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A classification system for split-hand/ foot malformation (SHFM): A proposal based on 3 pedigrees with *WNT10B* mutations

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ABSTRACT

SHFM6 (OMIM 225300) is caused by *WNT10B* pathogenic variants (12q13.12). It is one of the rarest forms of SHFM; with only seven pathogenic variants described in the world literature. Furthermore, it has not been determined if SHFM6 has specific phenotypic characteristics.

In this paper, we present a case series of three unrelated families with SHFM6 caused by three novel *WNT10B* pathogenic variants. The index patient of the first family was homozygous for the nonsense variant c.676C > T (p.Arg226*) in the *WNT10B* gene. The index case of the second family had a homozygous splice variant c.338-1G > C in the *WNT10B* gene. Finally, the index case of the third family carried two different variants in the *WNT10B* gene: A nonsense variant (p.Arg226*), and a missense variant (p.Gln86Pro). The latter represents the first compound heterozygous pathogenic variant related to SHFM6. We also offer a classification system for the hand/foot defects to illustrate the specific phenotypic characteristics of SHFM6. Based on this classification and a review of all previously reported cases, we demonstrate that SHFM6 caused by *WNT10B* pathogenic variants have the following characteristics: more severe feet defects (compared to the hand defects), polydactyly, severe flexion digital contractures, and phalangeal dysplasia.

1. Introduction

Split-hand/foot malformation (SHFM) is a complex malformation of the central rays of the hands and feet which is caused by different genetic variants (Sowinska-Siedler et al., 2014). Hence, SHFM has been classified into seven sub-types (SHFM 1 to 7).

SHFM6 (OMIM 225300) is caused by *WNT10B* pathogenic variants (12q13.12). It is one of the rarest forms of SHFM; with only seven pathogenic variants described in the world literature (Ugur and Tolun, 2008; Blattner et al., 2010; Khan et al., 2012; Aziz et al., 2014; Ullah et al., 2018; Kantaputra et al., 2018). Furthermore, it has not been determined if SHFM6 has specific phenotypic characteristics.

In this paper, we present a case series of three unrelated native Saudi families with SHFM6 caused by three novel *WNT10B* pathogenic variants including the first compound heterozygous variants related to SHFM6. We also offer a classification system for the hand/foot defects to illustrate the specific phenotypic characteristics of SHFM6.

1.1. Patients and methods

The index cases presented to Hand Surgeon (MMA) for consideration of surgical treatment of their hand defects. The Hand Surgeon has special interest in SHFM (Al-Qattan, 2014a) and has devised a classification system for the hand/foot defects aimed to investigate specific phenotypic characteristics of the various types of SHFM. This classification system will be utilized to investigate the unique features of SHFM6 phenotypes described in all previously reported cases including the current series.

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Table 1
Classification of the severity of the hand/foot defects in SHFM and identification of unique phenotypic characteristics.

Classification of severity/Unique features	Description
A) Classification of the severity of the defect	Grade I: Normal, Grade II: isolated syndactyly, Grade III: one missing central ray (phalanges only or the entire ray), Grade IV: two missing central rays, Grade V: three missing central rays (also known as lobster-claw deformity), Grade VI: mono-dactyly (a single postaxial ray). Concurrent syndactyly may occur in Grades III-V.
B) Unique features	
(i) The severity of the hand vs foot defect	The severity of hand defects are more severe than foot defects or vice versa.
(ii) The presence of polydactyly (hands/feet) or tri-phalangeal thumb	The presence of polydactyly and tri-phalangeal thumb (which is genetically a form of pre-axial polydactyly) are rare concurrent features in SHFM. Hence, their presence is considered a unique feature.
(iii) The presence of severe flexion contractures of the remaining digits in the hand	Most cases of SHFM have no or minor flexion contractures of the remaining digits in the hands. Hence, the presence of severe contractures is considered a unique feature.
(iv) The presence of dysplasia/hypoplasia/aplasia of the distal and middle phalanges of the remaining digits (hands/feet)	The remaining digits in the hands and feet are usually well-formed. Hence, the presence of dysplasia, hypoplasia, or aplasia of the phalanges of the remaining digits is a unique feature.
(v) The presence of radial ray deficiency (hands/forearm)	SHFM patients usually have well-developed radial rays. Occasionally, there is radial ray deficiency and this is considered a unique feature.
(vi) The presence of dorsalization of the digits (hands/feet)	Partial or complete dorsalization of the digits is a known concurrent feature in certain SHFM phenotypes.

