ELECTRONIC LETTER

Analphoid de novo marker chromosome inv dup(3)(q28qter) with neocentromere in a dysmorphic and developmentally retarded girl

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In the March 1999 issue of the journal, Portnoï *et al*[1] reported on a patient with a marker chromosome with neocentromere which originated from distal 3q. Here we describe a further example of this type of marker chromosome from distal 3q, which is substantially smaller.

CASE REPORT

The marker was ascertained during cytogenetic diagnosis of a 5 year old girl with marked developmental delay and mild facial dysmorphism. Body measurements were birth length 49 cm, birth weight 2965 g, and OFC 34 cm. At the age of 8.5 years, height was 131 cm, and weight was 37 kg (>97th centile) as a result of hyperphagia which developed two years ago. Dysmorphic signs included hypognathia, broad and flat nasal root, and abnormally shaped alae nasi (fig 1). The median upper lids showed atypical epicanthus. Furthermore, the girl showed slight hirsutism and a bilateral ichthyosiform hyperkeratosis of the palms and soles. On clinical investigation at the age of 5 years our proband presented with slight muscular hypotonia and hyporeflexia. Perceptive skills and visuomotor coordination were retarded corresponding to a developmetal age of 3-3.5 years. At the age of 8.5 years the girl attends a special school for mentally handicapped children. She cannot speak properly, searching for words, can recognise only between 10 and 15 letters, and is still not able to write.

Standard cytogenetic investigation showed a very small, nearly metacentric supernumerary de novo marker chromosome (fig 2), which was C band negative (not shown) but appeared mitotically stable in cultured lymphocytes, since it was present in all metaphases investigated. FISH with an alpha satellite probe detecting all human centromeres (fig 3) gave hybridisation signals on all chromosomes of the proband, except the marker chromosome. This finding implies that it is an analphoid marker. The chromosomal origin of the marker was clarified by reverse painting. Twenty copies of the marker chromosome were collected by microdissection, subsequently amplified, and labelled by DOP-PCR,[2 3] and the resulting FISH probe was hybridised to metaphase spreads of a male control (results not shown) and of the proband, respectively. DNA from this microdissection library hybridised exclusively on the marker and on distal 3q (fig 4). FISH with the Vysis subtelomeric probe 3QTEL05 of chromosome 3q showed two terminal signals on the marker chromosome (fig 5).

In order to characterise the extent of the marker chromosome along distal 3q, FISH with mapped YAC or BAC clones was performed (table 1). All clones tested gave either no FISH signal on the marker chromosome or a double signal, a finding which suggests a (presumably inverted) duplication of the terminal chromosome 3q segment (with no monosomic segment in between). Therefore, one can assume that this chromosome belongs to the expanding category of analphoid

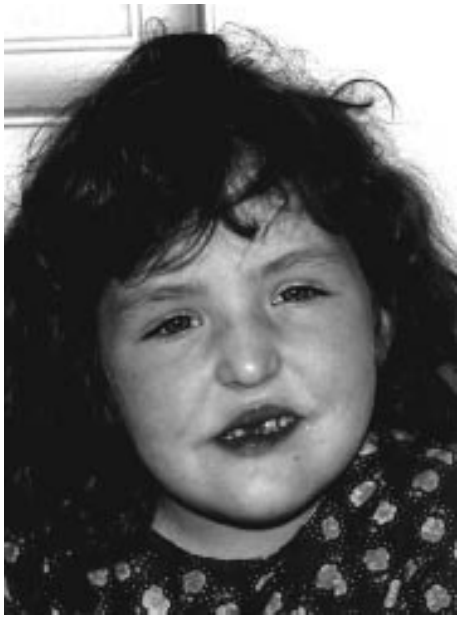

Figure 1 The proband aged 4 years.

