Original articles

High resolution comparative genomic hybridisation in clinical cytogenetics

Maria Kirchhoff, Hanne Rose, Claes Lundsteen

Abstract

High resolution comparative genomic hybridisation (HR-CGH) is a diagnostic tool in our clinical cytogenetics laboratory. The present survey reports the results of 253 clinical cases in which 47 abnormalities were detected. Among 144 dysmorphic and mentally retarded subjects with a normal conventional karyotype, 15 (10%) had small deletions or duplications, of which 11 were interstitial. In addition, a case of mosaic trisomy 9 was detected. Among 25 dysmorphic and mentally retarded subjects carrying apparently balanced de novo translocations, four had deletions at translocation breakpoints and two had deletions elsewhere in the genome. Seventeen of 19 complex rearrangements were clarified by HR-CGH. A small supernumerary marker chromosome occurring with low frequency and the breakpoint of a mosaic r(18) case could not be clarified. Three of 19 other abnormalities could not be confirmed by HR-CGH. One was a Williams syndrome deletion and two were DiGeorge syndrome deletions, which were apparently below the resolution of HR-CGH. However, we were able to confirm Angelman and Prader-Willi syndrome deletions, which are about 3-5 Mb. We conclude that HR-CGH should be used for the evaluation of (1) dysmorphic and mentally retarded subjects where normal karyotyping has failed to show abnormalities, (2) dysmorphic and mentally retarded subjects carrying apparently balanced de novo translocations, (3) apparently balanced de novo translocations detected prenatally, and (4) for clarification of complex structural rearrangements.

(*J Med Genet* 2001;**38**:740–744)

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

Cytogenetic Laboratory, Department of Clinical Genetics, Juliane Marie Centre, University Hospital, Copenhagen, Denmark
M Kirchhoff
H Rose
C Lundsteen

Correspondence to:
Dr Kirchhoff, Cytogenetic Laboratory, Rigshospitalet 4052, Blegdamsvej 9, DK-2100 Copenhagen Ø, Denmark, markir@rh.dk

Revised version received 21 August 2001
Accepted for publication 22 August 2001

Comparative genomic hybridisation (CGH) is a technique which screens the whole genome for imbalances. The major application of CGH has been in the field of cancer genetics. An increasing number of papers, however, show that CGH can also be successfully applied in clinical cytogenetics.[1–8] The sensitivity of CGH is usually considered to be relatively low; however, we developed the technique further in order to increase the sensitivity as well as the specificity.[9 10] This has enabled us to detect deletions as small as 3 Mb.[11] We have used the improved high resolution CGH technique (HR-CGH) in cancer projects as well as in our clinical cytogenetics laboratory. Here we present the HR-CGH results of 253 clinical cases performed from February 1997 to March 2001.

The results show that apart from being an excellent tool for clarification or confirmation of abnormal karyotypes, HR-CGH is well suited for investigation of dysmorphic and mentally retarded subjects with normal or apparently balanced karyotypes. Contrary to conventional CGH, HR-CGH enabled us to detect small chromosomal aberrations in a number of these patients. The HR-CGH technique has recently been implemented in CytoVision (Applied Imaging) and is thus commercially available.

Materials and methods

CASES

Most of the cases in this survey were referred to our laboratory for routine CGH analysis; however, some cases (especially apparently balanced translocations) were ascertained by us for research purposes.

Eighty-eight of the patients participated in an ongoing investigation of 100 dysmorphic and mentally retarded children with normal karyotypes. This investigation includes HR-**C**GH, FISH with **t**elomeric probes, and **S**KY karyotyping (CTS project). The detailed results of this investigation will be published elsewhere.

Cases were obtained as blood samples, amniotic fluid, chorionic villus samples, placental or fetal tissue samples, skin biopsies, or purified DNA. Case 12 was obtained from Coriell Cell Repositories (No GM10607), Coriell Institute for Medical Research, Camden, NJ, USA. All cases were karyotyped by conventional cytogenetics according to standard protocols.

