

Prospective Study Comparing HR-CGH and Subtelomeric FISH for Investigation of Individuals With Mental Retardation and Dysmorphic Features and an Update of a Study Using Only HR-CGH

Maria Kirchoff,^{1*} Søren Pedersen,² Eigil Kjeldsen,² Hanne Rose,¹ Morten Dunø,¹ Steen Kølvrå,² and Claes Lundsteen¹

¹Department of Clinical Genetics, Rigshospitalet, Copenhagen, Denmark

²Department of Clinical Genetics, Aarhus University Hospital, Aarhus, Denmark

In a prospective study 94 individuals with mental retardation (MR) and dysmorphic features with normal conventional karyotypes were investigated by both subtelomeric FISH and high resolution CGH (HR-CGH) in order to compare the potential of the two techniques in this application. A total of 9.6% abnormalities were found with HR-CGH and subtelomeric FISH, with HR-CGH detecting 8.5% (95% CI: 4.4–15.9) and FISH 3.2% (95% CI: 1.2–9.0). Thus, the techniques complemented each other, however, the diagnostic yield appeared higher of HR-CGH than of subtelomeric FISH, as most aberrations were interstitial. Another 330 individuals with MR and dysmorphic features with normal conventional karyotypes were investigated by HR-CGH on a routine basis. When added to the analyses of the prospective study a total of 51/424 (12%; 95% CI: 9.3–15.5) abnormalities were found, of which the majority were interstitial. We conclude that HR-CGH is well suited for routine screening for cryptic chromosomal imbalances in patients with MR and dysmorphic features. It is likely that the use of the technique in this application will reinforce the effort of defining new syndromes.

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KEY WORDS: high resolution CGH; subtelomeric FISH; mental retardation; developmental delay; dysmorphism; cryptic chromosome aberrations

INTRODUCTION

Although mental retardation (MR) is a common disorder and a large number of studies have focused on etiology, the cause remains unknown in more than half of the cases.

In recent years advances in molecular cytogenetics has resulted in a growing body of data, showing that cryptic chromosome abnormalities, which are too subtle to be detected

by conventional chromosome banding techniques, may be associated with MR [reviewed in Xu and Chen, 2003]. In most studies of cryptic chromosome abnormalities in patients with idiopathic MR, subtelomeric screening assays have been applied. The fact that telomeric regions are gene rich and often involved in chromosome rearrangements suggest that cytogenetically invisible subtelomeric translocations are a significant cause of genetic disease. Studies using subtelomeric assays have reported cryptic abnormalities in 0–23% of patients with idiopathic MR [Flint et al., 1995; Viot et al., 1998; Vorsanova et al., 1998; Knight et al., 1999; Lamb et al., 1999; Slavotinek et al., 1999; Ballif et al., 2000; Bonifacio et al., 2001; Borgione et al., 2001; Colleaux et al., 2001; Fan et al., 2001; Joyce et al., 2001; Riegel et al., 2001; Rosenberg et al., 2001; Rossi et al., 2001; Sismani et al., 2001; Anderlid et al., 2002; Baker et al., 2002; Clarkson et al., 2002; Popp et al., 2002; Rio et al., 2002; van Karnebeek et al., 2002; Jalal et al., 2003]. The criteria used for inclusion of patients in these studies are likely to contribute to this wide range in detection rate.

Yet, it is well known that MR may also be associated with interstitial chromosome aberrations. We have previously used the whole-genome screening technique HR-CGH for investigation of patients with MR and dysmorphic features with normal conventional karyotypes. In HR-CGH, CGH profiles are evaluated by the use of dynamic standard reference intervals instead of fixed thresholds [Kirchoff et al., 1998], which results in a three-fold increase in sensitivity compared to standard CGH [Kirchoff et al., 1999]. The improved CGH technique proved useful for investigating this group of patients, as cryptic chromosomal imbalances were found in 11% [Kirchoff et al., 2000]. The majority of the imbalances were interstitial.

Two other studies have used CGH in a similar application. Joly et al. [2001] used standard CGH for investigation of 17 patients (14 families) with MR, dymorphic features, and a normal karyotype. Five unbalanced terminal rearrangements were found in seven patients. Ness et al. [2002] investigated 50 children with various degrees of delayed psychomotor developments with or without dysmorphic features and congenital malformations by HR-CGH. Five chromosomal imbalances were detected of which only one was terminal.

