Original articles

Applications of comparative genomic hybridisation in constitutional chromosome studies

Claire J Breen, Lynn Barton, Aiveen Carey, Adam Dunlop, Mary Glancy, Keara Hall, Anne Marie Hegarty, M Tariq Khokhar, Monica Power, Karen Ryan, Andrew J Green, Raymond L Stallings

National Centre for Medical Genetics, Our Lady's Hospital for Sick Children, Crumlin, Dublin 12, Ireland
C J Breen
L Barton
A Carey
A Dunlop
M Glancy
K Hall
A M Hegarty
M T Khokhari
M Power
K Ryan
A J Green
R L Stallings

Children's Medical and Research Foundation, Crumlin, Dublin 12, Ireland
C J Breen

Faculty of Medicine, University College Dublin, Ireland
A J Green
R L Stallings

Correspondence to:
Dr Stallings.

Received 1 September 1998
Revised version accepted for publication 3 February 1999

Abstract
G band cytogenetic analysis often leads to the discovery of unbalanced karyotypes that require further characterisation by molecular cytogenetic studies. In particular, G band analysis usually does not show the chromosomal origin of small marker chromosomes or of a small amount of extra material detected on otherwise normal chromosomes. Comparative genomic hybridisation (CGH) is one of several molecular approaches that can be applied to ascertain the origin of extra chromosomal material. CGH is also capable of detecting loss of material and thus is also applicable to confirming or further characterising subtle deletions. We have used comparative genomic hybridisation to analyse 19 constitutional chromosome abnormalities detected by G band analysis, including seven deletions, five supernumerary marker chromosomes, two interstitial duplications, and five chromosomes presenting with abnormal terminal banding patterns. CGH was successful in elucidating the origin of extra chromosomal material in 10 out of 11 non-mosaic cases, and permitted further characterisation of all of the deletions that could be detected by GTG banding. CGH appears to be a useful adjunct tool for either confirming deletions or defining their breakpoints and for determining the origin of extra chromosomal material, even in cases where abnormalities are judged to be subtle. We discuss internal quality control measures, such as the mismatching of test and reference DNA in order to assess the quality of the competitive hybridisation effect on the X chromosome.
(*J Med Genet* 1999;**36**:511–517)

Keywords: comparative genomic hybridisation; constitutional chromosome studies

Comparative genomic hybridisation (CGH) is a dual fluorescence in situ hybridisation protocol capable of detecting global gains and losses of genomic regions by competitive DNA hybridisation. CGH has been widely applied to detect gains and losses of DNA sequences on specific chromosomes in the study of solid tumours,[1] but has not been extensively used to study unbalanced constitutional karyotypes. Constitutional chromosome studies involving conventional GTG band cytogenetic analysis, however, often show unbalanced karyotypes which cannot be fully defined because the extra material is too small to have a recognisable banding pattern. CGH is one of several molecular cytogenetic approaches available for further defining and confirming abnormal results detected by conventional banding techniques. Chromosomal identification of marker chromosomes and extra bands on chromosomes through molecular techniques is always of clinical interest.

Bryndorf *et al*[2] applied CGH to the study of unbalanced karyotypes in prenatal cases, including seven cases of unbalanced structural abnormalities and four cases of simple aneuploidies. Others have applied CGH to the study of constitutional chromosome abnormalities detected postnatally.[3 4] In all of these cases, CGH was successfully used to determine the origin of extra chromosomal material or to confirm a deletion. Although the structural chromosome abnormalities in these cases were too small to identify the origin of the extra genomic material based on banding pattern, none of the abnormalities described by these authors can be considered to be subtle or cryptic.

Ghaffari *et al*[5] used CGH to detect cryptic translocations in families with extensive histories of mental retardation. These authors successfully identified cryptic translocations in three families. The identification of cryptic translocations is of considerable importance since up to 6% of patients with idiopathic mental retardation may have unbalanced translocations involving the telomeres.[6] These translocations are either too small to detect by conventional light microscopy or do not produce a recognisable change in GTG banding pattern. Molecular cytogenetic approaches involving