Reference DNA for CGH was obtained from peripheral blood drawn from karyotypically normal males and females. High molecular

Table 1 Summary of 253 clinical cases analysed by HR-CGH during the period February 1997–March 2001

Indication	*No of analyses*	*No of abnormalities*
(1) Dysmorphic and mentally retarded with a normal karyotype	144	16
(2) Physically and mentally retarded with a normal karyotype	24	0
(3) Apparently balanced de novo translocation, prenatal	6	0
(4) Apparently balanced de novo translocation associated with disease	25	6
(5) Clarification of abnormal karyotype	19	12
(6) Confirmation of abnormal karyotype	19	13
(7) Others*	16	0
Total	253	47

*Investigation of parental chromosomes.
Familial balanced translocations associated with disease.

weight genomic DNA was prepared by extractions on Qiagen Genomic Tip columns (Qiagen, Hilden, Germany).

FISH

FISH was performed in order to confirm the CGH findings. The results have been described previously for cases 2, 3, 4, and 7 (cases 3, 10, 4, and 7, respectively, in Kirchhoff *et al*[12]). In case 6, FISH with probes for loci D2S447 and D2Z4 showed only one signal. In case 12, FISH with a probe for locus D5S23 showed only one signal. In case 8, whole chromosome painting probes for chromosomes 4 and 10 were used (Oncor, Gaithersburg, MD, USA).

CGH

CGH was performed as described previously.[10] Briefly, patient DNA and normal reference DNA were labelled with FITC-12-dUTP and Texas Red-5-dUTP (DuPont, Boston, MA), respectively. A total of 400-800 ng of DNA and 20-30 µg Cot1 DNA were hybridised to normal metaphase chromosomes. Slides were hybridised for three to four days, washed, and counterstained with 4,6-diamidino-2-phenylindole. CGH image capture was performed with a CytoVision (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. Aberrations were detected by standard reference intervals as described in Kirchhoff *et al*.[10] Briefly, along the mean ratio profiles, the 99.5% confidence interval of each mean ratio profile value was compared to 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. Where 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.

Table 2 Abnormalities detected in dysmorphic and mentally retarded subjects with normal or apparently balanced karyotypes

Case No	*Initial karyotype*	*HR-CGH analysis*	*Revised karyotype*	*Subsequent confirmation by*	
				G banding	*FISH*
1	46,XY	dim(1q22q22)	46,XY,del(1)(q22q22)	Yes	Not done
2	46,XY	dim(2p15p15)	46,XY.rev ish dim(2p15p15)	No	Yes
3	46,XX	enh(9pter→qter)	46,XX/47,XX+9	Yes	Yes
4	46,XY	enh(10q11q11)	46,XY.rev ish enh(10q11q11)	No	Yes
5	46,XX	dim(7p15p15)	46,XX,del(7)(p15p15)	Yes	Not done
6	46,XX	dim(2q37→qter)	46,XX,del(2)(q37→qter)	Yes	Yes
7	46,XX	dim(3q22q24)	46,XX,del(3)(q22q24)	Yes	Not done
8	46,XX	dim(4q35→qter) enh(10p15→pter)	46,XX.rev ish dim(4q35→qter) enh(10p→15pter)	No	Yes
9	46,XY,t(1;4)(q31;q21.2)t(3;13)(p14.1;q33)	dim(13q33q33)	46,XY,t(1;4)(q31;q21.2)t(3;13)(p14.1;q33).rev ish dim(13q33q33)	No	Yes
10	46,XX,t(1;6;5)(p13;q14;p13)	dim(6q14q14)	46,XX,t(1;6;5)(p13;q14;p13).rev ish dim(6q14q14)	No	Not done
11	46,XX,t(10;16)(p11;q21)	dim(4q12q12)	46,XX,del(4)(q12q12),t(10;16)(p11;q21)	Yes	Not done
12	46,XY,t(3;5)(p23;p13)	dim(2q24q24) dim(5p13p13)	46,XY,del(2)(q24q24),t(3;5)(p23;p13).rev ish dim(5p13p13)	Yes/no	Not done/yes
13	46,XY,t(1;5;12)(q25;p15;q21)	dim(5p14p14)	46,XY,t(1;5;12)(q25;p15;q21).rev ish dim(5p14p14)	No	Not done

Cases 1, 2, 3, 4, 5, 6, 9, 10, and 12 were published in Kirchhoff *et al*[12] as cases 2, 3, 10, 4, 6, 5, 7, 8, and 9, respectively.
Rev ish: reverse in situ hybridisation. Dim: diminished fluorescence ratio intensity ≈ deletion. Enh: enhanced fluorescence ratio intensity ≈ duplication.

