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ARTICLE

High resolution comparative genomic hybridisation analysis reveals imbalances in dyschromosomal patients with normal or apparently balanced conventional karyotypes

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A sensitive technique is needed for screening whole genome imbalances in dyschromosomal patients when G-banding shows normal karyotypes or apparently balanced translocations. In this study we performed highly sensitive comparative genomic hybridisation analysis on a number of such cases and revealed chromosomal imbalances in all. *European Journal of Human Genetics* (2000) **8**, 661–668.

Keywords: comparative genomic hybridisation; chromosome analysis; chromosome aberrations; dyschromosomal

Introduction

Chromosome aberrations are the most common known cause of mental and physical deficiencies. During the approximately 40 years that cytogenetic analysis has been available as a diagnostic tool a number of clinical syndromes have proved to be caused by chromosome anomalies (eg Down syndrome, Turner syndrome and Klinefelter syndrome). Banding techniques have made it possible to reveal a number of small structural chromosome aberrations. However, since some small structural chromosome abnormalities may be overlooked by G-banding analysis, additional techniques for screening for small imbalances in dyschromosomal patients are needed.

Such techniques may also be of value for apparently balanced *de novo* translocations detected by prenatal diagnostics. Of these 6–10% are pathogenic[1,2] and may actually be unbalanced, but they cannot be distinguished from true balanced translocations by conventional cytogenetic techniques.

Multiplex-FISH and spectral karyotyping are whole genome screening techniques that have been successfully used for cytogenetic diagnostics of constitutional chromosomal abnormalities in pre- and postnatal applications.[3,4] However, small deletions and duplications are in danger of being undetected by these techniques. YAC mapping of cryptic deletions in apparently balanced translocations in dyschromosomal patients have likewise shown to be successful.[5,6] This technique, however, is laborious and is not suitable for patients with normal G-banded karyotypes.

Comparative genomic hybridisation (CGH) is a screening technique for whole genome imbalances.[7] The sensitivity of CGH is usually considered to be relatively low; however, we developed the technique further in order to increase sensitivity as well as specificity.[8,9] This has enabled us to detect deletions below 10 Mbp with very high specificity (the smallest so far being 3 Mbp).[10] Thus the improved high resolution technique supplements the chromosome banding and FISH techniques mentioned above and it is probably especially advantageous for detecting small deletions and

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duplications. In this study high resolution CGH was applied to one embryonic tissue case and nine cases of dyschromosomal patients. The latter cases, along with a number of similar cases, were referred to our laboratory for CGH analysis because the phenotypes of the patients were characteristic for chromosomal imbalance, despite the fact that conventional G-banding showed normal karyotypes or apparently balanced translocations. The initial G-band analyses were either performed at our laboratory or at other cytogenetic laboratories. In contrast to the G-banding results, high resolution CGH analysis revealed chromosome imbalances in the 10 cases presented in this work.

We believe that the CGH technique will turn out to be an indispensable adjunct to conventional chromosome analysis.

Materials and methods

Cases

Case 1 was embryonic tissue. The cytogenetic abnormalities of this case were known prior to CGH analysis since a partial monosomy 18qter was accidentally found in a chorionic villus case by interphase FISH with a locus-specific probe for chromosome 18qter. However, *no abnormalities* were detected on the G-banded karyotype. The father of the foetus was shown by FISH to have a balanced 11;18 translocation which was also undetectable by G-banding. The father had a dyschromosomal brother with normal conventional karyotype.

Cases 2–10 were all blood samples derived from dyschromosomal patients. The phenotypes of the patients showed dysmorphic figures with congenital malformation and/or mental retardation. The imbalances of these cases were all revealed by CGH analysis. All cases were previously karyotyped by conventional cytogenetics according to standard protocols.

Karyotypes by G-banding: cases 1–6: normal karyotypes. Case 7: balanced t(1;4) *de novo*, t(3;13) *de novo*. Case 8: balanced t(1,6,5) *de novo*. Case 9: balanced t(3;5) *de novo*. Case 10: normal karyotype (see Table 1). Case 9 was obtained from Coriell Cell Repositories, Coriell Institute for Medical Research, Camden, NJ, USA (Repository number GM10607).

Prior to CGH, FISH was used in Case 1 – Whole chromosomes painting probes for chromosome 11 (BRL – now Oncor, Gaithersburg, MD, USA) and 18 (Oncor, Gaithersburg, MD, USA) and a locus specific probe for chromosome 18qter, (IG-18, obtained from former Integrated Genetics, Framingham, MA, USA).