Table 1 Data from GTG banded karyotypes, CGH, and FISH

Case No	G banded karyotype	CGH	FISH	Final karyotype
1	46,XX,add(16)(q24)	enh(16)(q23q24)	wcp16+	46,XX,inv dup(16)(q24q23)
2	46,XX,add(17)(q25)	enh(7)(p21-pter)	wcp7+	46,XX,der(17)t(7;17)(p21;q25)
3	46,XX,add(17)(q25)	enh(17)(q24q25)	wcp17+	46,XX,inv dup(17)(q25q24)
4	46,XY,ins(20;?)(q11.2;?)	enh(20)(q11.2q12)	wcp20+	46,XY,dup(20)(q11.2q12)
5	46,XX,add(3)(p25)	enh(8)(q24.1-qter)	wcp8+; D3S1442−	46,XX,der(3)t(3;8)(p25;q24.1)
6	46,XY,ins(2;?)(q24.2;?)	enh(2)(q24.2q31)	wcp2+	46,XY,dup(2)(q24.2q31)
7	46,XX,add(9)(q34.3)	Normal profile	wcp9−; TEL 11p+	46,XX,t(9;11)(q34.3;p15.3)
8	47,XX,+mar	enh(19)(p13.1q10)	wcp19+	47,XX,+der(19)(p13.1q10)
9	47,XX,+mar	Normal profile		47,XX,+mar
10	47,XY,+r[20]/46,XY[9]	Normal profile		47,XY,+r[20]/46,XY[9]
11	47,XX,+9p	enh(9)(p12-pter)	wcp9+	47,XX,+9p
12	47,XY,+mar	enh(15)(q11q13)	SNRPN+	47,XY,+inv dup(15)
13	46,XY,del(15)(q12q12)	dim(15)(q12q12)	SNRPN−	46,XY,del(15)(q12q12).ish del(15)(q12q12)(SNRPN−)
14	46,XX,del(15)(q12q12)	dim(15)(q12q12)	SNRPN−	46,XX,del(15)(q12q12).ish del(15)(q12q12)(SNRPN−)
15	46,XY	Normal profile	SNRPN−	46,XY.ish del(15)(q12q12)(SNRPN−)
16	46,XY	Normal profile	SNRPN−	46,XY.ish del(15)(q12q12)(SNRPN−)
17	46,XX,del(1)(p36.3)	dim(1)(p36.3)	wcp1+; D1S1615−	46,XX,del(1)(p36.3)
18	46,XY,del(11)(q13.5q21) or (q14.2q22.2) or (q21q23.1)	dim(11)(q22)	wcp11+	46,XY,del(11)(q21q23.1)
19	46,XY,del(11)(q13.5q21) or (q14.2q22.2) or (q21q23.1)	dim(11)(q14)	wcp11+	46,XX,del(11)(q14.2q22.2)

FISH analysis with chromosome specific subtelomeric probes have also been used to detect cryptic translocations.[7] Finally, some authors have used CGH to confirm the presence of subtle interstitial duplications.[8 9]

Here, we assess the utility of CGH for determining the origin of supernumerary marker chromosomes and the origin of extra G bands occurring interstitially and terminally. We have also applied CGH to confirm the presence of subtle deletions and to clarify breakpoints in two cases of chromosome 11q deletions.

Material and methods

Cases 1, 2, 3, 5, 6, 7, 8, 9, 11, 12, 18, and 19 were submitted for cytogenetic analysis primarily for developmental delay and dysmorphic features. Case 4 was submitted for clarification of results from another laboratory, case 10 for infertility, case 17 for small for dates and short upper limbs, and cases 13, 14, 15, and 16 for Prader-Willi syndrome. The GTG banded karyotypes for each of these cases is listed in table 1. The chromosome abnormalities detected in cases 1 to 4, 6 to 9, and 11 to 19 were de novo in origin. Parental cytogenetic analysis for cases 5 and 10 could not be carried out.

GTG banding and FISH were carried out on metaphases derived from short term lymphocyte cultures stimulated with phytohaemagglutinin M by standard techniques. Probes for whole chromosome paints (1, 2, 7, 8, 11, 16, and 17), SNRPN, D3S1442, D1S1615, and the 11p telomere (Tel 11p) were obtained from Oncor and hybridised under conditions recommended by the supplier. Whole chromosome paints for chromosomes 9 and 20 were obtained from Cytocell and hybridised under recommended conditions.

DNA from test and reference samples was carefully quantified using a fluorimeter with known standards. Exactly 1 μg of test DNA from a patient with an unbalanced karyotype was labelled with Spectrum green-dUTP (Vysis) and 1 μg of reference (normal) DNA with Spectrum red-dUTP(Vysis) using a CGH nick translation kit from Vysis. Ten μl of nick translation enzyme (DNAase and polymerase I) from this kit were used for each nick translation reaction carried out at 15°C for four hours. An aliquot of each reaction was run on a 1% agarose gel to determine the probe size, the optimum size required being 300 bp to 3 kb. Test and reference DNA were then ethanol precipitated in the presence of 50 × human Cot-1 DNA (to block highly repetitive sequences), and resuspended in 10 μl of hybridisation buffer (50% formamide, 1 × SSC, 10% dextran sulphate) overnight at 37°C to aid resuspension.

Hybridisation was performed on commercially prepared (Vysis) slides containing metaphases. The metaphase DNA was denatured by immersing slides in 70% formamide, 2 × SSC at 73°C for five minutes.

