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REVIEW ARTICLE

Genetic advances in skeletal disorders: an overview

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ABSTRACT

Genetic skeletal disorders (GSDs) are a large group of rare heterogeneous disorders characterized by abnormal development, remodeling, and growth of the human skeleton's cartilage and bones. GSDs have a high spectrum of phenotypes that range from disproportionate short stature (dwarfism) in childhood to osteoarthritis in old age. According to the latest nosology classification of skeletal dysplasias, 461 disorders under 42 groups are classified according to specific radiographic, clinical, and molecular standards. In addition, correct molecular diagnosis for these rare GSDs is important for genetic and psychological counseling and treatment. GSDs are also associated with many syndromic forms that affect other parts such as hearing, vision, neurological, pulmonary, renal, or cardiac function. This review highlights the importance of GSDs and details a few selected disorders and their management strategies.

Keywords: GSDs, osteogenesis imperfecta, chondrodysplasias; polydactyly, syndactyly, acromesomelic dysplasia, SHMF, diagnosis; genetics; management.

Introduction

The word skeleton is derived from the Greek "skeletos," meaning "dried up", and it is composed of bone and cartilage. It is a complex organ, which consists of 206 bones and divided into 6 ossicles, 74 axial, and 126 appendicular bones (1). The human skeleton is divided into axial and appendicular skeleton. The shoulder girdle, lower and upper limbs, and pelvic girdle constitute the appendicular skeleton. The axial skeleton includes the skull, associated bones, spinal column, and rib cage. The axial skeleton attaches the appendicular skeleton to the body through the pelvic and pectoral girdles (2). The main component of the skeleton is the bone. It is the main reservoir for accumulating minerals like calcium and phosphorous (1,3). It consists of three types of cells, osteocytes, osteoblasts, and osteoclasts (4). Skeletal disorders resolute during the embryonic development, such as their location, shape, growth, and differentiation rate. A process called intramembranous ossification, from where mesenchymal cells (MSCs) differentiate directly into osteoblasts, while the majority of skeletal elements are formed by endochondral ossification, such as the lateral halves of the clavicle and parts of the skull (3,4). The latter process starts with forming a cartilaginous template, which is eventually replaced by the bone. This requires co-regulation of differentiation of the cell types specific for chondrocytes and osteoblasts, respectively (5,6). During embryogenesis, the human skeleton originates from three different sites, such as the MSCs, which are responsible for the appendicular skeleton. The paraxial mesoderm originates in the axial skeleton, and the cranial neural crest gives rise to craniofacial bones (7). Special signaling pathways control skeletal development during embryonic stages for appropriate propagation and sharing of sclerotomes and lateral plate mesoderm (LPM) cranial neural crest by involving several genes to accumulate mesenchymal aggregate (8). During embryonic development, three germ layers, including ectoderm, endoderm, and mesoderm, transform into derivatives, including an ectoderm-derived neural tube, mesoderm-derived notochord, and LPM (9). The neural tube desquamates the neural crests. The cells differentiate into various types, such as neuronal cells and melanocytes. The LPM gives rise to

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the limb skeletal structure (appendicular skeleton), the sternum (axial skeleton), and nonskeletal elements. The paraxial mesoderm gives rise to somites, forming most axial skeleton, such as ribs and vertebrae (10).

Skeletal patterning

During endochondral bone formation, the limb skeleton builds up from cartilage anlagen. It begins when chondrocytes progenitors, which then develop into a cartilage template that is eventually substituted by bones. The crucial step in establishing limb skeletal "patterning" occurs throughout the cartilaginous anlagen (11). Skeletal patterning, condensation, and differentiation of MSCs into chondrocytes (cartilage formation), osteoblasts (bone formation), osteoclasts, and bone remodeling is under the tight control of several cytokines, growth factors, and intercellular signaling pathways including Wingless/Integrated (*Wnt*), Sonic hedgehog (*SHH*), Indian hedgehog, fibroblast growth factor (*FGF*), Notch, TGF-β, and bone morphogenetic protein (BMPs). Genetic variations in cytokines and growth factors lead to inherited skeletal disorders (11,12). During the skeletal patterning and remodeling, the size, shape, number, and skeletal primordial are defined in correct relationship to one another. Different skeletal elements like axial, craniofacial, and appendicular skeleton are formed during the skeletal patterning process in an organized manner (13).

Limb development

The development of limbs in vertebrates is controlled by genetic processes, which are impenetrable and still not fully understood. Experimental studies of the molecular genetics of human limb development theorize and manipulate the different genetic interactions and their concerned pathways. There are three principal zones for the development of limbs: the proximal stylopod, zeugopod, and distal autopod (14).

Initiation of limbs

The limb bud originates from the edge of the embryo, that is, LPM covered by a layer of ectoderm. It has lineages for all types of limb tissues except muscles. Muscle progenitors initiate from somites and rapidly migrate to the embryonic limb buds. The skeletal element elaborates when the tissue progenitors differentiate, and the limb bud grows toward the distal side. In the limb bud, when it grows toward the distal side, the varied tissue progenitors differentiate and establish the elaborated pattern of skeletal elements (15,16).

