A new approach to the elucidation of complex chromosome rearrangements illustrated by a case of Rieger syndrome

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Abstract

A patient with a complex chromosome rearrangement and unilateral Rieger syndrome is presented. This rearrangement involves four chromosomes and six breakpoints, one of which is at 4q25, the candidate region for Rieger syndrome. We discuss a novel approach to the elucidation of this case using a multiprobe fluorescence in situ hybridisation method to show rearrangements unpredictable from G banded analysis, and the clear and unambiguous presentation of the karyotype using computer generated colour ideograms.

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Complex chromosome rearrangements (CCRs) or "complex chromosome transloca-

tions" have been defined¹ as "those involving more than a reciprocal exchange of segments between two chromosomes", and more recently as "structural rearrangements involving at least three chromosomes and three or more chromosomal breakpoints".²

Batista *et al*³ have reviewed 93 cases of CCR (involving three or more chromosomes); 35 of these were familial, 51 de novo, and in seven cases the families were not studied completely. They concluded that with respect to transmission, subfertility in male carriers, segregation, and parental origin, CCRs follow the same basic principles as simpler rearrangements. The CCRs involved up to seven chromosomes and 10 breakpoints.

CCRs are normally identified in the first instance by standard chromosome banding techniques. However, complete characterisation is not always possible; in these cases, fluorescence in situ hybridisation (FISH, in particular with whole chromosome paints and chromosome specific centromeric and subtelomeric probes) can provide additional

Figure 1 (A) In situ hybridisation of a metaphase spread to whole chromosome paints specific for chromosomes 3 (green) and 13 (red) using the multiprobe FISH procedure; the arrow indicates chromosome 3 material on the derivative chromosome 17 and the arrowhead shows chromosome 3 material on the derivative chromosome 10. (B) In situ hybridisation with chromosome 17q subtelomeric probe; the arrow shows signal on the derivative chromosome 4 and the arrowhead shows the normal chromosome 17. (C) In situ hybridisation with chromosome 3p subtelomeric probe; the arrow shows the normal chromosome 3 and the arrowhead shows signal on the derivative chromosome 10. The identity of the derivatives in all three images was confirmed by enhancement using PSI Macprobe Powergene software to produce banded chromosomes.

information resulting in a more accurate definition of the rearrangement.³⁻⁶

We present a new approach to the elucidation of CCRs using a simple, flexible, and technically straightforward single slide multiprobe FISH procedure⁷ together with computer generated colour ideograms to provide a clear visual interpretation of the structural rearrangements involved. This approach is illustrated using a CCR case presenting with Rieger syndrome.

Case report

The proband was born after an uneventful twin pregnancy at 38 weeks' gestation by spontaneous vaginal delivery. No abnormalities were detected in the male twin but the proband (female) was found to have a mildly dysmorphic face with a long philtrum and a thin upper lip. The eyes were also abnormal with a congenital cataract of the right eye and a left coloboma. Ophthalmological examination under anaesthetic showed Rieger syndrome with a right sided Peter's anomaly. At the age of 1 year, the right intraocular pressure was raised and she developed buphthalmos. The right eye required enucleation following an injury but at

the age of 14 she retains vision (acuity 6/12) in her left eye with normal intraocular pressure and optic disc. Owing to absent dentition, she is currently awaiting surgery to her upper jaw to improve her dental malocclusion and cosmetic appearance. She attends normal school and has reached the same high academic level as her twin brother.

Materials and methods

CYTOGENETICS

Cytogenetic analysis was performed on GTG banded chromosomes from thymidine synchronised cultures of PHA stimulated peripheral lymphocytes, using standard techniques.

IN SITU HYBRIDISATION

For hybridisation of 24 paints to a single slide,⁷ the slide was first marked with a diamond pen on the underside to form a grid of 12 squares of sides approximately 0.8 cm. A total of 1.5 µl of fixed cell suspension was pipetted onto the top surface of the slide into the centre of each square; this typically gave 15-40 metaphase spreads per square, depending on the thickness of the original preparation. The slide was pretreated in 2 × SSC (pH 7.0) at 37°C for 30

Figure 2 Computer generated colour ideograms with images captured from G banded metaphase spreads. The full ISCN (1995)⁸ description is 46,XX,t(3;10;4)ins(17;3)?t(10;17)(3qter→3p22.2::4q25→4qter;10pter→10q11.22::3p24.2→3pter;4pter→4q25::10q11.22→10q26.3::17q25.3→17qter;17pter→17q25.3::3p24.2→3p22.2::?10q26.3→10qter).