The slides were then dehydrated in an ethanol series (70%, 85%, and 100%) and air dried. The probe DNA was denatured at 73°C for five minutes and added immediately to the slides. Slides were coverslipped, sealed with rubber cement, and placed in a moist container at 37°C for 72 hours.

Unbound DNA fragments were removed by washing in 0.4 × SSC/0.3% NP-40 at 73°C for two minutes and 2 × SSC/0.1% NP-40 at room temperature for 30 seconds. Slides were then counterstained with 4'-6-diamidino-2-phenylindole dihydrochloride (DAPI-antifade from Oncor).

Separate digitised grey level images of DAPI, Spectrum green, and Spectrum red fluorescence were acquired with a CCD camera coupled to an Olympus BX60 microscope. Image processing was carried out using Cytovision 3.52 software from Applied Imaging. Average green:red fluorescence ratio profiles were calculated for each chromosome from 8-10 metaphases. As an internal control, each CGH experiment was performed by mismatching the sexes between test and reference DNA. If the green labelled test DNA is male (single X chromosome) and the red labelled reference DNA is female (two X chromosomes), then we

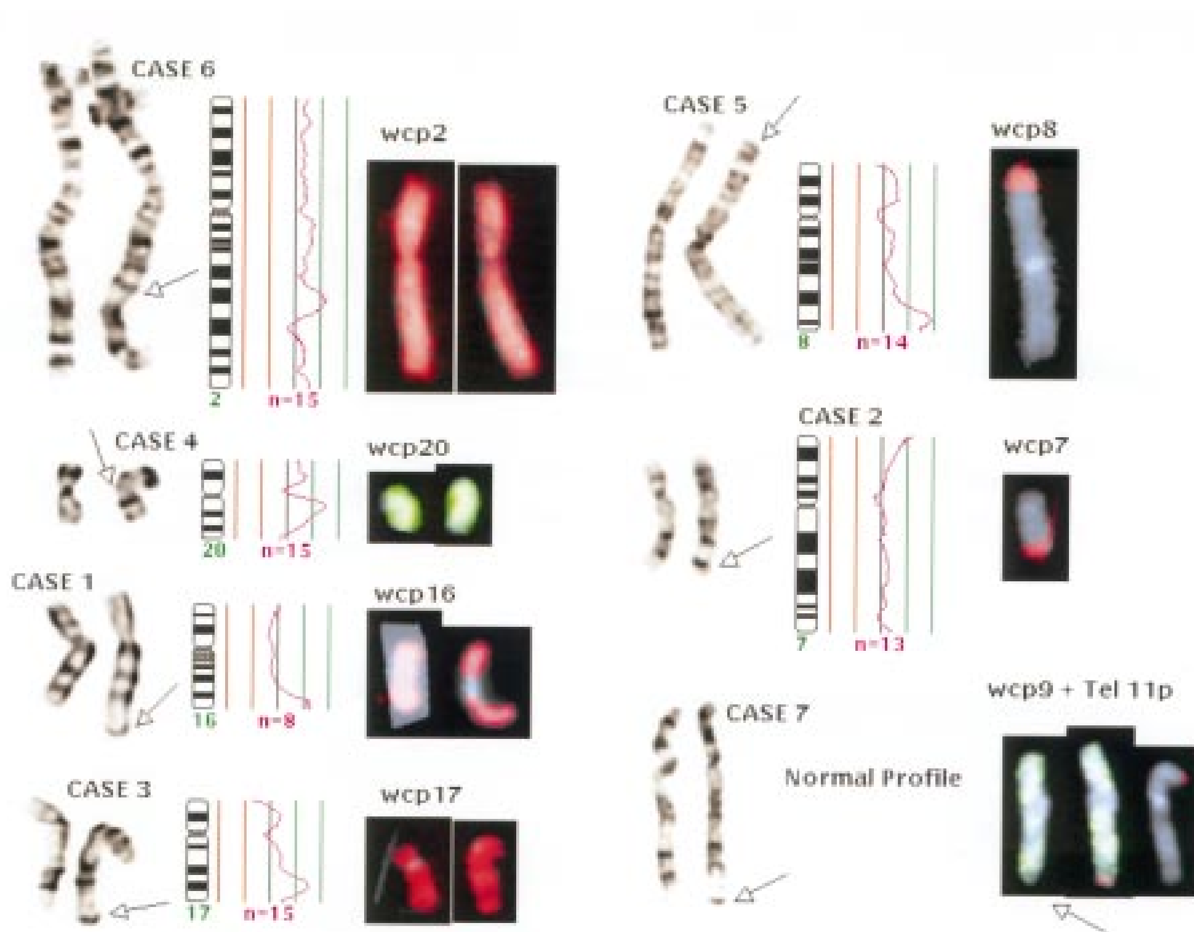

Figure 1 This illustration shows each G banded chromosome that had extra material, the related CGH profile, and FISH images that provided confirmation of CGH results. The abnormal G banded chromosome is on the right of each pair, with arrows pointing to abnormal regions. Lines, from left to right on CGH profiles, represent FR values of 0.5, 0.75, 1.0, 1.25, and 1.5. The probe used for each FISH experiment is designated above each FISH image. Two cases of interstitial duplications (cases 6 and 4) and five cases of chromosomes with abnormal terminal banding patterns (cases 1, 3, 5, 2, and 7) are presented. For case 7, wcp9 is labelled with FITC and the chromosome 11p telomere probe is labelled with digoxygenin and visualised with an antidigoxygenin-rhodamine antibody.