The *HOX* gene plays a fundamental role during embryonic development, generates morphological diversity with the body axis, and genetically determines the position of the limb buds (17,18). When the limb's position is decided, a series of interactions between epithelial-to-mesenchymal and the LPM, the ectoderm, is established. In this event, the establishment of the apical ectodermal ridge (AER), an epithelial thickened structure from limb ectoderm occurs, which facilitates the distal margins of the limbs bud to its posterior tip from its anterior side and is dorso-ventrally (DV) located along the border of the limb bud (14,18). Several studies have revealed that a number of molecules expressed in specific domains, either in dorsal or ventral ectoderm, are involved in limb developmental processes such as *WNT7A*, Radical fringe, Engrailed-1 *(EN-1)*, and *TGF*-β*/BMP* (19).

Limbs patterning

After the development of limb buds, the undifferentiated mesenchyme is targeted by a series of signals to determine the morphology of skeletal elements. The AER, the zone of polarizing activity (ZPA), and the dorsal ectoderm play a key role in controlling the limbs proximal to distal outgrowth, anterior-posterior (AP) patterning, and establishing DV polarity, respectively (15). The limb buds have mesoderm cells, homogenous masses covered with a layer of ectoderm. The initial mark of patterning is a thin epithelial thickening at the limb bud proximal tip, known as the AER (14). The AER is important for proximal to distal patterning, and it is revealed that when the AER in chick wings is removed, the wings become truncated (20). AER promotes the proliferation of mesodermal cells and stops apoptotic events by providing *FGF* signaling to the mesoderm cells (21). *FGF10* expression, which is required for maintaining FGF8 expression in the limb mesenchyme, is induced by the AER. Thus, *FGF8* and *FGF10* establish an epithelial-mesenchymal positive feedback loop during limb growth (22).

During limb development, abnormalities in AER maintenance lead to abnormal phenotypes, including split-hand/foot syndromes resulting from *TP63* mutations, whose expression is vital for AER maintenance (23). A cell colony termed the ZPA is localized in the posterior limb bud mesenchyme, showing posture activity. The molecular basis of ZPA was discovered when *SHH* was proposed to be the diffusible morphogen responsible for polarizing activity (24). Another gene reported was Glioma-associated oncogene 3 (*GLI3*), which has two isoforms, one with active full-length *GLI3* (*GLI3F*) and repressor truncated *GLI3* (*GLI3R*). *SHH* signaling promotes the expression of *GLI3F* in the posterior mesenchyme, while the absence of *SHH* signaling leads to the production of *GLI3R* (25). The importance of *GLI3* and *SHH* during vertebral limb growth was discovered in the mouse by gene inactivation; *SHH*_mutant mice had only one rudimentary digit while all other digits were absent.

On the other hand, *GLI3* mutant mice show polydactyly. *SHH* and GLI3 mutations in human leads to different limb anomalies, including preaxial or postaxial polydactyly (PAP) or even severe conditions like acheriopodia (26). *SHH* plays a key role in AP patterning, maintains limb bud proliferation, and expands the digit-forming field (25). *SHH*, *GLI3*, and other regulators promote digit numbers and identity. In this context, a BMP signaling gradient was also suggested as a mediator, while genetic analysis of mice did not prove its role. It was shown that patterning information in chicks is stored in the interdigital mesenchyme. Signals to the growing phalanges are directed from the interdigital mesenchyme,

which provides them with information necessary for reaching their final length (27).

DV patterning is mediated by LIM homeobox transcription factor-1 (*LMX-1*) in the dorsal mesenchyme with subsequent expression of *WNT-7A* in the dorsal ectoderm and EN-1 in the ventral ectoderm. In the ventral ectoderm, EN-1 inhibits the *WNT7A* expression (28). Acting as a morphogen, *WNT7A* diffuses to the dorsal mesoderm and induces expression of the *LMX1B* (transcriptional factor). In the limb bud mesenchyme, *LMX1B* is considered a key regulator of dorsal patterning. *LMX1B* mutation in human result in a syndrome (Focal segmental glomerulosclerosis 10; MIM 256020) characterized by a defect in DV patterning of the limb, which is known as a nail-patella syndrome [MIM 161200] (29).

Signaling pathways involved in limb patterning

This is instinctive that AER, dorsal ectoderm, and ZPA are the centers of signaling and have strong coordination in their functions, as AER removal results in cell death in the underlying mesenchyme and leads to loss of *SHH* expression. Interestingly, *FGF4* could reimburse this function of the AER. Similarly, SHH actively controls *FGF4* expression in the AER. Thus both (*SHH* and *FGF4*) molecules form a positive feedback loop. This feedback loop is the best example of a signal relay in epithelial-to-mesenchymal communication: *SHH* actively controls the Gremlin 1 (Grem1) expression, a BMP inhibitor. Taken together, *GREM1* inhibits BMP action, which has a negative effect on the AER (30). *WNT7A* is required to replace the removed ectoderm, while *SHH* is expressed in the dorsal mesoderm. In mammals, this function is highly conserved. There is a decrease in *SHH* expression by the inactivation of *WNT7A*, and the posterior digit is lost (19).

Genetic skeletal disorders (GSDs)

GSDs arise from complex skeletal development, growth, and homeostasis disturbances caused by gene mutations. These disorders represent a challenge in diagnosis and treatment due to their rarity and verity (31). GSDs are clinically and genetically heterogeneous groups of disorders affecting bone and cartilage growth (32). It has significant effects on muscles, tendons, and ligaments. The overall incidence of skeletal dysplasia is 1/500 to 1/1,000 live birth. It is mainly associated with abnormalities of the linear skeleton and results from somatic mosaicism, teratogen exposure, and imprinting errors (33). Mutation in metabolism signaling pathways or in the synthesis of structural proteins, degradation of macromolecules, receptors, growth factors, or transcription factors may cause skeletal dysplasia (34).

