

Short reports

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Delineation of a complex karyotypic rearrangement by microdissection and CGH in a family affected with split foot

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Abstract

We report on a male patient and members of his family with additional material in chromosome 3. This derivative chromosome 3 was transmitted from his mother who had a complex rearrangement between chromosomes 2, 3, and 7. It was possible to delineate her chromosomal rearrangement by microdissection and reverse painting and to exclude these aberrations from being responsible for neonatal deaths and several abortions in this family. Two members of this family suffer from ectrodactyly or split hand/foot malformations (SHFM) of the feet which

possibly correlates with the derivative chromosome 7 containing a breakpoint in the *SHFM1* critical region involving several homeobox genes.

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Split hand/foot malformation (SHFM) is a heterogeneous limb developmental disorder, characterised by missing digits, fusion of the remaining digits, and a deep median cleft in the hands and feet.^{1,2} The hereditary form of SHFM is often correlated with a chromosomal breakage in the *SHFM1* critical region 7q21.3-q22.1.^{1,2} The *DSS1* gene,³ as well as the distal-less homeobox genes *DLX5* and *DLX6*,¹ are located in this region. *DSS1* is possibly responsible for SHFM phenotypes.³ Marinoni *et al*⁴ reported the coexistence of split foot and developmental anomalies with a deletion of three microsatellite markers in 7q21.2-q22.1. We delineate a chromosomal rearrangement in a family with two members suffering from bilateral split feet but no other developmental anomalies, and one member with very mild deformities of the feet and other developmental anomalies. Also, several cases of neonatal death and abortions have occurred in this family.



Figure 1 Bilateral split feet of the patient's sister. The mother of the patient also had similar malformations of her feet, which were amputated and replaced by prostheses in infancy.

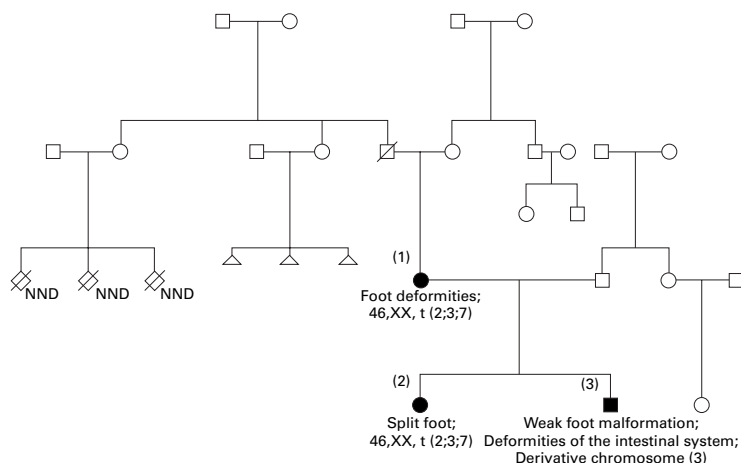


Figure 2 Pedigree of the patient's family. The mother (1) and sister (2) exhibit the same chromosomal aberration and both have bilateral deformities of their feet. The patient (3) exhibits only widely spaced first and second toes, intestinal malformations, and the maternally transmitted derivative chromosome 3. NND=neonatal death.

Case report

The 5 month old patient has minor malformations like widely spaced first and second toes, significant nail hyperplasia, disproportionate size of fingers to hand, a relatively long thorax, epicanthus medialis, intestinal malformations, and possibly a spina bifida occulta. So far he has no head control. Like his mother, his sister also has split feet (fig 1), whereas he himself only has widely spaced first and second toes. A hereditary connection between these dysmorphic symptoms and chromosomal aberrations seems very likely.

The patient's maternal grandfather has two childless sisters, one of whom had three abortions and the other three neonatal deaths (fig 2).

