Nonsyndromic Split-Hand/Foot Malformation: Recent Classification

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Abstract

Split-hand/foot malformation (SHFM) is a genetic limb anomaly disturbing the central rays of the autopod. SHFM is a genetically heterogeneous disorder with variable expressivity inherited as syndromic and nonsyndromic forms. We provide an update of the clinical and molecular aspects of nonsyndromic SHFM. This rare condition is highly complex due to the clinical variability and irregular genetic inheritance observed in the affected individuals. Nonsyndromic SHFM types have been reviewed in terms of major molecular genetic alterations reported to date. This updated overview will assist researchers, scientists, and clinicians in making an appropriate molecular diagnosis, providing an accurate recurrence risk assessment, and developing a management plan.

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Ectrodactyly or split-hand/foot malformations (SHFM) is a rare congenital malformation of the limbs, involving mostly the central rays of the autopods, median clefts in hands and feet, syndactyly, and metacarpal,

metatarsal and phalangeal aplasia or hypoplasia. The hands and/or feet appear split into 2 halves with aplasia (failure of development) of the phalanx, metacarpal, and/or metatarsal bones of one or more fingers and/or toes as well as hypoplasia (underdevelopment) of the phalanges, metacarpals, and metatarsals (the bones leading up to the toes) [Duijf et al., 2003]. SHFM is inherited as an autosomal dominant, recessive, or X-linked entity, with a prevalence of 1 per 90,000 live births, and its clinical severity varies from patient to patient as well as between the limbs of the same patient (Fig. 1A, B) [Duijf et al., 2003; Elliott and Evans, 2006].

SHFM Classification

Twelve different types of SHFM have been mapped to different human chromosomes, including SHFM1 located in 7q21 (OMIM 183600) [Scherer et al., 1994], SHFM2 in Xq32 (OMIM 313350) [Faiyaz ul Haque et al., 1993], SHFM3 located in 10q24 (OMIM 246560) [Nunes et al., 1995; Gurrieri et al., 1996], SHFM4 in chromosome 3q27 (OMIM 605289) [Ivanakiev et al., 2000], SHFM5 in 2q31 (OMIM 606708) [Boles et al., 1995], SHFM6 in 12q13.11q13 (OMIM 183600) [Ugur and Tolun, 2008], SHFM7 in 2q31.1 (MIM 616890) [Spielmann et al., 2016], SHFM8 in 19p13.11 [Umair et al., 2018] and a locus in

Table 1. SHFM current classification

SHFM type	Locus	OMIM	Causative gene/molecular mechanism	Chromosomal localization	Inheritance
Type 1	SHFM1	183600	Mutations in <i>DLX5</i> and <i>DLX6</i>	7q21.2q21.3	AD
Isolated SHFM	SHFM1	220600	Homozygous mutation in <i>DLX5</i>	7q21.3	AR
	SHFM2	313350	Unknown	Xq26	XL
	SHFM3	246560	Microduplications involving <i>BTRC</i> , <i>POLL</i> , and <i>FBXW4</i>	10q24	AD
	SHFM4	605289	<i>TP63</i> mutations	3q28	AD
	SHFM5	606708	Suspected dysregulation of <i>HOXD</i> cluster	2q31	AD
	SHFM6	225300	<i>WNT10B</i> mutations	12q13.12	AR
	SHFM7	616890	<i>ZAK</i> mutations	2q31.1	AR
	SHFM8	616826	<i>EPS15L1</i> microdeletions/mutations	19p13.11	AR
			Unknown	8q21.11q22.3	AR
Type 2	SHFLD1	119100	Unknown	1q42.2q43	AD
SHFM with long bone deficiency	SHFLD2	610685	Unknown	6q14.1	AD
	SHFLD3	612576	Microduplications involving <i>BHLHA9</i>	17p13.3	AD

AD, autosomal dominant; AR, autosomal recessive; SHFLD, split-hand/foot malformation with long bone deficiency; SHFM, split-hand/foot malformation; XL, X-linked.

Fig. 1. Schematic representation of different split-hand/foot malformation (SHFM) types based on the median cleavage. **A** SHFM showing aplasia of the central rays in hands and **B** aplasia of both preaxial and central rays, characterized as monodactyly. **C** Typical SHFM diagram showing a median cleft in the foot. **D** Diagram showing the area in the foot which may be affected in SHFM.

chromosome 8q21.11q22.3 [Gurnett et al., 2006]. For these 12 loci, 5 genes including *DLX5/DLX6* (MIM 600028, MIM 600030) for SHFM1 [Ullah et al., 2017], *TP63* (MIM 603273) for SHFM4 [Ianakiev et al., 2000], *WNT10B* (MIM 601906) for autosomal recessive SHFM6 [Ugur and Tolun, 2008], *ZAK* (MIM 609479) for autosomal recessive SHFM7 [Spielmann et al., 2016], and *EPS15L1* (MIM 616826) for autosomal recessive SHFM8 [Umair et al., 2018] have been identified (Table 1).

