Original Article

Application of Chromosomal Microarray for Evaluation of Idiopathic Short Stature in Asian Indian Children: A Pilot Study

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Abstract

Background: Human height is a classic polygenic trait and currently available data explains only 10% of the phenotypic variation in height. Almost 60%–80% of the children coming to pediatric and endocrinology outpatient department for the evaluation of short stature are still labeled as idiopathic. **Objectives:** The aim of this study is to identify various chromosomal alterations causing idiopathic short stature (ISS) and short stature with dysmorphic features not pertaining to known genetic syndromes. **Materials and Methods:** After exclusion of all nutritional, systemic, endocrine, and syndromic causes of short stature, 19 patients with height <2 standard deviation scores were subjected to chromosomal microarray (CMA) study using Affymetrix CytoScan 750K array and CMA Scanner 3000 platform. **Results:** We identified total 61 copy-number variant (CNV) and polymorphs (33 gains, 11 loss, and 17 gain-mosaics) not described as normal variants in database of genomic variations. We identified SHOX haploinsufficiency as a cause of short stature in two patients, whereas one patient was gain-mosaic for SHOX. All three had normal conventional karyotype. One of these patients also had deletion of PAX3, which could be the cause of both short stature and associated mild intellectual impairment in this patient. We also found a long noncoding RNA, namely, KIAA0125 and a pseudogene ADAM6 in 18 out of our 19 patients which might have a regulatory role. **Conclusion:** This study shows that CMA is a very promising tool for the identification of pathogenic CNVs in patients with ISS. It can also help to identify novel genes controlling height and can open up new insight into pathophysiologic mechanisms underlying ISS, and thus may help to unfold new therapeutic targets for treatment of this condition. The association of CNV having genes for long noncoding RNAs, such as KIAA0125 and pseudogene such as ADAM6 with ISS suggest that they may play a role in controlling the expression of height-related genes and it needs further investigations.

Keywords: Chromosomal microarray, copy-number variations, idiopathic short stature

Introduction

Human growth is a highly complex and multifactorial trait, with an estimated heritability of about 80%–90%.[1] Since 3% of the general population present with a body height below-2 standard deviation scores (2SDS), shortness of stature is one of the common medical concerns in childhood. Uncovering the genetic basis of short stature is not only important for clinical diagnosis, prognosis, and genetic counseling of affected individuals and their families but also a prerequisite for the future development of therapeutic approaches.

Idiopathic short stature (ISS) is defined as a condition, in which the height of an individual is more than 2SDS below the corresponding mean height for a given age, sex, and population group without evidence of systemic, endocrine, nutritional, or chromosomal abnormalities.[2] Specifically, children with ISS have normal birth weight and are GH sufficient. ISS describes a heterogeneous group of children consisting of many presently unidentified causes of short stature. It is estimated that approximately 60%–80% of all short children at or below-2 SDS fit the definition of ISS.[3,4] This definition includes children with constitutional delay of growth and puberty (CDGP), familial short stature (FSS), and short children who will not have delayed puberty and whose height is not consistent with parental heights. Therefore, this definition includes both normal, healthy children (those with

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Website: www.ijem.in

DOI: 10.4103/ijem.IJEM_202_17

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How to cite this article: Singh H, Tiwari P, Bhavi V, Chaudhary PS, Suravajhala P, Mohan MK, *et al*. Application of chromosomal microarray for evaluation of idiopathic short stature in Asian Indian children: A pilot study. Indian J Endocr Metab 2018;22:100-6.

FSS and CDGP), and children who are presumed to have an unidentified disorder impairing their growth.

Microarray-based genomic copy-number analysis is now a commonly ordered clinical genetic test for individuals with unexplained developmental delay/intellectual disability, autism spectrum disorders, or multiple congenital anomalies.[5] Many studies of copy-number variants (CNVs) in patients with neuropsychiatric conditions or multiple congenital anomalies showed that de novo or inherited CNVs are pathogenic in up to 20% of patients.[6] With an intermediate length of 1 Kb to several Mb, they include both duplications and deletions and can affect single exon, one or several genes, as well as regulatory sequences.

