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Sequence Variants in the *WNT10B* Underlying Non-Syndromic Split-Hand/Foot Malformation

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Split hand and foot malformation · Limb anomaly · *WNT10B* · Sequence variants

Abstract
Introduction: Split hand and foot malformation (SHFM) or ectrodactyly is a rare limb deformity characterized by median cleft of the hand and foot with impaired or missing central rays. It can occur as an isolated anomaly or in association with abnormalities of other body parts. ***Methods:*** After delineating the clinical features of two families (A–B), with non-syndromic SHFM, exome and Sanger sequencing were employed to search for the disease-causing variants. ***Results:*** Analysis of exome and Sanger sequencing data revealed two causative variants in the *WNT10B* gene in affected members of the two families. This included a novel missense change [c.338G>C; p.(Gly113Ala)] in family A and a previously reported frameshift variant [c.884-896delTCCAGCCCCGTCT; p.(Phe295Cysfs*87)] in family B. ***Conclusion:*** Our findings add a novel variant in *WNT10B* gene as the underlying cause of SHFM. The finding adds to the growing body of knowledge about the genetic basis of developmental disorders and provides valuable insights into the molecular mechanisms that regulate limb development.

Introduction

Ectrodactyly or split hand and foot malformation (SHFM) is a rare inherited bone disorder accounting for about 15% of all limb defects. The condition is also called lobster claw malformation. The clinical physiognomy of SHFM is highly variable in severity and ranges from complete truancy to under-development of one or more digits in hands and/or feet. Its severity varies even among individuals in the same family. In a few cases of SHFM, oligodactyly, monodactyly, and hemimelic deficiency of the distal long bones of limb including tibia and radius have been reported. Most likely, these phenotypic variations are the consequences of genetic background including epigenetic and environmental factors [Umair et al., 2017, 2019; Holder-Espinasse et al., 2019; Paththinige et al., 2019; Babbs et al., 2007].

SHFM may occur as an isolated anomaly or in syndromic fashion. Most of the SHFM cases show autosomal

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dominant pattern of inheritance. Pathogenic infirmity of SHFM is associated with maldevelopment of apical ectodermal ridge (AER). The AER is a major signaling center for limb development, located at the distal rim of the developing limb bud, and induced with the involvement of Wnt-Bmp-Fgf pathways. Disruption of these molecular events in the AER leads to ectrodactyly [Kantaputra et al., 2018].

To date, six genes have been found to be associated with autosomal recessive and autosomal dominant forms of SHFM. These included *DLX5* (MIM 600028), *DLX6* (MIM 600030), *TP63* (MIM 603273), *WNT10B* (MIM 601906), *ZAK* (MIM 194521), and *EPS15L1* (MIM 616826) [Umair et al., 2018]. In addition, three distinct types of SHFM with long bone deficiency (SHFLD1–3) have been mapped on different human chromosomes. However, only for SHFLD3, causative variants in *BHLHA9* have been identified as the underlying molecular correlate [Armour et al., 2011]. Homozygous intragenic variants in the same gene result in mesoaxial synostotic syndactyly associated with postaxial polydactyly [Malik et al., 2014; Ullah et al., 2017, 2019; Umair and Hayat, 2020].

In the present investigation, we have clinically and genetically characterized two families, segregating isolated SHFM in autosomal recessive manner. Whole-exome sequencing (WES), followed by Sanger sequencing, identified two causative variants in the *WNT10B*. These included a novel homozygous variant in family A and a previously reported variant in family B.

Methods

Study Approval and Genomic DNA Isolation

The study, presented here, was performed according to the Declaration of Helsinki protocols, approved by the Institutional Review Board (IRB) of Quaid-i-Azam University, Islamabad, Pakistan. Written informed consent for publication of radiographs and photographs was obtained from both affected and unaffected family members who participated in the study.

Peripheral blood samples were collected in EDTA containing vacutainer sets (BD, Franklin Lakes, NJ, USA). Genomic DNA was extracted using GenEluteTM Blood Genomic DNA Kit (Sigma-Aldrich, MO, USA). Extracted DNA was quantified using a NanoDrop-1000 spectrophotometer (Thermal Scientific, Wilmington, MA, USA).

