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ARTICLE

Array-CGH fine mapping of minor and cryptic HR-CGH detected genomic imbalances in 80 out of 590 patients with abnormal development

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During a 6-year period, 590 patients suspected of having a minor or cryptic genomic imbalance as the cause of mental retardation with dysmorphic signs +/− malformations have been investigated with high-resolution comparative genomic hybridisation (HR-CGH) in our diagnostic laboratory. Thirty-six patients had a small chromosomal aberration detected by routine karyotyping, and 554 patients had a normal G-banded karyotype. In the latter group, a genomic imbalance was detected by HR-CGH in 40 patients (7.2%): 29 deletions, 3 duplications, 4 unbalanced translocations, and 4 occult trisomy mosaicisms. When microarray-based comparative genomic hybridisation (array-CGH) became available, all HR-CGH-positive samples were also investigated by 1 Mb resolution array-CGH for more precise mapping. From the 514 patients with normal HR-CGH findings, a subset of 20 patients with particularly high suspicion of having a chromosomal imbalance was selected for array-CGH. In four of them (20%), an imbalance was detected: three deletions and one duplication. Of note, 73 out of the 80 array-CGH mapped patients had a *de novo* chromosomal rearrangement (91%). Taken together, this work provides phenotype–genotype information on 80 patients with minor and cryptic chromosomal imbalances.
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Introduction

In Europe, mental retardation with or without dysmorphic signs and malformations is recognised in around 1% of children.[1–3] The true incidence may be higher due to non-ascertainment. An unbalanced karyotype can be found in 10%[4] to 16%[5] of these individuals by routine chromosome analysis (microscopy of G-banded metaphases from a T-lymphocyte blood culture). Other patients have a cryptic (ie invisible) chromosomal imbalance detectable by chromosome-based high-resolution comparative genomic hybridisation (HR-CGH).[6,7] We and others have found that deletions above ~3 Mb can be detected by HR-CGH.[8] Duplications are usually more difficult to detect. In recent years, CGH profiles have been determined using genomic microarrays instead of metaphase chromosomes to obtain higher sensitivity.[9–14] The resolution is no longer limited by the quality of the normal metaphase spreads, but by the density of genomic clones. Many laboratories have employed bacterial artificial chromosome (BAC)-based arrays with an average clone distance of 1 Mb for screening patients with developmental delay and/or patients suspected for having a cryptic chromosomal imbalance, hereafter called 1 Mb array-CGH. The 1 Mb array-CGH pick-up rate for clinically relevant chromosomal

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imbalances in mentally retarded patients with normal karyotypes was 8.4% when data from five different studies with a total of 332 patients were taken into account.[10] Using a more limited 831-clone BAC array targeted to regions of known clinical relevance, a genomic alteration was found in 5.6% of 1500 consecutive cases with a variety of developmental problems.[15]

In our laboratory, we have used HR-CGH as a screening method for cryptic chromosomal imbalances for several years. In an initial study, we found a cryptic chromosomal imbalance in 5 out of 50 patients with abnormal development and normal karyotypes,[7] in line with other observations.[6] As an extension of this study and to map minor imbalances more accurately, we have recently used 1 Mb array-CGH to investigate all HR-CGH findings obtained during the past 6 years. Thus, this work gives detailed information on the position of the genomic imbalances in 80 patients with abnormal phenotypes, adding data on phenotype–genotype correlations.

Patients and methods

Patients

Blood samples from 590 patients (age range 1–58 years), all except 3 being mentally retarded, and most also having dysmorphic features and/or malformations, were received for analysis at the Center for Medical Genetics and Molecular Medicine, Haukeland University Hospital, during a 6-year period (2001–2006). Blood samples were referred to us from the whole of Norway by medical geneticists or experienced paediatricians. After conventional G-banded karyotyping, the samples were examined by HR-CGH analysis. This was performed either because the observed chromosomal abnormality was small and doubtful or difficult to classify, or because the patient phenotype was suggestive of a chromosomal abnormality despite the karyotype being normal. When array-CGH became available, all HR-CGH-positive samples were investigated by 1 Mb array-CGH for fine mapping. In addition, 1 Mb array-CGH analysis was carried out on a subgroup of 20 patients with strong clinical impression of having a genomic imbalance despite normal findings on G-banding and HR-CGH. The layout of the study is summarised in Figure 1. Parental analyses were carried out to investigate whether the finding was *de novo* or the result of a balanced rearrangement in one of the parents. Parental DNA was not available for case 32 (Table 1). Many patients were later referred to us for clinical evaluation and genetic counselling. In other patients, more detailed clinical information was asked for. The patients' phenotypes are summarised in Tables 1–3.

HR-CGH

Chromosome-based HR-CGH was performed as described previously.[7] Briefly, patient DNA and normal reference DNA (800 ng each) were labelled with fluorescein isothiocyanate-12-dUTP and Texas Red-5-dUTP (NEN Life Science Products Inc., USA), respectively. Commercial reference DNA was used, consisting of a pool from either 10 normal female donors or 10 normal male donors (Promega GmbH, Germany). The labelled DNAs together with 20 μg of human Cot-1 DNA (Invitrogen Inc., USA) were hybridised onto normal metaphase chromosomes (from the same individual). Sex-matched hybridisations were performed. Analysis was performed using the high-resolution CGH analysis software that is part of the CytoVision System (Applied Imaging, UK). Imbalances were scored as a deviation from the standard reference interval CGH ratio profiles at 99.5% confidence intervals.[16] The CGH results were verified either by re-examination of G-banded high-quality chromosomes (band level above 600) or by BAC-based fluorescence *in situ* hybridisation (FISH) analysis.[7]

