Duplication of 16q22→qter confirmed by fluorescence in situ hybridisation and molecular analysis

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Abstract

We report a female infant with congenital dislocation of the knee and dysmorphic features including a prominent forehead, midface hypoplasia, and micrognathia. Fluorescence in situ hybridisation and PCR amplification of microsatellite repeats were used to show that she had a de novo unbalanced translocation resulting in partial trisomy for 16q and partial monosomy for 15q (46,XX,-15,tder(15)t(15;16)(q26.1;q22). The consequences of partial aneuploidy of 16q are discussed.

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Trisomy 16 is the most common autosomal trisomy among spontaneous abortuses.^{1,2} While complete trisomy 16 is non-viable there are reports of liveborn infants with duplication of 16q. The majority of cases have involved virtually the entire long arm of chromosome 16 and have been associated with dysmorphic features and limited postnatal survival.³⁻¹⁹

We report an infant with a de novo duplication of 16q22→qter and deletion of 15q26.1→qter and compare the clinical features with other cases of 16q aneuploidy.

Case report

The proband, a female, was born at 38 weeks to non-consanguineous, healthy, Pakistani parents following a pregnancy complicated by intra-uterine growth retardation. Birth weight was

2100g (<3rd centile), length 49 cm (between the 25th and 50th centile), and head circumference 30.4 cm (<3rd centile). The Apgar score was 7 at one and 9 at five minutes respectively.

The mother was 24 and the father 25 years old. There was no family history of congenital malformations. There was a healthy female sib: however, the mother had previously had two late miscarriages at 5 and 7 months. In both cases the fetuses had been male and no abnormalities were found at necropsy.

Dislocation of the left knee and hip and stiffness of the elbow joints were immediately detected at birth. In addition the infant was noted to have dysmorphic features including a high forehead, small, narrow palpebral fissures with a slight antimongoloid slant, mild midface hypoplasia, and micrognathia. Unfortunately permission was not obtained to publish clinical photographs. Examination of the oral cavity showed a high arched palate and a sublingual frenulum. The fifth digits of the hands were small and there were single palmar creases. In the coccygeal area a large, blind ending sinus was noted and anal stenosis was found. Cardiovascular examination was normal and there were no urogenital or clinically detectable abdominal abnormalities.

Intermittent airway obstruction developed in the first few days of life. This was the result of choanal narrowing. Early indications are that motor development is delayed.

Chromosome analysis showed a female karyotype with additional material at the tip of the long arm of one chromosome 15 (fig 1). Parental chromosomes were normal. Fluorescence in situ hybridisation (FISH) was performed using a modification of the method of Pinkel *et al*²⁰ with commercially available chromosome 15 and 16 libraries (Cambio, Cambridge, UK) (fig 2). FISH with the chromosome 15 library showed one chromosome 15 consistently to have a region of signal free material at the end of the long arm (fig 2A). There was no evidence of translocated chromosome 15 material elsewhere in the karyotype. Re-evaluation of the G banded chromosomes suggested distal 16 long arm as a possible origin of the extra material, and therefore FISH was performed with a chromosome 16 library. This showed two normal chromosomes 16 and a region of chromosome 16 positive material at the end of the long arm of one D group chromosome (fig 2B). The

Figure 1 Partial karyotype showing a normal chromosome 15, the derived chromosome 15, and a normal chromosome 16. Arrows indicate the breakpoints.

Figure 2 (Top) Metaphase spread following in situ hybridisation with the Cambio chromosome 15 paint. The short arrow indicates the normal chromosome 15, and the long arrow indicates the derived chromosome 15 with a region lacking chromosome 15 signal at the distal end of the long arm. (Bottom) Metaphase spread following in situ hybridisation with the Cambio chromosome 16 paint. Pointers show the two normal chromosomes 16, and the short arrow indicates the derived chromosome 15 with 16 paint positive material at the end the long arm.

cytogenetic and FISH results therefore show that the infant had an unbalanced de novo translocation between the long arms of chromosomes 15 and 16, resulting in a duplication of the distal long arm of chromosome 16 (16q22→qter) and concomitant deletion of the most distal segment of the long arm of chromosome 15 (15q26.1→qter).

Molecular analysis of the long arm of chromosome 16 was by PCR amplification of DNA microsatellite repeats. The primer sequences used were Mfd49 (D15S87, mapping to 15q26→qter),²¹ 16AC1.15 (D16S305, mapping to 16q24.2→q24.3),²² AFMO31xa5 (D16S402, distal to 16q23.1), and AFM196xg1 (D16S413, distal to 16q23.1).²³ PCR conditions were those described by Hudson *et al.*²⁴ Results were visualised using a 6% denaturing polyacrylamide gel followed by autoradiography. Table 1 shows the results of PCR amplification of DNA microsatellite repeats in the proband and her parents. Fig 3 shows PCR amplification of DNA microsatellite repeat sequences from the proband and her parents using the primers AFM196xg1 at D16S413 and Mfd49 at D15S87. The results

Table 1 Results of PCR amplification of DNA microsatellite repeats at 16AC1.15 (D16S305), AFMO31xa5 (D16S402), AFM196xg1 (D16S413), and Mfd49 (D15S87) in the proband and parents

	D16S305	D16S402	D16S413	D15S87
Proband	2,3,3	1,3,3	1,2,2	3,—
Mother	1,3	2,3	1,2	2,2
Father	2,2	1,3	1,3	1,3

indicate that the proband appears to have inherited one paternal allele and two copies of one of the maternal alleles at the loci D16S305 and D16S413, based on the difference in band intensities. The results at D16S402 are consistent with a maternal duplication, but are not informative for the parental origin. However, the absence of a maternal contribution at D15S87 indicates that this structural chromosome abnormality is of maternal origin as well as confirming the deletion of the distal long arm of chromosome 15 suspected on cytogenetic grounds.

