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ARTICLE

Cryptic subtelomeric translocation t(2;16)(q37;q24) segregating in a family with unexplained stillbirths and a dysmorphic, slightly retarded child

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We here describe a submicroscopic translocation affecting the subtelomeric regions of chromosomes 2q and 16q, and segregating in a family with stillbirths, early pregnancy losses, and two dysmorphic and slightly retarded babies. FISH analysis showed a 46,XY,der(2)t(2;16)(q37.3;q24.3) in the propositus, and a balanced t(2;16) in his mother, her conceptus and maternal grandfather. FISH with YACs and BACs made it possible to map the 2q37 breakpoint precisely between the regions covered by y952E1 and y746H1, and the 16q breakpoint between the regions encompassed by bA 309g16 and bA 533d19. The contribution of 2q37.3 monosomy and 16q24.3 trisomy to the proband's phenotype is compared with that in reported patients with similar imbalances of either chromosome.
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Introduction

Cytogenetic molecular studies have demonstrated the presence of cryptic unbalanced chromosome rearrangements affecting chromosome telomeric bands in more than 7% of all cases with severe and 0.5% with mild mental retardation.[1,2] These chromosomal imbalances are due to a malsegregation of familial cryptic translocations in half of the cases.[3]

Screening approaches for the simultaneous analysis of all subtelomeric regions have been developed[4,5] and rearrangements of these regions, that may go undetected by conventional cytogenetic studies, have been identified by using subtelomeric FISH probes.

We here describe a family in which a submicroscopic translocation affecting the subtelomeric regions of chromosomes 2q and 16q was found to have segregated in an unbalanced form to a 15-month-old male with mild craniofacial dysmorphisms and psychomotor retardation: the mother and maternal grandfather carry the balanced form. A subsequent prenatal diagnosis on amniotic fluid cells allowed the detection of a balanced foetus.

Family report

The family tree is shown in Figure 1.

The index case (IV-1) was a 15-month-old male who presented microcephaly, bitemporal constriction, a flat occiput, inner epicanthal folds, short palpebral fissures, periorbital oedema, a beaked nose, a long philtrum, thin lips, a high and narrow palate, and long fingers. His serum

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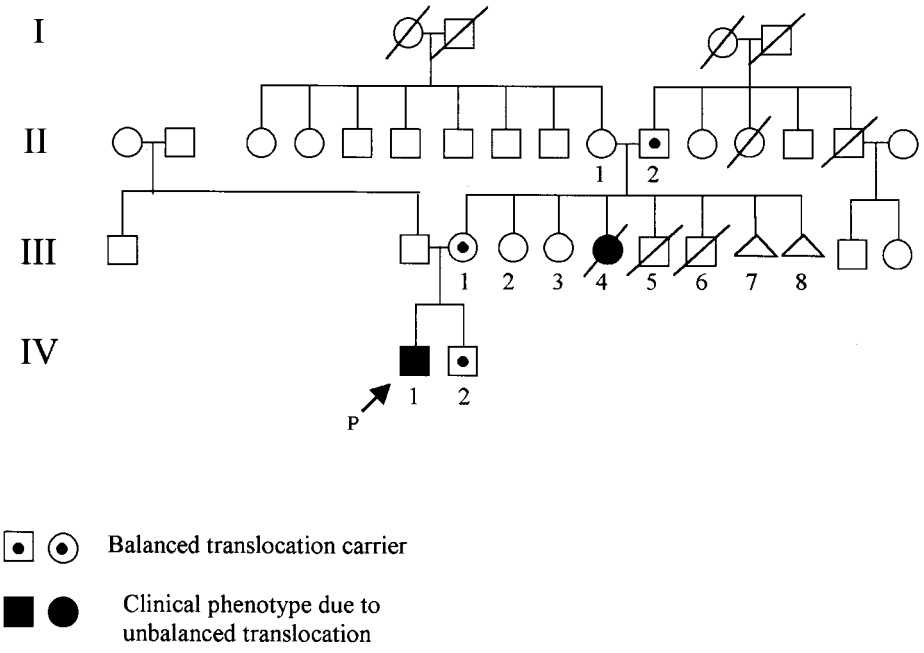