A comparison of results from HR-CGH studies and subtelomeric assays studies remains problematic, as detection rates are expected to be highly dependent on the strategy used for selection of patients. Subtelomeric screening offers high sensitivity but is confined to chromosome ends, while HR-CGH enables whole-genome screening albeit at lower sensitivity. In order to firmly establish the relative merits of these two techniques in the screening for cryptic chromosomal aberrations in individuals with MR and dysmorphic features with normal conventional karyotype, we performed a prospective

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*Correspondence to: Maria Kirchoff, Department of Clinical Genetics, 4052, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen Ø, Denmark. E-mail: markir@rh.dk

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study including 94 such patients. All patients were blindly investigated by both subtelomeric FISH probes and HR-CGH. The results showed that more aberrations were detected by HR-CGH and that most aberrations were interstitial. This article also reviews another 330 HR-CGH analyses of individuals with MR and dysmorphic features with normal conventional karyotype performed in our laboratory. The results suggest that patients with this indication and without a family history should be offered HR-CGH analysis prior to subtelomeric FISH analysis, as the former technique seems to offer the highest diagnostic yield.

MATERIALS AND METHODS

Cases

Ninety-four patients were investigated by both HR-CGH (in Copenhagen) and subtelomeric FISH probes (in Aarhus) in a prospective study. The inclusion criteria were: 1) age: 0–10 years; 2) uncomplicated pregnancy and birth; 3) clinical manifestations: psychomotor retardation and chromosomal stigmata defined as one or several of the following: growth retardation, dysmorphic features, congenital malformations of, e.g., heart, kidney, or central nervous system; and 4) a normal conventional karyotype. Patients with a non-chromosomal diagnosis were excluded. Informed consent from parents was obtained. All patients were recruited from pediatric departments in Denmark. Samples were obtained as blood or purified DNA.

No results were shared between the Copenhagen and the Aarhus laboratory until all results were available.

The remaining 330 patients with MR and/or dysmorphic features described in this survey were referred to the Copenhagen laboratory for routine HR-CGH analysis.

HR-CGH

CGH was performed as described previously [Kirchoff et al., 1997, 1998]. Briefly, patient DNA and normal reference DNA were labeled with FITC-12-dUTP and Texas Red-5-dUTP (DuPont, Boston, MA), respectively. Four to eight hundred nanogram of each DNA and 20–30 µg Cot1 DNA were hybridized to normal metaphase chromosomes. Slides were hybridized for 3–4 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 analyzed on the CytoVision or transferred to a Magiscan image analysis system (Applied Imaging)¹. In each case, 10 metaphases were analyzed. Aberrations were detected by standard reference intervals as described in [Kirchoff et al., 1998]. 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.

Subtelomeric FISH Assay

Simultaneous FISH for all 41 subtelomeric regions was performed using the Chromoprobe Multiprobe T system according

¹Analysis is now exclusively performed on the CytoVision, using a prototype version of the software where automatic axis fitting and background subtraction have been optimized for HR-CGH.

to the manufacturer's specifications (Cytocell, Oxford, UK). Hybridized metaphases were analyzed using a Leica DMRB fluorescence microscope (Leica, Germany) and images were captured by a Photometrics Sensus CCD camera and analyzed by with IPLab Spectrum software (Scanalytics, Inc., Fairfax, VA, USA). A minimum of three metaphases were examined for each chromosome. In cases of positive results a separate FISH analysis was performed for the suspected regions with the same probes either on a new Multirobe-T system slide or with the Aquarius telomeric specific probes (Cytocell).

Confirmative G-Banding Analysis

G-banding analysis was performed at ISCN band level 500–750 for confirmation of HR-CGH findings. The band level of individual cases are shown in Tables I and II.

Confirmative FISH

FISH was performed in order to confirm the HR-CGH findings. The results have been described previously for cases 12, 13, 24, and 30, (cases 10, 4, 3, and 5, respectively in Kirchoff et al. [2000]). In case 6 a Smith–Magenis probe (Appligene-Oncor, Illkirch Graffenstaden, France) was applied. In cases 17, 22, 45, 50, 51, and 52 subtelomeric probes from Appligene-Oncor was used.

Confirmative PCR

Quantitative PCR was used for confirmation of HR-CGH and subtelomeric FISH findings and was performed by an ABI Prism 7000 sequence detection system (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions.