Another form of SHFM known as SHFLD (split-hand/foot malformations with long bone deficiency) is dominantly inherited and genetically different from isolated forms of SHFM. To date, 3 types of SHFLD have been mapped to different human chromosomes including SHFLD1 in 1q42.2q43 (OMIM 119100) [Naveed et al., 2006], SHFLD2 in 6q14.1 (OMIM 610685) [Naveed et al., 2007], and SHFLD3 located in 17p13.1p13.3 (OMIM 612576) [Lezirovitz et al., 2008] (Table 1). SHFM has been described in association with other congenital malformations and is associated with more than 50 different syndromes (OMIM).

SHFM1

SHFM1 (MIM 183600) is characterized by deep median clefts, absence of central digital rays, and syndactyly. SHFM1 is dominantly transmitted and mapped for the first time to chromosome 7q21.3q22 [Scherer et al., 1994], harboring translocations, deletions, and inversions in this chromosomal region. The hallmark clinical features as-

Table 2. Clinical phenotypes associated with SHFM types

No.	SHFM type	Gene/locus	Inheritance	Phenotype
1a	SHFM1	<i>DLX5, DLX6</i>	AD	Ectrodactyly, split hand, aplasia of single digital ray, hypoplasia, triphalangeal thumbs, lower limbs with broad hallux and clinodactyly
1b	SHFM1 with sensorineural hearing loss	<i>DLX5</i>	AR	Short stature, mild scoliosis, sensorineural hearing loss, split hand/foot, cylindrical nails
2	SHFM2	Xq26	XL	Split hand/foot, monodactylous median cleft anomaly, partial syndactyly, metacarpal and phalangeal hypoplasia
3	SHFM3	10q24	AD	Maxillary hypoplasia and micrognathia, dysplastic ears with hearing loss, cleft palate, renal anomalies, ectrodactyly, clinodactyly, ridged and dystrophic nails, and intellectual disability observed in some patients
4	SHFM4	<i>TP63</i>	AD	Split hand/foot, missing phalanges, monodactyly, triphalangeal thumb, syndactyly, and missing metacarpals and metatarsals
5	SHFM5	2q31	AD	Monodactyly, penoscrotal hypoplasia, growth retardation, hypertelorism, cleft palate, microcephaly, microphthalmia, split hand malformation
6	SHFM6	<i>WNT10B</i>	AR	Ectrodactyly (split hand/foot), with additional variable phenotype such as complex syndactyly and polydactyly also reported
7	SHFM7 with mesoaxial polydactyly	<i>ZAK</i>	AR	Split foot malformation, normal hands, hearing impairment, cutaneous syndactyly, and duplication of finger nail bed of fourth digit
8	SHFM8	<i>EPS15L1</i>	AR	Mild–severe split foot, missing metacarpals and metatarsals, complex preaxial syndactyly, underdeveloped digits and missing nail
9	SHFLD1	1q42.2q43	AD	Cleft hand, absent tibia, absent middle finger, tetramonodactyly, transverse hemimelia, hypoplastic big toes, bifurcation of the femurs, cup-shaped ears and ulnar aplasia/hypoplasia
10	SHFLD2	6q14.1	AD	Mild–severe skeletal defects involving upper and lower limbs, split hand/foot, syndactyly of fingers/toes, hypoplastic big toes, absence of middle phalanges, hypoplastic tibiae, beaked nose, and no cleft lip/palate or ectodermal dysplasia observed
11	SHFLD3	17p13.3p13.1 (<i>BHLHA9</i> duplication)	AD	Ectrodactyly, oligodactyly, brachydactyly, syndactyly, camptodactyly, pes varus, club foot, tibial aplasia/hypoplasia, femoral bifurcation observed in some patients

AD, autosomal dominant; AR, autosomal recessive; SHFLD, split-hand/foot malformation with long bone deficiency; SHFM, split-hand/foot malformation; XL, X-linked.

sociated with SHFM1 include ectrodactyly, split hand/foot, aplasia or/and hypoplasia of single digital ray, triphalangeal thumbs, and lower limbs with broad hallux and clinodactyly. The disorder is associated with variable expressivity and incomplete penetrance. It is usually caused by duplication, deletion, or rearrangement involving the *DLX5*, *DSS1*, and *DLX6* genes as well as possible regulatory elements in the 7q21.3q22 chromosome region [Scherer et al., 1994].

A Yemeni family with 2 individuals affected by SHFM and hearing loss has been reported. The affected individuals had additional features such as a severe short stature, delayed walking, mild synophrys, cylindrical nails, tapered fingers, lower limb hypoplasia, clinodactyly, and asymmetrical short and deformed lower limbs. Molecular

analysis using autozygome and whole-exome sequencing (WES) [Shamseldin et al., 2012] identified a homozygous missense mutation (c.533A>C; p.Gln178Pro) in the *DLX5* gene that segregated with the disease phenotype. The disorder was termed as SHFM1 with sensorineural hearing loss (MIM 220600).

Ullah et al. [2017], using direct Sanger sequencing, identified a heterozygous missense variant (c.632T>A; p.Val211Glu) in the distal-less homeobox six (*DLX6*) gene, located in chromosome 7q21, causing SHFM1 in a Pakistani family. The affected individual exhibited features such as aplasia of carpals, metacarpals and phalanges with a classical central ray defect. Additional features included clubbed nails, short radius/ulna, anonychia, dental crowding, and synophrys [Ullah et al., 2017].