Array-CGH

For array-CGH, a BAC/PAC clone set consisting of approximately 3500 clones with an average interclonal distance of 1 Mb was obtained from the Wellcome Trust Sanger Institute (UK).[17] Production of genomic 1 Mb arrays based on the clone set was done by the Norwegian Microarray Consortium (NMC; www.microarray.no) at the RR-HF/UiO Microarray Core Facility in Oslo, Norway. Arrays were produced as described previously,[18] but tile path resolution on chromosome 1q was not included in our arrays. Each clone is represented by four DNA spots on the array. Array-CGH was performed as described previously.[18] Briefly, patient DNA and normal reference DNA (500 ng each) were labelled with Cy3-dCTP and Cy5-dCTP (NEN Life Science Products), respectively. Commercial reference DNA described above was used (Promega). The labelled DNAs together with 135 μg of human Cot-1 DNA (Invitrogen) were hybridised onto the array. Sex-matched hybridisations were carried out. Arrays were scanned in an Agilent G2565B scanner (Agilent Technologies, USA), and images were analysed using GenePix Pro 5.0 software (Molecular Devices Corp., USA). Further data processing, including normalisation and filtering, was performed using the M-CGH software.[19] In brief, spots with intensities lower than the background, or signal intensities below local background plus twice the standard deviation (SD) of the background, or replicates with SD > 0.2, were excluded from the analysis. Classification (gains, normals, and losses) and calculation of the log 2 ratio thresholds for the amplicons were computed by the M-CGH software.[19] BAC clone information was based on the Ensembl database v44 (www.ensembl.org), NCBI build 36.2.

Determination of the size of a deletion or duplication

The 1 Mb distance between the BAC clones is an average. The real distance varies depending on chromosomal location, and a breakpoint may also be in a region partly

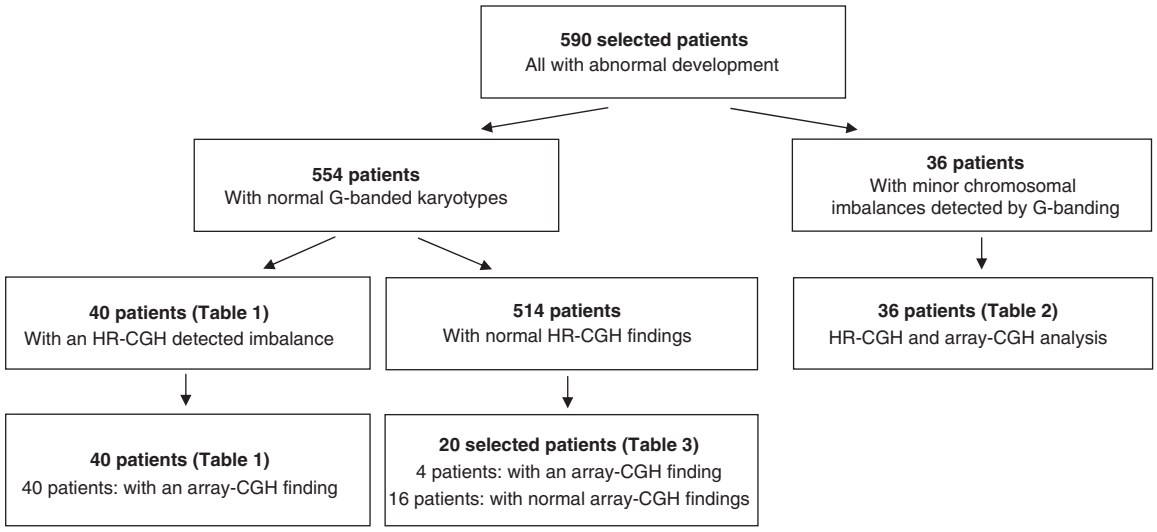

Figure 1 Study layout.

covered by an abnormal clone. Therefore, the sizes of the deletions and duplications (Tables 1–3) are given both as the minimum and maximum sizes. The terminal clones of the imbalance define the minimum size and the position of the imbalance, and the normal flanking clones define the maximum size. The end coordinates of the clones were used for size determination. Aberration calls were based on two or more consecutive clones.

Results

In our sample of 590 patients with abnormal development, which has been screened for minor or cryptic chromosomal imbalances by G-banding and HR-CGH, we applied array-CGH on a subset of 96 patients, using 1 Mb resolution BAC clone arrays. The patients' phenotypes are summarised in Tables 1–3. The specificity of the 1 Mb BAC arrays was tested in advance by running a total of 10 normal-versus-normal hybridisations with DNA from normal male or female blood donors (data not shown). A low level of background noise, that is, clones variably showing a signal intensity ratio outside the limits defined for normal DNA copy numbers, was detected. On average, 15 non-consecutive single clones (0.4%), behaving differently from experiment to experiment in the normal-versus-normal hybridisations, were detected. To test the sensitivity and reproducibility of the array slides, DNA samples from two patients with deletions of known size after FISH mapping (case 6 in Table 1 and case 69 in Table 2) were run for analysis four times each. The genomic ratio profiles of the four repeated experiments in both cases were consistent (data not shown).

Detection of cryptic chromosomal imbalances and occult trisomy mosaicisms

Among the 590 patients described above, 36 had a small visible chromosome aberration detected by G-banding and 554 patients had a normal karyotype. When genomic BAC arrays became available from the NMC, 1 Mb array-CGH analysis was used for fine mapping of all HR-CGH findings and for examination of selected patients with normal findings on both G-banding and HR-CGH. In the group of 554 patients having a normal karyotype, HR-CGH analysis detected a genomic imbalance in 40 of them (7.2%; Table 1). Of note, four patients had occult mosaicism: three for trisomy 9 and one for trisomy 14. The percentage of mosaicism in these patients (cases 25, 26, 27 and 33; Table 1) was determined by interphase FISH to be 21, 23, 15 and 36%, respectively.

Among the 40 patients, all imbalances were known to be *de novo* aberrations except cases 5, 11 and 20 (parent translocation carrier) and case 32 (unknown). Of the 40 patients, 72% (29/40) had deletions, 10% (4/40) had occult trisomy mosaicisms, 10% (4/40) had cryptic translocations and 8% (3/40) had duplications (Table 1). The mean minimum size of an HR-CGH-detected isolated deletion was 6.0 Mb (Table 1), whereas the mean maximum size was 8.5 Mb. For the three isolated duplications found (Table 1), the mean minimum size was 5.6 Mb and the mean maximum size was 8.9 Mb. The theoretical size maximum of the smallest duplication detected (case 12 in Table 1) was 5.3 Mb.

