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ARTICLE

A comparison of different metaphase CGH methods for the detection of cryptic chromosome aberrations of defined size

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An increasing body of evidence indicates that submicroscopic gene dose alterations may cause mental impairment and malformations. During the last decade, comparative genomic hybridization (CGH) has become a useful tool in the detection and mapping of chromosome aberrations. Modifications of CGH with increased resolution down to 3–5 Mb have been reported and CGH is now offered as a diagnostic procedure in the evaluation of patients with idiopathic mental retardation (MR). In order to increase the resolution, we modified the CGH protocol using freshly prepared high-quality metaphase slides and chemical labeling, and tested the method on a set of patients with well-defined submicroscopic chromosome abnormalities with confirmed size 1.3–20.5 Mb. Subsequently, a completely blinded test was performed to compare the performance of the chemical labeling CGH to the commercially available HR-CGH. Using the two different CGH methods, we were able to detect chromosome imbalances down to 2–3 Mb approximately. The HR-CGH method detected all aberrations >6 Mb and a few smaller, while the modified CGH method was able to detect all but three aberrations >1.8 Mb. The modified CGH method was superior in the detection of terminal imbalances, while the HR-CGH software was more successful in the detection of imbalances located very close to the centromeric regions. In conclusion, the resolution of metaphase CGH may be as high as 2–3 Mb but is most likely depending on the chromosomal region involved, a clear limitation when used as a screening method for chromosome aberrations in patients with idiopathic MR.

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Introduction

Although mental retardation (MR) is a common disorder, etiological factors are not found in more than half of the patients who are subjected to extensive clinical and laboratory evaluation. One important cause of MR is chromosome imbalance due to rearrangements that give rise to gene dose alterations. Some of these rearrangements have escaped detection since current screening methods to detect chromosome abnormalities have not been sensitive enough. Molecular cytogenetic methods, such as fluorescence *in situ* hybridization (FISH), comparative genomic hybridization (CGH) and multicolor karyotyping have made it possible to increase the resolution, and to identify very small chromosome rearrangements as the cause of

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MR.[1] Further development of more accurate and sensitive genome wide screening methods may facilitate the clinical diagnosis of patients with subtle rearrangements. In order to cover the whole genome, genome-wide screening for chromosomal imbalances in patients with idiopathic MR using microsatellite markers has been reported,[2,3] as well as metaphase CGH.[4–7]

The quality of CGH analysis is dependent on the slide quality as well as the labeling and hybridization efficiency.[8] In this report, the performance of CGH was tested after optimizing the protocol in order to increase the resolution. We used chemical labeling by Universal Linkage System (ULS®)[9] and freshly prepared slides (<3 days old) with good quality chromosomes (>500 bands). In addition, the method was compared to the commercially available HR-CGH performed on the Cytovision® System (Applied imaging, Newcastle, UK).

Material and methods

Material selection

In all, 17 cases with known cryptic aberrations were selected (12 cases with subtelomeric rearrangements, two cases with an interstitial deletion and four cases with microdeletion syndromes: DiGeorge syndrome, Prader Willi syndrome, Wolf Hirschhorn syndrome and a mosaic case with Smith Magenis syndrome, Table 2). The 17 cases contained altogether 26 rearrangements (21 terminal aberrations and five interstitial aberrations). Their sizes were ranging from 1.3 to 20.5 Mb (42% of the aberrations were smaller than 5 Mb), and 19 different chromosome arms were involved. Routine chromosome analysis (450–500 bands) had failed to detect the rearrangements in all cases. Cases 2–8, 12 and 14–17 (Table 1) were identified using subtelomeric FISH probes. Cases 1–10 have been published previously.[10–12] Case 7, with an interstitial deletion of 15q23–25.1, was serendipitously detected during subtelomeric screening, as the 15q control probe (LSI PML) in the subtelomeric screening kit (Vysis) was deleted. Cases 9, 10 and 13 were detected using microdeletion FISH probes and case 1 was identified by spectral karyotyping (SKY). Despite the large chromosome fragments involved in the unbalanced translocation in this case, repeated standard G-band analysis could not reveal the rearrangement due to the similarity in the banding pattern of the chromosome fragments involved. Case 11 was identified by HR-CGH. All chromosome imbalances were size mapped using BAC and PAC clones based on Ensembl human genome browser (http://ensembl.org, July 2003), except for cases 9 and 13. Case 9 was size mapped using a chromosome 22 specific microarray.[13] From case 13, no patients metaphase chromosomes were available and the size was estimated as a common Prader Willi syndrome 15q−deletion.[14]