SHFM2

SHFM2 (MIM 313350) is clinically characterized by a median cleft in both feet, with bidactyly or monodactyly of both hands, mostly involving all 4 limbs. The mode of inheritance of SHFM2 is X-linked. Ahmad et al. [1987] clinically characterized a Pakistani family with 36 affected individuals in 7 generations (33 males and 3 females). The affected individuals had features such as metacarpal and phalangeal hypoplasia, syndactyly, and bone malformations of the hands. The homozygous females and hemizygous males had typical severe SHFM, with heterozygous females displaying a mild clinical presentation. Later, in the same family using linkage analysis, Faiyaz-Ul-Haque et al. [2005] narrowed down and identified a previously reported 22-Mb genetic interval in chromosome Xq24q26 to a 5.1-Mb region. The candidate gene is still unknown.

SHFM3

SHFM3 (MIM 246560) is transmitted as an autosomal dominant trait and accounts for classical SHFM phenotypes. Typical clinical features of affected individuals reported include dysplastic ears with hearing loss, cleft palate, face with maxillary hypoplasia and micrognathia, renal anomalies, ectrodactyly, clinodactyly, triphalangeal thumbs, preaxial polydactyly as well as ridged and dystrophic nails. In addition, intellectual disability has been observed in some patients [de Mollerat et al., 2003].

About 20% of the SHFM3 cases are caused by duplications mapped to chromosome 10q24 (a 325 to 570-kb genomic region) and defined by submicroscopic duplications and complex rearrangements [de Mollerat et al., 2003]. Rearrangements in several genes contribute to SHFM3 phenotypes such as *FGF8*, *LBX1*, *BTRC*, and *DACTYLIN*. The SHFM3 locus at 10q24 shows conservation of the syntenic region with the *Dac* region in mice (chromosome 19). The *Dac* mice exhibit the ectrodactyly phenotype with only certain genetic backgrounds [Chai, 1981; Kano et al., 2007].

SHFM4

SHFM4 (MIM 605289) displays complex phenotypes of hands and feet such as aplasia of the phalangeal, metacarpal and metatarsal bones, with or without syndactyly and webbing [Ianakiev et al., 2000]. Any pathogenic mutations in the *TP63* gene (MIM 603273) cause SHFM4. The *TP63* gene encodes a p63 protein, involved in the differentiation and regulation of the apical ectoderm ridge (AER) and ectodermal development [van Bokhoven et al., 2001; Berdón-Zapata et al., 2004]. The clinical presentation of affected individuals includes monodactyly, missing phalanges,

metacarpals/metatarsals, thumb duplication, and syndactyly. To date, more than 120 mutations have been reported in the *TP63* gene associated with several disorders such as limb-mammary syndrome, SHFM, cleft lip, ADULT syndrome, EEC syndrome, kidney disorders, AEC syndrome, and Rapp-Hodgkin syndrome (Table 2).

SHFM5

SHFM5 (MIM 606708) has been mapped to chromosome 2q31 [Boles et al., 1995], with the *DLX1* and *DLX2* genes closely related to this genomic region, but no mutations have been reported yet. It is inherited as an autosomal dominant disorder. Defective development of AER and ectrodactyly in mouse hind limbs were reported in the *Dlx5* and *Dlx6* double-knockout mouse [Restelli et al., 2014]. Goodman et al. [2002] suggested that as *DLX1* and *DLX2* genes are expressed in AER, they may be new candidate genes for SHFM5. Affected individuals with chromosomal rearrangements at the SHFM5 locus presented with features such as monodactyly or zeugopod, penoscrotal hypoplasia, and several other anomalies such as short stature, microphthalmia, hypospadias, microcephaly, and cleft palate (Table 2).

SHFM6

WNT10B (MIM 601906) has been reported as a causative gene for SHFM6 (MIM 225300). It has an autosomal recessive inheritance pattern and is reported in several consanguineous families [Ugur and Tolun, 2008; Khan et al., 2012; Aziz et al., 2014; Ullah et al., 2018]. The *WNT10B* gene is a WNT gene family member consisting of 18 other genes. *WNT10B* encodes a 389 amino acid protein. WNT proteins act as ligands in a variety of signaling pathways and play a major role in limb development and morphogenesis [Yang, 2003]. These proteins bind the low-density lipoprotein receptor and cell surface frizzled related proteins, which activate a conserved “canonical” signaling pathway [Peifer and Polakis, 2000] (Fig. 2). Proteins such as WNT6, WNT10a, and WNT10b involved in Wnt signaling are important for the maintenance and development of many tissues and organs including bones [Cadigan and Nusse, 1997]. During the development of the limb bud, the Wnt signaling pathway influences various mechanisms such as limb morphogenesis and patterning [Cawthorn et al., 2012].

