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Phenotyping and genotyping of skeletal dysplasias: Evolution of a center and a decade of experience in India

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ABSTRACT

Genetic heterogeneity, high burden and the paucity of genetic testing for rare diseases challenge genomic healthcare for these disorders in India. Here we report our experience over the past decade, of establishing the genomic evaluation of skeletal dysplasia at a tertiary university hospital in India. Research or clinical genomic testing was carried out by Sanger sequencing and next-generation sequencing. Close national and international collaborations aided phenotyping and genotyping. We report 508 families (557 affected individuals) with the definitive molecular diagnosis of skeletal dysplasia. Dysostoses multiplex (n = 196), genetic inflammatory/rheumatoid-like osteoarthropathies (n = 114) and osteogenesis imperfecta and decreased bone density (n = 58) were the most common diagnoses. We enumerate the processes, clinical diagnoses and causal variants in the cohort with 48 novel variants in 21 genes. We summarize scientific contributions of the center to the description of clinical and mutation profiles and discovery of new phenotypes and genetic etiology. Our study illustrates the establishment and application of genomic testing tools for genetic disorders of skeleton in a large cohort. We believe this could be a model to emulate for other developing genetic centers.

1. Introduction

Skeletal dysplasias are a heterogeneous group of disorders with abnormal skeletal size, shape, density and cartilage growth. They often present with a variety of orthopedic, neurologic, pulmonary, cardiac, renal, visual, auditory and neurodevelopmental symptoms. Currently, the genetic skeletal disorders are a combination of skeletal dysplasias and dysostoses, and 436 distinct entities listed under 42 nosology groups [1]. Individually, skeletal dysplasias are very rare, but collectively their incidence is nearly 1 in 5000 live births [2]. Notably, their prevalence rates in India are not known. A tertiary medical center from Karnataka, India estimated the incidence of skeletal dysplasias among new-borns to be 19.6 per 10,000 births and 5.2 per 10,000 births for lethal skeletal dysplasias [3]. While clinical evaluation, ultrasonography (prenatal onset skeletal dysplasias), and radiography are traditionally used for diagnosis of skeletal dysplasias, a high degree of phenotypic variability necessitates the integration of genomic testing with clinical and radiological approaches.

India is one of the fastest developing countries with diverse ethnicities and sub-populations. Most studies concur on the presence of a very unique mutation spectrum of genetic disorders for Indians when compared to other ethnicities [4–6]. Presently, few clinical reports and mutation profiles of large cohort of single disorders have been reported in our country [7]. Paucity of reports on molecular testing for skeletal dysplasias reflects the scarcity of molecular testing and research facilities. The delineation of the mutation spectrum for skeletal dysplasias is imperative to facilitate accurate diagnosis, disease prognostication, prenatal diagnosis, medical management and genetic counseling for the affected individuals and their families.

In this context, here we present our experience of testing 508 families with skeletal dysplasias over a period of ten years (2008–2017) at the Medical Genetics unit of Kasturba Medical College and Hospital, Manipal, India. Our study illustrates the spectrum of genetic disorders of the skeleton tested at Manipal over the last decade, highlights the salient clinical and molecular findings and provides a model to emulate for establishing genomic diagnosis of rare disorders in developing countries.

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2. Materials and methods

2.1. Patients

Clinical services at the center in Manipal were initiated in 2008 with a team of a medical geneticists and orthopedists. A laboratory completely equipped for molecular testing was operational at Manipal by 2012. Next generation sequencing (NGS) based testing was provided from 2014 onwards.

Patients visiting our center were recruited for the study after detailed clinical evaluation, including pedigree analyses, radiological survey and informed consent. The clinical and radiographic details of the patients were recorded in a predetermined case proforma. We have also recruited patients for molecular testing referred by various clinical collaborators from 13 cities in 6 states of India (Uttar Pradesh, West Bengal, Maharashtra, Telangana, Karnataka, Tamil Nadu and Kerala) after gathering the clinical information (See list of collaborators, Table S2). Skeletal survey was obtained for probands in all families. 2–3 ml of EDTA blood was collected from proband and the available family members. The research protocols employed during the entire duration of study have been approved by the Ethics Committee of Kasturba Hospital, Manipal and were funded by various national and international agencies (Table S1).

2.2. Methods

Molecular genetic testing was offered, either as a clinical service or after recruitment in one of the ongoing research proposals. In families with specific clinical diagnosis such as achondroplasia, hypochondroplasia, Morquio syndrome A, GM1 gangliosidosis, progressive pseudorheumatoid dysplasia (PPD) and multicentric osteolysis nodulosis and arthropathy (MONA), in-house targeted genetic testing was carried out. Genomic DNA was extracted from whole blood of probands and parents using phenol-chloroform method. Primers were designed using Primer3web [49] and primer-BLAST [50] for the coding exons and flanking intronic regions. PCR amplification with the respective primers and Sanger sequencing were performed followed by the variant analysis using chromatogram files.

