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ORIGINAL PAPER

Detection of a concomitant distal deletion in an inverted duplication of chromosome 3. Is there an overall mechanism for the origin of such duplications/deficiencies?

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We describe the first inverted duplication of the p21.3p26 region of chromosome 3 in a child with phenotypic features of the trisomy 3p syndrome. This uncommon type of aberration was verified by multicolour fluorescence *in situ* hybridisation (FISH) using yeast artificial chromosome (YAC) clones from chromosome 3 (CEPH library). With a newly constructed YAC clone from the 3p26 region an unexpected subtelomeric deletion was diagnosed in the aberrant chromosome 3. Using the primed *in situ* labelling (PRINS) method, telomeres were found to be present on the recombinant chromosome 3. The repeated appearance of concomitant distal deletions in inverted duplications suggests that an overall mechanism exists for the origin of such duplications/deficiencies.

Keywords: inverted duplication; deletion; chromosome 3; fluorescence *in situ* hybridisation; YAC clones

Introduction

Cases of an inverted duplication of the short arm of a chromosome in association with a deficiency distal to the duplication itself have been reported for chromosomes 8,[1–8] 9,[9] and 7.[10] To define the location of the rearrangement breakpoints more precisely, Floridia *et al*[8] demonstrated by extensive molecular analysis that the inverted 8p duplications of all 16 cases studied were consistently accompanied by a subtelomeric deletion of the region spanning from D8S349 to the telomere. Teebi *et al*[9] reported a simultaneous deletion of p22pter in an inverted duplication of the short arm of chromosome 9 detected by the combined application of high resolution trypsin G banding and fluorescence *in situ* hybridisation (FISH) analysis. A similar type of an aberrant rearrangement resulting in a duplication was reported in the long arm of one chromosome 7(q21.2q36) in a second trimester foetus.[10] Part of band q36 was apparently deleted as shown by the absence of hybridisation with the DNA probe D7S427.

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We report on a *de novo* inverted duplication of chromosome 3. On the basis of cytogenetic studies alone, the proposita had the karyotype 46,XX,inv dup(3)(p21.3p26). Since complex mutational events associated with a *de novo* inverted duplication have been observed in three different chromosomes, we assumed that distal deletions might be a common feature of such rearrangements. Therefore we performed an extensive molecular cytogenetic analysis with DNA probes spanning the short arm of chromosome 3.

Methods

Patient

The proband exhibited many of the main clinical manifestations of the partial trisomy 3p syndrome: psychomotor retardation, microcephaly, square-shaped face, frontal bossing, temporal indentation, prominent cheeks, hypertelorism or telecanthus, down-turned corners of the mouth, cleft lip and palate, eversion of lips, micrognathia/retrognathia, short neck and congenital heart disease.(see reviews[11,12]) No apparent phenotypic abnormality related to the monosomy 3p syndrome was observed.

Cytogenetic Analysis

Chromosome analysis was performed on PHA stimulated lymphocytes using standard trypsin G banding techniques.

Chromosome Painting

Chromosome *in situ* suppression (CISS) hybridisation was performed with a whole painting DNA probe of chromosome 3 (AGS, Heidelberg, Germany) on metaphase spreads from the patient. The conditions used for hybridisation and detection of the resultant signal by an antibody/peroxidase system were described by Stock *et al*.[13]

Probes

Two yeast artificial chromosome (YAC) clones (806A7, 938G11) from the short arm of chromosome 3 were obtained from the Centre d'Etude du Polymorphisme Humain (CEPH) library. Information on the YACs can be found in the Genome Data Base. The YAC clone TYAC148,[14] which mapped to 3p26, was constructed and screened with the human specific telomere sequence TTAGGG. It was kindly provided by Helen Donis-Keller (Washington University School of Medicine, St Louis, USA).