In this report, we present the results of chromosomal microarray (CMA) in a study group of 19 patients with ISS. Our report highlights the ability of CMA to identify clinically important rare genetic disorders. This is also the first study from India using CMA as a diagnostic tool for evaluation of ISS.

Methods

This study was approved by the Institutional Review Board of the institution. All participants or their legal guardians provided written informed consent. Participants were recruited as part of a larger cohort searching for novel genetic etiologies of short stature in individuals with no known systemic, endocrine, nutritional, or syndromic abnormalities.

All the children <18 years of age presenting in SMS endocrine outpatient department for the evaluation of short stature were evaluated by complete history, physical examination, and anthropometry including height, weight, body mass index, arm span, upper segment: lower segment ratio and sexual maturation rate, presence of dysmorphic features, and signs of underlying systemic illness. Those with heights <3rd centile or <-2SDS on revised Indian Academy of Pediatrics 2015 charts were tested for complete blood count, erythrocyte sedimentation rate, red blood cell, renal function tests, liver function tests, serum calcium, phosphorus, alkaline phosphatase, albumin, bicarbonate, FT4, thyroid stimulating hormone, cortisol, follicle-stimulating hormone (for females), S. tTGIgA, Venous blood gas (VBG), urine pH, and X-ray hand for bone age. Karyotyping was done in all females with the features of Turner syndrome or with unexplained short stature. After exclusion of systemic diseases, and after confirmation of euthyroid and eucortisolemic status, patients were tested by S.IGF1 and clonidine stimulation test to rule out growth hormone deficiency. Priming with estrogen was done in pubertal age group patients if needed.

Those having dysmorphic features were evaluated thoroughly to find any known genetic syndrome associated with moderate-to-severe short stature[8] [Table 1] with additional investigations which included 2D-echo, USG abdomen and pelvis, audiometry, IQ assessment, fundus and MRI brain if needed. About 19 of those patients with normal test results and those with dysmorphic features who did not fit into any known short stature syndromes were then subjected to CMA using Affymetrix CytoScan 750K array and CMA Scanner3000 platform.

Table 1: Syndromes associated with moderate to severe short stature[7]

Syndromes with very short stature	Syndromes with moderate short stature
Turner syndrome and its variants	Smith-Lemli-Opitz syndrome
Brachmann-de Lange syndrome	Kabuki syndrome
Rubinstein-Taybi syndrome	Williams syndrome
Silver-Russell syndrome	Noonan syndrome
Mulibrey syndrome	Costello syndrome
Dubowitz syndrome	Cardiofacio-cutaneous syndrome
Bloom syndrome	Aarskog syndrome
Johannson-Blizzard syndrome	Robinow syndrome
Seckel syndrome	Opitz syndrome
Hallermann-Streiff syndrome	Floating-Harbor syndrome
Prader--Willi syndrome	

Chromosomal microarray methodology

First, the human genomic DNA was extracted from whole blood using Qiagen-DNeasy Blood and Tissue kit (Cat No. 69504). The concentration of DNA samples was determined using Nanospectrophotometer and quality has been estimated on agarose gel electrophoresis. Then, 50 ng/μL of genomic DNA was digested with the restriction enzyme Nsp I. Then, it was ligated to a common adaptor with T4 DNA ligase. Following ligation, the template underwent to PCR amplification using Titanium Taq DNA polymerase. The amplified PCR product was then pooled and purified using bead-based purification methods. Purified product was then quantified and fragmented with Fragmentation Reagent (DNAse I), and end-labeled using terminal deoxynucleotidyl transferase, and then, the labeled samples were hybridized by loading on array. After the completion of hybridization, the array were washed, stained and scanned. The raw data we got from the scanner was then analyzed using Chromosomal Analysis suite software.