NGS based testing (gene panel or whole exome sequencing) was employed wherever appropriate. Exome sequencing was outsourced to commercial service providers. Patient's sample, either dried blood spot or genomic DNA, was transported to the service provider's laboratory. The exome sequencing data was received in the form of fastq files through cloud. This raw data was analyzed using the in-house pipeline based on BWA (v0.7.15) [8] and GATK best practices (v3.6) [9]. The variant call format (vcf) files were annotated by using ANNOVAR [10] and in-house scripts. Variants with minor allele frequency $< 2\%$ were filtered with the help of population databases such as Exome Aggregation Consortium (ExAC) [11], gnomAD and an in-house variant database. Disease causing variants were prioritized by applying case specific variant filtering strategies combined with clinical correlation. Candidate variants were validated using Sanger sequencing.

Subsequently an in-house database of sequence variants observed in the local population was collated as there is a scarcity of publicly available high throughput sequence data from the Indian subcontinent [12,13]. This database significantly facilitated our variant prioritization and filtering processes for candidate gene identification.

Pathogenicity of the variants observed by targeted gene testing and exome sequencing was assessed by a systematic approach. The variants were checked against population data bases like gnomAD [51], ExAC [52] and an in-house exome database. Mutation databases HGMD [53] and ClinVar [54], and the existing literature for the respective genes, were queried to confirm the novelty of the variant. Multiple in-silico prediction tools, MutationTaster [57], SIFT [56] and PolyPhen2 [55] and analysis of splicing variants by human splice finder [58] and NetGene2 [59] were used to predict the pathogenicity of the variants. Segregation analysis with parental and family members' samples was done. The classification of variants was performed as per ACMG standards and guidelines and HGVS guidelines were implemented for variant nomenclature [14,15]. Concordance of clinical and molecular findings in patients was verified and clinical or research reports, as applicable were issued to the patients in prescribed formats, under the supervision of clinical geneticists and molecular genetics experts.

All the skeletal dysplasia patients diagnosed at our center were further categorized into different groups in accordance with the 'Nosology and Classification of Genetic Skeletal Disorder: 2015 revision'. [1] Here, we report only on the patients and families diagnosed at our facility with molecularly proven genetic skeletal disorders (without enumeration of non-diagnostic tests).

3. Results

Five hundred and eight (n = 508) families with a skeletal dysplasia (557 affected individuals) at our center from 2008 to 2017 have a molecular diagnosis. Clinical and research testing were performed using one of the following methods: Sanger sequencing (397 families), gene panel testing (38 families) or exome sequencing (73 families). Patients without a molecular diagnosis have been excluded in this report. Among the 508 families enumerated here, 290 were males and 210 were females. These families did not disclose any relatedness to each other. Carrier analysis and prenatal testing was offered to eight prenatal samples. Age of probands ranged from fetuses to 47 years. Most subjects were in the pediatric age group (Supplementary fig. 1A).

Confirmation of molecular diagnosis was achieved in probands from 508 families (and additional 49 affected family members). Most of the families had an autosomal recessive condition (n = 419, 82.5%) (Supplementary fig. 1B). Autosomal dominant conditions constituted 16.3% (n = 83) of the diagnoses and X-linked disorders were very rare (n = 6, 1.2%). Of these 508 families, the marriages in 187 families were consanguineous (37%), 242 were non-consanguineous (47.6%) and this data was not available for the other 79 families (15.5%). Among the 419 families with autosomal recessive conditions, 43% reported parental consanguinity and 43.4% denied the same. Consanguinity data was not available for 14% of those diagnosed with recessive conditions.

These families were classified as per the recommendations of the 'Nosology and Classification of Genetic Skeletal Disorder: 2015 Revision' and diagnoses are summarized in Table 1. The common group of disorders in our cohort includes: dysostoses multiplex group (n = 196), genetic inflammatory/rheumatoid-like osteoarthropathies (n = 114) followed by 'osteogenesis imperfecta and decreased bone density' (n = 58), FGFR3 chondrodysplasias (n = 16), osteolysis (n = 15) and 'filamin group and related disorders' (n = 14) (Fig. 1). Fifteen families could not be classified into any of the existing groups and have been listed as unclassified disorders in Table 1.

We present our work as genotype and phenotype series on large cohorts, validation of genes and phenotypes for extremely rare disorders, new phenotypes without a known genetic cause, new genes for known phenotypes and newly discovered phenotypes and genotypes below.

