Brief papers

Value of chromosome painting in determining the chromosomal outcome in offspring of a 12;16 translocation carrier

C A Brandt, T Lyngbye, S Pedersen, L Bolund, U Friedrich

Abstract

We currently use direct and reverse chromosome painting in prenatal diagnosis. In a family with a subtle 12;16 translocation, adjacent 1 segregation was diagnosed in the first child, a boy, in whom symptoms compatible with partial trisomy 16p and partial monosomy 12q were seen. In the next pregnancy, a chorionic villus biopsy was tested using chromosome painting. Only by supplementing conventional cytogenetic methods with molecular cytogenetic techniques could the true karyotype be unequivocally determined. Reverse painting, using DOP-PCR amplified, flow sorted paternal derivative chromosomes as a DNA library to paint the chorionic villus cells, was especially informative.

(J Med Genet 1994;31:234-237)

Partial trisomy of the short arm of chromosome 16 has been reported several times (a review of 14 cases is given by Léonard *et al*¹). The phenotype varies depending on the size of the triplicated segment of the short arm of chromosome 16 and the concomitant structural abnormalities in the other chromosomes owing to unbalanced inheritance of the translocation. Léonard *et al*¹ pointed out that in nearly half of the progeny with unbalanced translocations from the parents carrying the translocation, the breakpoint was in the telomeric region. When small chromosomal fragments are exchanged and the shape of the chromosomes involved are not changed, the diagnosis in an unbalanced translocation carrier may be difficult by conventional cytogenetic methods. However, with molecular cytogenetic methods subtle translocations are easily diagnosed, even in chorionic villus samples, in spite of the inferior quality of chromosomes. We describe the diagnostic evaluation using such methods in a family where a 12;16 translocation was segregating.

Case report

The proband, a boy, was the first child of healthy white parents who did not have any history of genetic disease. At the time of his

birth the mother was 25 and the father 26 years of age.

On routine fetal ultrasound scanning in the 16th week of pregnancy a cyst was found in the nuchal region. Consequently, a chorionic villus biopsy was performed, which showed a normal karyotype; re-examination by ultrasound in the 28th week showed that the cyst had disappeared. The findings were considered to represent spontaneous regression of a cystic hygroma. Otherwise, the pregnancy was uneventful.

The delivery was spontaneous after 35 weeks and four days of gestation. The amniotic fluid was stained with meconium. Apart from this, the birth was normal with an Apgar score of 9 at five minutes. The birth weight was 2440 g. The child was vigorous with a loud cry. He had increased flexor responses in his arms and hands and extensor tone of the body. The shape of the neurocranium was remarkably round. He had hypertelorism as well as blepharophimosis and microphthalmia (fig 1), and bilateral cheilognathopalatoschisis although the gnathoschisis was not complete on the left side. The mandible was small. There was a "tag" in front of the right ear and both helix and tragus of the left ear was smaller than the right. The skin was abundant in the glabella region and at the back of the neck, and he also had oedema on the back of the feet. These three findings were considered to be suggestive of hygroma. The hands exhibited flexion contractures of all proximal interphalangeal joints, and there was a tendency to overlapping of the fingers. The nails on both hands and feet were dysplastic and the fingernails were also slender and hyperconvex. The feet showed a "sandal gap" between the first and the second toes. A closed sinus was seen over the sacral area.

For the first two months he required diuretic treatment for uncompensated heart disease, which proved to be the result of both atrial and ventricular septal defects. When he was 3 months old he was operated on for bilateral inguinal hernias. He showed muscular hypertonia. From the beginning it was clear that his rate of development was below 50%. His ability to use his sight developed despite the microphthalmia. At the age of 8 months he developed seizures and exhibited electroencephalographic hypsarrhythmia.

Institute of Human Genetics, The Bartholin Building, University of Aarhus, DK-8000 Aarhus C, Denmark
C A Brandt
S Pedersen
L Bolund
U Friedrich

Paediatric Department, Aarhus Municipal Hospital, Denmark
T Lyngbye

Cytogenetic Laboratory, Institute of Basic Research in Psychiatry, Psychiatric Hospital, Aarhus, Denmark
U Friedrich

Correspondence to Dr Friedrich.

Received 1 September 1993
Accepted for publication 7 October 1993

Figure 1 The appearance of the proband in the first month after birth.