Genotyping and Sanger Sequencing

To test linkage of the families to three previously reported causative genes (*WNT10B*, *ZAK*, and *EPS15L1*), genotyping using highly polymorphic microsatellite markers was performed as reported previously [Aziz et al., 2014]. After failing to establish linkage of the family A to the tested markers, three other genes (*DLX5*, *DLX6*, and *TP63*) involved in causing dominantly inherited SHFM, were sequenced in affected members.

Whole-Exome Sequencing

DNA of affected member in family A was subjected to WES (Fig. 1a) using a NovaSeq 6000 system (Illumina, San Diego, CA, USA) as described previously [Umair et al., 2017; Falb et al., 2023; Umair, 2023]. WES was performed at institute for medical genetics and applied genomics, University of Tubingen, Germany. Enrichment of coding sequences was carried out using SureSelect XT Human All Exon kit version 7 (Agilent Technologies, CA, USA). Considering an autosomal recessive inheritance of the disorder in the family A (Fig. 1a), we queried the database to search for rare homozygous variants in the affected individual.

Bioinformatics Analysis and Sanger Validation

Frequency of the selected variants was cross-checked with >15,000 exome and genome in-house datasets including 160 exomes of Pakistani origin, Exome Variant Server (EVS), Exome Aggregation Consortium (ExAC), gnomAD, 1000 Genomes, and dbSNP. Segregation of the selected sequence variants with the disorder was validated by bidirectional Sanger sequencing performed on DNA extracted from blood of additional family members. Primers, for PCR amplification of the variants, were designed using Exon Primer (http://ihg.gsf.de) (genomic build number: GRCh38/hg38) (WNT10B: Gene ID: 7480; Transcript ID: NM_003394.4).

Results

Clinical Description of Families

Family A: This family originated from Sindh province of the country. The family pedigree drawing presented autosomal recessive pattern inheritance of the disorder. Two affected members (II-5 and III-2) and three unaffected members (II-9, II-10, and III-1) participated in the research study (Fig. 1a). Clinical investigation showed a severe form of SHFM anomaly in the affected members. The affected individual (III-2) had mild cleft hand abnormality, while severe cleft of central type was observed in the feet. The dorsal view of hand revealed well-developed nails, missing digits, and complex syndactyly of 3rd and 4th digit. The palmer view of the hand showed mild cleft in both hands and complex pre-axil syndactyly in the right hand thumb (Fig. 1b–d). The same affected individual had broad feet with severe bilateral SHFM. In the feet, 2nd and 3rd digits were missing. Nails were normal in the remaining digits. Other bones in the feet such as cuneiform, cuboid, navicular, talus, and calcaneus were unaffected (Fig. 1b–d).

X-rays of the affected individual (II-5) revealed complex unilateral SHFM and complex pre-axial syndactyly in the right hand. The 3rd–4th digits were fused in a complex fashion, exhibiting syndactyly (Fig. 1e). The right foot showed missing 3rd digit and complex syndactyly of the 3rd and 4th tarsal. The left foot showed severe SHFM with missing 2nd and 3rd digits.

