Split Hand/Foot Malformation Associated with 7q21.3 Microdeletion: A Case Report

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Established Facts

- A girl diagnosed with split hand/foot malformation (SHFM) type I showed a microdeletion within the sub-band 7q21.3 using FISH with BAC probes and array CGH.
- This case confirms the involvement of the *DLX5*, *DLX6*, *DSS1*, *SLC25A13*, and *DYNC111* genes localized to this sub-band and reported to be responsible for the phenotypic features.

Novel Insights

- The proband also exhibited a larger deletion involving the bands 7q21.11q21.2 confirmed by microarray analysis.
- The significance of genes encoding the class-3 semaphorins SEMA3A, SEMA3D and SEMA3E, and PlexinA2 needs to be explored further.

Key Words

BAC-FISH · Chromosomal microarray · Developmental delay · Ectrodactyly · High-resolution banding · SHFM1

Abstract

Split hand/foot malformation (SHFM) or ectrodactyly is a rare genetic condition affecting limb development. SHFM shows clinical and genetic heterogeneity. It can present as an iso-

lated form or in combination with additional anomalies affecting the long bones (nonsyndromic form) or other organ systems including the craniofacial, genitourinary and ectodermal structures (syndromic ectrodactyly). This study reports a girl with SHFM who also exhibited developmental delay, mild dysmorphic facial features and sensorineural hearing loss. High-resolution banding analysis indicated an interstitial deletion within the 7q21 band. FISH using locus-specific BAC probes confirmed the microdeletion of 7q21.3.

Chromosomal microarray analysis also revealed a microdeletion of 1.856 Mb in 7q21.3. However, a larger 8.44-Mb deletion involving bands 7q21.11q21.2 was observed, and the breakpoints were refined. The phenotype and the candidate genes underlying the pathogenesis of this disorder are discussed.

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Split hand/foot malformation (SHFM) or ectrodactyly (or lobster claw deformity) is a rare congenital limb deformity. It is characterized by the absence of central digital rays with syndactyly of the remaining digits, deep median cleft, and aplasia or hypoplasia of the phalanges, metacarpals and metatarsals [Scherer et al., 1994b]. The overall prevalence of SHFM is ~1/8,500–25,000 newborns accounting for 8–17% of all limb reduction defects [Gurrieri and Everman, 2013].

The most common mode of inheritance is the autosomal dominant form, with autosomal recessive and X-linked forms occurring more rarely. Segregation distortion characterized by excessive transmission from affected males to sons has also been observed [de Mollerat et al., 2003]. SHFM shows variability in penetrance and expression of the phenotype. Highly variable phenotypes have been observed within affected members of the same family as well as between limbs of a single patient, ranging from mild syndactyly to severe central clefting of the autopods, oligodactyly or monodactyly [Dujif et al., 2003]. Around 40% of individuals presenting with SHFM have associated non-limb congenital anomalies that include intellectual disability (ID), cleft palate and ectodermal dysplasia. The disorder is genetically heterogeneous involving several loci including 7q21.3, Xq26, 10q24, 3q27, 2q31, and 12q13 [Gurrieri and Everman, 2013; Sowińska-Seidler et al., 2014].

SHFM type I (SHFM1) has been known to occur in an isolated form or with additional anomalies affecting the long bones, referred to as the nonsyndromic form or as part of the ectrodactyly-ectodermal dysplasia-cleft syndrome [Crackower et al., 1996]. Both forms were frequently found to be associated with chromosomal rearrangements such as deletions or translocations involving 7q21q22. Besides the ectrodactyly-ectodermal dysplasia-cleft syndrome, many other syndromes including SHFM have been described [Gurrieri and Everman, 2013]. This study reports a syndromic form of SHFM1 in a patient associated with a microdeletion of the sub-band 7q21.3, which was confirmed by FISH using BAC clones and array CGH.

Patient and Methods

Patient

The proband is an 8-month-old, first-born female child to third-degree consanguineous parents (first cousins). It was a normal full-term delivery, and she weighed 2.5 kg at birth. She was referred to the Department of Genetics, Dr. ALM PGIBMS, at the age of 6 years. The proband exhibited ectrodactyly of the right hand and both feet (fig. 1a, b). Physical examination also revealed facial dysmorphism including a flat occiput, microcephaly, corneal opacity of the left eye and low-set ears. She underwent surgical treatment for patent ductus arteriosus. Follow-up assessment after 2 years revealed developmental delay and bilateral sensorineural hearing loss (fig. 1c, d).