RESOLUTION

HR-CGH

The resolution of the present HR-CGH technique is approximately 3 Mb.[11] The resolution is, however, expected to show some variation depending on the chromosomal location of the abnormality. Thus, the resolution of telomeric regions may be somewhat lower than 3 Mb owing to wider standard reference intervals in the telomeres compared to the rest of the genome.

G banding

The majority of the conventional cytogenetic analyses which preceded the HR-CGH analyses of the dysmorphic and mentally retarded patients with normal or apparently balanced karyotypes were performed in other laboratories. Accordingly we have few data regarding the resolution of these analyses. We consider the resolution of the G banding analyses to be in the range of 300-600 bands corresponding to ½-1 band or 5-10 Mb.

Results

A total of 253 clinical cases was analysed by HR-CGH during the period February 1997 to March 2001 and 47 abnormalities were detected. They included 16 (11%) abnormalities in a group of dysmorphic and mentally retarded subjects with a normal karyotype and six abnormalities in five subjects (21%) in a group of dysmorphic and mentally retarded subjects with an apparently balanced karyotype. Twelve abnormalities were found in a group of abnormal karyotypes where HR-CGH was used for clarification, and 13 abnormalities were found in a group of abnormal karyotypes where HR-CGH was used for confirmation. A summary of all cases including indications is shown in table 1.

Table 3 Unbalanced abnormalities clarified or not clarified by HR-CGH analysis

Case No	Clinical information	Initial karyotype	CGH analysis	Comments or revised karyotype
Translocations				
14	Turner syndrome	46,X,add(Xp)	enh(Yq11→qter)	46,X,add(Xp).rev ish der(X)t(X;Y)(p22.3;q11)enh(Yq11→qter)
15	Prenatal diagnosis	46,XX,inv(9),add(9p)	dim(9p22→pter) enh(18q11.1→qter)	46,XX,inv(9),add(9)(p22).rev ish der(9)t(9;18)(p22;q11.1)enh(18q11.1→qter)
16	Mentally retarded male	46,X,add(Xp)	enh(Yp10→pter)	46,X,add(X)(p).rev ish der(X)t(X;Y)(p22.3;p10)enh(Yp10→pter)
Duplications				
17	Morbus cordis and growth retarded	46,XX,add(2p)de novo	enh(2p21p23)	46,XX,add(2p).rev ish enh(2p21p23)
18	Prenatal diagnosis	46,XX,add(18q)	enh(18q11.2→qter)	46,XX,add(18q).rev ish enh(18q11.2→qter)
19	Dysmorphic with failure to thrive	46,XY,+mar. Ish add(5)(wcp5+)	enh(5p15-pter)×2	46,XY,add(5p).rev ish enh(5p15→pter)×2
20	Prenatal diagnosis	46,XX,ins(12;?)(q21;?) de novo	Normal	wcp12 paints the entire chromosome 12, pregnancy resulted in a healthy child
Deletions				
21	Dysmorphic with malformations	46,XX,r(13)	dim(13q22-qter)	46,XX,r(13).rev ish dim(13p11.2q22)
22	Dysmorphic and mentally retarded	46,XX,del(11q?)	dim(11q23.2)	46,XX,del(11q?).rev ish dim(11q23.2)
Numerical				
23	Prenatal diagnosis	47,XY,+mar	enh(15q12q12)	47,XY,+mar.rev ish enh(15)(q12q12)pat, pregnancy continued
24	Missed abortion, suspected trisomy 18	47,XY,+mar	Normal	The marker is assumed to be inactive and of no clinical significance
25	Prenatal diagnosis	47,XY,+mar	enh(14q12q12)	47,XY,+mar.rev ish enh(14q12q12), pregnancy terminated
26	Prenatal diagnosis	46,XY/47,XY,+mar	Normal	The marker is assumed to be inactive, pregnancy resulted in a healthy child
27	Prenatal diagnosis	46,XY,+mar	Normal	The marker is assumed to be inactive
Others				
28	Missed abortion	46,XX,add(12p)	dim(12p13→pter) enh(12q13→qter)	46,XX,add(12p). rev ish rec(12)dup(12)inv(12)(p13q13)dim(12p12→pter)enh(12q13→qter)mat
29	Induced abortion of severely hydropic fetus	46,XX,add(21p)	Normal	The material on 21q is assumed to be inactive and without clinical significance
30	Prenatal diagnosis	46,XY, abnormal Y?	Normal	Father had a similar Y chromosome
Unbalanced abnormalities failed to be clarified by CGH				
31	Dysmorphic?	45,XX,-18/46,XX,r(18)	dim(18pter→qter)	Size of r(18) could not be estimated from the CGH analysis
32	Dysmorphic and delayed development	47,XXY/48,XXY,+mar	enh(Xper→pter)	Marker was only present in 7% of the cells and could not be detected with CGH