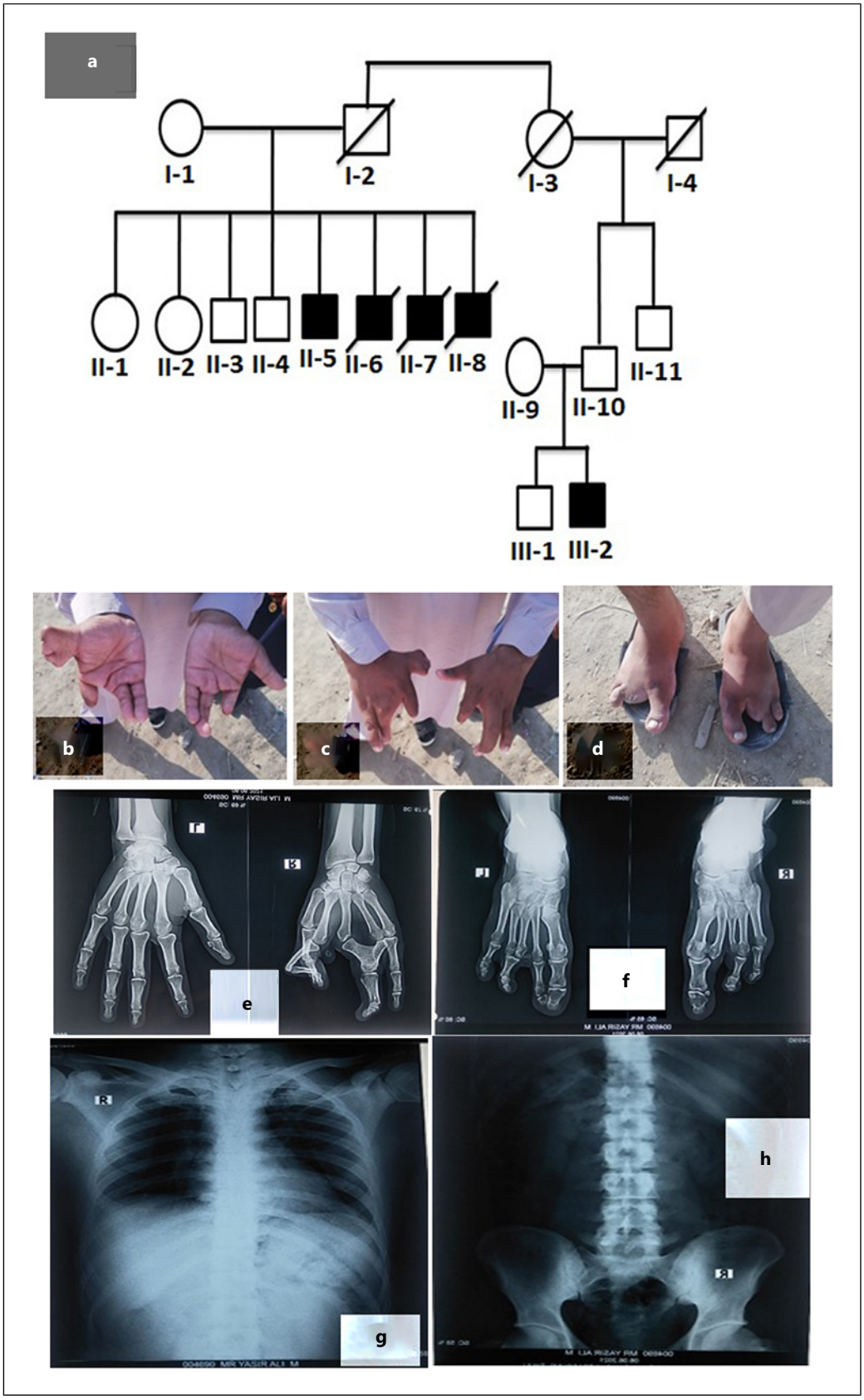

Fig. 1. a Pedigree drawing of family A segregating SHFM in autosomal recessive manner. **b** Hands of affected individual (III-2) in palmer view. **c** Hands of affected individual (III-2) in dorsal view. **d** Feet of affected individual III-2. **e, f** X-ray of hands and feet of affected member II-5. **g, h** X-rays of the chest and spine of affected member II-5.