would expect a green to red fluorescence ratio (FR) on the target X chromosome of 0.5. For the converse situation where the green labelled test DNA is female and the red labelled reference DNA is male, we would expect a green to red fluorescence ratio (FR) on the target X chromosome of 2.0. Significant deviation from these theoretical FRs indicates a poor CGH experiment.

Abnormalities on the X chromosome can still be observed using FR values of either 0.5 or 2.0 as baselines.

Results

CGH analysis has been applied to 19 different cases of constitutional chromosome abnormalities. These cases fall into several broad categories, including deletions, supernumerary marker chromosomes, and chromosomes presenting with abnormal terminal or interstitial banding patterns. CGH was applied to supernumerary marker chromosomes and to chromosomes possessing additional bands in order to elucidate the origin of the additional chromosomal material. CGH was applied to deletions in order to confirm or further define the deleted regions. The abnormalities were determined to have arisen de novo in all cases where parental chromosome analysis could be undertaken (table 1).

CGH ANALYSIS OF CHROMOSOMES PRESENTING WITH ADDITIONAL CHROMOSOMAL MATERIAL

CGH analysis was performed on seven cases where additional chromosomal material was detected on chromosomes. In cases 6 and 4, the additional bands occurred interstitially at 2q31 and 20q11.2, respectively. CGH analysis indicated increases in fluorescence ratio (FR) that exceeded 1.25 for the region 2q24-q31 in case 6 and for the region 20q11.2-q12 in case 4 (fig 1). FISH analysis with whole chromosome painting probes (wcp) confirmed that the extra material originated from chromosomes 2 and 20. We conclude that the extra material found on chromosomes 2q and 20q arose through a tandem duplication event.

The other five cases involved additional abnormal terminal bands detected on chromosomes 3 (add 3p25 in case 5), 9 (add 9q34.3 in case 7), 16 (add 16q24 in case 1), and 17 (add 17q25 in cases 2 and 3) (table 1). For cases 1 and 3, CGH analysis indicated sharp increases in FR values at the terminal region of the chro-

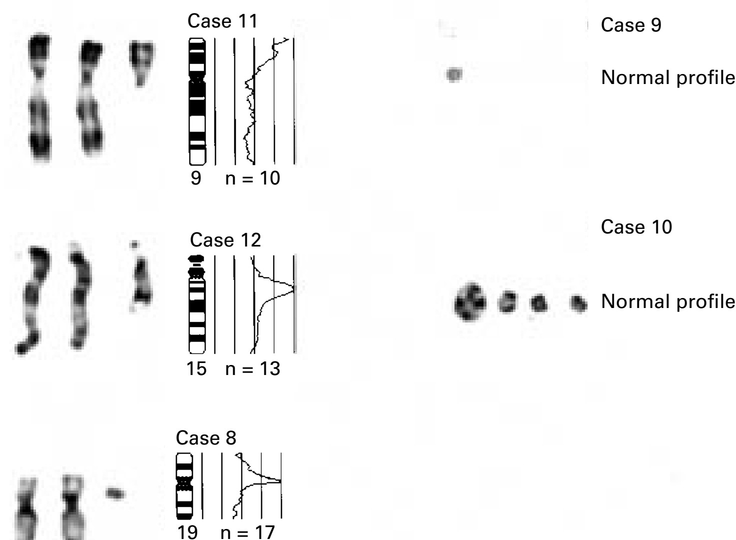

Figure 2 G banded marker chromosomes and their CGH profiles. For case 10, the marker appeared to be a ring chromosome of varying size. Lines, from left to right on CGH profiles, represent FR values of 0.5, 0.75, 1.0, 1.25, and 1.5.

mosome containing the abnormality, that is, 16q23-qter for case 1 and 17q24-qter for case 3 (fig 1). FISH analysis with whole chromosome painting probes 16 and 17 confirmed that the additional material in case 1 and 3 originated from chromosomes 16 and 17, respectively. This information, together with the G banding pattern, indicate that the abnormal bands on chromosomes 16 and 17 arose through inverted duplications.

