

## ELECTRONIC LETTER

## Refinement of the deletion in 7q21.3 associated with split hand/foot malformation type 1 and Mondini dysplasia

I Wieland, P Muschke, S Jakubiczka, M Volleth, B Freigang, P F Wieacker

*J Med Genet* 2004;41:e54 (<http://www.jmedgenet.com/cgi/content/full/41/5/e54>). doi: 10.1136/jmg.2003.010587

Split hand/foot malformation type I (SHFM1, OMIM \*183600) is an autosomal dominant developmental disorder of limb formation that results in the absence of the central digital rays, deep median clefts, and syndactyly of the remaining digits. Patients with SHFM1 harbour deletions, translocations, and inversions in chromosomal region 7q21–q22.<sup>1</sup> The deletions at 7q21–q22 encompass different genomic regions and probably result in a contiguous gene syndrome that includes growth impairment, microcephaly, craniofacial manifestations, hernias, hearing loss, and mental retardation.<sup>2–5</sup> Cases with translocations do not show this broad pattern of abnormalities but are associated with hearing loss in most cases.<sup>4–5</sup> Split hand/foot malformation type I is the only form of split hand/foot malformation associated with sensorineural hearing loss, and it has been designated SHFM1D (OMIM \*605617).<sup>5–6</sup> Recently, SHFM1D was shown to result from Mondini dysplasia in a boy with a *de novo* deletion of about 8.9–17 cM of the paternal chromosome 7q21.1–q21.3.<sup>7</sup> So far, microdeletions at 7q21.3 have been described in only two cases: one in a boy with split hand/foot malformation plus mild mental retardation, growth retardation of post-natal onset, and hypotonia and another in a patient with ectrodactyly, ectodermal dysplasia, and cleft lip/palate (EEC) syndrome.<sup>8,9</sup> Mapping of the deletion and translocation breakpoints in several patients showed a critical interval of about 1 Mb for the SHFM1 locus at 7q21.3.<sup>9</sup> This interval included a 500 kb region that spanned five of seven known translocation breakpoints. In this region, the candidate genes *DLX5* and *DLX6* (human homologues of the *Drosophila* *distal-less* homeobox gene family) and *DSS1* (deleted in the split hand/split foot SHFM1 region) were identified. No mutations were detected in patients with sporadic split hand/foot malformation with translocations or two families with split hand/foot malformation, sensorineural deafness, and normal chromosomes who showed linkage to 7q21.<sup>6,9,10</sup> Haploinsufficiency through interruption of the gene's regulatory elements was considered therefore to be the cause of SHFM1. Recently, an animal model of human SHFM1 has been produced in mice by targeted inactivation of *Dlx5* and *Dlx6* candidate genes.<sup>11,12</sup> In this mouse model, the *Dlx5/Dlx6*<sup>-/-</sup> genotype resulted in inner ear and severe limb malformations and craniofacial and axial skeletal defects.<sup>11</sup>

## CLINICAL SUMMARY

The index patient was the four year old son of a 28 year old mother and a 32 year old father who were healthy and not consanguineous. The child was born in the 42nd week of pregnancy. His weight at birth was 3630 g, length at birth 50 cm, and fronto-occipital circumference 36.0 cm. The patient was referred to our department at the age of four weeks because of split hand/split foot malformation. He had ectrodactyly of the left hand and both feet, with typical lobster claw (fig 1). The right hand had syndactyly of the third and fourth digits. Further dysmorphic features were

## Key points

- Split hand/foot malformation type I (SHFM1, OMIM \*183600) is characterised by missing digits, syndactyly, and deep median clefts in the hands and feet, and it may be associated with sensorineural hearing loss (SHFM1D, OMIM \*605617). The disease locus has been located to chromosome 7q21.3–q22 by the use of deletion mapping and linkage analysis.
- We report a boy with a *de novo* microdeletion of 0.9–1.8 Mb in the SHFM1 region, with classical symptoms of SHFM1 and deafness caused by Mondini dysplasia. Haplotype analysis with microsatellite markers showed loss of the paternal alleles D7821, D7S491, and D7624. The microdeletion included the candidate genes *DSS1*, *DLX5*, and *DLX6*, whereas two copies of the flanking *DNC11* gene were retained in the patient's genome. In all three cases of SHFM1 investigated by haplotype analysis, the paternal alleles were missing.

dysplastic ears and retrognathia. A hearing test showed profound deafness. Examination by magnetic resonance tomography showed a malformation of the inner ear typical for Mondini dysplasia, including enlargement of the vestibulum and a plump cochlea structure. Cochlea implantation was performed at the left side. To date, psychomotoric development seems normal.