FISH mapping

Based on human clone physical map (http://www.ensembl.org July 2003 http://genome.ucsc.edu, April 2003), 328 BAC and PAC clones were selected for breakpoint mapping of the chromosome aberrations. The size of the aberrations was estimated to 100 kb accuracy approximately (Table 2), using the location of the clones that spanned over each breakpoint. The clones were obtained from Resources for

Table 1 CGH analysis performed blinded and semiblinded

CGH analysis by ULS® labeling and Quips software				*CGH analysis by nick translation and HR-CGH software*			
Semiblinded		*Completely blinded*		*Semiblinded*		*Completely blinded*	
Case	*Abnormality*	*Case*	*Abnormality*	*Case*	*Abnormality*	*Case*	*Abnormality*
1	Monosomy 7q Trisomy 10q	11	Deletion 10q25	11	Deletion 10q25	1	Monosomy 7q Trisomy 10q
2	Monosomy 18p Trisomy 13q	12	Deletion 4p	12	Deletion 4p	2	Monosomy 18p Trisomy 13q
3	Monosomy 21q Trisomy 9q	13	Deletion 15q11–q13	13	Deletion 15q11–q13	3	Monosomy 21q Trisomy 9q
4	Trisomy 17q Monosomy 12q	14	monosomy 9q Trisomy 22q	14	Monosomy 9q Trisomy 22q	4	Trisomy 17q Monosomy 12q
5	Monosomy 4q Trisomy 2q	15	Deletion 1p	15	Deletion 1p	5	Monosomy 4q Trisomy 2q
6	Monosomy 6q Trisomy 6p	16	Monosomy 13q Trisomy 5q	16	Monosomy 13q Trisomy 5q	6	Monosomy 6q Trisomy 6p
7	deletion 15q24	17	Monosomy 13q Trisomy 2p	17	Monosomy 13q Trisomy 2p	7	Deletion 15q24
8	Deletion 6p	18	Normal			8	Deletion 6p
9	Deletion 22q11.2					9	Deletion 22q11.2
10	Deletion 17p11					10	Deletion 17p11
						18	Normal

Cases 1–10 were obtained from the by Karolinska Hospital, while cases 11–17 were contributed by the JF Kennedy Institute.

Table 2 CGH result

Case	Karyotype	Chromosome imbalances	Detection method	Size (Mb)	ULS labeling Quips software	Nicktranslation HR-CGH software	Nicktranslation quips software	ULS labeling HR-CGH software
1	46,XY,der(7)t(7;10)(q35;q25.3)	Monosomy 7q	S	13.2	D	D	—	D
		Trisomy 10q		19.1	D	D	—	D
2	46,XY,der(18)t(13;18)(q33.3;p11.31)	Monosomy 18p	T	5.8	D	D	D	—
		Trisomy 13q		5.9	D	ND	D	—
3	46,XY,der(21)t(9;21)(q33.3;q22.3)	Monosomy 21q	T	2.5	D	ND	—	—
		Trisomy 9q		11.2	D	D	—	—
4	46;XY,der(12)t(12;17)(q24.33;q25.3)	Trisomy 17q	T	1.3	ND	ND	ND	—
		Monosomy 12q		2.1	D	ND	D	—
5	46,XX,der(4)t(2;4)(q36.1;q34.3)	Monosomy 4q	T	8.7	D	D	—	—
		Trisomy 2q		20.5	D	D	—	—
6	46,XX,rec(6)dup(6p)inv(6)(p23q27)	Monosomy 6q	T	3.5	D	ND	ND	—
		Trisomy 6p		14.7	D	D	D	—
7	46,XX,del(15)(q23q25.1)	deletion 15q24	T[a]	10.3	D	D	—	—
8	46,XX,del(6)(p25.3→pter)	deletion 6p	T	1.8	D	ND	ND	—
9	46,XY,del(22)(q11.21q11.21)	Deletion 22q11.2	M	2.5	ND	ND	—	—
10	46,XY,del(17)(p11.2p11.2)[30]/46,XY[20]	Deletion 17p11	M	3.6	D	D	—	D
11	46,XX,del(10)(q25.1;q25.3)	Deletion 10q25	H	8.1	D	D	—	—
12	46,XY,del(4)(p16.1→pter)	Deletion 4p	M	6.9	D	D	—	—
13	46,XX,del(15)(q11q13)	Deletion 15q11–q13	M	~4[b]	D	D	—	—
14	46,XX, der(9)t(9;22)(q34.2;q13.31)	Monosomy 9q	T	4.1	D	ND	D	ND
		Trisomy 22q		5.7	ND	ND	ND	ND
15	46,XX,del(1)(p36.23→pter)	Deletion 1p	T	8	D	D	—	—
16	46,XX,der(13)t(5;13)(q35.2;q34)	Monosomy 13q	T	3.9	D	ND	D	ND
		Trisomy 5q		6.5	D	D	D	D
17	46,XX,der(13)t(2;13)(p25.3;q32.1)	Monosomy 13q	T	19.6	D	D	—	—
		Trisomy 2p		3.5	ND	ND	—	—

