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Confirmation of a suspected 16q deletion in a dysmorphic child by flow karyotype analysis

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SUMMARY Cytogenetic examination of a dysmorphic infant with multiple congenital abnormalities revealed a possible de novo interstitial deletion in the long arm of chromosome 16. Conclusive proof of the deletion was obtained by flow karyotype analysis of the patient and both parents, which showed that the deleted segment was approximately 7000 kb in size.

There have been at least nine previous published cases of children with a visible chromosomal deletion in the long arm of chromosome 16. In the first report, Fryns *et al*[1] described the clinical features associated with monosomy for the region distal to 16q21 and subsequent reports described similar features even in infants with different deletions of 16q.[2–7] Elder *et al*[5] reported identical twins with a deletion from 16q12·2→q13 defined by high resolution chromosome banding techniques. They proposed that deletion of this region was critical to the production of the 16q− phenotype and considered that conflicting reports of the site of the 'critical region' were due to inadequate resolution in conventional metaphase preparations. Thus, it is desirable to have an alternative technique for characterising very small chromosome aberrations which may only be visible to an experienced cytogeneticist examining the best chromosome preparations. In this report, we describe how the technique of flow cytometry may be used to confirm the presence and also to estimate the size of a very small de novo deletion of chromosome 16 discovered in an infant born with multiple congenital abnormalities.

Case report

The female infant was the second child of unrelated Scottish parents who were both 28 years old. The birth weight at term was 2·98 kg, length was 43 cm, and head circumference (OFC) was 29 cm. The infant fed poorly and at 10 days a systolic murmur and central cyanosis were noted. At two months generalised seizures developed and at four months she developed right heart failure. On examination the following features were noted: microcephaly (OFC 35 cm, 5 SD below the mean) with diastasis of the metopic suture and an occipital naevus with an underlying bony defect, short palpebral fissures with a mongoloid slant, a flat nasal bridge, posterior rotation of the ears, a high palate, and a short webbed neck (fig 1). The finger print patterns comprised six ulnar loops and four whorls. The crown-heel length (52 cm) and weight (4·2 kg) were well below the 3rd centile.

The results of investigations were as follows: plasma urea 12 mmol/l, creatinine 135 μmol/l, and uric acid 460 μmol/l were all raised. Chest *x* ray showed a large heart with prominent lung vessels and a skull *x* ray showed multiple wormian bones. ECG showed signs of biventricular hypertrophy. The echocardiogram was difficult to interpret; four chambers were seen and the overall picture was of non-obstructed total anomalous pulmonary venous drainage. Renal ultrasound and intravenous pyelography showed a hydronephrotic or cystic, poorly functioning left kidney. A barium swallow was normal in appearance, as was an echoencephalogram.

Her clinical condition remained poor during infancy and she had recurrent chest infections with worsening cardiac failure. She made little developmental progress and died at the age of 19 months.

CYTOGENETIC ANALYSIS

Cytogenetic studies were carried out by standard

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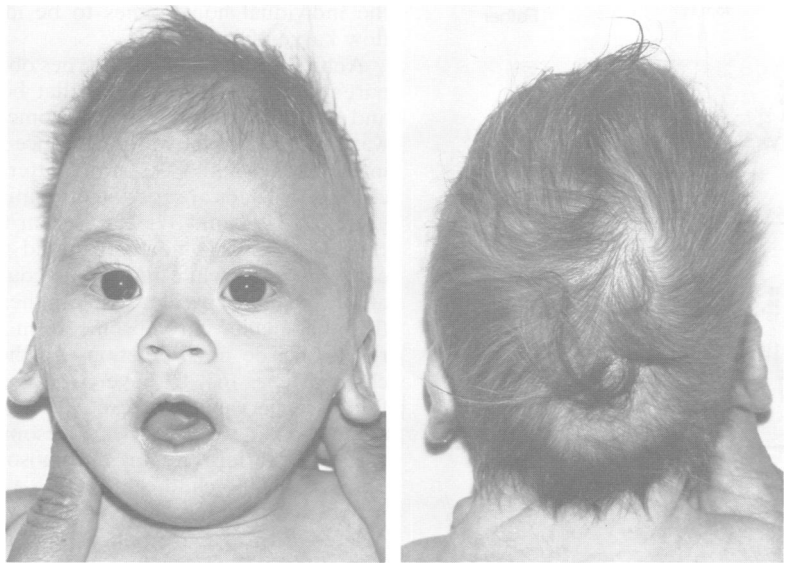