Key points

- A very small supernumerary de novo marker chromosome was ascertained during cytogenetic diagnosis of a 5 year old girl with developmental delay and mild facial dysmorphism. The marker was C band negative but appeared mitotically stable in cultured lymphocytes.
- FISH with an alpha satellite probe detecting all human centromeres gave hybridisation signals on all of the proband's chromosomes except the marker chromosome. The chromosomal origin of the marker was clarified by microdissection and reverse painting; DNA collected from the marker hybridised exclusively on the marker and on distal 3q.
- The extent of the marker chromosome was characterised by FISH using mapped YAC or BAC clones. Each positive clone gave a double signal on the marker. Thus the marker belongs to the expanding category of analphoid marker chromosomes originating from duplication of a distal chromosome arm, which must have gained centromere function by neocentromere formation.
- According to the ENSEMBL database, the most proximal clone present on the marker, RP11-634I24, maps to chromosome band 3q28, and the extent of the duplicated segment is approximately 9 Mb.

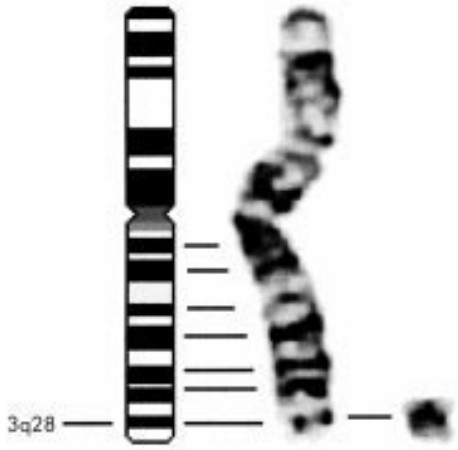

Figure 2 The marker chromosome after G banding in comparison with one homologue of chromosome 3 from the same cell and with the ideogram.

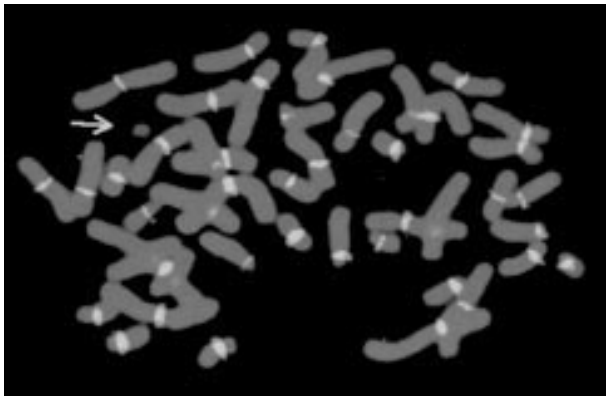

Figure 3 FISH with an alpha satellite DNA probe detecting all human centromeres (Oncor, Gaithersburg, MD, USA) gives signals on each chromosome, except the marker chromosome (arrow).

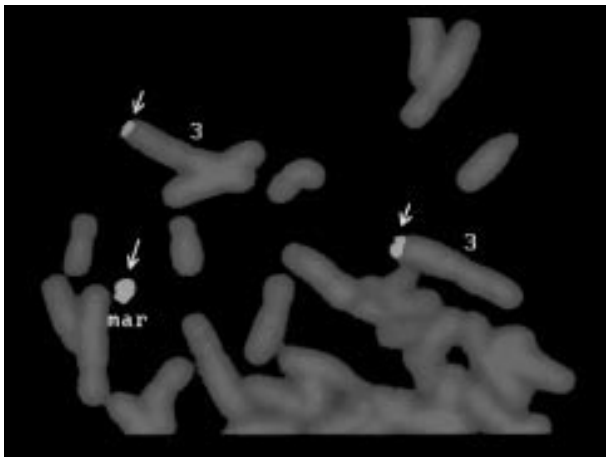

Figure 4 FISH with a microdissection library representing the total marker chromosome shows signals on the marker chromosome and on distal chromosome 3q.

marker chromosomes with a neocentromere, the majority of which have derived from inversely duplicated terminal chromosome arms.[4] According to the ENSEMBL database the most proximal clone RP11-634l24 maps to chromosome band 3q28. The G banding pattern of the marker chromosome is in agreement with an inverse duplication of the distal G bands 3q28 and 3q29 (fig 2).