3.1. Genotype and phenotype series

Dysostosis multiplex group constituted the largest series in our patient cohort, including Morquio syndrome (MPS IVA, n = 123), GM1 gangliosidosis (n = 71), Sanfilippo syndrome (Mucopolysaccharidosis type III, n = 1) and Mucolipidosis type α/β (n = 1). Analysis of *GALNS* revealed that the sequence variants, p.Phe216Ser (9%), p.Ser287Leu (6%), c.1003-3C > G (6%), p.Asn32Thr (6%), p.Leu36Arg (6%), p.Cys79Arg (6%), p.Arg251Gln (5%), p.Ala291Ser (4%), p.Met1Ile (3%), p.Pro151Leu (3%), p.Asn204Thr (3%) and p.Gln414Ter (3%) accounted for nearly 60% of the total pathogenic variants in *GALNS* gene in the studied population. The pathogenic variants in exon 1, 7

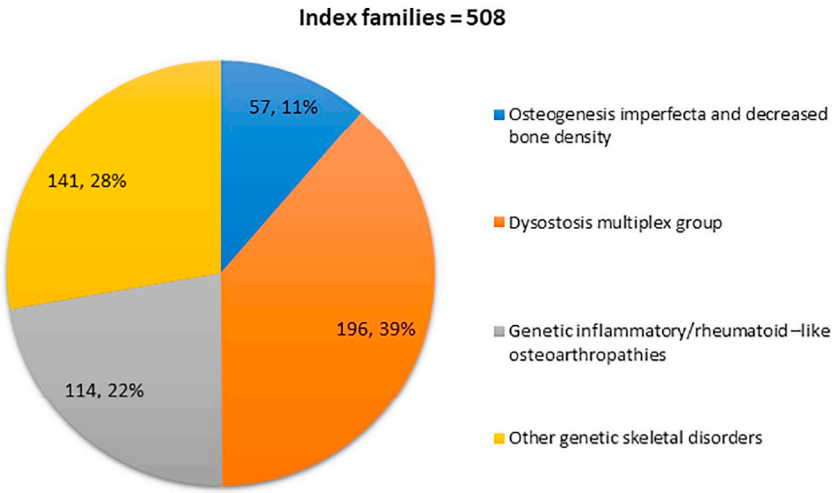

Fig. 1. Referral pattern of genetic skeletal disorders at Manipal.

and 8 comprise 44.8% of the total variants, and thus may be considered as a cost-effective first line diagnostic strategy in Asian Indian population [16]. Seventy one families with GM1 gangliosidosis (and 8 couples from unrelated families for carrier screening followed by prenatal screening) were sequenced for variants in *GLB1*. The variants, c.75 + 2dupT (15%), p.Leu337Pro (10%), p.Trp527LeufsTer5 (9%), p.Arg457Ter (6%), p. Phe357LfsTer26 (5%), p.Val360TyrfsTer23 (5%) and p.Arg482His (5%) constituted approximately 54% of all pathogenic variants detected [17].

Progressive pseudorheumatoid dysplasia (PPD) is the second largest series (n = 106 families, 21%) in our cohort. Pathogenic sequence variants, p.Cys52Ter and p.Cys78Tyr in exon 2, and p.Cys337Tyr in exon 5 of *WISP3* gene accounted for approximately 70% of all variants. Mutation profiles have been published for seventy-nine of these families (95 patients) with PPD. [18,19] Founder event was identified for the variant, p.Cys78Tyr by genome-wide SNP genotyping studies [20].

Probands of 57 families with osteogenesis imperfecta (OI) were tested by either next generation sequencing based panel or exome sequencing. Pathogenic variants were observed in eight genes: *COL1A1* (n = 18), *COL1A2* (n = 10), *WNT1* (n = 9), *SERPINF1* (n = 6), *LEPRE1* (n = 6), *FKBP10* (n = 4), *BMP1* (n = 2) and *IFITM5* (n = 2). Pathogenic variants in *COL1A1* and *COL1A2* accounted for 32% (n = 18) and 17% (n = 10) respectively. A high percentage (48%) of autosomal recessive forms of OI due to pathogenic variants in *BMP1, FKBP10*, *LEPRE1, SERPINF1*, and *WNT1* was due to a high degree of consanguinity and founder events owing to the several inbred subpopulations and endogamous marriages practiced over several centuries [21–23].

Molecular analysis of fifteen patients with multicentric osteolysis nodulosis and arthropathy (MONA) showed that three variants, p.Asn430ThrfsTer68, p.Arg101Cys and p.Arg101His were recurrent in *MMP2* [24]. Seven novel deleterious biallelic truncating variants in *FLNB* gene in 10 patients from 7 families with spondylocarpotarsal synostosis syndrome (SCT) have expanded the clinical and molecular repertoire of this syndrome [25]. Four novel missense variants were seen in *FLNB* gene in four families with Larsen syndrome [26]. Three novel and a known pathogenic variant were identified in *CTSK* gene in seven patients from four unrelated families with pycnodysostosis [27].

