SHORT REPORTS

Elucidation of the centromere involvement in an inversion (13) by fluorescent in situ hybridisation

Patricia L Gordon, James D Dalton, Paula R Martens, Avirachan T Tharapel, R Sid Wilroy

Abstract

A newborn infant with phenotypic features of trisomy for distal 13q was found to have recombinant inversion duplication involving the (13)(q22→qter) region. Parental karyotypes showed that the mother had a normal 46,XX complement and the father had an apparently balanced pericentric inversion of a chromosome 13. Because of the unusual nature of the inversion, the exact position of the centromere on the father's inverted chromosome 13 was difficult to assign by GTG banding, even on prometaphase chromosomes. CBG and NOR banding were not informative in determining the location of the centromere. Fluorescent in situ hybridisation with an α satellite DNA probe for D13Z1/D21Z1 helped in confirming the exact position of the centromere in the rearranged paternal chromosome. Thus, the origin of the proband's abnormal chromosome 13 was clarified.

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Partial trisomy for the proximal and distal regions of chromosome 13 constitute two distinct clinical entities.¹⁻⁴ Partial duplications of distal 13(q21 or 22→qter) are known to result from familial balanced pericentric inversions of chromosome 13.⁵⁻⁸ The mechanism leading to the duplication of a segment in an inversion heterozygote is crossing over within the inversion loop during meiosis. In this report we describe a patient with partial duplication of chromosome 13(q22→qter) and the use of fluorescent in situ hybridisation (FISH) with an α satellite DNA probe to delineate a balanced pericentric inversion 13(p11.2q22) in her father. α satellite DNA is composed of heterogeneous repeated sequences which are unique to most human chromosomes. Exceptions such as the 13/21 α satellite probe that hybridises to the centromeres of more than one chromosome pair are known. Yet even in these cases, the size differences of chromosomes 12 and 21 would allow the detection of the presence and location of centromeres. In the family presented here, we used a 13/21 α satellite probe for FISH which allowed the confirmation of

the centromere position on the paternal inverted 13.

Case report

The proband was referred for genetic evaluation at 3 days of age because of dysmorphic features and multiple congenital anomalies. She was born to an 18 year old mother and a 24 year old father. Both parents were in good health at the time of the proband's conception. Labour was induced because of post-term status and meconium stained amniotic fluid. Birth weight was 3745 g (90th centile) and length was 51.5 cm (75th centile). Respiratory distress occurred shortly after birth and a congenital heart defect was suspected. However, cardiac evaluation showed no anomalies. Review of the family history indicated that the maternal grandmother had one spontaneous fetal loss at six months' gestation. Dysmorphic physical features of the proband included the following: flat occiput, large anterior fontanelle, open sagittal suture, capillary haemangiomas of the face, forehead, nasal bridge, eyelids, upper lip, and back, colobomas of the irides, left microphthalmos, sparse eyelashes and eyebrows, low set, large ears with broadly rolled helices, transverse nasal crease, long, bulbous nose, micrognathia, short neck, prominent sternum, narrow thorax, prominent labia majora, hirsutism of the lower back, pilonidal dimple, rocker bottom feet, postaxial hexadactyly of the hands and feet, and long, narrow fingers. She was hypertonic with incomplete extension of the hips, knees, and elbows. Renal ultrasonogram showed enlarged kidneys with duplication of the collecting system on the left. Further ophthalmological evaluation showed left optic nerve coloboma with left optic nerve agenesis.

CYTOGENETICS

Chromosome analysis using conventional GTG banding showed a recombinant inversion duplication of the distal 13q region with a 46,XX, rec(13), inv dup (q22→qter) complement (fig 1A). Parental karyotypes showed the mother had a normal 46,XX complement and

Division of Genetics,
Department of
Pediatrics, University
of Tennessee,
Memphis, 711
Jefferson Avenue,
Suite 523, Memphis,
Tennessee 38163, USA.
P L Gordon
J D Dalton
P R Martens
A T Tharapel
R S Wilroy

Correspondence to
Dr Gordon.