Classification of skeletal disorders

Nomenclature and classification of Osteochondrodysplasias termed as "taxonomy." The dysostoses have been incorporated into the nomenclature, also called "nosology" (35). From 1977 to 1997, several efforts were made to classify the nosology (skeletal dysplasia). Categorizing different skeletal disorders based on clinical diagnosis, metabolism, and radiology was challenging. The list of genetic disorders mentioned in nosology helps to diagnose and delineate variants or newly recognized genetic disorders (36). The latest classification was performed in 2019, "Nosology and classification of GSDs: 2019 revision" (37). They classified the known GSDs into 461 disorders, organized into 42 groups. The classification was based on the involvement of 337 different genes in establishing molecular pathways, genetic, and radiographic criteria, and the role of biochemical was defined as the cause of these disorders.

The growth and development of the limbs involve several genetic pathways, and disruption of these genetic pathways leads to various anomalies in size, shape, and structure of the limbs, collectively known as congenital limb deformities. Limb deformities involve an odd number of digits in hands and feet, anomalous separation of the digits, or deviation of central rays of the autopods. The congenital limb malformations rate is 1 per 500 to 1 in 1,000 live births for upper limbs (37). Based on the clinical radiological manifestations, many congenital limb deformities have been discussed here, including osteogenesis imperfect (OI), Acromesomelic Dysplasia (AMD), split hand foot malformations (SHFM), Bardet–Biedl syndrome (BBS), and polydactyly. Such reviews might be helpful for clinicians and researchers to get an overview regarding rare GSDs that might help in genetic screening of the culprit gene involved and correct molecular diagnosis.

Osteogenesis imperfecta

(OI; MIM 166200) is a rare skeletal dysplasia characterized by growth deficiency, reduced bone mass, and fragility (38). The term "OI" refers to imperfect bone formation and disorder of the connective tissue matrix. The condition's hallmark lies in bone fragility and easy fractures caused by decreased bone mass (38,39). OI patients may have short stature, blue sclerae, joint laxity, scoliosis, skeletal deformity, and dentinogenesis imperfect, which are the secondary clinical features of OI (39,40).

OI is also known as "brittle bone disease," as it is a genetically and clinically heterogeneous heritable connective tissue disorder resulting from defects in type I collagen biosynthesis (41). Defects of collagen type I include abnormalities of primary collagen structure, unusual folding, abnormal post-translational modification, and matrix incorporation (41,42). The affection range is spread from mild osteopenia to moderate and severe forms, including limb deformity and lethal cases (42). According to the definition, mutations in one of the two genes, i.e., *COL1A1* and *COL1A2*, are responsible for OI as a heritable disorder. *COL1A1* and *COL1A2* encode the two chains, proα-1(I) and proα-2(I), respectively, of type I pro-collagen. In bones, type I collagen is most abundantly present; normally, collagen is composed of alpha chains (42). Overall, the incidence of OI ranges from 1 in 15,000–20,000 births, and autosomal dominant inheritance is the major source of causation. Phenotype depends on the type of mutation present; thus,

a genotype–phenotype relation does exist up to a certain level (43).

About 90% of the patients have OI, which is dominantly inherited. The set of genes involved in *COL1A1* or *COL1A2* encodes the α-1 and α-2 chains of type I collagen (44). Due to mutations in these genes, the structure or the amount of type I collagen is altered, leading to the severe skeletal phenotype that ranges from subclinical to lethal (44).

Consanguineous marriages are the major reason for autosomal recessive (AR) OI, which accounts for only 10%. Recent advances in molecular diagnosis have increased the number of novel candidate genes associated with recessive OI (Table 1). Genes responsible for AR OI usually encode proteins responsible for the assembly of the triple helix, chaperoning of the type I pro-collagen hetero trimer (42,45). Only three genes have been associated with dominant OI, accounting for 90% of the disease, while 17 recessive OI have been characterized. Those account for only 10% of the disease pathogenesis (45).

Acromesomelic dysplasias

AMD are severe skeletal dysplasias that constitute disproportionate shortening of skeletal elements, mainly affecting the hands and feet [distal segments] and the forearms and forelegs [middle segments] of the appendicular skeleton. Three main types of AMD have been reported in the literature (46; Table 2).

AMD type 1 (Maroteaux)

Acromesomelic dysplasia maroteaux (AMDM), also known as AMDM (MIM 602875) caused by homozygous or compound heterozygous variants in the *NRP2* gene located on chromosome 9p13.3. Associated clinical features include disproportionate short stature, bowed forearms, increased lumbar lordosis, short tubular bones, metaphyseal flaring of long bones, flattened midface, short, broad digits, bowing of the radius, short nails, and other associated phenotypes (46). It has been observed that the individual's carrier for the mutation is shorter than normal (47). Natriuretic peptide-natriuretic peptide receptor B (NPR-B) acts as a receptor for the C-type natriuretic peptide, which performs the function of an autocrine regulator in several different human tissues. The structure of NPR-B constitutes a ligand-binding domain [extracellular; 23-441 amino acids (a.a)], a transmembrane domain [hydrophobic; 442-512 a.a], protein kinase homology domain [intracellular; 512-826 a.a], and nucleotide cyclase domain [826-1046] (47; Figure 1).