FISH analysis with wcp 3, wcp 9, and wcp 17 probes in cases 5, 7, and 2, respectively, indicated that the extra chromosomal material did not originate from the chromosome upon which it was attached. CGH analysis of cases 5 and 2 indicated that the extra bands on chromosomes 3 and 17 arose from the regions 8q24.1-qter and 7p21-pter respectively. These results were confirmed with wcp 7 and wcp 8 probes.

A completely normal CGH profile was obtained for case 7, which had additional material at 9q34.3. In this experiment, test and reference DNA was derived from subjects of opposite sex. Therefore, we can rule out the possibility that there was a hybridisation problem since an extremely good competitive hybridisation was detected for the X chromosome. This region on 9q was C band and Ag-NOR staining negative, indicating that it did not originate from either a centromere or NOR satellite region which are difficult to assess by CGH. This case was originally reported as an add(9)(q34.3) by another laboratory, and was referred to our laboratory for further clarification of the abnormality. Our original G band analysis could only be accomplished at a 450 band resolution owing to poor growth of the patient's lymphocytes, so that the normal CGH profile prompted us to obtain a second blood sample. A 700 band resolution analysis allowed us to redefine the abnormality as a balanced reciprocal translocation, t(9;11)(q34.3;p15.3). FISH analysis with a whole chromosome 9 painting probe and chromosome 11p specific subtelomere sequences confirmed the reciprocal translocation (fig 1).

CGH ANALYSIS OF MARKER CHROMOSOMES

CGH analysis was performed on five marker chromosomes (fig 2). The first marker (case 11) was of considerable size and resembled by banding pattern a chromosome 9p. The green to red FR ratio showed a highly significant increase on chromosome 9p and, as expected, whole chromosome 9 painting probe (wcp 9) also showed a positive signal on the marker. The second marker (case 12) was bisatellited, of considerable size, and resembled an inverted duplicated 15. CGH indicated gain of the region 15q11-q13. This result was confirmed by FISH analysis with the SNRPN probe.

The marker chromosome in case 10 was present as a ring chromosome of varying size in 75% of cells (fig 2). A completely normal CGH profile was obtained. Mosaicism for this marker, in addition to its small size, is likely to have contributed to the failure of CGH to determine its origin. The origin of this marker remains to be resolved.

The marker chromosome from case 9 was also extremely small (fig 2), and a completely normal CGH profile was obtained. A large portion of the DNA from this marker is likely to be composed of centromeric repeat sequences. Many centromeric regions display considerable variation in green to red fluorescence ratios, sometimes making it impossible to identify abnormalities from these regions. The origin of this marker requires resolution by other molecular cytogenetic methods.

The last marker, case 8, was too small to have any recognisable banding pattern and was of similar size to the marker found in case 9 (fig 2). However, CGH analysis showed an increase in FR at the centromeric region of chromosome 19. This chromosome is not noted for large fluctuations in FR at the centromeric region, so FISH analysis with a whole chromosome 19 painting probe was performed. The wcp 19 probe confirmed that the marker originated from chromosome 19. This result leads us to conclude that the origins of even very small marker chromosomes can sometimes be ascertained by CGH. This would be particularly true in instances where the derivative chromosome has a centromeric region that usually does not vary greatly in FR.

CGH ANALYSIS FOR CONFIRMATION OF SUBTLE DELETIONS

An additional application of CGH in constitutional chromosome studies is to detect or to confirm the existence of a subtle deletion. We have examined the sensitivity of CGH for the confirmation of subtle deletions from four Prader-Willi syndrome patients (15q11q13), and from 1 patient with a del(1)(p36.1). Each of these deletions were problematical in the sense that one is proximal to a centromeric region while the other is telomeric.

Two of the four del(15)(q11q13) from the PWS patients (cases 13 and case 14) were cytologically visible from G banded prepara-