After CGH, FISH was used in Case 1 – telomeric probes for chromosome 11 (TL1102–15 (band 11q25)) and chromosome 18 (TL1802–15 (band 18q23)) both obtained from AL Technologies, Arlington, VA, USA; Case 3 – YAC probes for the following loci: D2S2227, D2S123, D2S2251, D2S378, *D2S370*, *D2S2198*, D2S147, D2S380, D2S2293 (CEPH, Paris, France), loci in italics were found to be deleted; Case 4 – YAC probe 885d10 (CEPH); Case 7 – YAC probes for the following loci: D13S159, D13S277, *D13S1267*, *D13S174*, *D13S259*, *D13S274*, D13S173, 13WI–9114, 13WI–6500, D13S261 (CEPH), loci in italics were found to be deleted; and Case 10–chromosome 9 centromere specific probe (D9Z1, Oncor)).

Reference DNAs for CGH analysis were obtained from peripheral blood drawn from karyotypically normal males and females.

High molecular weight genomic DNA was prepared by extractions on Qiagen Genomic Tip column (Qiagen, Hilden, Germany), or Puregene DNA isolation kit (Puregene, Gentra Systems; Minneapolis, USA).

CGH

CGH was performed as described previously.[8,9] Briefly, patient DNA and normal reference DNA were labelled with FITC-12-dUTP and Texas Red-5-dUTP (DuPont, Boston, MA, USA), respectively. Four hundred ng of each DNA and 20 μg Cot1 DNA were hybridised to normal metaphase chromosomes. Slides were hybridised for 4 days, washed, and counterstained with 4,6-diamidino-2-phenylindole. CGH

Table 1 Summary of G-banding, CGH and FISH data

Case no.	*G-banding analysis*	*CGH analysis*	*Confirmation of CGH results by G-banding*	*Conformation of CGH results by FISH*
1	46,XX *de novo*	rev ish enh (11q25)	no	yes
		rev ish dim (18q23)	no	yes
2	46,XY *de novo*	rev ish dim (1q22)	yes	n.d.
3	46,XY *de novo*	rev ish dim (2p15)	no	yes
4	46,XY *de novo*	rev ish enh (10q11)	no	yes
5	46,XX	rev ish dim (2q37)	yes	n.d.
6	46,XX	rev ish dim (7p15)	yes	n.d.
7	46,XY,t(1;4) (q31;q21.2) t(3;13)(p14.1; q33) *de novo*	rev ish dim (13q33)	no	yes
8	46,XX,t(1,6,5) (p13;q14;p13) *de novo*	rev ish dim (6q14)	no	n.d.
9	46,XY,t(3;5) (p23;p13) *de novo*	rev ish dim (2q24)	yes	n.d.
		rev ish dim (5p13)	no	n.d.
10	46,XX	rev ish enh (9)	yes[a]	yes[b]

n.d.: not done; [a]trisomy 9 was detected in 4% of cells; [b]trisomy 9 was detected in 46% of uncultured cells by interphase FISH.

image capture was performed with a Cyto Vision (Applied Imaging, Sunderland, UK) interfaced to a DM RBE fluorescence microscope (Leica, Heerbrugg, Switzerland) and images were transferred to a Magiscan image analysis system (Applied Imaging, Sunderland, UK). In each case, 10 metaphases were analysed. Detection of aberrations was performed by standard reference intervals as described in Kirchhoff *et al*.[9]

Briefly, along the mean ratio profiles the 99.5% confidence interval of each mean ratio profile value was compared with a corresponding 99.5% standard reference interval based on an average of 17 normal cases. The standard reference interval is especially wide at profile areas where CGH measurements are known to be unreliable. Whenever no overlap existed between the two intervals, the corresponding chromosome region was designated 'aberrant'. The standard reference interval was scaled automatically to fit the individual test case.

Results

Table 1 summarises the results of the G-banding analysis and the CGH analysis and states whether or not the CGH results could be confirmed by reinspection of the G-banded karyotype or by FISH. Chromosome aberrations were designated to bands according to the DAPI banding patterns. Figure 1 shows the CGH profiles and G-banding of the relevant chromosomes of all analyses. The following results were obtained.

Case 1: CGH analysis showed gain of chromosome 11q25 and loss of chromosome 18q23. Reinspection of the G-banded karyotype showed no imbalances. Whole chromosomes painting probes for chromosomes 11 and 18 showed that the duplicated part of chromosome 11 was translocated to the truncated chromosome 18 (Figure 2, A). Interphase FISH with a chromosome 18qter locus specific probe showed only one signal from chromosome 18 (Figure 2, B). A telomere probe for chromosomal band 11q25 showed that the translocation breakpoint of chromosome 11 was within this band (Figure 2, C). A telomere probe for chromosome band 18q23 showed that the translocation breakpoint of chromosome 18 was within this band (Figure 2, D).