Primers were designed by the use of Primer Express Software (Applied Biosystems) and DNA sequence information were obtained from the public UCSC database. In each case primer sets were designed in DNA sequences annotated to the chromosomal region/band that was found abnormal by HR-CGH or subtelomeric FISH. Especially for small aberrations, designation to specific bands by HR-CGH was not highly accurate and a number of primer sets were applied. A minimum of two sets of primers confirmed the aberration in every case.

SYBR Green PCR master mix (Applied Biosystems) was used for PCR according to the manufacturer's instructions. Relative copy numbers were measured relative to GAPDH. Two normal individuals were included. Each assay was duplexed and evaluated by a comparative method validated by Applied Biosystems with the formula $2^{-\Delta\Delta Ct}$. Care was taken that nearly exact amounts of reference and patient DNA was compared, illustrated by the GAPDH Ct value.

Beside confirmation, quantitative PCR was also used in a few cases for mapping breakpoints in order to estimate the sizes of aberrations.

RESULTS

In the prospective study 94 mentally retarded and/or dysmorphic individuals with normal conventional karyotype were successfully analyzed by both subtelomeric FISH and HR-CGH. As seen in Table I a total of nine aberrations were detected, three by subtelomeric FISH, eight by HR-CGH, and two by both techniques. All aberrations were confirmed by a second technique. Aberrations detected by HR-CGH were confirmed by G-banding, FISH, or quantitative PCR, while aberrations detected by subtelomeric FISH were confirmed by quantitative PCR (two aberrations were detected by both techniques hereby providing extra confirmation). In five cases

TABLE I. Aberrations Detected in 94 Individuals With Mental Retardation (MR) and Dysmorphic Features by HR-CGH and Subtelomeric FISH

Case no.	HR-CGH findings	Subtelomeric FISH	Subsequent confirmation by			Origin
			G-banding*	FISH	Quantitative PCR	
Duplications						
1	enh(1q21q21)		Yes (550)			de novo
2	enh(5q13q15)		Yes (700)			de novo
Deletions						
3	dim(3q23q23)				Yes	de novo
4	dim(3q25q26)		Yes (500)			de novo
5	dim(10q25q25)		Yes (550)			de novo
6	dim(17p11p12)		Yes (500)	Yes		de novo
7	dim(22q13 → qter)	del(22)(qtel)(D22S1726 × 1)			Yes	de novo
8		del(22)(qtel)(D22S1726 × 1)			Yes	de novo
Translocations						
9	enh(7p22 → pter)	del(4)(ptel)(D4S3360 × 1) dup(7)(ptel)(G3141 × 3)			Yes	de novo

Dim, diminished fluorescence ratio intensity ~ deletion; Enh, enhanced fluorescence ratio intensity ~ duplication.

*Band resolution of confirmative analysis is noted in brackets.

the subtelomeric assay indicated a deletion of 2qter, however, quantitative PCR showed a balanced state for this chromosome region in each case.

In Table II another 43 chromosome abnormalities found among 330 individuals with MR and/or dysmorphic features analyzed by HR-CGH are listed. Except for one case where sample material was lacking, all abnormalities were confirmed. When an aberration was shown to be inherited this was counted as a confirmation. Figure 1 shows the HR-CGH results of all analyses performed, including those of the prospective study. Chromosome aberrations were detected in 51/424 patients = 12%. Twenty-seven abnormalities were found in males while 24 were found in females. The parents of the patients shown to carry chromosome aberrations by either HR-CGH or subtelomeric FISH were investigated by HR-CGH, FISH or quantitative PCR when sample material was available. In cases 47, 48, 49, and 52 a parent was shown to carry a balanced translocation; in cases 15, 16, 19, 25, 42, and 43 a parent was shown to carry a chromosomal imbalance apparently similar to that of the child; in cases 11, 14, 27, and 30 sample material from one or both parents was not available while the abnormalities in the rest of the cases were of de novo origin. A limited number of findings were not included since they were considered to be either false positive results or normal chromosomal variations. These were a duplication of chromosome 15q12, and 11 duplications or deletions of 1q21, 9p11, and 16p11. Table III shows the types of structural abnormalities detected in 50 mentally retarded and dysmorphic individuals by HR-CGH and in Table IV the known microdeletion syndromes that were detected among all the investigated patients are shown.