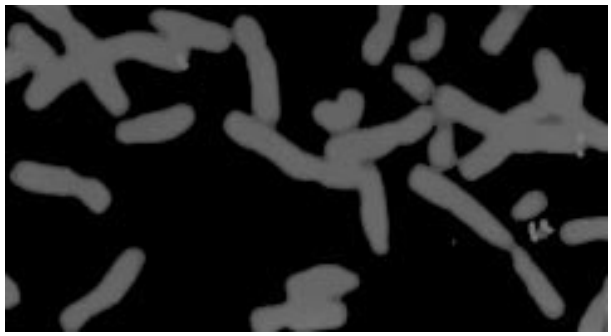

Figure 5 Single signals on the normal homologous chromosomes 3 at distal 3q, and a double signal on the marker chromosome after FISH with a subtelomeric probe representing STS 3QTEL05 (Vysis, Downers Grove, IL, USA).

DISCUSSION

To our knowledge there are only two reports on marker chromosomes representing a duplication of distal 3q. Portnoï *et al*[1] described a marker chromosome with inverse duplication of 3q27→qter, which is substantially larger than the marker reported here. Cockwell *et al*[5] reported on an analphoid supernumerary marker chromosome derived from terminal 3q in a fetus with multiple malformations. The breakpoint of this marker was assigned to band 3q26.2 according to its banding pattern but was not precisely determined by molecular genetic methods. Kroisel *et al*[6] reported on partial tetrasomy 3q resulting from triplication within a single chromosome. A comparison of the extent of the resulting tetrasomic segment of our proband with those of the reported cases with more precise determination of the breakpoint is shown in table 1.

The marker chromosome reported here is smaller than the one described by Portnoï *et al*[1] and the resulting tetrasomic segment is also smaller than on the derivative chromosome 3 described by Kroisel *et al*.[6] Even standard banding analysis shows that the marker reported by Cockwell *et al*[5] is also substantially larger, since it exceeds the size of a G chromosome. According to the FISH analysis, the marker described here contains at least 9 Mb from the distal chromosome 3q28-29.

In the female patient described by Portnoï *et al*,[1] the clinical picture is restricted to pigmentary anomalies of the skin following the lines of Blaschko.[7] Mental development is not impaired and she has no dysmorphic features. In this girl the mild clinical manifestation and the skin pigmentary pattern might both be explained by the finding that she represents a mosaic with a euploid cell line. The patient reported by Kroisel *et al*[6] displays a very similar pattern of skin hyperpigmentation to the patient of Portnoï *et al*[1] and he also represents mosaic tetrasomy 3q. However, this patient is developmentally retarded. Contrary to these cases, in the patients described here and by Cockwell *et al*[5] no mosaicism was detected. Though the patient reported by Kroisel *et al*[6] is a mosaic, he can be compared with our proband. Both patients are mentally retarded and both share some typical non-specific symptoms commonly observed in patients with unbalanced karyotypes, namely coarse facial features and a prominent supraorbital region. However, a broad nasal root, long philtrum, and large, prominent ears were only reported in the patient of Kroisel *et al*[6] and not in the girl reported here. Particularly, the skin anomalies observed in our patient are distinct from the pigmentary anomalies observed in the patients described by Portnoï *et al*[1] and Kroisel *et al*.[6] In the latter patient, hyperpigmentation of the skin was already manifest at birth, whereas in the patient of Portnoï *et al*[1] this phenotype developed only after the age of 10-12 years.

