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

Hema Singh¹, Pradeep Tiwari¹,², Vijay Bhavi¹, Praveen Singh Chaudhary¹, Prashanth Suravajhala³, M Krishna Mohan¹, Sandeep Kumar Mathur¹

¹Department of Endocrinology, SMS Medical College, ²Department of Chemistry, Manipal University, Jaipur, ³Department of Biotechnology and Bioinformatics, Birla Institute of Scientific Research, Statue Circle, Jaipur, Rajasthan, India

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 haplinsufficiency 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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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. tGtGA, 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.

**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 (57.9%) 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 52,802 kbps. 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 also found KIAA0125, a long noncoding RNA and ADAM6.

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 |

Table 3: Copy-number variant profiles of the study group

| Number   | Gain          | Loss          | Mosaic        |
|----------|---------------|---------------|---------------|
| Pi1      | 17            | 11            | 3             |
| Pi2      | 13            | 17            | 1             |
| Pi3      | 11            | 11            | 1             |
| Pi4      | 13            | 13            | 1             |
| Pi5      | 11            | 12            | 2             |
| Pi6      | 11            | 11            | 1             |

SDS: Standard deviation score

The potential candidate genes identified in the CNV regions were TBX1, SHOX, TMEM165, 4q12, and Yp11.32-p11.2. Including deletions at 22q11.21, duplications at 4q11-q13.1, 4q12, and Yp11.32-p11.2. The potential candidate genes located in the CNV regions were TBX1, SHOX, TMEM165, POLR2B, and PDGFRα. Zahnleiter et al. searched for rare CNVs in 200 families, 92 sporadic, and 108 familial, with

**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. The potential candidate genes located in the CNV regions were TBX1, SHOX, TMEM165, POLR2B, and PDGFRα. Zahnleiter et al. searched for rare CNVs in 200 families, 92 sporadic, and 108 familial, with

![Figure 1: Standard deviation score for mean height across different patients](image1)

![Figure 2: Copy-number variants found in study group](image2)
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| Patient | Sex  | Height (cm) | Height (SDS) | Weight (kg) | CA (year) | HA (year) | WA (year) | BA (year) | Arm span | US: LS | SMR | Physical features |
|---------|------|-------------|--------------|-------------|-----------|-----------|-----------|-----------|----------|-------|-----|------------------|
| 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 |
| 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, saddle gap |
| 3       | Female | 138     | −2.53        | 27          | 14        | 10.5      | 10.3      | 10        | 140      | 0.69  | A+P2B2 | Short stature, pigmented nevi, short fourth metacarpal |
| 4       | Female | 108     | −2.81        | 15          | 8         | 5.5       | 3.5       | 7         | 108      | 0.96  | A-P1B1 | Short stature, delayed puberty, dry, shiny, hairless skin, micrognathia, wide-spaced nipples, hyper convex nails, increased carrying angle, mild intellectual impairment |
| 5       | Female | 130     | −4.21        | 32          | 17        | 9         | 11.6      | 131       | 0.874    | A-P1B1 | Short stature, IUGR, microcephaly, prognathism, VSD-PAH, rickets |
| 6       | Female | 86      | −5.47        | 9.5         | 7         | 2.5       | 1         | 2.5       | 86       | 1.2   | A-P1B1 | Short stature, isolated short stature |
| 7       | Male | 132      | −2.47        | 28          | 13        | 10        | 10        | 11        | 131      | 0.97  | A2P2G2 | Short stature, isolated short stature |
| 8       | Male | 115      | −3.64        | 24.5        | 11        | 6.5       | 9         | 6         | 115      | 0.91  | A-P2G1 | Short stature, isolated short stature |
| 9       | Female | 114     | −3          | 17          | 10        | 6         | 4.5       | 7         | 117      | 0.96  | A-P1B1 | Short stature, isolated short stature |
| 10      | Female | 128     | −2.8        | 22          | 11.5      | 8.5       | 8.5       | 11        | 127      | 0.91  | A-P2B1 | 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 |
| 11      | Male | 142      | −3.62        | 35          | 16        | 11.5      | 12        | 16        | 150      | 1     | A-P5G3 | Isolated short stature |
| 12      | Male | 133      | −2.36        | 25          | 13        | 9.6       | 9         | 9         | 130      | 0.88  | A-P1G1 | Isolated short stature |
| 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 |
| 14      | Female | 107     | −2.96        | 15          | 8         | 5         | 3.5       | 6.5       | 107      | 0.86  | A-P1B1 | Isolated short stature |
| 15      | Female | 143     | −2.21        | 38          | 17        | 11        | 13        | 17.6      | 145      | 0.86  | A-P4B4 | Isolated short stature |
| 16      | Female | 124     | −3.48        | 20          | 11.9      | 8         | 7         | 10        | 124      | 0.94  | A-P1B1 | Short stature, widely spaced nipples |
| 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 |
| 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 |
| 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