Figure 1 Family pedigree. The symbols with a dot refer to individuals carrying a balanced (2;16) translocation; the full symbol refers to the dysmorphic proband carrying an unbalanced form of the translocation. Stillborn (III-5 and 6), a stillbirth (III-7) and a spontaneous abortion (III-8) occurred in the progeny of the maternal grandfather.

calcium and phosphate levels were normal, as were the X-ray findings.

During the neonatal period, a cardiological evaluation revealed an ostium secundum inter-atrial defect and a neurological examination showed mild trunk and neck hypotonia. The baby has undergone physiotherapy and is now able to dram himself and say his first words. During his first year of life, he presented a number of respiratory infections.

The family history includes two stillbirths (with suspected cardiac and cerebral malformation) (III-5; III-6), a child who died at 14 months-of-age (III-4) with the same phenotype as the index case (IV-1) and early pregnancy losses (III-7; III-8). The maternal grandfather (II-2) is affected by age-related macular degeneration (AMD).

Cytogenetic studies

Conventional karyotyping of the proband at birth did not reveal any chromosome abnormality. At the time of our evaluation, a cytogenetic analysis was made using routine QFQ-banding techniques on cultured peripheral lymphocytes. Simultaneous FISH with chromosome-specific subtelomeric probes was performed according to the manufacturer's specifications (Cytocell, Ltd., Oxford, UK).

FISH of spreads from the proband's mother showed that one chromosome 2q was devoid of the specific hybridisation signal, which was observed on chromosome 16q (Figure 2a), whereas the 16q telomeric probe did not show any signal on one 16q telomere, but gave a signal on 2q (Figure 2b). The mother's karyotype could therefore be defined as 46,XX,t(2;16)(q37.3;q24.3).

FISH on the proband's metaphases using the 2q subtelomeric probe revealed a lack of hybridisation on both chromosomes 2 (Figure 2c), which is due to the deletion of the 2q telomere as a result of translocation malsegregation and the simultaneous presence of a variable number tandem repeat (VNTR) polymorphism on paternal chromosome 2 which, as reported,[6,7] hampers the detection of hybridisation signals. FISH using the 16q probe revealed three hybridisation spots: two on chromosomes 16q and one on 2q, corresponding to the 16q translocated segment (Figure 2d). The patient's karyotype could then be corrected to 46,XY, der(2)t(2;16)(q37.3;q24.3).

The propositus therefore inherited the unbalanced 2;16 translocation that led to monosomy for the distal 2q and trisomy for the distal 16q from his mother.

In order to define the 2q37 breakpoint, FISH studies were performed using YAC clones belonging to the WC2.16 contig, which were selected from the Genome Database (http:/www.genome.wi.mit.edu). All of the probes were labelled by means of nick-translation with biotin or digoxigenin (Roche, Switzerland). The FISH analysis was performed according to Lichter and Cremer,[8] with minor modifications. Table 1 lists the YACs used ordered from cen to tel, their anchored markers, and the FISH results on der(2). As can be seen in Table 1 and Figure 3a,b, the 2q37 breakpoint

