Insertion or inversion: that is the question

B Pearce[1], K Watts[1], V Maloney[2], T Boyle[1], M Collinson[1], J Barber[1,2,3]

1 Wessex Regional Genetics Laboratory, Salisbury NHS Foundation Trust, Salisbury, SP2 8BJ, UK
2 National Genetics Reference Laboratory (Wessex), Salisbury Hospital NHS Trust, Salisbury, Wiltshire, SP2 8BJ, UK
3 Human Genetics Division, Southampton University School of Medicine, Southampton General Hospital, Southampton

Introduction

Correct characterisation of balanced structural rearrangements is essential in order to give carriers an accurate recurrence risk. The risk of unbalanced offspring for different structural rearrangements varies greatly, from a negligible risk for most paracentric inversion carriers to an average risk of 15% for carriers of an intrachromosomal insertion (Madan and Nieuwint, 2002) .

Distinguishing insertions from inversions in the long arm of chromosome 13 can be difficult especially when one (or more) of the breakpoints is in a G-dark band. Four cases have recently been identified at the Wessex Regional Genetics Laboratory which on initial G-banded analysis were interpreted as intrachromosomal insertions within the long arm of chromosome 13 (figure 1), but which were later identified as paracentric inversions after further analysis using fluorescent *in situ* hybridisation (FISH) with a panel of BACs from 13q.

Figure 1. A karyogram of G-banded chromosomes 13 showing the normal (left) and the abnormal chromosome (right).

Patient Details

Patient 1 – A prenatal sample was received from a 20 year old female who was referred due to an increased serum screened risk of 1:40. Parental studies showed a maternal rearrangement of 13q.

Patient 2 – A 15 year old female was referred with possible Turner syndrome; parental studies showed the rearrangement had been inherited maternally.

Patient 3 – A 36 year old male was referred as his brother was shown in another lab to have a structural rearrangement of one chromosome 13.

Patient 4 – A 31 year old female was referred due to infertility; parental studies showed a paternal rearrangement of 13q.

Results

A panel of dual colour BAC probes from the RP-11 library on the Ensembl Genomebrowser (www.ensembl.org/Homo_sapiens/cytoview) were used to study the rearrangement on chromosome 13 in patient 1; these probes span the long arm of chromosome 13. Figure 2 shows some of the pairs of probes used in establishing this rearrangement as an inversion.

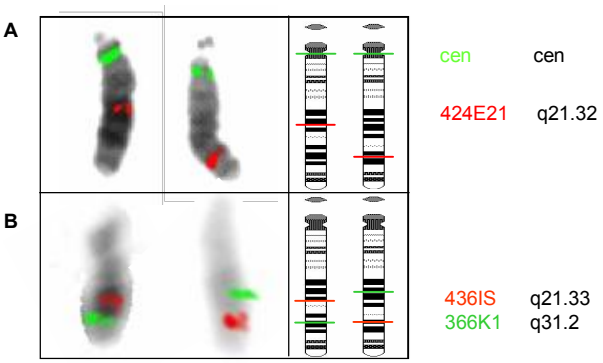

Figure 2. Examples of the dual colour BAC FISH used to establish the breakpoints and that this rearrangement is an inversion. The normal chromosome is on the left and the inverted chromosome on the right in each panel.

A: The q21.32 BAC 424E21 (red) has moved to a more distal location indicating that the breakpoint must be proximal to this band.
B: The q21.33 BAC 436I5 (red) and the q31.2 BAC 366K1 (green) are inverted with respect to each other consistent with an inversion and breakpoints proximal to q21.33 and distal to this part of q31.2.

We concluded that patients 1, 2, and 3 have paracentric inversions of 13q that looked similar cytogenetically with common breakpoints in 13q21.3 and 13q32.1 - inv(13)(q21.3q32.1). A more distal paracentric inversion was identified in patient 4 with breakpoints in 13q32.1 and 13q34 – inv(13)(q32.1q34).

Retrospectively we have been able to identify the most informative BAC pairs with which to establish the presence of a paracentric inversion in each case (Figure 3). The probe pairs marked in blue and yellow would be inverted with respect to each other, whereas the probe pairs marked in orange, green and pink would be further apart in an inversion.

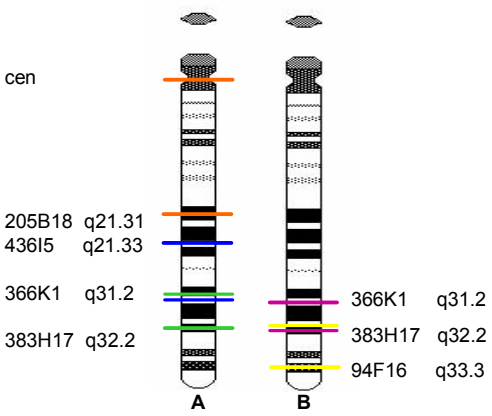

Figure 3. Ideograms showing the best combinations of probes to identify the rearrangements as inversions.
A: shows probes for identifying inversions in regions q21.3 – q32.1.
B: shows probes for identifying inversions in regions q32.1 – q34.

From the panel of probes used, two have retrospectively been identified that could have been useful if used in combination to identify the inversion in patients 1, 2 and 3 - RP11-366K1 and RP11-424E21. Therefore this pair of probes may be useful in the future to identify inversions within the same region as the ones seen here.

Discussion

All four inversions were initially identified as intrachromosomal insertions on G-banded cytogenetic analysis. This illustrates the difficulty in correctly defining rearrangements of chromosome 13 through G-banded analysis alone. It is important to distinguish these forms of balanced rearrangements, as the risks of unbalanced offspring for these carriers varies greatly.

The presence of common breakpoints in three apparently unrelated families suggests unsuspected identity by descent (Gilling et al, 2006) or a recurrent inversion arising from non-allelic homologous recombination between predisposing sequences. The common breakpoint at 13q21.3 in all four families suggests that there may be common predisposing sequences within this band but further detailed work would be needed to map these breakpoints at the molecular level.

Conclusion

We have identified pairs of probes that can be used to distinguish inversions from insertions in future structural rearrangements of 13q.

References

Gilling M et al (2006) Breakpoint cloning and haplotype analysis indicate a single origin of the common Inv(10)(p11.2q21.2) mutation among northern Europeans. Am J Hum Genet. 78(5):878-83.

Madan K and Nieuwint AW (2002) Reproductive risks for paracentric inversion heterozygotes: Inversion or insertion? That is the question. Am J Med Genet. 107(4):340-3.

Ensembl Genomebrowser (www.ensembl.org/Homo_sapiens/cytoview)