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Molecular investigation of a dicentric 13;17 chromosome found in a 21-week gestation fetus with multiple congenital abnormalities

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Abstract. We report a 21-week gestation fetus terminated because of multiple congenital abnormalities seen on ultrasound scan, including ventriculomegaly, possible clefting of the hard palate, cervical hemivertebrae, micrognathia, abnormal heart, horseshoe kidney and a 2-vessel umbilical cord. On cytogenetic examination, the fetus was found to have a male karyotype with 45 chromosomes with a dicentric chromosome, which appeared to consist of the long arms of chromosomes 13 and 17. Molecular genetic investigations and fluorescence in situ hybridization (FISH) unexpectedly showed that the derivative chromosome contained two interstitial blocks of chromosome 17 short arm sequences, totalling approximately 7 Mb, between the two centromeres. This effectively made the fetus monosomic for ~ 15 Mb of 17p without the concurrent trisomy for another chromosome normally seen following malsegregation of reciprocal translocations. It also illustrates the complexity involved in the formation of some structurally abnormal chromosomes, which can only be resolved by detailed molecular investigations.

Genome databases can be used to precisely determine the probe pattern in structural chromosome abnormalities. Recent research using tiling path probes has found that some structural chromosome abnormalities, previously thought to be balanced, have duplications, deletions and inversions around the breakpoint regions that could not be picked up even on a high resolution conventional cytogenetic analysis. These findings show that the formation of some structural abnormalities, especially those associated with an abnormal phenotype, is not as straightforward as previously thought (Wirth et al., 1999; Fantes et al., 2003; Gribble et al., 2005).

Excluding the Robertsonian translocations, autosomal constitutional dicentric chromosomes are rare and the majority of these involve an acrocentric chromosome with a short arm breakpoint (Dewald, 1988) fused to another chromosome in which there is a single breakpoint.

We describe a dicentric 13;17 chromosome found in a 21-week fetus terminated because of multiple congenital abnormalities. During molecular genetic investigations to determine the parental origin of the abnormal chromosome, it was found that an interstitial block from the middle of the chromosome 17 short arm was present between the two centromeres. The PCR markers allowed us to select suitable FISH probes to investigate the breakpoints further.

Materials and methods

Clinical history

An ultrasound scan at 21 weeks gestation showed multiple congenital abnormalities in the fetus of a 24-year-old G_2P_1 woman and as a result the pregnancy was terminated. Although the body size and weight was consistent with a 21-week fetus, the foot length was more in keeping with 17 to 18 weeks gestation. The head was globular and the face was small in comparison indi-

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cating craniofacial disproportion. The eyes were widely spaced and the eyelids were fused. The bridge of the nose was flat and wide with an upturned end. Both ears were small with thickened helical ridges. The philtrum was long and the chin was small suggesting micrognathia. There was clefting of the hard and soft palates and the tongue was small and curved. All the fingers were flexed, with the 5th digit overlapping the 4th and postaxial polydactyly on the left hand. On the right hand the 2nd and 5th digits overlapped the 3rd and 4th. Both feet showed "rockerbottom" feet with outward rotation. No spina bifida could be felt on palpation. The testes were not present in the scrotal sac.

There was a complex of cardiac abnormalities with an ostium secundum type atrial septal defect and a double outflow tract from the right ventricle supplying a large dilated aortic arch. The interventricular septum showed a 2-mm perimembranous ventricular septal defect and there was pulmonary atresia. All the intestines were placed on the left with the caecum located on the midline which was indicative of malrotation. A horseshoe kidney joined at the lower pole was found. The umbilical cord was inserted marginally into the placental disc, but had only a single umbilical artery. The placenta was otherwise normal.

Cytogenetic, FISH and molecular genetic studies

The chromosomes of the abortus were examined from fibroblasts derived from fetal skin. The solid tissue biopsies had been digested by collagenase prior to being seeded onto in situ coverslips and incubated in an open 5% CO_2 and 5% O_2 culture system (Fisher et al., 1996). The metaphases from the fetal fibroblasts and the parental bloods were GTL banded using a modification of Seabright's (1971) method.

FISH (Pinkel et al., 1988) using the probes L1.26 (Dr P. Devilee, University of Leiden, The Netherlands) and 17H8 (Dr H. Willard, Case Western Reserve University, Cleveland) which are specific for the centromeres of chromosomes 13/21 and 17 respectively were used to determine the centromere status of the "fused" chromosome. Microsatellite molecular genetic analysis using DNA extracted from cultured fetal skin fibroblasts and from the parental bloods was used to establish the parental origin of the abnormal chromosome and to identify any chromosome 17 short arm euchromatin. For this, fluorescently labelled primer sets (Table 1) were used to amplify polymorphic microsatellite repeat sequences along the length of the short arm of chromosome 17 (Sambrook et al., 1989) and were analysed on an ABI automated DNA sequencer.