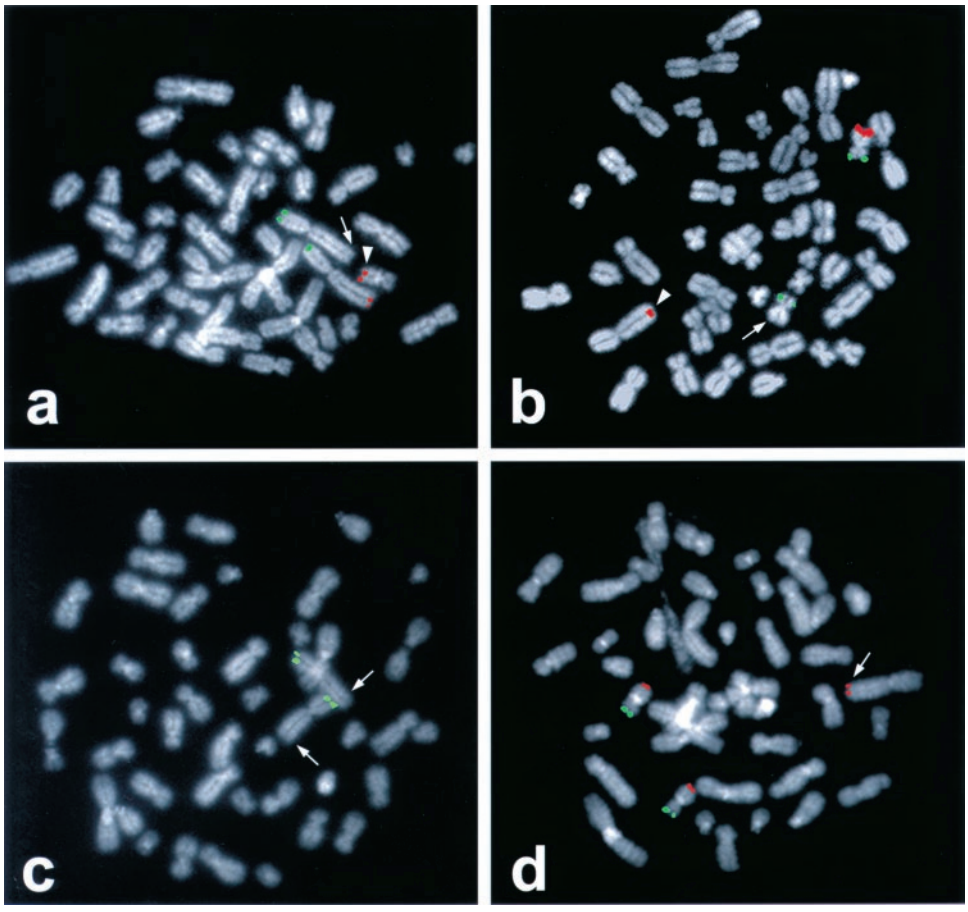

Figure 2 FISH on chromosomal spreads from mother (**a;b**) and proband (**c;d**) with 2pter/2qter (green signals) and 16pter/16qter probes (red signals). (**a**) As indicated by the arrow, the expected red signal on chromosome 2q is missing and translocated to distal 16q (arrowhead); (**b**) the expected red signal on chromosome 16q is missing (arrowed) and can be seen on 2q (arrowhead). (**c**) No red fluorescent signals can be seen on both 2q telomeres (arrowed): a benign paternally derived 2q telomeric polymorphism account for the lack of fluorescence on normal homologue. The expected green signals are present on 2p; (**d**) an extra 16qter specific red signal can be seen on chromosome 2q (arrowed).

could be localised between the regions covered by y952E1 (to which AFMB341XG5 is anchored) and y746H11, which contains the WI-11222, WI-932, D2S1283 and WI-4332 loci. FISH of YAC 792E1 (16q23), belonging to the WC16.11 contig revealed a hybridisation signal on der(16), thus indicating that the 16q breakpoint is distal to the region covered by the clone (data not shown). Following FISH of BACs A 309g16 (16q24.1) and A 533d19 (16q24.3) (from CCAP library and selected from: http://biologia.uniba.it/rmc) on the proband's metaphases, bA 309g16 hybridised on both chromosomes 16 but not on der(2) (Figure 3c), whereas bA 533d19 showed an additional signal corresponding to the translocated 16q segment on der(2) (Figure 3d). We were therefore able to map the 16q breakpoint to between WI-81647 (anchored to bA 309g16) and SG-31080 (anchored to bA 533d19), and to restrict the proband's trisomic region to the segment between SG-31080 and the 16q telomere.