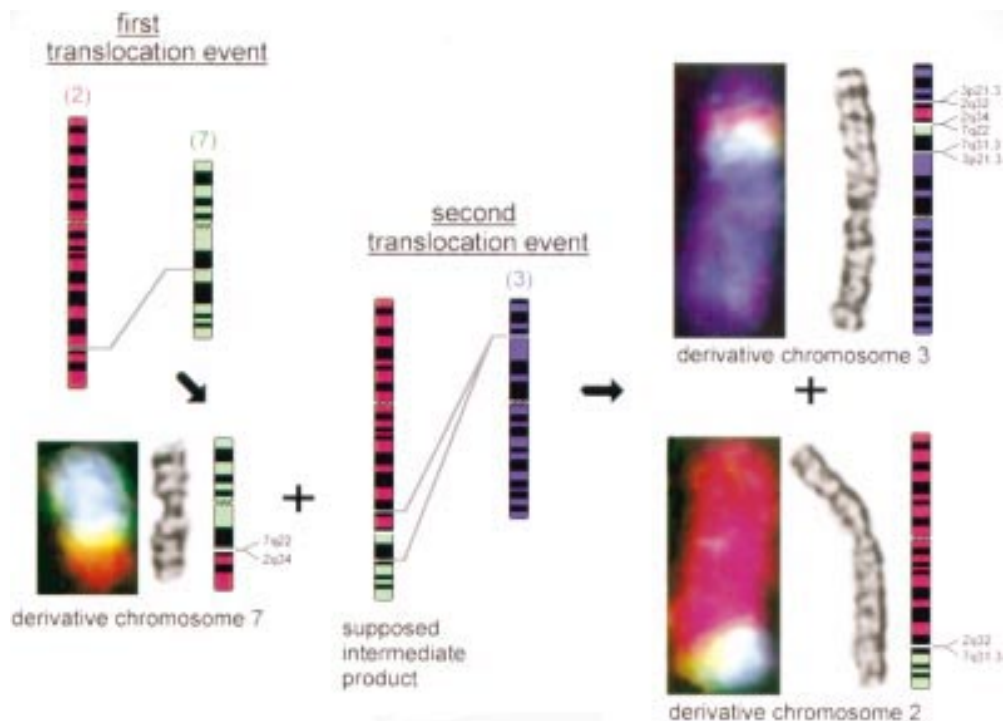


Figure 3 Presumed mechanism of translocation in G banded ideograms.²⁰ The fluorescence images are M-FISH pictures of the derivative chromosomes 2, 3, and 7 compared with their G banded chromosomes.

CYTOGENETIC ANALYSIS

Initially, GTG banding of this newborn boy with mild dysmorphic features showed a 46,XY,der ins(3)(p?) karyotype with unspecified breakpoints (see G banding of the derivative chromosome 3 in fig 3). Analysis of the

maternal karyotype and the karyotype of the patient's sister shows an identical complex translocation pattern including chromosomes 2, 3, and 7. Fluorescence in situ hybridisation with commercial human whole chromosome painting probes (WCP) of chromosomes 2, 3, and 7 indicates an insertion of additional chromosomal material from chromosomes 2 and 7 into the short arm of chromosome 3 of the patient (see M-FISH pictures in fig 3). The chromosome 2 material was distally inserted next to the proximally inserted chromosome 7 material in the derivative chromosome 3. In his mother's karyotype the same composition of signals of her derivative chromosome 3 were found. The transmitted derivative chromosome 3 of the patient is the same as in his mother's and sister's karyotype. Furthermore, translocation products of chromosomes 2 and 7 were detected in the mother's and sister's karyotype.

Comparative genomic hybridisation (CGH)^{6,7} analysis of the patient's DNA was performed to define the contents of the additional inserted chromosomal material of the derivative chromosome 3 (fig 4), and thus the inserted regions could almost be limited to 2q33–2q34 and 7q22–7q31. Chromosome microdissection^{8,9} followed by reverse painting^{10,11} was applied to characterise the exact breakpoints of the balanced translocation in the patient's mother. We generated whole chromosome painting probes of the derivative chromosomes 2, 3, and 7 by microdissection. The multicolour fluorescence in situ hybridisation (M-FISH)¹² results of the reverse painting (fig 5) identified the translocated areas. To detect the point of insertion in chromosome 3, a further experiment was necessary. The best strategy to identify insertion points is to dissect