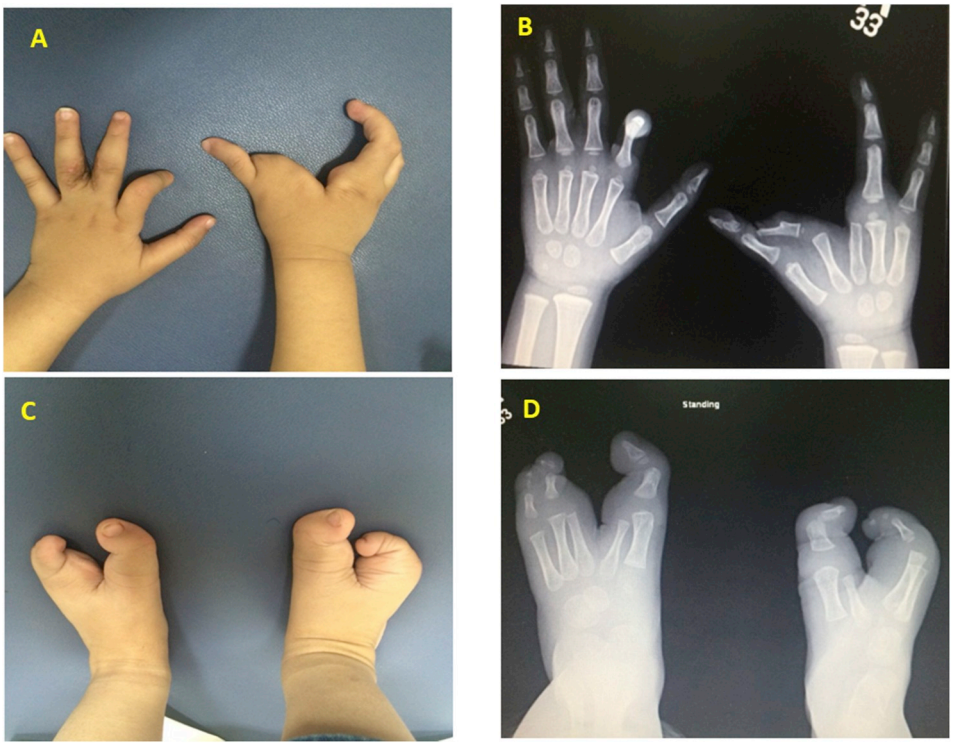

Fig. 1. The index case of the first family
a) Clinical appearance of the hands, b) radiological appearance of the hands. Note that the right hand has: middle finger ectrodactyly, thumb index syndactyly, triphalangeal thumb, and a dysplastic index finger. The left hand has: middle finger syndactyly (operated), flexion contracture of the index finger, and dysplastic distal phalanx of the index finger.
c) Clinical appearance of the feet, d) radiological appearance of the feet. Note the missing central rays bilaterally as well as the dysplasia of the distal and middle phalanges of the 4th & 5th toes.

1.2. Classification of the hand/foot defects and identification of unique phenotypic characteristics

Although the presence of ectrodactyly (also known as central clefts in the hand surgery literature) is an essential feature of SHFM, it is well known that one or more limbs may be normal or manifest with syndactyly without ectrodactyly (Al-Qattan, 2014a). Furthermore, the number of the missing central rays may vary from 1 to 3 rays; and most severe cases present with monodactyly. It has been noted that the preserved digit in SHFM patients with monodactyly has always been the postaxial (and not the preaxial) digit (Al-Qattan, 2014a). Hence, the severity of the defect may be classified into the grades shown in Table 1.

Identification of unique phenotypic characteristic are also shown in Table 1; and these may then be used to investigate genotype-phenotype correlations with various SHFM sub-types.

1.3. Clinical and radiological features

The first family was a tribal native Saudi consanguineous family with one affected boy and one unaffected girl. The index case (Fig. 1) had a Grade II left hand, a Grade III right hand, a Grade IV left foot, and a Grade V right foot. Characteristic features included: the foot defect is more severe, polydactyly (a tri-phalangeal right thumb), flexion contracture of the left index finger, dysplasia of the distal/middle phalanges of the right index finger, and dysplasia of the postaxial toes of both feet.