Alu PCR

The agarose plugs containing the YAC DNA were prepared as described by Sheehan and Weiss,[15] with minor modifications. Briefly, yeast cells were grown to $OD_{600} = 7$ in SD medium[16] and washed in ET buffer (10 × ET: 100 mM Tris HCI, 500 mM EDTA, pH 7.5). Cells were spheroplasted with lyticase (L-8012, Sigma, Deisenhofen, Germany), embedded in 1% low melting point agarose solution (GibcoBRL, Eggenstein, Germany) and put into plug moulds. After 20 min hardening at 4°C, the plugs were removed from the moulds and incubated at 60°C in ET buffer containing 5% SDS for 2 h, followed by incubation in the same buffer containing proteinase K (P-6556, Sigma, Germany) instead of SDS at 50°C overnight. After three washes in ET buffer, the plugs were stored at 4°C. The FISH probes were prepared from the YAC plugs by means of Alu-PCR, as described by Lengauer *et al*.[17] The PCR products were biotinylated (BioPrime-Kit, GibcoBRL, Germany) or digoxigenin labelled (DIG-DNA-Labeling-Kit, Boehringer Mannheim GmbH, Mannheim, Germany) by random primed labelling according to the manufacturer's instructions.

Fluorescence in situ *hybridisation with YAC clones*

For FISH, the techniques of Pinkel *et al*[18] and Lengauer *et al*[17] were modified. A small amount (2 µl) of hybridisation mix (per µl: 10ng of each of the two differently labelled YAC probes, 10 µg of unlabelled human Cot 1 DNA, 50% formamide, 10% dextran sulfate and 1 × SSC, pH 7.5) was placed on the cell containing area of the slides, which was covered with a round 10 mm cover slip and sealed with rubber cement. The slides were denatured for 5 min at 75°C and hybridised overnight at 37°C. Post-hybridisation washes were performed three times in 0.1 × SSC at 60°C for 7 min. In multicolour FISH experiments biotin and digoxigenin conjugated probes were detected simultaneously by a mixture of Cy3 conjugated avidin (red fluorochrome) (Dianova, Hamburg, Germany) and FITC conjugated anti-digoxigenin (green fluorochrome) (Boehringer Mannheim GmbH, Mannheim, Germany), followed by a mixture of anti-avidin (Camon, Wiesbaden, Germany) and monoclonal mouse anti-FITC (DAKO, Hamburg, Germany). A third layer included a mixture of Cy3 conjugated avidin and FITC-conjugated donkey anti-mouse (Dianova, Hamburg, Germany). Amplification was performed by repeating the last two detection steps. Chromosomes were counterstained with 1 µg/ml 4',6–diamidino–2–phenylindole–dihydrochloride (DAPI, Sigma, Germany). Slides were mounted in antifade solution (0.2% p-phenylenediamine dihydrochloride, Sigma, in 80% glycerol, 20 mM Tris-HCI, pH 8.0). Signals were visualised with a ZEISS Axiophot fluorescence microscope (Zeiss, Oberkochem, Germany) by changing the filter sets 09 (FITC), 00(Cy3) and 02(DAPI), and digitised for documentation with the ISIS system (Metasystems, Sandhausen, Germany). Ten metaphase spreads were analysed for each FISH experiment.

Primed in situ *labelling*

The telomeres were visualised with the primed *in situ* (PRINS) labelling method, as described in detail by Therkelsen *et al*,[19] using the telomere sequence $(CCCTAA)_7$ as primer.

Results

Cytogenetic Analysis

All 30 analysed metaphases from the child revealed the karyotype 46,XX,invdup(3)(p21.3p26)(Figure 1). Both parents had normal karyotypes.

Chromosome Painting

After CISS hybridisation with a whole painting probe of chromosome 3, both the normal chromosome 3 and