S = SKY; T = subtelomeric FISH; M = microdeletion FISH; H = HR-CGH; D = detected; ND = not detected, -not performed.
[a]Control probe was deleted. [b]Data from literature.

Molecular Cytogenetics (Bari, Italy), The Wellcome Trust Sanger Institute (Cambridge, UK) and BACPAC Resource Center Children's Hospital (Oakland Research Institute, Oakland, CA, USA). Bacterial cultures and DNA isolation were performed according to the BAC-PAC miniprep protocol (http://www.biologia.uniba.it/rmc). Probes were labeled by nick translation with FITC-dUTP (NEN Life Science Products, Boston, MA, USA) or SpectrumRed-dUTP (Vysis Inc., Downers Grove, IL, USA), and FISH analyses were performed according to a standard protocol. The slides were analyzed on a Zeiss Axioplan 2 (Carl Zeiss, Göttingen, Germany) epifluorescence microscope, and images were captured using a cooled CCD camera (Sensys Photometrics, München, Germany) and SmartCapture 2 software (DigitalScientific Ltd, Cambridge, UK). Inverted 4,6-diamidino-2-phenylindole (DAPI) staining was used for chromosome identification during FISH analysis.

DNA preparation

Genomic DNA was extracted from blood samples, EBV transformed lymphocytes or cultured fibroblast cells using Puregene blood kit (Gentra systems Inc., Minneapolis, MN, USA) or Qiagen genomic tip (Qiagen GmbH, Hilden, Germany) according to the manufacturer's protocol.

CGH by chemical labeling (ULS®)

CGH was performed essentially as described previously[15] with a few modifications. Briefly, genomic DNA from the patients and reference DNA was digested into fragments of 100–2000 bp size by overnight incubation with *Dpn*II (New England Biolabs Inc., Beverly, MA, USA) at 37°C and checked on a 2% agarose gel. The DNA fragments were purified using Qiaquick PCR purification kit (Qiagen GmbH). *Dpn*II digested DNA (1 μg) was directly labeled by Universal Linkage System ULS® (Q-BIOgene, Illkirch, France) according to the manufacturer's protocol. The reference DNA (Promega GmbH, Mannheim, Germany) consisting of a pool of 10 normal individuals was labeled with rhodamine (Q-BIOgene) and patient DNA was labeled with dGreen (Q-BIOgene). They were mixed and repurified using the PCR purification kit (Qiagen GmbH). Cot-1 DNA (20–40 μg) (Invitrogen, Carlsbad, CA, USA) was added before ethanol precipitation. The pellet was air-dried and resuspended in 10 μl Hybrisol VI (Q-BIOgene). Labeled DNA was denatured and applied onto denatured, freshly