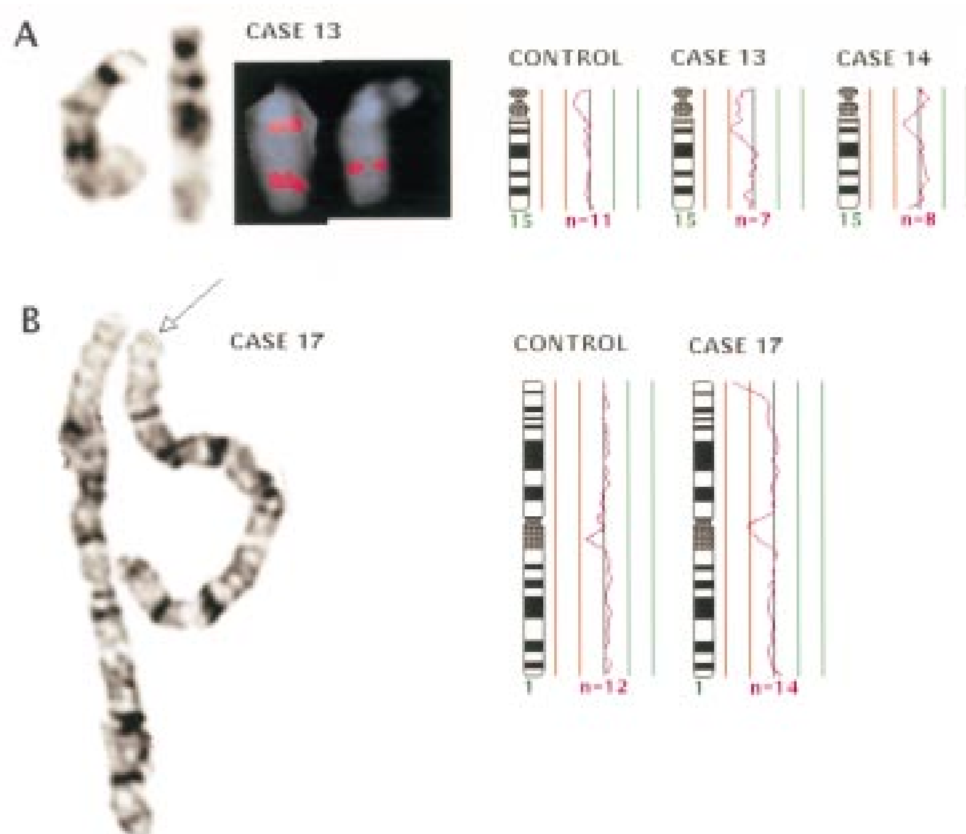

Figure 3 (A) CGH profiles from two PWS cases which exhibited 15q12 deletions that were detectable by both GTG banding and FISH analysis with SNRPN (deleted chromosome 15 on right). CGH also indicated a deletion in both cases. The decline in FR for the 15q12 region was distinctly different from the decline in FR normally occurring at the centromere region (one profile of normal control presented at far left). (B) The subtle deletion of 1p detected in case 17. Normal chromosome 1 on left, deleted 1p on right. CGH profile showed a distinct decline in FR at the terminal end of the p arm of chromosome 1 compared to the profile of a normal control. Lines, from left to right on CGH profiles, represent FR values of 0.5, 0.75, 1.0, 1.25, and 1.5.

tions, while the other two deletions from cases 15 and 16 could only be detected by FISH analysis with the SNRPN probe. As expected, the two microdeletions showed no indication of a deletion by CGH analysis. However, as indicated in fig 3A, the two macroscopically visible deletions showed a distinct decline in the FR value in the 15q12 region, indicating a deletion. The FR in case 13 went to 0.75, while case 14 showed a distinct decline, but did not touch the 0.75 significance line. We compared these CGH profiles to chromosome 15 profiles from 10 subjects who did not have a del(15)(q11q13), and in all instances the decline in FR from the two Prader-Willi syndome patients, cases 13 and 14, could be distinguished from the decline at the centromere region detected on the normal chromosome 15 profiles. Comparison of putatively abnormal profiles with a large number of normal profiles is one method that can allow increased confidence as to whether or not a profile really reflects an abnormality. This is particularly true for chromosomal regions that show wide variation in FR values.

In case 17, illustrated in fig 3B, CGH was useful in confirming that the patient had a subtle terminal deletion of 1p. Deletions in this region are difficult to detect because the region is usually very faintly stained, with little contrast between negative and positive G bands. The G banding pattern was abnormal from 1p36.1 to pter, with a terminal dark G band (fig 3B). The presence of the deletion was confirmed by CGH (fig 3B). No other chromosome in the CGH profile had an apparent telomeric deletion, and a decreased green to red ratio indicating the loss of chromosome 1p material was apparent on both homologues in a high proportion of cells in which both chromosomes 1 were amenable to CGH analysis (five out of five). These results indicate that the loss of material detected at distal 1p by CGH is not an artefact related to close proximity to a telomeric region

FISH analysis with the subtelomeric probe D1S1615 also indicated hemizygosity for the region covered by the probe.

CGH ANALYSIS FOR DEFINING DELETION BREAKPOINTS

Although deletions in the range of 5-10 megabases are usually easy to detect at moderate banding resolution (550 bands), exact breakpoints are sometimes difficult to determine as alternative possibilities may exist. An example would involve deletions of 11q14 and 11q22, which are cytologically indistinguishable.[10] Two examples of interstitial deletion of chromosome 11q are illustrated in fig 4. For these cases, three alternative breakpoints are possible: del(11)(q13.5q21) or (q14.2q22.2) or