The second family was a tribal native Saudi consanguineous family with one affected boy. The patient (Fig. 2) had a Grade III left hand, a Grade III right hand, a Grade IV left foot and a Grade V right foot. Characteristic features included: the foot defect is more severe, polydactyly (partial duplication of the left ring finger), severe flexion contractures of both index fingers, dysplasia of the phalanges of both index fingers, and dysplasia of the postaxial toes of both feet.

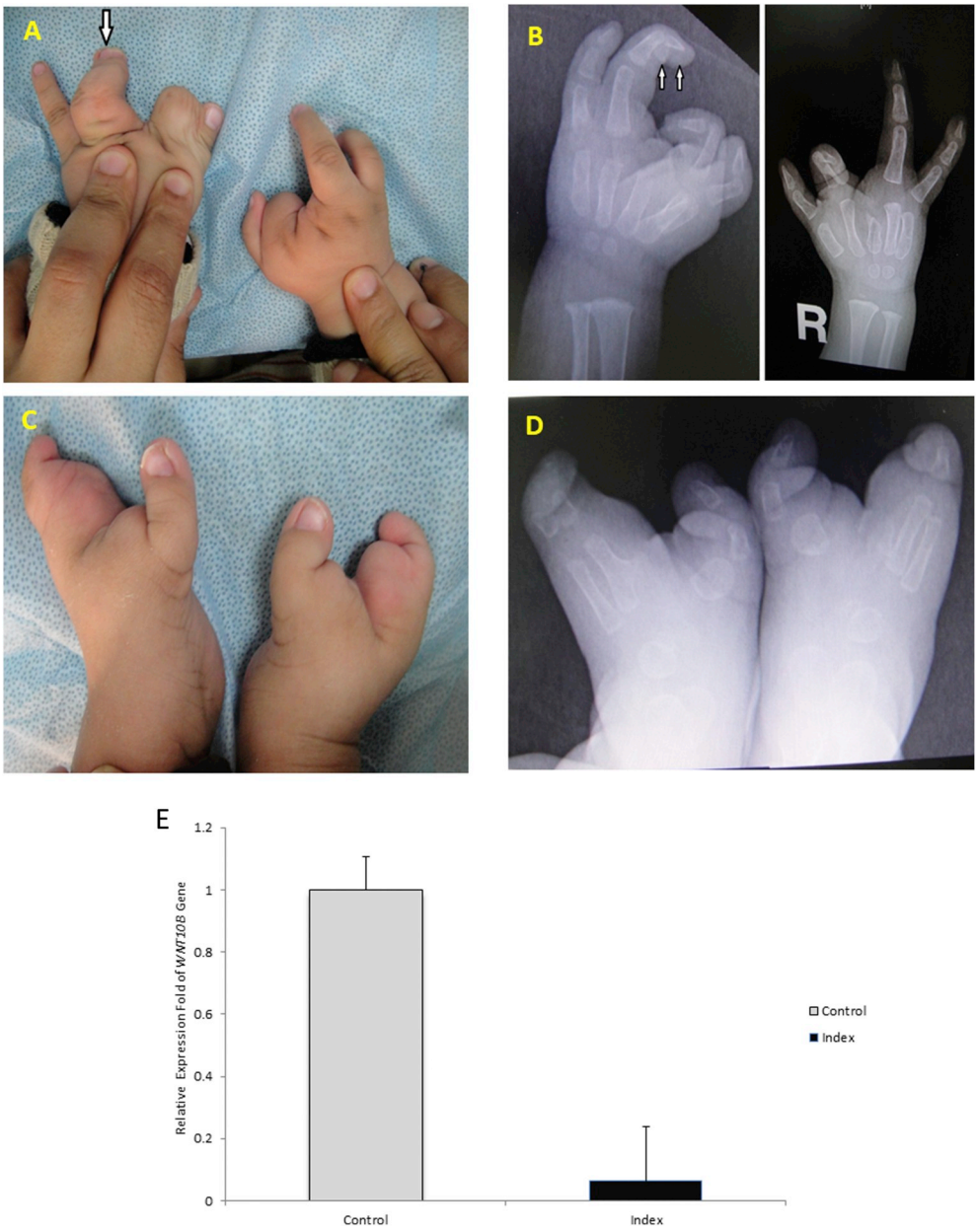

Fig. 2. The index of the second family
a) Clinical appearance of the hands, b) radiological appearance of the hands. Note that the middle finger ectrodactyly bilaterally. Both index fingers have severe flexion contractures with hypoplasia in the distal phalanx. The left hand has syndactyly of the thumb and index finger. Also note the duplicated nail in the left ring finger which is associated with dysplastic and incompletely ossified distal and middle phalanges (arrows).
c) Clinical appearance of the feet, d) radiological appearance of the feet. Note the missing central rays, the broad 1st metatarsal and the dysplastic distal/middle phalanges of the 4th & 5th toes.
e*) WNT10B* gene quantitative real-time PCR expression. Index case showed highly reduced expression compared to control sample.