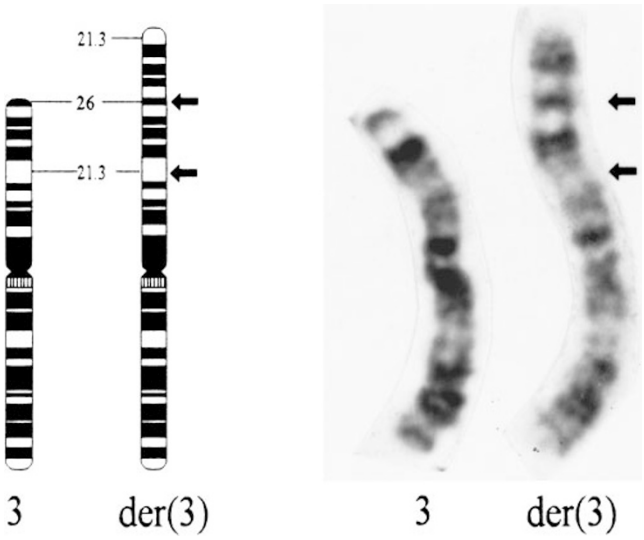

Figure 1 *Trypsin G banding of chromosomes from the female carrier showing the inv dup(3)(p21.3p26). Idiograms of the normal chromosome 3 and the recombinant chromosome 3 are shown on the left, high resolution banding of the chromosomes 3 (IARC: 600 bands) on the right. The inverted duplication is indicated by two arrows.*

the recombinant chromosome 3 showed clear, uninterrupted hybridisation signals. Therefore the additional chromosome material in the short arm must have been of chromosome 3 origin (Figure 2a).

FISH using YAC clones

In multicolour FISH, the YAC clone 806A7 hybridised more telomerically (green hybridisation signals) than the YAC clone 938G11 (red hybridisation signals) in the normal chromosome 3 (cen-938G11-806A7-tel), whereas in the aberrant chromosome 3 the two YACs had the following order: cen-938G11-806A7-806A7-938G11-tel (Figure 2b). In single FISH the YAC clone 806A7 mapped to 3p26 and the YAC clone 938G11 to p22p23, respectively (data not shown). The newly constructed YAC clone TYAC148 hybridised to the most distal region of the short arm of chromosome 3 in the band p26. Hybridisation of this YAC clone (in combination with YAC 806A7) to the chromosomes of the child, however, gave a single hybridisation signal only on the normal chromosome 3 and not on the aberrant chromosome 3. The validity of this result was confirmed in ten patient metaphases which all gave the same result (Figure 2c).

PRINS

By the PRINS labelling technique it was shown that the telomeres were present in all chromosomes (Figure 2d).

As the data accumulated, it became apparent that the recombinant chromosome 3 had a partial duplication 3p as well as a deletion of 3p26, the karyotype being 46,XX,invdup(3)(qter > p26::p26- > p21.3::p26), del(3)(p26).

Discussion

Three mechanisms have been proposed to explain the origin of inverted duplication chromosomes leading to partial monosomy and partial trisomy. (see reviews [5,8]) Weleber *et al* [1] suggested that an unusual intrachromosomal meiotic recombination occurs in two homologous chromosomes (Figure 3a). This is an initial event in which the ends of the two symmetric broken chromosomes fuse together. The recombination process is called a U-type exchange (Figure 3b) and creates a dicentric chromosome (Figure 3c). After division one daughter cell contains a recombinant chromosome with one centromere and a region with a symmetric inverted duplication accompanied by a concomitant distal deficiency together with the lack of telomeric sequences (Figure 3d).

New data on inverted 8p duplications/deficiencies proposed a similar, but modified mechanism. During pairing an abnormal misalignment occurs between two homologous chromosomes, which is followed by an abnormal crossover (Figure 3e). However, the resulting dicentric chromosome is not symmetric, since the duplicated regions are separated by a single copy region (Figure 3f). During the subsequent anaphase I, breakage can occur at the centromere or anywhere in the short arm region between the two centromeres of the dicentric chromosome, forming two different chromosomes either with one centromere or with a second centromere at the tip of the short arm (Figures 3g and 3h).[8] In contrast to the lack of telomeric sequences as proposed by Weleber *et al*,[11] Floridia *et al* [8] observed new telomeres after FISH with the all-human telomeres probe (Onkor).