Case 2: CGH analysis showed loss of chromosome 1q22 and this finding was confirmed by reinspection of the G-banded karyotype (Figure 1).

Case 3: CGH analysis showed loss of chromosome 2p15. This was not evident by reinspection of the G-banded karyotype but FISH studies with YAC probes confirmed the deletion, which was mapped to be 4–5 Mbp (Figure 3, A, B and C).

Case 4: CGH analysis showed a gain of chromosome 10q11. The gain was not confirmed by reinspection of the G-banded karyotype; however, FISH analysis with a chromosome 10 locus specific probe showed increased or two signals on one of the chromosomes 10 (Figure 3, D and E).

Case 5: CGH analysis showed loss of chromosome 2q37 and this finding was confirmed by reinspection of the G-banded karyotype (Figure 1).

Case 6: CGH analysis showed loss of chromosome 7p15 and this finding was confirmed by reinspection of the G-banded karyotype (Figure 1).

Case 7: CGH analysis showed loss of chromosome 13q33. This deletion was located in the breakpoint of the chromosome 13 involved in the 3;13 *de novo* translocation of this case. This was not evident by reinspection of the G-banded karyotype, however FISH with YAC probes confirmed the deletion, which was mapped to be approximately 5 Mbp (Figure 3, F, G and H).

Case 8: CGH results showed a deletion of chromosome 6q14. The deletion was not evident by reinspection of the G-banded karyotype and FISH studies have not been possible to carry out due to lack of sample material. The deletion of 6q14 was located in the breakpoint of the chromosome 6 involved in the 1;6;5 *de novo* translocation of this case.

Case 9: CGH analysis showed deletions of chromosomes 2q24 and 5p13. Only the deletion on chromosome 2 was confirmed by reinspection of the G-banded karyotype (Figure 1) and FISH studies could not be carried out due to lack of sample material. The deletion of 5p13 was located in the breakpoint of the chromosome 5 involved in the 3;5 *de novo* translocation of this case.

Case 10: CGH results showed gain of chromosome 9. Screening of 100 G-banded metaphases showed trisomy 9 in 4 cells (chromosomes 9 from one of the trisomic cells is shown in Figure 1). Interphase FISH with a chromosome 9 centromere specific probe performed on uncultured cells showed three signals in 46 of 100 counted cells (not shown).

No other aberrations were detected by CGH in any of the cases apart from the ones listed in Table 1 and no chromosome regions were excluded from analysis. The chromosomes of the parents were analysed with G-banding analysis and/or CGH whenever blood samples were obtainable. The analyses all showed that the chromosome aberrations found in the dyschromosomal children were *de novo* (see Table 1).

Discussion

The cases in this study (except for case 1) were analysed by CGH due to suspicion of chromosomal imbalance despite the fact that conventional G-banding showed normal karyotypes or apparently balanced translocations. The CGH analyses showed abnormalities in all cases. However, since the experience with high resolution CGH was limited, it was desirable to confirm the findings by other methods. Ten of the 12 imbalances found by CGH have been confirmed by either FISH or reinspection of the G-banded karyotype or both. It could not be excluded that the two unconfirmed deletions (Cases 8 and 9) were false positive results. However, as previously shown[9] false positive results are practically non-

existing at the level of confidence used in the CGH analyses of this work. Moreover, the reliability of the two deletions found in Cases 8 and 9 is strengthened by the fact that the deletions were found at the breakpoints of chromosomes involved in apparently balanced *de novo* translocations. Furthermore, the patient with the deletion on chromosome 5p (Case 9) was clinically suspected of cri-du-chat syndrome.

The success of CGH analysis in detecting imbalances in this study can be related to a number of factors. The sensitivity of the CGH technique seems higher than that of G-banding at the 300–600 band level, since for some Cases (1,3,4,7),