Rev ish: reverse in situ hybridisation. Dim: diminished fluorescence ratio intensity ≈ deletion. Enh: enhanced fluorescence ratio intensity ≈ duplication.

Of the 22 abnormalities found in dysmorphic and mentally retarded subjects with a normal or apparently balanced karyotype (indication groups 1 and 4, table 1), 12 were confirmed by reinspection of the G banded karyotypes, 10 by FISH, three by both techniques, one by microsatellite analysis (a patient from the CTS project), and two abnormalities have not yet been confirmed owing to lack of sample material.

Seventeen of the 22 abnormalities were interstitial, four were terminal, and one was a trisomy.

Table 2 outlines abnormalities detected in dysmorphic and mentally retarded subjects with normal or apparently balanced G banded karyotypes. Patients participating in the CTS project are not included. As indicated in table 2, 10 of the cases were previously reported in Kirchhoff *et al*.[12] The parents of the patients in table 2 were investigated when sample material was available. In case 8, the mother was shown to carry a balanced translocation (see Materials and methods). In eight cases (1, 2, 3, 4, 7, 9, 10, and 11), G banding, CGH, or FISH showed that the abnormalities were of de novo origin.

Table 3 shows 17 of 19 unbalanced abnormalities clarified by HR-CGH and two which could not be clarified, and table 4 describes 16 of 19 other unbalanced abnormalities confirmed by HR-CGH and three which could not be confirmed.

Five findings were not included since they were considered to be either false positive results or normal chromosomal variations. These were three duplications of chromosome 15q12, a duplication of chromosome 9p11, and a deletion of chromosome 16p11.

Discussion

The cases where HR-CGH analysis provided essential information fall into two categories: (1) cases where G banded karyotypes were normal or apparently balanced and chromosomal disease was suspected (table 1, indication groups 1 and 4), and (2) cases with unbalanced G banded karyotypes where clarification or confirmation of the abnormalities by additional techniques was attempted (table 1, indication groups 5 and 6).

We have previously shown that HR-CGH is capable of detecting abnormalities in the former category.[12] However, the present survey shows that abnormalities were found in remarkably high numbers, since chromosomal imbalances were detected in 11% of 144 dysmorphic and mentally retarded subjects with a normal conventional karyotype and in 20% of 25 dysmorphic and mentally retarded subjects carrying an apparently balanced de novo translocation. The two unconfirmed deletions in table 2 (cases 10 and 13) were found at the breakpoints of chromosomes involved in apparently balanced de novo translocations, which strengthens the reliability of these findings. Although these deletions could be false positive results, we have previously shown that false positives are very rare at the