Discussion

To our knowledge only 21 cases of infants with duplications involving 16q have been described previously.^{3–19} Of the reports which specify the trisomic region six cases have involved complete duplication of the long arm (16p11→16qter),^{6–9} five cases of duplication of the long arm except for the proximal heterochromatic region (q13→qter),^{10–13} three cases involving q21→ter,^{14–16} and four cases with a duplication of q22→ter.^{17–19} In all previous cases one of the parents has carried a balanced translocation.

A number of other chromosomes have been involved with 16 in the translocation including 9p,¹⁰ 11p,¹⁷ 11q,¹³ 13p,¹⁰ 20p,¹² 21,¹⁸ 22p,¹⁴ 22q,⁴ and 15.¹⁹ While the phenotypic effects of partial trisomy 16q may be influenced by secondary chromosome rearrangements resulting from concurrent monosomies some trends do emerge. Table 2 summarises the most frequent clinical findings in these patients divided into groups according to the length of the duplicated segment of 16q. As might be expected, infants with the largest duplication have died shortly after birth. This reflects in part the severe internal malformations present when the long arm duplication of chromosome 16 is virtually complete. These include CNS malformations such as porencephaly, congenital heart disease, and intestinal malformations including malrotation and shortened small bowel. It is of interest that anorectal defects have been described in each of these groups. As shown in table 2 there are no striking differences in the facial features between the groups: prominent forehead, mid-face hypoplasia, and small palpebral fissures with antimongoloid slant. From the classification in table 2, we suggest that duplication of 16q22→qter is a critical region for the facial features in partial trisomy 16q syndrome.

This patient also had a small deletion of chromosome 15 (q26.1→ter). There is little precedent for understanding the role this played in her phenotype. Roback *et al.*²⁵ reported a case

Figure 3 PCR amplification of DNA microsatellite repeat sequences from the proband and her parents. Alleles are marked with arrows. (A) Using the primers AFM196xg1 at D16S413, genotypes: proband 1,2,2; mother 1,2; father 1,3. (B) Using Mfd49 primers, genotypes: proband 3,-; mother 2,2; father 1,3.

of 15q26.1→ter and reviewed seven published cases. While most of these cases had some features common to the case we report (intrauterine growth retardation, microcephaly, micrognathia, high arched palate, renal an-

omalies, and failure to thrive), facial features did not include midface hypoplasia or small palpebral fissures with an antimongoloid slant. Furthermore, joint dislocation was not a feature.

The case reported by Nyhan *et al*¹⁹ is karyotypically very similar to ours with a duplication of 16q22→qter and deletion of chromosome 15q26.1→qter. Multiple congenital joint contractures of the hands, wrists, ankles, and feet were a presenting feature of their case. Joint contractures have also been present in a number of other cases reported suggesting a role for genes in this region in the development of distal arthrogryposis. Although joint contractures were not present in our case there was dislocation of more than one joint. Previous cases with congenital dislocation have involved only the hip joints but in our case the knee was also affected. Although our patient had no abnormalities of the distal digits we note some similarity with Larsen syndrome (McKusick No 15025 and 24560), characterised by midface hypoplasia with depressed nasal bridge and prominent forehead, and joint dislocations especially of the knees.²⁶ This may suggest a possible candidate region for the localisation of the abnormal gene responsible for this condition which can be both recessively and dominantly inherited. Alternative, Larsen-like features may have heterogeneous causes. Piequin *et al*²⁷ have previously reported Larsen syndrome in association with two unrelated patients with similar but distinct unbalanced translocations resulting in partial trisomy for 1q and partial monosomy for 6p.

Table 2 Comparison of cases of trisomy 16q with virtually complete or partial duplication of 16q

	Complete		Partial		This case
	p11→qter	q13→qter	q21→qter	q22→qter	
Reference	6-9	10-13	14-16	17-19	0/1
Sex M/F	4/2	2/3	3/0	2/2	0/1
Low birth weight	3	2		1	+
Age of death/last observation	<4mth	<6mth	(2)<3mth	<9y, (1) 3.5y* (1) 22mth*	14d* (1) 3y*
<i>General</i>					
Failure to thrive	6	4	2	2	+
Hypotonia	2	1	1	2	-
Sparse subcutaneous fat	3	1	2	1	-
<i>Head/neck</i>					
Abnormal skull shape	5	2	3	2	-
Antimongoloid slant	6	2	2	1	+
Broad/depressed nasal bridge	4	1	2	3	+
High arched palate	4	2	1		+
High/prominent forehead	5	2	3	4	+
Long, poorly defined philtrum	1	1	1	1	-
Low set/dysplastic ears	6	5	3	1	-
Micrognathia	6	4	1	1	+
Sublingual frenulum	1		1		+
Small palpable fissures	4	2	3	3	+
Thin vermilion borders					+
Thin upper lip	3	1	1	2	+
<i>Extremities</i>					
Congenital dislocation	1		1	3	+
Club feet	2	1	1	1	-
Genital hypoplasia	6				-
Flexion of fingers + other joint contractures	6	2	2	2	-
Rocker bottom heels	1	1			-
Sacral dimple/coccygeal sinus					+
Simian crease	3				+
Small fifth finger/clinodactyly	4	1		2	+
<i>Thorax/abdomen</i>					
Anorectal anomalies	2	1	1	1	+
CHD	4	5	1		-
Undescended testis	3	1	2		-
Intestinal anomalies	2	2			-
Choanal atresia	1				+

CHD = congenital defect. *Alive.

It is clear that further cases of partial trisomy 16q will help to define the karyotype-phenotype relationship and ultimately the genes of importance in this region.

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