Further FISH was used to refine the 17p breakpoints within the abnormal chromosome. The probes were made from glycerol stocks from which the probe DNA was extracted using rapid alkaline lysis (Sambrook et al., 1989). The DNA was labelled using a nick translation reaction with biotin-16-dUTP for the 17 centromere probe (17H8) and digoxigenin-11-dUTP (Boehringer Mannheim, UK) for the 17 short arm probes (Table 1).

The microsatellite markers and FISH probes were taken from the Ensembl genome browser, release version: V25.34e.1 (1st October 2004), URL: www.ensembl.org/.

Table 1. FISH probes and molecular genetic marker results. Ensembl release version: V25.34e.1 (1st October 2004); URL: www.ensembl.org/

17 band[a]	FISH probe	Marker	Mb from 17pter	Result[b]
p telomere				
p13.3		D17S1866	0.12	Mat deletion
p13.3		D17S849	0.17	Mat deletion
p13.3		D17S926	0.61	Mat deletion
p13.3		D17S1840	0.94	Mat deletion
p13.3		D17S1529	1.03	n/i
p13.3		D17S831	2.11	Mat deletion
p13.3		D17S1798	2.76	n/i
p13.2		D17S1583	2.90	n/i
p13.2		D17S829	3.75	n/i
p13.2		D17S1828	4.01	Mat deletion
p13.2		D17S1584	4.60	Biparental
p13.2	373N8		5.73–5.79	Present
p13.2		D17S1854	5.86	Biparental
p13.2		D17S513	6.03	Biparental
p13.2		D17S1832	6.17	Biparental
p13.2	243K12		6.32–6.47	Present
p13.1	p53		7.77–7.79	Present
p13.1		D17S1353	7.81	Biparental
p13.1	859020		8.90–9.02	Present
p13.1		D17S786	9.01	n/i
p12	165H21		11.45–11.62	Deleted
p12		D17S1875	11.58	n/i
p12	409023		12.93–13.01	Deleted
p12	388F14		13.48–13.65	Deleted
p12	131K5		14.00–14.16	Deleted
p12		D17S1856	14.11	n/i
p12		D17S900	14.39	n/i
CMT		D17S921	14.46	Mat deletion
p11.2		D17S1843	16.26	n/i
p11.2	836L9		20.23–20.35	Deleted
p11.2	381P6		20.86–21.04	Deleted
p11.2	218E15		20.60–20.79	Deleted
p11.2	283C24		20.66–20.86	Deleted
p11.2	64J19		21.23–21.41	Deleted
p11.2	45G12		21.41–21.56	Deleted
p11.2		D17S953	21.69	Mat deletion
p11.2	305L6		21.84–22.04	Deleted
p11.2		D17S1871	22.20	n/i
p11.2	299G20		22.39	Present
centromere				

[a] *CMT*: Charcot-Marie-Tooth type 1 critical region.
[b] Mat: maternal; n/i: non-informative.

Results

Ten metaphases (ISCN 400 bands) examined from fetal skin showed a male karyotype with 45 chromosomes including a "fused" chromosome consisting of what appeared to be the long arms of chromosomes 13 and 17 (Fig. 1a). Both parental karyotypes (10 metaphases each at ISCN 550 bands) showed apparently normal chromosome complements and so the abnormality had arisen de novo in the fetus. The cytogenetic karyotype was reported as 45,XY,dic(13;17)(q10;q10)de novo.

FISH with the 13/21 (L1.26) and 17 (17H8) centromere probes showed that the abnormal chromosome contained both the 13 and 17 centromeres and that there was intervening chromatin between them (Fig. 1b). The 17 centromere probe was consistently seen as a "double chromatid" signal, whereas the 13 centromere looked more like the signal seen in a monocentric chromosome. This suggests that the 17 centromere was suppressed and the 13 centromere was active.