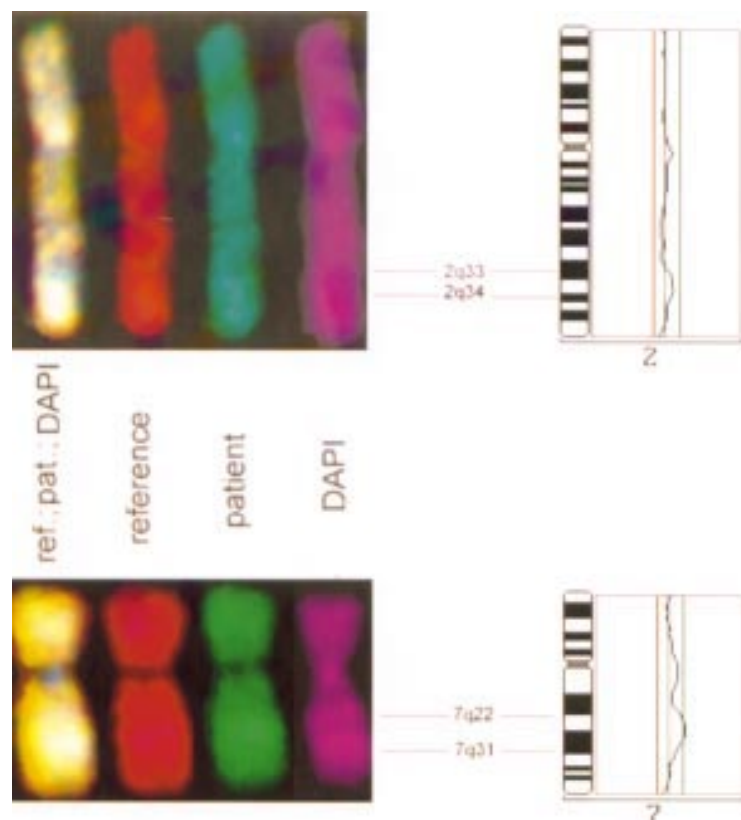


Figure 4 CGH results with DNA of the patient. The ideogram shows a gain limited to 2q33–2q34 and 7q22–7q31. The fluorescence signals are given on the left.

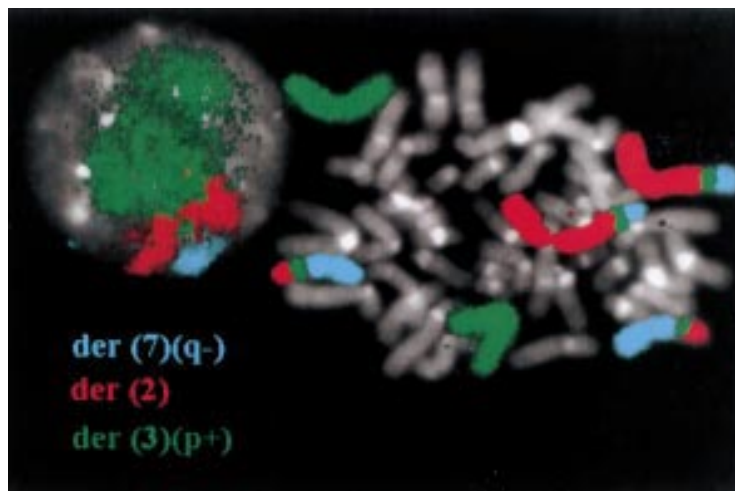


Figure 5 Reverse painting of the maternal derivative chromosomes *der(2)*, *der(3)*, and *der(7)* generated by microdissection on normal metaphases.

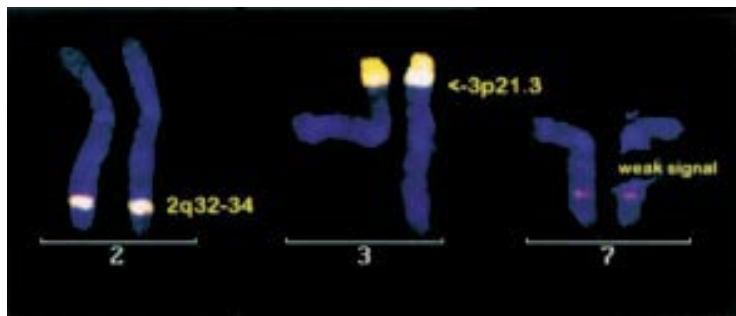


Figure 6 Reverse painting of a distal part of the maternal aberrant arm of the derivative chromosome 3 (*3pter*→*3p21.3*::*2q32*→*2q34*::*7q22*→*7q22*) isolated by microdissection and hybridised on normal metaphases.

proximally or distally to the inserted region. We used a partial chromosome painting probe of the distal p arm of the derivative chromosome 3, *der(3)(3pter*→?:*2q32*→*2q34*::*7q22*), obtained by microdissection,^{13 14} to determine the breakpoint of the insertion into the derivative chromosome 3. The results of this microdissection and reverse painting (fig 6) showed a very distinct signal on chromosome 3 distal to the insertion breakpoint at the telomeric end of *der(3p)* as well as on *2q32*→*34*, but almost no signal on chromosome 7. Therefore, the insertion breakpoint of the derivative chromosome 3 could be determined as *3p21.3*. The results of microdissection and reverse painting pinpoint the inserted areas in chromosome 3 as *2q32*→*2q34*::*7q22*→*7q31.3* (figs 5 and 6). All

Table 1 Known genes and FISH signal distribution of cosmid signals located in the *SHFM1* critical region of the mother