The parents of the third index case were unrelated (each parent was from a different Saudi native tribe), and there was a family history of SHFM in both families as shown in Fig. 3. The index case (Fig. 4) had a Grade III left hand, a Grade III right hand, a Grade V left foot and a Grade V right foot. Characteristic features included: the foot defect is more severe, polydactyly of the right thumb and index finger, severe flexion contractures of the right ring and left index fingers, and dysplasia of the phalanges of the little toes. The brother of the index case had normal hands (Grade I) and a Grade III left foot and a Grade IV right foot.

1.4. Genetic analysis

Peripheral blood samples were obtained after signing a written consent. The Hand Surgeon (MMA) works at two different government hospitals and hence the approach to genetic analysis varied according to the approach of the genetics team of the hospital.

A) The Ampli-Seq panel used in families # 1 and 2:

Genomic DNA was extracted using standard Qiagen Kit. Sample sequencing by Ion AmpliSeq technology was done with the Ion PGM sequencer instrument, which performs ultra-high multiplex PCR enrichment of the targeted regions of the genes. Ion AmpliSeq custom on-demand design panel was made with the following genes: *DLX5*, *DLX6, EVC, EVC2, SUFU, TP63, BTRC, WNT10B, FGF13, DLX2, DLX1, FBXW4.* After sequencing, analysis was carried out with automated streamlined software's Torrent Suite Software v5.6 and Ion Reporter to identify variants along with annotation. All disease-causing variants were checked against HGMD, ClinVar, Genome Aggregation Database (gnomAD) and Exome Aggregation Consortium (ExAC).

B) Family 2-index Gene Expression Quantitative Real-time PCR (qRT-PCR) Analysis

Total RNA were extracted from index and control blood samples using QIAamp RNA Blood Mini Kit (Qiagen, Germany) and cDNA were then prepared using Reverse Transcription Kit (Applied Biosystems, USA) according to the manufacturer's instructions.

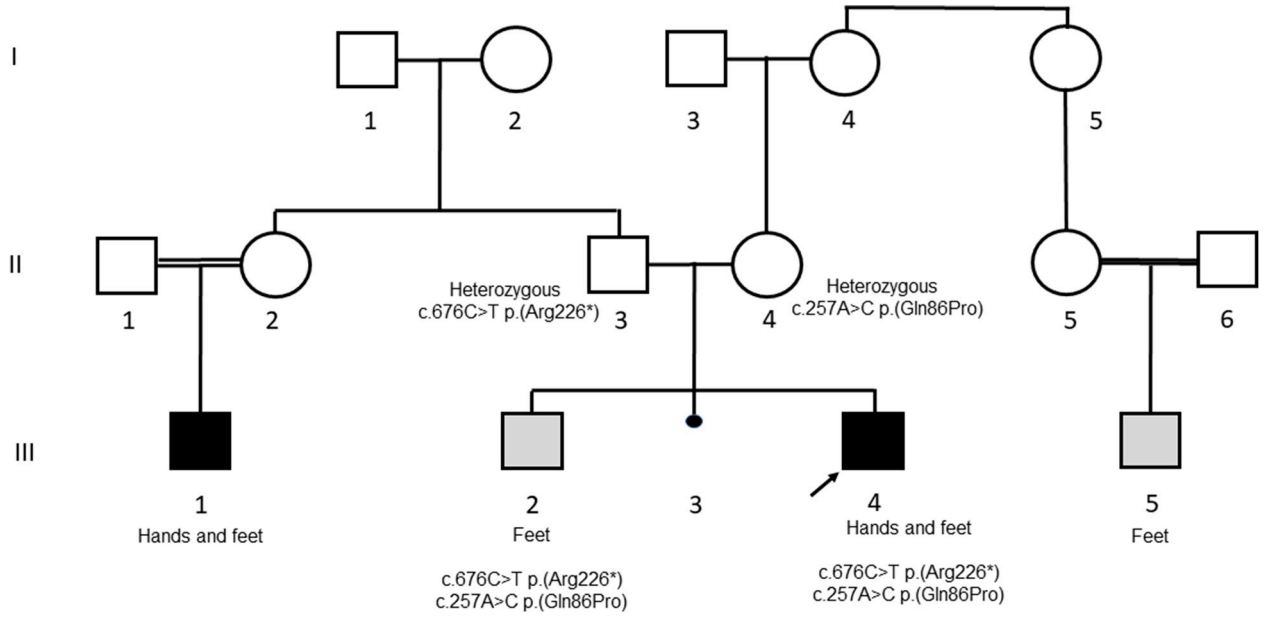

Fig. 3. The pedigree of the third family.