Table 1. *OI classification.*

Phenotype	Inheritance	MIM number	Gene/Locus	Location
OI, type I	AD	166200	*COL1A1*	17q21.33
OI, type II	AD	166210	*COL1A2*	7q21.3
OI, type III	AD	259420	*COL1A2*	7q21.3
OI, type IV	AD	166220	*COL1A2*	7q21.3
OI, type V	AD	610967	*IFITM5*	11p15.5
OI, type VI	AR	613982	*SERPINF1*	17p13.3
OI, type VII	AR	610682	*CRTAP*	3p22.3
OI, type VIII	AR	610915	*P3H1*	1p34.2
OI, type IX	AR	259440	*PPIB*	15q22.31
OI, type X	AR	613848	*SERPINH1*	11q13.5
OI, type XI	AR	610968	*FKBP10*	17q21.2
OI, type XII	AR	613849	*SP7*	12q13.13
OI, type XIII	AR	614856	*BMP1*	8p21.3
OI, type XIV	AR	615066	*TMEM38B*	9q31.2
OI, type XV	AR	615220	*WNT1*	12q13.12
OI, type XVI	AR	616229	*CREB3L1*	11p11.2
OI, type XVII	AR	616507	*SPARC*	5q33.1
OI, type XVIII	AR	617952	*TENT5A*	6q14.1
OI, type XIX	XLR	301014	*MBTPS2*	Xp22.12
OI, type XX	AR	618644	*MESD*	15q25.1
OI, type XXI	AR	619131	*KDELR2*	7p22.1
OI, type XXII	AR	619795	*CCDC134*	22q13.2

Abbreviations: AR, autosomal recessive; AD, autosomal dominant; XLR, X-linked recessive.

Table 2. *AMD classification.*

Phenotype	Inheritance	MIM number	Gene/Locus	Location
AMD 1, Maroteaux type	AR	602875	*NPR2*	9p13.3
AMD 2A	AR	200700	*GDF5*	20q11.22
AMD 2B	AR	228900	*GDF5*	20q11.22
AMD 2C, Hunter-Thompson type	AR	201250	*GDF5*	20q11.22
AMD 3	AR	609441	*BMPR1B*	4q22.3
AMD 4	AR	619636	*PRKG2*	4q21.21

Abbreviations: AR, autosomal recessive.

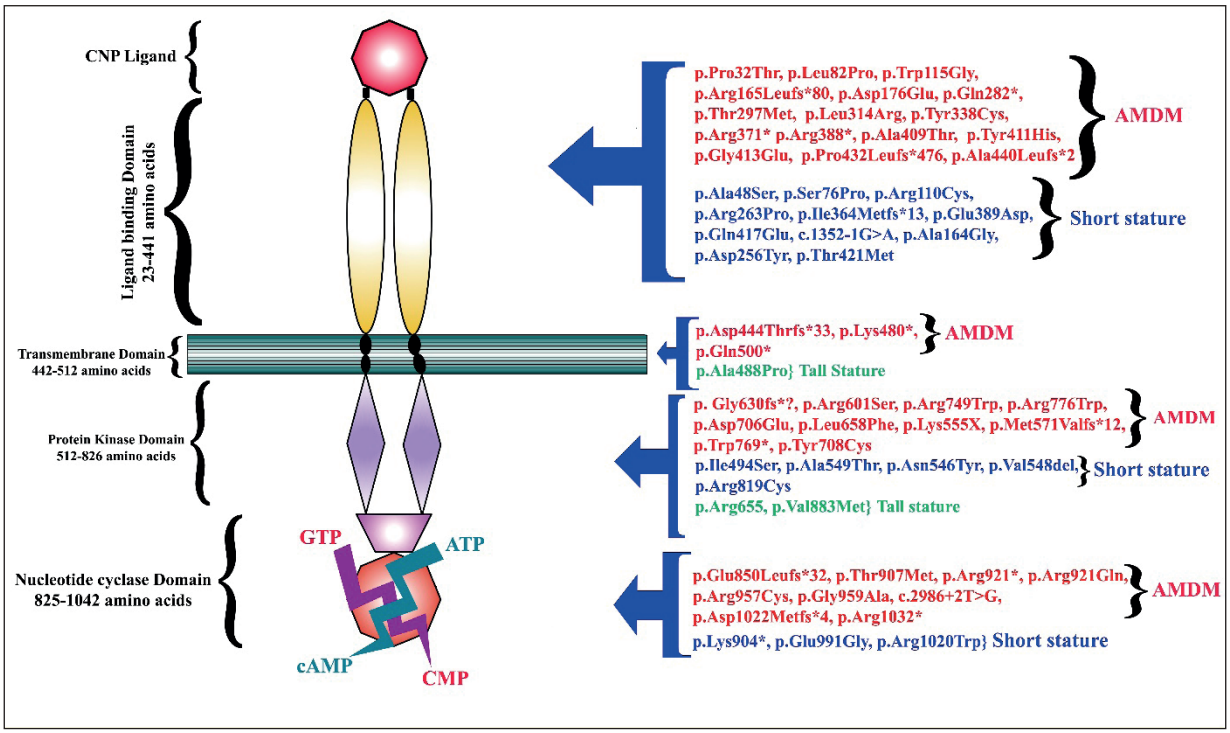

Figure 1. *Structure of NPR2 and position of previously reported variants in different domains.*

AMD type 2 (AMD2)

AMD2 (OMIM) is a recessively inherited distinct limb developmental disorder. AMDG is further divided into three categories such as AMD 2A, also known as Grebe dysplasia (AMDG; OMIM 200700), AMD 2B, also known as Du pan syndrome (OMIM 228900) and AMD 2C also known as Hunter-Thompson type (OMIM 201250). All the AMD2 types are caused by homozygous or compound heterozygous variants in the *GDF5* gene located on chromosome 20q11.22 (48-50).