In the group of 36 patients with small chromosome aberrations detected by G-banding, HR-CGH and array-CGH were applied for better characterisation (Table 2). Among these patients, all imbalances were known to be *de novo* aberrations except cases 62 (parent translocation carrier) and 69 (familial inversion). Of the 36 patients, 13 had deletions, 12 had duplications and 11 had translocations/complex single-chromosome rearrangements. Thus, in the group of 40 patients with normal G-banded karyotypes (Table 1), a deletion (29/40) was a 10 times

Table 1 Array-CGH fine mapping of chromosomal imbalances detected by HR-CGH, but not by routine G-banding

No.	*Clinical information*						*CGH result*	*Position*	*Size (Mb)*
	YoB	*MR*	*GR*	*DF*	*BP*	*Other features*	*HR-CGH/array-CGH*	*Aberration and terminal aberrant clones (Mb from pter)*	*Minimum/ maximum*
1	−05	+	+	+	?	Cleft palate, small VSD, hydrocele testis	dim(1)(p36.22pter)	1p del: 0–9.3	9.3/10.0
2	−97	1	+	1	++	ADHD, poor language development	dim(1)(p31.1p31.2)	1p del: 66.7–76.7	10.0/14.6
3	−95	2	+	1	+	Atactic gait, autistic traits, paresis of left nervus abducens	dim(2)(q32.3q33.1)	2q del: 193.0–202.3	9.3/12.0
4	−86	2	–	3	++	Autistic with destructive behaviour, tetralogy of Fallot	dim(2)(q37.3qter)	2q del: 237.1–242.8	5.7/6.5
5	−97	2	+	2	+	VSD, CLP, microcephaly, low hairline	enh(2)(q37.2qter) dim(12)(p13.32pter)	2q dup: 236.1–242.8 12p del: 0–4.3	6.7/8.0 4.3/5.2
6	−92	1	–	2	–	Adipositas (PWS-like), cutis marmorata, microcephaly	dim(3)(p21.31p22.1)	3p del: 41.2–45.3	4.1/6.3
7	−02	1	–	2	–	Overgrowth, hydrocephalus, cryptorchism, 4p− like facies	dim(3)(p21.31p21.31)	3p del: 47.9–50.0	2.1/5.3
8	−05	?	?	?	?	No clinical information received	dim(3)(q22.3q24)	3q del: 137.8–149.7	11.9/14.4
9	−04	2	–	1	–	Long slender hands/feet, large nose and mouth	dim(3)(q24q25.33)	3q del: 150.3–161.3	11.0/12.5
10[a]	−90	3	+	2	+	Autistic features, ASD	dim(4)(q21.21q21.22)	4q del: 81.3–83.4	2.1/8.1
11	−94	3	+	2	–	Dystrophic nails, short fifth finger, no malformations	dim(4)(q33qter) enh(9)(p22.1pter)	4q del: 172.0–191.3 9p dup: 0–19.4	19.3/23.2 19.4/20.2
12	−02	+	–	1	–	Large mouth, hypertelorism	enh(5)(q13.2q13.2)	5q dup: 69.7–71.6	1.9/5.3
13	−01	+	–	2	–	Occipital encephalocele, epicanthus inversus	dim(5)(q35.1qter)	5q dup: 171.0–181.0	10.0/11.2
14	−96	+	–	1	–	Deep-set eyes, hypotelorism	dim(6)(p22.3p24.1)	6p del: 13.3–17.8	4.5/6.5
15[a]	−88	3	+	2	–	Autistic features, ASD, prognatia, midface hypoplasia	dim(6)(q14.3q15)	6q del: 85.0–90.4	5.4/10.5
16	−01	3	+	1	–	GR after birth, delayed myelinisation, epilepsy	dim(6)(q26qter)	6q del: 163.4–170.9	7.5/8.0
17	−90	3	+	3	–	Epilepsy, angiomatous nevi, bifid thumbs and first toes, small penis	dim(7)(p13p14.1)	7p del: 39.3–45.9	6.6/9.0
18	−96	+	++	2	–	Craniosynostosis, cleft palate, hearing loss, dysplasic hips	dim(7)(q21.13q21.3)	7q del: 90.3–97.2	6.9/9.9
19	−02	2	+	0	–	Microcoria, anterior synechiae, ocular apraxia, relative macrocephaly.	dim(7)(q22.1q31.1)	7q del: 102.3–103.8	1.5/4.3
20	−97	1	–	2	–	SGA, brachydactyly type 1C, social but uncritical behaviour	dim(8)(p23.1pter) enh(19)(q13.42qter)	8p del: 0–8.2 19q dup: 59.0–63.8	8.2/9.8 4.8/6.0
21	−01	+	+	1	–	Delayed language development	dim(8)(p23.1pter)	8p del: 0–8.7	8.7/9.8
22	−89	1	+	0	–	ASD primum, VSD, jejunal atresia, microcephaly	dim(8)(p23.1p23.1)	8p del: 8.0–11.8	3.8/7.6
23	−89	2	–	1	–	Spherocytosis, bilateral CLP, hypogonadotropic hypogonadism	dim(8)(p11.21p12)	8p del: 36.3–42.7	6.4/7.7
24	−03	+	?	2	?	Brachycephaly, hypotonia	dim(8)(q21.13q21.13)	8q del: 80.7–82.7	2.0/5.5
25	−96	3	++	2	–	SGA, ASD, hip dysplasia, club foot, micrognatia, body asymmetry	mos +9	mos +9	
26	−93	3	+	2	–	SGA, tracheomalacia, hip dysplasia, large chin	mos +9	mos +9	
27	−93	+	?	+	–	Micrognatia	mos +9	mos +9	
28	−04	+	–	1	–	Cryptorchism, left double kidneys	dim(9)(p24.1p24.3)	9p del: 2.1–7.4	5.3/7.6
29	−01	+	–	2	–	Pulmonary vein anomaly, cryptorchism, frontal bossing	dim(10)(p11.23q12.1)	10p del: 26.8–31.1	4.3/5.5
30	−04	2	–	1	–	Fallot tetralogy, microcephaly, marked eyebrows, hypotelorism.	dim(10)(q26.11q26.12)	10q del: 120.4–122.6	2.2/4.4
31	−95	2	+	2	–	Microcephaly, micrognatia, pointed chin, hearing loss	dim(10)(q26.13qter)	10q del: 125.0–135.4	10.4/11.1
32	−04	+	?	+	?	Frontal bossing, large anterior fontanel, deep-set eyes	dim(11)(q25qter) enh(17)(q25.1qter)	11q del: 132.2–134.5 17q dup: 69.8–78.3	2.3/6.5 8.5/12.1

