

ELECTRONIC LETTER

Multicolour FISH fine mapping unravels an insertion as a complex chromosomal rearrangement involving six breakpoints and a 5.89 Mb large deletion

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Occam's razor is a logical principle attributed to the medieval philosopher William of Occam. It is also referred to as the principle of parsimony, which states that one should always choose the simplest explanation of a phenomenon.¹ This principle is usually applied in chromosome banding analyses, as interpretations with a minimal number of breakpoints are usually favoured to explain observed chromosomal rearrangements. However, here we present a case in which this principle was not applicable.

In a patient seen because of developmental delay and several craniofacial dysmorphic features, chromosome analysis showed an insertion of 7q material into the short arm of one chromosome 5. This seemingly simple rearrangement was unravelled by advanced molecular cytogenetic tools to be in fact a highly complex rearrangement. We present the clinical data and describe the entire molecular cytogenetic examinations, which included seven fluorochrome multiplex fluorescence in situ hybridisation (M-FISH)² and the application of 61 BAC probes for the fine mapping of breakpoints within kb resolution. Altogether we identified six breakpoints and found inside the inserted material a deletion with a size of 5.89 Mb.

This is a case that shows that apparent balanced chromosomal rearrangements, which are difficult to corroborate with clinical findings, should be double checked by high resolution approaches.

CASE REPORT

The patient was born as the second child to a 32 year old mother and her non-consanguineous 39 year old husband after an uneventful pregnancy. Birth weight (2690 g, >10th centile), length (53 cm, >90th centile), and head circumference (33 cm, 25th centile) were within the normal range. She had a 4 year old, healthy sister.

At the age of 4 months insufficient control of head movement was noted. Physiotherapy treatment was started at the age of 7 months. At 10 months the patient could sit unassisted and spoke several syllables. At 13 months she had febrile convulsions. On physical examination muscular hypotonia and joint laxity were noted. At 19 months her motor and neurological development were judged to be mildly delayed but still within normal limits. At 20 months she again had three febrile convulsions.

At the age of 23 months, her height (83 cm, 25th centile), weight (11.8 kg, 50th–75th centile), and head circumference (46.5 cm, >10th centile) continued to be within normal limits. Several dysmorphic signs were noted (no consent was given to show pictures of this child), including brachycephaly, a high anterior hair line, slightly reduced inner canthal distance of the eyes, epicanthic folds, upward slanting palpebral fissures, a slight left strabismus divergens; medially broad and laterally rather diffuse eyebrows, broad nasal bridge and bulbous tip of the nose, wide and flattened philtrum, and elongated, low set, and abnormally shaped ears. There was no malformation of any internal organ.

Key points

- An apparent balanced insertion of chromosome 7 material into the short arm of one chromosome 5 was unravelled by advanced molecular cytogenetic tools and was shown to be a highly complex rearrangement.
- The molecular cytogenetic tools used in this study included seven fluorochrome multiplex-FISH (M-FISH) and 61 BAC probes, which were hybridised in a multi-colour fashion to fine map the breakpoints within the kb resolution.
- This chromosomal rearrangement, which was initially thought to be simple, involved six breakpoints and contained a deletion of 5.89 Mb within chromosome bands 7q34 and 7q35.
- The clinical features of our patient (developmental delay and several craniofacial dysmorphic features) were similar to a previously described patient with an interstitial deletion del(7)(q35).
- This case shows that apparently balanced chromosomal rearrangements, difficult to corroborate with clinical findings, should be double checked by higher resolution approaches.
- High resolution breakpoint mapping as presented here should increase the efforts to establish more accurate genotype-phenotype correlations.

METHODS

Banding analysis

Preparation of metaphase spreads and G banding analysis was done according to standard protocols.

Seven fluorochrome multiplex FISH

Multiplex-FISH³ was done with seven fluorochromes as described previously.²

FISH with YAC, BAC, and PAC probes for high resolution breakpoint mapping

For the detailed characterisation of the chromosomal rearrangement, a multitude of different probes was used.

To check the integrity of the 7q subtelomere region we used the 7q subtelomeric PAC 3K23.⁴ For an initial screen based on the banding information we selected several YAC probes, 882a10 (5p13), 761h12 (7q31), 964f3 (7q34), 761h5 (7q35), and 942g9 (7q36).