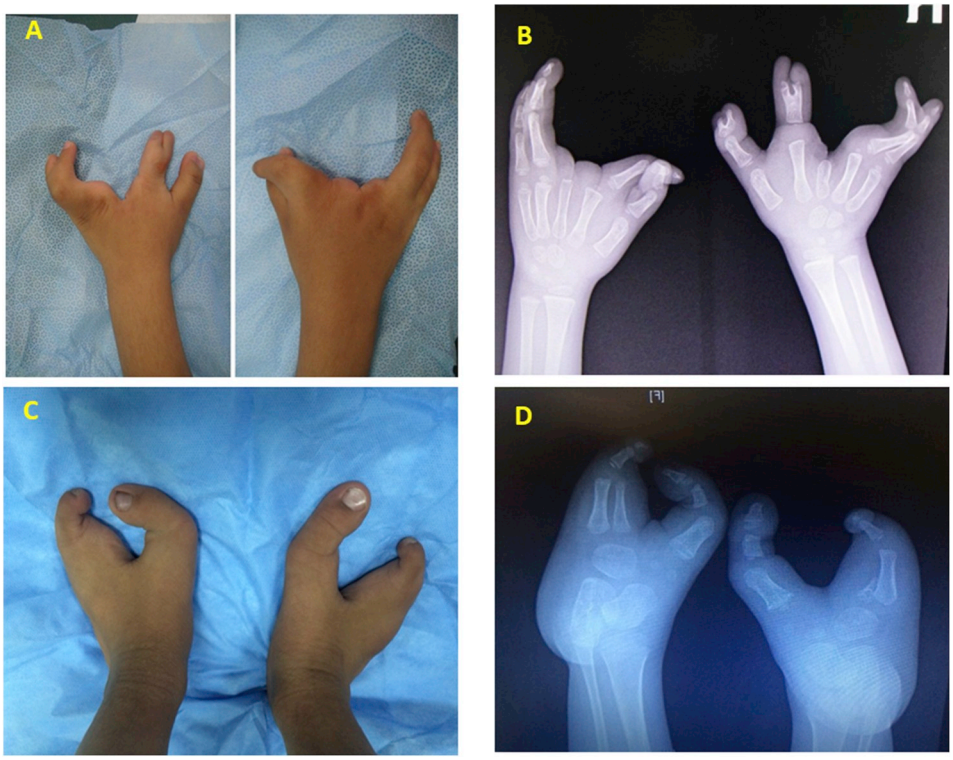

Fig. 4. The index case of the third family
a) Clinical appearance of the hands, b) radiological appearance of the hands. Note the middle finger ectrodactyly bilaterally. There is duplication of the right thumb and index finger; and flexion contractures of the right ring and left index fingers.
c) Clinical appearance of the feet, d) radiological appearance of the feet. Note the missing three central rays with Lobster-claw deformity bilaterally. There is also hypoplasia of the distal phalanges of the little toes.

Quantitative Real-time PCR (qRT-PCR) gene expression was performed in triplicate experiments for patient and control using *WNT10B* exon3-exon4 primers, 5′-CACAGCGCCATCCTCAA-'3, 5'-CTCTTGCCTCGGGACAG-'3 and *GAPDH* (internal control), 5′-TGAT GAC ATC AAG AAGG TGGT GAAG-3′ and 5′-TCCT TGGA GGCC ATGT GGGC CAT-3′. The reaction was done using the SYBR master mix on QuantaStudio 6 Flex (Applied Biosystems, Foster City, USA) and the relative quantification gene expression of *WNT10B* and *GAPDH* genes were analyzed by SDS Software version 2.4 (Applied Biosystems).

C) Exome sequencing used in family # 3.

