ELECTRONIC LETTER

Interstitial telomeres of an inv(9)(p11.2;q34) involved in a jumping translocation found in a woman through a stable unbalanced translocation in her malformed child

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According to the original definition by Lejeune *et al*,[1] a constitutional jumping translocation (JT) is a non-reciprocal, unstable translocation of a specific chromosomal segment onto the ends of various chromosomes, which leads to a rearranged chromosome with interstitial telomeres. More than 20 cases of constitutional JTs have been reported so far,[2–6] and JTs have also been detected in chromosomal instability syndromes[7 8] and in about 20 tumours, mainly of the haematological type.[9]

Most cases of constitutional JTs involve acrocentric chromosomes as either donors or recipients, with a few balanced and unbalanced Robertsonian translocations. Whatever the partner chromosomes, the chromosomal regions around the majority of JT breakpoints are pericentromeric and subtelomeric and are built up of arrays of tandemly repeated sequences.[4] In particular, the qh region of chromosome 1 seems be non-randomly involved in the JTs detected in most haematological neoplasms.[9]

We report here the case of a woman carrying JTs in which the donor segment originated from the short arm of chromosome 9. A particular aspect of this case is the presence of an inv(9)(p11.2;q34) chromosome in the constitutional karyotype, and the transmission of the most often represented JT (9;17)(p11.2;qter) to her malformed child.

MATERIALS AND METHODS

Case report

The infant is the first and only child, spontaneously delivered at term after an uneventful pregnancy when the mother was 38 years old. His birth weight was 2720 g (<10th centile), length 49 cm (<25th centile), and head circumference 35 cm (50th centile). As a newborn, he showed facial dysmorphism including hypertelorism, a flat nasal bridge, and low set ears, bony abnormalities of both hands and feet (camptodactyly and talipes equinovarus with partial syndactyly of the second and third fingers), and unilateral cryptorchidism. During the first postnatal week, he experienced mild respiratory distress and oliguria. Echocardiography showed a patent foramen ovale and ductus arteriosus, but these were subsequently spontaneously repaired. At the age of 3 years, psychomotor and growth retardation and lack of verbal ability and visual contact became evident. At the age of 4 years, both his weight and height were between the 3rd and 10th centile; an evaluation according to Griffith's scale showed abilities equivalent to those of a child aged less than 2 years and severe language impairment with pluridyslalia. His face was still dysmorphic with scaphoplagiocephaly, genu valgum, and flat feet.

Pedigree analysis did not indicate any genetic disease or congenital defects. The family was not available for clinical and laboratory examination.

Cell cultures and chromosome preparations

Phytohaemagglutinin stimulated peripheral blood lymphocytes were set up in culture from independent samples using Chromosome Kit "Synchro"(Celbio) and modified RPMI (Irvine Scientific) plus 5% fetal calf serum (Gibco). The cultures were stopped with colchicine after 72 hours. The chromosome preparations were obtained using a standard technique and analysed by GTG, RBA, and QFQ banding. The karyotypes were reconstructed following the guidelines of the International System for Chromosome Nomenclature 1995.[10]

YAC preparation

The YAC clones 727d12, 750h11, 882f4, 930f8, 922g2, 929g12, 819h8, 823g12, and 763a12 from the CEPH library (http://www-genome.wi.mit.edu/) were obtained from the DIBIT-HSR resource centre (Milan, Italy). The cultures from single colonies were grown in selective AHC medium and resuspended in 100 µl LMP (Biorad) agarose plugs containing 7×10^7 cells.