AMD type 3 (AMD3)

AMD with genital or without anomalies, also known as "Demirhan type," have overlapping features with Grebe, Hunter-Thompson, and DuPan syndrome (fibular hypoplasia and complex brachydactyly) patients (OMIM 609441). Demirhan *et al*. (51) reported a homozygous mutation in *BMPR1B* in a patient having severe AMD and ovarian dysfunction. Later, Ullah *et al.* (48) reported a novel homozygous missense variant (c.1190T > G, p.Met397Arg) in the *BMPR1B* gene associated with AMD Hunter-Thompson type. BMPR1B acts as the major receptor for CDMP1, which play a major role in the signaling process for the bone morphogenbetic pathway, suggesting a critical role in skeletal development, digit patterning, chondrocyte differentiation, and joint development (48).

Split hand-foot malformation

SHFM is an extremely rare limb developmental abnormality that results in improper patterning and development of both upper and lower limbs. It results in the development of deep median clefts unilaterally or bilaterally in hands and feet, resulting in aplasia or hypoplasia of the digits. SHFM can occur as a part of a complex syndrome or as an isolated entity, and the phenotypic presentation can range from mild-severe phenotypes depending on the gene/variant involved (Figure 2), (52).

Nonsyndromic SHFM is further characterized into eight different types in humans. These include four types

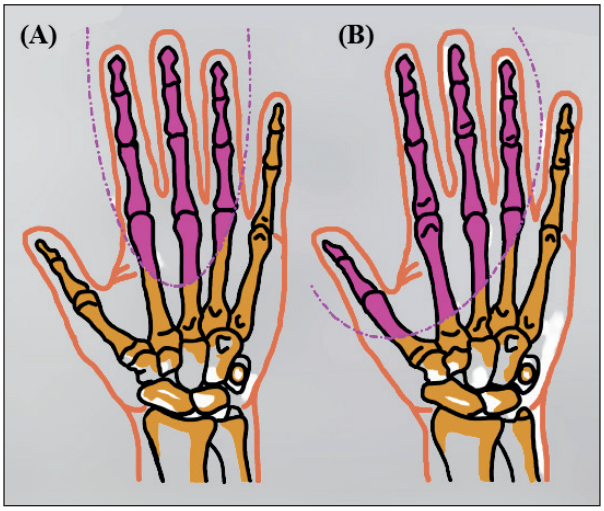

Figure 2. *A: Exhibiting type of SHFM in which fingers colored in pink are missing. B: Manifesting monodactyly in which all fingers are missing except little finger.*

inherited in an autosomal dominant fashion, caused by heterozygous variants in the concerned gene, including SHFM1 (*DLX5* gene, *DLX6* gene), SHFM3 (SHFM3 locus 10q24), SHFM4 (*TP63* gene), SHFM5 (SHFM5 locus 2q31), three types inherited in AR fashion caused by homozygous variants including *SHFM6* Wnt Family Member 10B (WNT10B gene), SHFM7 (*ZAK* gene, SHFM8 (*EPS15L1* gene) and only one type associated with X-linked SHFM (SHFM2 (SHFM2 locus Xq26) (52-58) (Table 3). To date, disease-causing variants have been associated with only 6 genes causing SHFM, including *TP63, DLX5, DLX5, WNT10B, ZAK, and EPS15L1* (52) (Table 3).

SHFM type 1

SHFM1 (OMIM 183600) results in the deep median clefts, lack of the central digital rays, and complex syndactyly, mapping to 7q21.3. Microdeletion and variants in the *DLX5* and *DLX6* have been associated with SHFM1 (53,59).

SHFM type 2

SHFM2 (OMIM 313350) is inherited in an X-linked fashion, mapped to chromosome Xq26.3. It is characterized by bilateral lobster-claw deformity, metacarpal hypoplasia, partial syndactyly, and phalangeal hypoplasia involving both upper and lower limbs (52).

SHFM type 3

SHFM3 (OMIM 246560), characterized by maxillary hypoplasia, hearing loss, cleft palate, ectrodactyly, phalangeal hypoplasia, and intellectual disability, was observed in some patients. SHFM3 was mapped to human chromosome 10q24 (60).

SHFM type 4

SHFM4 (OMIM 605289) exhibits variable features such as lobster-claw anomaly, monodactyly, syndactyly, missing phalanges, and triphalangeal thumb. Affected individuals in different families and even within the same family have been observed to have reduced penetrance. SHFM4 is associated with disease-causing variants in the *TP63* gene located on chromosome 3q28. *TP63* is a transcription factor that encodes the p53 family of transcription factors. The p53 family proteins have several domains, including an N-terminal transactivation domain, a central DNA-binding domain, and an oligomerization domain (57).

SHFM type 5

SHFM5 (OMIM 606708) has an autosomal dominant inheritance mode. It was mapped on chromosome 2 with a genetic address of 2q31 (52). It includes closely related genes *DLX1* and *DLX2* with no pathogenic variants yet reported (61). No associated genes have been identified so far for SHFM5.