Bioinformatics approach

The CNVs thus detected were then searched in database of genomic variations (DGV)[16] to look for physiological variations. Only those CNVs which were not described as physiological were then selected, and the genes present in those CNVs were then matched with those reported in International Standards for cytogenomic arrays (ISCA) consortium database[15] and ClinVar database to be associated with ISS sibling ontologies. Genes already reported in database associated with height were considered causative in that patient. Genes not reported in database were then studied for their signaling pathways and interactions with other height-related genes and possible mechanisms that can lead to short stature.

Results

Our study group included 19 patients with ISS [Table 2]. Six (31.5%) were male and 13 (68.42%) were female.

Eight (42.1%) had isolated short stature while 11 (57.89%) individuals presented with additional features such as a mild dysmorphic facial gestalt. The mean height SDS was 3.272 [Figure 1]. None had a positive family history of delayed puberty or short stature. Out of them, 17 (89.47%) patients had proportionate short stature, whereas two (10.52%) patients had disproportionate short stature but without radiographic signs suggestive of skeletal dysplasias. A borderline low IQ in the range of learning disability was observed in three patients (15.7%).

The anthropometric measurements and other phenotypic features of the study group are described in Table 3. After excluding the CNVs described as normal physiological variation in DGV, we identified total 61 potential pathogenic CNVs out of which 33 were gain in copy number, 11 were loss and 17 were gain-mosaics [Figure 2]. The minimum size of CNVs was 130 kbp, whereas maximum size was 1, 52,802 kbp. About 14 CNVs were of size between 100 and 500 kbps, 19 between 500 and 1000 kbps, 5 between 1000 and 2000 kbps, and 14 >2000 kbps. After looking for the genes lying in these CNV regions, we identified two genes in 3 of our patients tightly linked to a GWAS SNP implicated in human height variations. These are SHOX and PAK3. These genes are reported in database to be causally related to short stature. We also found KIAA0125, a long noncoding RNA and ADAM6, a pseudogene common in most (18 out of 19) of our cohort of ISS. An organization chart illustrating the identified CNVs and genetic findings is shown in Table 4.

Table 2: Overview of the phenotypic characteristics of the patient group

Features	Patients (%)
Total	19
Male/female	6/13 (31.5/68.42)
Mean height SDS	−3.272
Isolated short stature/associated other abnormalities	8/11 (42.1/57.89)
Intellectual status normal/mild learning disability	16/3 (84.2/15.7)
Proportionate/disproportionate	17/2 (89.47/10.52)
Positive family history of short stature/negative family history	0/19

SDS: Standard deviation score

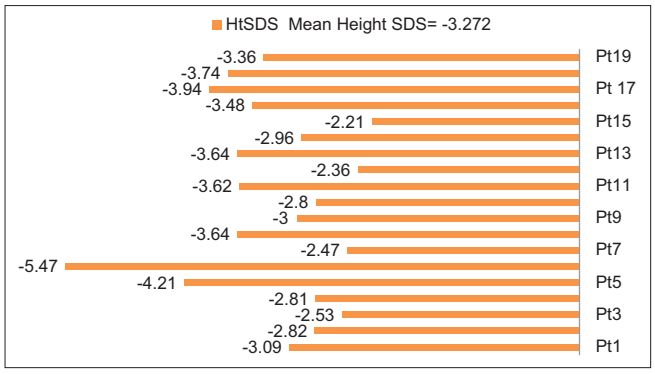

Figure 1: Standard deviation score for mean height across different patients

Discussion

CMA analysis in 19 patients with ISS in this study identified 61 novel CNVs, which are not otherwise reported as normal variant in DGV.[12] Out of these 19 participants, only 3 had CNV polymorphs containing genes otherwise known to be associated with short stature. However, an interesting finding of this study is identification of a novel CNV having genes KIAA0125 and ADAM6.