DISCUSSION

In recent years technical improvement of molecular cytogenetics has proved that cryptic chromosomal rearrangements are a significant cause of MR. As none of the available techniques for detecting cryptic rearrangements are fully informative on their own it is important to clarify which investigations would be most beneficial for a particular type of patients. In the present study we compared subtelomeric FISH with HR-CGH for investigation of mentally retarded and dysmorphic patients with normal conventional karyotypes. Among the 94 patients investigated by both techniques, 9 abnormalities were found, and as seen in Table I, 8 abnormalities were detected by HR-CGH, 3 by subtelomeric FISH, and 2 by both techniques. Thus,

only one of the nine abnormalities was not detected by HR-CGH, while five were undetected by subtelomeric FISH. These five abnormalities were obviously not uncovered because they were interstitial. The 22qter deletion not detected by HR-CGH (see Table I) would be expected to be smaller than the resolution of HR-CGH, which is approximately 3 Mb [Kirchhoff et al., 1999]. Quantitative PCR, however, revealed that the deletion was approximately 4.5 Mb. Further PCR studies showed that a duplication of approximately 8 Mb was present at chromosome 22q just proximal to the deletion (the detailed results will be published elsewhere). It seems likely that the averaging of CGH profiles with relatively small deletions and duplications right next to each other would smear the signals and decrease the sensitivity. In case 9 only the 7pter duplication and not the 4pter deletion was detected by HR-CGH, while the entire translocation was detected by subtelomeric FISH. This is obviously due to the higher resolution of subtelomeric FISH which is equal to the size of the probes (30–100 kb).

The two techniques complemented one another in the present study, however, the results suggest that for investigation of mentally retarded and dysmorphic individuals, where where a G-banding analysis of at least 500 band resolution has shown normal results, HR-CGH should be applied prior to subtelomeric FISH as more aberrations are detected by the former technique. For patients with a family history subtelomeric FISH might be offered as a first choice as unbalanced translocations are likely to be involved in such cases. Among the 94 patients investigated in this study, 5 were reported to have other affected members in the family. None of the abnormalities were, however, found among those five patients.

With regard to practical considerations both techniques are labor-intensive and require follow-up, as results should be confirmed by a second method. It may be necessary to investigate potential polymorphisms when subtelomeric FISH is performed. In the present study the subtelomeric assay indicated that five cases had 2qter deletions. Follow-up with FISH on these cases showed that in three cases one of the healthy parents had a similar deletion. In two cases sample material from the parents was not available. However, quantitative PCR showed that none of the five patients had 2qter deletions. This is in accordance with previous reports on a common polymorphism in this region with a frequency ranging from 1.6–6% [Ballif et al., 2000; Fan et al., 2001; Anderlid et al., 2002; Baker et al., 2002; Clarkson et al., 2002; van Karnebeek et al., 2002].

TABLE II. Aberrations Detected in 330 Individuals With Mental Retardation and Dysmorphic Features by HR-CGH

Case no.	HR-CGH findings	Subsequent confirmation by			Origin
		G-banding*	FISH	Quantitative PCR	
Duplications					
10	enh(6q22q22)	Yes (750)			de novo
11	enh(7q36q36)	nd			nd
12	enh(9pter → qter)	Yes (550)	nd	nd	de novo
13	enh(10q11q11)		Yes		de novo
14	enh(22q10q11.2)		Yes		de novo
15	enh(Xq28 → qter)			Yes	Maternal normal
16	enh(Xq26q26)				Maternal
Deletions					
17	dim(1p36.3 → pter)		Yes		de novo
18	dim(1p36.2 → pter)	Yes (550)			de novo
19	dim(1p31p32)			Yes	Maternal
20	dim(1p13p21)	Yes (550)			de novo
21	dim(1q23q25)	Yes (550)			de novo
22	dim(1q43 → qter)	Yes (600)	Yes		de novo
23	dim(2p24p24)	Yes (550)			de novo
24	dim(2p15p15)		Yes		de novo
25	dim(2p10p12)			Yes	de novo
26	dim(2q23q24)	Yes (700)		Yes	Maternal
27	dim(2q24q24) dim(6q23q25)**			Yes	de novo
28	dim(2q31q32)			Yes	nd
29	dim(2q33q35)	Yes (600)		Yes	de novo
30	dim(2q37 → qter)	Yes (650)	Yes		de novo
31	dim(3q22q24)	Yes (550)			Maternal normal
32	dim(6q25q25)	Yes (600)			de novo
33	dim(6q26q26)			Yes	de novo
34	dim(7p21 → pter)			Yes	de novo
35	dim(7p15p15)	Yes (550)		Yes	de novo
36	dim(7p14p15)	Yes (650)		Yes	de novo
37	dim(7q22q22)			Yes	de novo
38	dim(7q32 → qter)	Yes (650)		Yes	de novo
39	dim(9q13q21)	Yes (550)			de novo
40	dim(10q21q21)	Yes (500)			de novo
41	dim(10q24q24)			Yes	de novo
42	dim(13q31.1q31.1)			Yes	de novo
43	dim(18q12q21)			Yes	Paternal
44	dim(18q21q22)			Yes	Paternal
45	dim(22q13.3 → qter)	Yes (500)			de novo
Translocations					
46	enh(4p16 → pter) dim(8p23 → pter)	Yes (550)	Yes		de novo
47	enh(4p14 → pter) dim(9p13 → pter)				Paternal
48	dim(4q35 → qter) enh(10p15 → pter)				Maternal
49	enh(5q35 → pter) dim(13q34 → qter)				Paternal
50	dim(12p → pter) enh(17p → pter)		Yes	Yes	de novo
51	dim(18p11.3 → pter) enh(21q22 → qter)	Yes (600)	Yes		Maternal
52	dim(21q22 → qter) enh(22q13 → qter)		Yes		Maternal