3.2. Validation of genes and phenotypes

Our reports on the identification of pathogenic variants in *LRRK1* resulting in osteosclerotic metaphyseal dysplasia (OSMD), *SFRP4* in Pyle's disease, *COL27A1* in Steel syndrome and *BMPER* in ischiospinal dysostosis (ISD) have further validated their genotype and phenotype associations [28–31]. We have also reported novel *RAB33B* pathogenic variants in three additional patients with Smith-McCort Dysplasia (SMC), further validating the pathogenic relationship between *RAB33B* and SMC [32]. Biallelic pathogenic variants identified in an Indian patient in *KIAA0753* gene and further functional characterization in zebrafish embryos have confirmed its role in short-rib thoracic dysplasia, thus extending the phenotypes of skeletal ciliopathies [33].

3.3. New phenotypes

Four novel phenotypes have been reported from our center. A new entity with multiple swellings on skull due to massive cranial osteolysis, wrinkly skin with mosaic hypopigmentation, growth retardation, facial anomalies, severe hypercalcemia and developmental delay, suggestive of a Gorham-like syndrome, was reported in a 16-months-old girl [34]. We have also reported on a novel syndrome with overlapping clinical features of Larsen (OMIM 150250) and otopalatodigital syndromes (OPD; OMIM 311300 and 304120) in an adolescent girl with sparse scalp hair, cubital pterygium, facial dysmorphism, scoliosis, short distal phalanges and swan neck deformity of fingers [35]. Another rare condition with symmetrical and bilateral terminal transverse limb reduction defect was observed in two fetuses [36]. A novel phenotype of growth deficiency, intellectual disability, distinctive dysmorphic features, cataract, pigmentary retinopathy, hypoplastic thorax, kyphoscoliosis, and unusual skeletal changes was also reported in a 14-year-old girl [37]. Genomic testing was not carried out in any of these individuals.

3.4. New genes

Two novel genes were discovered in collaboration with national and international experts. A new candidate gene, *sFRP4* responsible for cortical-bone fragility was identified in subjects with Pyle's disease [38]. Exome sequencing in three families including one from our center with an X-linked spondyloepimetaphyseal dysplasia (XLR-SEMD) detected a recurrent pathogenic variant in *BGN* and this nosologic entity has now been defined by Cho et al., as, XLR SEMD, BGN type [39].

3.5. Novel genes and phenotypes

Exome sequencing analysis resulted in the discovery of two novel genes and phenotypes from our cohort. We reported a homozygous nonsense variant, p.Arg142Ter in *IFT52,* a component of the intraflagellar transport (IFT) complex B of primary cilia, causing a novel form of human skeletal ciliopathy (OMIM 617102) [40]. Recently,

Table 1
Summary of molecularly proven skeletal dysplasias at Manipal.