DNA was extracted from dried blood spots in filter cards (CentoCard®) using standard, spin column-based methods. Approximately 60 Mb of the Human Exome (targeting > 99% of regions in CCDS, RefSeq and Gencode databases) was enriched from fragmented genomic DNA with Agilent's SureSelect Human All Exon V6 kit. The generated library was sequenced on an Illumina platform for CentoXome Gold to an average coverage depth 70–100X. An end to end in-house bioinformatics pipelines including base calling, primary filtering of low-quality reads and probable artefacts, and annotation of variants is applied. All disease-causing variants reported in HGMD, in ClinVar or in CentoMD (class 1) as well as all variants with minor allele frequency (MAF) of less than 1% in ExAc

Table 2
Summary of the detected variant mutations within *WNT10B* gene.

Family numbers/ members	Phenotype	Genomic position (GRCh37/hg19) Chr12	Mutation in cDNA (GenBank) NM_003394.3	Consequence (Protein)	Mutation Status	Annotation	CADD score (v1.4)	GnomAD	Pathogenicity
Family # 1									
Index	SHFM (hand/feet)	g.49361764G > A	c.676C > T	p.(Arg226*)	Homozygous	stop gained	36	Reported	Likely pathogenic
Sister	No abnormalities	g.49361764G > A	c.676C > T	p.(Arg226*)	Heterozygous			Reported	
Mother	No abnormalities	g.49361764G > A	c.676C > T	p.(Arg226*)	Heterozygous			Reported	
Father	No abnormalities	g.49361764G > A	c.676C > T	p.(Arg226*)	Heterozygous			Reported	
Family #2									
Index	SHFM (hand/feet)	g.49362103C > G	c.338-1G > C		Homozygous	splice acceptor	33	Not reported	Likely pathogenic
Mother	No abnormalities	g.49362103C > G	c.338-1G > C		Heterozygous			Not reported	
Father	No abnormalities	g.49362103C > G	c.338-1G > C		Heterozygous			Not reported	
Family #3									
Index	SHFM (hand/feet)	g.49361764G > A/ g.49363952T > G	c.676C > T/ c.257A > C	p.(Arg226*)/ p.(Gln86Pro)	Compound Heterozygous	stop gained and missense	36 28.7	Not reported	Likely pathogenic
Brother	SHFM (feet only)	g.49361764G > A/ g.49363952T > G	c.676C > T/ c.257A > C	p.(Arg226*)/ p.(Gln86Pro)	Compound Heterozygous		36 28.7	Not reported	
Mother	No abnormalities	g.49363952T > G	c.257A > C	p.(Arg226*)	Heterozygous			Not reported	
Father	No abnormalities	g.49361764G > A	c.676C > T	p.(Arg226*)	Heterozygous			Not reported	

Abbreviations are as follows: Chr-chromosome, CADD- Combined Annotation Dependent Depletion (score > 20), GnomAD- Genome Aggregation Database.

database and Genome Aggregation Database (gnomAD) are considered. Evaluation is focused on exons and intron boundaries ± 20.

2. Results

The results are shown in Table 2. The index patient of the first family was homozygous for the nonsense variant c.676C > T (p.Arg226*) in the *WNT10B* gene. It is classified as likely pathogenic (Class 2) according to ACMG. The parents and the sister were carriers and showed no abnormalities.

The index case of the second family had a homozygous splice variant c. 338-1G > C in the *WNT10B* gene. The variant was at the splice acceptor site in intron 3 and this is expected to result in: retention of the intronic region, inclusion of an intronic fragment, or removal of an exonic fragment. RNA exon3/exon4 *WNT10B* gene expression studies were confirmed the splice acceptor variant affect and result revealed a highly reduced expression of exon3/exon4 (mutant) *WNT10B* once compared to the wild type expression on control (Fig. 2C). Both parents were heterozygous for the same variant and had no abnormalities.

The index case of the third family carried two different variants in the *WNT10B* gene: A nonsense variant: p.Arg226* (classified as likely pathogenic or Class 2 according to ACMG); and a missense variant: p.Gln86Pro (classified as variant of uncertain significance or class 3 according to ACMG). The affected brother (with SHFM6 phenotype of the feet only) had the same compound heterozygous pathogenic variants. The unaffected father was heterozygous for the former variant and the unaffected mother was heterozygous for the latter variant.

