RESEARCH PAPER

Clinical and Genetic Profile of Children With Short Stature Presenting to a Genetic Clinic in Northern India

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Received: November 21,2021;
Initial review: December 29,2021;
Accepted: April 26,2022.

Objective: To define the spectrum of genetic disorders in patients with short stature visiting the genetic out-patient department in a tertiary care hospital. **Methods:** A chart review was done for 455 individuals (10 months-16 yrs) with short stature, who were evaluated at the genetic clinic from 1 January, 2017 upto 31 October, 2018. 226 patients who needed detailed evaluation, the spectrum of genetic diagnosis is presented. **Results**: Proportionate short stature was identified in 63% individuals (*n*=142) of which 93 (65%) were recognizable syndromes such as Turner syndrome, and William syndrome, and RASopathies. In clinically undefined syndromes (39, 27%), a diagnosis could be made by karyotype (*n*=3/10), chromosomal microarray (6/12) and exome sequencing (1/6). In the 84 children in the disproportionate short stature group (37%), lysosomal storage disorders (LSDs) (45%, *n*=38) were identified by enzyme analysis in 86.8% and skeletal dysplasias (44%, *n*=37) identified by skeletal survey in 89% cases. **Conclusions:** In undefined syndromic short stature, chromosomal microarray may be the first investigation of choice if phenotyping is not suggestive of a specific genetic syndrome. Exome sequencing can be useful in identifying newer genes among idiopathic and familial short stature cohorts.

Key words: *Chromosomal microarray analysis, Etiology, Exome sequencing.*

Linear growth is a complex polygenic trait due to the combined effect of many different genes as well as influenced by several environmental factors prenatally and postnatally [1]. However, single genes contribute to the etiology in many patients with significant short stature. The guidelines for genetic testing in short stature have been recently updated by the American College of Medical Genetics (ACMG) [2], along with the likely yield of different genetic tests. Till recently, genetic testing in short stature was limited to testing for Achondroplasia, Turner syndrome and Russell Silver syndrome. The availability of exome sequencing has allowed many new genes like *ACAN, NPR2, FBN1, IHH* and *BMP2* amongst others to be identified and many individuals with milder phenotypes of known monogenic disorders like RASopathies [3] are being diagnosed. We analysed the spectrum of genetic diagnosis in patients with short stature presenting to a tertiary care hospital in northern India.

METHODS

This is a chart review of patients with height less than 3rd centile referred for genetic evaluation of short stature from 1st January, 2017 through 31st October, 2018 (22 months) to the genetic clinic. In addition to short stature, many patients also had additional phenotypes such as developmental delay, cardiac defects, hepatosplenomegaly or bony deformities. Clinical data and investigations including genetic tests were tabulated on a structured, predefined format and analyzed. The anthropometric details were measured as per guidelines [4,5], and data was collated. Two cohorts were defined - patients with proportionate short stature and those with disproportionate short stature on the basis of upper segment and lower segment ratios (US:LS) [6] and were then assessed for etiology. Where standing height could not be taken, length was measured. Patients with incomplete/missing data were excluded from the study.

All patients with disproportionate short stature were clinically evaluated and skeletal survey (antero-posterior views of long bones, hands and feet, pelvis with hip joint, and lateral spine radiographs) was done. In some patients, additionally, a skull radiograph was also taken. Patients with coarse facial features with or without hepatosplenomegaly and dysostosis multiplex were evaluated for lysosomal storage disorders using urine glycosaminoglycans/oligosaccharides and enzyme assay in blood. For those suspected to have achondroplasia or

hypochondroplasia, targeted *FGFR3* gene study was done. In the others, exome sequencing was carried out to evaluate genes of the different skeletal dysplasias based on the clinical and radiographic differential diagnosis [7].

Patients with proportionate short stature were assessed for dysmorphism and associated abnormalities of eye/ear/skin/hair, which could help in assigning the syndrome. Assessment for developmental delay was done by evaluating age dependent skills attained in the gross motor/fine motor, cognitive, language and personal/ social domains. Echocardiography was performed as necessitated on clinical evaluation. Syndrome search was done as per standard practice [8-10]. Karyotyping (50 metaphases counted and five analysed) was done in girls suspected to have Turner syndrome. In patients with features suggestive of William syndrome/22 q deletion syndrome, fluorescence in situ hybridization (FISH) for the specific microdeletion was the first investigation of choice. In children who were small for gestation with a clinical diagnosis of Russell-Silver syndrome (RSS), methylation sensitive multiplex ligation-dependent probe amplification (MS-MLPA) for 11p15 was performed. Maternal uniparental disomy for chromosome 7 was not tested in this study. For the other short stature syndromes, exome sequencing was performed where feasible. Neonates and infants with hypotonia and feeding difficulties with a clinical suspicion of Prader-Willi syndrome (PWS) or those with the characteristic phenotype were evaluated by MS-MLPA. Children suspected of RASopathies and other single gene syndromes were mostly advised clinical or whole exome sequencing to evaluate the genes linked to this pathway, except one who had undergone single gene *PTPN11* sequencing, the commonest gene for Noonan syndrome.