There are only a few published reports on application of CMA for the investigation of ISS. In a study on a genome-wide association analysis of CNV and stature by Dauber A *et al*., it was revealed that children with short stature had a greater global burden of lower frequency and rare deletions and a greater average CNV length than controls.[8] These observations suggest that CNVs might contribute to genetic variation in stature in the general population. Van Duyvenvoorde *et al*. performed genome-wide analysis for CNVs,[9] in 162 patients (149 families) with short stature. CNVs were detected in 40 families. In six families, a known cause of short stature was found (SHOX deletion or duplication, IGF1R deletion), in two combined with a *de novo* potentially pathogenic CNV. Thirty-three families had one or more potentially pathogenic CNVs (n = 40). In 24 of these families, segregation analysis could be performed; identifying three-*de novo* CNVs and nine CNVs segregating with short stature. Four were located near loci associated with height in GWAS (ADAMTS17, TULP4, PRKG2/BMP3, and PAPPA). Besides six CNVs known to be causative for short stature, 40 CNVs with possible pathogenicity were identified. Hu *et al*. studied the applicability of the custom microarray and to analyze CNVs in Chinese ISS children and identified sixty nonpolymorphic CNVs including five pathogenic or possibly pathogenic CNVs in five patients, including deletions at 22q11.21, duplications at 4q11-q13.1, 4q12, and Yp11.32-p11.2.[10] The potential candidate genes located in the CNV regions were TBX1, SHOX, TMEM165, POLR2B, and PDGFRA. Zahnleiter *et al*. searched for rare CNVs in 200 families, 92 sporadic, and 108 familial, with