Table 1 (*Continued*)

No.	Clinical information						CGH result	Position	Size (Mb)
	YoB	*MR*	*GR*	*DF*	*BP*	*Other features*	*HR-CGH/array-CGH*	*Aberration and terminal aberrant clones (Mb from pter)*	*Minimum/maximum*
33	−00	+	++	3	?	Complex heart malformation, duodenal atresia	mos +14 (ratio around 1.25)	mos +14	
34	−95	+	+	?	?	CLP, athetosis, dyskinesias, many pneumonias	dim(14)(q13q21.1)	14q del: 35.6–40.6	5.0/9.0
35	−99	1	++	2	–	SGA, normal HC, delayed bone age, late dentition	dim(15)(q25.2q26.1)	15q del: 79.7–87.5	7.8/9.7
36	−01	1	+	1	–	Feeding difficulties first year, normal HC	dim(16)(p12.1p12.1)	16p del: 21.9–26.3	4.4/8.1
37	−99	3	–	1	–	Delayed myelinisation, corpus callosum agenesia	dim(18)(q21.2q21.31)	18q del: 49.8–52.9	3.1/4.9
38	−01	+	–	?	–	SGA, ASD, lung hypoplasia, renal cysts, hypospadias	dim(20)(p12.2p13)	20p del: 3.5–10.6	7.1/9.3
39	−82	+	+	+	–	ASD, NF type II, choanal atresia, CHARGE-like patient	dim(22)(q11.23q12.2)	22q del: 23.8–29.9	6.1/7.4
40	−54	+	–	1	+	Man with large habitus, HC 59 cm, prognatia, self-mutilator	enh(X)(p21.2p22.11)	Xp dup: 24.3–29.3	5.0/10.1

YoB = year of birth; MR = mental retardation: + present, 1 mild, 2 moderate and 3 severe/profound; GR = growth retardation: − normal range, + growth retarded and ++ severely growth retarded; DF = dysmorphic features: + present, 0 not reported, 1 mild, 2 moderate and 3 severe; BP = behavioural problems; MCA = multiple congenital anomalies; HC = head circumference; CLP = cleft lip and palate; SGA = small for gestational age; ND = neonatal death.
All imbalances were *de novo* except in cases 5, 11, 20 and 32.
[a]Case previously described by Ness-GO *et al*. *Am J Med Genet* 2002; **113**:125–136.

more common finding than duplication (3/40), whereas in the group of 36 patients with aberrations detectable by G-banding (Table 2), small deletions and duplications were equally common findings. In Table 1, all six non-mosaic patients known to have severe mental retardation had deletions (mean minimum size 7.3 Mb). In contrast, in Table 2, all six patients known to have severe mental retardation had duplications (mean minimum size = 15.7 Mb).

In Figure 2, the location and size of all deletions and duplications are shown apart from the four cases with occult trisomy mosaicism. A total of 91% of the imbalances were *de novo*. Except for two patients (case 69; Table 2 and case 80; Table 3), all single-chromosome imbalances were *de novo* (Figure 2). Imbalances were found on all chromosomes. The size and number of imbalances varied greatly between chromosomes, and the majority of the imbalances were found on chromosomes 2–10, 13 and 14. When only the interstitial chromosome rearrangements were counted, the imbalances were more equally spread on all chromosomes.

As expected, the array-CGH results showed that the plots made by the HR-CGH software could be misleading. In 3 of the 40 patients with cryptic imbalances (Table 1), HR-CGH indicated the position of a deletion or duplication that missed its true position: The 5q13 duplication in case 12, the subterminal 9p deletion in case 28, and the 10q26 deletion in case 30. In case 58 (Table 2), the HR-CGH finding was both incorrect and incomplete. Here, a terminal deletion combined with a subterminal duplication on 7q was only shown as a subterminal 7q duplication by the HR-CGH software. The finding of a terminal deletion combined with a subterminal duplication suggests that the mechanism might have been a breakage–fusion–bridge cycle after 7q arm fusion.[20] In two patients with small but visible imbalances (Table 2), the HR-CGH software excluded the subtelomeric regions from the imbalance,[16] for example, case 72, where a terminal 19q duplication that appeared to be interstitial (Figure 3a) and, case 74, where the HR-CGH software suppressed the terminal part of a 22q13 deletion. This demonstrates that HR-CGH software may overlook even visible subtelomeric imbalances.

Array-CGH on a subset of patients with normal karyotype and HR-CGH

Twenty patients with a phenotype strongly suggesting a chromosomal abnormality despite normal findings on both G-banding and HR-CGH were selected for 1 Mb array-CGH. In four of them (20%), a chromosomal imbalance was found: three deletions (mean minimum size 3.2 Mb, range 3.1–3.3 Mb) and one duplication (minimum size 8.7 Mb) (Table 3). All findings were verified by array-CGH analysis on CytoChip-arrays (BlueGnome, UK), a commercial BAC array with 1 Mb resolution (data not

Table 2 Array-CGH fine-mapping of chromosomal aberrations detected by routine G-banding