SHFM type 6

SHFM6 (OMIM 225300) is inherited in an AR fashion, characterized by hallmark features such as ectrodactyly, split foot, split hands, complex syndactyly, polydactyly (some patients), and other variable phenotypes. SHFM6 is mapped at chromosome 12q13.11-q13, and disease-causing variants in *WNT10B* have been associated with the phenotype (55-57). Wnt Family Member 10B (*WNT10B*) gene is a member of the *WNT* gene family, and its protein signaling is a molecular switch that governs adipogenesis. WNT signaling is involved in the translocation of β-catenin to the nucleus and binds to several transcription factors, resulting in the regulation of osteoblastogenesis (52,61).

SHFM type 7

SHFM7 is characterized by split-foot malformation, cutaneous syndactyly, digit duplication, and sensorineural hearing impairment. Disease-causing variants in the *ZAK* gene have been associated with SHFM7, located on chromosome 2q31.1 (58). ZAK is a serine-threonine kinase that belongs to the MAPKKK family of signal transduction molecules. It has been observed that Zak is a positive mediator of cell hypertrophy in cultured rat cardiac myocytes and mediates TGF-beta-induced cardiac hypertrophy via a TGF-beta-ZAK-MKK7-ANF signaling pathway (62).

SHFM type 8

SHFM8 (OMIM 616826) is characterized by features such as mild SHFM, cutaneous syndactyly, aplasia, and hypoplasia of carpals and metacarpals. Epidermal Growth Factor Receptor Pathway Substrate 15 Like 1 (*EPS15L1*) is involved in the endocytosis of integrin beta-1 (ITGB1) and transferrin receptor; internalization of ITGB1 as DAB2-dependent cargo. Disease-causing variants in the *EPS15L1* gene have been associated with SHFM8, located on chromosome 19p13.11 (54). Only a single family with two affected individuals has been associated with SHFM8 having a homozygous frameshift variant (c.409delA) in exon 7 of the *EPS15L1* (54).

Table 3. *SHFM current classification.*

SHFM type	Locus	OMIM	Causative gene/Molecular mechanism	Chromosomal localization	Inheritance
Isolated SHFM	SHFM1	183600	Mutations in *DLX5* and *DLX6*	7q21.2-q21.3	AD
	SHFM1D	220600	Suspected dysregulation of *DLX5* and *DLX6*	7q21.2–q21.3	AD
	SHFM2	313350	Unknown	Xq26	XL
	SHFM3	246560	Microduplications involving *BTRC*, *POLL*, and *FBXW4*	10q24	AD
	SHFM4	605289	*TP63* mutations	3q28	AD
	SHFM5	606708	Suspected dysregulation of *HOXD* cluster	2q31	AD
	SHFM6	225300	*WNT10B* mutations	12q13.12	AR
	SHFM7	616890	*ZAK* mutations	2q31.1	AR
	SHFM8	616826	*EPS15L1* microdeletions/mutations	19p13.11	AR

Abbreviations: AR, autosomal recessive; AD, autosomal dominant; XLR, X-linked recessive.

Bardet-Biedl syndrome

BBS is a multi-systemic recessive syndrome characterized by hallmark features such as intellectual disability, obesity, renal anomalies, retinal cone-rod dystrophy, hexadaxtyly, and hypogenitalism. If the affected individual presents four out of the six significant features or three major features and two minor features, he is classified as having BBS. Other associated features include cardiovascular anomalies, hearing loss, oral/dental abnormalities, neurodevelopmental abnormalities, metabolic defects, and diabetes mellitus (63).

BBS exhibits extensive clinical heterogeneity, and the occurrence of digenic and transgenic inheritance has been observed. BBS has been associated with disease-causing mutations in 23 different genes mapped on different chromosomes, including *BBS1* (OMIM 209901), *BBS2* (OMIM 606151), *BBS3 (ARL6; O*MIM 608845), *BBS4* (OMIM 600374), *BBS5* (OMIM 603650), *BBS6* (*MKKS*; OMIM 604896), *BBS7* (OMIM607590), *BBS8* (*TTC8*) (OMIM 608132), *BBS9* (OMIM 607968), *BBS10* (OMIM 610148), *BBS11* (*TRIM32*; OMIM 602290), *BBS12* (OMIM 610683), BBS13 (*MKS1*; OMIM 609883), BBS14 (*CEP290*; OMIM 610142), BBS15 (*C2ORF86*; OMIM 613580), BBS16 (*SDCCAG8* (OMIM 613524), BBS17 (*LZTFL1*; OMIM 606568)**,** BBS18 (*BBIP1*; OMIM 615995)**,** BBS19 (*IFT27*; OMIM 615996)**,** BBS20 (*IFT172*; OMIM 619471)**,** BBS21 (*CFAP418*; OMIM 617406)**,** BBS22 (*IFT74*; OMIM 617119), and BBS23 (*CEP19*; OMIM615586) (64,65) (Table 4).

BBS is a genetically heterogeneous syndrome with overlapping clinical features with other ciliopathies disorders. Thus, molecular testing through a ciliopathy gene panel or whole exome sequencing (WES) is the correct method for proper molecular diagnosis. To date, no successful therapy has been suggested for BBS, as the disorder is multi-systemic. Thus several organs are affected; therefore, the patient requires multidisciplinary care, proper coordinated management, and extensive therapeutic interventions (65,66).

Polydactyly

Polydactyly, also termed hexadactyly, is the development of supernumerary digits or toes. Polydactyly is an inherited condition and one of the most common inherited digital anomalies, manifesting in various forms. It might range from complete duplication of a limb or limb part to complete duplication of a digit. Polydactyly can occur as an isolated entity or be associated with a complex syndrome (syndromic forms; 67).