prepared (<3 days old) and pepsin-treated, good quality (>500 bands) metaphase slides from normal lymphocyte cultures. After overnight hybridization at 37°C, the slides were washed in 0.4 × SSC/0.1% Tween-20 at 73°C for 3 min and in 4 × SSC/0.1% Tween-20 at room temperature for 1 min. They were counterstained with 0.15 μg/ml DAPI in antifade solution (Vectashield, Vector Inc., Burlingame, CA, USA). All reasonably straight, nonoverlapping chromosomes from 15 to 20 metaphases were analyzed on a Zeiss Axioplan 2 (Zeiss) epifluorescence microscope, and images were captured using a cooled CCD camera (Sensys Photometrics) and SmartCapture 2 software (DigitalScientific Ltd). Images were analyzed using the Quips™ software (Vysis Inc.). In the CGH analysis using the Quips software, chromosome regions were considered to be lost if the hybridization green:red ratios were <0.85 or <0.90 and hybridization ratios >1.15 or >1.10 were considered to be gained. When false-positive gains or losses on other chromosome regions were observed, the aberration was considered not detectable.

CGH by HR-CGH software

CGH was performed essentially as described previously[15] with some modifications. Briefly, 1.5 μg patient DNA was labeled with SpectrumGreen-dUTP (Vysis) and 1 μg normal reference DNA was labeled with Spectrum Red-dUTP (Vysis) using nick translation (fragment length obtained was 600–2000 bp). 1.2 μg patient and 0.8 μg reference DNA were combined with 100 μg of Cot-1 DNA (Invitrogen) and ethanol precipitated. The pellet was air-dried and resuspended in 10 μl hybridization buffer. Labeled DNA was denatured and applied onto denatured good quality (>500 bands) metaphase slides of normal lymphocytes, which were chemically aged by incubation in by 99% ethanol at −18°C and pepsin treated prior to hybridization. After 48–72 h hybridization at 37°C, the slides were washed in 1 × SSC at 72°C for 5 min and in 2 × SSC at 72°C for 5 min. The slides were then counterstained with 0.15 μg/ml DAPI in an antifade solution (Vectashield, Vector Inc.). All reasonably straight, nonoverlapping chromosomes from 15 metaphases were analyzed on a Zeiss Axioplan (Zeiss) fluorescence microscope, and images were captured and analyzed using the Cytovision System, version 2.7 High Resolution CGH analysis software (Applied Imaging Ltd). Chromosome regions were considered to be lost or gained when the green:red ratio exceeded the ratios from the dynamic reference interval, using confidence interval 95% or 99.5%. The analyses were mainly performed using the dynamic reference interval that is included in the software. It is recommended by Applied Imaging to create a user-specific dynamic reference interval by using a number of normal DNA. This will adjust the dynamic reference interval to the user's labeling and hybridization conditions. In our study, however, the user-specific dynamic reference interval needed more optimizations and better results were obtained using the dynamic reference interval included in the software.

Results

All cases were analyzed by both CGH methods. Cases 1–10 were obtained from the same laboratory that performed the chemical labeling CGH using the Quips software, and these samples were therefore not considered to be analyzed completely blinded since it was known that they contained some kind of aberrations. This knowledge could bias the analysis and these samples are listed as semiblinded in Table 1. Cases 11–18 were obtained from the laboratory that performed the HR-CGH method and were analyzed completely blinded without any knowledge about the sample, using the chemical labeling GCH and Quips software. For the performance of the HR-CGH method, the situation was reversed with regard to blinded and semiblinded analysis (Table 1). Case 18 was a normal DNA sample and no imbalances were found in this case using either of the methods.

CGH results for each case are listed in Table 2. Using the chemical labeling CGH and Quips software, we were able to detect 22 of the 26 aberrations studied. Four aberrations were not detected without detecting multiple false-positive losses or gains on other chromosome regions; a terminal 1.3 Mb duplication on chromosome 17q, an interstitial 2.5 Mb deletion and a terminal 5.7 Mb duplication on chromosome 22q, in addition to a terminal 3.5 Mb duplication on chromosome 2p. The smallest aberration detected by this CGH method was a terminal deletion of 1.8 Mb on chromosome 6p (Figure 1b).

This aberration was correctly identified using a threshold of 1.10–0.90 and despite the narrow threshold no false-positive results were detected on other chromosome regions (Figure 4).