## MATERIALS AND METHODS

## Microsatellite analysis

Fourteen microsatellite markers from chromosome 7q21.3–q22.1 retrieved from the NCBI STS map ([www.ncbi.nlm.nih.gov/](http://www.ncbi.nlm.nih.gov/)) were informative in the family. Primers for polymerase chain reaction (PCR) were synthesised commercially (MWG-Biotech, Ebersberg, Germany), and standard PCR analysis was performed.<sup>13</sup>

## Southern blot hybridisation

We cleaved high molecular weight DNA with restriction endonuclease *Hind III*. We resolved the digests on 0.8% agarose gel and blotted them on a nylon membrane (Hybond-N+, Amersham Biosciences, Little Chalfont, UK). We produced hybridisation probes *DNC11* (accession number NM\_004411), *DLX5* (accession number NM\_005221), and *DLX6* (accession number NM\_005222) by amplification of cDNA templates from placenta and fetal brain tissue (Clontech, Palo Alto, CA, USA) with PCR primers *DNC11* for 5'AGAAGAGAAGAAACGGAAGG3' and *DNC11*rev 5'CAGG AACACATTTGCCATC3' to give a 1188 bp PCR product, *DLX5* for 5'CGTCTCAGGAATCGCCAAC3' and *DLX5*rev

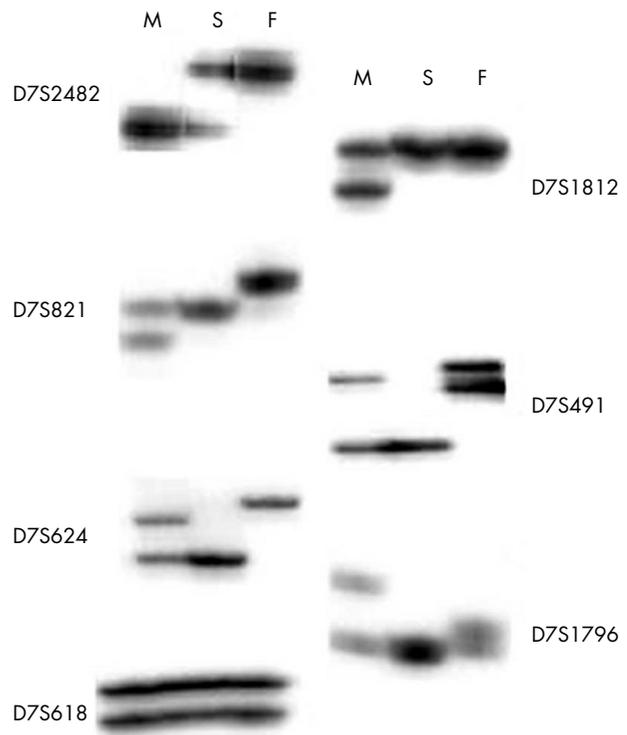
5'ACTGGTTGGAGGTCGGAGG3' to give a 651 bp PCR product, and DLX6 for 5'ACTCGCAGCACAGCCCTTAC3' and DLX6rev 5'CTGCATCGTGTCTGGTGT3' to give a 513 bp PCR product. Annealing temperatures were 55°C for DNC11 and 61°C for DLX5 and DLX6. We performed PCR amplifications with the Enhanced High Fidelity PCR System (Roche Diagnostics, Basel, Switzerland). We purified PCR products with a QIAEX II Gel Extraction Kit (Qiagen, Hilden, Germany) and radiolabelled them by random priming with alpha-<sup>32</sup>P dCTP. We carried out hybridisations, washings, and autoradiography according to standard protocols.<sup>14</sup>