An alternative mechanism involving an inverted duplication with partial monosomy was favoured by Gorinati *et al*.[20] This more complex interaction includes two abnormal events: a paracentric inversion is found in the short arm of a chromosome in one parent. During meiosis the chromosome carrying the paracentric inversion together with its normal homologous chromosome forms an inversion loop. A crossing-over occurs in the inversion loop and the repair process creates a U-type exchange. After division the daughter

cell contains a monocentric recombinant chromosome with an inverted duplication region and a concomitant distal deficiency together with normal telomeric sequences (the other daughter cell contains a chromosome with an interstitial deletion). We did not detect a paracentric inversion in our patient's parents' chromosomes. Nevertheless, we observed an inverted duplication of the 3p21p26 region accompanied by a subtle deletion distal to the cytogenetically proposed 3p26 breakpoint and telomeric repeats (Figure 3f). Therefore we favour either a symmetric or asymmetric recombination process involving the main steps (Figure 3) as discussed by Weleber *et al*[1] or Floridia.[8] Our findings that telomeres were found to be present on broken chromosomes are in agreement with the study of Floridia *et al*[8] and with several other reports.[10,21]

Theoretically our patient should express clinical symptoms of both the trisomy 3p syndrome because of the duplication of the 3p21p26 region, and the monosomy 3p syndrome because of the deletion in 3p26. The characteristic growth retardation and ptosis seen in patients with the monosomy 3p syndrome are lacking in our patient, whereas the main features of the trisomy 3p syndrome, i.e. the typical square-shaped face and temporal indentations, are present. Most of the other symptoms of these two syndromes overlap, namely