Nonsyndromic polydactyly is further divided into three types, (a) preaxial polydactyly (PPD), having an extra digit at the side of the thumb or great toe, (b) PAP, with extra digits at the side of the 5th finger or toe and (c) complex polydactyly, where the extra digit originates from the middles of the hand (67-69).

PPD is further divided into four types. Type 1 is characterized by an extra digit with the first finger, polydactyly of the triphalangeal first digit is included in type 2, type 3 is polydactyly of the second digit. In contrast, type 4 is polysyndactyly (67,70).

PAP is classified into type A, and type B. In type A, the extra digit is fully developed, with fully developed bone (both functional or nonfunctional), and in type B, where the extra digit is not well formed and occurs in the form of a nonfunctional skin tag (67,66) (Figure 3).

Nonsyndromic (isolated) polydactyly segregates in autosomal dominant and recessive fashion. PPD is further classified into four types such as PPD1, caused by variants in the *GLI* gene located on chromosome 12q13.3 (OMIM165220) (71,72), PPD2 caused due by mutations in the *LMBR1* gene located on 7q36.3 (OMIM174500), PPD3, whose locus is not mapped up till now (OMIM174600), PPD4 inherited in dominant fashion and *GLI3* associated mutations have been linked to the disease phenotypes (73). Triphalangeal thumb, type I, caused by variants in the *LMBR1* gene located on 7q36.3 (OMIM174500), and PPD5 inherited in AR fashion and homozygous mutation in *STKLD1* has

Table 4. *BBS classification.*

Phenotype	Inheritance	MIM number	Gene/Locus	Location
BBS 1	AR	209900	*BBS1*	11q13.2
BBS 1	AR	209900	*CCDC28B*	1p35.2
BBS 1	AR	209900	*ARL6*	3q11.2
BBS 2	AR	615981	*BBS2*	16q13
BBS 3	AR	600151	*ARL6*	3q11.2
BBS 4	AR	615982	*BBS4*	15q24.1
BBS 5	AR	615983	*BBS5*	2q31.1
BBS 6	AR	605231	*MKKS*	20p12.2
BBS 7	AR	615984	*BBS7*	4q27
BBS 8	AR	615985	*TTC8*	14q31.3
BBS 9	AR	615986	*PTHB1*	7p14.3
BBS 10	AR	615987	*BBS10*	12q21.2
BBS 11	AR	615988	*TRIM32*	9q33.1
BBS 12	AR	615989	*BBS12*	4q27
BBS 13	AR	615990	*MKS1*	17q22
BBS 14	AR	615991	*CEP290*	12q21.32
BBS 14	AR	615991	*TMEM67*	8q22.1
BBS 15	AR	615992	*WDPCP*	2p15
BBS 16	AR	615993	*SDCCAG8*	1q43-q44
BBS 17	AR	615994	*LZTFL1*	3p21.31
BBS 18	AR	615995	*BBIP1*	10q25.2
BBS 19	AR	615996	*IFT27*	22q12.3
BBS 20	AR	619471	*IFT172*	2p23.3
BBS 21	AR	617406	*CFAP418*	8q22.1
BBS 22	AR	617119	*IFT74*	9p21.2
BBS 23	AR	615586	*CEP19*	3q29

Abbreviations: AR, autosomal recessive.

been associated with the disease phenotype located on chromosome 9q34.2 (OMIM618530) (67).

PAP is associated with 11 genes/loci located on different human chromosomes (Table 5). PAP1 mapped on chromosome 7p13 with *GLI3* gene mutations (74), PAP2 having a chromosomal address of 13q21-q32 (no gene identified), PAPA3 with characteristics of PAP-A/B mapped on chromosome 19p13.1-13.2 (no gene identified) and PAPA4 have an autosomal dominant inheritance with PAP-A/B phenotypes and partial cutaneous syndactyly mapped on chromosome 7q21-q34 (no gene identified). PAPA5 was mapped in a large Pakistani family having AR on chromosome 13q13.3-q21.2 (no gene identified). PAPA6 has AR inheritance, and the associated gene is *ZNF141*, located on chromosome 4p16.3. A disease-causing variant in the *IQCE* gene has been associated with PAPA7, located on chromosome 7p22.3 (70). A disease-causing variant in the *GLI1* gene has been associated with PAPA8 with *EVC* overlapping features on chromosome 12q13.3 (75). PAPA9 having recessive inheritance has been associated with *FAM92A* gene variants. A disease-causing variant in the *KIAA0825* gene has been associated with PAPA10, located on chromosome 5q15 (76). Similarly, PAPA11

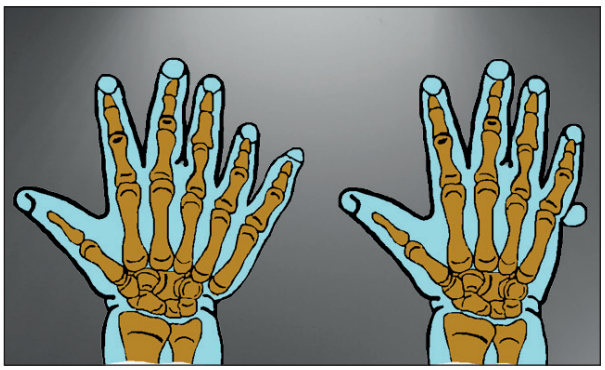

Figure 3. *A: Type of fully developed polydactyly with metacarpal bone. B: Manifesting features of polydactyly type B.*

has been associated with homozygous *DACH1* variants on chromosome 13q21.33 (77).