FIG 1 *The affected child at six months of age.*

techniques using PHA stimulated lymphocyte cultures and Giemsa banding. Analysis revealed an apparently abnormal banding pattern in the long arm of chromosome 16 with bands q21 and q23 appearing unusually close together. This was interpreted as a probable interstitial deletion involving band 16q22 (fig 2). The parental karyotypes were normal.

Epstein-Barr virus transformed lymphoblastoid cell lines from the patient and her parents were established and grown up in RPM1 medium with 15% fetal bovine serum. Cytogenetic analysis of these lines was carried out before flow karyotyping to exclude the possibility of any additional chromosome changes arising in culture.

CHROMOSOME ISOLATION AND FLOW KARYOTYPE ANALYSIS

Metaphase chromosomes were isolated as previously described,[8–10] stained with ethidium bromide at a concentration of 40 μg/ml, and examined using FACS 440 and the 514·5 nm laser line. The relative fluorescence values for the chromosome peaks in the flow karyotype were standardised by assigning the value 560 to the central peak containing chromosomes 10, 11, 12 (and sometimes 9) and the actual position and area of each peak calculated by computer fitted Gaussian distributions.[11]

Chromosome preparations derived from lymphoblastoid cell lines for the child and both parents were examined using the FACS 440 (fig 3). The flow karyotype obtained from the child showed that her two chromosome 16s were different in size, with the larger forming a separate peak (at a relative fluorescence of 379·9) and the smaller being incorporated in the chromosome 17 peak (at a relative fluorescence of 351·2). In addition, six other chromosomes, namely numbers 1, 9, 13, 15, 18, and 19, exhibited polymorphic size variations allowing

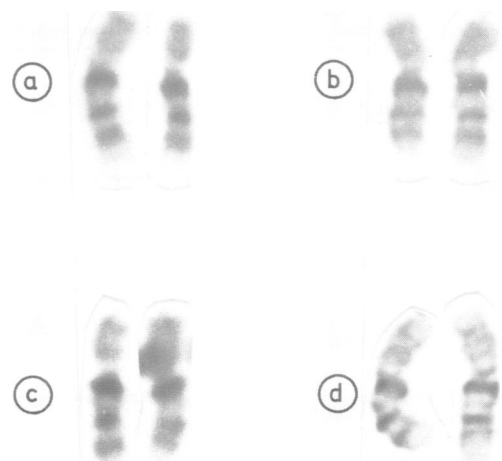

FIG 2 *G banded chromosomes 16 of (a) mother, (b) father, (c) and (d) child, with normal chromosome on the left.*

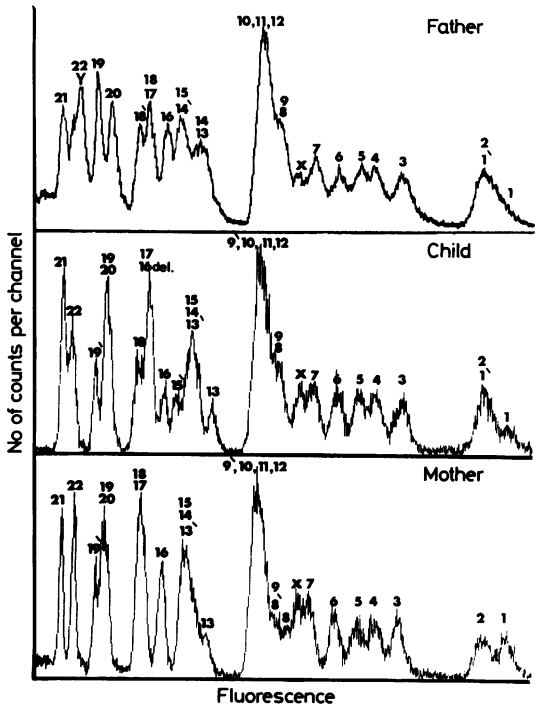

FIG 3 *Flow karyotypes of the child and her parents. For detailed analysis see table 1.*

the individual homologues to be identified in the flow karyotype (table 1).