Group	Nosology	Number of families (total = 508)	SKD class and No. of patients (n = 557)	MIM#	Gene	MIM*	Inheritance pattern	PMID of published manuscript
1	FGFR3 chondrodysplasia group	16	a) Achondroplasia (13)	100800	*FGFR3*	134934	AD	NA
			b) Hypochondroplasia (3)	146000	*FGFR3*	134934	AD	NA
2	Type 2 collagen group	4	a) Kniest dysplasia (2)	156550	*COL2A1*	120140	AD	NA
			b) Spondyloepimetaphyseal dysplasia Strudwick type (2)	184250	*COL2A1*	120140	AD	NA
			c) Spondyloepiphyseal dysplasia congenita (1)	183900	*COL2A1*	120140	AD	NA
3	Type 11 collagen group	1	Stickler syndrome type III (1)	604841	*COL11A1*	120280	AD	NA
4	Sulfation disorders group	2	Diastrophic dysplasia (2)	222600	*SLC26A2*	606718	AR	NA
7	Filamin group and related disorders	14	a) Larsen syndrome (4)	150250	*FLNB*	603381	AD	27048506
			b) Spondylocarpotarsal synostosis syndrome (12)	272460	*FLNB*	603381	AR	29566257
			c) Frank-Ter-Haar syndrome (3)	249420	*SH3PXD2B*	613293	AR	NA
8	TRPV4 group	5	a) Metatropic dysplasia (1)	156530	*TRPV4*	605427	AD	27567651
			b) Spondylometaphyseal dysplasia, Kozlowski type (4)	184252	*TRPV4*	605427	AD	NA
9	Ciliopathies with major skeletal involvement	5	a) Short-rib thoracic dysplasia type 3 with or without polydactyly (1)	613091	*DYNC2H1*	603297	AR	NA
			b) Ellis-van Creveld Syndrome (1)	225500	*EVC*	604831	AR	NA
			c) Short-rib thoracic dysplasia 2 with or without polydactyly (1)	611263	*IFT80*	611177	AR	NA
			e) Short-rib thoracic dysplasia type 6 with or without polydactyly (2)	263520	*NEK1*	604588	AR	NA
10	Multiple epiphyseal dysplasia and pseudoachondroplasia group	10	a) Multiple epiphyseal dysplasia (1)	607078	*MATN3*	602109	AD	NA
			b) Pseudoachondroplasia (9)	177170	*COMP*	600310	AD	NA
11	Metaphyseal dysplasias	8	a) Metaphyseal dysplasia, Schmid type (2)	156500	*COL10A1*	120110	AD	NA
			b) Cartilage hair hypoplasia (7)	250250	*RMRP*	157660	AR	NA
			c) Metaphyseal dysplasia CHH like, POP1 type (1)	617396	*POP1*	602486	AR	NA
13	Spondylo-epi-(meta)-physeal dysplasias (SE(M)D)	9	a) Dyggve-Melchoir-Clausen dysplasia (6)	223800	*DYM*	607461	AR	NA
			b) Smith-McCort dysplasia (2)	615222	*RAB33B*	605950	AR	28127940
			c) X-linked spondyloepiphyseal dysplasia tarda (4)	313400	*TRAPPC2*	300202	XLR	NA
15	Acromelic dysplasias	1	Acromicric dysplasia (1)	102370	*FBN1*	134797	AD	NA
16	Acromesomelic dysplasias	1	Acromesomelic dysplasia type Maroteaux (AMDM) Grebe dysplasia (1)	602875	*NPR2*	108961	AR	NA
18	Campomelic dysplasia and related disorders	1	Campomelic dysplasia (1)	114290	SOX9	608160	AD	NA
20	Dysplasias with multiple joint dislocations	1	SEMD with joint laxity (SEMD-JL) leptodactylic or Hall type (1)	603546	*KIF22*	603213	AD	NA
21	Chondrodysplasia punctata group	1	Chondrodysplasia punctata 2, Conradi-Hunermann type (1)	302960	*EBP*	300205	XLD	NA
23	Osteopetrosis and related disorders	4	Pycnodysostosis (6)	265800	*CTSK*	601105	AR	NA
24	Other sclerosing bone disorders	5	Pyle disease (2)	265900	*sFRP4*	606570	AR	28100910; 27355534;
			Hypertrophic osteoarthropathy, primary (3)	259100	*HPGD*	601688	AR	NA
25	Osteogenesis imperfecta and decreased bone density group	58	a) Osteogenesis imperfecta (59)	610915	*P3H1/LEPRE1* (6)	610339	AR	25691190;
					COL1A1 (18)	120150	AD	24668929;
					SERPINF1 (6)	172860	AR	29499418
					COL1A2 (10)	120160	AD	
					FKBP10 (4)	607063	AR	
					WNT1 (10)	164820	AR	
					BMP1 (3)	112264	AR	
					IFITM5 (2)	614757	AD	
			b) Singleton-Merten Syndrome (1)	182250	*IFIH1*	606951	AD	NA
26	Abnormal mineralization group	2	Hypophosphatemic rickets with hypercalciuria (2)	241530	*SLC34A3*	609826	AR	NA

(*continued on next page*)

Table 1 (*continued*)