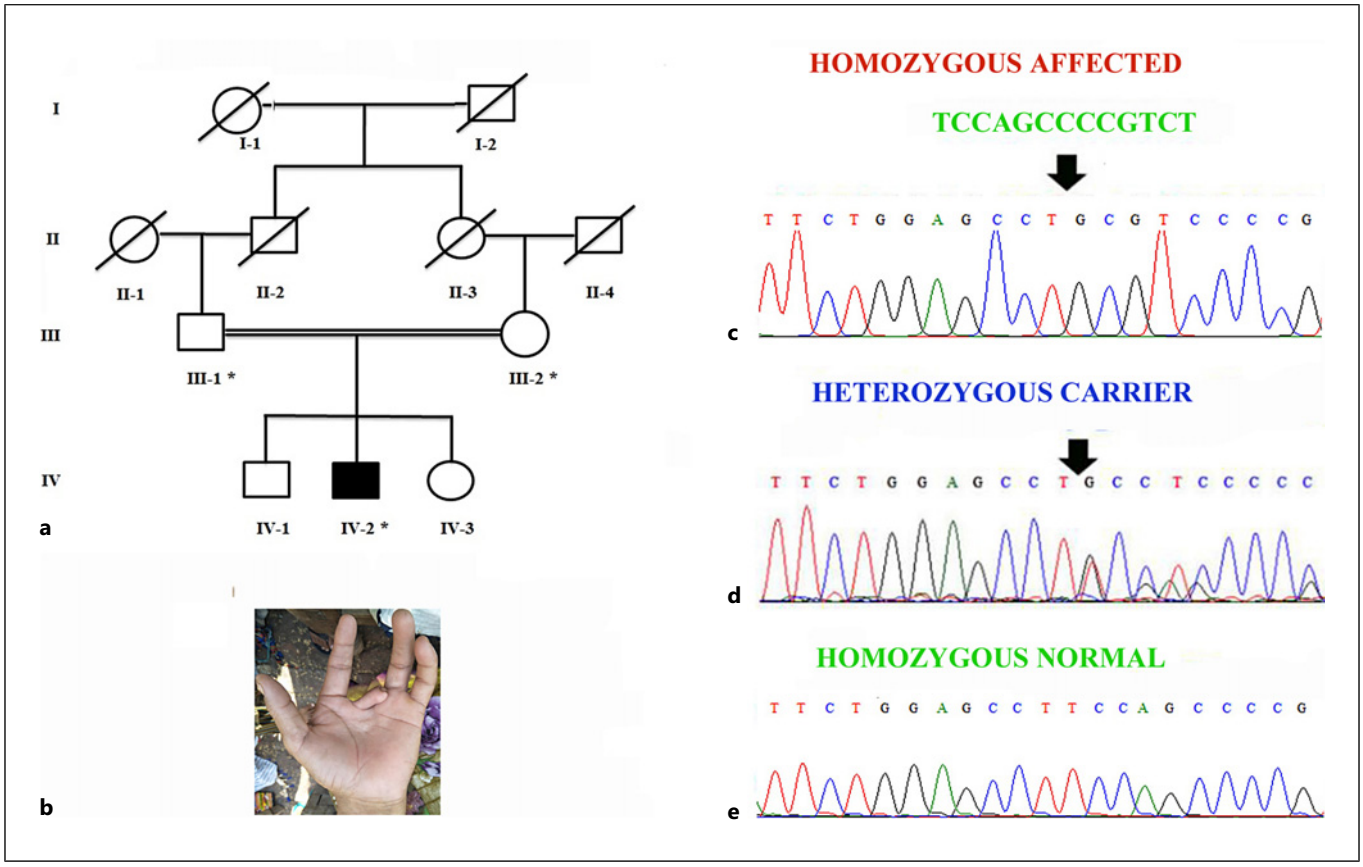

Fig. 2. a Pedigree drawing of family B segregating SHFM in autosomal recessive manner. **b** Hands of an affected individual (IV-2). **c–e** Chromatograms showing nucleotide sequence of the *WNT10B* in affected, carrier, and normal individual.

Complex fusion of the 2nd tarsal with the first tarsal was observed. Similarly, the 3rd tarsal was fused with the 4th tarsal in a highly complex fashion. Furthermore, the right foot had syndactyly of the 1st and 2nd digits (Fig. 1f). He had normal chest, spine, and lower long bones (Fig. 1g, h).

Family B: This family was recruited from a highly poor area in the Sindh province of the country. It had only one affected member (IV-2, Fig. 2a). Clinical examination, at local government hospital, showed a mild form of SHFM in the affected member. History of the patient disclosed that he was born of a normal pregnancy, and the limb abnormality was apparent at birth. He had a cleft left hand and defective middle finger. Phalanges of the middle fingers were also missing in the left hand. Other fingers, including thumb were normal. Limb abnormality was not found in the right hand. The feet of the patient were also normal. Facial deformity, heart issue, cleft palate, and teeth problems were not found in the family (Fig. 2b).