The microsatellite molecular genetic analysis showed that the dicentric chromosome was maternal in origin. However, although it showed that the 17p proximal breakpoint was near the centromere/euchromatin border, unexpectedly, it also showed that there was also disomy for a block of euchromatin derived from 17p13 (Table 1). The results obtained from the markers used for PCR facilitated the selection of probes for the additional FISH studies (Fig. 1). Using probes from the Ensembl tiling path clones, the proximal breakpoint was refined to being less than 0.35 Mb from the centromere/17p euchromatin border. Furthermore, the approximate 7 Mb interstitial block from 17p13 was found to be present between the centromeres of the dicentric chromosome, with the breakpoints lying between 4.01–4.6 Mb (distal) and 9.02–11.45 Mb (proximal) measured from the telomere of the short arm. The molecular

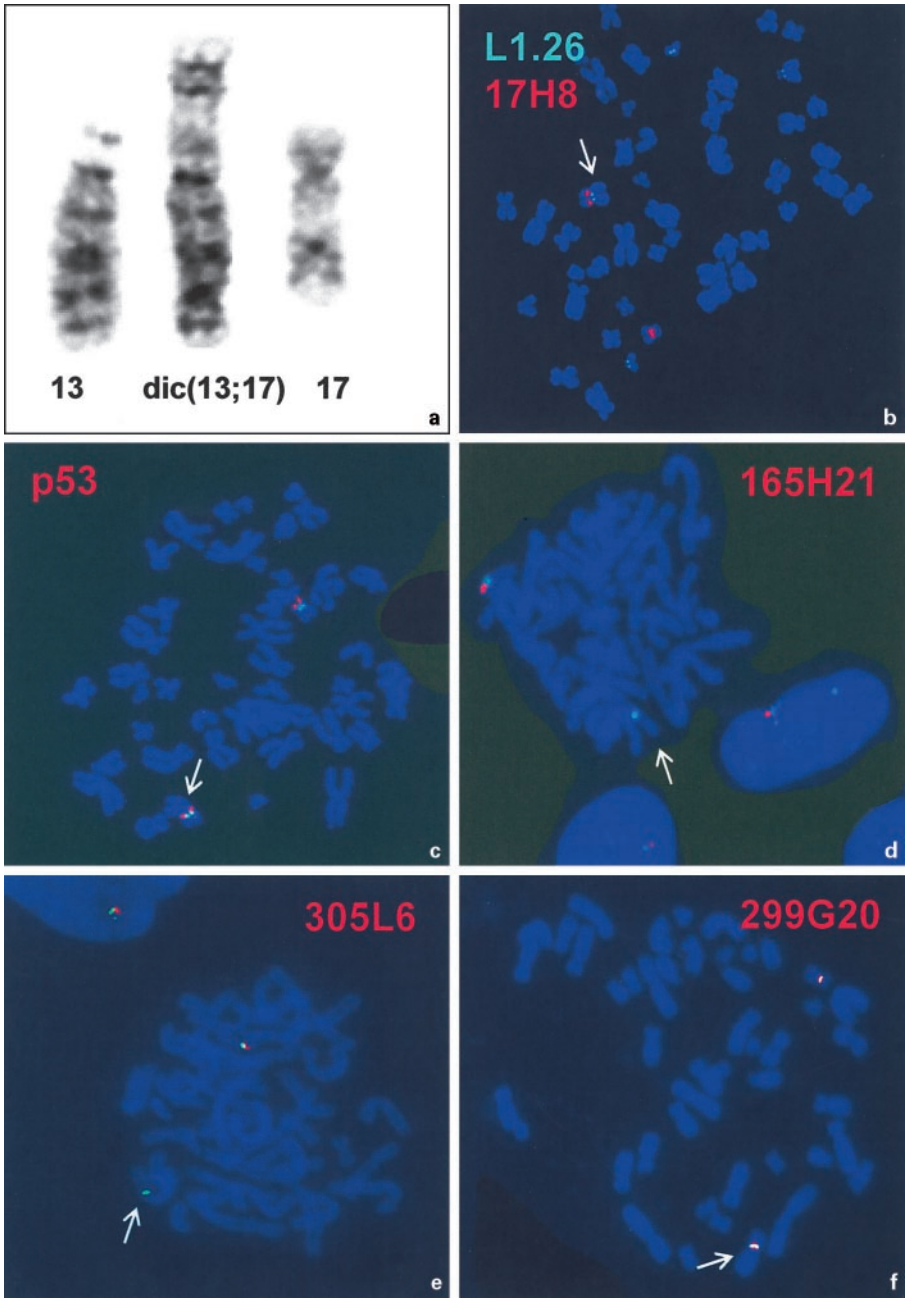

Fig. 1. (**a**) Partial G-banded karyotype showing the dicentric 13;17 chromosome and the normal homologues. (**b**) FISH with chromosome 13 (L1.26) and 17 (17H8) centromere probes showing the chromosome is dicentric. The splayed 17 centromere signal indicates that this centromere is probably suppressed. Note that probe L1.26 cross hybridizes to chromosome 21. (**c–f**) The 17 centromere probe (17H8) is labelled green, the 17p probes are red. (**c**) Probe for *TP53* gene is present. (**d**) Probe 165H21 deleted proximal to the interstitial block. (**e**) Probe 305L6 deleted distal to the centromeric/euchromatic border region. (**f**) Probe 299G20 is present at the centromeric/euchromatic border, the 17 centromere probe and 299G20 being so close together that they appear as a fused signal. The dic(13;17) is indicated by arrows.