In patients with clinically undefined dysmorphic syndromes chromosomal microarray (CMA) was done to examine chromosomal microdeletions and microduplications. Karyotyping was done if the microarray was not possible. All tests were performed at the in-house laboratory except urine oligosaccharide test and exome sequencing, which was outsourced. Institutional ethics committee cleared the study, and informed written consent was obtained from the legal guardians/adults before genetic testing and photography when done.

Statistical analysis: Data recording and analysing was done in MS Excel format. Descriptive statistics have been used to present the data.

RESULTS

Of a total of 455 patients with short stature in this study period, 226 patients required detailed phenotyping and genetic testing for confirmation of the etiology while 229 were identified on preliminary history/examination and investigations. Of these, 63 % (n=142) had proportionate short stature (**Fig. 1**).

The height of 84 patients with disproportionate short stature ranged from -2 to -3 z-score (60.7%, n=51), -3 to -4 z-score (33.3%, n=28) -4 to -5 z-score (5.9%, n=5). Mean (SD) age was 3.8 (1.9) years and mean (SD) height was 88.8 (10.6) cm. A lysosomal storage disorder (LSD) (45%, n=38) or a skeletal dysplasia (44%, n=37) was present in an almost equal proportion of patients. In patients with LSDs, enzyme analysis confirmed the disorder in 86.8% (n=33) and molecular confirmation was available in 73.6% (n= 28) (**Table I**). Skeletal survey helped to define 89% (n=33) cases of skeletal dysplasia and molecular confirmation could be done in 86.4% (n=32). Nine patients could not be classified (exome sequencing had been done in three of these). Ten patients confirmed with a skeletal dysplasia had proportionate short stature. Overall, a molecular diagnosis was available in 71% (n=60) in this group.

The height of the 142 patients in proportionate short stature group ranged from -2 to -3 z-scores (52.1%, n=74) -3 to -4 z-scores (47.8%, n=68), mean (SD) age was 4.1 (2.5) year and mean (SD) height was 90 (11.5) cms. Recognizable syndromes (excluding Downs syndrome) constituted 65.5% (n=93); 27.4% (n=39) could not be identified on phenotyping and ten cases had skeletal dysplasia (**Fig. 1**). Among the recognizable syndromes, 59% (n=55) were confirmed by the respective cytogene-tic/molecular test and the remaining could not be tested. The molecular and cytogenetic profile of children with a confirmed genetic diagnosis is shown in **Table I**. Testing by MS-MLPA confirmed hypermethylation with 15q11.2 deletion in seven patients with PWS, hypermethylation without deletion in one patient with PWS, hypomethy-lation of 11p15.5 in one patient with RSS. One patient with RSS could not be confirmed on MS-MLPA and karyotype. Two phenotypically male children with DSD and 46 XX karyotype who underwent CMA were found to have deletion of Xp involving *SHOX* and other genes with presence of translocated *SRY* gene.

A stepwise testing identified an etiology in ten patients with a clinically undefined syndrome. Karyotype was done in ten patients and identified an etiology in three (yield = 30%), CMA was done in twelve and identified an etiology in six patients (yield=50%) and exome sequencing was performed in six patients with a definite diagnosis in one patient.

DISCUSSION

In this study we present the genetic spectrum of patients referred to a genetic clinic who had short stature as one of the presenting phenotypes. The utility of CMA in