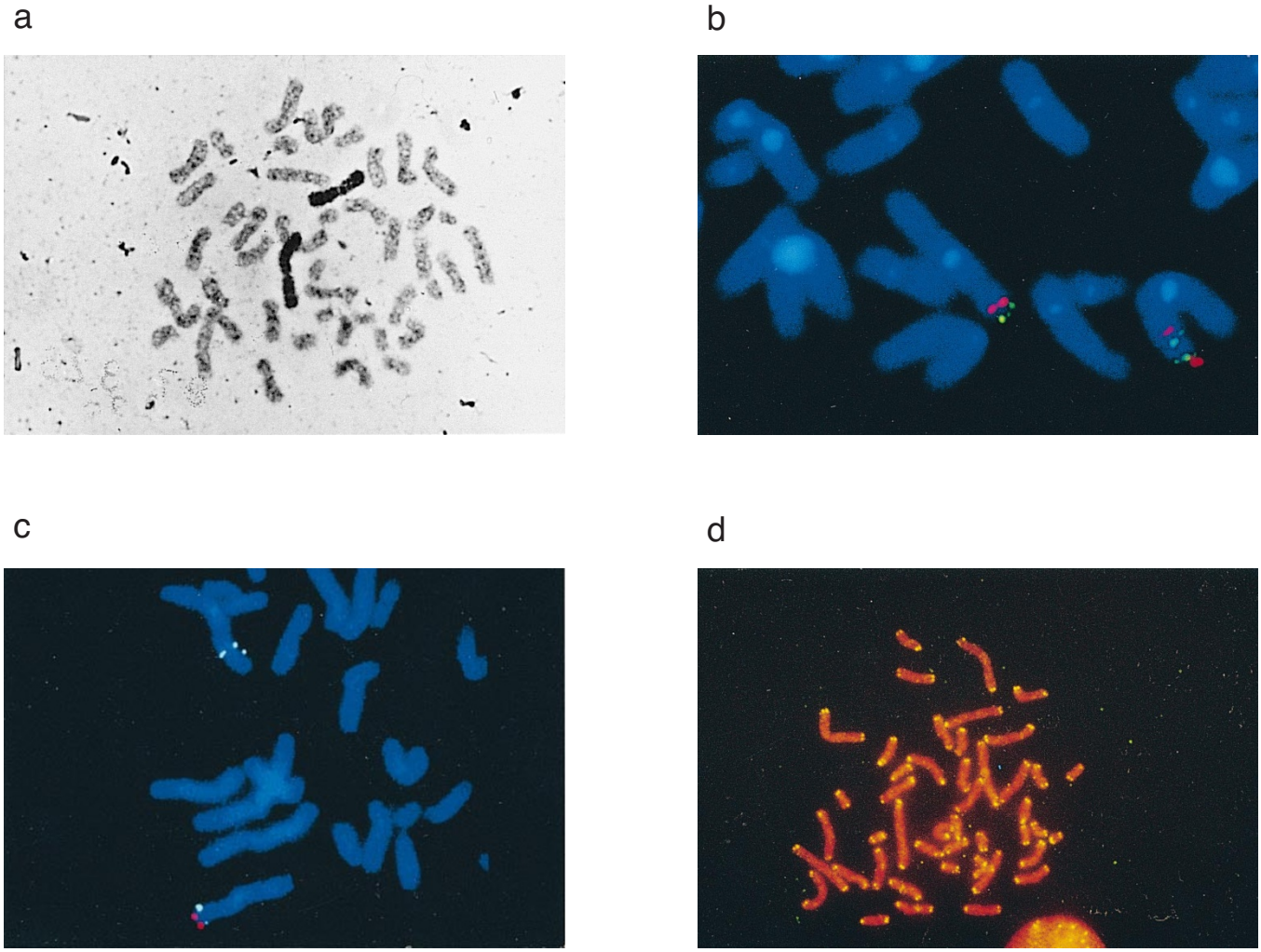

Figure 2 **(a)** *Metaphase from the patient showing the normal chromosome 3 and the recombinant chromosome 3 after* in situ *hybridisation with a painting probe of chromosome 3 (AGS, Heidelberg, Germany) using indirect peroxidase with diaminobenzidine staining and hematoxylin-eosin counterstain.* **(b)** *Partial metaphase after multicolour* in situ *hybridisation with the YAC clone 806A7 (green hybridisation signals) and the YAC clone 938G11 (red hybridisation signals).* **(c)** *Partial metaphase after multicolour FISH with the YAC clone 806A7 (green hybridisation signals) and the subtelomerspecific YAC clone TYAC148 (red hybridisation signals). The aberrant chromosome 3 does not show any signal with the YAC clone TYAC148, whereas the normal chromosome 3 has one signal.* **(d)** *The telomeres of all chromosomes were visualised with the PRINS labelling method.*

mental retardation, microcephaly, micrognathia, congenital heart disease, gastrointestinal malformation, and renal abnormalities.[11,12,22,23]

It is interesting to note that the deletion related to TYAC148 has a size of 300 Kb and includes not only repeated sequences but also the chromosome-specific STS sAKB8, indicating the presence of heteropolymorphism of chromosome telomere regions. The cytogenetic breakpoint associated with the monosomy 3p syndrome has been identified at band p25. According to our data the inverted 3p duplication shared a more distal p26 deletion, perhaps resulting in the lack of any additional detectable phenotypic consequences. In fact, a 3p25.3 terminal deletion with no apparent abnormalities has been demonstrated in a mother and her child.[24]

Similarly, a characteristic phenotype associated with deletions at 7q36 was not found in a second trimester foetus with an inverted duplication of chromosome 7 in association with a deletion within 7q36.[10] However, the loss of the *DEF1* gene in all cases of inverted 8p duplication/deficiencies supports the assumption that during the process of repair of an aberrant recombination, important genes are both duplicated and deleted. In addition, the loss of the distal 9p26 region in an inverted duplication made a detectable contribution to the phenotype, because the presence of upslanting palpebral fissures and dolichomesopalangy in the patient was in agreement with the diagnosis of a monosomy 9p syndrome.