| Patient | 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: 0-3,722,038 | 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: 106,171,686-106,968,257 | Gain | q32.33 | 728.295 |
|         |     | chrX: 144,046,272-144,993,055 | Gain | q27.3 | 1010.363 |
|         |     | chrX: 140,706,625-141,418,197 | Gain | q27.2 | 547.364 |
|         |     | chrX: 148,631,322-149,918,448 | Gain | q28 | 990.098 |
|         |     | chrX: 151,365,806-138,689,495 | Gain | q26.3 | 1018.223 |
| Patient 6 | Female | chr10: 46,018,645-48,401,505 | Gain | q27.3 | 485.923 |
|         |     | chr14: 106,163,525-107,031,454 | Gain | q32.33 | 726.743 |
| Patient 7 | Male | chrY: 27,301,744-28,246,509 | Gain | q11.23 | 726.743 |
|         |     | chr14: 106,191,586-106,780,631 | Gain | q11.22 | 594.341 |
|         |     | chr14: 22,581,094-22,987,206 | Gain | q11.2 | 453.113 |
| Patient 8 | Male | chr14: 106,176,903-106,782,547 | Gain | q32.33 | 465.88 |
|         |     | chrY: 0-3,722,038 | Gain-mosaic | p11.31 | 26,149.23 |
| Patient 9 | Female | chr14: 106,161,758-106,939,405 | Gain | q32.33 | 598.191 |
| Patient 10 | Female | chr14: 106,132,366-106,780,963 | Gain | q32.33 | 598.191 |
| Patient 11 | Male | chr14: 106,181,830-106,781,904 | Gain | q32.33 | 498.921 |
|         |     | chr1: 144,273,391-149,206,051 | Loss | p25.3 | 130.489 |
| 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: 22,581,094-22,987,206 | Loss | q11.2 | 312.394 |
| Patient 13 | Female | chr14: 106,161,758-106,939,405 | Gain | q32.33 | 598.191 |
|         |     | chrY: 0-3,722,038 | Gain-mosaic | p11.31 | 26,149.23 |
| 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 | chr14: 106,172,186-106,885,843 | Gain | q32.33 | 1128.508 |
|         |     | chrX: 51,282,352-52,002,388 | Gain | q11.22 | 553.874 |
|         |     | chr14: 106,016,599-106,958,339 | Gain | q32.33 | 724.416 |
| Patient 16 | Female | chr15: 22,639,758-23,772,175 | Loss | q11.2 | 871.091 |
|         |     | chrX: 14,165,318-155,270,560 | Loss | q28 | 122,667.635 |
| Patient 17 | Female | chr22: 22,841,357-23,361,473 | Gain | q11.22 | 400.09 |
|         |     | chr14: 106,168,324-106,848,325 | Gain | q32.33 | 523.079 |
|         |     | chrX: 0-12,052,026 | Gain-mosaic | p22.33 | 10,333.457 |
| 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. 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. 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. 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.

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. 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

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

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

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**Conflicts of interest**

There are no conflicts of interest.

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