Familial four breakpoint complex chromosomal rearrangement as a cause of monosomy 9p22→pter and trisomy 10p11.2→pter and 11q21 analysed by dual and triple colour FISH

P Stankiewicz, E Kostyk, E Bocian, H Stańczak, J Parczewska, E Piątkowska, T Mazurczak, J J Pietrzyk

Abstract

A familial four breakpoint complex chromosomal rearrangement involving chromosomes 9, 10, and 11 was ascertained through a child with dysmorphic features, hypertrophic cardiomyopathy, and hypotonia. A cryptic insertion, invisible in G banded chromosomes was identified by fluorescence in situ hybridisation (FISH) using chromosome specific libraries. Possible mechanisms of its formation as well as karyotype-phenotype correlation are discussed.

(J Med Genet 1997;34:696-699)

Keywords: complex chromosomal rearrangement; cryptic insertion; multicolour FISH

Complex chromosomal rearrangements (CCR) are defined as involving more than two chromosome breakpoints with reciprocal exchange of segments.^{1,2} They can be divided into familial and de novo or categorised according to the number of breakpoints. Those with four or fewer breaks belong to group I and those with more than four breaks to group II.³ Congenital CCRs are rarely compatible with life, in contrast to acquired ones frequently seen in certain leukaemias and cancers.

Until recently it was difficult to identify and characterise aberrations with a small amount of translocated material. Now, detection and interpretation of such subtle, as well as complex, chromosomal rearrangements is easier with the use of fluorescence in situ hybridisation (FISH).^{4,5}

We report on a family with a CCR consisting of a three way reciprocal translocation (9;11;10) and an insertion (9;11), and show that painting probes may be very useful in clarifying the G banding results. We also try to explain the mechanisms of its formation and correlate it with the phenotype.

Patients and methods

The proband, a girl, was referred to the Genetic Counselling Department at the age of 3 months. She was the product of the fourth, uncomplicated pregnancy of a healthy mother and father aged 34 and 38 years, respectively. Caesarean section was performed because of previous neonatal deaths. The baby's birth weight was 4400 g, length 60 cm, and head cir-

cumference 38 cm. Her Apgar score was 7 at one minute and 8 at five minutes. The main symptoms were cyanosis and dyspnoea, with visible dysmorphic features (not specified in the medical reports from that period of her life). The child was immediately referred to the Cardiology Unit, where the diagnosis of hypertrophic cardiomyopathy was made.

Three previous pregnancies produced one healthy boy, a macerated stillborn female, and a boy who died after two hours as a result of intracranial haemorrhage (fig 1). From the medical reports, the boy from the third pregnancy showed some dysmorphic features, including congenital heart malformation and hypospadias. In the pedigree, there were neither malformed (stillborn) children nor miscarriages among other family members.

On physical examination at the age of 3 months, the proband showed a dysmorphic face with a prominent forehead, large anterior fontanelle (4 × 6 cm), low set ears, epicanthic

Figure 1 The pedigree of the family. Karyotypes of II.2 and II.3 are unknown.

Department of
Genetics, National
Research Institute of
Mother and Child, ul
Kasprzaka 17A, 01-211
Warsaw, Poland
P Stankiewicz
E Bocian
H Stańczak
T Mazurczak

Department of
Medical Genetics,
Polish-American
Children's Hospital,
Collegium Medicum,
Jagiellonian University,
Wielicka 265, 30-663
Cracow, Poland
E Kostyk
J Parczewska
E Piątkowska
J J Pietrzyk

Correspondence to:
Dr Bocian.

Received 22 October 1996
Revised version accepted for
publication 5 March 1997

Figure 2 Facial appearance of the proband at the age of 2 years. (Photograph reproduced with permission.)

folds with mild hypotelorism, convergent strabismus, flat nasal bridge, flat and wide philtrum, thin upper lip, a large tongue, and high arched palate (fig 2). She was hypotonic with contractures of the knees and elbows. She

had widely spaced nipples and a heart murmur, 3/6 to 4/6 on Levin score. Cardiolog- ical examination showed hypertrophic cardio- myopathy with mitral insufficiency and arterial hypertension. The dermatoglyphic pattern of the child was abnormal: her fingertips showed whorls and axial triradii in the t' position. Both hands showed aberrant Sydney lines. There was a high degree of ridge hypoplasia and an excessive number of fine secondary creases. The proband's dermatoglyphics were very similar to those observed in her mother, who also showed a high degree of ridge dysplasia.