Cases 12, 13, 21, 24, 30, and 35 were published as cases 10, 4, 2, 3, 5, and 6, respectively in Kirchoff et al. [2000], and cases 31, and 48 were published as cases 7, and 8, respectively in Kirchoff et al. [2001].

Dim, diminished fluorescence ratio intensity ~ deletion; Enh, enhanced fluorescence ratio intensity ~ duplication; nd, not determined.

*Band resolution of confirmative analysis is noted in brackets.

**The aberrations are counted as a single aberration.

Another 330 patients with the indication of MR and/or dysmorphic features and normal karyotypes were investigated by HR-CGH (Table II). A total of 51 abnormalities were found among 424 patients (including those of the prospective study) resulting in a detection rate of 12%. As seen in Table III and Figure 1, the majority of the aberrations found by HR-CGH were interstitial and more deletions than duplications were found. Similar results were found by Ness et al. [2002], who used HR-CGH for investigation of 50 patients with indications comparable to those of our study. Five (10%) aberrations were found, of which four were interstitial. Joly et al. [2001] performed standard CGH analysis of 17 patients (14 families) with MR, dysmorphic features, and a normal karyotype. Five

subtle unbalanced translocations were identified in seven patients. These were all telomeric and would most likely have been detected by application of a subtelomeric FISH assay. This is in contrast to our results and the study of Ness et al. [2002], where most of the aberrations were interstitial and a subtelomeric screening would have shown different results from those of HR-CGH. Compared to the HR-CGH studies the study of Joly et al. [2001] was biased in favor of patients with a family history, thereby increasing the possibility of detecting cryptic segregating translocations.

The detection rate of 12% seems quite high, especially as the inclusion criteria eventually have become less strict. Thus, some patients were only mildly retarded and some had no or

TABLE III. Structural Abnormalities Detected in 50 Individuals With Mental Retardation and Dysmorphic Features by HR-CGH

Abnormality	Interstitial	Terminal	Total
Duplication	7	2	9
Deletion	27	7	34
Translocation	0	7	7
Total	34	16	50

scarcely any dysmorphic features. Still, the detection rate is to some extent also dependent on the quality of the preceding G-banding analysis. The majority of these analyses were performed in other laboratories, thus we have few data regarding the resolution of individual analyses. The band level of analyses performed in these laboratories is between 400–550 (Departments of Clinical Genetics at Kennedy Institute, Aarhus University Hospital, Odense University Hospital, Vejle Hospital, Denmark). Approximately one-half of the abnormalities were retrospectively confirmed by G-banding analysis at 500–750 resolution level. Thus these aberrations were not truly submicroscopic, but they were only detected once it was known where to look for them. If the banding resolution had been higher in the first place, it is likely that some of the larger abnormalities detected by HR-CGH in this

study, would initially have been detected by G-banding. We estimate that approximately 15/51 (30%) aberrations might have been found at the 500–750 band level used for confirmation, given no prior knowledge of their chromosomal location. The fact that they were not detected, may reflect that the quality of G-banding analysis is at risk of being compromised when high out-put screening is performed and/or that the analysis is highly dependant on the expertise and actual performance of the cytogeneticist.