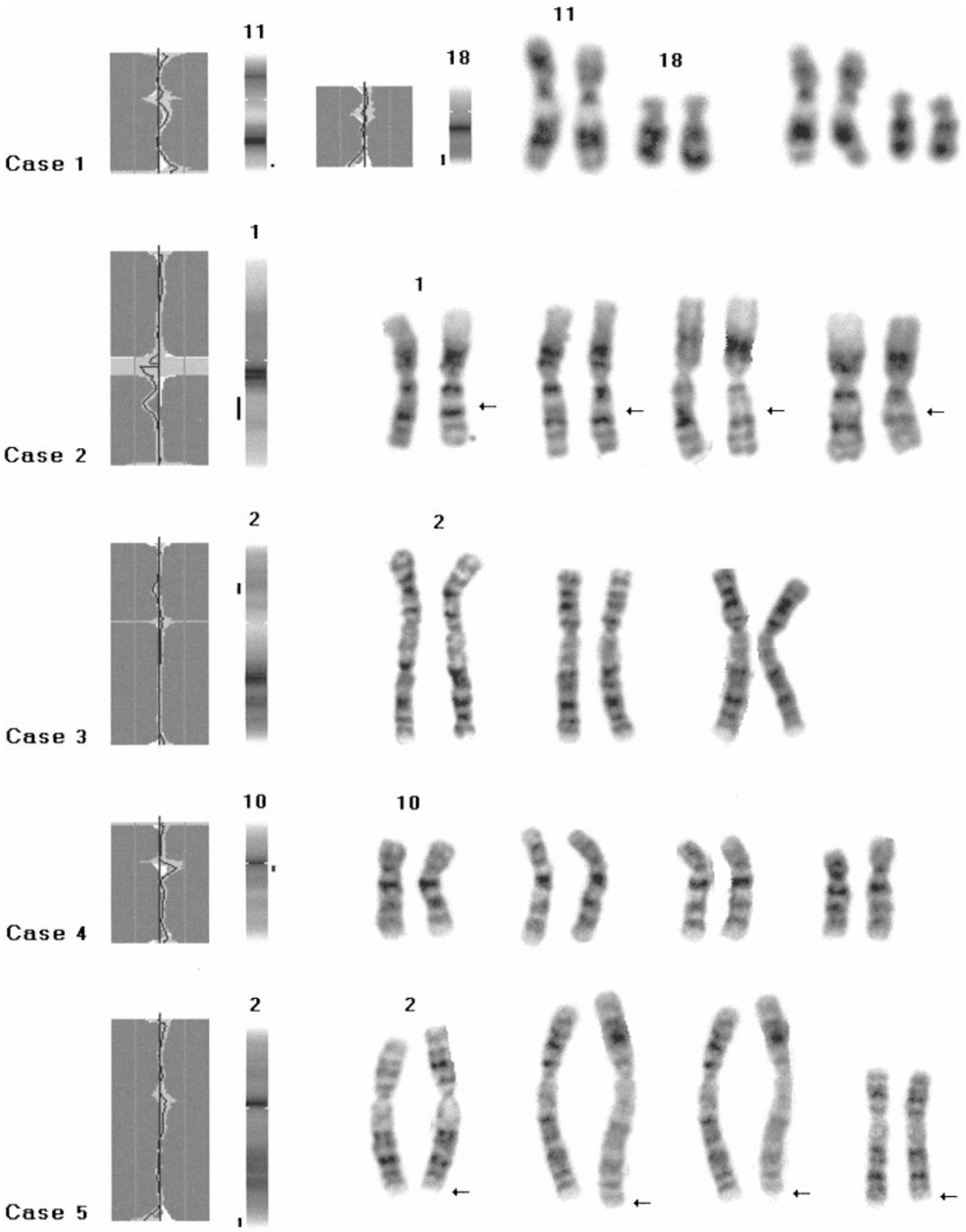

Figure 1 *Continued on next page.*

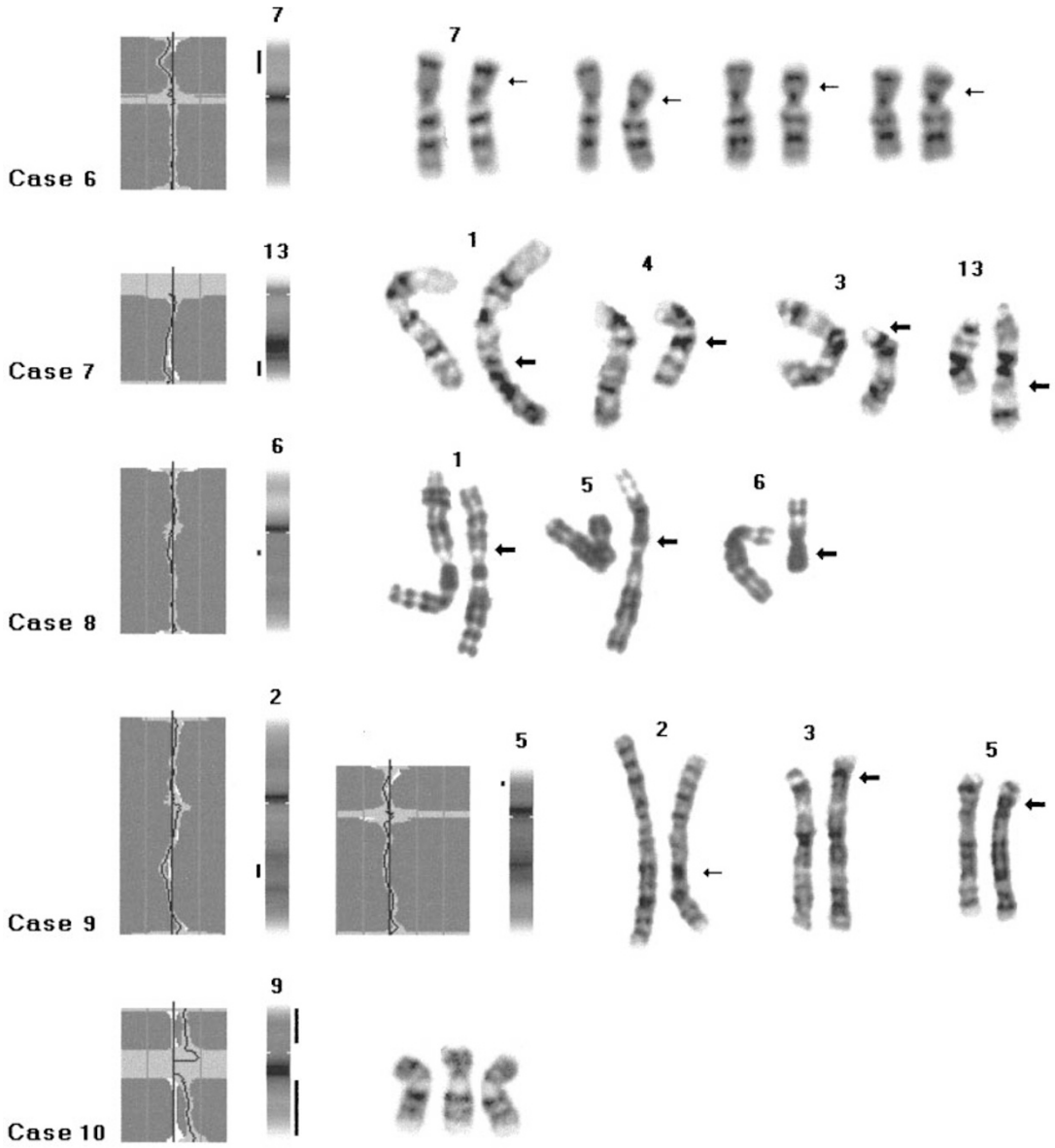

Figure 1 CGH results and G-banding analysis of selected chromosomes of all 10 cases. Mean ratio-profiles and 99.5% confidence intervals (grey) of the cases are shown. 99.5% standard reference intervals (white) are positioned underneath the confidence intervals. Aberrations are detected whenever the two intervals do not overlap. Vertical lines represent ratio 0.5, 1.0 and 1.5. Gains and losses are shown as black bars positioned respectively to right and left of the mean inverted DAPI banded pattern. Bold arrows indicate breakpoints in translocations and light arrows indicate chromosome aberrations confirmed by reinspection of the G-banded karyotype.

confirmed by FISH, it was not possible subsequently to confirm the CGH findings by reinspection of the G-banded karyotype. We know from a previous study that detection of deletions of 3 Mbp is feasible by high resolution CGH[10] and we have previously detected a number of Prader Willi/Angelman deletions by CGH (see Kirchhoff *et al*,[9] for example). These deletions are usually considered to be approximately 4 Mbp,[11] and are thus likely to be undetected by normal G-banding at the 300–600 band level.

For some of the cases reinspection of the G-banded karyotype confirmed the CGH findings and for two Cases (2 and 6) the abnormalities were actually quite conspicuous once it was decided on where to look. This clearly illustrates a weakness of the G-banding technique in comparison to CGH. G-band analysis is dependent on the qualifications and the actual performance of the cytogeneticist and this makes the technique somewhat subjective. Even highly skilled cytogeneticists may overlook small imbalances if no prior