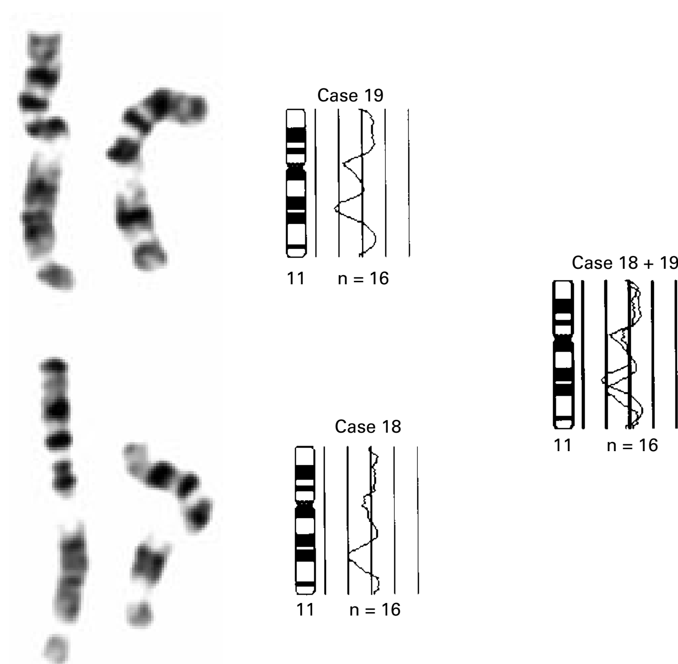

Figure 4 Examples of del(11)(q14 or q22). Deleted chromosomes are on right. CGH profiles confirm the deletion and show that the deletion in case 19 overlaps case 18, but is more proximal. CGH profiles on right side show positions of both deletions when the profiles are superimposed on each other. Lines, from left to right on CGH profiles, represent FR values of 0.5, 0.75, 1.0, 1.25, and 1.5.

(q21q23.1). CGH analysis with DNA from these two patients, however, resolved the breakpoints of both deletions, with case 19 having a more proximal breakpoint than that found in case 18, that is, del(11)(q14.2q22.2) *v* del(11)(q21q23.1), respectively. In this particular instance, CGH resolved deletion breakpoints that could not be determined by G banding alone.

Discussion

CGH is an extremely useful tool for determining the origin of extra genetic material found on chromosomes. Our results indicate that a success rate approaching 100% can be expected for this category of case. Although approaches based on painting probes, such as M-FISH,[11] will also provide information on the chromosomal origin of marker chromosomes etc, CGH has the added advantage of indicating subregional localisation of the extra chromosomal material. In addition, CGH can confirm the presence of a subtle deletion.

In one case that was originally considered to be an unbalanced karyotype (case 7), CGH gave a normal profile. A normal CGH profile suggested a balanced rearrangement, which was confirmed by high resolution GTG banding and FISH analysis on a second blood sample from which high resolution banding could be obtained. The inability of CGH to detect balanced rearrangements is often considered to be its primary disadvantage. However, in this instance, the capability of CGH to indicate balanced genomes proved to be a major advantage.

Overall, our results indicate that CGH will provide novel or confirmatory results in more than 90% of unbalanced, non-mosaic constitutional cases to which it might be applied. This overall success rate would be considerably lower for cases involving very small supernumerary marker chromosomes. This is because very small marker chromosomes are composed primarily of centromeric repeat sequences and that FRs at centromeric regions show great variation. This normal fluctuation at centromeric regions often makes it difficult to identify the chromosomal origin of any euchromatic sequences located on small markers. Nevertheless, CGH analysis correctly identified the origin of one out of the two very small, non-mosaic markers studied here. It is likely that the CGH success rate on small markers will be chromosome dependent, that is, chromosomes possessing centromeres that "behave well" will probably be amenable to CGH analysis. The origin of larger size markers, such as that observed for the inverted duplicated chromosome 15, appear to be easily identified by CGH analysis.

Among the 19 cases studied, CGH analysis was used to determine or confirm the origin of the extra chromosomal material in 10 cases (cases 1, 2, 3, 4, 7, 8, 5, 6, 11, and 12). In all instances, we have confirmed our CGH results by FISH analysis with either painting probes or locus specific probes, with 100% concordance. We conclude that in the general practice of clinical cytogenetics, confirmation of CGH results by FISH may not be necessary in cases where an abnormality was detected by GTG banding analysis. This is particularly true in instances where a high quality CGH result was obtained, as assessed by mismatching test and reference DNA and examining the fluorescence ratio along the length of the X chromosome.