Group	Nosology	Number of families (total = 508)	SKD class and No. of patients (n = 557)	MIM#	Gene	MIM*	Inheritance pattern	PMID of published manuscript
27	Lysosomal Storage Diseases with Skeletal Involvement	196	a) Morquio A syndrome (127)	253000	*GALNS*	612222	AR	25252036
			b) GM1 gangliosidosis (72)	230500	*GLB1*	611458	AR	25936995
			c) Sanfilippo syndrome type 3B (2)	252920	*NAGLU*	609701	AR	NA
			d) Mucolipidosis type α/β (1)	252500	*GNPTAB*	607840	AR	22495880
28	Osteolysis group	15	Multicentric osteolysis nodulosis and arthropathy (18)	259600	*MMP2*	120360	AR	26601801
29	Disorganized development of skeletal components group	1	Hereditary multiple cartilaginous exostoses 1 (1)	133700	*EXT1*	608177	AD	NA
31	Genetic inflammatory/rheumatoid –like osteoarthropathies	114	a) Hyaline fibromatosis syndrome (4)	228600	*ANTXR2*	608041	AR	NA
			b) Progressive pseudorheumatoid dysplasia (127)	208230	*WISP3*	603400	AR	22987568; 25988854
			c) Chronic neurologic cutaneous and articular syndrome (1)	607115	*NLRP3/CIAS1*	606416	AD	NA
			d) Hyperostosis/hyperphosphatemia syndrome, tumoral calcinosis (3)	211900	*GALNT3*	610233	AR	NA
32	Cleidocranial dysplasia and related disorders	1	Cleidocranial dysplasia (1)	119600	*RUNX2*	600211	AD	27177937
33	Craniosynostosis syndrome	2	Apert syndrome (2)	101200	*FGFR2*	176943	AD	NA
34	Dysostoses with predominant craniofacial involvement	2	Craniofrontonasal dysplasia (2)	304110	*EFNB1*	300035	XLD	NA
35	Dysostoses with predominant vertebral with and without costal involvement	8	a) Spondylocostal dysostosis I (8)	277300	*DLL3*	602768	AR	NA
			b) Spondylocostal dysostosis II (1)	608681	*MESP2*	605195	AR	NA
37	Brachydactylies (without extra skeletal manifestations)	1	Brachydactyly type 1 (1)	112500	*IHH*	600726	AD	28794911
39	Limb hypoplasia-reduction defects group	1	a) Werner mesomelia (1)	188740	*LMBR1*	605522	AD	24478176
41	Polydactyly-Syndactyly-Triphalangism group	4	a) Synpolydactyly (2)	186000	*HOXD13*	142989	AR	28794915
			b) Meckel syndrome 3 (1)	607361	TMEM67	609884	AR	NA
			c) Meckel syndrome 4 (1)	611134	CEP290	610142	AR	NA
			d) Meckel syndrome 6 (1)	612284	*CC2D2A*	612013	AR	NA
NA	Unclassified autosomal recessive skeletal dysplasia	15	a) Osteosclerotic metaphyseal dysplasia (1)	615198	*LRRK1*	610986	AR	27829680
			b) Steel syndrome (1)	615198	*COL27A1*	608461	AR	28276056
			c) Vitamin D resistant rickets (1)	600081	*CYP2R1*	608713	AR	NA
			d) Ischiospinal dysostosis (1)	NA	*BMPER*	608699	AR	NA
			e) New skeletal ciliopathy (1)	617102	*IFT52*	617094	AR	26880018
			f) Short rib-type skeletal dysplasia (1)	NA	*KIAA0753*	617112	AR	29138412
			g) Spondyloepimetaphyseal dysplasia (SEMD) with multiple joint laxity and dislocations (2)	NA	*EXOC6B*	NA	AR	26669664
			h) Oral-facial-digital syndrome 6, Varadi-PAPP syndrome (3)	277170	*C5ORF42*	614571	AR	NA
			i) Bardet-Biedl syndrome (1)	615987	*BBS10*	610148	AR	NA
			j) Tetra Amelia syndrome (1)	273395	*RSPO2*	610575	AD	29977062
			k) X-linked recessive spondyloepimetaphyseal dysplasias (XLR-SEMD), BGN type (1)	300106	*BGN*	301870	XLR	27236923
			l) Short-rib Thoracic Dysplasia (1)	NA	*SYNJ2*	609410	AR	NA
			m) Meckel syndrome type 1 (1)	249000	TXNDC15	617778	AR	NA
			n) Meckel syndrome 10 (1)	614175	*B9D2*	611951	AR	NA

NA: Not applicable.
MIM# - A descriptive entry, usually of a phenotype in Online Mendelian Inheritance in Man (OMIM).
MIM* - A gene in Online Mendelian Inheritance in Man (OMIM).

another individual with compound heterozygous sequence variants in *IFT52* along with functional evidence of its vital role in ciliary biogenesis, transport and maintenance has been reported, thereby confirming the association of *IFT52* with a skeletal ciliopathy [41]. We also reported a homozygous nonsense p.Tyr302Ter variant in *EXOC6B* in a family with an uncharacterized autosomal recessive spondyloepimetaphyseal dysplasia (SEMD) with scoliosis, severe joint laxity and dislocations [42].

3.6. Novel variants

Here we are also listing the 48 unreported disease causing variants (Table 2) in twenty-one distinct genes, *ANTXR2* (n = 2), *BBS10* (n = 1), *COL1A1* (n = 1), *COL2A1* (n = 2), *COL10A1* (n = 2), *COL11A1* (n = 1), *COMP* (n = 4), *CPLANE1* (n = 3), *DYM* (n = 2), *EFNB1* (n = 2), *GALNS* (n = 6), *GALNT3* (n = 1), *GLB1* (n = 5), *KIF22* (n = 1), *NAGLU* (n = 1), *NEK1* (n = 2), *NPR2* (n = 1), *POP1* (n = 1), *RMRP* (n = 4), *SOX9* (n = 1) and *WISP3* (n = 5) in our cohort. Mendelian segregation was confirmed for all these variants.