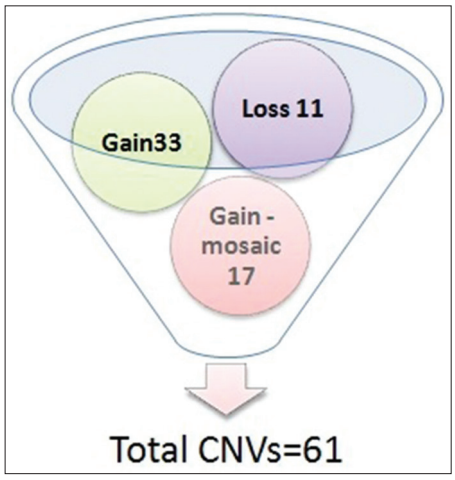

Figure 2: Copy-number variants found in study group

Table 3: Anthropometric measurements and other features

	Sex	Height (cm)	Height (SDS)	Weight (kg)	CA (year)	HA (year)	WA (year)	BA (year)	Arm span	US: LS	SMR	Physical features
Patient 1	Male	112	−3.09	18	9	6	5.5	6	113	0.9	A-P1G1	Short stature, almond-shaped eyes, hypertelorism, small ears, mild mental disability
Patient 2	Female	121	−2.82	25	11	7.5	9.5	7	120	0.959	A-P1B1	Short stature, mild mental disability (IQ 55), almond-shaped eyes, hypertelorism, small ears, clinodactyly, sandle gap
Patient 3	Female	138	−2.53	27	14	10.5	10.3	10	140	0.69	A+P2B2	Short stature, pigmented nevi, short fourth metacarpal
Patient 4	Female	108	−2.81	15	8	5.5	3.5	7	108	0.96	A-P1B1	Isolated short stature
Patient 5	Female	130	−4.21	32	17	9	11.6		131	0.874	A+P1B1	Short stature, delayed puberty, dry, shiny, hairless skin, micrognathia, wide-spaced nipples, hyper convex nails, increased carrying angle, mild intellectual impairment
Patient 6	Female	86	−5.47	9.5	7	2.5	1	2.5	86	1.2	A-P1B1	Short stature, IUGR, microcephaly, prognathism, VSD-PAH, rickets
Patient 7	Male	132	−2.47	28	13	10	10	11	131	0.97	A2P2G2	Isolated short stature
Patient 8	Male	115	−3.64	24.5	11	6.5	9	6	115	0.91	A+P2G1	Isolated short stature
Patient 9	Female	114	−3	17	10	6	4.5	7	117	0.96	A-P1B1	Short stature, micrognathia short philtrum, antimongoloid slant
Patient 10	Female	128	−2.8	22	11.5	8.5	8.5	11	127	0.91	A+P2B1	Isolated short stature
Patient 11	Male	142	−3.62	35	16	11.5	12	16	150	1	A+P5G3	Low set ears, gap between upper and lower incisor, flattened midline facies, exaggerated knee and ankle reflex, mild mental disability (IQ 78), short stature, sleep disturbance, history of polyhydramnios
Patient 12	Male	133	−2.36	25	13	9.6	9	9	130	0.88	A-P1G1	Isolated short stature
Patient 13	Female	134	−3.64	27	14.6	9.5	10	10	134.5	0.81	A+P3B1	Short stature, LBW <1.5, hypertelorism, flat foot, almond-shaped eyes, hyperconvex nails, short stature, delayed puberty
Patient 14	Female	107	−2.96	15	8	5	3.5	6.5	107	0.86	A-P1B1	Isolated short stature
Patient 15	Female	143	−2.21	38	17	11	13	17.6	145	0.86	A+P4B4	Isolated short stature
Patient 16	Female	124	−3.48	20	11.9	8	7	10	124	0.94	A-P1B1	Short stature, widely spaced nipples
Patient 17	Female	125	−3.94	27	13	8	10	8	129	0.98	A-P1B1	Short stature, delayed puberty, Multiple pigmented nevi, high-arched palate, wide carrying angle
Patient 18	Female	130	−3.74	23	14.3	9.5	9	11	131	0.86	A+P2B2	Short stature, delayed puberty, prominent upper incisors, micrognathia, wide carrying angle, greying of hairs
Patient 19	Female	129	−3.36	27	13	8	10	8	129	0.98	A-P1B1	Isolated short stature

IUGR: Intrauterine growth retardation, IQ: Intelligence quotient, SDS: Standard deviation score, LBW: Low birth weight, US: Upper segment, LS: Lower segment, CA: Chronological age, HA: Height Age, WA: Weight Age, BA: Bone Age, SMR: Sexual Maturity Rating, VSD-PAH: ventricular septal defect-predicted adult height