The majority of constitutional chromosome studies to which CGH has been applied involve situations where abnormalities were detected by GTG banded analysis.[1–3 8 9] However, Ghaffari *et al*[5] have shown that CGH can detect cryptic chromosome abnormalities in some instances where G banding indicated a normal karyotype. Thus, CGH analysis may have a role in prospective chromosome studies. Among the 17 cases studied in this report that had macroscopically visible abnormalities, 14 out of 17 abnormalities could potentially have been detected by CGH analysis without previous knowledge of the G banding result. The three cases displaying normal profiles (a balanced reciprocal translocation, a small marker, and a small mosaic marker) would have escaped detection. Examination of the average fluorescence ratios for all chromosomes from each of our cases indicated that a number of false positive results would have been obtained. These false positives are instances where the fluorescence ratios deviated from the normal range (that is, <0.75 and >1.25) at specific regions. Some cases showed no false positives, while others had as many as four. Overall, the average number of false positives would be one per case. This false positive rate was very similar to the number of false positives detected by Gaffari *et al*,[5] although their criteria for defining abnormalities was more stringent (FR <0.5 and >1.5).

The use of higher and lower FR thresholds for defining abnormalities may eliminate many false positive results, but it is also more likely to increase the numbers of false negatives. This would be of major concern for prospective chromosome studies involving CGH. If we applied the stringency (FR <0.5 and >1.5) used by Gaffari *et al*[5] for defining abnormalities to our results, we would have detected abnormalities in only two out of the 17 cases. Given that the numbers of false positives are relatively low, 0 to 4 per case, we would prefer to use a lower threshold for detecting abnormalities, and then to verify the abnormalities with chromosome specific FISH probes. An abnormality detected by CGH in cases where G banding failed to indicate an abnormality would always have to be independently verified by FISH. We further propose that the mismatching of sex and reference DNA, followed by examination of the competitive hybridisation effect on the X chromosome, provides an important quality control measure that would be particularly useful in prospective CGH studies such as those carried out by Gaffari *et al*.[5]

At present, CGH has been used only to identify the origin of additional chromosomal material or to detect/confirm the presence of deleted material. Our use of CGH to identify deletion breakpoints on chromosome 11 is a novel use of the CGH technique that will most likely lead to more precise genotype:phenotype correlations in cases where GTG banding can not resolve different chromosome breakpoint possiblities. In this respect, CGH software capable of relating CGH results to other types of FISH mapping data, such as FLpter measurements,[12] would be useful.

This work was supported in part by the Children's Medical and Research Foundation, Dublin. The technical support of Mr Dan Barrell is gratefully acknowledged.

1 Forozan F, Karhu R, Kononen J, Kallioniemi A, Kallioniemi, O. Genome screening by comparative genomic hybridization. *Trends Genet* 1997;**13**:405-9.
2 Bryndorf T, Kirchhoff M, Rose H, *et al*. Comparative genomic hybridization in clinical cytogenetics. *Am J Hum Genet* 1995;**57**:1211-20.
3 Erdel E, Duba H, Verdorfer I, *et al*. Comparative genomic hybridization reveals a partial de novo trisomy 6q23-qter in an infant with congenital malformations: delineation of the phenotype. *Hum Genet* 1997;**99**:596-601.
4 Levy B, Gershin IF, Desnick RJ, *et al*. Characterization of a de novo unbalanced chromosome rearrangement by comparative genomic hybridization and fluorescence in situ hybridization. *Cytogenet Cell Genet* 1997;**76**:68-71.
5 Ghaffari SR, Boyd E, Tolmie JL, Crow YJ, Trainer AH, Connor JM. A new strategy for cryptic telomeric translocation screening in patients with idiopathic mental retardation. *J Med Genet* 1998;**35**:225-33.
6 Flint J, Wilkie AOM, Buckle VJ, Winter RM, Holland AJ, McDermaid HE. The detection of subtelomeric chromosomal rearrangements in idiopathic mental retardation. *Nat Genet* 1995;**9**:132-40.
7 Knight SJL, Horsley SW, Lawrie NM, *et al*. Development and clinical application of an innovative fluorescence in situ hybridization technique which detects submicroscopic rearrangements involving telomeres. *Eur J Hum Genet* 1997;**5**:1-8.
8 Browne CE, Dennis NR, Maher E, *et al*. Inherited interstitial duplications of proximal 15q: genotype-phenotype correlations. *Am J Hum Genet* 1997:**61**:1342-52.
9 Barber JC, Joyce CA, Collinson MN, *et al*. Duplication of 8p23.1: a cytogenetic anomaly with no established clinical significance. *J Med Genet* 1998;**35**:491-6.
10 Bateman JB, Maumenee IH, Sparkes RS. Peters' anomaly associated with partial deletion of the long arm of chromosome 11. *Am J Ophthalmol* 1984;**97**:11-15.
11 Speicher MR, Ballard SG, Ward DC. Karyotyping human chromosomes by combinatorial multi-fluor FISH. *Nat Genet* 1996;**12**:368-75.
12 Lichter P, Tang CC, Cal K, *et al*. High resolution mapping of human chromosome 11 by in situ hybridization with cosmid clones. *Science* 1990;**247**:64-9.