 11.3			
Significance level	$p = 0.067$			
Begg's test				
Kendall's Tau	0.4			
Significance level	$p = 0.059$			

CI: confidence interval

Table 5. Prevalence of *SHOX* mutation in South, West and East Asian population

Study	Prevalence (%)	LCI 95%	HCI 95%	Fixed effect weight (%)
South Asia	10.4	0.1	0.2	13.7
West Asia	7.5	0.0	0.1	23.1
East Asia	8.4	0.1	0.1	63.2
Pooled	8.5	0.1	0.1	100.0
Statistics				
I-squared	0.0	0	78.3	
Cochran's Q	0.9			
Chi^2, p	0.6			

Musebeck et al. (17) investigated 35 patients with ISS, none of whom had the *SHOX* deletions.

This meta-analysis aimed to investigate the association between the *SHOX* variation and ISS in Asia. Data on 979 ISS cases was analysed. The overall prevalence of *SHOX* variation in Asia was 14.3%. Subgroup analysis showed the presence of *SHOX* variations in 10.4% of the patients with ISS in South Asia. Among South Asians, Kumar et al. (18) reported pathogenic heterozygous variants in 4 children (6.5%) out of 61, exon 5 duplication, splice site variation c.278-1G>C, one partial deletion and complete deletion of *SHOX*. Singh et al. (19) showed *SHOX* haploinsufficiency in two patients (10.5%), while one patient (5.2%) had mosaic gain in *SHOX* out of 19 patients.

In a West Asian population, Delil et al. (20) identified one patient (2.6%) with *SHOX* variation. In contrast, Bakır et al. (21) found no variation in the *SHOX* gene in 37 patients. Alharthi et al. (22) found only one variation in exon 4 of *SHOX* while rest of patients had polymorphisms in exons 1, 2, 4, and 6. Gürsoy et al. (23) found three point variations and one whole gene deletion in 15 patients from four different families. The overall prevalence of *SHOX* variants in ISS in West Asia was around 7.5%.

In Eastern Asia, Fukami et al. (5), reported six rare CNVs in PAR1 in 245 patients. Lee et al. (24), confirmed *SHOX* deficiency in 23 patients from 15 unrelated families. In a study by Tung et al. (25), *SHOX* intragenic deletions were found in five patients, one deletion in the regulatory region, and a missense variation at exon 5. Prevalence of variation in *SHOX* gene varied between different geographical regions of Asia, being highest in South Asia. Shima et al. (26) reported *SHOX* abnormalities in 3.8% of ISS and 50.0% of LWD cases. These results indicate the difference in the prevalence of the *SHOX* variations based on selection criteria, methodology, different sample size, and ethnicity.

Study Limitations

This was a hospital-based study and results should be interpreted in that context. Our study had a relatively small sample size, as it was a single centre study, and as it was a dissertation study, had to be concluded over a limited time period.

Conclusion

In the cohort of North Indian children with ISS, the prevalence of SHOX variants was 12.9%. This was consistent with the subgroup analysis of studies from this region. The meta-analysis, a compilation of findings from the last decade across western, southern and eastern Asia, presented an updated picture of overall prevalence of *SHOX* variations in Asians, underscoring its potential as a main target in ISS patients. Further investigations of higher quality, large cohort size with functional validation are warranted to validate this association.

Acknowledgements

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Ethics

Ethics Committee Approval: This was a prospective study conducted in a tertiary hospital from July 2020 to March 2022, with approval from the Ethics Committee of the Postgraduate Institute of Medical Education & Research (PGIMER) (approval no: NK/6656/MS/315, date: 01.12.2020).

Informed Consent: Written informed consent was obtained from the participants.