CYTOGENETIC STUDIES

Chromosomes from the proband were obtained both from cultured amniocytes and from peripheral blood lymphocytes after birth. The metaphase chromosome preparations were Q banded according to standard techniques. During routine prenatal diagnosis a normal male karyotype was found. After birth, the clinical findings prompted prometaphase analysis. Prometaphase chromosomes were stained with acridine orange and the banding pattern of chromosome 12 disclosed a surplus band on the long arm of unknown origin. Re-examination of metaphase chromosomes showed an apparently normal karyotype except that the distal light region of one chromosome 12 seemed to be enlarged (fig 2). Chromosome analysis of both parents showed a normal karyotype in the mother and a balanced 12;16 translocation in the father (fig 2). His karyotype was 46,XY,t(12;16)(q24;p12).

Chromosome painting using a DNA library generated from flow sorted normal chromosomes 16 by the DOP-PCR technique was performed as described by Pedersen *et al.*² A translocated short segment of chromosome 16 could easily be recognised on the long arm of one chromosome 12 in the father and in the proband. At the same time, one chromosome 16 was too short in the father, whereas two normal chromosomes 16 were observed in the proband. The proband has thus inherited an unbalanced translocation from his father with partial trisomy 16p and partial monosomy 12q.

Telomeric staining was performed by the PRINS technique as described elsewhere.³⁻⁵ An oligonucleotide (CCCTAA)_n, representing telomeric sequences was used to stain the telomeres of the chromosomes. The result shows normal telomeres on all chromosomes including the derivative chromosomes der(12) and der(16) from the father and the proband and no signs of telomeres at the breakpoints. The chromosomes were identified by Q banding after telomeric staining (data not shown).

In the next pregnancy, a chorionic villus biopsy was taken in the ninth week. Chromosome preparations were made after short term incubation. The conventional cytogenetic analysis disclosed an apparently normal karyotype. The chromosomes obtained from short term incubation were of inferior quality. Nevertheless, direct chromosome painting with the DNA library specific for chromosome 16 showed two apparently normal chromosomes 16 and, more importantly, no chromosome 16 material on the long arm of chromosome 12 (fig 3c), supporting the diagnosis of a normal karyotype. The result was confirmed by the reverse chromosome painting technique⁶ using the DNA library generated by flow sorting of the paternal derivative chromosome der(12) applied to metaphases obtained from the cultured chorionic cells (fig 3d). The reverse painting signals on pter of both chromosomes 16 unequivocally showed the presence of terminal chromosome 16 material and therefore the absence of the der(16) chromosome. Thus, a normal 46,XY karyotype could be confirmed in the fetus.

Discussion

Detection of subtle reciprocal translocations by fluorescence in situ hybridisation (FISH) has recently been described.⁷ The application of whole chromosome libraries for chromosome in situ suppression (CISS) hybridisation has great advantages^{8,9} in the detection and characterisation of chromosomal rearrangements. We are currently applying both direct and reverse chromosome painting to the practice of prenatal diagnosis.

In the first pregnancy in this family an unbalanced subtle translocation was overlooked, since the size, proportion, and banding pattern of the terminal rearrangement appeared almost unchanged compared to the normal homologous chromosome. Several clinical signs in the proband after birth indicated, however, a chromosomal imbalance. This suspicion was enhanced by the finding of a slight difference in staining intensity in the faintly banded chromosome region of the distal long arm of chromosome 12. Thus, more refined cytogenetic methods were applied. Still, only when the exact nature of the balanced karyotype in the father was known was it possible to perform prenatal diagnosis in this family using molecular cytogenetic methods. A combination of direct painting using a DNA library generated from flow sorted chromosomes 16 (a DNA library from flow chromosome 12 is not obtainable by flow

Figure 2 Q banded metaphase and R banded prometaphase chromosomes 12 and 16 from the father and son. Arrows indicate the translocated segments.

Figure 3 Direct chromosome painting with a chromosome 16 library showing (a) one normal chromosome 16, the shorter der(16) chromosome, and the der(12) in the father who has a karyotype 46,XY,t(12;16)(q24;p12); (b) painting of two normal chromosomes 16 in combination with der(12) indicating an unbalanced karyotype in the son; and (c) painting (short term incubated chorionic villus cells) of two normal chromosomes 16. There are no signals on chromosome 12, indicating a normal karyotype in the fetus. (d) Reverse painting using the DNA library generated from the derivative chromosome der(12) from the father confirmed the normal karyotype in the fetus in chorionic villus preparations cultured for 10 days. The picture was taken from the computer screen of the confocal scanning equipment.

sorting, because chromosomes 9, 10, 11, and 12 cannot be distinguished by conventional flow fluorometric analysis) and reverse painting using a DNA library generated from flow sorting of the paternal, slightly enlarged der(12) chromosome were used. This strategy proved to be more sensitive in establishing the segregation of the 12;16 translocation from the father than conventional banding analysis and therefore indispensable in this prenatal dia-

gnosis, because of the small size of the translocated segment.