At the age of 2 years dysmorphic features were still present with evidence of severe phys- ical and mental retardation. The height of the girl was -1.2 SD with a weight deficiency of 20%; the head circumference was appropriate for her height. The child was hypotonic and could neither control her head nor sit even with help. She had some eye contact with her parents, but was not able to communicate. There was chronic prurigo on the skin. Her cardiolog- ical status had not changed.

Metaphase and prometaphase chromosomes were prepared from PHA stimulated peripheral blood lymphocytes. They were used for GTG banding as well as for FISH analysis. In situ hybridisation with chromosome 9, 10, and 11 specific paint probes on slides from the proband, her healthy mother, and her brother was performed following the procedure of Pin- kel *et al*⁶ and molecular probes were used according to the manufacturer's instructions. Chromosome 10 paint probe from Oncor was labelled with digoxigenin and detected using FITC conjugated layers of antibodies (Oncor). In dual colour FISH experiments, Cambio libraries labelled with biotin or FITC were applied for simultaneous visualisation of chro- mosomes 9 and 11, respectively. Biotin was visualised with rhodamine conjugated avidin and biotinylated antiavidin goat antibodies (Vector). Chromosome preparations were counterstained with DAPI and propidium iodide diluted in Vectashield antifade (Vector). In the triple colour FISH version the following differentially labelled Cambio probes were applied: chromosome 10 with biotin, chro- some 11 directly with FITC, and chromosome 9 with Cy3. Biotin labelled probe was detected with AMCA conjugated avidin and biotin- ylated antiavidin goat antibodies (Vector). No counterstaining with DAPI or propidium iodide was applied.

Metaphases were analysed under a Nikon epifluorescence microscope. The filter combi- nation used was dual band pass filter (Chroma Tech Corp) for FITC and Cy3 (or PI) and UV-1A (Nikon) for AMCA (or DAPI). For documentation photomicrographs were ob- tained with Kodak EKTAR 1000 ASA using dual exposure.

Cell lines from the proband and from the balanced translocation carrier are not available.

Results

In the routine cytogenetic studies the proband's karyotype was described as 46,XX,9p+, while the karyotype of her healthy

Figure 3 Ideograms and GTG banded prometaphase chromosomes showing t(9;11;10). The derivative chromosome is shown on the right of each chromosome pair. Breakpoints are denoted by arrows.

Figure 4 (A) FISH with the library for chromosome 10 shows a signal clearly painting the normal chromosome 10, the whole but distal part of the short arm of its homologue, and the translocated fragment. The abnormal der(10) is indicated by an arrow. (B) Application of both chromosome 9 and 11 paints showed that the small, distal part of chromosome 9 short arm was translocated onto the long arm of the abnormal chromosome 11. Note the der(9) chromosome with the thin insertion chromosome 11 segment transmitted to the proband. Abnormal chromosomes are indicated by arrows. (C) Simultaneous application of chromosome 9 (red), 10 (blue), and 11 (green) specific paints to chromosomes of the proband's mother. The karyotype of the proband's mother described according to ISCN 1995 is 46,XX,t(9;11;10)(p22;q21;p11.2). ish ins(9;11)(p22;q21q21)t(9;11;10)(wcp9+,wcp11+,wcp10+,wcp11+,wcp9+,wcp10-,wcp10+,wcp11+,wcp9-).

mother and brother was 46,XX (or 46,XY), 9p+,10p+,11q-. High resolution studies at the level of 850 bands showed a complex three breakpoint translocation allowing the mother's karyotype to be defined as 46,XX,t(9;11;10)(9qter→9p22::10p11.2→10pter;11pter11q21::9p22→9pter;10qter→10p11.2::11q21→11qter) (fig 3).