Molecular Analysis

Family A: Exome sequencing was used to search for the candidate gene in the family. Candidate variant prioritization was based on a presumed autosomal recessive model of inheritance. Initially, 229 homozygous variants were selected after filtering steps which included MAF <0.01% in ExAC, gnomAD, and CADD. Functional characterization of the screened variants led to the selection of a variant in the previously reported SHFM causative gene *WNT10B*. Sanger sequencing, using DNA of rest of the affected and unaffected members, validated segregation of a novel missense variant [c.338G>C; p.(Gly113Ala)] (NM_003394.4) in the *WNT10B* gene with the disorder within the family (Fig. 3a–c). To exclude polymorphic nature of the identified variant, it was cross-checked in 160 in-house exomes. HomoloGene revealed the amino acid Gly113 highly conserved in the WNT10B orthologs (Fig. 3d). WNT10B variant showed uncertain significance having conservation score (phylop100 = 7.844). Variant was pathogenic moderate (MetaRNN = 0.9152).

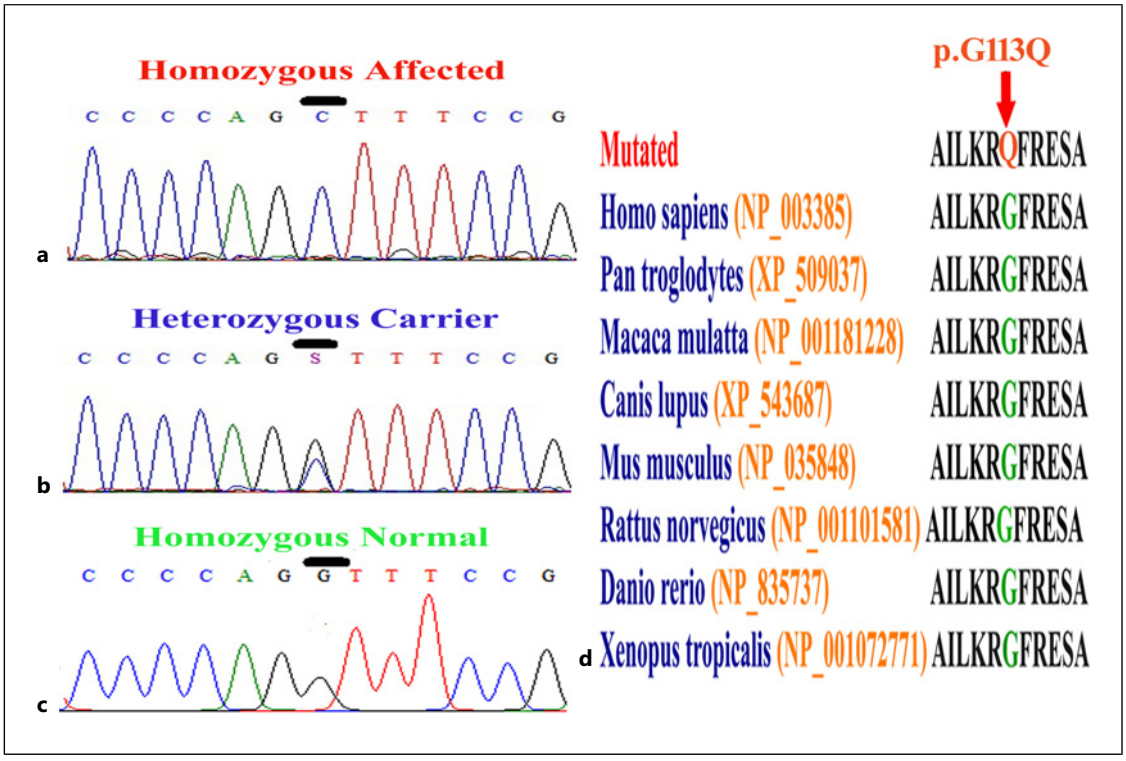

Fig. 3. Chromatograms of family A showing wild type nucleotide sequence of *WNT10B* in homozygous affected (**a**), heterozygous carrier (**b**), and homozygous normal member (**c**). **d** Conservation of a mutated amino acid glycine across different species.