Analysis of the flow karyotypes obtained from the parents of the child showed that both the mother and father had a single chromosome 16 peak which was present at relative fluorescence values of 378·5 in the former and 380·2 in the latter. As the child's chromosome 16s are present at relative fluorescence values of 379·9 and 351·2, it is clear that the smaller chromosome does not correspond to the parental chromosomes, while the larger could have originated from either parent. One can therefore conclude that the smaller chromosome 16 in the child is the result of a de novo deletion. As the four parental chromosome 16s all have a very similar relative fluorescence value (corresponding to similar DNA content[11]), it would appear reasonable to assume that the difference in chromosome 16 values observed in the child can provide a measure of the proportion of the chromosome which has been deleted. The figure calculated from the flow karyotype is 7·55% which corresponds reasonably well with the 8·70% difference in DNA content between chromosomes 16 and 17 obtained in normal subjects by CYDAC analysis[12] and previously by flow cytometry.[11] Assuming that the total amount of DNA in the haploid genome amounts to 3 million kb, chromosome 16 will contain approximately

TABLE 1 *Analysis of flow karyotypes from the child and her parents giving standardised peak positions and numerical chromosome allocations determined by computer fitted Gaussian distributions.*

Father		*Child*		*Mother*	
Relative fluorescence	*Chromosome No*	*Relative fluorescence*	*Chromosome No*	*Relative fluorescence*	*Chromosome No*
1011·9	1(x1)	1026·7	1(x1)M	1035·6	1
974·1	1(x1)C,2	982·1	1(x1)P,2	990·1	2
824·3	3	827·6	3	828·9	3
775·0	4	778·2	4	787·1	4
736·2	5	744·9	5	750·9	5
703·3	6	706·5	6	708·8	6
660·3	7	662·2	7	660·6	7
631·8	X	634·5	X	636·7	X
				615·5	8(x1)
593·5	8,9	592·4	8,9(x1)P	591·1	8(x1)C,9(x1)
560	10,11,12	560	9(x1)M,10–12	560	9(x1)C,10–12
		470·3	13(x1)M	465·0	13(x1)C
449·6	13				
429·4	14(x1)C	430·8	13(x1)P,14, 15(x1)M	434·5	13(x1),14
406·5	14(x1),15	401·9	15(x1)P	418·3	15
380·2	16	379·9	16(x1)	378·5	16
347·6	17,18(x1)	351·2	16(x1),17	347·7	17(x1)C
		336·1	18(x1)M	337·6	17(x1),18(x1)C
327·5	18(x1)C	325·9	18(x1)P	330·7	18(x1)
277·6	20	271·7	20,19(x1)M	268·3	20,19(x1)C
251·6	19	249·9	19(x1)P	251·2	19(x1)
219·3	Y,22(x1)				
203·5	22(x1)C	205·9	22	211	22
186·3	21	189·5	21	187·3	21

C=homologue inherited by child. P=paternal. M=maternal.

3·22% or 97 000 kb. Therefore, a 7·55% deletion would amount to 7300 kb.

Using the relative fluorescence values for each chromosome peak and the area proportion encompassed by each peak to give a measure of chromosome abundance, it is possible to determine the parental origin of all six chromosomes showing homologue size variation in the child (that is, chromosomes 1, 9, 13, 15, 18, and 19, table 1). It is also possible to deduce that the father has passed on the smaller of his chromosome 22 homologues and the larger of his chromosome 14 homologues to the child, while the mother has contributed her larger chromosome 17 homologue and smaller chromosome 8 homologue.

Discussion

This family illustrates how flow karyotype analysis may complement traditional cytogenetic techniques in elucidating very small chromosome aberrations. The detection in the child of homologue size variation in a highly polymorphic chromosome, such as chromosome 16, by flow karyotyping would normally be regarded as of little significance. However, concurrent examination of the flow karyotypes from both parents provided irrefutable confirmation of our initial impression that the karyotype showed a de novo deletion of chromosome 16. This finding also fitted the clinical features observed in the child which were in keeping with partial 16q monosomy (table 2).