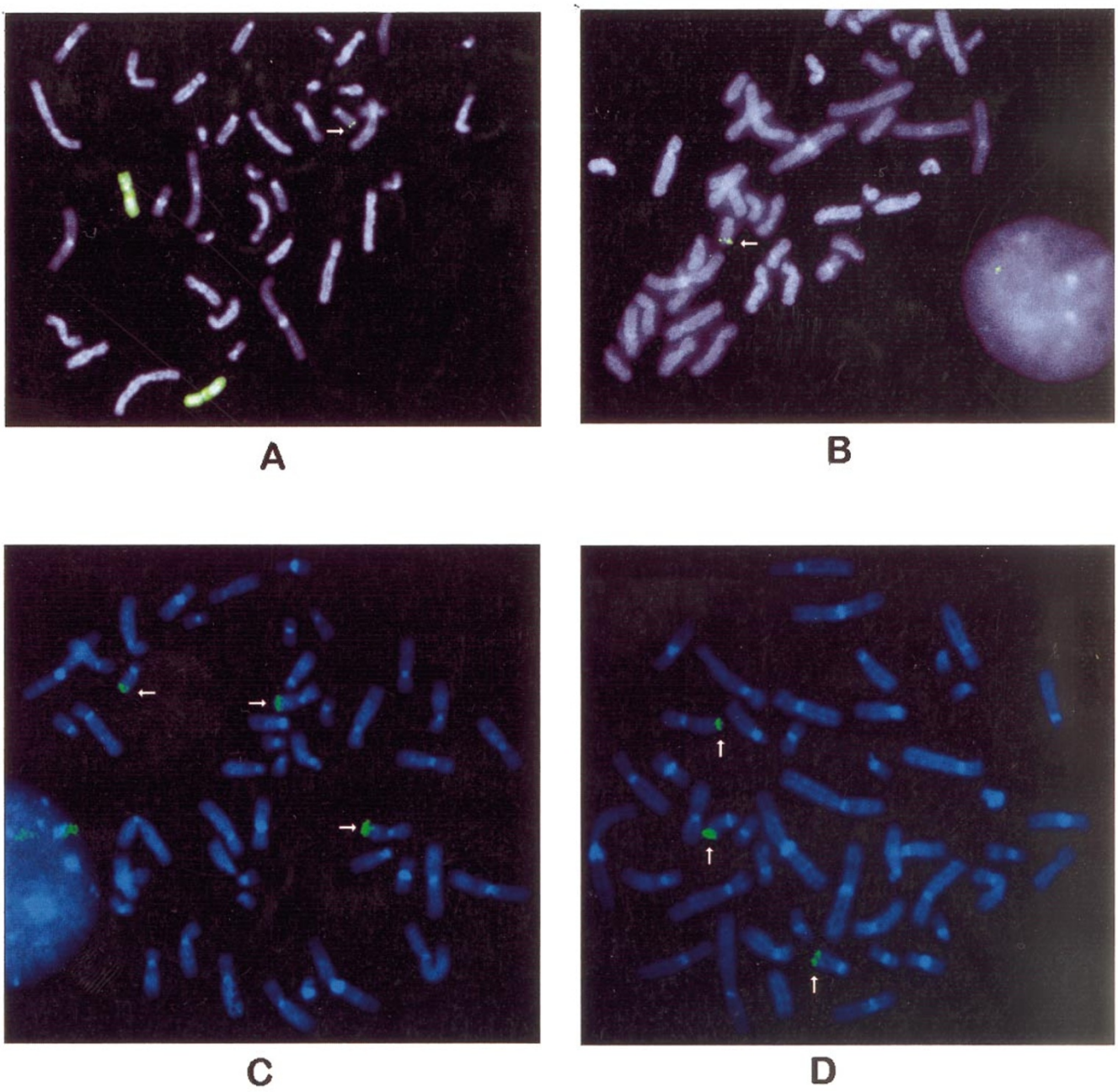

Figure 2 FISH analyses of case 1: **A** Whole chromosome painting probe for chromosome 11 shows chromosome 11 material at the distal end of chromosome 18q. **B** Locus specific probe for chromosome 18qter shows signal from only one chromosome 18. **C** Telomere probe for chromosome band 11q25 shows that the translocation breakpoint is within this band. **D** Telomere probe for chromosome band 18q23 shows that the translocation breakpoint is within this band.

knowledge of the abnormality is available, and as to the cases carrying the translocations, it may be impossible to detect small imbalances since exchange of chromosomal material may alter the appearance of the bands at the breakpoints making it difficult to detect deletions.

It is possible that the finding of a translocation in a G-banded karyotype may distract the cytogeneticists attention from detecting other abnormalities. This might have been so when Case 9 was karyotyped. A chromosome 3;5 translocation was detected in this case but an interstitial