<i>SHFM1</i> critical region	Gene	Cosmid probes	Distance to <i>DSS1</i>	Distribution of signals
7q21.3	<i>PDK4</i>	cos426/61d4		<i>der(7)</i> chromosome 7 <i>der(3)</i>
	<i>DNC11</i>	cos437/237d7		+ +
	<i>SLC25A13</i>	cos405/24g11	400 kb	+ +
		cos274/64b4	100 kb	+ +
	<i>DSS1</i>	cos256/191f10		+ +
7q22.1	<i>DLX6, DLX5</i>	cos DLX		+ +

PDK4 = pyruvate dehydrogenase kinase isoenzyme 4. *DNC11* = dynein cytoplasmic intermediate polypeptide 1. *SLC25A13* = adult onset type II citrullinaemia (CTLN2). *DSS1* = deleted in split hand/split foot 1 region. *DLX5* and *DLX6* = distal less homeobox gene 5 and 6. Additional information on the cosmid probes can be found at: <http://www.genet.sickkids.on.ca/chromosome7/>

reverse painting results showed the following complex karyotype: 46,XX,rev ish t(2;3;7;) (*2pter*→*2q32*::*7q31.3*→*7qter*; *3pter*→*3p21.3*::*2q32*→*2q34*::*7q22*→*7q31.3*::*3p21.3*→*3qter*; *7pter*→*7q22*::*2q34*→*2qter*). All identified breakpoints are shown in fig 3.

Further investigations were performed to confirm the breakpoint location of the derivative chromosome 7 in the *SHFM1* critical region. Using six cosmids located in the *SHFM1* critical region, the breakpoint could be narrowed down to *7q22.1* (table 1). The breakpoint is distal to cos405/24g11 and proximal to cos274/64b4 in the *SHFM1* critical region.

Discussion

We suggest two possibilities for the development of this complex aberration. The first event is a reciprocal translocation between chromosomes 2 and 7 with the breakpoints at *2q34* and *7q22* (fig 3). One product of this translocation event is identical to one derivative chromosome in the karyotype of the patient's mother and sister (derivative chromosome 7). The other derivative product is the starting point for a second translocation with a chromosome 3. The chromosomal region (*2q32*→*2q34*::*7q22*→*7q31.3*) of this intermediate product is inserted into *3p21.3* (fig 6). The derivative chromosomes 2 and 3 in the patient's mother are derived from the second translocation event. Another event with breakpoints in *2q32* and *7q31* in the first translocation is a possibility. If so, the second translocation event would have happened between the derivative chromosome 7 as a presumed intermediate product and a chromosome 3 with an inverted insertion. Regarding the G banding of the derivative chromosome 3, we prefer the mechanism in fig 3, since the G banding of the derivative chromosome 3 is in accordance with the ideogram of the rearrangement model.

Split hand/foot malformation (*SHFM*) is an autosomal dominant developmental malformation. Variations of *SHFM* where patients have deformities of the feet without hand deformities have been described.^{4 15} Ectrodactyly has been described in several patients, often associated with cytogenetically visible rearrangements involving the chromosomal region (*7*)(*q21.2-22.1*).^{4 15 16} We could confirm one breakpoint of the complex aberration in the (*7*)(*q21.3-22.1*) *SHFM1* region,^{15 16} next proximally to the *DSS1* and the distal-less homeobox genes like *DLX5* and *DLX6*.³ Neither *DSS1* nor the *DLX* genes are directly affected by the breakpoint on the derivative chromosome 7 of the mother and daughter. If *DSS1* is not affected indirectly by the close location of the breakpoint (between around 100 kb for cos274/64b4 and 400 kb for cos405/24g11 proximal to *DSS1*⁵), a further developmental gene for split foot could exist. A close balanced reunion junction near *DSS1* could influence the transcription of *DSS1* in the developmental process and cause malformations of the feet.

The malformations in our patient are possibly the result of trisomy of genes in the inserted

regions like *DSS1*, *DLX6*, and *DLX5*. The derivative chromosome 7 showing a breakpoint in 7q22 could have caused the foot malformations of the patient's mother and sister if the supposed gene for ectrodactyly, *SHFM1*, is influenced.

From this hypothesis, further SHFM phenotypes should be present in this family (fig 2) if both translocation events of the complex rearrangement between chromosomes 2, 3, and 7 occurred in more than one meiotic cell division. The derivative chromosome 7 would be one of the first results of the first translocation event. Then the second translocation event could have happened at gametogenesis of the next generation. However, no foot malformations have been reported in the patient's grandparents and their sisters (fig 2). Therefore, it is very likely that both meiotic translocation events happened at gametogenesis of one maternal grandparent simultaneously. However, it should be noted that ectrodactyly sometimes skips a generation.¹⁷⁻¹⁹ The patient's grandfather's sisters with three abortions and three neonatal deaths definitely did not harbour the translocation.

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