Abbreviations: FISH, fluorescence in situ hybridisation; M-FISH, multiplex-FISH

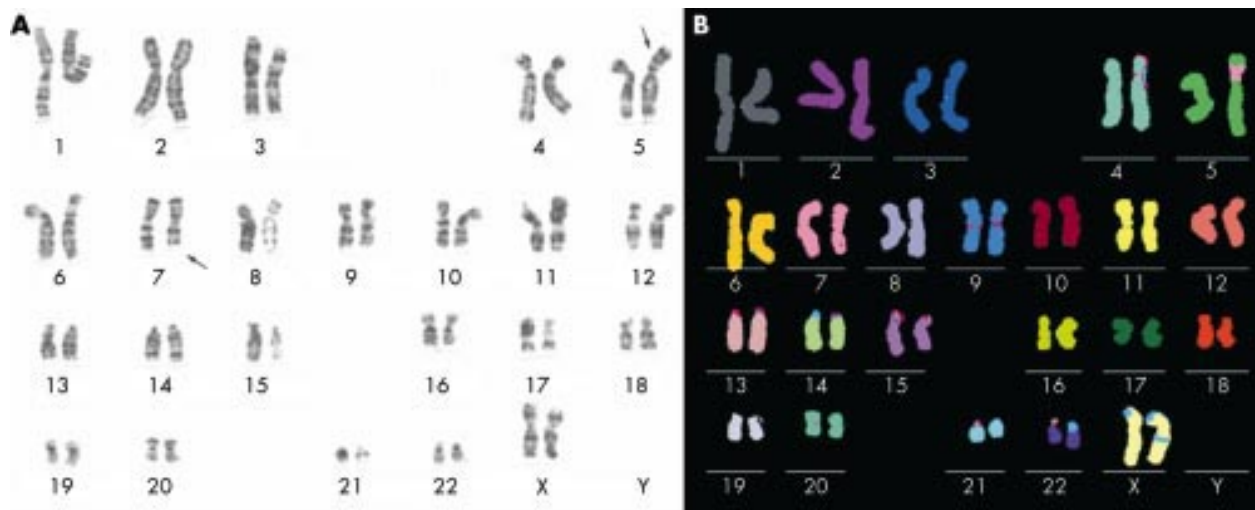


Figure 1 (A) Banding analysis of the patient's chromosomes showing a de novo insertion of chromosome 7q material into one of the 5p arms. (B) Confirmation of the $\text{ins}(5;7)$ by seven fluorochrome M-FISH.

For the high resolution breakpoint mapping, 7q probes were selected from the Ensembl genome browser of the Sanger Institute (<http://www.ensembl.org>).⁵ This is a constantly updated server; the data presented here are based on release 8.30a.1.

We selected 61 BAC clones within the physical chromosome 7 segment 110.97 Mb to 157.43 Mb, which corresponds to chromosome bands 7q31 to 7q36. These BAC clones included the probes RP5-1062J16, RP11-448H19, RP11-560I19, RP5-1101C3, RP5-850I1, RP11-395G17, RP5-1166A24, RP11-106F1, RP11-390E23, RP5-902E20, RP5-907C10, RP11-563O3, RP11-420H19, RP11-24C13, RP11-391H5, RP11-128A6, RP11-36B6, RP11-329I5, RP11-355K3, RP11-221G19, RP11-371N6, RP11-88K4, RP11-102G17, RP11-269N18, RP11-383F6, RP5-886O8, RP4-659J6, RP11-561D8, RP11-47C1, RP4-592P3, RP4-762N20, RP5-839B19, RP4-813F11, RP5-1007M8, RP5-1173P7, RP4-733H1, RP5-914M6, RP5-842K16, RP11-237G17, RP5-894A10, RP11-1220K2, RP11-282G13, RP11-298A10, RP11-272P13, RP11-13M3, RP11-334C6, RP5-832O14, RP4-630F1, RP5-968I16, RP11-301O24, RP5-819O4, RP4-558L10, RP4-811H12, RP5-1136G13, RP4-584D14, RP4-548K24, RP11-354O2, RP11-93F2, RP4-708P22, RP5-1058P19, and RP4-764O12.