For some of the aberrations it remains unclear whether they are responsible for the clinical findings in the patients. Thus, detailed knowledge of the diagnostic implications of all the imbalances is unlikely to be obtained until the particular chromosome regions can be characterized further or until additional cases are identified. The first steps necessary for further evaluation of the significance of the findings are to confirm them by another technique and to investigate the parents to find out whether the aberrations are inherited or de novo. Only 1 of the 51 abnormalities has not yet been confirmed due to lack of sample material and so far no confirmations have failed.

A duplication of chromosome 15q12 was excluded from the results. It was found in an affected child, whose father had the same duplication. Variation of region 15q11-12 has been described in a number of normal subjects [Ludowese et al., 1991; Browne et al., 1997; Barber et al., 1998; Fantes et al.,

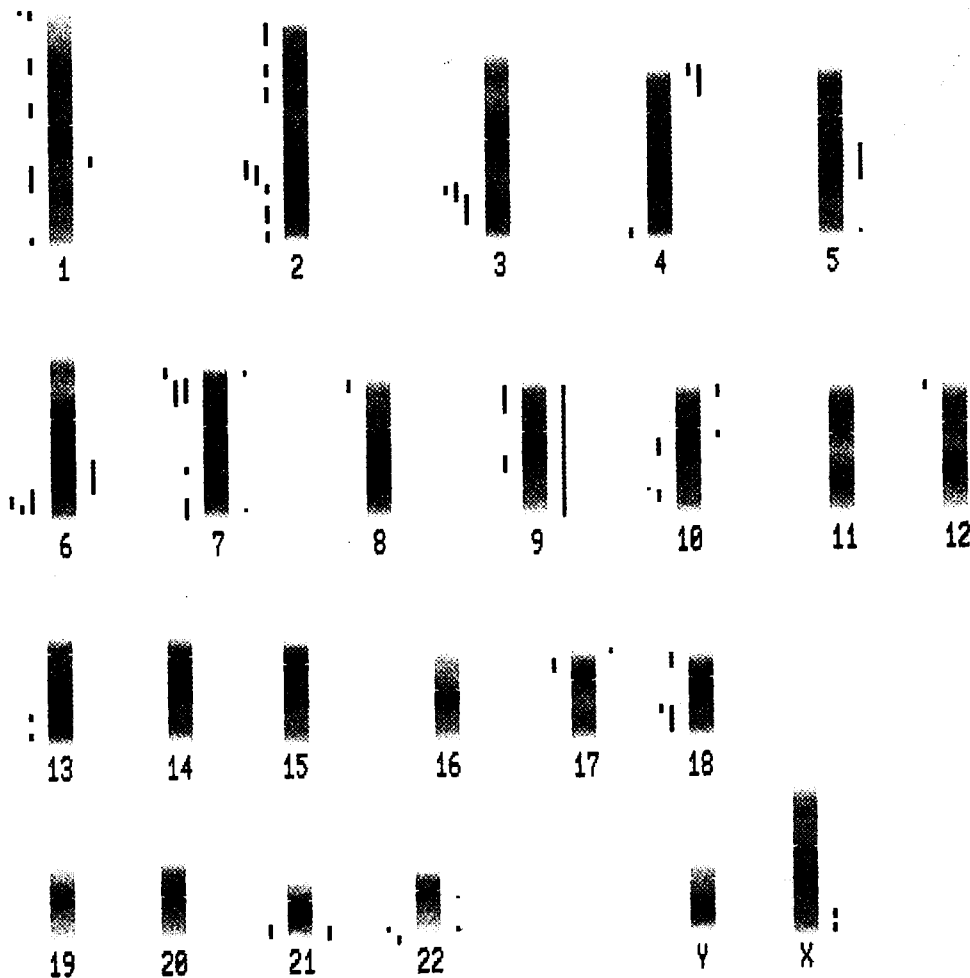


Fig. 1. Fifty-one abnormalities detected in 424 mentally retarded and dysmorphic individuals with high resolution CGH (HR-CGH). Gains and losses are shown as black bars on the right and left of the DAPI-banded chromosomes respectively. For technical reasons the bars are somewhat longer than the actual size of the aberrations.