## RESULTS

Chromosome analysis from the phytohemagglutinin stimulated lymphocytes of the patient showed a normal karyotype, apart from a heteromorphism in chromosome 9 (karyotype: 46,XY,9qh-). This heteromorphism was also detected in the mother (46,XX,9qh-). The karyotype of the father was inconspicuous (46,XY). In the patient, no deletion in chromosome 7 could be detected at a banding level of 400, and no abnormal comparative genomic hybridisation pattern was seen (data not shown), which suggests the possibility of a split-hand/foot malformation microdeletion in this patient. Haplotype analysis of the SHFM1 critical region at 7q21.3–q22.1 of the parents and their affected son showed unambiguous loss of the paternal markers D7S821, D7S491, and D7S624, whereas the flanking markers D7S2482 and D7S618 retained heterozygosity (table 1, fig 2). Additional markers within this interval were found to be uninformative. To delineate the deletion further, we performed quantitative Southern blot analysis of the genes *DLX5*, *DLX6*, and *DNC11* (dynein cytoplasmic intermediate polypeptide 1) within the interval. For the *DNC11* gene, signal intensity was the same in the affected boy, his parents, and a normal control person (fig 3). In contrast, for the *DLX5* and *DLX6* genes, signal intensities in the affected boy were reduced clearly to about half those of his parents and the control person (fig 3). These data suggest retention of both parental copies of the *DNC11* gene but loss of one copy of each of the *DLX5* and *DLX6* genes in the affected boy. From these results, the deletion breakpoints are located between the *DNC11* gene and marker D7S821 at the proximal border and between the *DLX5* gene and marker D7S618 at the distal border. This narrows the deletion to 0.9–1.8 Mb (table 1).

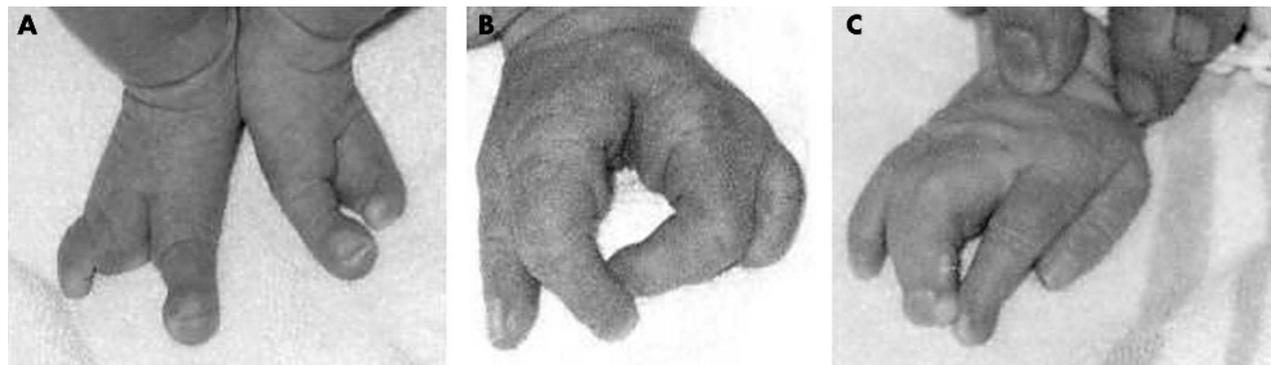
## DISCUSSION

We report the first patient with a microdeletion in 7q21.3 who shows SHFM1 with Mondini dysplasia. This microdeletion includes the SHFM1 candidate genes *DLX5*, *DLX6*, and *DSS1*. In mice, all three candidate genes apparently are