No	*Clinical information*						*Karyotype*	*Position*	*Size (Mb)*
	YoB	*MR*	*GR*	*DF*	*BP*	*Other features*	*Refined karyotype after mapping by array-CGH*	*Aberration and terminal aberrant clones (Mb from pter)*	*Minimum/maximum*
41[a]	−00	+	+	3	?	MCA, CLP, ND	46,XY,der(5)t(1;5)(q42.13;p14.1)	1q dup: 225.9–247.2	21.3/21.9
								5p del: 0–27.6	27.6/28.7
42	−01	2	–	2	?	Hydrocephalus, optic atrophy	46,XY,der(Y)t(Y;2)(p11.2;p24.1)	Yp del: n.d.	
								2p dup: 0–20.2	20.2/21.3
43[a]	−01	+	+	1	?	Frontal bossing	46,XX,dup(2)(p14p21)	2p dup: 42.4–65.8	23.4/25.9
44[a]	−87	2	–	1	–	Anteverted nostrils	46,XY,der(3)del(3)(p26.1) dup(3)(q26.31qter)	3p del: 0–5.2	5.2/7.1
								3q dup: 173.2–199.5	26.3/28.6
45	−01	2	–	1	+	Growth >97.5 centile, autistic, ADHD	46,XX,der(4)dup(4p)ins(4)(q35p16.2p16.3)	4p dup: 0–5.3	5.3/6.8
46	−04	2	–	2	?	ASD, VSD, hip dysplasia, scoliosis	46,XX,der(4)del(4)(q32.3) ins(4;4)(q32.3p16.3pter)	4q del: 166.3–191.0	24.7/26.3
								4p dup: n.d.	
47	−65	+	–	+	–	Full lips	46,XX,del(5)(p15.1)	5q del: 0–15.3	15.3/16.4
48	−78	2	+	2	–	Choanal atresia, large HC (97.5 centile)	46,XX,del(5)(q11.2q13.1)	5q del: 54.0–67.6	13.6/16.2
49	−04	2	+	0	–	Deaf (cochlea implant installed), epilepsy	46,XY,del(5)(q12q13.2)	5q del: 60.0–71.3	11.3/13.5
50	−87	3	++	2	–	Hypospadias, hypotelorism	46,XY,dup(5)(q14.3q22.3)	5q dup: 86.3–113.4	27.1/31.7
51	−97	+	?	2	?	Choanal atresia, club feet	46,XX,dup(6)(p21.3p12.3)	6p dup: 37.1–49.8	12.7/13.9
52	−48	+	?	2	?	Severe myopia	46,XY,dup(6)(p21.31p22.1)	6p dup: 28.2–34.0	5.8/7.4
53	−02	1	–	1	–	Microcephaly, no malformations	46,XX,der(6)inv(6)(p12q16) del(6)(q16.3q21)	6p del: 105.1–111.8	6.7/8.0
54	−95	2	–	1	–	Diaphragmatic hernia	46,XX,der(1)t(1;7)(q44;p21.1)	1q del: n.d.	
								7p dup: 0–15.9	15.9/17.8
55	−97	2	–	1	–	Pectus excavatum, flat midface, webbed neck	46,XX,dup(7)(p15.1p21.1)	7p dup: 19.2–29.4	10.2/13.4
56	−04	+	?	?	?	Lack further information	46,XY,der(8)t(7;8)(q31;p23)	7q dup: 123.7–159.0	35.3/36.3
								8p del: 0–6.0	6.0/6.7
57	−92	3	+	3	++	TRPS-like face, no malformations	46,XX,dup(7)(q33q34)	7q dup: 133.6–142.1	8.5/11.6
58	−92	3	++	3	–	Ptosis, blepharophimosis, central upper incisor	46,XX,der(7)dup(7)(q35q36.1) del(7)(q36.2qter)	7q dup: 144.2–152.2	8.0/12.0
								7q del: 154.3–158.8	4.5/6.6
59	−95	+	–	?	?	Prone to infections, excessive drooling	mos47,XX,+der(8) del(8)(p12)[26] /46,XX[4]	8 dup: 42.5–146.3	103.8/104.2
60	−90	1	–	1	–	Cryptorchism (dxt)	46,XY,del(8)(p12p21.1)	8p del: 30.8–32.7	1.9/7.9
61[a]	−00	+	?	1	?	ASD, feeding problems	46,XY,der(6)t(6;9)(q27;p21)	6q del: n.d.	
								9p dup: 0–26.7	26.7/29.4
62	−03	3	?	2	?	Dandy–Walker, no hydrocephalus	46,XY,+der(14)t(9;14)(p23;q21)	9p dup: 0–11.3	11.3/12.9
								14q dup: 15.5–41.5	26.0/26.5
63[a]	−96	2	+	2	–	Club feet, dysplastic hips, epilepsy	46,XX,t(10;11)(q21.1;q22.3)	10q del: 60.1–65.9	5.8/10.4
								11q del:104.9–108.4	3.5/7.2
64	−97	+	+	+	+	Autistic traits	46,XY,del(10)(q26.13)	10q del: 126.0–135.4	9.4/10.3
65	−83	3	–	3	–	Severe coloboma, Pierre Robin sequence	46,XY,dup(12)(q14.2q21.32)	12q dup: 62.8–85.7	22.9/29.4
66	−05	+	?	2	?	Ventrally placed anus, obstipation	46,XY,del(13)(q33.3)	13q del: 109.3–114.0	4.7/7.4
67	−02	2	+	2	–	Metachromatic leukodystrophy (unrelated)	del(13)(q21.1q31.1)	13q del: 56.5–80.8	24.3/27.2
68	−95	2	++	3	–	AVSD, right-sided hernia inguinalis	46,XY,t(2;4)(p21;q33) *de novo*, del(13)(q14q22)	13q del: 59.7–90.5	30.8/33.5
69	−63	1	–	0	–	Hereditary spherocytosis	46,XX,inv(14)(q23.2q23.3)	14q del: 63.4–65.1	1.7/3.7
70	−97	+	–	?	+	Epilepsy, obesity, normal MRI	47,XX,+der(15)del(15)(q15)	15q dup: 17.0–27.6	10.6/11.2
71	−97	1	+	1	+	PDA, hernia inguinalis, ADHD	46,XY,dup(18)(p11.21p11.32), inv(20)(p13q11) *de novo*	18p dup: 1.7–11.9	10.2/14.9