Initially, the analysis of cases 10 and 13 gave inconclusive results using the Quips software due to insufficient suppression of the repetitive sequences by Cot-1 DNA.

When repeated using the same amount of Cot-1 DNA but with another batch, the analysis revealed deletions located close to the centromere (Figure 3).

Using nick translation labeling and the commercially available HR-CGH software (Applied Imaging), 15 of 26 aberrations studied were detected. Of these, 11 chromosome imbalances were not detected without detecting multiple false-positive losses or gains on other chromosome regions. Six of these undetected aberrations had a size larger than 3 Mb; four terminal duplications (size ranging from 1.3 to 5.9 Mb), six terminal deletions (size ranging from 1.8 to 4.1 Mb) and a 2.5 Mb interstitial deletion on chromosome 22. The smallest aberration detected was an interstitial 3.6 Mb deletion on chromosome 17 that was present in 60% of the cells (Table 2).

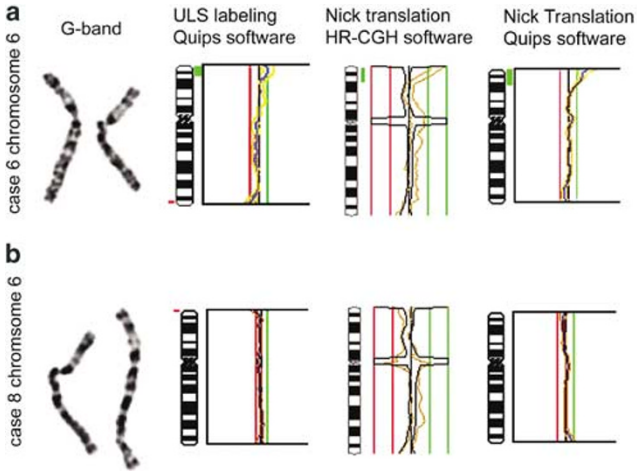

Figure 1 (**a**) Case 6 chromosome 6: Giemsa-stained chromosomes, (no abnormalities were found). CGH profile of DNA sample labeled by ULS® analyzed with the Quips software. Both the 14.7 Mb duplication at 6p and the 3.5 deletion at 6q were detected using a threshold of 0.85–1.15. CGH profile of DNA sample labeled by nick translation, analyzed on the Cytovision® using HR-CGH software. The 14.7 Mb duplication was clearly visible, but the deletion at 6q was not detected. The black lines represent the dynamic reference interval (CI 99.5%), while the brown lines represent the slide average ratio of the patient. At 6q the brown lines do not exceed the ratios of the dynamic reference interval and no deletion is detected. CGH profile of DNA sample labeled by nick translation analyzed with the Quips software (slide exchange test). The duplication on 6p was clearly visible, but the deletion on 6q is not, since the slide average ratio (blue lines) had a value close to 1.0 (normal). (**b**) Case 8 chromosome 6: Giemsa-stained chromosomes, (no abnormalities were found). CGH profile of DNA sample labeled by ULS® analyzed with the Quips software. A 1.8 Mb deletion at 6p was visible using a threshold of 1.10–0.9. No false-positive results on other chromosome regions were observed. CGH profile of DNA sample labeled by nick translation, analyzed on the Cytovision® using HR-CGH software. The 1.8 Mb deletion on 6p was not detectable. CGH profile of DNA sample labeled by nick translation analyzed with the Quips software (slide exchange test). The 1.8 Mb deletion on 6p was not detectable using a threshold of 1.15–1.85. Narrowing down the threshold to 0.90–1.10 resulted in false-positive duplication of 6q and no deletion of 6p could be detected.