The disease-causing mutation in the *WNT10B* gene was first described by Ugur and Tolun [2008], identifying a homozygous missense mutation (Arg332Trp) in a Turkish consanguineous family. Later, a Swiss patient with a homozygous 4-bp duplication in *WNT10B* exhib-

Table 3. Mutations known to date in different genes responsible for nonsyndromic SHFM

Gene mutation	cDNA position	Protein position	Effect	Phenotype
DLX5				
Nonsense	c.115G>T	p.Glu39*	Stop codon	SHFM
Missense	c.505G>A	p. Glu169Lys	Substitution	Hypogonadotropic hypogonadism
Missense	c.533A>C	p.Gln178Pro	Substitution	SHFM
Missense	c.558G>T	p. Gln186His	Substitution	SHFM
Missense	c.576C>G	p.Ile192Met	Substitution	Pierre Robin sequence
Missense	c.593A>C	p. Asn198Thr	Substitution	Hypogonadotropic hypogonadism
Frame shift	c.482_485dupACCT	–	FS and PTC	SHFM
Gross deletions	~1 Mb incl. entire gene and <i>DLX6</i>	–	FS and PTC	SHFM
Gross deletions	~8.478 Mb incl. entire gene, <i>DLX6</i> and >50 others	–	FS and PTC	SHFM
Gross deletions	0.9–1.8 Mb incl. entire gene, <i>DLX6</i> and <i>DSS1</i>	–	FS and PTC	SHFM
Gross insertion	719 kb incl. entire gene and <i>DLX6</i>	–	FS and PTC	SHFM
WNT10B				
Missense	c.265G>A	p.Asp89Asn	Substitution	Dental anomalies
Missense	c.475G>C	p.Ala159Pro	Substitution	Dental anomalies
Missense	c.569C>G	p.Pro190Arg	Substitution	Oligodontia
Missense	c.632G>A	p.Arg211Gln	Substitution	Oligodontia
Missense	c.661C>T	p.Arg221Trp	Substitution	SHFM
Missense	c.767G>A	p.Cys256Tyr	Substitution	Obesity
Nonsense	c.786G>A	p.Trp262T*	Substitution	Oligodontia
Missense	c.849C>A	p.Ile283Ile	Substitution	Oligodontia
Missense	c.851T>G	p.Phe284Cys	Substitution	Oligodontia
Missense	c.986C>G	p. Thr329Arg	Substitution	SHFM
Missense	c.986C>A	p. Thr329Lys	Substitution	SHFM
Missense	c.994C>T	p. Arg332Trp	Substitution	SHFM
Missense	c.1052G>A	p. Arg351His	Substitution	Dental anomalies
Missense	c.1087C>T	p. Arg363Cys	Substitution	Dental anomalies
Splice site	c.338–1G>C	–	Substitution	SHFM
Frame shift	c.695_697delACA	p. Asn232del	Small deletions	SHFM
Frame shift	c.293_299dupAGGGCGG	–	Small deletions	SHFM
Frame shift	c.458_461dupAGCA	–	Small deletions	SHFM
TP63				
Missense	c.191A>G	p. Gln25Arg	Substitution	Heterotaxy
Missense	c.289C>T	p. Arg58Cys	Substitution	SHFM
Missense	c.343G>T	p. Gly76Trp	Substitution	Limb-mammary syndrome
Missense	c.386C>T	p. Ser90Leu	Substitution	Cleft lip
Missense	c.386C>G	p. Ser90Trp	Substitution	Limb-mammary syndrome
Missense	c.448G>A	p. Ala111Thr	Substitution	Cleft palate
Missense	c.497C>T	p. c.497C>T	Substitution	ADULT syndrome
Missense	c.518G>A	p. Gly134Asp	Substitution	Limb-mammary syndrome
Missense	c.518G>T	p. Gly134Val	Substitution	ADULT syndrome
Missense	c.598A>G	p. Lys161Glu	Substitution	SHFM
Missense	c.602T>C	p. Leu162Pro	Substitution	EEC syndrome
Missense	c.605A>G	p. Tyr163Cys	Substitution	EEC syndrome
Missense	c.691T>G	p. Tyr192Asp	Substitution	EEC syndrome
Missense	c.692A>G	p. Tyr192Cys	Substitution	EEC syndrome
Missense	c.697A>G	p.Lys194Glu	Substitution	SHFM
Missense	c.721G>A	p. Val202Met	Substitution	EEC syndrome
Missense	c.728G>A	p. Arg204Gln	Substitution	EEC syndrome
Missense	c.728G>T	p. Arg204Leu	Substitution	EEC syndrome
Missense	c.727C>T	p. Arg204Trp	Substitution	EEC syndrome
Missense	c.740A>G	p. His208Arg	Substitution	EEC syndrome
Missense	c.739C>G	p. His208Asp	Substitution	EEC syndrome
Missense	c.739C>T	p. His208Tyr	Substitution	EEC syndrome
Missense	c.797G>A	p. Arg227Gln	Substitution	EEC syndrome
Missense	c.797G>C	p. Arg227Pro	Substitution	EEC syndrome
Missense	c.799G>A	p. Val228Ile	Substitution	Congenital anomalies of the kidney and urinary track
Missense	c.923G>A	p. Cys269Tyr	Substitution	EEC syndrome
Missense	c.929G>C	p. Ser271Thr	Substitution	EEC syndrome
Missense	c.932G>A	p. Ser272Asn	Substitution	EEC syndrome
Missense	c.932G>C	p. Ser272Thr	Substitution	EEC syndrome
Missense	c.935G>A	p. Cys273Tyr	Substitution	EEC syndrome