Table 1 Results of the FISH analysis to characterise the extent of chromosome 3 derived DNA on the marker described here in comparison with the marker chromosomes described by Portnoï *et al*[1] and Kroisel *et al*[6]

Probes used for FISH analysis	Marker	GenBank Accession number	Assignment to band on chromosome 3	Absence (–)/presence (+) of FISH signal on the der(3) chromosome of the patient described		
				This study	Portnoï *et al*[1]	Kroisel *et al*[6]
YAC 806d8	D3S3520		q26.31	ND	–	+*
YAC 760f3	D3S3699		q26.32	–	++	+*
YAC 781f8	D3S513			ND	++	+++*
YAC 906c11	D3S1571		q27.1	–	ND	ND
YAC 909d10	*KNG*†		q28	–	ND	ND
	D3S1314		q28			
YAC 883d12	*KNG*†		q28	ND	++	+++*
BAC RP11-814o5	D3S3076	AC062008	q28	–	ND	ND
BAC RP11-634l24		AC041034	q28	++		
BAC RP11-69j10		AC026518	q29	++		
BAC RP11-221n10		AC067896	q29	++		
BAC RP11-496h1		AC024560	q29	++		
YAC 763e7	D3S1272		q29	++	ND	ND
YAC 919f12	D3S1272		q29	ND	ND	ND
pVYS223B	3QTEL05		q29	++	ND	+++*

*The additional material is attached distally to the subtelomeric end at 3q thus forming an elongated 3q+ chromosome.
†*KNG* (Kininogen precursor gene).
ND: not determined.
+, ++, +++ single, double, triple FISH signal.

The marker chromosomes of Portnoï *et al*,[1] Cockwell *et al*,[5] and the one described in this report most probably represent inverse duplications with a neocentromere acquired to stabilise a de novo acentric fragment. The different breakpoints in these three examples suggest that such "acentric fragments stabilised by neocentromere formation" can originate at many, if not any, chromosomal sites. This is in accordance with the observation of variable breakpoints within the growing collection of this type of marker chromosome representing the different chromosome arms[4] and even within the same chromosome arm.[8]

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REFERENCES

1 **Portnoï MF**, Boutchneï S, Bouscarat F, Morlier G, Nizard S, Dersarkissian H, Crickx B, Nouchy M, Taillemite JL, Belaich S. Skin pigmentary anomalies and mosaicism for an acentric marker chromosome originating from 3q. *J Med Genet* 1999;**36**:246-50.
2 **Senger G**, Luedecke HJ, Horsthemke B, Claussen U. Microdissection of banded chromosomes. *Hum Genet* 1990;**84**:507-11.
3 **Chudoba I**, Rubtsov N, Senger G, Junker K, Bleck C, Claussen U. Improved detection of chromosome 16 rearrangements in acute myeloid leukemias using 16p and 16q specific microdissection libraries. *Oncol Rep* 1996;**3**:829-32.
4 **Depinet TW**, Zackowski JL, Earnshaw WC, Kaffe S, Sekhon GS, Stallard R, Sullivan BA, Vance GH, Van Dyke DL, Willard HF, Zinn AB, Schwartz S. Characterization of neo-centromeres in marker chromosomes lacking detectable alpha-satellite DNA. *Hum Mol Genet* 1997;**6**:1195-204.
5 **Cockwell AE**, Gibbons B, Moore IE, Crolla JA. An analphoid supernumerary marker chromosome derived from chromosome 3 ascertained in a fetus with multiple malformations. *J Med Genet* 2000;**37**:807-9.
6 **Kroisel PM**, Petek E, Wagner K. Skin pigmentary anomalies in a mosaic form of partial tetrasomy 3q. *J Med Genet* 2000;**37**:723-5.
7 **Happle R**. Lyonization and the lines of Blaschko. *Hum Genet* 1985;**70**:200-6.
8 **Warburton PE**, Dolled M, Mahmood R, Alonso A, Li S, Naritomi K, Tohma T, Nagai T, Hasegawa T, Ohashi H, Govaerts LC, Eussen BH, Van Hemel JO, Lozzio C, Schwartz S, Dowhanick-Morrissette JJ, Spinner NB, Rivera H, Crolla JA, Yu C, Warburton D. Molecular cytogenetic analysis of eight inversion duplications of human chromosome 13q that each contain a neocentromere. *Am J Hum Genet* 2000;**66**:1794-8.