FISH studies of other family members revealed the presence of the balanced translocation in the maternal grandfather's karyotype, whereas a normal karyotype was found in the two maternal aunts (III-2; III-3). The proband's mother has undergone a prenatal diagnosis: FISH analyses of amniocyte metaphases using the chromosome-specific 16qter probe (Cytocell, Ltd., Oxford, UK) and the 2q subtelomeric YAC 890F11 showed that the male foetus has inherited the maternal balanced translocation t(2;16)(q37;q24).

Discussion

The role of cryptic rearrangements involving the telomeres as a major cause of unexplained mental retardation and congenital anomalies is well documented.[2,9,10] Since the first description,[11] the number of

reports has rapidly increased and also include prenatal diagnoses.[12–14] Pitfalls of the telomere specific probes have also been pointed out, suggesting caution in the interpretation of the results to exclude the possibility of a benign familial polymorphism segregating in the family.[15]

In our case, conventional karyotyping at birth did not reveal any chromosomal abnormality. However, the family

Table 1 YAC FISH results on der(2) chromosome

YACs name	*Localisation*	*Markers*	*FISH signals on der(2)*
929G1	2q36	D2S2158-CHLC.ATA20H03-WI-5779-WI-7328	+
961F11	2q36	D2S2213-D2S2297-D2S341	+
885A11	2q36	WI-9093-WI-8692-D2S172-NIB1194	+
964A8	2q36	CHLC.GATA67B01-D2S2276-WI-7767-WI-8964	+
783D10	2q36	D2S331-D2S206-D2S2348-AFMB341XG5-	+
847E5	2q36	D2S2348-AFMB341XG5	+
952E1*	2q37	AFMB341XG5	+
746H11*	2q37	WI-11222-WI-932-D2S1283-WI-4332	–
854C9	2q37	D2S338-WI-3425-WI-6437	–
890F11	2q37	D2S338-WI-3425-WI-6437	–

Loci are reciprocally ordered as shown in the MIT web site (http://www.genome.wi.mit.edu). According to ISCN 95, the following symbols are used: + Stationary signal (signal remaining at original location); – Absent signal; * YACs used for FISH analyses shown in Figure 3.