Most of the other chromosomes exhibiting polymorphic size variation in the infant's flow karyotype can be confidently assigned to one or other of the parents by virtue of coincident or very similar relative fluorescence values. The chromosome 16 peaks in both parents have essentially the same relative fluorescence value which is also coincident with one of the child's chromosomes 16, thus allowing a reasonably accurate estimate of the size of the deletion in the abnormal chromosome corresponding to approximately 7000 kb of DNA.

Thus, although flow karyotyping does not approach the levels of sensitivity achievable with DNA hybridisation techniques, it does provide a useful and relatively rapid adjunct to conventional cytogenetic analysis with the potential to detect small abnormalities at or just beyond the limit of microscopic resolution. The unequivocal demonstration of an abnormality by flow cytometry provides justification for detailed studies (by cytogenetic or biochemical techniques) to localise it further on the particular chromosome concerned.

The previous cases of partial deletion of 16q have been apparently caused by deletions of slightly different sizes, which have been mapped by chromosome banding studies to different regions of the long arm (fig 4). When clinically similar patients have overlapping deletions it may be argued that the overlapping segment is the 'critical segment', deletion of which alone results in the phenotype. However, it is often difficult to define accurately the limits of a small deletion. Indeed one case with the clinical features of partial 16q deletion has been reported where there was no visible deletion at all.[13] When there is debate about the significance of a thinner than normal chromosome band, flow karyotype analysis offers an alternative means of confirming the presence of an abnormality and in this case has provided firm evidence for the presence and de novo origin of the deletion, thus permitting precise genetic counselling.

TABLE 2 *Clinical features of the present case compared to nine previous cases (adapted from Elder et al[5]).*

Feature	*Previous cases*	*Present case*
Growth		
Small for dates	7/9	+
Postnatal growth <3rd centile	8/9	+
Microcephaly	6/9	+
Failure to thrive	8/8	+
CNS and development		
Psychomotor retardation	7/7	+
Hypotonia	8/9	+
Feeble suck	4/4	+
Hydrocephalus	2/6	−
Craniofacial		
Large anterior fontanelle	9/9	+
High forehead	9/9	+
Diastasis cranial sutures	4/8	+
Prominent metopic sutures	8/9	+
Broad flat nasal bridge	7/9	+
Hypertelorism	5/9	−
Low set dysmorphic ears	9/9	+
Small palpebral fissures	5/9	−
Upward slanting palpebral fissures	4/9	+
Micrognathia	4/9	+
High arched palate	5/9	+
Short neck	9/9	?+
Thorax and abdomen		
Congenital heart defect	4/9	+
Narrow thorax	7/8	+
Ectopic anus	4/9	+
Renal cystic dysplasia/hypoplasia	2/6	+
Musculoskeletal		
Flexed fingers	2/8	+
Small hands and feet	5/9	−
Bilateral simian creases	3/8	−
Malposition of toes	4/9	+
Talipes eqinovarus/calcaneovalgus	4/9	+
Broad first toe	6/8	+

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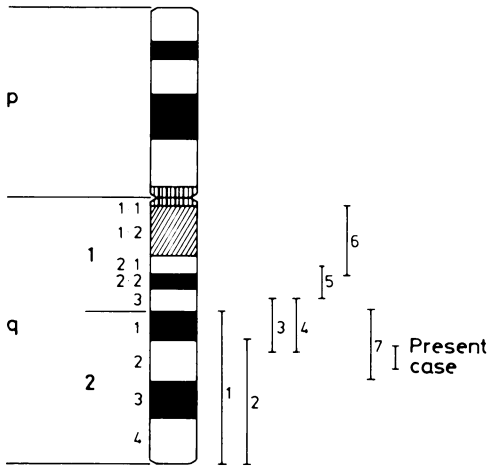

FIG 4 *Diagram of chromosome 16 showing the extent of reported deletions which have produced some (or all) of the features of the 16q− phenotype. Numbers refer to references.*

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