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Figure 1 (A) Partial karyotype of the proband's normal and abnormal chromosome 13 with inversion duplication. (B) Partial karyotype of proband's father's normal and inverted chromosome 13. (C, D) A partial metaphase from the father stained with GTG and NOR banding, respectively. Note the NOR positive area at the terminus of the inverted 13.

the father had an apparently balanced pericentric inversion of one chromosome 13 (fig 1B). The inverted 13 had a satellite and satellite stalk at one terminus. This made the localisation of the centromere all the more difficult despite GTG, CBG, and NOR banding analyses. A partial metaphase spread stained by GTG and NOR banding showing the inverted and normal chromosome 13s of the father is shown in fig 1C and D. FISH with an α satellite DNA probe for D13Z1/D21Z1 was useful in resolving the position of the centromere in the rearranged chromosome 13 of the father. The inversion was interpreted as pericentric with a 46,XY,inv(13)(p11q22) (fig 2). The probe for D13Z1/D21Z1 was obtained commercially (Oncor Inc, Gaithersburg, MD) and was used according to the manufacturer's instructions. The hybridisation was detected

Figure 2 Fluorescent in situ hybridisation with the probe for D13Z1/D21Z1. Long arrow shows the normal chromosome 13 and short arrow shows the inverted chromosome 13 with the centromere located in the middle of the chromosome. Arrowhead shows the two chromosome 21s with cross hybridisation.

by FITC labelled avidin, signals were amplified by antiavidin antibody, and chromosomal DNA was counterstained by propidium iodide. The slides were viewed and photographed under a Zeiss Fluorescence Photomicroscope II using Kodacolor Gold 200 film.

Discussion

A patient with a recombinant inversion duplication resulting in partial trisomy 13 led to the identification of a parent with a pericentric inversion of chromosome 13. The inversion duplication of (13)(q21 or 22→qter) usually originates through synaptic crossing over within the inversion loop in an inv(13)(p11q21) heterozygote. Morphologically, the inverted chromosome may rarely appear as an acrocentric itself⁵ or may appear as metacentric.⁶ Using the conventional chromosome banding techniques the centromere position in the inverted chromosome may be difficult to determine precisely. The GTG banded chromosome 13 in the parent of our patient had an acrocentric appearance with a satellite on one terminus (fig 1C). On NOR banding silver stain positivity was seen at the same terminus of the inverted chromosome (fig 1D). A clear definition of the centromere and the pericentric nature of the inversion in the father was possible only through FISH with a 13/21 α satellite DNA probe (fig 2).

The use of FISH to determine the chromosomal origin of the centromeres in whole arm translocations and to define the origin of ring and marker chromosomes has been reported previously.^{9,10} When dealing with certain pericentric inversions, it may be difficult to determine the location of the centromere. This can lead to questionable cytogenetic interpretations. Accuracy in determining the area involved is important in many cases, such as partial trisomy 13q in which phenotypic variability occurs. The delineation of the mechanisms responsible for this variability can only be enhanced by more consistent nomenclature and by more accurate clinical descriptions.

From a clinical standpoint, it is important to address the fact that most of the features seen in our proband are consistent with trisomy for the distal portion of 13q. In distal 13q partial trisomy, the most consistent features noted in published reports are polydactyly, haemangiomas, high arched palate, and cryptorchidism.² The latter does not apply as our patient is female. Polydactyly and haemangiomas were present but high arched palate was not. Polydactyly has been associated with trisomy of 13q31 and haemangiomas with trisomy of 13q32→qter. Both features are consistent with the inversion duplication of 13q22→qter in our patient. Noel *et al*⁴ suggested that the critical factor in the development of colobomas and microphthalmos is the presence of trisomy for the 13q14.2 or q14.3 regions. These regions are not present in triplicate in our patient who had colobomas and microphthalmos. Also, our patient had sparse eyebrows and eyelashes, which is in contrast to the review of Bonioli *et al*¹¹ of 33 patients with partial 13q

trisomy, all of whom had long, upward curved lashes.¹¹

Rogers² has previously addressed the phenotypic variation in cases of partial 13q trisomy and discussed several explanations including that by Martin-Lucas *et al*¹² suggesting that the expression of a chromosome abnormality may be under the control of the entire genome. It has also been proposed that regulatory genes may undergo position changes during chromosome rearrangement and thus affect gene expression resulting in phenotypic variation.² He also noted that inconsistent clinical information and breakpoint identification is likely to be responsible for some 'phenotypic variation'. Detailed systematic clinical evaluation of these patients and the use of more sensitive cytogenetic techniques, including non-isotopic in situ hybridisation, as exemplified in this report, would further ensure the accuracy of future phenotype-karyotype correlations.

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