4. Discussion

Skeletal dysplasias have been largely diagnosed by clinical and radiographic evaluation in India with a very limited literature on prevalence, spectrum and genetic etiology [3,5,6]. Here we describe data on phenotyping and genotyping of skeletal dysplasias from a tertiary university hospital in Manipal, India over the last decade (2008–2017) in a large cohort of 508 families.

There are very few publications outlining the prevalence and mutation spectrum of skeletal dysplasias in India. One of the first hospital-based studies from India reported 169 individuals with skeletal dysplasias. The clinical diagnosis ascertained that 59% of the patients corresponded to osteochondrodysplasias and the remaining 41% were diagnosed with dysostoses and other conditions with predominant skeletal involvement [3]. A subsequent retrospective analysis of 273 fetal autopsies by Puri et al. showed the preponderance of short-rib thoracic dysplasia (33%) among the lethal skeletal dysplasias in their patient cohort [6]. Recently Nampoothiri et al., have reported a large series of 514 consecutive diagnoses of skeletal dysplasias from Kerala, India, with clinical, radiological, biochemical and molecular evidences. One hundred and nine patients out of 514 (21.2%) had a definitive molecular diagnosis [5]. A Turkish retrospective study of 417 patients with genetic skeletal disorders mainly osteochondrodysplasias (along with 10 fetal autopsies) carried over a five-years period was the first study to be reported from the Middle East and Eastern Mediterranean region. This study had considered the patients with osteochondrodysplasias and excluded patients with overgrowth syndromes, dysostoses and craniofacial syndromes. Further, it established definitive diagnosis in 79% of the patients and 18% of them received a probable diagnosis, while no diagnosis was achieved for remaining the 3% of the patients. Finally, a molecular diagnosis was attained for 17% of the patients evaluated [4].

The Turkish study had showed a higher rate of overall consanguinity (53%) in their population [4]. However, overall consanguinity rate observed in our patient cohort is 37% while 13.6% consanguinity has been reported in the Indian patient cohort from Kerala by Nampoothiri et al. The consanguinity rate reported in all over India by Bittles and his colleagues was only 11.6% of consanguinity rate whereas the South Indian population (Andhra Pradesh, Karnataka and Kerala) demonstrated an increased incidence of consanguineous marriages (23%) [43,44]. When only recessive skeletal dysplasias were considered, we noted 43% consanguinity which is comparable to 31% of consanguinity observed in Kerala. Thus, our study illustrates supporting evidence for the high rate of consanguineous marriages observed in South India. Further, we observed a higher incidence of recessive conditions and homozygous sequence variants in 43.3% of families despite consanguinity being denied by them. Of interest is a higher proportion of recessive genes in the osteogenesis imperfecta pool. Recent studies have shown increased incidence of autosomal recessive conditions in India. This is likely attributed to the unique social structure of India where endogamy is practised within small communities, as well as to founder effect [20,45–47].

The identification of specific mutation spectrum of rare genetic disorders in specific populations and ethnic groups aids in devising appropriate diagnostic and management strategies [4–6]. Our work delineated mutation spectrum of several recessive skeletal dysplasias in India, including Morquio A syndrome, GM1 gangliosidosis, progressive pseudorheumatoid dysplasia and multicentric osteolysis nodulosis arthropathy, in addition to identifying the recurrent pathogenic variants in the population. The delineation of these recurrent variants has helped in designing cost-effective diagnostic approaches for our population (unpublished data).

We have also given the details of 48 unreported variants in twenty-one distinct genes (Table 2) in patients with skeletal dysplasia. Nineteen variants were missense (n = 19), seven nonsense (n = 7), six affecting splice sites (n = 6), twelve causing frameshift (n = 12; 7 small deletions, 2 insertions and 3 indels) and four small duplications in non-coding RNA of *RMRP* (n = 4) were detected in our cohort. The molecular genetic testing data of all the patients was detailed in Supplementary table S4. These would aid molecular evaluation of skeletal dysplasia in general and Asian Indians in particular.

Based on the nosology and classification of genetics skeletal disorders, 2015 revision, “dysostoses multiplex”, “genetic inflammatory/rheumatoid-like osteoarthropathies” and “osteolysis” groups were the most common in our cohort. Similar findings were reported by Nampoothiri et al., with “dysostoses multiplex”, “*FGFR3* chondrodysplasias”, “osteogenesis imperfecta and decreased bone density” groups among the largest in their series using an earlier classification [48]. The Turkish study showed “*FGFR3* chondrodysplasias”, “osteogenesis imperfecta with decreased bone density” and “lysosomal diseases with skeletal involvement (dysostoses multiplex)” as the most frequently diagnosed groups in accordance with the 2010 nosology classification. The large number of individuals with the “genetic inflammatory/rheumatoid-like osteoarthropathies” seen in our cohort may not be the representation of high prevalence of this group of disorders and may be attributed to referral bias for research testing [5].