Table 4 Unbalanced abnormalities confirmed or not confirmed by HR-CGH analysis

Case No	Clinical information	Initial karyotye	CGH analysis	Comments
Larger structural and numerical abnormalities confirmed by CGH				
33	Dysmorphic with malformations	47,XX,+der(22)t(11;22)(q23;q11.2)	enh (11q23→qter)	
34	Turner syndrome?	45,X/46,X,-X,+der(X)t(X;X)(p11.4;q12)	dim(Xpter→qter) dim(Xp11.4→pter)	The ratio from Xp11.4→pter was more reduced than the rest of the X chromosome
35	Prenatal diagnosis	46,XX,-4,+der(4)t(4;8)(p15.3;p22)pat	dim(4p15.3→pter) enh(8p22→pter)	
36	Prenatal diagnosis	46,XY,der(6)t(5;6)(q34;q27)mat	dim(5q34→qter)	
37	Severely growth retarded	46,XY,del(11)(q14.2;q23.3)	dim(11q14.2q23.3)	
38	Prenatal diagnosis	46,XY,der(7)ins(13;7)(q32;q32q34)mat	dim(7q32→qter)	
39	Microcephaly	46,XX/46,X,i(X)(q10)	dim(Xp10→pter) enh(Xq10→qter)	
40	Prenatal diagnosis	47,XY,+mar mat	Normal	Marker assumed to be inactive
41	Mental retardation	47,XY,+mar.ish+idic(15)(q11)	Normal	Marker assumed to be inactive
42	Prenatal diagnosis	45,X/46,X+mar.nuc ishYp(probe x2)	dim(Xpter→qter) enh(Yp10→pter)	Retrospectively, 20% mosaicism of an i(Yp) was found on G banding
43	Prenatal diagnosis	46,XY,9qh+mat	Normal	The light stained 9qh region was divided by a dark band like a G band
44	Phenotypic male	46,XX	46,XX	
Cryptic abnormalities confirmed by CGH				
45	Prenatal diagnosis	46,XX,-18,+der(18)t(11;18)(q25;q23)pat	enh(11q25q25) dim(18q23q23)	This cryptic translocation was incidentally detected by interphase FISH[28]
46	Mentally retarded	46,XX,-11,+der(11)t(11;18)(q25;q23)mat	dim(11q25q25) enh(18q23q23)	––
47	Angelman syndrome	46,XX.ish del(15)(q11q13)	dim(15q11q13)	
48	Prader-Willi syndrome	46,XX.ish del(15)(q11q13)	dim(15q11q13)	
Cryptic abnormalities failed to be detected by CGH				
49	DiGeorge syndrome	46,XY.ish del(22)(q11.2q11.2)	Normal	
50	Williams syndrome	46,XX.ish del(7)(q11.23q11.23)	Normal	
51	DiGeorge syndrome	46,XY.ish del(22)(q11.2q11.2)	Normal	

Rev ish: reverse in situ hybridisation. Dim: diminished fluorescence ratio intensity ≈ deletion. Enh: enhanced fluorescence ratio intensity ≈ duplication.

level of confidence used in the CGH analyses of this work.[10]

In the present study, an amplification of chromosome 9p11 and a deletion of chromosome 16p11 were regarded as false positive results or normal variations and were excluded. Normal variations in these chromosomal regions have previously been described.[13 14] We have, however, also noticed that, contrary to the rest of the genome, imbalances are on rare occasions detected in normal subjects in 9p11, 16p11, and 1q21, which may disappear when the analyses are repeated. Generally, the ratio deviations in two or all three of these regions are correlated in the individual analyses, raising considerably suspicion that they may represent technical artefacts. Three duplications of chromosome 15q12 were regarded as normal variations. They were found in analyses of an affected boy and his healthy father and in a girl with an apparently balanced t(3;21). Variation of region 15q11-12 has been described in a number of normal subjects.[15–17]

So far, it is unclear if the abnormalities detected in dysmorphic and mentally retarded subjects with a normal or apparently balanced karyotype are responsible for the clinical findings in all the patients. Analysis of the parents is obviously important, since de novo imbalances in subjects with parents carrying normal or balanced karyotypes are more likely to be associated with an affected phenotype. Detailed knowledge of the diagnostic implications of all the imbalances is, however, unlikely to be obtained until the particular chromosomal regions can be characterised further or until additional cases are identified.

A total of 7.6% of the 144 dysmorphic and mentally retarded patients with a normal G banded karyotype carried an interstitial abnormality while only 2.8% of the patients had terminal abnormalities. These figures are interesting since subtelomeric abnormalities have been shown in approximately 7.5% of mentally retarded subjects in several investigations with subtelomeric FISH probes.[18–23] Knight *et al*[20] concluded that: "once recognizable syndromes have been excluded, abnormalities that include the ends of chromosomes are the commonest cause of mental retardation in children with undiagnosed moderate to severe mental retardation". The results of our survey indicate that in dysmorphic and mentally retarded patients interstitial imbalances are found in numbers close to the number of abnormalities found in subtelomeric regions by FISH probes.

The present data suggest that chromosomal abnormalities may be detected in approximately 15% (7.5% + 7.6%) of mentally retarded and dysmorphic patients if both subtelomeric screening and HR-CGH are applied. However, it may be that these figures are not additive since the primary criterion for the investigations with subtelomeric probes was mental retardation,[20] while dysmorphic features were also required in our cases. Nevertheless, it is likely that for most of the patients both these criteria were fulfilled.