Key points

- Jumping translocations (JTs) are rarely detected in the constitutional karyotype, but may represent the fortuitous outcome of low grade mosaicism shown by means of their deleterious reproductive consequences. Following the birth of a malformed newborn carrying a der(17)t(9;17)(p11.2;qter) leading to 9pter-p1.2 trisomy, a 46,XX,inv(9)(p11.2;q34) karyotype was observed in the mother, prompting us to further study.
- The finding in the mother of a cell with the same t(9;17) along with a 9pter-p11.2 deletion of the inverted chromosome and subsequent scoring of 2598 metaphases allowed the detection of nine different JTs, of which t(9;17) was the most frequent (2%). In all JTs, the same donor chromosomal segment generated from the q34 site harbouring interstitial telomeric sequences of the unstable inv(9) was found to be involved.
- FISH characterisation of the mother's inv(9) made it possible to map the 9p inversion breakpoint between the centromere and p11.2.
- Double FISH with a p11.2 localised y763a12 and the 9q telomere specific probe showed overlapping signals on the rearranged maternal 9 and the child's der(17). FISH with the telomeric-like sequence (T_2AG_3) showed signals on the normal chromosome 9 at p11.2, indicating the involvement of internal telomeric DNA in the rearrangement leading to the unstable inv(9). The putative sequence of events leading from the inverted chromosome 9 to JTs on different recipient chromosomes is discussed. Fixation of the prevalent JT 9;17 in a primordial maternal germ cell traces its origin back to the early blastocyst stage.