Table 2 (*Continued*)

No	Clinical information						Karyotype	Position	Size (Mb)
	YoB	MR	GR	DF	BP	Other features	Refined karyotype after mapping by array-CGH	Aberration and terminal aberrant clones (Mb from pter)	Minimum/maximum
72	−93	3	?	+	?	Epilepsy	46,XY,der(6)t(6;19)(q27;q13.41)	19q dup: 57.5–63.8	6.3/7.3
73	−83	0	–	0	–	Poor balance, ataxia, learning difficulties	46,XX,del(21)(q11q21)	21q del: 14.6–24.6	10.0/11.9
74	−99	+	+	2	–	Thin corpus callosum, poor myelinisation	46,XY,del(22)(q13.2)	22q del: 41.5–49.6	8.1/8.8
75	−01	+	–	0	–	Hearing loss	46,XY,del(22)(q13.31)	22q del: 45.6–49.6	4.0/5.1
76	−84	0	+	–	–	Turner-like	46,X,der(X)del(X)(p11.3) dup(X)(q24qter)	Xp del: 0–39.9 Xq dup: 122.2–154.9	39.9/40.8 32.7/35.3

YoB = year of birth; MR = mental retardation: + present, 1 mild, 2 moderate and 3 severe/profound; GR = growth retardation: – normal range, + growth retarded and ++ severely growth retarded; DF = dysmorphic features: + present, 0 not reported, 1 mild, 2 moderate and 3 severe; BP = behavioural problems; MCA = multiple congenital anomalies; HC = head circumference; CLP = cleft lip and palate; ND = neonatal death; n.d. = not detected.
All array-CGH imbalances, except case 69, could also be detected by HR-CGH. All imbalances were *de novo* except cases 62 and 69.
[a]Case previously described by Ness-GO *et al*. *Am J Med Genet* 2002; **113**:125–136.

shown). Among the four patients, three imbalances were known to be *de novo*, whereas the aberration in case 80 was caused by a parental between-arm insertion. Even though the deletion on chromosome 14 in case 79 is close to the telomere, it would not have been detected by subtelomere-FISH screening (Figure 3b). In case 77, the finding of a deletion involving the *ZFHX1B* gene was expected on clinical grounds because the patient had Mowat–Wilson syndrome, known to be caused by haploinsufficiency of this gene.[21] This finding was also verified by a BAC-FISH probe against the *ZFHX1B* locus. None of the deletions were picked up by HR-CGH analysis, even at lower confidence intervals, but the duplication in case 80 was seen when the confidence intervals were set at 95%. However, the amount of noise on the HR-CGH profiles at such low confidence intervals makes analysis at this level of sensitivity unfeasible.

Discussion

The main purpose of this study was fine mapping for improved genotype–phenotype information on a well-characterised group of patients with minor and cryptic chromosomal imbalances diagnosed during a 6-year period. In addition, the performance of the two CGH techniques could be compared.

In patients with normal karyotypes and a combination of mental retardation and dysmorphic signs and/or malformations, the detection rate of HR-CGH was 7.2%. Our results are comparable to a group of 332 patients from five different European studies, where the 1 Mb array-CGH diagnostic detection rate, all data combined, was 8.4%.[10] This does not indicate that array-CGH is only slightly better in detecting clinically relevant genomic imbalances than HR-CGH. The yield of different diagnostic approaches is dependent on patient ascertainment, although the influence of patient selection on the detection rate might be smaller than previously thought.[22] In the five European studies summarised above, the 1 Mb array-CGH diagnostic detection rates varied from 8%[10] to 16%.[23] Of note, some of the patients in these studies had been pre-screened for subtelomeric imbalances, and elimination of these ten patients from our cohort would have reduced our diagnostic detection rate to 5.4%. In addition, our results show that HR-CGH may overlook even visible subtelomeric imbalances. Therefore, HR-CGH should be combined with conventional G-banding whenever feasible. In a subgroup of 20 patients with normal findings on G-banding and HR-CGH but with a strong clinical suspicion of a genomic imbalance, four patients (20%) had a genomic imbalance. The sample is too small to conclude if this high detection rate is a random finding or due to skilled patient selection.

Table 3 Chromosomal imbalances detected by array-CGH only

No.	*Clinical information*						*Array-CGH result*	*Position*	*Size (Mb)*
	YoB	*MR*	*GR*	*DF*	*BP*	*Other features*	*HR-CGH/array-CGH*	*Aberration and terminal aberrant clones (Mb from pter)*	*Minimum/maximum*
77	−01	+	+	+	−	Mowat–Wilson syndrome, died unexpectedly when 2 years old	dim(2)(q22.2q22.3)	2q del: 141.6–144.7	3.1/6.9
78	−52	2	+	2	?	Micrognatia	dim(2)(q23.3q24.1)	2q del: 153.5–156.6	3.1/4.5
79	−95	3	−	3	−	Micropenis, turricephaly, craniosynostosis	dim(14)(q32.2q32.32)	14q del: 99.1–102.4	3.3/5.2
80	−03	+	+	2	−	SGA, hip dysplasia, lacks several phalanges	enh(19)(p13.2p13.3)	19p dup: 1.0–9.7	8.7/10.3

YoB = year of birth; MR = mental retardation: + present, 1 mild, 2 moderate and 3 severe/profound; GR = growth retardation: − normal range, + growth retarded and ++ severely growth retarded; DF = dysmorphic features: + present, 0 not reported, 1 mild, 2 moderate and 3 severe; BP = behavioural problems.
Findings were observed in 4 out of 20 cases highly suspicious of having a chromosomal aberration. All imbalances were *de novo* except the one found in case 80.