3. Discussion

Our report added to the world literature three novel pathogenic variants of the *WNT10B* gene associated with SHFM6. We believe this is a significant contribution since the literature on the topic only reported seven others pathogenic variants (Ugur and Tolun, 2008; Blattner et al., 2010; Khan et al., 2012; Aziz et al., 2014; Ullah et al., 2018; Kantaputra et al., 2018). Furthermore, we offered a classification system for the hand/foot defects to illustrate specific phenotypic characteristics of SHFM6.

Table 3 summarizes all reported pathogenic variants of *WNT10B* associated with SHFM6 including the phenotypic characteristics of these variants. In all 10 reported pathogenic variants, the feet were more severely affected than the hands. Polydactyly/triphalangeal thumbs were seen with 8 variants (80% of reported variants). Severe flexion contractures of the remaining digits in the hands were seen in 9 variants and were not specified in the 10th variant. Dysplasia/hypoplasia of the phalanges of the remaining digits were seen in 8 pathogenic variants (80% of reported pathogenic variants). Hence, more severe feet defects, polydactyly, severe flexion digital contractures, and phalangeal dysplasia are commonly seen in the SHFM6 phenotype and may be considered as characteristic features. In contrast, radial ray deficiency and dorsalization of the digits are not commonly seen with *WNT10B* pathogenic variants.

Dorsalization of the digits is known in the hand surgery literature as “dorsal dimelia” (Al-Qattan, 2013). The degree of dorsalization varies from partial to complete. Partial dorsalization may manifest as thickening of the hyponychium, duplication of the nail bed, or the appearance of a hypoplastic palmar nail. Complete dorsalization involves the entire digit and includes the presence of a well-developed palmar nail, dorsalization of the palmar skin of the digit, and loss of digital flexion since the flexor tendons become replaced with hypoplastic extensor tendons (Al-Qattan, 2013). *ZAK* pathogenic variants associated with SHFM are known to be associated with partial digital dorsalization (Spielmann et al., 2016).; while intragenic *DLX5* pathogenic variants associated with SHFM are known to be associated with complete dorsalization (Shamseldin et al., 2012).

SHFM3 is one of the most common types of SHFM and is caused by duplications at the 10q24 locus. A recent review article on SHFM3 including 32 new index cases was reported (Holder-Espinasse et al., 2019). Table 4 shows the comparison between the clinical features of SHFM6 and SHFM3. The main characteristic feature of SHFM3 is the high frequency of monodactyly (Grade VI), and this is seen in the hands and feet. Another characteristic feature of SHFM3 is the common occurrence of preaxial polydactyly. Although polydactyly is also a characteristic feature of SHFM6, it is seen both pre- and post-axially.

The pathogenesis of SHFM is well described in the literature (Al-Qattan, 2014a; Kantaputra and Carlson, 2019). SHFM is caused by loss of maintenance (rather than lack of development) of the central part of the apical ectodermal ridge (AER). The following protein expression in the central AER contribute to its maintenance: FGF8, P63, DLX5/6, WNT10B and EPS15L1 (Kantaputra and Carlson, 2019; Umair et al., 2018). Fig. 5 shows that all known pathogenic variants associated with SHFM eventually lead to suppression of the levels of FGF8 in the central AER. One mystery regarding the pathogenesis of SHFM is the frequent occurrence of central syndactyly in the hands instead of having central ray deficiency. Syndactyly is associated with increased FGF activity of the overlying AER (Al-Qattan, 2014b; Al-Qattan and Alkuraya, 2019).

Table 3
WNT10B mutations associated with SHFM6: A summary of all previously reported mutations including those in the current series.