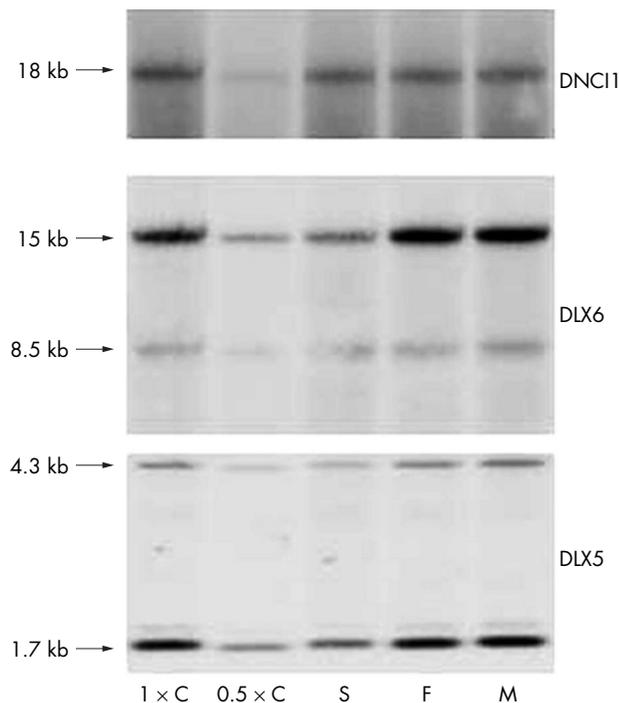


**Figure 2** Analysis of microsatellite markers from the critical SHFM1 region. Marker positions are listed in table 1. Genotypes of the mother (M), son (S), and father (F) for the indicated markers are shown.

involved in limb formation.<sup>9 11 12</sup> The *Dlx5* and *Dlx6* genes mostly are coexpressed in a unique spatial and temporal pattern. Knockout mice that solely lack the *Dlx5* gene show no apparent limb abnormalities.<sup>15</sup> Furthermore, the SHFM1 phenotype was apparent only in *Dlx5/6*<sup>-/-</sup> double knockout mice and could be rescued by transgenic overexpression of the *Dlx5* gene.<sup>11</sup> This suggests that the *Dlx5* and *Dlx6* genes functionally are redundant in controlling limb and craniofacial development in mice.<sup>11 16 17</sup> Interestingly, *Dlx5/Dlx6*<sup>-/-</sup> mice have disturbed development of the inner ear. From the mouse model, it seems possible that deletion of *DLX5* and *DLX6* also causes Mondini dysplasia in SHFM1D. The role of the *DSS1* gene in limb formation is less clear. In yeast, deletion of the *DSS1* homologue *SEM1* induces pseudohypheal growth under differentiating conditions.<sup>18</sup> *SEM1* and *DSS1*



**Figure 1** Ectrodactyly of both feet (A) and the left hand (B), with lobster claw malformation. The right hand (C) had syndactyly of the third and fourth fingers.



**Figure 3** Southern blot analysis of the *DNCI1*, *DLX6*, and *DLX5* genes with corresponding cDNA probes. Equal amounts of DNA from the son (S), father (F), mother (M), and a control person (1×C) and half the amount (0.5×C) of the control DNA, respectively, were separated by agarose gel electrophoresis. *Hind III* cleaved phage λ DNA was used to calculate the fragment sizes shown on the left.

functionally are conserved, as *DSS1* was able to restore the pseudohyphal phenotype in yeast. *SEMI* is a vesicle transport protein and seems to act as a negative regulator for exocyst function. The role of membrane traffic in mammalian development, however, is poorly understood.

**Table 1** Genotype analysis in the SHFM1 interval on 7q21.3–q22.1

Marker*	Marker's position on chromosome 7 (kbp)	Genotype		
		Mother	Son	Father
D7S2410	88 915	1–2	1–3	3–3
D7S657	91 342	1–2	1–2	1–2
D7S3050	92 238	1–2	1–2	1–1
D7S2482	93 658	2–2	1–2	1–1
D7S527	94 151	1–2	2–2	2–2
DNCI1†	~94 000	++	++	++
D7S1812	94 293	1–2	1–?	1–1
D7S821	94 593	2–3	2–del	1–1
D7S491	95 031	2–4	4–del	1–3
D7S624	95 062	2–3	3–del	1–1
D7S1796	95 088	1–3	3–?del	2–3
AFM284ZC1	95 089	1–2	2–?del	1–2
DLX6†	~95 200	++	+	++
DLX5†	~95 200	++	+	++
D7S618	95 854	1–2	1–2	1–2
D7S554	95 860	3–3	2–3	1–2
D7S651	97 083	1–2	2–3	2–3

del, deletion of the paternal allele.

\*Microsatellite markers used for haplotype analysis and their corresponding position on chromosome 7 in kbp are from NCBI maps ([www.ncbi.nlm.nih.gov/](http://www.ncbi.nlm.nih.gov/)).