To investigate the influence of the software *versus* slide quality four cases were analyzed (1, 10, 14 and 16) on the Cytovision® with HR-CGH software using the same slide previously analyzed on the Quips software. Two aberrations (a 4.1 Mb deletion on 9q and a 3.9 Mb deletion on 13q) were not detected (Figure 2), while they were previously detectable using the Quips software. We also reanalyzed six slides (cases 2, 4, 6, 8, 14 and 16) using the Quips software, which were previously analyzed with HR-CGH software on the Cytovision® (Figures 1 and 2). Four

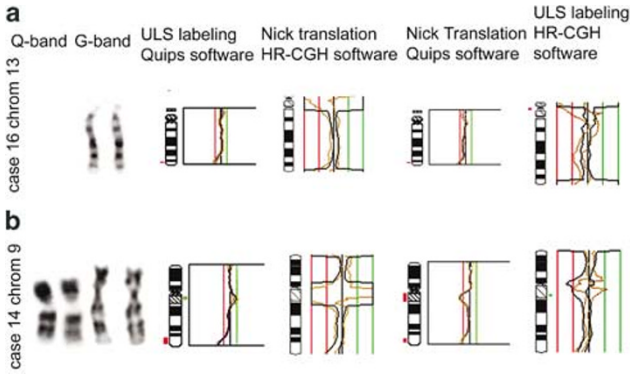

Figure 2 (**a**) Case 16 chromosome 13: Giemsa-stained chromosomes (no abnormalities were found). CGH profile of DNA sample labeled by ULS® analyzed with the Quips software. A 3.9 Mb deletion on 13q was visible using a threshold of 1.15–0.85. CGH profile of DNA sample labeled by nick translation, analyzed on the Cytovision® using HR-CGH software. The 3.9 Mb deletion at 13q was not visible probably due to the dynamic reference interval (CI 95%) at 13q (black lines). CGH profile of DNA sample labeled by nick translation analyzed with the Quips software (slide exchange test). The 3.9 Mb deletion on 13q was detected using a threshold of 1.15–1.85. CGH profile of DNA sample labeled by ULS® analyzed on the Cytovision® using HR-CGH software (slide exchange test). The 3.9 Mb deletion on 13q was not detected using CI 95%. (**b**) Case 14 chromosome 9: Quinacrine-stained chromosomes, original chromosome analysis (no abnormalities were found). Giemsa-stained chromosomes (no abnormalities were found). CGH profile of DNA sample labeled by ULS® analyzed with the Quips software. A 4.1 Mb deletion on 9q was visible using a threshold of 1.15–0.85. The repetitive sequences are not completely suppressed in the heterochromatin region of chromosome 9. CGH profile of DNA sample labeled by nick translation, analyzed on the Cytovision® using HR-CGH software. The 4.1 Mb deletion on 9q was not visible since the ratio of dynamic reference interval deviated as much from normal (ratio 1.0) as the ratio of the patient sample. CGH profile of DNA sample labeled by nick translation analyzed with the Quips software (slide exchange test). The 4.1 Mb deletion on 9q was detected using a threshold of 1.15–1.85. The repetitive sequences were not completely suppressed in the heterochromatic region of chromosome 9. CGH profile of DNA sample labeled by ULS®, analyzed on the Cytovision® using HR-CGH software (slide exchange test). The 4.1 Mb deletion on 9q was not detected, incomplete suppression of the repetitive sequences was not fully corrected by the dynamic reference interval (CI 95%).

aberrations, a 5.9 Mb duplication on 13q, a 2.1 Mb deletion on 12q, a 4.1 Mb deletion on 9q and a 3.9 Mb deletion on13q, were detected using the Quips software, but not using the HR-CGH software. This 'exchange slide' test was performed only on recently hybridized slides, since old hybridizations did not have sufficient quality for reanalysis (Table 2).

Double hybridizations using different reference DNA are always performed on diagnostic samples, but not consequently in these experiments.

Conclusion and discussion

A large number of studies have demonstrated that small duplications or deletions of chromosomal segments may cause multiple anomalies. However, most studies include only screening for subtelomeric rearrangements,[16] and reliable and sensitive high-resolution genome-wide screening methods have not been widely implemented in the clinical setting. The resolution of methods such as CGH is comparatively low (5–10 Mb) and thus in most cases a high-quality karyotype has a better resolution. Instead, its important use has been to outline gene dose alterations in cancer, where good karyotypes are difficult to obtain, or where only archived material has been available. However, recent improvements of the metaphase CGH technique has made it possible to detect segmental aneusomy for chromosome regions as small as 3 Mb,[17] and previously reported software simulations indicated that the theoretical resolution of metaphase CGH might be as high as 2 Mb.[18] A HR-CGH software is now commercially available and several clinical laboratories are offering the method as a diagnostic test in the evaluation of MR and dysmorphism.[5,7]