All probes were labelled by standard nick translation. Probes were labelled directly or indirectly. Fluorochromes used for the direct labelling were DEAC, Cy3, and Cy5. Biotin or digoxigenin were used as haptens for the indirect labelling. For each probe 200 ng were precipitated with ethanol together with unlabelled Cot-1 DNA (Roche Diagnostics) and salmon testis DNA (Sigma) and resuspended in 50% formamide and 15% dextran sulphate. This mix was hybridised overnight at 37°C. Slides were washed with $4 \times \text{SSC}/\text{Tween}$ (3×5 minutes at 37°C), $1 \times \text{SSC}$ (3×5 minutes at 60°C) and blocked with 3% bovine serum albumin (30 minutes at 37°C). Avidin-Cy3.5 (Rockland) and antidigoxigenin-FITC (Roche Diagnostics) were used for detection. Chromosomes were counterstained with 4',6-diamidino-2-phenylindole.

Fluorescence microscopy and image analysis

Images were captured with a charge coupled device camera (Sensys, Photometrics, Kodak KAF1400 chip) on a Leica DMRXA-RF8 epifluorescence microscope (Leica, Wetzlar, Germany). Automatic acquisition and overlaying of grey scale images were done with Leica QFISH software (Leica Microsystems Imaging Solutions, Cambridge, UK) as previously described.⁶

RESULTS

Banding analysis of the patient's chromosomes showed an insertion of chromosome 7q material into one of the 5p arms (fig 1A). The initial interpretation of the karyotype was $46,XX,\text{inv ins}(5;7)(p13;qterq31)$. The chromosomes of the parents were normal.

Seven fluorochrome M-FISH² confirmed the $\text{ins}(5;7)$ and therefore excluded the involvement of other possible chromosomal rearrangements which could have contributed to the phenotype of our patient (fig 1B).

To analyse the involvement of the 7q subtelomeric region, we hybridised the 7q subtelomere probe 3K23. This probe showed signals on the distal q arms of both the normal chromosome 7 and the der(7) (fig 2A).

For a first assessment several YAC probes were hybridised. The 5p13-YAC 882a10 spanned the breakpoint on the der(5) chromosome (fig 2B).

Hybridisation with a panel of four differently labelled YAC probes specific for chromosome bands 7q31, 7q34, 7q35, and 7q36, showed the expected order of hybridisation signals on the normal chromosome 7 (fig 2C). However, the hybridisation pattern of the inserted material within the der(5) surprised us. Firstly, no signal was detectable for the 7q35 band specific YAC (fig 2D). Secondly, the hybridisation pattern of the three remaining YAC clones appeared instead of the expected sequence $7q34 \rightarrow 7q36 \rightarrow 7q31$.

These unexpected findings prompted us to pursue a detailed, high resolution analysis of the aberration. Sixty-one BAC probes were used for a high resolution analysis of the inserted chromosome 7 material. The sequence of hybridisation signals on the normal chromosome 7 was compared with the signal sequence within the inserted region.

To achieve an accurate comparison between the order of BAC clones on the normal chromosome 7 and within the inserted chromosome 7 region, several probes were hybridised simultaneously in a multicolour fashion. This allowed the identification of four different 7q fragments within the inserted material, based on the altered order of hybridisation signals. The fragments were enumerated according to their mapping positions from centromere to telomere on the normal chromosome 7. The first fragment ranged from BAC clones RP11-560I19 to RP11-390E23, encompassing 1.59 Mb, representing Mb 120.28 to 121.87 of chromosome 7, which are localised on chromosome band 7q31.32 (fig 3). The second fragment ranged from BAC clones RP5-902E20 to RP4-592P3, representing a size of 16.40 Mb (122.28–138.68) and spanning chromosome bands 7q31.32 to 7q34. The third fragment was