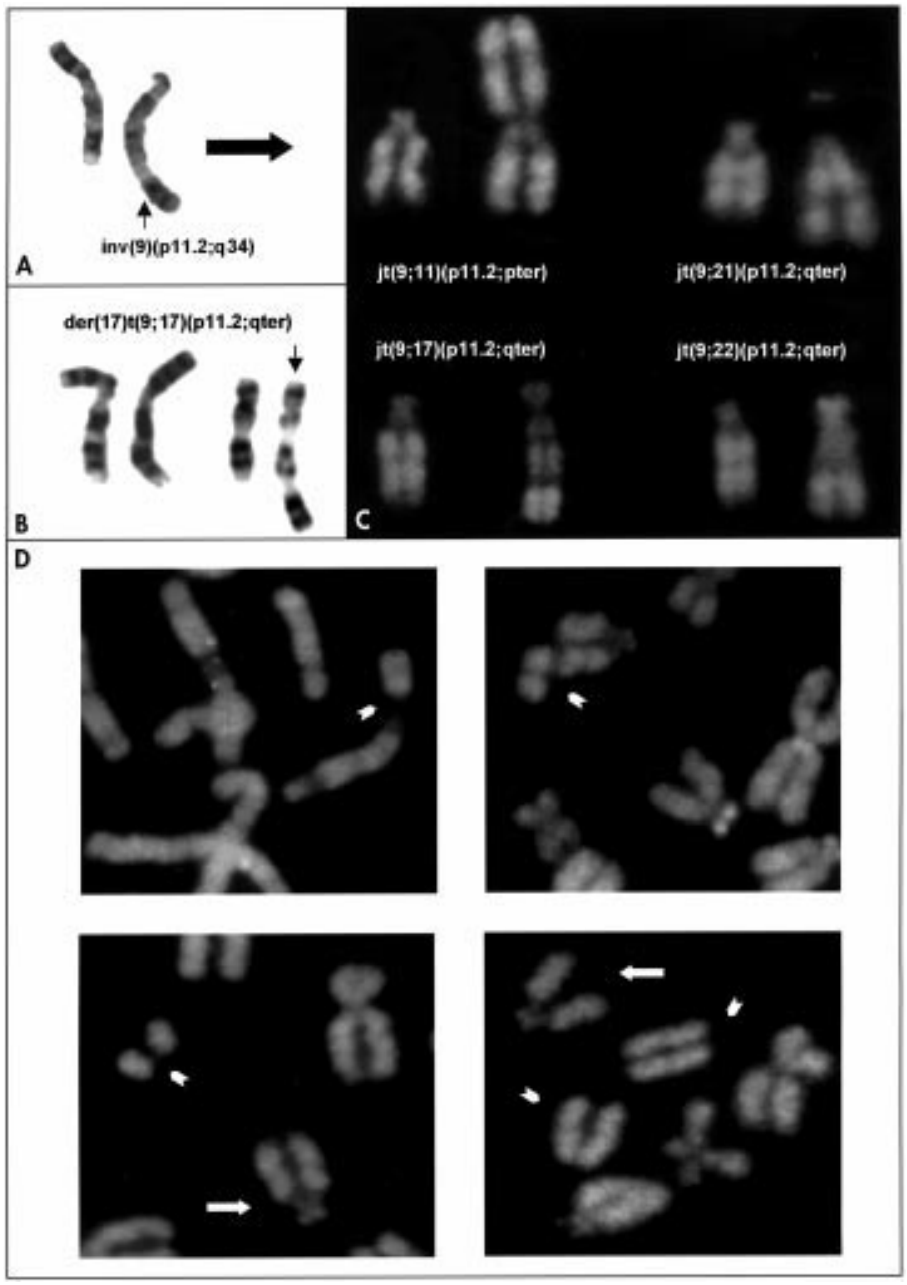

Figure 1 (A) Partial constitutional GTG karyotype from the normal woman showing the inv(9) to the right. (B) GTG banded chromosome 9 and 17 pairs from the woman's child showing the der(17) chromosome leading to 9p trisomy. (C) Partial QFQ chromosome pairs showing a del(9) on the left and, from top to bottom on the right, a few JTs including the one transmitted to the child. (D) Partial QFQ metaphases showing fragility manifestations at inv(9): the arrows indicate inv(9) with a chromosome gap at 9q34 (top left), inv(9) with a chromatid break at 9q34 (top right), del (9p) (bottom left) and the ace in the same metaphase, del(9p) and two ace i(9)(p10) (confirmed by FISH using specific 9p telomeric probes) (bottom right).

The Alu 153, 154, 451, 450 primers[11] were used for the specific amplification of YAC DNA from a total yeast DNA preparation. The PCR reactions contained 2 µl of the molten yeast plug as a template, 1 µmol/l of Alu (153, 154, 451, 450) primers, 1 U *Taq* polymerase (Bioline), 1.5 mmol/l $MgCl_2$, and 200 µmol/l dNTPs in a total reaction volume of 50 µl. Thirty-five PCR cycles were performed: 94°C for one minute, 55°C for one minute, and 72°C for two minutes.

FISH

DNA from YACs and a mixture of long synthetic $(T_2AG_3)n$ fragments (1-20 kb) (able to detect very short repeats as well), provided by Dr E Giulotto,[12] were resuspended in 100 µl of Tris 10 mmol/l, EDTA 1 mmol/l, and 1 µg of each probe was labelled with digoxigenin-dUTP (Boehringer Mannheim) using a nick translation kit (Boehringer Mannheim). One hundred nanograms of labelled probe were precipitated with 10 µg of salmon sperm and 5 µg of Cot-1 DNA (Boehringer Mannheim), and resuspended in 15 µl of hybridisation buffer (50% formamide, 2 × SSC, 10% dextran sulphate, 0.1% Tween 20). The probes were denatured at 72°C for seven minutes and then preincubated at 37°C for 30 minutes. Chromosomal spreads from peripheral blood lymphocytes were denatured in 70% formamide, 2 × SSC for two minutes, and then immediately dehydrated in an ethanol series (70%, 90%, and 100%).

We performed FISH with the following commercial probes: whole chromosome painting (WCP) 9 and 17 (Oncor), 9 specific α satellite (Vysis), 9 specific β satellite (Oncor), 9 specific classic satellite (Oncor), all human centromeres (Oncor), all human telomeres (Oncor), and 9p and 9q specific subtelomeres (Cytocell).[13]

The FISH experiments were performed according to standard procedures.[14] Only the first layer of the detection protocol was followed using the fluorescein labelled detection of digoxigenin labelled probes (Oncor). The chromosomes were counterstained with DAPI 0.1 µg/ml in antifade (Oncor), and then visualised using a Leitz DM-RB microscope equipped for DAPI and FITC/TRITC epifluorescence optics. The images were

Table 1 Jumping translocations and chromosome aberrations in peripheral blood and lymphoblastoid cell line from the proband's mother

	Metaphases No (%)				
	9;inv(9)	JTs* all types	JT 9;17	Fragility	Total No
PBL cultures (1st sample) RPMI medium	680 (96.9)	5† (0.7)	14 (2)	3 (0.4)	702
PBL cultures (2nd sample) folic free medium	1284 (96.9)	5‡ (0.5)	30 (2.2)	4 (0.3)	1322
Lymphoblastoid cell line	572 (99.4)	2§ (0.5)	0	0 (0)	574

*JT recipient chromosomes:
†5qter, 8pter, 11pter, 21qter, 22qter.
‡1qter (2 cells), 2qter (2 cells), 19pter.
§2qter (2 cells).