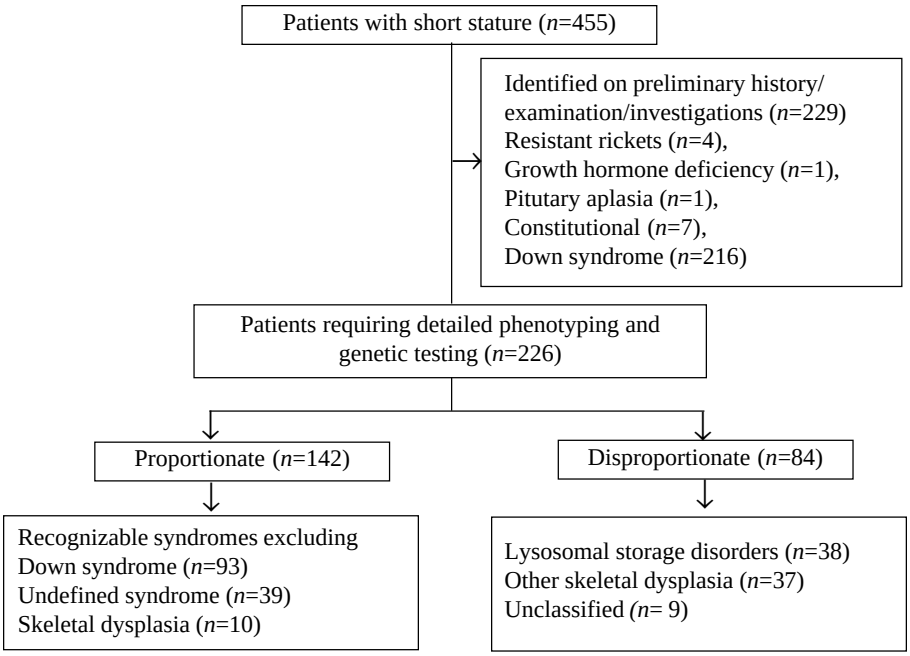

Fig. 1 Study flow chart and results.

syndromic short stature is reported to vary between 10-15% [3]. In the current study the small cohort evaluated by CMA could account for the high yield of 50%. We identified two individuals with *SHOX* gene deletion by CMA as a part of a larger deletion on X chromosome and translocation of the *SRY* gene. Kumar, et al. [11] reported *SHOX* gene deletion in 6.5% of Indian children with idiopathic short stature [11], and worldwide it is reported in 2-15% of such children [1]. However, we could not test all patients of nonsyndromic short stature for *SHOX* gene deletion to derive a diagnostic yield from this study. In this study, a diverse cytogenetic profile including mosai-cism and isochromosome Xq was seen in Turner synd-rome patients, similar to that reported in literature [12]. However, previous studies evaluating karyotype-pheno-type correlations in Turner syndrome have shown conflicting results [12,13].

The yield of exome sequencing in cases of short stature with a negative chromosomal analysis and gene panel testing varies between 16.5% and 46% [2]. In this study, exome sequencing could be done in only six patients with an unexpected diagnosis of histiocytosis and lymphadenopathy syndrome in one patient who presented with only short stature and myopia. The identification of this disorder had important implications for monitoring and comorbid diagnosis in this child. It is possible that decreasing costs of exome sequencing since the time of this study would enable testing of a larger cohort of 'idiopathic' short stature patients and improve patient management.

Traditionally, skeletal dysplasias are diagnosed on clinical examination and skeletal survey as was seen in 89% of the study cohort. However, we feel that with recognition of short stature in heterozygous carriers, exome sequencing could increase the genetic diagnosis, as seen in one patient with familial short stature and pathogenic variant in the recently implicated *ACAN* gene [3]. In this study, a definite molecular diagnosis of skeletal dysplasia (including LSDs) was achieved in 71% cases.

While individuals with short stature and defects in the GH-IGF-1 axis were under represented in our study (two patients), as they were probably referred to endocrinology clinics, it remains an important cause of proportionate short stature to be evaluated. Its incidence of 1.5-5.1% has been reported previously [11,14].

The limitation of this study is that due to its retrospective nature and bias of a genetic clinic referral, patients with hypothyroidism, GH deficiency, celiac disease, constitutional growth delay may be under represented and individuals with idiopathic short stature were not a part of this cohort.

Clinical evaluation within the spectrum of proportion-ate and disproportionate short stature helps to categorize the kind of genetic tests to be performed from the armamen-tarium of urine analysis for glycosaminoglycans and

WHAT THIS STUDY ADDS?

- In clinically undefined syndromes of short stature with developmental delay and/or dysmorphology chromosomal microarray may be the first investigation of choice.
- In familial short stature or idiopathic short stature exome sequencing may identify rare monogenic short stature syndromes.

oligosaccharides, metabolic enzyme testing, karyotype, chromosomal microarray, targeted gene testing and next generation sequencing tests including panel and exome tests. Through this study, we have attempted to represent the appropriate testing indications. This become more relevant with the increasing availability of the tests and decreasing costs since the time of this data. Achieving a definitive diagnosis can help to guide prognosis, explore utility of recombinant human growth hormone therapy [15] and provide genetic counselling to families.

Ethics clearance: EIC;Sir Ganga Ram Hospital; No: EC/04/21/1862, dated June 8, 2021.
Contributors: RDP,KS,SBM,ICV: conceptualization and writing original draft; RDP, KS: methodology; ML,PS: cytogenetic analysis of cases; JV,DT: enzyme analysis of cases; RS,SK: molecular analysis of cases. All authors approved the final version of manuscript, and are accountable for all aspects related to the study.
Funding: None; *Competing interests*: None stated

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