Table 3 (continued)

Gene mutation	cDNA position	Protein position	Effect	Phenotype
Missense	c.946A>T	p. Met277Leu	Substitution	Skeletal abnormality
Missense	c.952C>T	p. Arg279Cys	Substitution	EEC syndrome
Missense	c.953G>A	p. Arg279His	Substitution	EEC syndrome
Missense	c.952C>A	p. Arg279Ser	Substitution	EEC syndrome
Missense	c.955C>T	p. Arg280Cys	Substitution	SHFM
Missense	c.956G>A	p.Arg280His	Substitution	SHFM
Missense	c.956G>T	p. Arg280Leu	Substitution	SHFM
Missense	c.955C>A	p.Arg280Ser	Substitution	EEC syndrome
Missense	c.1010G>A	p. Arg298Gln	Substitution	ADULT syndrome
Missense	c.1009C>G	p. Arg298Gly	Substitution	ADULT syndrome
Missense	c.1028G>A	p.Arg304Gln	Substitution	EEC syndrome
Missense	c.1028G>C	p. Arg304Pro	Substitution	EEC syndrome
Missense	c.1027C>T	p. Arg304Trp	Substitution	EEC syndrome
Missense	c.1033T>C	p. Cys306Arg	Substitution	EEC syndrome
Missense	c.1034G>A	p. Cys306Tyr	Substitution	EEC syndrome
Missense	c.1037C>A	p. Ala307Asp	Substitution	EEC syndrome
Missense	c.1037C>G	p. Ala307Gly	Substitution	EEC syndrome
Missense	c.1039T>A	p. Cys308Ser	Substitution	EEC syndrome
Missense	c.1040G>A	p. Cys308Tyr	Substitution	AEC syndrome
Missense	c.1042C>T	p. Pro309Ser	Substitution	EEC syndrome
Missense	c.1046G>A	p. Gly310Glu	Substitution	SHFM
Missense	c.1048A>G	p. Arg311Gly	Substitution	EEC syndrome
Missense	c.1051G>A	p. Asp312Asn	Substitution	EEC syndrome
Missense	c.1053C>A	p. Asp312Glu	Substitution	EEC syndrome
Missense	c.1052A>G	p. Asp312Gly	Substitution	EEC syndrome
Missense	c.1051G>C	p. Asp312His	Substitution	EEC syndrome
Missense	c.1054A>G	p. Arg313Gly	Substitution	Cleft lip
Missense	c.1061C>A	p. Ala315Glu	Substitution	EEC syndrome
Missense	c.1063G>C	p. Asp316His	Substitution	AEC syndrome
Missense	c.1646T>C	p. Ile510Thr	Substitution	Rapp-Hodgkin syndrome
Missense	c.1655T>C	p. Phe513Ser	Substitution	AEC syndrome
Missense	c.1654T>G	p. Phe513Val	Substitution	Rapp-Hodgkin syndrome
Missense	c.1659A>T	p. Leu514Phe	Substitution	AEC syndrome
Missense	c.1658T>C	p. Leu514Ser	Substitution	AEC syndrome
Missense	c.1657T>G	p. Leu514Val	Substitution	AEC syndrome
Missense	c.1670G>T	p. Gly518Val	Substitution	AEC syndrome
Missense	c.1672T>C	p. Cys519Arg	Substitution	AEC syndrome
Missense	c.1681T>C	p. Cys522Arg	Substitution	AEC syndrome
Missense	c.1681T>G	p. Cys522Gly	Substitution	AEC syndrome
Missense	c.1683T>G	p. Cys522Trp	Substitution	AEC syndrome
Missense	c.1685T>C	p. Leu523Pro	Substitution	AEC syndrome
Missense	c.1695C>A	p. Phe526Leu	Substitution	AEC syndrome
Missense	c.1706G>T	p. Gly530Val	Substitution	AEC syndrome
Missense	c.1709T>C	p. Leu531Pro	Substitution	Ankyloblepharon filiforme adnatum associated with Hay-Wells syndrome
Missense	c.1714A>C	p. Thr533Pro	Substitution	AEC syndrome
Missense	c.1724A>T	p. Gln536Leu	Substitution	AEC syndrome
Missense	c.1727T>C	p. Ile537Thr	Substitution	AEC syndrome
Missense	c.1739C>T	p. Ser541Phe	Substitution	AEC syndrome
Missense	c.1739C>A	p. Ser541Tyr	Substitution	Rapp-Hodgkin syndrome
Missense	c.1747G>T	p. Asp544Tyr	Substitution	AEC syndrome
Missense	c.1751T>C	p. Leu545Pro	Substitution	AEC syndrome
Missense	c.1766T>A	p. Ile550Asn	Substitution	EEC syndrome
Missense	c.1769C>A	p.Pro551His	Substitution	AEC syndrome
Missense	c.1769C>T	p. Pro551Leu	Substitution	AEC syndrome
Missense	c.1781G>C	p. Arg555Pro	Substitution	AEC syndrome
Missense	c.1790T>C	p. Ile558Thr	Substitution	AEC syndrome
Missense	c.1799G>A	p. Gly561Asp	Substitution	Rapp-Hodgkin syndrome
Missense	c.1799G>T	p. Gly561Val	Substitution	AEC syndrome
Missense	c.1805T>C	p. Leu563Pro	Substitution	EEC syndrome
Missense	c.1807G>C	p. Asp564His	Substitution	Cleft lip