Cytogenetic Analysis

After having obtained written informed consent from the proband's father, heparinized blood was drawn from the proband and her parents. Elongated metaphase chromosomes were obtained from phytohemagglutinin-stimulated lymphocyte cultures by adding ethidium bromide for 1 h prior to harvest. Twenty-five metaphases were analyzed from each individual. About 5 well-banded metaphases were documented and karyotyped using Applied Spectral Imaging Systems karyotyping software, BandView version 6.0 (ASI Inc., Carlsbad, Calif., USA). Chromosomal anomalies were designated using standard ISCN nomenclature [ISCN, 2013].

FISH Analysis

FISH was performed on metaphase chromosome spreads prepared from lymphoblastoid cells using probes of DNA isolated from 5 BAC clones spanning the sub-band 7q21.3 and localized to the 7q21.2q22.1 region following manufacturer's protocol. These clones were selected from the human DNA RP11 library in the UCSC genome browser and provided by BACPAC Resources (<http://bacpac.chori.org/home.htm>). The BAC probes were each ~180–200 kb in size and included RP11-1080O8, RP11-737I16 and RP11-991E7 localized to 7q21.3, RP11-998E13 (7q21.2) and RP11-794O22 (7q22.1). DNA extracted from these BAC clones were labeled with digoxigenin 11-dUTP by nick translation according to standard protocols. FISH probes were hybridized to the slides and detected using anti-DIG antibody tagged with rhodamine. The commercially available probe specific for the centromeric region of chromosome 7 was also used for reference.

Whole-Genome Array-CGH Analysis

Genomic DNA was isolated from lymphocytes using the phenol-chloroform-isoamyl alcohol method. Whole-genome array-CGH analysis was performed using Affymetrix CytoScan® 750K microarray (Affymetrix Inc., Santa Clara, Calif., USA) consisting of 750,000 oligonucleotide probes across the genome (including 550K unique non-polymorphic probes and 200K SNP probes) following manufacturer's instructions at Innovative Life Discoveries Pvt. Ltd., Gurgaon, Harayana, India. Approximately 250 ng of DNA was digested with Nsp1 and then ligated by Nsp1 adapter. Titanium Taq amplified PCR products were purified using AMP pure beads. Purified DNA was fragmented to the product size of 25–125 bp, biotin labeled, hybridized on CytoScan 750K array, and then scanned for cell file generation. Detailed analysis was done using Affymetrix Chromosome Analysis Suite (ChAS) software ver. 1.1. Localization of gains and losses in copy number was done using the UCSC Genome Browser Assembly.

Fig. 1. Both feet (a) and the right hand (b) of the proband show ectrodactyly. Photographs of the proband at about 2½ (c), 4½ (d) and 6 years of age (e). Photograph of the girl's face at her present age of 6 years (f).

Results

Chromosomal analysis at 650-band resolution showed an interstitial deletion within the band 7q21 of the proband (fig. 2a). Both parents showed a normal karyotype (fig. 2b, c). FISH analysis using the BAC clone RP11-737I16 (95,059,137–95,262,009) together with a probe for the centromeric region of chromosome 7 (control) disclosed a microdeletion within the sub-band 7q21.3 (fig. 3e). Two signals were viewed for the remaining clones, RP11-1080O8 and RP11-991E7 mapped to 7q21.3, RP11-998E13 to 7q21.2 and RP11-794O22 to 7q22.1 (fig. 3a–d). Chromosomal microarray (CMA) analysis using Affymetrix CytoScan 750K microarray chip further confirmed and revealed a microdeletion of 1.856 Mb within 7q21.3 (arr[hg19] 7q21.3(94,883,839–96,739,875)×1) besides a larger deletion encompassing 8.44 Mb at 7q21.11q21.2 (arr[hg19] 7q21.11q21.2(83,236,518–91,680,906)×1) (fig. 4a). Based

on these results, the karyotype was rewritten as 46,XX, del(7)(q21.11q21.2).ish del(7)(q21.3q21.3)(RP11-737I16–). The CMA analysis also revealed a 35-Mb long region of homozygosity on chromosome 18 (arr[hg19] 18q12.1q22.1(29,156,998–64,771,932)×2 hmz) (fig. 4b).