Authorship Contributions

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References

1. Cohen P, Rogol AD, Deal CL, Saenger P, Reiter EO, Ross JL, Chernausek SD, Savage MO, Wit JM; 2007 ISS Consensus Workshop participants. Consensus statement on the diagnosis and treatment of children with idiopathic short stature: a summary of the Growth Hormone Research Society, the Lawson Wilkins Pediatric Endocrine Society, and the European Society for Paediatric Endocrinology Workshop. J Clin Endocrinol Metab 2008;93:4210-4217. Epub 2008 Sep 9
2. Rosenfeld RG, Hwa V. Toward a molecular basis for idiopathic short stature. J Clin Endocrinol Metab 2004;89:1066-1067.
3. Rao E, Blaschke RJ, Marchini A, Niesler B, Burnett M, Rappold GA. The Leri-Weill and Turner syndrome homeobox gene SHOX encodes a cell-type specific transcriptional activator. Hum Mol Genet 2001;10:3083-3091.
4. Rao E, Weiss B, Fukami M, Rump A, Niesler B, Mertz A, Muroya K, Binder G, Kirsch S, Winkelmann M, Nordsiek G, Heinrich U, Breuning MH, Ranke MB, Rosenthal A, Ogata T, Rappold GA. Pseudoautosomal deletions encompassing a novel homeobox gene cause growth

failure in idiopathic short stature and Turner syndrome. Nat Genet 1997;16:54-63.