† Approximate position of genes in kbp ([www.ncbi.nlm.nih.gov/](http://www.ncbi.nlm.nih.gov/)) and copy number for genes (++, 2 copies; +, 1 copy); the *DSS1* gene is located between markers D7S821 and D7S491.

In contrast to the situation in mice, contiguous loss of one allele of the SHFM1 candidate genes seems to be enough to cause the SHFM1 phenotype in humans. Most deletions in SHFM1 were detected cytogenetically, and only in two previously reported cases was haplotype analysis performed.<sup>3–7</sup> Surprisingly, in these two cases, as well as in the case presented here, the *de novo* deletions occurred on the paternal chromosome 7. This may indicate preferential loss of the paternal alleles in SHFM1. Recently, a new imprinted gene cluster on human chromosome 7q21, which includes the  $\epsilon$ -sarcoglycan gene and the paternally expressed 10 (PEG 10) gene, has been suggested.<sup>19–20</sup> Both genes are located about 2 Mb proximal of the SHFM1 critical region and seem to be imprinted maternally. Linkage analysis in one of two SHFM1D families, however, does not support a role of maternal imprinting in SHFM1 because maternal transmission of the disease was observed for six of nine female family members.<sup>6</sup> Haplotype analysis of a larger number of patients with SHFM1 with *de novo* deletions on chromosome 7 could show whether the paternal chromosome 7 is prone to deletion of the SHFM1 region.

**ACKNOWLEDGEMENTS**

We thank Dr G Klingebiel, Berlin, for his expertise on magnetic resonance topography scanning, and G Koch and G Weilepp for technical assistance.

**Authors' affiliations**

**I Wieland, P Muschke, S Jakubiczka, M Volleth, P F Wieacker**, Institut für Humangenetik, Universitätsklinikum, Otto-von-Guericke-Universität, Leipziger Str. 44, 39120 Magdeburg, Germany

**B Freigang**, Universitätsklinik für HNO-Heilkunde, Otto-von-Guericke-Universität, Leipziger Str. 44, 39120 Magdeburg, Germany

Conflicts of interest: none declared.

Correspondence to: Professor P Wieacker, Institut für Humangenetik, Universitätsklinikum, Otto-von-Guericke-Universität, Leipziger Str. 44, 39120 Magdeburg, Germany; peter.wieacker@medizin.uni-magdeburg.de

Received 19 May 2003

Accepted for publication 19 June 2003

**REFERENCES**

- Duijf PHG, van Bokhoven H, Brunner HG. Pathogenesis of split-hand/split-foot malformation. *Hum Mol Genet* 2003;**12**:R51–60.
- Morey MA, Higgins RR. Ectro-amelia syndrome associated with an interstitial deletion on 7q. *Am J Med Genet* 1990;**35**:95–9.
- Marinoni JC, Stevenson RE, Evans JP, Geshuri D, Phelan MC, Schwartz CE. Split foot and developmental retardation associated with a deletion of three microsatellite markers in 7q21.2–q22.1. *Clin Genet* 1995;**47**:90–5.
- Ignatius J, Knuutila S, Scherer SW, Trask B, Kere J. Split hand/split foot malformation, deafness, and mental retardation with a complex cytogenetic rearrangement involving 7q21.3. *J Med Genet* 1996;**33**:507–10.
- Genuardi M, Pomponi MG, Sammito V, Bellussi A, Zollino M, Neri G. Split/hand split foot anomaly in a family segregating a balanced translocation with a break point on 7q22.1. *Am J Med Genet* 1993;**47**:823–31.
- Tackels-Horne D, Toburen A, Sangiorgi E, Gurreri F, de Mollerat X, Fischetto R, Causio F, Clarkson K, Stevenson RE, Schwartz CE. Split hand/split foot malformation with hearing loss: first report of families linked to SHFM1 locus in 7q21. *Clin Genet* 2001;**59**:28–36.
- Haberlandt E, Löffler J, Hirst-Stadlmann A, Stöckel B, Judmaier W, Fischer H, Heinz-Erian P, Müller T, Utermann G, Smith RJH, Janecke AR. Split hand/split foot malformation associated with sensorineural deafness, inner and middle ear malformation, hypodontia, congenital vertical talus, and deletion of eight microsatellite markers in 7q21.1–q21.3. *J Med Genet* 2001;**38**:405–9.
- Hudgins L, Massa H, Distèche C, Scherer SW, Tsui L-C, Trask B, Evans JP. Identification of a microdeletion at 7q21.3 with fluorescence in situ hybridization with split hand/split foot (ectrodactyly). *Am J Hum Genet* 1994;**3**:A107.
- Crackower MA, Scherer SW, Rommens JM, Hui CC, Poorkaj P, Soder S, Cobben JM, Hudgins L, Evans JP, Tsui LC. Characterization of the split hand/split foot malformation locus SHFM1 at 7q21.3–q22.1 and analysis of a candidate gene for its expression during limb development. *Hum Mol Genet* 1996;**5**:571–9.
- Scherer SW, Poorkaj P, Allen T, Kim J, Geshuri D, Nunez M, Soder S, Pagon R, Stephens K, Patton M, Rivera H, Berg MA, Pfeiffer RA, Naritomi K,