Table 4: Description of the copy number variants identified in the study group

	Sex	Chromosome position	Type	Cytob and start	Size (kbp)	Variant present in ISCA and related to ISS
Patient 1	Male	chr14: 106,164,141-107,027,146	Gain	q32.33	663.851	
		chr15: 43,847,725-44,028,382	Loss	q15.3	138.967	
		chrY: 0-32,722,038	Gain-mosaic	p11.31	26,149.23	
Patient 2	Female	chr10: 46,018,645-48,401,505	Gain	q11.22	1832.97	
		chr14: 106,163,525-107,031,454	Gain	q32.33	667.639	
Patient 3	Female	chr14: 106,171,550-106,953,762	Gain	q32.33	601.702	
Patient 4	Female	chr14: 106,171,555-106,938,127	Gain	q32.33	589.672	
Patient 5	Female	chrX: 144,046,272-144,993,055	Gain	q27.3	728.295	SHOX
		chrX: 81,699,194-83,012,665	Gain	q21.1	1010.363	PAK3
		chrX: 91,023,931-93,198,599	Gain	q21.31	1672.822	
		chrX: 140,706,625-141,418,197	Gain	q27.2	547.364	
		chrX: 148,631,322-149,918,448	Gain	q28	990.098	
		chrX: 137,365,806-138,689,495	Gain	q26.3	1018.223	
		chrX: 125,726,040-127,477,142	Gain	q25	1347.002	
		chr14: 106,171,768-106,968,257	Gain	q32.33	612.685	
		chrX: 145,005,905-145,637,604	Gain	q27.3	485.923	
		chrX: 0-66,858,038	Loss	p22.33	57,990.859	
		chrX: 68,706,095-155,270,560	Gain-mosaic	q21.1	75,240.873	
Patient 6	Female	chr14: 22,648,530-22,979,540	Loss	q11.2	254.624	
Patient 7	Male	chrY: 27,301,744-28,246,509	Gain	q11.23	726.743	
		chrY: 25,746,742-26,519,385	Gain	q11.22	594.341	
		chr14: 106,191,586-106,780,631	Gain	q32.33	453.113	
		chr14: 22,581,094-22,987,206	Loss	q11.2	312.394	
		chrY: 0-32,722,038	Gain-mosaic	p11.31	26,149.23	
Patient 8	Male	chr14: 106,176,903-106,782,547	Gain	q32.33	465.88	
		chrY: 0-32,722,038	Gain-mosaic	p11.31	26,149.23	
Patient 9	Female	chr14: 106,161,758-106,939,405	Gain	q32.33	598.191	
		chrY: 0-32,722,038	Gain-mosaic	p11.31	26,149.23	
Patient 10	Female	chr14: 106,132,366-106,780,963	Gain	q32.33	498.921	
Patient 11	Male	chr14: 106,181,830-106,781,904	Gain	q32.33	461.596	
		chr1: 144,273,391-149,206,051	Loss	q21.1	3794.354	
		chr6: 234,680-404,315	Loss	p25.3	130.489	
		chr1: 101,445,630-168,454,782	Loss mosaic	p13.3	51,545.502	
Patient 12	Male	chr14: 106,188,959-106,780,974	Gain	q32.33	455.397	
		chr2: 51,101,271-51,448,992	Loss	p16.3	267.479	
		chr14: 22,590,235-22,984,085	Loss	q11.2	302.962	
		chrY: 0-32,722,038	Gain-mosaic	p11.31	26,149.23	
Patient 13	Female	chr19: 527,854-1,753,772	Gain	p13.3	943.014	
		chr14: 106,065,267-106,951,991	Gain	q32.33	682.096	
Patient 14	Female	chr14: 106,003,519-106,599,191	Gain	q32.33	458.21	
		chr15: 85,961,982-89,810,512	Loss	q25.3	2960.408	
Patient 15	Female	chr19: 378,599-1,845,659	Gain	p13.3	1128.508	
		chr14: 106,172,186-106,855,843	Gain	q32.33	525.891	
Patient 16	Female	chrX: 51,282,352-52,002,388	Gain	p11.22	553.874	SHOX
		chr14: 106,016,599-106,958,339	Gain	q32.33	724.416	
		chr15: 22,639,758-23,772,175	Loss	q11.2	871.091	
		chrX: 0-12,052,026	Gain-mosaic	p22.33	10,333.457	
		chrX: 14,165,318-155,270,560	Gain-mosaic	p21.1	122,667.635	
Patient 17	Female	chr22: 22,841,357-23,361,473	Gain	q11.22	400.09	SHOX
		chr14: 106,168,324-106,848,325	Gain	q32.33	523.079	
		chrX: 0-155,270,560	Loss	p22.33	152,801.925	
		chrX: 152,668,486-155,270,560	Loss	q28	2230.098	
Patient 18	Female	chr14: 106,003,519-106,599,191	Gain	q32.33	458.21	
Patient 19	Female	chr14: 105,924,259-107,206,850	Gain	q32.33	986.609	

ISS: Idiopathic short stature, ISCA: International Standards for Cytogenomic Arrays (ISCA) consortium database

ISS compared to 820 control individuals.[11] They identified 10 duplications and 10 deletions ranging in size from 109 kb to 14 Mb, of which 7 were *de novo* and 13 inherited from the likewise affected parent but absent in controls. Eleven (55%) of these CNVs either overlapped with known microaberration syndromes associated with short stature or contained GWAS loci for height. The findings of all these studies are partly consistent with results of the present study in sense that CNVs are associated with ISS in a significant number of participants. However, the CNVs identified in all these studies were different from those found in the present study. SHOX is the only gene, known to be associated with height, located on a CNV region identified to be implicated in ISS in all of these studies.