genetic and FISH results were concordant and are shown fully in Table 1. The definitive karyotype was given as 45,XY, dic(13;17)(13qter→13p11::17p13.2→17p13.1or12::17p11.2→17qter).ishdic(13;17)(L1.26+,373N8+,p53+,859O20+,165H21–,305L6–,299G20+,17H8+).

Discussion

The most common dicentric chromosomes ascertained in man are the non-homologous Robertsonian translocations, which are seen in newborn surveys at a frequency of approximately 1 in 1000 (e.g. Hammerton et al., 1975). These consist of the long arms of two acrocentric chromosomes which are usually joined at their short arms (Bandyopadhyay et al., 2002) and are effectively genetically balanced. The Robertsonians are followed in frequency by dicentric isochromosomes formed by the sex chromosomes. Other constitutional dicentric chromosomes involving autosomes are found only rarely in patients and the vast majority involve an acrocentric chromosome with a short arm breakpoint as in our case (Dewald, 1988). However, all dicentrics show both centromere signals using in situ hybridization with the appropriate centromere probes. The two centromeres may be close enough that they act as one or they may even be fused together. If there is a separation between them, one will usually be suppressed or otherwise they may

both be active during cell division which may result in instability of the dicentrics in the daughter cells.

A dicentric chromosome is thought to form by a single break occurring in an arm of each chromosome and the pair fusing to become one chromosome, with the acentric chromatin being lost. In contrast to this simple model, our dicentric chromosome has three breakpoints in the 17 short arm and a minimum of one in the chromosome 13 short arm, resulting in the retention of a small (less than 0.35 Mb chromatin) block of 17p euchromatin at the centromere/euchromatin border and another larger (approximately 7 Mb) block from 17p13.1 in the intervening space between the 13 and 17 centromeres. Recent research using tiling path probes has found that some structural chromosome abnormalities, previously thought to be balanced, have submicroscopic deletions, inversions and duplications around the breakpoint regions not visible on high resolution cytogenetic analysis (Gribble et al., 2005). These findings show that the formation of some structural abnormalities, are more complex than previously thought (Wirth et al., 1999; Fantes et al., 2003).

The dicentrics seen in patients with congenital abnormalities provide a rare opportunity to study the phenotypic effects of chromatin deletion without a concurrent trisomy as seen with the malsegregation of the majority of reciprocal translocations. Two major cytogenetic deletion syndromes are located on the short arm of chromosome 17. One is Smith-Magenis syndrome (Smith et al., 1986), which is a contiguous gene syndrome associated with a deletion of band 17p11.2. The phenotype commonly includes sleep disturbances, self destructive and aggressive behaviour as well as eye, ear, genitourinary and cardiovascular anomalies, differences in the phenotype being attributed to the varying number of contiguous genes that are deleted. The second, Miller-Dieker syndrome (Miller, 1963; Dieker et al., 1969) is characterized by lissencephaly and characteristic facial features including prominent forehead, flat midface, short nose with upturned nares, protuberant upper lip and small jaw. It is associated with a visible or submicroscopic deletion of band 17p13.3. The genes responsible for the above two syndromes are deleted in the dicentric chromosome in our patient. Although some of the features in the above syndromes, for example the behavioural characteristics, would not be manifest until after birth and others such as lissencephaly would not be evident in a fetus of this gestational age, the heart (Greenberg et al., 1996) and renal defects and some facial features, including cleft lip and palate, of both the Smith-Magenis and Miller-Dieker syndromes are present. Conversely, the tumour suppressor gene *TP53*, located at band 17p13.1, is present within the interstitial block of euchromatin present between the two centromeres. Mutations of this gene are thought to be present in the majority of human cancers. It acts to eliminate cells with DNA damage and tumour cells in which it has been inactivated have a selective growth advantage.

The use of a genome database allowed us to precisely determine which genes are monosomic, and the pregnancy we describe is monosomic for approximately 15 of the 22.4 Mb euchromatin comprising the short arm of chromosome 17. This loss amounts to approximately 0.6% of the haploid autosomal length. Given the phenotypic abnormalities present in our case, even though the fetus was still viable with normal growth rates until the termination of pregnancy at 21 weeks gestation, it is unlikely that he would have survived to term.

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