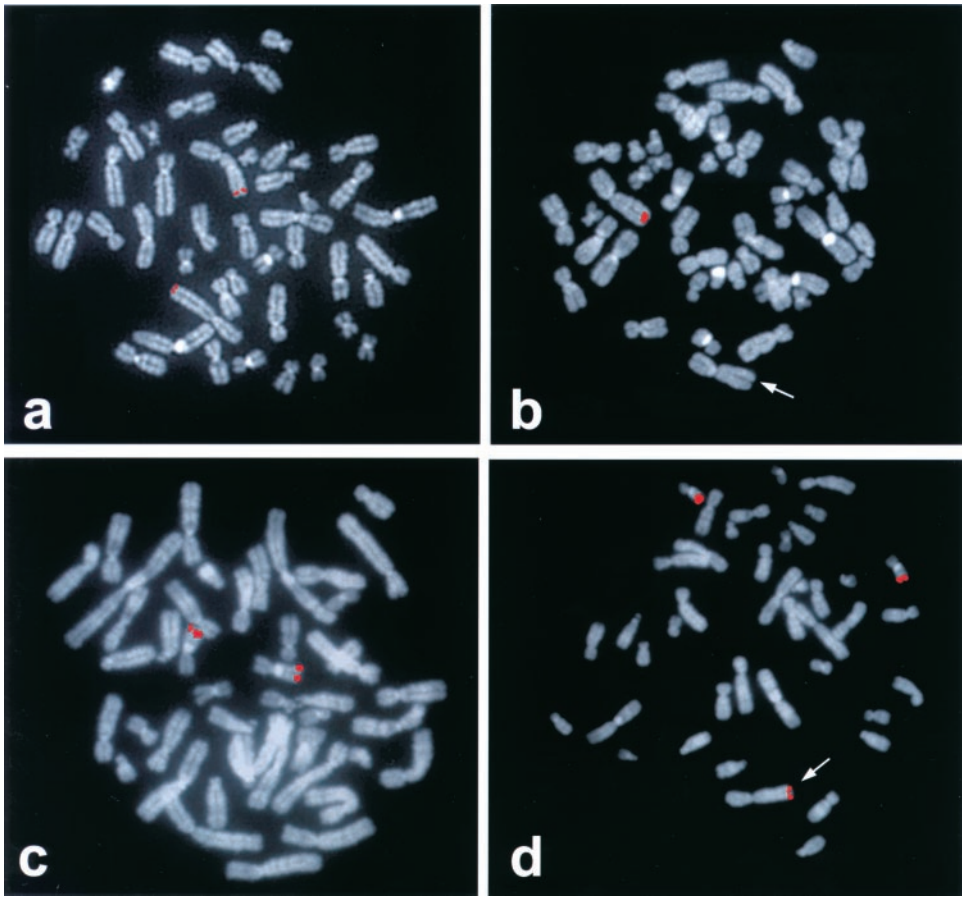

Figure 3 FISH mapping of 2q37 and 16q24 breakpoints on proband metaphases. The breakpoint on 2q37 can be localised between the regions covered by (**a**) y952E1, which shows a signal on both chromosomes 2, and (**b**) y746H11, which has no signal on one chromosome 2 (arrowed). The breakpoint on 16q24 is presumed to be between the regions covered by (**c**) bA309g16, which shows a signal on both chromosomes 16 but not on der(2), and (**d**) bA533d19, which shows three signals: two on chromosomes 16q and another on distal 2q (arrowed).

history of stillbirths, early pregnancy losses and a dysmorphic child suggested the segregation of a familial translocation. FISH confirmed this hypothesis and led to the identification of an unbalanced cryptic t(2q;16q) translocation in the proband. The same unbalanced translocation causing monosomy of distal 2q region and concurrent trisomy of distal 16q may have been present in his maternal aunt (III-4, in Figure 1), who had the same craniofacial dysmorphisms.

Unrelated patients with a 2q37 deletion and a phenotype consistent with Albright's hereditary osteodystrophy (AHO) have recently been described.[16–18] However, the absence of AHO signs or an unclear association between AHO and a terminal 2q37 deletions have been reported.[3,19]

Some of the cases with a distal 2q deletion show aggressive behaviour with self-mutilation,[18,20,21] and it has been assumed that a dose-sensitive gene affecting behaviour is located in the distal 10 cM of 2q.[21] Our patient currently shows no clinical signs of AHO and his behaviour is not aggressive. However, it is known that, like aggressive behaviour, the craniofacial dysmorphism and radiological characteristics of AHO usually become gradually apparent during later childhood. The contribution of trisomy 16q to our patient's phenotype is difficult to assess because all the previously reported cases had a larger trisomic region. To the best of our knowledge, trisomy 16q23-qter is the smallest so far described without associated monosomies.[22] This patient presented with hypotonia and dysmorphic features (a high forehead, a small and narrow nose with a depressed nasal bridge, and hypertelorism), only a few of which are shown by our patient.

The proband carried also a paternally derived chromosome 2 benign variant , consisting in a deletion in the 2q subtelomeric region, which has been reported as the most frequent telomeric polymorphism affecting $\sim$5% of the population.[15]

Our study confirms the usefulness of investigating cryptic rearrangements in families with a history of unexplained pregnancy losses, stillbirths and progeny with mental retardation with or without associated dysmorphisms, in order to define the genetic mechanism responsible for all these events. As in the case of our family, it can offer the practical advantage of a prenatal diagnosis. Furthermore, the refined mapping of both subtelomeric breakpoints achieved in our patient may help to assess the contribution of 2q37 and 16q24 bands to the clinical phenotype of this and other patients carrying similar imbalances.

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