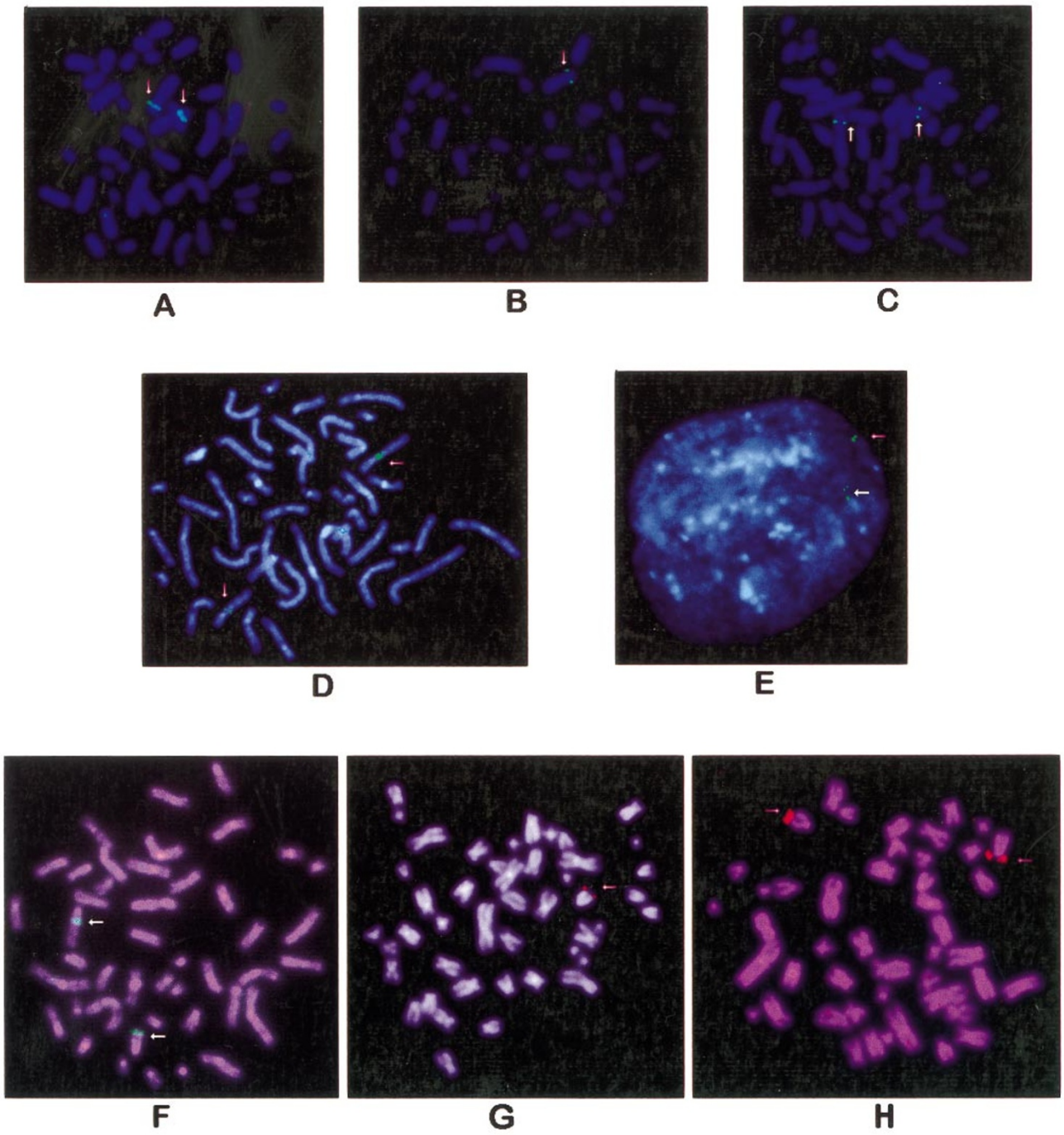

Figure 3 Fish analyses of Cases 2, 4 and 7: **A** Case 3. Chromosome 2 YAC probe for locus D2S123 shows signals from both chromosomes 2. The locus is proximal to the deletion. **B** Case 3. Chromosome 2 YAC probe for locus D2S370 shows signal from the normal chromosome 2. **C** Case 3. Chromosome 2 YAC probe for locus D2S147 shows signals from both chromosomes 2. The locus is distal to the deletion. **D** Case 4. Chromosome 10 YAC probe 885d10 shows enhanced signal from the chromosome 10 with the duplication. **E** Case 4. Chromosome 10 YAC probe 885d10 shows distinct signals from the chromosome 10 with the duplication. **F** Case 7. Chromosome 13 YAC probe for locus D13S277 shows signal from the normal chromosome 13 and the derivative chromosome 13. The locus is proximal to the deletion. **G** Case 7. Chromosome 13 YAC probe for locus D13S259 shows only signal from the normal chromosome 13. **H** Case 7. Chromosome 13 YAC probe for locus D13S173 shows signal from the normal chromosome 13 and from the derivative chromosome 3. The locus is distal to the deletion.

deletion on chromosome 2q was overlooked. This deletion was, however, confirmed by G-banding after CGH analysis.

Variability of the quality of G-banded chromosomes is an additional problem that is experienced by all laboratories working with the technique and it is also apparent in Figure 1. The G-banded chromosomes shown in this figure are derived from four different laboratories.

CGH analysis is characterised by a higher degree of objectivity than G-banding analysis. The metaphases used for the analysis are chosen by the individual performing the analysis, but the final detection of abnormalities is performed by statistical analysis. This objectivity paired with high sensitivity and specificity makes CGH analysis an excellent choice for detection for chromosomal aberrations in dyschromosomal patients when conventional G-banding shows normal chromosomes or apparently balanced translocations.