All six imbalances detected in the patients with apparently balanced translocations (table 2) were interstitial and, interestingly, two of them were detected outside the regions involved in the translocations. Since these two abnormalities were retrospectively confirmed by G banding, it is likely that the finding of a translocation in a G banded karyotype may distract the cytogeneticist's attention from other abnormalities.

HR-CGH was also applied to de novo apparently balanced translocations detected prenatally. No abnormalities were detected in the six prenatal cases investigated in this survey (table 1, indication group 3). When, however, analyses of more such cases are performed, imbalances are bound to be found in some of them. Naturally they will not appear as frequently as in the dysmorphic and mentally retarded patients with apparently balanced karyotypes, since the majority of the prenatal cases are not expected to be associated with disease.[24 25]

The cases listed in tables 3 and 4 show that HR-CGH is well suited to the confirmation or clarification of abnormal G banded karyotypes. An increasing number of papers similarly show that this application of CGH is becoming more widely recognised.[2 3 8 26 27] The cases that were not confirmed or clarified by HR-CGH had abnormalities that were typically too small (DiGeorge and Williams syndrome deletions, table 4, and probably case 31, table 3) or were present in a low level mosaic state (case 32, table 3).

The present survey shows that HR-CGH is likely to make a significant contribution to the difficult and laborious task of associating chromosome abnormalities with clinical manifestations. Hopefully, an increasing number of laboratories will participate in this work. HR-CGH is commercially available on the CytoVision from Applied Imaging Corporation who has the sole and exclusive rights to the marketing of the HR-CGH software.

We wish to thank colleagues from the Institute of Medical Biochemistry and Genetics, Panum Institute, the Department of Clinical Genetics, Århus Kommunehospital, The Kennedy Institute, and Dr James Lespinasse, Central Hospital, Chambry, France, and Dr Ying Guo, Beijing Hospital, Beijing, China for providing samples and clinical data. This work was supported by the Ivan Nielsens Fond for personer med specielle sindslidelser.