- Hughes H, Genuardi M, Gurrieri F, Neri G, Lovrein E, Magenis E, Tsui L-C, Evans JP. Fine mapping of the autosomal dominant split hand/split foot locus on chromosome 7, band q21.3–q22.1. *Am J Hum Genet* 1994;**55**:12–20.
- 11 **Robledo RF**, Rajan L, Li X, Lufkin T. The *Dlx5* and *Dlx6* homeobox genes are essential for craniofacial, axial, and appendicular skeletal development. *Genes Dev* 2002;**16**:1089–101.
  - 12 **Merlo GR**, Paleari L, Mantero S, Genova F, Beverdam A, Palmisano GL, Barbieri O, Levi G. Mouse model of split hand/foot malformation type I. *Genesis* 2002;**2**:97–101.
  - 13 **Wieland I**, Muschke P, Wieacker P. Further delineation of Wittwer syndrome and refinement of the mapping region. *Am J Med Genet* 2003;**116**:57–60.
  - 14 **Sambrook J**, Fritsch EF, Maniatis T. *Molecular cloning: a laboratory manual*. Cold Spring Harbor: Cold Spring Harbor Laboratory Press, 1989.
  - 15 **Acampora D**, Merlo GR, Paleari L, Zerega B, Postiglione MP, Mantero S, Bober E, Barbieri O, Simeone A, Levi G. Craniofacial, vestibular and bone defects in mice lacking the Distal-less-related gene *Dlx5*. *Development* 1999;**126**:3795–809.
  - 16 **Depew MJ**, Liu JK, Long JE, Presley JE, Meneses JJ, Pedersen RA, Rubenstein JL. *Dlx5* regulates regional development of the branchial arches and sensory capsules. *Development* 1999;**126**:3831–46.
  - 17 **Charite J**, Mc Fadden DG, Merlo G, Levi G, Clouthier DE, Yanagisawa M, Richardson JA, Olson EN. Role of *Dlx6* in regulation of an endothelin-1-dependent, dHAND branchial arch enhancer. *Genes Dev* 2001;**15**:3039–49.
  - 18 **Jääntti J**, Lahdenranta J, Olkkonen VM, Söderlund H, Keränen S. SEM1, a homologue of the split hand/split foot malformation candidate gene *Dss1*, regulates exocytosis and pseudohyphal differentiation in yeast. *Proc Natl Acad Sci USA* 1999;**96**:909–14.
  - 19 **Zimprich A**, Grabowski M, Asmus F, Naumann M, Berg D, Bertram M, Scheidtmann K, Kern P, Winkelmann J, Müller-Myhsok B, Riedel L, Bauer M, Müller T, Castro M, Meitinger T, Strom TM, Gasser T. Mutations in the gene encoding  $\epsilon$ -sarcoglycan cause myoclonus-dystonia syndrome. *Nature Genet* 2001;**29**:66–9.
  - 20 **Ono R**, Kobayashi S, Wagatsuma H, Aisaka K, Kohda T, Kaneko-Ishino T, Ishino F. A retrotransposon-derived gene, PEG 10, is a novel imprinted gene located on human chromosome 7q21. *Genomics* 2001;**73**:232–7.