Similar to the earlier studies, our experience over a period of ten years has shown the cohesive scientific collaborations within India and across the world aiding detection of rare and novel causative sequence variants and elucidation of their role in human skeletal disease. Our work also established an in-house database of variants from exome data of 519 individuals.

5. Conclusions

In conclusion, this study demonstrates how clinical expertise, research collaborations, funding and application of the high throughput approaches were utilized in a low-and middle-income country with resource limitations for creation of a center for comprehensive care of skeletal dysplasias. Emulation of this model by other centers would aid in strengthening the healthcare of rare disorders for genetic counseling, efficacious and appropriate management and therapeutics.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bone.2018.10.026.

Declarations of interest

None.

Table 2
Novel variants observed in our cohort.

S. No.	Gene	MIM*	Ref Seq transcript ID	Nucleotide change	Amino acid change
1	*ANTXR2*	608041	NM_058172.5	c. 1A > G	p.(M1?)
				c.797-1G > A	p.?
2	*BBS10*	610148	NM_024685.3	c.1337_1338del	p.(Phe446TyrfsTer3)
3	*COL1A1*	120150	NM_000088.3	c.1877G > A	p.(Gly626Asp)
4	*COL2A1*	120140	NM_001844.4	c.1680 + 2dup	p.?
				c.2627G > A	p.(Gly876Asp)
5	*COL10A1*	120110	NM_000493.3	c.1989C > A	p.(Tyr663Ter)
				c.2018C > A	p.(Ser673Ter)
6	*COL11A1*	120280	NM_080629.2	c.1057G > A	p.(Glu353Lys)
7	*COMP*	600310	NM_000095.2	c.874 T > C	p.(Cys292Arg)
				c.976G > A	p.(Asp326Asn)
				c.1309G > A	p.(Asp437Asn)
				c.1445A > T	p.(Asp482Val)
8	*CPLANE1*	614571	NM_023073.3	c.2666C > T	p.(Ala889Val)
				c.7020_7021insA	p.(Leu2341ThrfsTer3)
				c.8710C > T	p.(Arg2904Ter)
9	*DYM*	607461	NM_017653.4	c.946 + 1G > A	p.?
				c.1563 + 2 T > C	p.?
10	*EFNB1*	300035	NM_004429.4	c.561_562insACTGC	p.(Val189AlafsTer26)
				c.857C > T	p.(Ala286Val)
11	*GALNS*	612222	NM_000512.4	c.475 T > C	p.(Trp159Arg)
				c.700del	p.(Ala234ProfsTer85)
				c.902G > A	p.(Gly301Asp)
				c.906del	p.(Ser302ArgfsTer17)
				c.1140 + 1G > A	p.?
				c.1523dup	p.(Thr509fs)
12	*GALNT3*	610233	NM_004482.3	c.1576G > T	p.(Gly526Ter)
13	*GLB1*	611458	NM_000404.3	c.11_12delinsGA	p.(Phe4Ter)
			NM_000404.3	c.326G > C	p.(Arg109Pro)
			NM_000404.3	c.527del	p.(Asn176MetfsTer6)
			NM_000404.3	c.1071_1073delinsGG	p.(Phe357LeufsTer26)
			NM_000404.3	c.1099C > T	p.(Pro367Ser)
14	*KIF22*	603213	NM_001256270.1	c.457 T > C	p.(Phe153Leu)
15	*NAGLU*	609701	NM_000263.3	c.384-3C > A	p.?
16	*NEK1*	604588	NM_001199397.1	c.1957C > T	p.(Arg653Ter)
				c.3391G > C	p.(Glu1131Gln)
17	*NPR2*	108961	NM_003995.3	c.2966G > A	p.(Arg989Gln)
18	*POP1*	602486	NM_001145860.1	c.2087 T > A	p.(Val696Asp)
19	*RMRP*	157660	NR_003051.3	n.-4_-19dup; r.?	NA
				n.-6_-22dup; r.?	NA
				n.-8_-20dup; r.?	NA
				n.2_-18dup; r.?	NA
20	*SOX9*	608160	NM_000346	c.804_805del	p.(Asp269LeufsTer26)
21	*WISP3*	603400	NM_003880.3	c.135delinsGGTGAGGGCATTTG	p.(Gln46ValfsTer35)
				c.275G > A	p.(Cys92Tyr)
				c.286A > T	p.(Lys96Ter)
				c.629del	p.(Val211CysfsTer21)
				c.699del	p.(Asn233LysfsTer8)

NA: Not applicable.
MIM* - A gene in Online Mendelian Inheritance in Man (OMIM).

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