In conclusion, precise characterisation of the recombinant chromosome 3 was possible by the combined application of cytogenetics and various molecular cytogenetic techniques. Concomitant deletions due to an inverted duplication are most probably more frequent than cytogenetic methods alone have been able

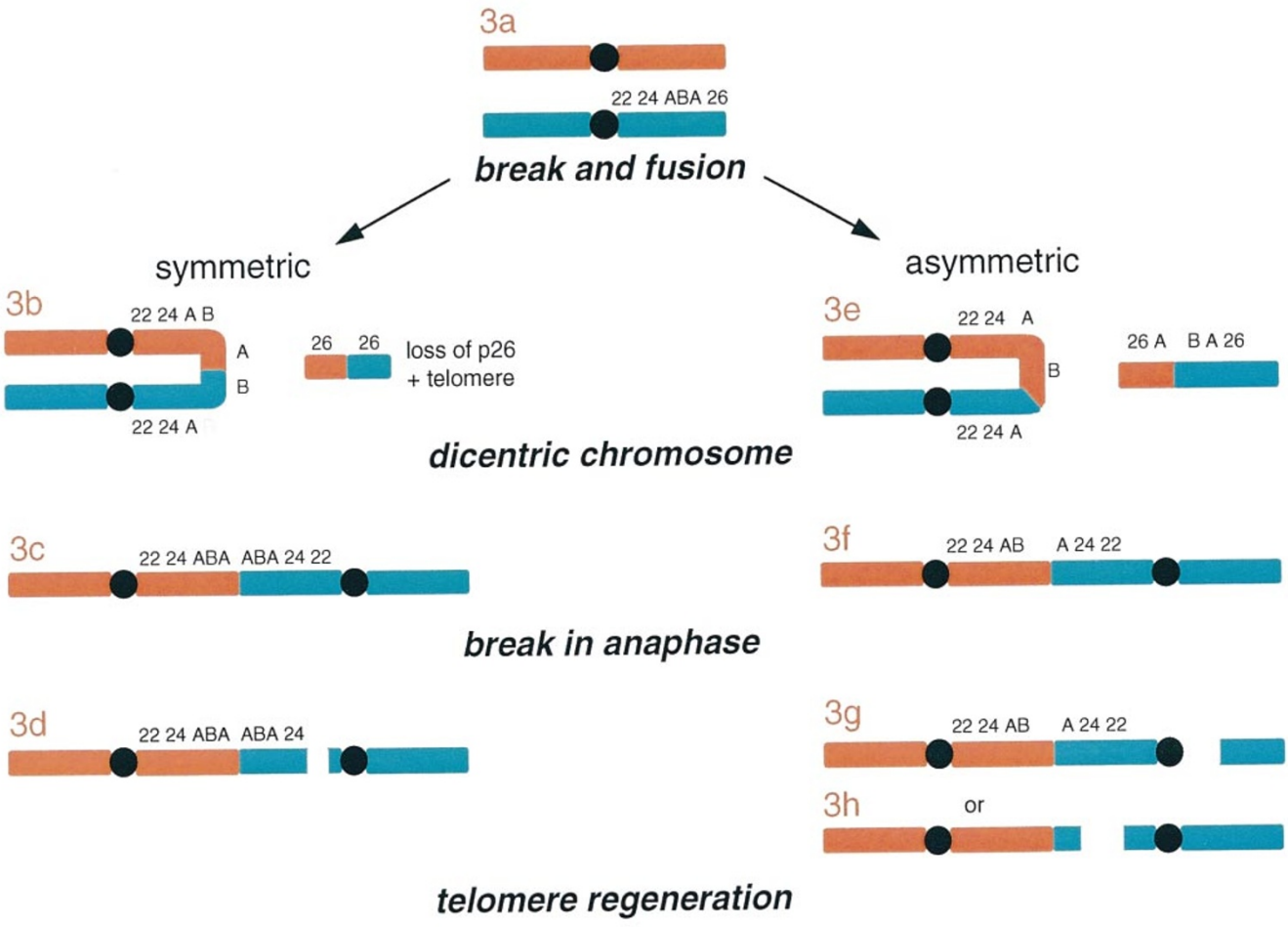

Figure 3 *Schematic presentation of a classical (Weleber* et al[1]*) and modified (Floridia* et al[8]*) proposed mechanism to explain the origin of a* de novo *inverted duplication chromosome with a concomitant deletion (see Discussion).*

to demonstrate. The occurrence of the complex rearrangement in a further chromosome suggests that an overall mechanism exists for such inverted duplications leading to partial trisomy and partial monosomy.

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