Among the 80 patients with genomic imbalances, findings were on all chromosomes, in agreement with other 1 Mb array-CGH studies.[10] The largest numbers of aberrations, with deletions being as common as duplications, were seen on chromosomes 7 and 8. On chromosome 19, our most gene-dense chromosome, only duplications were found. Another interesting observation was that minor but visible duplications were as common as deletions, whereas cryptic deletions were 10 times more common than cryptic duplications. One explanation may be that deletions are easier to detect than duplications because the actual change in DNA amount is 100% for deletions and 50% for duplications. It is also possible that small duplications are less likely to cause a phenotype that makes the patient look 'chromosomal', that is, reduces the chance of ascertainment.

Although useful, it is difficult to evaluate the clinical significance of deletions and duplications based only on size, especially until we have a more extensive and in-depth knowledge about copy number variations (CNVs) in the human genome. The number of microdeletion syndromes that are solely or largely caused by the absence of one gene among many is increasing (eg *EHMT1* in 9q34 deletions,[24] *RAI1* in Smith–Magenis syndrome,[25] and *LIS1* in Miller–Dieker syndrome). Therefore, parental DNAs should be available to verify that a finding is *de novo*. Using different types of arrays, others have found that CNVs occur probably in all healthy individuals.[26–28] It is not yet clear how much of the human genome that is quite tolerant to CNV, but it appears to be at least 12%.[28] Eventually, we will know the identity of most genes that cannot be duplicated or deleted without affecting normal development or brain function.

Looking at individual patients with interesting phenotypes, it is noted that in case 3, an ~9.3 Mb deletion of 2q32.3–2q33.1 was associated with Duane anomaly, autistic traits and ataxia. This deletion on chromosome 2 is ~5 Mb distal to the published locus for Duane retraction syndrome type 2 (DURS2), mapped by linkage analysis to 2q31.[29] Maybe the deletion is associated with an inversion disrupting the DURS2 gene. In case 4 with severe autism and self-destructive behaviour, a terminal deletion on 2q was found, a region previously found to be deleted in autistic patients.[30] A patient with a similar-sized terminal 2q deletion who had severe behavioural disturbance was recently described.[31] The deletion in case 17, where bifid thumbs and bifid first toes were found in addition to epilepsy and a small penis, includes the *GLI3* gene. Deletions or mutations in this gene are associated with Greig cephalopolysyndactyly (OMIM 175700) and Pallister–Hall syndromes (OMIM 146510), and epilepsy and a small penis are also features of the latter. The small deletion of 7q22.1–7q31.1 in case 19 includes reelin (*RELN*), a gene that may be mutated in Norman–Roberts-type lissencephaly (OMIM 257320) and thought to be associated with autism

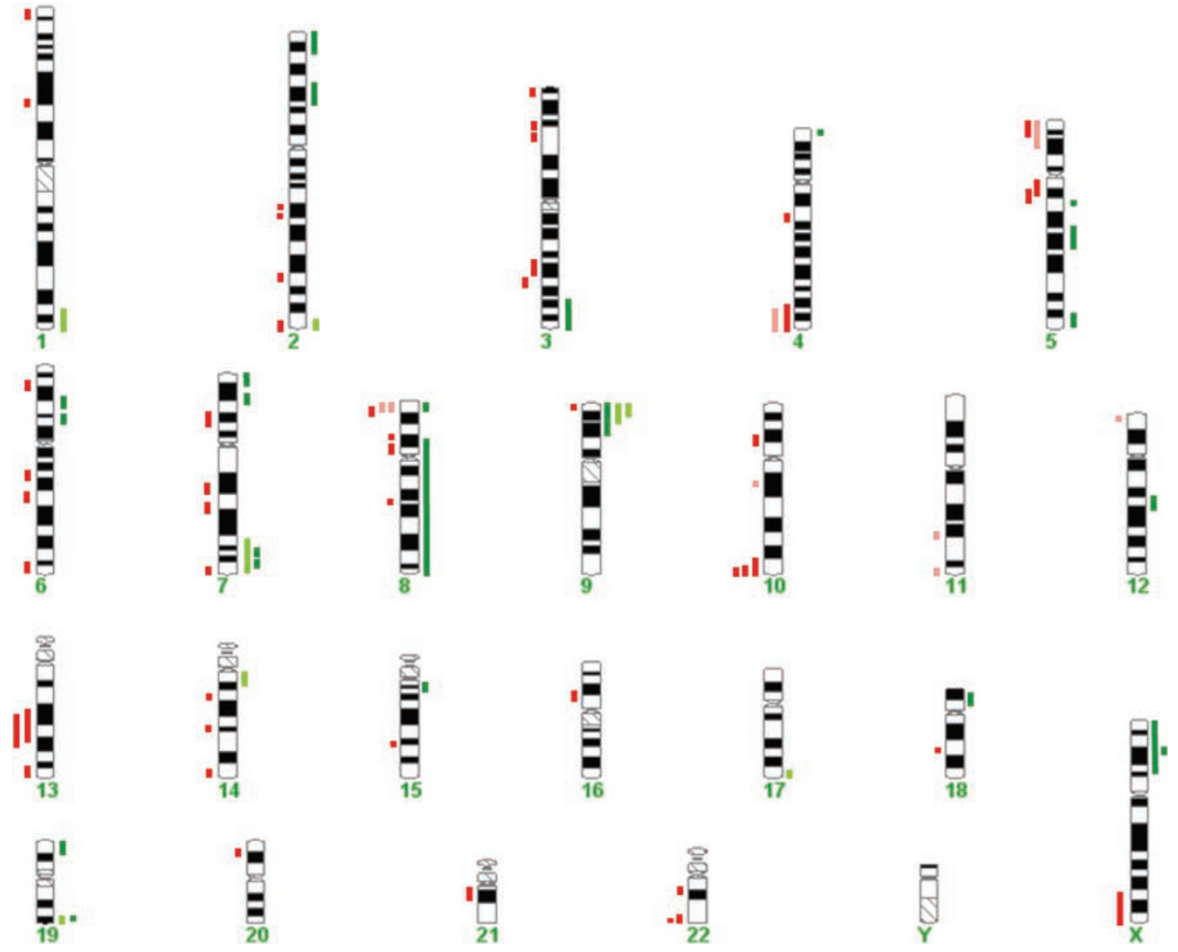

Figure 2 Overview of chromosomal imbalances mapped by 1 Mb array-CGH: 400-band chromosome ideograms are shown with duplications drawn as green lines and deletions as red lines. Imbalances caused by translocations are shown in a lighter colour.