Table 3 (continued)

Gene mutation	cDNA position	Protein position	Effect	Phenotype
Missense	c.1904G>T	p. Gly596Val	Substitution	Ectodermal dysplasia
Missense	c.1910G>T	p. Arg598Leu	Substitution	AEC syndrome
Missense	c.1919A>T	p. Asp601Val	Substitution	AEC syndrome
Nonsense	c.1974G>A	p. Trp619*	PTC	SHFM
Nonsense	c.2011A>T	p. Lys632*	PTC	Limb-mammary syndrome
Nonsense	c.2017C>T	Gln634*	PTC	SHFM
Nonsense	c.2032G>T	p. Glu639*	PTC	SHFM
Splice site	c.63–1G>C	–	Intronic	Prostate carcinoma
Splice site	c.580–2A>C	–	Intronic	SHFM
Splice site	c.580–2A>G	–	Intronic	EEC syndrome
Splice site	c.1350–2A>G	–	Intronic	EEC syndrome
Splice site	c.1747G>T	–	Intronic	AEC syndrome
Regulatory sequence	c.*374G>A	–	Stop loss	Bladder cancer
Regulatory sequence	c.*2345C>T	–	Stop loss	Bladder cancer
Regulatory sequence	c.62+6895C>T	–	Stop loss	Lung adenocarcinoma
Regulatory sequence	c.*20609A>G	–	Stop loss	Bladder cancer
Regulatory sequence	c.62+33817C>T	–	Stop loss	Lung adenocarcinoma
Small deletion	c.970_972delATT	p.Ile324del	FS	EECUT plus syndrome
Small deletion	c.1338_1341delACTT	p.Leu446Phefs*20	FS and PTC	Orofacial clefting
Small deletion	c.1693_1694delTT	p.Phe565Hisfs*12	FS and PTC	EEC syndrome
Small deletion	c.1815delG	p.Gln606Serfs*98	FS and PTC	Rapp-Hodgkin syndrome
Small deletion	c.1827delA	p.Glu609Aspfs*95	FS and PTC	Rapp-Hodgkin syndrome
Small deletion	c.1838delC	p.Pro613Leufs*91	FS and PTC	Rapp-Hodgkin syndrome
Small deletion	c.1859delC	p.Pro620Glnfs*84	FS and PTC	AEC syndrome
Small deletion	c.1860_1861delAA	p.Ser621Glnfs*11	FS and PTC	EEC syndrome
Small deletion	c.1900delC	p.Arg634Glyfs*70	FS and PTC	Rapp-Hodgkin syndrome
Small deletion	c.1904delG	p.Gly635Valfs*69	FS and PTC	Rapp-Hodgkin syndrome
Small deletion	c.1963delC	p.Arg655Glufs*49	FS and PTC	AEC syndrome
Small deletion	c.1976delA	p.Asn659Metfs*45	FS and PTC	Rapp-Hodgkin syndrome
Small insertions	c.819_820dupCC	–	FS and PTC	Cleft lip and palate
Small insertions	c.1572dupA	–	FS and PTC	Rapp-Hodgkin syndrome
Small insertions	c.1689_1690insA	–	FS and PTC	EEC syndrome
Small insertions	c.1718_1720dupTCT	–	FS and PTC	AEC syndrome
Small insertions	c.1833_1843dup11	–	FS and PTC	AEC/Rapp-Hodgkin syndrome
Small indel	c.953_954delGCinsAA	–	FS and PTC	EEC syndrome
Gross deletion	>19,1059 bp incl. exons 1–4	–	FS and PTC	EEC syndrome
DLX6				
Missense	c.632T>A	p.Val211Glu	Substitution	SHFM
ZAK				
Missense	c.1103T>G	p.Phe368Cys	Substitution	SHFM
Gross deletion	exons 12–16del	–	Small protein	SHFM
EPS15L1				
Small deletion	c.409delA	p.Ser137Alafs*19	FS and PTC	SHFM

FS, frame shift; PTC, premature termination codon; SHFM, split-hand/foot malformation.

ited typical SHFM features [Blattner et al., 2010]. A novel homozygous mutation (Arg332Trp) in the *WNT10B* gene was detected by Khan et al. [2012] in a consanguineous Pakistani family, and Aziz et al. [2014] reported 2 Pakistani families with a homozygous 4-bp deletion (c.1165_1168delAAGT) and a homozygous 7-bp duplication (c.300_306dupAGGGCGG). Recently, Ullah et al. [2018] reported a recurrent duplication and a nonsense mutation in 4 Pakistani families segregating autosomal

recessive SHFM6. To date, only 20 homozygous mutations have been reported in the *WNT10B* gene, with 8 being associated with SHFM (HGMD, 2018; Table 3). Affected individuals with a mutation in *WNT10B* present with, e.g., oligodontia, dental anomalies, and SHFM. Affected individuals with SHFM phenotypes include additional features such as polydactyly, complex cutaneous syndactyly, hypoplasia, aplasia of radial ray of the hands, and fixed flexion contractures (Table 2).