Our previous CGH protocol consisted of labeling with FITC-dUTP (NEN Life Science) and Spectrum Red-dUTP (Vysis) by nick translation and we used commercially available metaphase slides (Vysis). In order to increase the resolution, we modified our CGH protocol using chemical labeling and fresh high-quality metaphases. By using chemical labeling (ULS®), we received a very strong and uniform signal over the whole chromosome, and the hybridization on fresh, high-quality metaphase slides, which were easy to denature, improved our CGH quality tremendously. We were able to detect deletions as small as 1.8 Mb. However, four aberrations were missed. Three out of four undetected aberrations were duplications and the smallest duplication that could be detected was three times larger than the smallest deletion. This might indicate that small heterozygous duplications are more difficult to detect than deletions, which might be explained by the fact that the green:red ratio for duplications is 3:2, while the ratio is 1:2 for deletions.

Other possible explanations to the missed aberrations may be varying hybridization conditions between different chromosome regions due to chromosome condensation and the presence of different repeats, for example, the region on 22q, which contains a large number of low copy repeats[13] and where a deletion of 2.5 Mb and a duplication of 5.7 Mb were missed (by both CGH methods). Already early CGH studies reported that it was more difficult to obtain reliable results for some chromosome regions (among them chromosome 22).[8] This of course hampers the analysis and explains the variations in the sensitivity of the method. The HR-CGH could detect approximately half of the subtelomeric aberrations while half of them remained undetected (10 of 21). In order to increase the resolution without increasing the false-positive results, the HR-software uses a dynamic reference interval to correct for the regions that contain highly repetitive sequences (as the centromeric and heterochromatin regions), but also for regions that are variable in signal intensity (as the subtelomeric regions (Figures 1 and 2). This seems to lower the detection rate in the subtelomeric regions, but improves the detection in regions close to the centromere.

In order to test the software performance regardless of the slide quality, slides were exchanged and analyzed between the systems. The Quips software performed clearly better in the detection in the subtelomeric regions using both chemically labeled DNA as well as enzymatically labeled DNA, since seven terminal aberrations were detected by Quips, but not by HR-CGH software. In addition, four aberrations that were previously not detected by the HR-CGH software were detected by the Quips software in the 'slide exchange test' (Table 2). However, using the Quips software, the results were difficult to interpret when the repetitive sequences were insufficiently suppressed (Figures 3), a problem that sometime occurs due to variable quality of Cot-1 DNA. This problem could only be resolved by rehybridizing with another batch of Cot-1 DNA. All interstitial deletions were

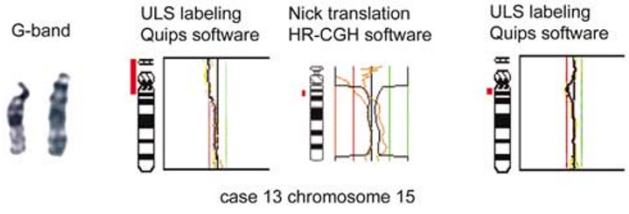

Figure 3 Case 13 chromosome 15. Giemsa-stained chromosomes (no abnormalities were found). CGH profile of first hybridization of DNA sample labeled by ULS® analyzed with the Quips software. The deletion on 15q11–q13 was not detected due to the insufficient suppression of the repetitive sequences, closely located to the deletion. Incomplete suppression of repetitive sequence was also observed on many other chromosomes in this hybridization and this made the analysis uninterpretable. CGH profile of DNA sample labeled by nick translation analyzed on the Cytovision® using HR-CGH software. The deletion on chromosome 15 was easily detected, as the software uses a standard reference interval (CI 99.5%) that is excluding the region containing repetitive sequences (black lines) from analysis. Second hybridization with DNA sample labeled by ULS® and analyzed with the Quips software CGH. The deletion was easily detected when the repetitive sequences were completely suppressed by Cot-1-DNA.