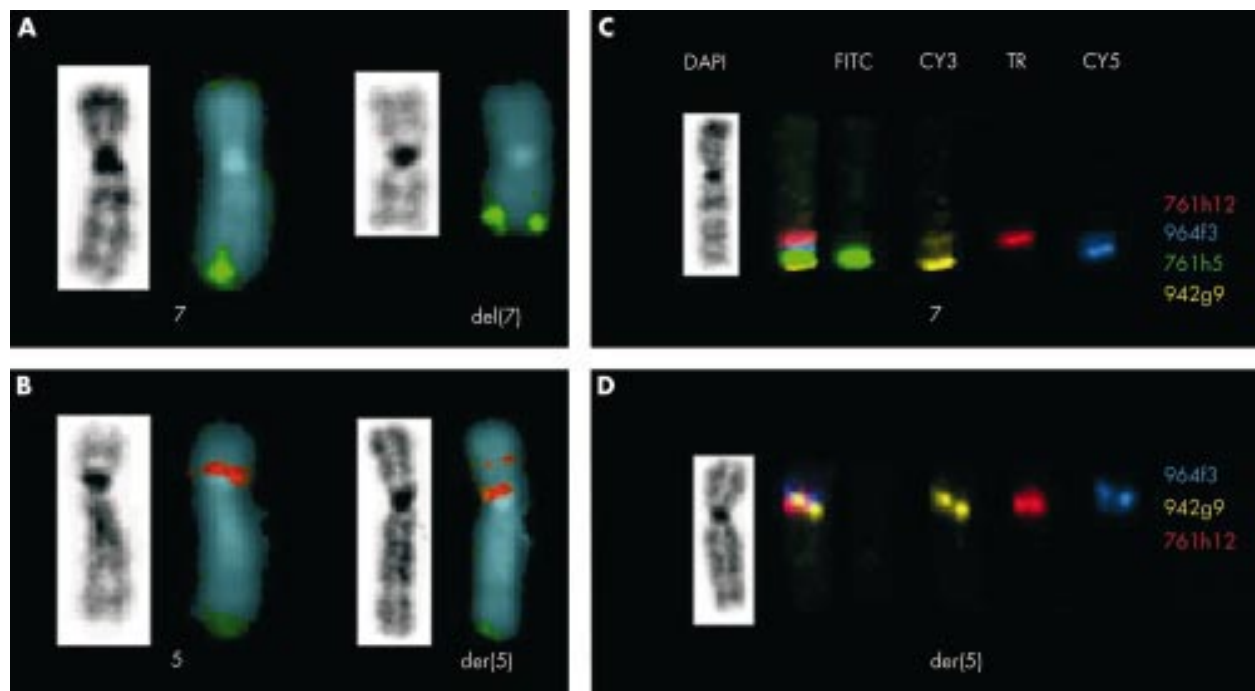


Figure 2 (A) Hybridisation of the 7q subtelomere probe 3K23 showing signals on the distal q arms of the normal chromosome 7 and the der(7). (B) The 5p13 YAC clone 882a10 spans the breakpoint on the der(5) chromosome. (C) Hybridisation with a panel of four differently labelled YAC probes specific for chromosome bands 7q31 (761h12 in red), 7q34 (964f3 in blue), 7q35 (761h5 in green), and 7q36 (942g9 in yellow), showing the expected order of hybridisation signals on the normal chromosome 7. (D) The inserted chromosome 7 material showed a different hybridisation pattern. The signal for the 7q35 band specific YAC is missing. The order of the three remaining YAC clones is 761h12 (7q31)→942g9 (7q36)→964f3 (7q34) from centromere to telomere of the derivative chromosome 5.

defined by BAC clones RP5-839B19 and RP5-968I16 which corresponds to a fragment within chromosome bands 7q34 and 7q35 with a size of 5.89 Mb (138.68–144.57). The fourth fragment had a size of 12.57 Mb (144.57–157.14) and was characterised by BAC clones RP4-558L10 and RP4-708P22, localised in chromosome bands 7q35 to 7q36.

The third fragment was deleted. Thus, the deletion spans the distal 3 Mb of the G light band 7q34 and the proximal 3 Mb of the G dark band 7q35. The order of the remaining three fragments on the short arm of the der(5) chromosome was 5cen→1→4→2→5pter (fig 3). Altogether six breakpoints

were identified, one each in chromosome bands 5p13, 7q34, 7q35, and 7q36 and two in 7q31.32. Based on these extensive mapping efforts the karyotype could be refined to: 46,XX,der(5)(5pter→5p13::7q31.32→7q34::7q35→7q36::7q31.32→7q31.32::5p13→5 qter),der(7)(7pter→7q31.32::7q36→7qter).