1 Daniely M, Aviram-Goldring A, Barkai G, Goldman B. Detection of chromosomal aberration in fetuses arising from recurrent spontaneous abortion by comparative genomic hybridization. *Hum Reprod* 1998;**13**:805-9.
2 Levy B, Dunn TM, Kaffe S, Kardon N, Hirschhorn K. Clinical applications of comparative genomic hybridization. *Genet Med* 1998;**1**:4-12.
3 Airam-Goldring A, Fritz B, Bartsch C, Steuber E, Daniely M, Lev D, Chaki R, Barkai G, Frydman M, Rehder H. Molecular cytogenetic studies in three patients with partial trisomy 2p, including CGH from paraffin-embedded tissue. *Am J Med Genet* 2000;**91**:74-82.
4 Christianes CG, Vissers J, Poddighe PJ, de Pater JM. Comparative genomic hybridization for cytogenetic evaluation of stillbirth. *Obstet Gynecol* 2000;**96**:281-6.
5 Lapierre JM, Cacheux V, Luton D, Collot N, Oury JF, Aurias A, Tachdjian G. Analysis of uncultured amniocytes by comparative genomic hybridization: a prospective prenatal study. *Prenat Diagn* 2000;**20**:123-31.
6 Lestou VS, Desilets V, Lomax BL, Barrett IJ, Wilson RD, Langlois S, Kalousek DK. Comparative genomic hybridization: a new approach to screening for intrauterine complete or mosaic aneuploidy. *Am J Med Genet* 2000;**92**: 281-4.
7 Lomax B, Tang S, Separovic E, Phillips D, Hillard E, Thomson T, Kalousek DK. Comparative genomic hybridization in combination with flow cytometry improves results of cytogenetic analysis of spontaneous abortions. *Am J Hum Genet* 2000;**66**:1516-21.
8 Turleau C, Lapierre JM, Joly G, Prieur O, Raoul O, de Blois MC, Morichon-Devallez N, Gosset P, Romana S, Vekemans M. CGH as a routine procedure in a clinical cytogenetic laboratory: a one-year assessment. *Am J Hum Genet Suppl* 2000;**67**:152.
9 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**:130-4.
10 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**:163-73.
11 Kirchhoff M, Gerdes T, Maahr J, Rose H, Bentz M, Döhner H, Lundsteen C. Deletions below 10 megabasepairs are detected in comparative genomic hybridization by standard reference intervals. *Genes Chrom Cancer* 1999;**25**:410-13.
12 Kirchhoff M, Rose H, Maahr J, Gerdes T, Bugge M, Tommerup N, Tümer Z, Lespinasse J, Jensen PKA, Wirth J, Lundsteen C. High resolution comparative genomic hybridisation analysis reveals imbalances in dysmorphic patients with normal or apparently balanced karyotypes. *Eur J Hum Genet* 2000;**8**:661-8.
13 Sutherland GR, Eyre H. Two unusual G-band variants of the short arm of chromosome 9. *Clin Genet* 1981;**19**:331-4.
14 Barber JCK, Reed CJ, Dahoun SP, Joyce CA. Amplification of a pseudogene cassette underlies euchromatic variation of 16p at the cytogenetic level. *Hum Genet* 1999;**104**:211-18.
15 Barber JCK, Cross IE, Douglas F, Nicholson JC, Moore KJ, Browne CE. Neurofibromatosis pseudogene amplification underlies euchromatic cytogenetic duplications and triplications of proximal 15q. *Hum Genet* 1998;**103**:600-7.
16 Browne CE, Dennis NR, Maher E, Long FL, Nicholson JC, Sillibourne J, Barber JCK. Inherited interstitial duplications of proximal 15q: genotype-phenotype correlations. *Am J Hum Genet* 1997;**61**:1342-52.
17 Ludowese CJ, Thompson KJ, Sekhon GS, Pauli RM. Absence of predictable phenotypic expression in proximal 15q duplications. *Clin Genet* 1991;**40**:194-201.
18 Flint J, Wilkie AO, Buckle VJ, Winter RM, Holland AJ, McDermid HE. The detection of subtelomeric chromosomal rearrangements in idiopathic mental retardation. *Nat Genet* 1995;**9**:132-40.
19 Anderlid B, Annerén G, Blennow E, Nordenskjold M. Subtelomeric rearrangements detected by FISH in patients with idiopathic mental retardation. *Am J Hum Genet Suppl* 1999;**65**:A67.
20 Knight SJ, Regan R, Nicod A, Horsley SW, Kearney L, Homfray T, Winter RM, Bolton P, Flint J. Subtle chromosomal rearrangements in children with unexplained mental retardation. *Lancet* 1999;**354**:1676-81.
21 Huang XL, Wyandt HE, Milunsky JM. Subtelomeric testing for cryptic chromosomal rearrangements in 68 patients with idiopathic mental retardation and dysmorphology. *Am J Hum Genet Suppl* 2000;**67**:162.
22 Jalal SM, Harwood A, Anderson M, Lorentz C, Law M, Lindor N, Karnes P, Kulharya A, Sekhon G, Michels V. Screening for subtle structural anomalies by use of subtelomere specific FISH probe set. *Am J Hum Genet Suppl* 2000;**67**:148.
23 Knight S, Flint J. Perfect endings: a review of subtelomeric probes and their use in clinical diagnosis. *J Med Genet* 2000;**37**:401-9.
24 Wassman ER, Cheyovich DL, Nakahara Y. "Possibly" de novo translocations: prenatal risk counseling. *Am J Obstet Gynecol* 1989;**161**:698-702.
25 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.
26 Lapierre JM, Cacheux V, Collot N, Da Silva E, Hervy N, Rivet D, Romana S, Wiss J, Benzaken B, Aurias A, Tachdjian G. Comparison of comparative genomic hybridization with conventional karyotype and classical fluorescence in situ hybridization for prenatal and postnatal diagnosis of unbalanced chromosome abnormalities. *Ann Genet* 1998; **41**:133-40.
27 Daniely M, Barkai G, Goldman B, Aviram-Goldring A. Structural unbalanced chromosome rearrangements resolved by comparative genomic hybridization. *Cytogenet Cell Genet* 1999;**86**:51-5.
28 Schultz LN, Schmidt P, Tabor A, Bryndorf T, Lundsteen C. Cryptic familial t(11;18)(q25;q23) incidentally detected by interphase FISH. *Clin Genet* 2001;**59**:279-83.