The G banding interpretations were confirmed with FISH using chromosome 9, 10, and 11 paints. However, the chromosome 11 specific paint yielded unexpected results. This probe appeared to hybridise to the structurally normal chromosome 11, to the distal part of the aberrant chromosome 10, as well as to the breakpoint region of chromosome 9 (fig 4). Thus, a four breakpoint complex chromosomal rearrangement has been identified. The karyotype of the mother was defined as 46,XX,ins(9;11),t(9;11;10)(9qter→9p22::11q21q21::10p11.2→10pter;11pter→11q21::9p22→9pter;10qter→10p11.2::11q21→11qter). The same rearrangement was found in the proband's healthy brother. The karyotype of the proband was finally designated as 46,XX,der(9)ins(9;11),t(9;11;10)(9qter→9p22::11q21q21::10p11.2→10pter)mat proving partial monosomy of chromosome 9 and partial trisomy of chromosomes 10 and 11.

Figure 5 Schematic representation of suggested simultaneous rearrangement mechanism. Note the chromosome 11 "tearing" insertion fragment.

Discussion

In our case, as in other similar cases, chromosome analysis using FISH was helpful in clarifying the G banding results.^{4 7 8} Combined classical and molecular cytogenetic data allowed us to reinterpret this complex chromosomal rearrangement as both a reciprocal translocation and an insertion.

According to data collected by Schinzel,⁹ one can assume that hypotelorism, a large tongue, and an aberrant dermatoglyphic pattern may be caused by del(9) and a large anterior fontanelle and mitral insufficiency by dup(10). Hypertrophic cardiomyopathy, hypertension, and chronic prurigo match none of the three aberration components although it is very difficult to assess the contribution of dup(11). The other features of the proband are common and not specific to any particular chromosome aberration.

We suggest that three different mechanisms could lead to chromosome 11 insertion. It could arise in gametogenesis during simultaneous fragment exchange among involved chromosomes as shown in fig 5. The other possibilities assume two step events. In the second mechanism a reciprocal translocation between chromosomes 10 and 11 may have occurred, t(10;11)(p11.2;q21), and was subsequently followed by the reciprocal translocation between chromosomes 9 and 11, t(9;11)(p22;q21), with a very close but different breakpoint in chromosome 11. In the third mechanism, the insertion of chromosome 11 would have been the first event. It may be possible to assign breakpoints more precisely with unique sequence probes that are more specific than chromosome paints. Three colour high resolution (fibre) FISH with probes mapping within and outside the inserted segment could produce evidence for direct or inverted insertion helping to elucidate the way in which the

abnormality arose. Unfortunately we are not able to perform such an analysis at present. In the mother of the proband, theoretically only two (alternate) of 20 possible meiotic 3:3 segregations of multivalent formation can be balanced. It cannot be excluded that balanced translocations in females favour non-disjunction owing to interchromosomal effect. However, no evidence for such an effect during paternal meiosis was found.¹⁰ Thus, asymmetrical segregations (4:2, 5:1, or 6:0) as well as crossing over events make this unlikely. This explains the high risk of miscarriages and abnormal outcomes of pregnancy in balanced CCR carriers, estimated as ~50% at amniocentesis.¹¹

Our case of CCR fits quite well with four conclusions concerning complex chromosomal rearrangements drawn by Batista *et al.*⁷ It is a familial, female transmitted, four breakpoint aberration resulting from meiotic 3:3 adjacent 1 segregation. Unfortunately, we were unable to define whether it had arisen during spermatogenesis in her father, as has been suggested for most CCR cases. What contradicts the observations of Batista *et al.* is the fact that of the offspring of our CCR carrier, it is the male who carries the balanced aberration and the proband with the unbalanced karyotype is a female.

In the present case the chromosomal insertion could be detected only with FISH which shows that this technique is a very useful adjunct to classical cytogenetics.

We wish to thank Professor Ewa Bartnik and Professor Ingo Hansmann for helpful discussion. The work was supported by the State Committee for Scientific Research, grant No 4 P05E 036 11.

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