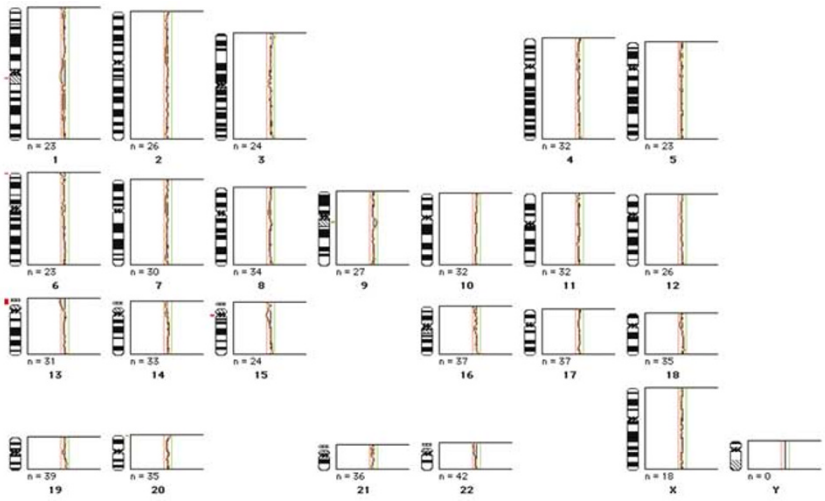

Figure 4 CGH profile of case 8, DNA sample labeled by ULS® and analyzed with the Quips software CGH. A 1.8 Mb deletion on 6p is visible using a threshold of 1.10–0.90. No false-positive results were observed on other chromosome regions. Repetitive sequences were incompletely blocked by Cot-1 DNA on chromosome 1, 9, 13 and 15.

detected by both systems, except for the 2.5 Mb deletion on chromosome 22q, which escaped detection with both methods.

Nick translation is an enzyme-mediated labeling based on the incorporation of reporter molecules covalently attached to nucleotide triphosphate substrates in polymerization, while ULS labeling is based on a marker/platinum complex that links to the purines of DNA. The platinum-based nucleic acid probes have been shown to perform well in multicolor FISH and microarray experiments.[19–21] When comparing the performance of these labeling techniques, we observed two terminal aberrations that were detectable when DNA was labeled by ULS, but not when labeled by nick translation (Figure 1). This might possibly indicate that the chemical labeling was more efficient in the subtelomeric regions. However, larger studies are needed to draw any reliable conclusions. In this study, ULS labeling did not increase the resolution when the slides were analyzed by the HR-CGH software, since two aberrations were not detected by HR-CGH regardless of the labeling method (Table 2). However, the dynamic reference interval was not optimized for ULS labeling, by hybridizing normal DNA samples labeled by ULS.

In summary, high-resolution CGH can be used to detect submicrosopic chromosome imbalances down to 2 Mb approximately. Optimization of the CGH protocol by using chemical labeling and freshly prepared high-quality metaphase spreads increased the detection rate, especially in the subtelomeric regions, which often have been difficult to interpret. As is shown in this study, very small deletions (1.8 Mb) can be detected by high-resolution CGH, while other larger aberrations (>5 Mb) will remain undetected. Hence, the resolution of metaphase CGH varies, and most likely depends on the chromosome region involved. In general, metaphase CGH has limitations in sensitivity and robustness, and it is not very accurate in the delineation of the size or location of the chromosome imbalance. Furthermore, it does not identify balanced translocations or inversions.

In this report, we show that metaphase CGH has clear limitations as a screening method for cryptic aberrations in patients with MR of unknown cause and dysmorphic features. The method was not reliable, in particular, for HR-CGH screening of the subtelomeric regions, and rearrangements are often found in these regions. For clinical purposes, metaphase CGH may, however, be useful as a supplement to multisubtelomeric FISH analysis, but a rather rigorous quality control must be set up. Hence, when nick translation HR-CGH is used for diagnostic purpose a fixed procedure has been established, including a defined confidence interval, double hybridizations with male and female reference DNA, repeat samples in doubtful cases and supplementary FISH analysis. Moreover, the recent development of array-based CGH[22] opens new opportunities for rapid high-resolution whole genome screening for chromosome imbalances. It does not only detect very small gene dose alterations but it is also very useful for size mapping of the aberrations,[12] which facilitates phenotype–genotype correlations. It is therefore likely that array CGH will have a greater impact than metaphase CGH as a test in the diagnostic setting in the future.

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