DISCUSSION

Here, we have presented a cytogenetic aberration, the complexity of which could not have been deduced or imagined from banding analysis. Several cytogeneticists did not see the 5.89 Mb deletion and the true complexity could only be

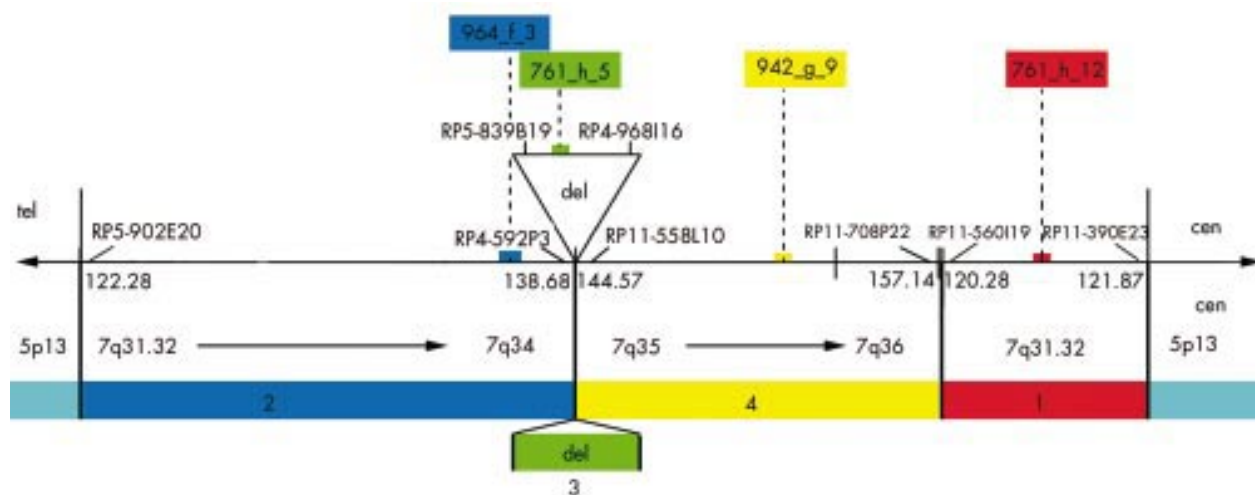


Figure 3 The hybridisation of a panel of 61 BAC clones allowed the identification of four chromosome 7 fragments within the inserted material. The order of these fragments is shown. One fragment, 5.89 Mb in size, on chromosome 7 ranging from bp 138.68 to 144.57 Mb, was lost. For details see text.

uncovered by a detailed molecular cytogenetic analysis. Several lessons can be learnt from this case.

Firstly, the rule of parsimony should not always be applied in cytogenetics. Seemingly simple chromosomal aberrations may be surprisingly complex. In particular cases in which a phenotype may be hard to explain by a de novo, balanced appearing rearrangement demand the exclusion of hidden small deletions, duplications, and complex rearrangements.

Secondly, owing to publicly available BAC clone resources, chromosomal deletions or duplications can now be mapped with an unprecedented accuracy. Furthermore, the application of BAC clones should allow a standardised report of chromosomal aberrations.

Thirdly, high resolution fine mapping, as described here, is a prerequisite to achieve precise genotype-phenotype correlations. The Ensembl database predicts 78 genes in the 5.89 Mb large deleted fragment. The effect of the loss of these genes on the phenotype is relatively mild. So far, only one case with a pure interstitial deletion del(7)(q35) with comparable dysmorphic features to our patient has been reported.⁷ In cases with larger deletions, such as del(7)(q33→q35), del(7)(q34→q36), or a del(7)(q34→qter),⁸⁻¹¹ some similar dysmorphic features were described including epicanthic folds, broad nasal bridge and bulbous tip, wide and flattened philtrum, and low set and abnormally shaped ears. As expected from the larger deletions these patients presented with additional features and more severe morphological defects. In the future, mapping efforts, as described here, should greatly facilitate the comparability of genotype-phenotype correlations reported from different clinics or laboratories.

Fourthly, the precise fine mapping and the accurate assessment of which genes were lost has the potential to shed some light on the possible function of these genes. This should in particular be true if more cases with comparable deletions are added.

Lastly, no method other than looking at metaphase chromosomes would have delineated the true nature of this complex rearrangement. The advancing array-comparative genomic hybridisation technologies^{12,13} would merely have detected the deletion. Thus, complex tools for a high resolution analysis of metaphase chromosomes will remain an indispensable cornerstone in cytogenetics.

Cases such as this, with apparently balanced chromosomal rearrangements which are difficult to corroborate with clinical findings, should be double checked by higher resolution approaches. This may unravel unexpected complexities and furthermore may identify small aneusomies, which may help to explain the phenotype.

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