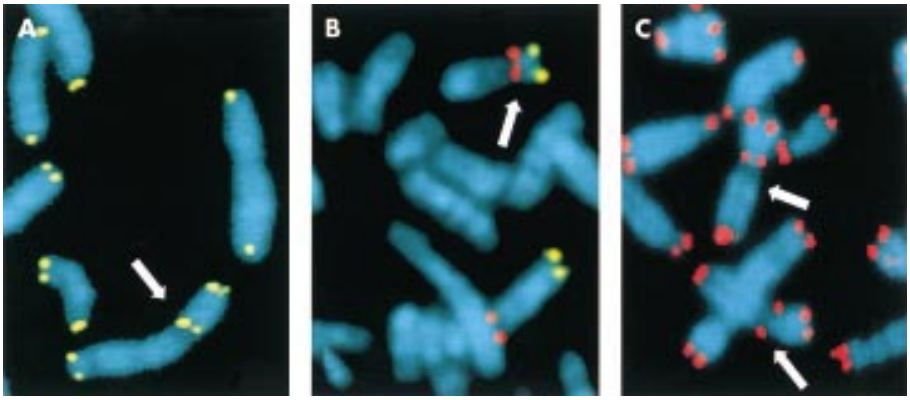

Figure 2 Distribution and specificity of telomeric sequences. (A) FISH with all human telomeric probes gives the expected signals on the tips of the inv(9) chromosome plus an interstitial signal at 9q34 (arrowed). (B) Dual FISH with specific probes for 9p (green fluorescence) and 9q (red fluorescence) subtelomeres shows that the p telomere in the inv(9) chromosome has moved to qter and the q telomere is interstitial; the pter of the inv(9) chromosome is devoid of 9 specific telomeric signals. (C) FISH of the synthetic polynucleotide T_2AG_3 probe, which hybridises all telomeres and also detects target sequences on a normal chromosome 9 at 9p11.2 in a few cells.

captured by means of a CCD camera (Hamamatsu 3CCD Camera, C5810) and visualised using Highfish software (Casti Imaging).

RESULTS

Cytogenetic analysis

Karyotype analysis of the child showed a 46,XY,der(17)t(9;17)(p11.2;qter) karyotype: the unbalanced 9;17 translocation was found in more than 100 cells analysed (fig 1B). The involvement of chromosomes 9p and 17q was suggested by QFQ, RBA, and GTG banding. The father's karyotype was normal showing the rare non-heterochromatic 9p12 variant[15] inherited by the child (right hand chromosome 9 in fig 1B); in contrast the mother's included a pericentric inversion of chromosome 9. The 9p breakpoint was apparently located near the centromere and the 9q breakage affected the 9q34 band. Consequently a nearly whole 9p arm was transposed to the tip of 9q (fig 1A).

The fortuitous finding of a cell with the same t(9;17) in the mother's chromosome spreads stimulated further investigations. The scoring of 702 metaphases from the woman's lymphocyte chromosome spreads showed six JTs having the 9p fragment atypically positioned on the rearranged chromosome 9 of different chromosomes, as indicated in table 1 and shown in fig 1C. The transposition of 9p to (mainly q) telomeric regions apparently led to the generation of balanced rearranged chromosomes (fig 1C). All of the JTs were found in single cells, with the exception of t(9;17) which was identified in 2% of the metaphases (table 1). Fragility figures documenting the transition pathway from the unstable inverted chromosome 9 to the JTs were also detected (table 1, fig 1D). After three months, the scoring of 1322 metaphases from lymphocytes cultured in folic free medium confirmed the previous cytogenetic findings; a few variations were apparent, but the JT (9;17) was observed with similar frequency (table 1). The establishment of a lymphoblastoid cell line and the analysis of a sample of 574 metaphases made it possible to detect only the JT 2qter/9p, which was observed twice in the second sample (table 1).