Mutations	Nationality of Family	Authors	The severity of hand vs foot defect	Concurrent features				
				Polydactyly or tri-phalangeal thumb	Severe flexion contractures of the digits in the hands	Hypoplasia/dysplasia of phalanges in the remaining digits	Radial ray aplasia/ hypoplasia	Dorsalization of digits
p.Arg332Trp (Homozygous missense)	Turkish	Ugur and Tolun, 2008	More severe in feet	Thumb polydactyly	Yes	–	–	–
p.Asp155AlafsX47 (Homozygous 4-bp duplication) leading to a frame shift	Swiss	Blattner et al. (2010)	More severe in feet	Little toe polydactyly	Not-specified	In hands	–	–
p.Thr329Arg (Homozygous missense)	Pakistan	Khan et al. (2012)	More severe in feet	Thumb polydactyly	Yes	In hands	Yes	–
p.Lys388Glufs*36 (Homozygous 4-bp duplication) leading to a frame shift	Pakistan	Aziz et al. (2014)	More severe in feet	6 metacarpals	Yes	In hands	–	–
p.Leu103Argfs*53 (Homozygous 7-bp duplication) leading to a frame shift	Pakistan	Aziz et al. (2014); Ullah et al. (2018)	More severe in feet	–	Yes	In hands	Yes	–
p.GLn154* (Homozygous nonsense)	Pakistan	Ullah et al. (2018)	More severe in feet	3rd toe polydactyly	Yes	In hands	Yes	–
p.Asn232del (Homozygous deletion)	India	Kantaputra et al. (2018)	More severe in feet	–	Yes	–	–	–
p.Arg226* (Homozygous nonsense)	Saudi	First family in the current study	More severe in feet	Tri-phalangeal thumb	Yes	In hands and feet	–	–
c.338-1G > C (Homozygous splice site)	Saudi	Second family of the current study	More severe in feet	Left right finger polydactyly	Yes	In hands and feet	–	–
c.676C > T (p.Arg 226*) and c.257A > C (p.GLn86Pro) (Compound heterozygous)	Saudi	Third family of the current study	More severe in feet	Right thumb and index finger polydactyly	Yes	Both little toes	–	–

Table 4
Comparison between the features of SHFM6 and SHFM3.

Feature	SHFM6	SHFM3
Classification of the severity of the defect	Mostly Grades III-V	Monodactyly (Grade VI) is seen in 31% of cases
Unique features:		
(i) Severity of hand vs foot defect	The feet are more severe	Variable
(ii) Polydactyly	A characteristic feature and is seen either as preaxial or postaxial polydactyly	A characteristic feature but is almost always seen as preaxial polydactyly
(iii) Severe flexion contractures of the digits	A characteristic feature	Not a characteristic feature
(iv) Dysplasia/Hypoplasia/Aplasia of the distal/middle phalanges	A characteristic feature	Not a characteristic feature
(v) Radial ray deficiency	Not a characteristic feature	Not a characteristic feature
(vi) Dorsalization of the digits	Not a characteristic feature	Not a characteristic feature

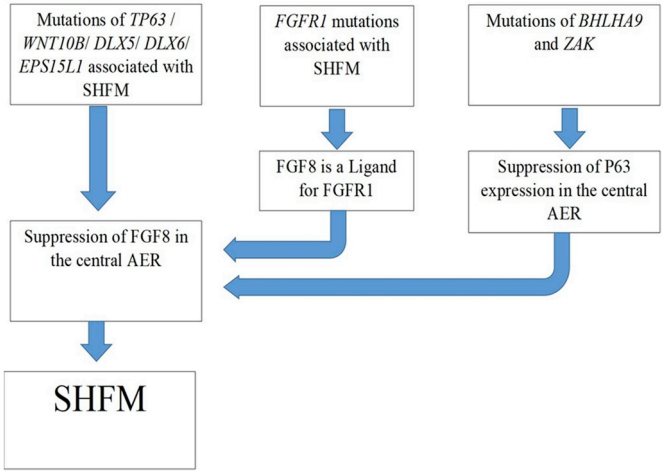

Fig. 5. The pathogenesis of SHFM is mainly through the suppression of FGF8 in the central AER.

This is interesting since ectrodactyly and syndactyly are frequently seen in the same patient in all subtypes of SHFM including SHFM Type 6 (Fig. 1). One theory of pathogenesis of SHFM – related syndactyly is that the initial reduction of FGF8 in the central AER will induce a secondary over-expression of FGF4 in the central AER; which in turn leads to syndactyly instead of ectrodactyly (Al-Qattan, 2014a). This theory was based on experimental observations (Lewandoski et al., 2000; Lu et al., 2006). FGF8 is normally expressed along the entire AER; while FGF4 is normally expressed in the posterior AER. When the expression of FGF8 is reduced in an area of the AER, secondary increased expression of FGF4 is noted (Lewandoski et al., 2000). When FGF4 is expressed in place of FGF8, all of the skeletal defects caused by inactivation of FGF8 are rescued. Furthermore, syndactyly and occasional polydactyly are seen in the phenotype of experimental animals (Lu et al., 2006). This may explain the presence of syndactyly (and also polydactyly) in the SHFM phenotype.

In conclusion, we present a case series of three families with SHFM6 and three novel *WNT10B* pathogenic variants We also offer a classification system for hand/foot defects. Finally, we review the literature to illustrate that SHFM6 has specific phenotypic characteristics.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmg.2019.103738.

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