Fig. 2. Canonical Wnt/ β -catenin pathway. **A** Absent Wnt signal. **B** Present Wnt signal.

WNT signaling plays a key role in the vertebrate limb development [Yang, 2003]. WNT forms a family of 19 highly conserved cysteine-rich signaling molecules, which plays an important role in osteoblastogenesis and bone formation [Pandur et al., 2002; Logan and Nusse, 2004]. The WNT name is derived from the first 2 members of the family: int-1 (mouse) and wingless (*Drosophila*) [Wodarz and Nusse, 1998].

The key functional role of the WNT pathway has been exclusively studied in the developing limb bud, which controls limb developmental processes such as dorsoventral limb identity, limb patterning, and limb morphogenesis [Galceran et al., 1999]. WNT signaling during late limb morphogenesis regulates the morphology and position of the limb development such as skeletal elements, tendons, and muscles [Yang, 2003]. Bone formation,

through regulating chondrogenic differentiation as well as osteoblast proliferation, has also been associated with WNT signaling [Rudnicki and Brown, 1997; Hartmann and Tabin, 2001].

WNTs are secreted glycoproteins involved in the determination of cell fate and growth as well as acting as ligands in different pathways. Among these, canonical Wnt/ β -catenin is the best understood. WNT10B plays an important role in the β -catenin/canonical Wnt pathway. In the process of WNT10B glycoproteins production, several players are involved [Miller et al., 1999]. The WNT10B protein is inactivated in the intercellular portion of the secreted frizzled-related protein or the Wnt inhibitory factor. Thus, WNT10B signals are absent, and the β -catenin is captured by the degradation complex containing the axin/conductin, glycogen synthase kinase

3b (GSK3b), and adenomatous polyposis coli (APC) as well as casein kinase 1a (CK1a). β -catenin is ubiquitinated, phosphorylated, and then degraded with the assistance of proteasome (Fig. 2A). Furthermore, in the nucleus, the Groucho (a transcriptional inhibitor) binds to lymphoid enhancer factor/T-cell factor transcription factors at the Wnt-responsive element and prevents transcription of Wnt target genes (Fig. 2A) [Miller et al., 1999].

Similarly, when the WNT10B molecules are secreted by the Wntless cells, it initiates the canonical Wnt signaling pathway. WNT10B binds and activates the low-density lipoprotein-related receptor protein and the Frizzled receptors. The degradation complex and the GSK3b are inactivated by the interface of axin with phosphorylated DVL and LRP5/6. The accumulation of β -catenin takes place in the cytoplasm, and it is translocated into the nucleus, where it forms a heterometric complex and activates Wnt target genes transcription (Fig. 2B). The interaction of WNT10B with other proteins has been illustrated (Fig. 3), demonstrating a strong interaction with other key players involved in skeletal development.

SHFM7/SFMMP

Split-foot malformation with mesoaxial polydactyly (SFMMP; 616890) was reported for the first time by Spielmann et al. [2016] in a Pakistani consanguineous family with 3 affected individuals and a Tunisian boy with unilateral/bilateral cutaneous syndactyly and SHFM. All affected individuals reported in the Pakistani family also had a bilateral sensorineural hearing impairment, but the Tunisian boy had normal hearing and normal psychomotor development. The affected individuals presented clinical features such as duplication of finger nail bed of the 4th digit, absent 3rd toe, and cutaneous syndactyly of the 1st and 2nd as well as the 4th and 5th toes. Interfamilial phenotypic variability was observed among the affected individuals of the same family.

Using SNP array, genotyping, and WES, Spielmann et al. [2016] identified homozygosity for a missense mutation in the *ZAK* gene in a Pakistani family that segregated with the disease phenotype, but it was not found in 180 Pakistani controls. In addition, a homozygous intragenic deletion in the *ZAK* gene was observed in the boy from Tunisia. Spielmann et al. [2016] showed that *Zak* is expressed in developing mice limbs and CRISPR/Cas-mediated knockout of the 2 *Zak* isoforms is embryonically lethal in mice. CRISPR/Cas-mediated knockout showed that *ZAK* is a key player in mammalian limb patterning and development [Spielmann et al., 2016].

Fig. 3. Schematic representation of WNT10B interaction with other key players involved in limb development in humans. Purple-shaded proteins indicate those involved in skeletal development, and brown-shaded proteins show the players involved in the Wnt signaling pathway (<https://genemania.org/>).