Discussion

Chromosomal rearrangements of the 7q21.3q22.1 region are associated with SHFM1 and occur mostly as a sporadic event. Besides limb anomalies, this region is associated with a wide spectrum of phenotypes, including growth impairment, microcephaly, craniofacial manifestations, hearing loss, and ID that differ in affected individuals (table 1). This critical region has also been implicated in ectrodactyly-ectodermal dysplasia-cleft lip/palate syndrome. Although the proband had hearing loss

Fig. 2. GTG-banded karyogram showing an interstitial deletion within the band 7q21 (arrow) in the proband (**a**) and normal patterns in her parents (**b, c**).

with ectrodactyly, she did not exhibit a cleft palate. This is indicative of variable expression and is consistent with existing literature.

It is interesting to note that ID is more often associated with deletions than translocations. Initially, 3 genes present in the 7q21.3q22.1 region, *DLX5*, *DLX6* and *DSS1*, were thought to play a role in limb development. The *DSS1* gene (deleted in SHFM1) was found to be expressed in regions of rapid cell growth (limb bud, branchial arches, genital bud, and skin) and excluded from regions of cell differentiation [Crackower et al., 1996]. Though the *DSS1* gene is involved in many biological and cellular processes including development, differentiation, cellu-

lar proliferation, neoplastic transformation, and ubiquitin dependent proteolysis, an involvement of this gene in the manifestation of this phenotype was unlikely as *Dss1* expression was normal in the combined homozygous *Dlx5*^{-/-} mice exhibiting a SHFM1 phenotype [Merlo et al., 2002; Dujif et al., 2003].

Molecular characterization of the genomic rearrangements involving the 7q21.3 sub-band led to the determination of a probable role of *DLX5* and *DLX6* genes, the *Drosophila* distal-less-related homeobox genes, in limb development. Both were expressed in the apical ectodermal ridge of the developing limbs, and a disruption in this ridge signaling was suggested to be a major cause of

Fig. 3. FISH analysis with the BAC clone RP11-737I16 (7q21.3) (red) and control probe for chromosome 7 centromeric region (green) confirms the microdeletion (arrow) (e). The other BAC clones (red) RP11-1080O8 and RP11-991E7 (7q21.3), RP11-998E13 (7q21.2) and RP11-794O22 (7q22.1) showed a normal hybridization pattern (a–d).

SHFM [Dujif et al., 2003]. Knockout studies in mice showed that an SHFM-like phenotype was observed only when both *Dlx5* and *Dlx6* were deleted [Crackower et al., 1996]. This indicated that the 2 genes participate co-operatively in a common developmental pathway [Kowenhoven et al., 2010]. Moreover, they were also expressed in the craniofacial prominence, the otic vesicle and in the brain, which correlates well with the sensorineural hearing loss and ID that are each present in about 30% of the SHFM1 patients [Tayebi et al., 2014]. These features were

also observed in the proband. However, recently mutations in only the *DLX5* gene have been reported in affected individuals. Shamseldin et al. [2012] reported a novel intragenic missense mutation in a highly conserved residue of the homeobox domain of *DLX5*. Likewise, a novel heterozygous mutation, c.558G>T (p.Gln186His) in *DLX5* was reported in both mother and son affected with SHFM1 [Wang et al., 2014].

A SHFM1 phenotype was recently reported in 2 affected members of the same family carrying a 106-kb de-

Fig. 4. Array CGH. A 1.856-Mb microdeletion of 7q21.3 and an 8.44-Mb deletion at 7q21.11q21.2 (**a**). A 35-Mb long region of homozygosity at 18q12.1q22.1 (**b**).

letion that did not disrupt the *DLX5/6* pair. It is the smallest deletion identified so far within the SHFM1 region. The deletion was found to encompass exons 11–18 of the *SLC25A13* (solute carrier family 25 member 13) gene and exons 14–17 of the *DYNC1I1* (dynein cytoplasmic 1 intermediate chain 1) gene [Lango Allen et al., 2014]. Multi-tissue enhancers have been reported earlier to reside in exons 15 and 17 of the *DYNC1I1* gene and intron 14 of the *SLC25A13* gene with the latter playing a role in the otic vesicle [Birnbaum et al., 2012]. The phenotype can thus be attributed to true haploinsufficiency of *DLX5/6* genes or functional haploinsufficiency resulting from the disruption of *cis*-acting regulatory elements through physical separation from their target *DLX5/6* genes [van