FISH studies

Dual FISH with WCP9 and WCP17 showed that the t(9;17) present in all of the metaphases from the child and a few cells (2%) from the mother was painted by WCP9 in distal 17q. In order to characterise the structure of the maternal inverted chromosome 9, FISH studies were performed using a set of probes, including 9 specific α satellite, β satellite, classic satellite, and the alphoid sequence conserved among all human centromeres, and which all together recognise different classes of repeated sequences within the centromeric and pericentromeric regions of chromosome 9. The results obtained indicated that none of the investigated sequences are involved in the t(9;17) chromosomal rearrangement (data not shown). Conversely, FISH with the probe for all human telomeres showed the two expected pter and qter hybridisation signals on the mother's inverted chromosome 9, and an additional interstitial signal at 9q34 (fig 2A). Interstitial signals were also apparent on the JTs (9;17)(p11.2;qter), inherited by the malformed child, (9;2)(p11.2;qter), and (9;19)(p11.2;qter) (data not shown). Furthermore, FISH experiments using 9p and 9q specific subtelomeric probes showed the lack of any hybridisation signal on 9p of the mother's inv(9) chromosome, an interstitial subtelomeric 9q specific signal, and a 9p specific signal at the tip of the long arm (fig 2B). Two 9pter YACs (727d12 and 750h11) gave signals at the qter region of inv(9), thus confirming the inverted translocation of 9p onto 9q (data not shown).

As a telomeric-like sequence (T_2AG_3) has been reported to be present in interstitial regions of specific chromosomes,

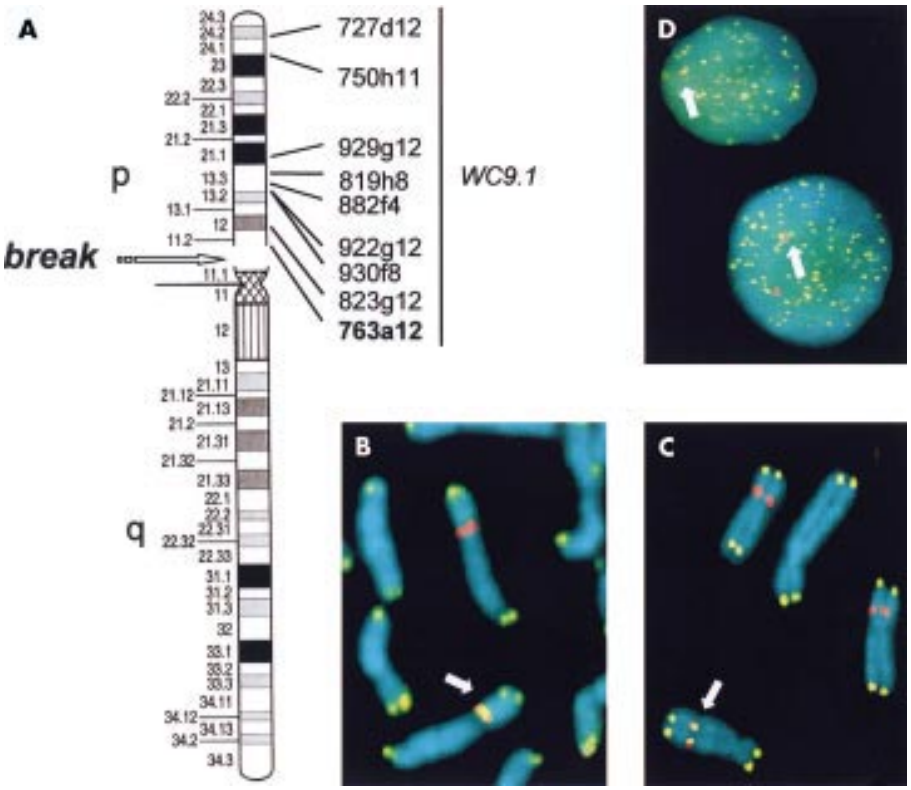

Figure 3 (A) Ideogram of chromosome 9: the arrow indicates the p breakpoint underlying the formation of inv(9). The YAC clones from the WC9.1 contig used in the FISH experiments are indicated; YAC y763a12 (which is close to the p breakpoint) is in bold. Dual FISH of y763a12 and all of the human telomere probes show two overlapping interstitial signals on mother inv(9) (B) and child der(17) (C). Merging of the two signals is also observed on the mother's interphase cells (D), thus showing the close contiguity of the target sequences.