To the best of our knowledge, this is probably the first report on application of CMA in identification CNVs implicated in the cause of ISS in Indian population. The finding of unique CNVs in this pilot study, which were not found in other races, suggests that we need to create a race specific database of ISS-related CNVs. Furthermore, there is scope of further biological investigation and pharmacogenomic studies, so that ISS can be further subclassified.

SHOX (OMIM312865) located at chromosome position Xp22.33 and Yp11.2, is part of a large family of homeobox genes, which act during early embryonic development to control the formation of many body structures. Specifically, the *SHOX* gene is essential for the development of the skeleton. It plays a particularly important role in the growth and maturation of bones in the arms and legs. Haploinsufficiency causes Turner syndrome and Léri–Weill dyschondrosteosis while loss of both copies of SHOX gene results in Langer mesomelic dysplasia. Morizio *et al*.[12] described SHOX haploinsufficiency by FISH in 4 out of 56 patients with ISS using a probe specific for the SHOX gene. None of these four patients had any skeletal abnormalities. Fukami *et al*.[13] reported six rare copy-number variations (CNVs) in PAR1 identified through copy-number analyzes of 245 ISS/LWD patients and 15 unaffected individuals. The six CNVs consisted of three microduplications encompassing SHOX and some of the CNEs, two microduplications in the SHOX 3'-region affecting one or four of the downstream CNEs, and a microdeletion involving SHOX exon 6b and its neighboring CNE. The amplified DNA fragments of two SHOX-containing duplications were detected at chromosomal regions adjacent to the original positions. The breakpoints of a SHOX-containing duplication resided within Alu repeats. A microduplication encompassing four downstream CNEs was identified in an unaffected father–daughter pair, whereas the other five CNVs were detected in ISS patients. Out of 19 patients, 3 of our patients (all females) had abnormalities related to SHOX, 2 had loss while one patient was gain mosaic for SHOX. Table 5 compares the CNVs related to SHOX in our study group with other similar studies. Two of these patients had a wide carrying angle while the third had no skeletal abnormality. These patients also had other stigmata of Turner such as multiple nevi and widely spaced nipples. All the three patients had normal conventional karyotype. This finding indicates that CMA may be a more sensitive tool than karyotype for the diagnosis of ISS due to copy-number variations (CNVs) involving SHOX and/or the highly evolutionarily conserved noncoding DNA elements (CNEs) flanking this gene. It is also interesting to note here that all the ISS patients with SHOX gene CNVs had subtle-isolated features of Turner syndrome, instead of complete clinical picture and normal karyotype. Therefore, we suggest that CMA can be an important investigative tool for such children. However, there is need of more data using a large sample size to confirm these findings.

Table 5: Comparison of copy-number variants found in present study group (SHOX) with other studies

Present study cohort	van Duyvenvoorde *et al*.[9]	Fukami *et al*.[13]
Xp22.33 (0-66,858,038) loss Xp22.33 (0-12,052,026) gain-mosaic Xp22.33 (0-155,270,560) loss	Xp22.33 (1-1,522,908) loss Xp22.33 (1-2,320,027) loss Xp22.33 (1-727,565) gain Yp11.32 (1-2,640,827) gain	Xp22.33 (486,700-757,437) gain Xp22.33 (520,681-1,314,734) gain Xp22.33 (1-596,006) gain Xp22.33 (798,435-1,474,970) gain Xp22.33 (619,112-743,611) loss Xp22.33 (617,949-1,497,274) gain