Silfhout et al., 2009; Birnbaum et al., 2012; Lango Allen et al., 2014]. Tayebi et al. [2014] reported overlapping deletions of the exonic enhancers 15 and 17 of *DYNC1I1* in affected individuals from 3 families with an autosomal dominant nonsyndromic, bilateral SHFM, and deletion of the last 3 exons of the *SLC25A13* gene in addition in the fourth family with hearing loss. CMA analysis showed a 1.856-Mb microdeletion in 7q21.3 which encompassed all the 5 genes, *DLX5*, *DLX6*, *DSS1*, *SLC25A13*, and *DYNC1I1*, in the proband. The development of molecular diagnostic testing, including array CGH, greatly contributes to the determination of the genetic variation underlying this clinically heterogeneous congenital defect and consequently to genetic counseling, and also to

Table 1. Phenotype associated with chromosome rearrangements involving 7q21

Karyotype	Limb anomalies	Other anomalies	Reference
46,XX,del(7)(q11.2q22.1)	unilateral SH, bilateral SF	cleft palate, severe ID, microcephaly	Pfeiffer, 1984
46,XY,del(7)(q21.3;q22)	unilateral SH, bilateral SF	genito-urinary anomalies, developmental delay, failure to thrive, microcephaly, seizures	Rivera et al., 1991
46,XY,ins(7),del(7)(pter→p15.1::q11.2l→q11.23::p15.1→q21.2::q22.1→qter)	bilateral SHSF	hypertelorism with epicanthic folds, posterior rotation of the ears, mild speech delay, mildly dysmorphic, mildly delayed speech, IQ 84	Roberts et al., 1991
46,XY,t(5;9;7)(5pter→5q11.2::5q34→5qter;9pter→9q22.1::7q31.3→7q21.2::5q34→5q11.2::7q31.3→7qter;7pter→7q21.2::9q22.1→9qter)	bilateral SHSF	mildly dysmorphic, low-set ears	Sharland et al., 1991
46,XX,inv(7)(q22.1q36.3)	bilateral SHSF	light-colored, sparse hair, bilateral cleft lip and palate, fused incisors, bilateral accessory nipples	Akita et al., 1993
46,XX,t(7;9)(q21.3;p12)	syndactyly of right hand, bilateral SF	light-colored, sparse hair, high-arched palate, abnormal ears	Fukushima et al., 1993
46,XX,t(2;7)(q21.1;q22.1)	unilateral SH, normal feet	hearing loss	Genuardi et al., 1993
46,XY,ins(3;7)(q21;q34q22)	unilateral SH, bilateral SF	high-arched palate, bifid uvula	Naritomi et al., 1993
46,XX,t(7;12)(q22.1;q24.2)	bilateral SHSF	mild speech delay	Scherer et al., 1994a
46,XY,del(7)(q21.12q22.1)	normal hands, bilateral SF	ID, seizures, microcephaly, coloboma, short stature	Scherer et al., 1994a
46,XY,inv(1)(q2q3),t(4;7)(q2,q21.3), inv(11)(p15q23)	bilateral SHSF	submucous cleft palate, deafness, ID, microcephaly, abnormal ears	Scherer et al., 1994b
46,XYdel(7)(q21.12;q21.3)	bilateral SHSF	micrognathia, low-set ears	Scherer et al., 1994b
46,XY,inv(7)(p22q21.3)	bilateral SHSF	NA	Cobben et al., 1995
46,XY,inv(1)(q21q32),t(4;7)(q31.1;q21.3), inv(11)(p15.1q23)	bilateral SHSF	severe ID, microcephaly, triangular face, downward slanting palpebral fissures, small ears, prominent lower jaw with prognathism	Ignatius et al., 1996
46,XX,der(7)ins(22;7) (q13.3;q21.2q22.1)mat	unilateral SH, normal feet	positional plagiocephaly, microcephaly, low-set ears, micrognathia	Slavotinek et al., 1997
46,XX,t(6;7)(q23.3;q32.3), del(7)(q21.1q21.3)	normal hands and feet	severe mental retardation, short stature, microcephaly, deafness	Tzschach et al., 2007
46,XX,inv(7)(q22.1q31.2), t(7;8)(q21.3q22.1;q23.3q24.12)	bilateral SHSF	psychomotor delay, dysmorphic features, cleft palate, deafness, tetralogy of Fallot	Bernardini et al., 2008
46,XX,del(7)(q21.13q22.1)	bilateral SHSF	frontal bossing, micrognathia, small dysplastic ears, long philtrum, deafness	van Silfhout et al., 2009