Family B: Considering there was only a single affected family member, linkage in the family was tested to previously reported SHFM causative genes. This was carried out by genotyping microsatellite markers mapped in the region encompassing SHFM genes. Haplotype analysis of the genotypes revealed a homozygous pattern of *WNT10B*-linked alleles (SHFM6) in the affected and heterozygous in the unaffected members. Sequence analysis revealed a homozygous 13-bp deletion [c.884-896 delTCCAGCCCCGTCT, p.(Phe295Cysfs*87)] in exon 5 of the *WNT10B* gene (Fig. 2c–e). This variant was previously reported by Holder-Espinasse et al. [2019] in another family of Pakistani origin.

Discussion

SHFM in humans is both genetically and phenotypically heterogeneous disorders. Identification of novel genes implicated in the congenital SHFM is important to understand the development of limbs for patient's management and development of therapeutic strategies.

The present study is based on investigation of two families segregating SHFM in autosomal recessive pattern. In order to characterize SHFM, presented here, at least two affected members in family A and a single affected member in family B were clinically investigated by medical officers at local government hospitals. Clinical examination of affected members in both families presented most of the SHFM-related features which were previously reported in families of Pakistani and other origins of the world. This included syndactyly, polydactyly, cleft hand/foot malformation, hallux valgus deformities, aplasia, hypoplasia, radial ray malformation, hypoplastic finger, and missing phalanges [Umair et al., 2018; Aziz et al., 2014; Ullah et al., 2016; Kantaputra et al., 2018; Khan et al., 2012; Ugur and Tolun 2008]. However, oligodontia/dental anomalies, brachydactyly, ectrodactyly, fusion of thumb with index finger, cardiac defects, retrognathia, thin lips, low set ears, and deafness reported previously in few SHFM cases [Ullah et al., 2016; Kantaputra et al., 2018; Khan et al., 2012; Umair et al., 2019] were not found in the present families. Variants in the *WNT10B* have also been associated with obesity

Table 1. Clinical comparison of two families with SHFM

Features observed	Family A	Family B
Consanguineous pedigree	Yes	Yes
Mutation type	Missense	Deletion
Variant	c.338G>C	c.884-896del TCCAGCCCCGTCT
Protein change	p.Gly113Ala	p.Phe295Cysfs*87
Ethnicity	Pakistani	Pakistani
Split hands	Yes	Yes
Split feet	Yes	No
Missing pre-axial side	No	No
Fusion if thumb	No	No
Polydactyly	No	No
Syndactyly	Yes	No
Long bones of forearm affected	No	No
Obesity	No	No

Table 2. Mutations reported to date in *WNT10B* gene

Publication	*WNT10B* variants
Ugur and Tolun, 2008	Homozygous c.994C>T, p.(Arg332Trp)
Aziz et al., 2014	Homozygous c.1165_1168delAAGT
	Homozygous c.300_306dupAGGGCGG, p.(Leu103Argfs*53)
Khan et al., 2012	Homozygous c.986C>G, p.(Thr329Arg)
Blattner et al., 2010	Homozygous c.458_461dupAGCA, p.(Asp155Alafs*47)
Kantaputra et al., 2018	Homozygous c.695_697delACA, p.(Asn232del)
Monies et al., 2017	Homozygous c.338-1G>C
Meng et al., 2017	Presumably compound heterozygous c.[661C>T; ?;986C>A], p.(Arg221Trp);?;(Thr329Lys)
Ullah et al., 2018	Homozygous c.460C>G, p.(Gln154*)
Brunelle et al., 2019	Homozygous c.817delG
	Compound heterozygous c.[746G>T]; [817dupG]
	Homozygous c.949T>A, p.(Phe317Ile)
Bilal et al., 2020	Homozygous c.338G>A, p.Gly113Asp*
	Homozygous c.884-896delTCCAGCCCCGTCT, p.(Phe295Cysfs*87)

[Christodoulides et al., 2006]; however, all the patients reported in this study had normal BMI. Clinical comparison of both families has been presented in Table 1.