SHFM8

Recently, Umair et al. [2018] presented the first direct evidence of involvement of the *EPS15L1* gene (MIM 616826) causing mild to severe SHFM phenotypes in a consanguineous Pakistani family. The family had 2 affected individuals exhibiting SHFM features such as cleft hand deformity, agenesis at the metacarpal joint, dysplastic middle and distal phalanx of the lesser toe as well as preaxial and postaxial syndactyly. Whole-genome SNP array and WES identified a frameshift deletion (c.409delA) in exon 7 of the *EPS15L1* gene that led to the formation of a premature stop codon (p.Ser137Alafs*19), which may have resulted in nonsense-mediated mRNA decay [Umair et al., 2018].

EPS15L1 is composed of 3 domains. Domain I consists of approximately 300 amino acids and an EF-hand-type calcium-binding domain. Domain II has heptad repeats of the coiled-coil domain, and Domain III exhibits a proline-rich region, consisting of a repeated aspartic acid-proline phenylalanine motif [Umair et al., 2018]. *EPS15L1* chiefly functions as a substrate for tyrosine kinase activity of the epidermal growth factor receptor (EGFR) which

is generally allied with limb morphogenesis [Seiler et al., 2015]. The EGFR signaling pathway is primarily involved in survival, growth, differentiation, and proliferation and is associated with limb development via AER. Both environmental factors and genetic defects may cause SHFM phenotypes by interfering with AER [Hsueh et al., 2015]. Any pathogenic mutation in the *EPS15L1* gene may change the EPS15L1 protein dosage, reducing the substrate concentration. The reduced substrate may lead to a decreased tyrosine kinase activity of the EGFR.

Recently, it has been found that EPS15L1 displays a unique nonredundant role in the nervous system. In addition, in *Eps15/Eps15l1* double-knockout mice, it has been shown to play a fundamental role during embryo development. All the developing embryos showed severe developmental delay, fused somites, a reduced midbrain-hindbrain boundary, and the absence of the limb bud [Milesi et al., 2019].

SHFLD

SHFLD known as split-hand/foot malformation with long-bone deficiency is dominantly inherited and genetically distinct from isolated forms of SHFM. SHFLD is mostly involved the deformity of the tibia and fibula and is associated with the duplication of the 17p13.3 locus. Currently, 3 types of SHFLD have been mapped to different human chromosomes including SHFLD1 located at chromosome 1q42.2q43 (OMIM 119 100) [Naveed et al., 2006], SHFLD2 at 6q14.1 (OMIM 610685) [Naveed et al., 2007], and SHFLD3 located in chromosomal region 17p13.1p13.3 (OMIM 612576) [Lezirovitz, et al., 2008].

Lezirovitz et al. [2008] mapped SHFLD for the first time in a large Brazilian family to an 841-kb interval at 17p13.1p13.3 (15). Later, Klopocki et al. [2012] revealed a defect in tandem duplication and narrowed down the region to only the single *BHLHA9* gene. The *Bhlha9* mouse and zebra fish expression pattern was restricted to the AER of the limb bud mesenchyme. Further, *bhlha9* knockdown in zebrafish embryos revealed shortening of the pectoral fins and suggested an important role in limb development [Klopocki et al., 2012].

Diagnostic Aspects and Genetic Counseling

Patients presenting with SHFM features should be carefully diagnosed, clinically examined, and submitted to relevant cytogenetic and/or molecular testing. As described above, at least 12 SHFM types have been described

in the literature [Umair et al., 2018]. Sporadic cases are mostly caused by de novo mutations exhibiting isolated SHFM features.

Considering the pathophysiology of variable expression, reduced penetrance, non-mendelian inheritance, and segregation falsification [Klopocki et al., 2012], genetic counseling, correct molecular diagnosis, and prenatal testing in SHFM cases are difficult and extremely challenging. Furthermore, variability of the phenotype between affected individuals of the same family makes it very difficult to diagnose the exact molecular etiology.

In the majority of isolated single cases with SHFM, conventional karyotyping can identify large chromosomal aberrations and thus reveal the disease phenotype. [Duijf et al., 2003; Elliott and Evans, 2006].

Direct Sanger sequencing of the *TP63*, *DLX5*, and *DLX6* genes can solve most of the dominant cases [Ullah et al., 2017]. The *TP63* mutations show highly variable expressivity and complete penetrance [Faiyaz-Ul-Haque et al., 2005]. Similarly, direct Sanger sequencing would be a suitable choice for families exhibiting rare autosomal recessive inheritance such as SHFM6 (*WNT10B*), SHFM7 (*ZAK*), and SHFM 8 (*EPS15L1*). Should Sanger sequencing fail to identify the disease-causing variant, whole-genome SNP array or WES could be used to identify the gene responsible for the disease.

Conclusion

The clinical and genetic heterogeneity of SHFM contributes to extremely challenging and difficult genetic counseling. Genetic alteration and appropriate molecular diagnosis responsible for SHFM is important for the entire family. Firstly, it would help the family to understand the genetic nature of the disease and develop proper risk management strategies for the disease. Secondly, molecular diagnosis would facilitate conscious family planning and support prenatal or preimplantation diagnosis. Finally, excluding all known disease-causing alterations, molecular diagnostic testing using next-generation sequencing provides an opportunity to solve unresolved cases (whole-exome or whole-genome sequencing), contributing to the identification of novel disease-causing candidate genes associated with SHFM.

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