PAK 3 (OMIM *300142) located at chromosome position Xq23, encodes a protein which is a serine-threonine kinase that plays a role in a variety of different signaling pathways including cytoskeleton regulation, cell migration, or cell cycle regulation. It plays a role in dendrite spine morphogenesis as well as synapse formation and plasticity. It is also involved in activation of MAP-kinase pathway. Various mutations in this gene have been linked to X-linked mental retardation.[14] A CNV (83.24Mb) Chromosome X: 69,722,080-152,960,691 containing duplication of PAX3 has been reported definitely pathogenic for short stature in DECIPHER. This CNV polymorph was present in one of our patient who also had SHOX mutation. PAX3 could be the reason for mild intellectual impairment in our patient. It could also be a contributing factor for short stature along with SHOX.

We also searched for concurrence of the most common CNV (chr14: 106,181,830-106,781,904) found in our study with CNV data of patients with diabetes with normal height (>175 cm in males and > 162 cm in females) from our country and found only one person with normal height having the same CNV, but it was loss in copy number rather than the gain in copy number seen in our study cohort. What remains interesting is that a long noncoding RNA, namely, KIAA0125

and a pseudogene ADAM6 were found in almost all (18/19) of our study population which provides a deep insight into the role of a nongenic/noncoding sequences in ISS phenotype. Both are juxtaposed in the same chromosome (chromosome14) and might be involved in regulatory role. These two have also been found to be associated with mental retardation in other unpublished study currently going on in our center.

The select 3 genes found in our patients have been shown in interaction map [Figure 3] with the pink edges indicating the physical interactions and the violet indicating coexpression and blue associated with the similar pathways. Considering the fact that there is pleiotropy, further experimentation on this may augment the association of these genes with ISS. Seldom do we find a gene and a long noncoding RNA associated with a disease. In this case, an experimental evidence of interaction between KIAA00125 and a pseudogene ADAM6 could be an interesting story to further explore.

Conclusion

We identified definite genetic cause of short stature in 3 of our patients with ISS using CMA. We also suggests that CMA is a more sensitive test for chromosomal disorders such as Turner syndrome than conventional karyotype which may have important clinical implications in the future like better screening of these patients for associated diseases as well as early diagnosis, and commencement of treatment with growth hormone which has a very positive impact on final adult height of such children in addition to reducing the total cost of treatment. Furthermore, we hypothesize that long noncoding RNAs, like KIAA0125 may play a role in controlling the expression of known height-related genes which needs to be proven experimentally.

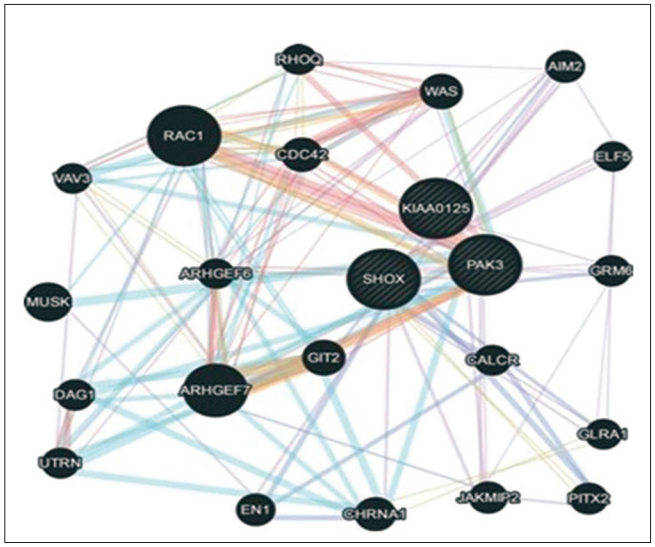

Figure 3: Interaction map of potential candidate genes found in this study

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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