To search for the disease-causing genes, the DNA of one affected member in family A was subjected to exome sequencing. In family B, the search for the disease-causing gene was based on genotyping polymorphic microsatellite markers linked to candidate genes. In both families, segregation of the identified variants was analyzed by Sanger sequencing. Sequence analysis of *WNT10B* gene revealed a novel homozygous missense variant p.(Gly113Ala) in family A and a previously reported 13-bp frameshift deletion p.(Phe295Cysfs*87) in family B [Bilal et al., 2020].

The *WNT10B* gene, located on human chromosome 12q13.12, contains five exons, spanning 6.42 kb of genomic DNA, and encodes 389 amino acids protein (UniProtKB/Swiss-Prot accession 000744). To date, 14 mutations causing SHFM have been reported in the *WNT10B* gene (Table 2). The variant missense p.(Gly113Ala) is located in WNT domain of the WNT10B protein. It is highly likely that the variant disrupted protein binding to the receptor FZD8, leading to abnormal pathway. The variant p.(Phe295Cysfs*87), identified in family B, resulted in the frameshift, is expected to degrade the gene product through a nonsense-mediated mRNA decay.

Wnt ligands have a prime role that is important in bone homeostasis and development of skeleton. Expression of various WNTs plays a significant role in development of human and mouse bone [Zhou et al., 2008]. The WNT family consists of 19 different WNT genes. In the development and upkeep of several tissues as well as organs including bones, WNT proteins including WNT10B, WNT10A, and WNT6

play an important role [Cadigan and Nusse, 1997; Cawthorn et al., 2012]. These 19 genes encode different proteins that are involved in the activation of "canonical" signaling mechanism and specifically bind cell surface receptors and frizzled-related proteins [Peifer and Polakis, 2000].

Wnt10b and Wnt3a are involved in canonical Wnt signaling, which acts upstream of FGFs to regulate AER gene expression. During chick limb bud outgrowth, Wnt3a acts upstream of Fgf8. Wnt3a also plays role in AER formation through Wnt3a/βeta-catenin signaling. When different Wnt genes activate the same intracellular signaling pathways, they can substitute for one another. Patients with SHFM may have deformed digits as a result of aberrant Shh expression which is controlled by the combinative influence of Wnt/Fgf signaling [Yamaguchi et al., 1999; Barrow et al., 2003; Geetha-Loganathan et al., 2008].

In conclusion, our study identified a novel variant in the *WNT10B* gene, which is implicated in the pathogenesis of SHFM. Our findings highlighted the importance of genetic mutations in the *WNT10B* gene as a potential causative factor for SHFM, expanding our understanding of the molecular mechanisms underlying this rare congenital limb anomaly. The discovery of this novel variant contributes to the growing body of knowledge on the genetic basis of SHFM and may have implications for diagnosis, genetic counseling, and potential therapeutic interventions in affected individuals. Although in the present report we have presented two families, screening additional families with SHFM will facilitate genotype/phenotype relation of the disorder.

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Statement of Ethics

The study was conducted in accordance with the Declaration of Helsinki protocols, approved by the Institutional Review Board (IRB) (IRB-QA-176) of Quaid-i-Azam University, Islamabad, Pakistan. Both affected and unaffected members in the two families provided their written informed consent for publishing their photographs and radiographs.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

No funding was received.

Author Contributions

Muhammad Bilal performed the laboratory experiments, analyzed the genomic data, and drafted the manuscript. Muhammad Bilal, Tobias Haack, Rebecca Buchert, Susana Peralta, Imtiaz Ahmad, Sanaullah Abbasi, and Faisal collected samples and analyzed clinical and genomic data. Wasim Ahmad designed the project, edited the manuscript, and provided funds for the study.

Data Availability Statement

Any additional data required are available on request.

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