If we type the mesh "Polydactyly" in OMIM (https://omim.org/), we receive "496" entries, thus showing its involvement in many different disorders. These syndromic polydactyly disorders/syndromes present diverse phenotypes and are very severe. Polydactyly can

Table 5. *Polydactyly classification.*

Disease	Genes	Inheritance	Locus	OMIM
PPD1	*GLI*	AR	12q13.3	165220
PPD2	*LMBR1*	AD	7q36.3	174500
PPD3	*PPD3*	AD	**U**	174600
PPD4	*GLI3*	AD	7p14.1	174200
PPD5	*STKLD1*	AR	9q34.2	618530
Triphalangeal thumb, type I	*LMBR1*	AD	7q36.3	174500
PAPA1	*GLI3*	AD, AR	7p14.1	174200
PAPA2	*U*	AD	13q21-q32	602085
PAPA3	*U*	AD	19p13.1-p13.2	607324
PAPA4	*U*	AD	7q21-q34	608562
PAPA5	*U*	AR	13q13.3-q21.2	263450
PAPA6	*ZNF141*	AR	4p16.3	615226
PAPA7	*IQCE*	AR	7p22.3	617642
PAPA8	*GLI1*	AR	12q13.3	165220
PAPA9	*FAM92A*	AR	8q22.1	617273
PAPA10	*KIAA0825*	AR	5q15	617266
PAPA11	*DACH1*	AR	13q21.33	603803

Abbreviations: AR, autosomal recessive; AD, autosomal dominant; U, unknown.

be identified at the early stage using ultrasound, which might give time for the clinicians to start management strategies for the severe syndromic form of the disorder (68,78,79).

Diagnosis and genetic counseling

GSDs are diagnosed mainly by the radiological features in association with either targeted gene, panel sequencing, or next-generation sequencing (NGS), either WES or whole genome sequencing. Phenotypic appearance and radiographic analysis of the affected individuals can be the first step of diagnosis; however, for a multi-systemic disorder such as BBS, molecular diagnosis is required to identify the culprit genetic variant. Once mutation in the specific gene is identified, carrier testing and proper genetic counseling of the family can be performed (80,81).

Discussion

GSDs are characterized by inconsistent growth, severe bone malformations, and distortion of individual bones or groups of bones that results in either nonsyndromic (isolated) or as a part of a complex syndrome (syndromic form) (81). Disruption of specific developmental pathways results in GSDs that can be either due to disruption of the intricate processes of growth, development, and/or homeostasis of the skeletal system. With the advent of the latest NGS technology and the development of new machines, the molecular diagnosis of GSDs is now accurate, quick, and cost-effective (82).

Many GSDs are very severe and result in the death of the affected individuals. Thus, genetic counseling, newborn screening, and molecular diagnosis are necessary (2). Genetic screening can be either targeted gene sequencing, panel gene sequencing in case there are more genes associated with a particular disorder, or WES of two or three individuals from each family. Recently, molecular diagnostic techniques such as prenatal genetic testing (PGT), especially pre-natal genetic screening for monogenetic disorders (PGT-M), served an excellent deal in the future management of monogenetic disorders (83,84). PGT, in association with *in vitro* fertilization, is an option for parents wishing to have future pregnancies (85). Disorders such as frontonasal dysplasias can be dealt with plastic surgeries so that the affected individuals can live a normal life (86-88). However, proper disease management and therapeutic interventions are only possible if the concerned clinicians receive a correct molecular diagnosis. In such a scenario, knowledge about the molecular etiology and pathophysiology of the disorder is a must to implement and draw future therapeutic interventions.

A total of 437 different genes are involved in causing 461 different GSDs, making it a complex heterogeneous group of disorders, thus making diagnosis difficult. Monogenetic disorders are best to study as loss of function in these genes presents a perfect model and help us to track down the proper gene function and associated pathways. Studying rare genetic disorders and the pathogenic mutations involved provide insight into different preventive measures and diagnostic applications and, finally, helps in therapeutic strategies. Furthermore, the number of increased patients associated with a particular disorder can be subjected to clinical trials using Food and Drug Administration-approved drugs (89).

As a result, large-scale DNA sequencing using NGS is mostly performed, which might help researchers to

diagnose easily, which is a prerequisite for accurate genetic counseling (90). Establishing a proper medical policy is vital, which would significantly reduce the risk of misdiagnosis and improve/develop a treatment for GSDs. A strong network and collaboration among international scientists from different institutes should be established to find an ultimate treatment for GSDs.

Conclusion

In developing countries, proper genetic testing and establishing newborn screening are still a great issue, and thus rare GSDs receive less attention. In such countries, a database development should be developed to save the data for patients with such severe conditions. Furthermore, there should be a register for GSDs that might provide information about the prevalent mutation in the community and tribe. Due to the unitability of such resources and documentation, this creates diagnostics issues for clinicians and researchers.

In such a situation, a systematic bibliographic study of GSDs might help to estimate the prevalence or occurrence of GSDs in a community and pinpoint hotshot variants. Knowledge regarding the pathophysiologic nature of the disorders, the disease mechanism, unrevealing the biomarkers, and the disease pathway is mandatory to proceed with gene therapy.

List of Abbreviations

AMD	Acromesomelic dysplasias
BBS	Bardet–Biedl syndrome
GSD	Genetic skeletal disorders
OI	Osteogenesis Imperfecta
PAP	Postaxial polydactyly
PPD	Preaxial polydactyly

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