5. Fukami M, Naiki Y, Muroya K, Hamajima T, Soneda S, Horikawa R, Jinno T, Katsumi M, Nakamura A, Asakura Y, Adachi M, Ogata T, Kanzaki S; Japanese SHOX study group. Rare pseudoautosomal copy-number variations involving SHOX and/or its flanking regions in individuals with and without short stature. J Hum Genet 2015;60:553-556. Epub 2015 Jun 4
6. Benito-Sanz S, del Blanco DG, Aza-Carmona M, Magano LF, Lapunzina P, Argente J, Campos-Barros A, Heath KE. PAR1 deletions downstream of SHOX are the most frequent defect in a Spanish cohort of Léri-Weill dyschondrosteosis (LWD) probands. Hum Mutat 2006;27:1062.
7. Schneider KU, Marchini A, Sabherwal N, Röth R, Niesler B, Marttila T, Blaschke RJ, Lawson M, Dumic M, Rappold G. Alteration of DNA binding, dimerization, and nuclear translocation of SHOX homeodomain mutations identified in idiopathic short stature and Leri-Weill dyschondrosteosis. Hum Mutat 2005;26:44-52.
8. 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 2006;43:735-739. Epub 2006 Apr 5
9. Jorge AA, Souza SC, Nishi MY, Billerbeck AE, Libório DC, Kim CA, Arnhold IJ, Mendonca BB. SHOX mutations in idiopathic short stature and Leri-Weill dyschondrosteosis: frequency and phenotypic variability. Clin Endocrinol (Oxf) 2007;66:130-135.
10. Hirschfeldova K, Solc R, Baxova A, Zapletalova J, Kebrdlova V, Gaillyova R, Prasilova S, Soukalova J, Mihalova R, Lnenicka P, Florianova M, Stekrova J. SHOX gene defects and selected dysmorphic signs in patients of idiopathic short stature and Léri-Weill dyschondrosteosis. Gene 2012;491:123-127. Epub 2011 Oct 14
11. Binder G. Short stature due to SHOX deficiency: genotype, phenotype, and therapy. Horm Res Paediatr 2011;75:81-89. Epub 2011 Feb 4
12. Rappold G, Blum WF, Shavrikova EP, Crowe BJ, Roeth R, Quigley CA, Ross JL, Niesler B. Genotypes and phenotypes in children with short stature: clinical indicators of SHOX haploinsufficiency. J Med Genet 2007;44:306-313. Epub 2006 Dec 20
13. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JP, Clarke M, Devereaux PJ, Kleijnen J, Moher D. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. BMJ 2019;339:b2700.
14. Wells GA, Shea B, O'Connell D, Peterson J, Welch V, Losos M, Tugwell P. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. Last Accessed Date: 2611.2022. Available from: https://www.ohri.ca/programs/clinical_epidemiology/oxford.asp
15. Bunyan DJ, Gevers E, Hobbs JI, Duncan-Flavell PJ, Howarth RJ, Holder-Espinasse M, Klee P, Van-Heurk R, Lemmens L, Carminho-Rodrigues MT, Mohamed Z, Goturu A, Hughes CR, Ajzensztejn M, Thomas NS. Rare dosage abnormalities flanking the SHOX gene. Egypt J Med Hum Genet 2021;22:89.
16. Stuppia L, Calabrese G, Gatta V, Pintor S, Morizio E, Fantasia D, Guanciali Franchi P, Rinaldi MM, Scarano G, Concolino D, Giannotti A, Petreschi F, Anzellotti MT, Pomilio M, Chiarelli F, Tumini S, Palka G. SHOX mutations detected by FISH and direct sequencing in patients with short stature. J Med Genet 2003;40:11.
17. Musebeck J, Mohnike K, Beye P, Tönnies H, Neitzel H, Schnabel D, Grüters A, Wieacker PF, Stumm M. Short stature homeobox-containing gene deletion screening by fluorescence in situ hybridisation in patients with short stature. Eur J Pediatr 2001;160:561-565.
18. Kumar A, Jain V, Chowdhury MR, Kumar M, Kaur P, Kabra M. Pathogenic/likely pathogenic variants in the SHOX, GHR and IGFALS genes among Indian children with idiopathic short stature. J Pediatr Endocrinol Metab 2020;33:79-88.
19. Singh H, Tiwari P, Bhavi V, Chaudhary PS, Suravajhala P, Mohan MK, Mathur SK. Application of Chromosomal Microarray for Evaluation of Idiopathic Short Stature in Asian Indian Children: A Pilot Study. Indian J Endocrinol Metab 2018;22:100-106.
20. Delil K, Karabulut HG, Hacıhamdioğlu B, Şıklar Z, Berberoğlu M, Öçal G, Tükün A, Ruhi HI. Investigation of SHOX Gene Mutations in Turkish Patients with Idiopathic Short Stature. J Clin Res Pediatr Endocrinol 2016;8:144-149. Epub 2015 Dec 18
21. Bakır A, Yirmibeş Karaoğuz M, Perçin FE, Tuğ E, Cinaz P, Ergün MA. Microdeletion and mutation analysis of the SHOX gene in patients with idiopathic short stature with FISH and sequencing. Turk J Med Sci 2018;48:386-390.
22. Alharthi AA, El-Hallous EI, Talaat IM, Alghamdi HA, Almalki MI, Gaber A. Screening of SHOX gene sequence variants in Saudi Arabian children with idiopathic short stature. Korean J Pediatr 2017;60:327-332. Epub 2017 Oct 20
23. Gürsoy S, Hazan F, Aykut A, Nalbantoğlu Ö, Korkmaz HA, Demir K, Özkan B, Çoğulu Ö. Detection of SHOX Gene Variations in Patients with Skeletal Abnormalities with or without Short Stature. J Clin Res Pediatr Endocrinol 2020;12:358-365. Epub 2020 Apr 16
24. Lee JS, Kim HY, Lee YA, Lee SY, Cho TJ, Ko JM. Clinical and Genetic Characteristics of 23 Korean Patients with Haploinsufficiency of the Short-stature Homeobox-containing Gene. Exp Clin Endocrinol Diabetes 2021;129:611-620. Epub 2020 Sep 15
25. Tung YC, Lee NC, Hwu WL, Liu SY, Lee CT, Chien YH, Tsai WY. SHOX deficiency in short Taiwanese children: A single-center experience. J Formos Med Assoc 2018;117:909-914. Epub 2017 Dec 16
26. Shima H, Tanaka T, Kamimaki T, Dateki S, Muroya K, Horikawa R, Kanno J, Adachi M, Naiki Y, Tanaka H, Mabe H, Yagasaki H, Kure S, Matsubara Y, Tajima T, Kashimada K, Ishii T, Asakura Y, Fujiwara I, Soneda S, Nagasaki K, Hamajima T, Kanzaki S, Jinno T, Ogata T, Fukami M; Japanese SHOX study group. Systematic molecular analyses of SHOX in Japanese patients with idiopathic short stature and Leri-Weill dyschondrosteosis. J Hum Genet 2016;61:585-591. Epub 2016 Mar 17

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