among which is the pericentromeric region of chromosome 9,[12] we used a long synthetic polynucleotide probe $(T_2AG_3)n$ in FISH experiments as a means of checking the putative localisation of telomere-like sequences in coincidence with the 9p breakpoint. An analysis of a normal subject with the screening of 110 cells identified two cells with signals on both chromatids at 9p11.2, the band involved in the rearrangement leading to the constitutionally rearranged chromosome 9 in the patient's mother (fig 2(C)). We then used a further set of seven YACs belonging to the WC9.1 contig and spanning the interval 9p21-9p11.2 in FISH experiments aimed at precisely mapping the 9p breakpoint. As shown in the ideogram in fig 3A, all of the YACs mapped distally to the breakpoint, thus confirming that it lies very close to the chromosome 9 centromere. Dual

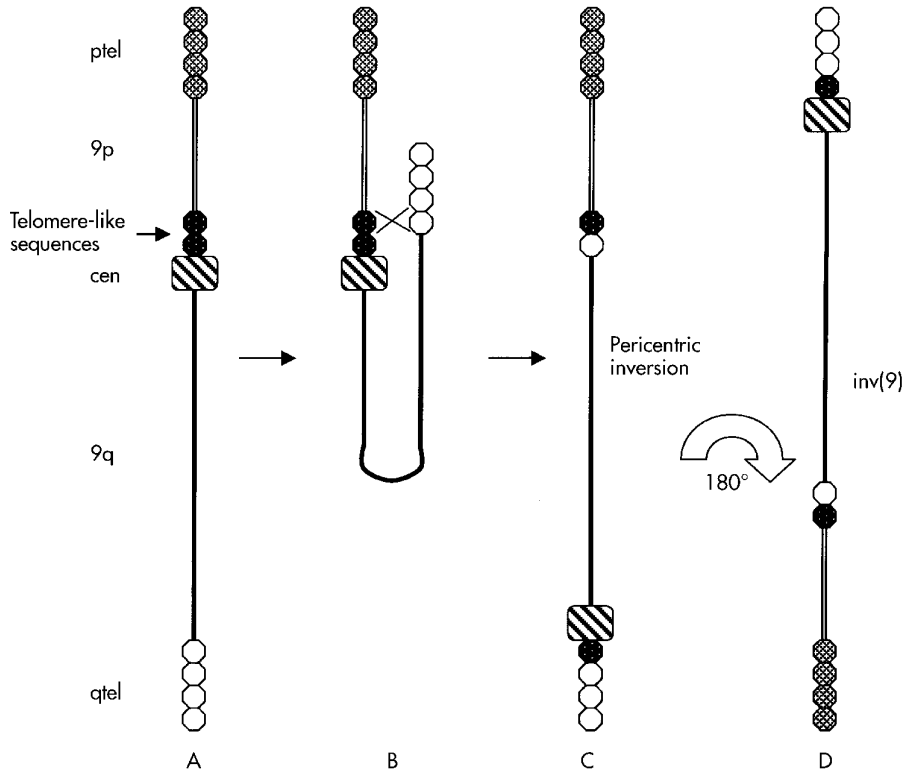

Figure 4 Interstitial telomere-like sequences located at 9p11.2 may (A) undergo intrachromosomal pairing and chromatid exchange with 9q telomere sequences (B), leading to a chromosome with a pericentric inversion (C), which shows remnant 9q telomeric sequences close to the centromere, a few of which have been integrated at the original interstitial telomeric site. The inverted chromosome 9 with an upright centromere is shown in (D) according to the conventional chromosomal representation. 9p telomeric sequences, cross hatched hexagons; 9q telomeric sequences, white hexagons; interstitial telomeric-like sequences, spotted black hexagons.