The fact that Léonard *et al*¹ found most cases of trisomy 16p to be caused by inheritance of translocation chromosomes makes the diagnosis more feasible in cases of subtle translocations. On the other hand, the location of the breakpoint in the telomeric region makes the diagnosis more difficult, especially when testing cultured tissue as in prenatal diagnosis.

Direct painting has proven its value in characterising the constitution of the derivative chromosome in cases of terminal rearrangements. Furthermore, in cases of 3:1 segregation, which Léonard *et al*¹ found in eight out of 14 cases with dup(16p), reverse chromosome painting using a DNA library generated from a flow sorted preparation of the supernumerary chromosome can establish the origin and composition of the extra chromosome.

The presence of interstitial telomeric sequences has been suggested in several reports involving terminal rearrangements.¹⁰ The hexanucleotide sequence, a human telomere consensus (TTAGGG)_n, identified by Moyzis *et al*,¹¹ was used as a priming oligonucleotide ((CCCTAA)₇) to visualise the telomeres by PRINS of the derivative chromosomes involved in our case. The presence of the sequence only at the terminus of the chromosomes indicates that the breakpoint in the reciprocal translocation 12;16 was proximal to the region where the telomeric sequences are placed.

Molecular cytogenetics has become an integrated part of our cytogenetic procedures. Time and economy preclude the use of these techniques as routine tools as yet. However, molecular cytogenetics should be used as a supplement to routine cytogenetic studies, if a karyotype appears to be normal under circumstances where there is particular suspicion of chromosomal aberration. This is so in cases like the one presented here, when fetal ultrasound or clinical investigation raise suspicion.

We have, in the present case, significantly

increased the diagnostic sensitivity with the introduction of direct and reverse chromosome painting to prenatal diagnosis of terminal rearrangements.

The expert technical assistance of Monna Caprani and Helle Strømkjær is appreciated. Kim Keilberg and Kirsten Millgaard are thanked for the photographic work, Anette Sørensen for typing the manuscript, and Tracey Flint for language correction. This work was supported by grant 5.18.10.03 from the Danish Human Genome Research Programme (CAB, LB).

- 1 Léonard C, Huret JL, Imbert M-C, *et al*. Trisomy 16p in a liveborn offspring due to maternal translocation t(16;21)(q11;p11) and review of the literature. *Am J Med Genet* 1992;43:621-5.
- 2 Pedersen S, Hindkjær J, Brandt CA, Bolund L, Kolvraa S. Reverse chromosome painting. In: Choo KHA, ed. *Methods in molecular biology. In situ hybridization protocols*. New Jersey: The Humana Press 99-102.
- 3 Hindkjær J, Koch J, Mogensen J, *et al*. Primed in situ labelling of nucleic acids. *Int J Biotech* 1991;12:752-6.
- 4 Brandt CA, Kierkegaard O, Hindkjær J, *et al*. Ring chromosome 20 with loss of telomeric sequences detected by multicolour PRINS. *Clin Genet* 1993;44:26-31.
- 5 Brandt CA, Djernes B, Strømkjær H, *et al*. Pseudodicentric chromosome 18 diagnosed by chromosome painting and primed in situ labelling (PRINS). *J Med Genet* 1994;31:99-102.
- 6 Telenius H, Palmear AH, Tunnacliffe A, *et al*. Cytogenetic analysis by chromosome painting using DOP-PCR amplified flow-sorted chromosomes. *Genes Chrom Cancer* 1992;4:257-63.
- 7 Speleman F, van Roy N, Wiegant J, *et al*. Detection of subtle reciprocal translocations by fluorescence in situ hybridization. *Clin Genet* 1992;41:169-74.
- 8 Pinkel D, Landegent J, Collins C, *et al*. Fluorescence in situ hybridization with human chromosome-specific libraries: detection of trisomy 21 and translocation of chromosome 4. *Proc Natl Acad Sci USA* 1988;85:9138-42.
- 9 Jauch A, Daumer C, Lichter P, *et al*. Chromosomal in situ suppression hybridization of human gonosomes and autosomes and its use in clinical cytogenetics. *Hum Genet* 1990;85:145-50.
- 10 Park VM, Gustashaw KM, Wathen TM. The presence of interstitial telomeric sequences in constitutional chromosome abnormalities. *Am J Hum Genet* 1992;50:914-23.
- 11 Moyzis RK, Buckingham JM, Cram LS, *et al*. A highly conserved repetitive DNA sequence, (TTAGGG)_n, present at the telomeres of human chromosomes. *Proc Natl Acad Sci USA* 1988;85:6622-6.