Case 10 exemplifies a different problem from the rest of the cases since the abnormality in it was not small. However, the trisomy 9 mosaic almost entirely disappeared during cultivation. CGH has gained great popularity in cancer research and one of the reasons for this is that clonal selection due to cultivation is avoided. It may be that clonal selection during cultivation of abnormal mosaic blood cases is a more widespread phenomenon than previously suspected.

It is still unclear if the aberrations found in the cases of this study are responsible for the clinical presentations of the patients. Loss of chromosomal material is usually associated with a seriously affected phenotype. Nevertheless, it cannot be ruled out that some findings may be of no apparent clinical significance. The small duplication on chromosome arm 10q in Case 4 may be a chromosomal variation present in some normal individuals. A likewise small duplication at 15q has been described in normal individuals.[12] Since conventional banding techniques do not detect small imbalances, the extension of such chromosomal variations in normal populations is unknown, and it is possible that several types of euchromatic variants may be transmitted in families, without reproductive or clinical effect.

It is of crucial importance to be able to diagnose dyschromosomal children. These children often go through a number of examinations with normal results, and without a diagnosis the parents are left in a very difficult situation in case of a new pregnancy. The use of CGH as an objective and sensitive screening technique for revealing chromosomal imbalances in dyschromosomal patients with normal or apparently balanced G-banded karyotypes seems promising. However, the CGH technique is both laborious and expensive to perform and economy may be a limiting factor for routine use. The crucial question is how often will imbalances be detected in dyschromosomal patients by CGH. We do not know the answer to this question since the imbalances presented in this work were found among a limited number of cases which were not referred to us on an organised basis.

In order to address the question and further characterise the potential of the technique for this specific clinical application we are performing a larger prospective study of dyschromosomal patients with either normal karyotypes or apparently balanced *de novo* translocations referred to our laboratory from paediatric departments all over Denmark.

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References

1 Wassman ER, Cheyovich DL, Nakahara Y: 'Possibly' *de novo* translocations: prenatal risk counseling. *Am J Obstet Gynecol* 1989; **161**: 698–702.
2 Warburton D: *De novo* balanced chromosome rearrangements and extra marker chromosomes identified at prenatal diagnosis: clinical significance and distribution of breakpoints. *Am J Hum Genet* 1991; **49**: 995–1013.
3 Uhrig S, Schuffenhauer S, Fauth C *et al*: Multiplex-FISH for pre- and postnatal diagnostic applications. *Am J Hum Genet* 1999; **65**: 448–462.
4 Schröck E, Veldman T, Padilla-Nash H *et al*: Spectral karyotyping refines cytogenetic diagnostics of constitutional chromosomal abnormalities. *Hum Genet* 1997; **101**: 255–262.
5 Kumar A, Becker LA, Depinet TW *et al*: Molecular characterization and delineation of subtle deletions in *de novo* 'balanced' chromosomal rearrangements. *Hum Genet* 1998; **103**: 173–178.
6 Wirth J, Nothwang HG, van der Maarel S *et al*: Systematic characterisation of disease associated balanced chromosome rearrangements by FISH: cytogenetically anchored YACs identify microdeletions and candidate regions for mental retardation genes. *Med Genet* 1999; **35**: 271–278.
7 Kallioniemi A, Kallioniemi OP, Sudar D *et al*: Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. *Science* 1992; **258**: 818–821.
8 Kirchhoff M, Gerdes T, Maahr J, Rose H, Lundsteen C: Automatic correction of the interfering effect of unsuppressed interspersed repetitive sequences in comparative genomic hybridization analysis. *Cytometry* 1997; **28**: 130–134.
9 Kirchhoff M, Gerdes T, Rose H, Maahr J, Ottesen AM, Lundsteen C: Detection of chromosomal gains and losses in comparative genomic hybridization analysis based on standard reference intervals. *Cytometry* 1998; **31**: 163–173.
10 Kirchhoff M, Gerdes T, Maahr J *et al*: Deletions below 10 megabasepairs are detected in comparative genomic hybridization by standard reference intervals. *Genes Chrom Cancer* 1999; **25**: 410–413.
11 Christian SL, Bhatt NK, Martin SA *et al*: Integrated YAC contig map of the Prader-Willi/Angelman region on chromosome 15q11–q13 with an average STS spacing of 35 kb. *Genome Res* 1998; **8**: 146–157.
12 Browne CE, Dennis NR, Maher E *et al*: Inherited interstitial duplications of proximal 15q: genotype-phenotype correlations. *Am J Hum Genet* 1997; **61**: 1342–1352.