colour FISH with the most centromeric y763a12 cohybridised with a probe specific for all human telomeres allowed two overlapping signals to be detected in the metaphases from the mother and child, on the 9q and 17q interstitial telomeres respectively (fig 3B, C). The close contiguity of the sequences hybridised by the two probes is further proven by the adjacent signals observed on the interphase chromatin (fig 3D), a finding indicating that y763a12 (anchored to D9S1874 in 9p11.2) is very near to the breakpoint and that the breakpoint is therefore sublocalised between the centromere and p11.2 (fig 3A). It is worth noting the perfect coincidence of the breakpoints underlying the inverted chromosome 9 and the 9;17 JT inherited by the child. The scoring of 620 metaphases from the child did not show any fragility figures or JTs, thus confirming that he currently has an abnormal but stable karyotype.

DISCUSSION

Our findings extend and confirm the observations relating to previously reported constitutional JTs,[4] and also highlight a distinctive feature that deserves some comment. Our case can be added to the restricted repertoire of structurally rearranged chromosomes whose deleterious consequences on progeny seem to be the result of the intermediate formation of JTs. An unusual inheritance of a reciprocal t(11;22)(p11;p12)mat translocation has been discovered in a family with one daughter having a different t(11;15)(p11;p12) translocation.[16] The other more recently described case is a phenotypically normal woman carrying an unbalanced cell line with an i(21q) and a balanced cell line with a rob(21q22q)[2]; according to the authors, although not clear from the cytogenetic data presented, the unbalanced i(21q) is the progenitor rearrangement that subsequently participates in a non-reciprocal rearrangement as in the case of JTs. In our case, the unstable chromosome from which the jumping donor segment stems is a balanced inv(9)(p11.2;q34). Like the jumping translocations mentioned above, our case shows a germ cell line fixation of one of the multiple JTs. Interestingly, the 9p;17q detected in 2% of the cells is that fixed in the primordial germ cell leading to the unbalanced conceptus through adjacent 1 segregation (table 1), and this relatively high frequency allows us to infer that the 9p;17q occurred very early in the postzygotic development of the woman carrying the inverted chromosome 9 (probably the blastocyst stage). This view is supported by the fact that the pachytene diagram of a pericentromeric inversion does not predict the formation of acentric chromosomal fragments with sticky ends prone to interchromosomal rearrangement. To the best of our knowledge, this is the first reported case of a chromosome bearing an intrachromosomal balanced rearrangement acting as a JT donor. The carrier of the inv(9), the rearranged progenitor chromosome of all observed JTs, displays features of chromosomal instability, such as breaks and gaps (fig 1D) and different clonal JTs (table 1). However, the rearranged chromosomes resulting from jumping of 9p are stable; evidence for this is the inheritance of der(17)t(9;17)(p11.2;qter) by the affected child. The putative mechanism involved in the formation of inv(9)(p11.2;q34) is shown in fig 4. The presence of interstitial telomere-like sequences at 9p11.2 shown by FISH with a $(T_2AG_3)n$ probe (fig 2C) probably generates a site of polymorphic variation that is highly unstable, as has been shown in the case of other internal telomeric repeats.[17] Variations in the size of the repeats may favour unequal crossovers with the telomeric sequence of the same chromosome (fig 4A, B), the outcome of which is the pericentromeric inversion shown in fig 4C. The rearranged chromosome has a novel telomere adjacent to the centromeric constriction, and a recombinant shortened interstitial telomere at 9q34. This latter site is highly unstable and prone to further breakage. A mechanism shortening telomeric sequences and leading to the loss of telomeric function has been postulated following the cloning and sequencing of the fusion region of 1q21;7p22, which was observed as the main JT in a patient with acute myelomonocytic leukaemia.[18] It is no accident that the number of JTs detected in tumours is nearly as high as that found in constitutional karyotypes, insofar as the latter cases come to light as a result of the birth of a malformed child. It is therefore likely that JTs represent an underestimated phenomenon that is rarely uncovered by current karyotypic analyses.

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