Cytogenetic analysis of a familial case of split-hand /foot malformation (SHFM)
Introduction

Split-hand/split-foot malformation (SHFM), also known as ectrodactyly or “lobster claw hand,” is a limb malformation involving the central rays of the autopod and presenting with syndactyly, median clefts of the hands and feet and aplasia/or hypoplasia of the phalanges, metacarpals and metatarsals (Jindal et al., 2009). Its global incidence has been reported to be about 1 in 90,000 babies with no sex predeliction. It can occur as non-syndromic or the syndromic form, with associated anomalies such as tibial aplasia, mental retardation, ectodermal and craniofacial findings, orofacial clefting and deafness (Pinette et al., 2006). The split-hand /foot malformation (SHFM) is a complex disorder with much heterogeneity in phenotypes affecting both hands and feet to various degrees and accounts for 8–17% of all limb reduction defects (de Mollerat XJ, 2003). In most cases it runs through generations into families and despite the great range of variability within families, predominantly it shows an autosomal dominant pattern of inheritance with high penetrance.

Gene responsible for SHFM3 is not yet revealed. In 20% of the cases microduplication of the SHFM3 critical region (10q24) is reported (Anna Sowińska-Seidler et al., 2014; Li Dai et al., 2013; Dimitrov et al., 2010).

Six loci for non-syndromic SHFM have been reported: 7q21-q22 (SHFM1), Xq26 (SHFM2), 10q24-q25 (SHFM3), 3q27 (SHFM4), 2q31 (SHFM5), and 12q13.11 (SHFM6). At the gene level, mutations in TP63 and WNT10B can lead to SHFM4 and SHFM6, respectively. A point mutation of DLX5 gene was reported to be responsible for autosomal recessive SHFM (Shamseldin et al., 2012). However, genetic causes for SHFM2 and SHFM5 are still unknown.

The SHFM3 had been mapped to chromosome 10q24 by linkage analysis (Ianakiev et al., 1999; Sidow et al., 1999; de Mollerat XJ et al., 2003; Khan et al., 2012). Further fine mapping narrowed down to a 0.5-Mb region. Genomic microduplications in 10q24 involving LBX1, BTRC and FBXW4 were observed into the region for SHFM3. Many studies tried to identify mutation in candidate genes within or flanking duplicated regions but no intragenic mutations were detected.
Materials and Methods

Clinical Presentation

A 5-generation family with split hand/foot malformation involving monodactyly of hands (type-II SHFM) and adactyly of legs in most of the affected members have been recruited for the study (Fig. 9.1A). One of the females (IV-8) and her two affected sons (V12, 13) have typical crab-claw like ectrodactyly (type-I SHFM) of hand and foot. Members also have malformed phalanges, metacarpals, metatarsals and short arm. The disease in the family has passed through at least 4 generations, suggesting an autosomal dominant pattern with full penetrance and much phenotypic variability. Apparently, severity of the phenotype seemed to decline through generations III and IV. Details of the phenotype and pictures of some of the affected members are given in table-1 and Fig. 9.1B.

Fig. 9.1: A. Family pedigree showing autosomal dominant pattern of inheritance B. Clinical photographs of the SHFM patient
Cytogenetic analysis

For cytogenetic analysis short term whole blood culture was performed in RPMI 1640 medium supplemented with 10% fetal bovine serum. G-banding of chromosome was performed by Saline–Trypsin–Giemsa (STG) method. Metaphases with 450 G-band resolution were observed under microscope (Carl Zeiss Microscopy GmbH, Göttingen, Germany) and karyotyping was done with the help of Ikaros karyotyping system—Metasystems software (Carl Zeiss Microscopy GmbH, Göttingen, Germany).

Genome-wide linkage mapping

Genome-wide linkage scan followed by microsatellite analysis of the candidate region was performed previously in our laboratory (Ali et al., 2012).

Whole genome sequencing

Whole genome sequencing was performed in three affected and one unaffected member of the family. Library preparation was done using TruSeq DNA Sample Preparation Kit (Illumina) and sequencing was carried out using Illumina HiSeq 2500 platform with 10X depth CASAVA software v1.8.2 (Illumina) was used for base calling while Human reference Sequence v19 (NCBI) database was used for variant calling using Avadis NGS v.1.5.1 (Strand scientific intelligence, Inc).

Cytogenetic microarray analysis

Cytogenetic microarray experiment was performed in affected member of the family using 2.7 M cytogenetics array (Affymetrix, Inc. Santa Clara, CA, USA) according to manufacturers’ protocol. Chromosome Analysis Suite (ChAS) software (Affymetrix, Inc. Santa Clara, CA, USA) was used to analyze the data.

Results and Discussion

Cytogenetic analysis did not reveal any chromosomal anomaly as karyotyping was normal (450 G-band resolution). Genome-wide linkage scan mapped a 10 cM locus at 10q25.1-q25.3 (within SHFM3 locus) in the family. Whole genome sequencing by NGS in revealed a gain of 4 Mb region on 10q24.31 in all affected members. This region contains 4 genes \textit{LBX1}, \textit{BTRC}, \textit{POLL} and \textit{FBXW4} (Fig. 9.2).
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Fig. 9.2: A. NGS data analysis showing unaffected member of the family with normal copy. B,C,D. Gain of 4Mb region on 10q24.31 in 3 affected members.

The gain of 4 Mb region was further confirmed by cytogenetic microarray analysis (Fig. 9.3).

Fig. 9.3: Cytogenetic microarray showing gain of 4 Mb region on 10q24.31
The overlapping regions of this chromosomal rearrangement was also reported earlier in SHFM3 (de Mollerat et al. 2003; Dimitrov et al. 2010). This chromosomal imbalance was analysed by Decipher database on human genome browser and found that similar imbalances were reported in database with similar phenotype (Fig. 9.4).

**Fig. 9.4:** Analysis of chromosomal imbalance using Decipher database on UCSC Human browser view

The mechanism by which the duplication of the SHFM3 critical region gives rise to the limb defect remains unknown. Any of the gene or the combination of gene might be serve for clinical phenotype in affected members in the family. *LBX1* is a homeobox gene and plays an important function in developmental processes (Jagla et al., 1995). However, this gene is mainly expressed in the central nervous system and its expression in the limb bud is restricted to the early myogenic cells, likely ruling out this gene as responsible for SHFM3 (de Mollerat XJ et al., 2003). BTRC functions as an ubiquitination factor of proteins involved in several signaling transduction pathways such as Wnt/β-catenin, SHH, and NF-κB (Maniatis 1999). All these pathways are involved in limb development, therefore dysregulation of BTRC expression in a dosage dependent manner may be responsible for the SHFM3 phenotype (de Mollerat et al. 2003). Poll encodes DNA polymerases, catalyze DNA-template-directed extension of the 3’-end of a DNA strand. This particular polymerase, which is a member of the X family of DNA polymerases, likely plays a role in non-homologous end joining and other DNA repair processes. Poll expression...
was not observed in limb bud RNA, given that there is no previous correlation with limb development. *FBXW4* gene is a member of the F-box/WD-40 gene family and in mouse, a highly similar protein is thought to be responsible for maintaining the apical ectodermal ridge of developing limb buds. Disruption of the mouse gene results in phenotype remarkably similar to split hand-split foot malformation in humans, a clinically heterogeneous condition with a variety of modes of transmission (Chryst-Ladd et al., 2008).

By analysis the above information *BTRC* and *FBXW4* were seems to be a strong candidate for SHFM3 in the family while ruling out the possibilities of *Poll* and *LBX1*. Although role of these gene cannot be ignored fully in phenotype manifestation.