2002]. In addition 11 duplications or deletions of 1q21, 9p11 and 16p11 were regarded as false positive results or normal variations and were excluded [Kirchhoff et al., 2001]. As seen in Table II it has not been possible to investigate all parents due to lack of sample material, however, the majority of the abnormalities were shown to be of de novo origin. Interestingly though, six of the inherited abnormalities were also unbalanced in the parent (cases 15, 16, 19, 25, 42, and 43, Table II). In all cases the parent was either unaffected or much less affected than the child (further details will be published elsewhere). In two cases the abnormalities were located on the X chromosome and inherited from mothers to sons, which may explain the discrepancies in phenotypic manifestations. In the remaining four cases the abnormalities were located on autosomes and other explanations must be sought. A straightforward one is that the abnormalities are merely accidental findings with no correlation to the clinical findings of the affected children. Little is known of the extent of submicroscopic normal chromosome variations in the population. With the introduction of a new technique like HR-CGH it is likely that normal variations will be revealed among those of clinical significance. Genomic imprinting may also explain the presence or absence of a phenotype depending on the sex of the transmitting parent. Another conceivable explanation would be that the exact size of the deletions in the parent and the child diverge, so that for instance more genetic material is lost in the child than in the parent. We are presently in the process of investigating this possibility by the use of molecular techniques. Finally, it may be that these deletions cause wide phenotypic variability. This has been described previously for 22q11 deletions, which may be found in apparently phenotypic normal individuals and individuals with DiGeorge syndrome within the same family [Leana-Cox et al., 1996; Digilio et al., 1997].

In eight cases the aberrations found were associated with known microdeletion syndromes (Table IV) [Wilson et al., 1995; Costa et al., 1998; Phelan et al., 2001; Heilstedt et al., 2003; Vlangos et al., 2003]. These syndromes were obviously not recognized in the clinical examinations of the patients and HR-CGH seems well-suited for revealing such cases.

The HR-CGH technique is well suited for routine analysis of mentally retarded and dysmorphic individuals with normal conventional karyotype and is likely to contribute to the definition of new microdeletions syndromes. Yet, the technique has limitations. It does not detect balanced translocations, and it does not replace ordinary FISH analysis when a known syndrome is suspected, as a number of such syndromes are caused by aberrations, that are below the resolution of HR-CGH.

It is of crucial importance to families and medical personnel to be able to diagnose a mentally retarded child. Many children go through numerous investigations without achieving a diagnosis, and despite the fact that many disorders have no specific therapeutic interventions, the determination of a

cause has implications for prognosis and treatment as well as counseling of families with regard to recurrence risk. According to our results about 12% of these children may get a diagnosis by HR-CGH analysis. In order to evaluate the clinical significance of the HR-CGH findings we use a follow-up strategy where the aberrations are rapidly confirmed by a second technique and investigation of the parents are performed. Molecular techniques are used for further characterization of the aberrations with regard to exact location, which is very important when individual cases are compared and required in the search for genes with critical impact on the clinical manifestations. Finally, we search in the literature, public databases, and collaborating cytogenetic laboratories for other patients with similar phenotypes and/or with imbalances or translocations overlapping the chromosomal regions affected in our patients. This will hopefully result in the definition of new syndromes.

Array-CGH is a newly developed technique which seems ready to implement in diagnostics [Veltman et al., 2003]. It is more sensitive than HR-CGH and it is expected to reveal imbalances in a higher number. Like in our investigation, a number of aberrations detected by array-CGH will probably represent normal variations of which some may be inherited. Molecular characterization and investigation of the clinical significance of submicroscopic chromosome aberrations is a laborious and challenging task, but it will inevitably succeed in more diagnosed patients and increase our knowledge of genes involved in the development of the phenotype.

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TABLE IV. Known Microdeletion Syndromes Detected Among 424 Patients With Mental Retardation and Dysmorphic Features Patients by HR-CGH

Smith Magenis ^a	1
BPES ^b	2
1pter deletion ^c	2
2qter deletion ^d	1
22qter deletion ^e	2
Total	8 = 19%

BPES: blepharophimosis-ptosis-epicanthus inversus syndrome.

^aVlangos et al., 2003.

^bCosta et al., 1998.

^cHeilstedt et al., 2003.

^dWilson et al., 1995.

^ePhelan et al., 2001.

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