Table 1 (continued)

Karyotype	Limb anomalies	Other anomalies	Reference
46,XX,del(7)(q21.11q21.3)	syndactyly of right foot	low-set ears, upslanting and short palpebral fissures, small mouth and retrognathia, sensorineural hearing loss	van Silfhout et al., 2009
46,XY,inv(7)(p22q21.3)	bilateral SHSF	NA	van Silfhout et al., 2009
46,XY,t(7;13)(q21;q32.1)	SHFM1	NA	Obenauf et al., 2010
t(2;7)(p25.1;q21.3)	unilateral SHSF, syndactyly of the left foot	NA	Lango Allen et al., 2014

NA = Not available; SF = split foot; SH = split hand; SHSF = split hand, split foot.

the discovery of novel genes in cases of uncertain origin [Sowińska-Seidler et al., 2014].

The proband was also found to exhibit a larger deletion involving the bands 7q21.11q21.2, which localizes genes encoding the class-3 semaphorins SEMA3A, SEMA3D and SEMA3E. These secreted proteins have been known to be involved in neuronal functions such as axon attraction and repulsion, apoptosis, cell migration, and growth cone collapse. Sema3s require binding to transmembrane receptors that comprise of heteromeric complexes of neuropilins, plexins and cell adhesion molecules [Sharma et al., 2012]. Plexins have established roles in regulating Rho-family GTPases. Some of the cell adhesion molecules and genes regulating Rho signaling have been implicated in various forms of ID [Govek et al., 2005]. It is probable that haploinsufficiency of the semaphorin genes may be responsible for the reduced cognitive function observed in the proband. SEMA3E has been proved to play an important role in ensuring the specificity of synapse formation [Ding et al., 2011]. Semaphorin signaling also plays a critical role in neural crest-mediated heart development through its interaction with PlexinA2 [Brown et al., 2001] and may explain the cardiac defect seen in our proband. Congenital heart defects are reported in 10% of SHFM1 patients [Elliot and Evans, 2008].

Additionally, CMA analysis revealed a 35-Mb region of homozygosity at 18q12.1q22.1 in the proband which could be attributed to the consanguinity of her parents. This region also contains the genes *PIK3C3*, *RIT2* and *SYT4* that have been associated with individuals affected with ID [Cody et al., 2007; Buysse et al., 2008; Vulto-van

Silfhout et al., 2013]. The genes *RNF165* and *EPG5* have been related to neuronal damage with the latter being linked to the multisystem disorder Vici syndrome [Kelly et al., 2013; Zhao et al., 2013]. The involvement of SMAD2 and ST8SIA5 proteins in neural induction and differentiation also makes them ideal candidates for the phenotype of the proband [Chang and Harland, 2007; Kwak et al., 2011]. Further, the *LOXHDI* gene present in the same locus is associated with nonsyndromic hearing loss [Dror and Avraham, 2010]. The involvement of these genes in the phenotype of the proband is uncertain and requires further analysis including parental testing to rule out uniparental disomy or identity by descent.

In conclusion, application of CMA analysis supported the precise delineation of the 7q breakpoint in our patient. This report on molecular characterization of the chromosomal abnormality in the SHFM patient will not only confirm the role of known candidate genes, but also will help in the identification of novel genes and, in turn, the pathogenetic mechanisms underlying the clinical heterogeneity associated with this disorder. This will also have important implications for risk assessment, management and genetic counseling.

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Statement of Ethics

This study was approved by the Institutional Ethics Committee. Written informed consent from the proband's father to participate in this study, including the publication of images, was obtained.

Disclosure Statement

The authors have no conflicts of interest to declare.

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