minutes, then dehydrated through an ethanol series (70%, 90%, 100%, for two minutes); denaturation was at 70°C in 70% deionised formamide/2 × SSC (pH 7.0) for two minutes, followed by dehydration in an ice cold ethanol series (concentrations as above). Biotinylated and FITC labelled whole chromosome paints were obtained from Cambio. Aliquots (2 µl) of each of the 24 paints were combined in pairs (one biotinylated with one FITC labelled) in 12 0.5 ml Eppendorf tubes. After mixing, the paints were denatured by incubation at 70°C for two minutes, followed by 30–60 minutes prehybridisation at 37°C. A rubber solution grid was formed on the denatured, dehydrated slide by the extrusion of Cow Gum through a micropipette tip using a 1 ml disposable syringe. The lines marked on the underside of the slide were followed to ensure that the squares created by the grid contained the fixed chromosome material. Each 4 µl mixture of paints was carefully pipetted into the individual squares created by the rubber solution grid. A silicone rubber rim of the same dimensions as the clear glass area of the slide and width 0.2 cm was applied to the slide with Cow Gum and another slide laid on top. This provided a seal but ensured that the covering slide did not touch the surface of the paints and thus cause mixing of the paints across the top of the grid. Hybridisation was at 37°C overnight in a humidified chamber. The covering slide, silicone rubber rim, and rubber solution grid were removed as one piece and stringent washes and detection were according to standard protocols, that is, stringent washes: 65% formamide/2 × SSC (pH 7.0) at 43°C for 15 minutes, 2 × SSC (pH 7.0) at 37°C for eight minutes; detection using the Cambio protocol which entails TR/avidin, α-avidin, TR/avidin for the biotinylated paints, in conjunction with a two layer detection system for the FITC labelled paints. Chromosome specific subtelomeric probes were obtained from Oncor and used according to the manufacturer's instructions. (Subtelomeric probes may also be tested using the multiprobe system described above, either alone or in combination with whole chromosome paints.) Chromosomes were counterstained with DAPI in Vectashield and viewed using a fluorescence microscope attached to a PSI MacProbe Image Enhancement system.

Results

G banded chromosome analysis of this case initially established a CCR involving chromosomes 3, 4, 10, and 17. It was not clear at this stage, however, whether or not the karyotype was balanced; in addition, the origin of the extra material on the long arm of chromosome 17 could not be determined. The multipoint FISH procedure confirmed the major rearrangements detected on G banding and identified the extra material on chromosome 17 as originating from chromosome 3 (fig 1A). Material from chromosome 3 was thus present on both the derivative 10 and the derivative 17. In order to determine which of these derivative chromosomes had the translocated 3pter,

chromosome specific subtelomeric probes were tested for chromosomes 3p and 17q. The chromosome 3p specific subtelomeric probe hybridised to the normal 3, as expected, and also to the derivative 10 (fig 1C), so it was logical to assume that the derivative 17 was the result of an insertional translocation of chromosome 3 material into the long arm of chromosome 17. It was therefore a completely unexpected finding that the 17q specific subtelomeric probe hybridised to the derivative 4 (fig 1B), in place of the 10q telomere. The 10q specific subtelomeric probe is not available to us, but we assume that it would hybridise to the derivative 17 (fig 2).

The patient's karyotype is therefore balanced and according to standard ISCN (1995)⁸ nomenclature may be written as:

46,XX,t(3;10;4)ins(17;3)?t(10;17)(3qter→3p22.2::4q25→4qter;10pter→10q11.22::3p24.2→3pter;4pter→4q25::10q11.22→10q26.3::17q25.3→17qter;17pter→17q25.3::3p24.2→3p22.2::?10q26.3→10qter). This may also be described in a slightly more concise form as: 46,XX,der(3,4,10,17)t(3;10;4)(p22.2;q11.22;q25)ins(17;3)(q25.3;p24.2p22.2)?t(10;17)(q26.3;q25.3).

Parental karyotypes were normal on G banded chromosome analysis; FISH with the 17q subtelomeric probe showed signal on the terminal region of the two homologues of chromosome 17 in both parents, indicating that the unexpected subtelomeric rearrangement in the proband did not originate from a parental subtelomeric translocation between chromosomes 10 and 17.

Discussion

The mechanism of formation of CCRs is unclear; cases must by definition have at least three breakpoints, but more breakpoints are usual, and two cases have been reported with as many as 10.^{5,9} In cases where FISH has been used for re-evaluation of G banded analysis, this has usually resulted in an increased estimate of the number of breaks.^{3,4,10} However, the probes chosen for testing are usually determined by information from G banded analysis concerning the chromosomes involved. The case presented here underlines the need for multiprobe analysis of these cases, as some of the rearrangements involved may be unpredictable. The availability of both a commercial product (Cytocell Ltd) and the fast, cheap, and technically straightforward method described here means that either may be used to test the complete set of chromosome paints. It is our understanding that complete sets of chromosome specific subtelomeric probes will also be available commercially in the near future, and we would recommend that these are applied, using a multiprobe technique, to uncover cryptic subtelomeric translocations. The more complex a rearrangement, the higher would be the expected proportion of unbalanced gametes produced at meiosis. A full characterisation of complex rearrangements is therefore important for a more precise estimate of risk of progeny with unbalanced

karyotypes and is absolutely essential for accurate prenatal diagnosis.

Both alternative forms of karyotype described above are cumbersome and difficult for the patient and clinician to understand. In addition, the standard ISCN conventions for complex karyotypes are ambiguous (a point also made by others⁵) and may result in different interpretations by different centres. The ISCN therefore recommends either the illustration of complex rearrangements or verbal descriptions thereof; visual representation is clearly essential in such a complicated case as that described here. Previous authors have either used partial karyotypes⁵ or black and white images, cut and pasted, either with breakpoints indicated with arrows and band numbers^{3,5} or coloured in to represent the rearrangements.⁶ The computer generated colour ideograms (CGCIs) presented here (fig 2) are clear and simple, and are easily manipulated to represent most chromosome rearrangements. This department uses them routinely when reporting translocations, inversions, and insertions, as well as CCR cases. It is also our policy to provide clinicians with CGCI representations of the possible outcomes of pregnancies for patients with balanced rearrangements.

Interestingly, there are several published case reports of patients with Rieger syndrome who also have interstitial deletions in the region 4q25-4q27^{11,12} or balanced translocations involving the 4q25-26 region.¹²⁻¹⁴ The case presented here therefore lends further support to 4q25 as a candidate region for the gene involved in Rieger syndrome.

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