and schizophrenia. This patient is growth retarded (length just below the 2.5 centile) but has a head circumference in the upper normal range (75–90 centile). Anterior synechiae and microcoria are peculiar features. In case 48, an ~13.6 Mb deletion of 5q11.2–5q13.1 was associated with choanal atresia, macrocephaly and pronounced dysmorphism. In case 49, a similar-sized (~11.3 Mb) but ~6 Mb more distal deletion (5q12–5q13.2) was not associated with dysmorphic features but congenital deafness. The deletions overlap with ~8 Mb. No dominant deafness genes or genes associated with choanal atresia or macrocephaly are known to be located in the interval. One gene with high expression in the central nervous system (CNS) that is located in the 3–4 Mb region deleted in case 49 but not in case 48 is *MAP1B* (microtubule-associated protein 1B). Heterozygosity for an insertional knockout in this gene leads to severe CNS affection in mice.[32] Finally, case 33 (Table 1), with occult mosaicism for trisomy 14, deserves to be mentioned, as this is a rare type of trisomy mosaicism.[33] It illustrates that such mosaicism, similar to our three patients with mosaic trisomy 9 (cases 25–27; Table 1), can be detected by CGH using blood sample DNA even though metaphase studies of blood lymphocyte cultures are completely normal.[10,34] The lowest HR-CGH-detected mosaicism grade was 15%, and this was found in a patient with trisomy 9 (case 27). Previously, mosaicism grade as low as 8% has been detected by 1 Mb array-CGH analysis.[10]

This work adds to our knowledge on minor and cryptic chromosome imbalances that cause varying degrees of mental impairment, malformations and/or dysmorphism. Mapping of genomic imbalances having distinct phenotypes may be useful not only for evaluation of the clinical importance of a *de novo* imbalance, but also for narrowing down regions of particular interest when searching for genes whose dosage is critical for normal development.

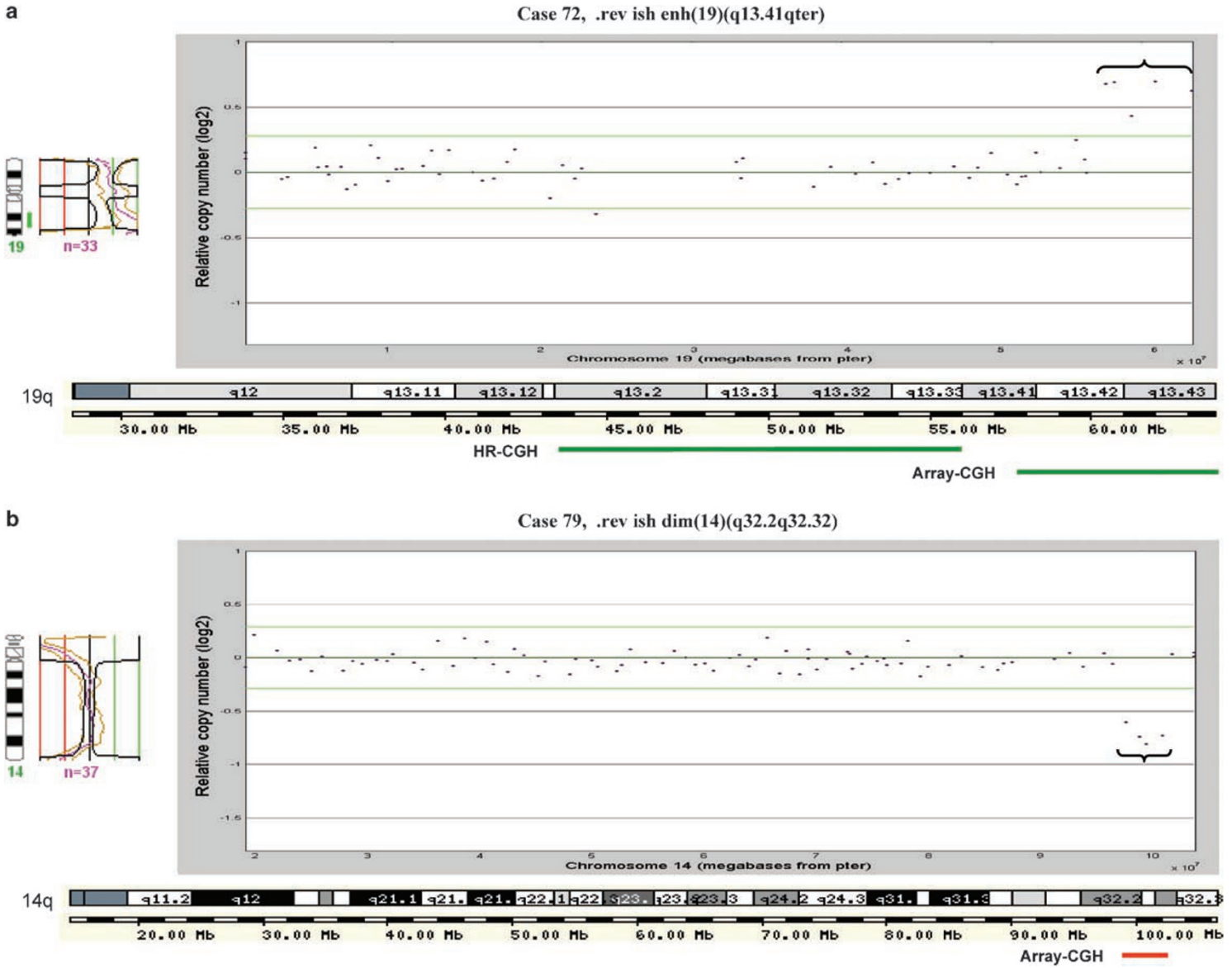

Figure 3 Inaccurate mapping of a chromosomal imbalance by HR-CGH (**a**) and array-CGH detection of a chromosomal imbalance in a case with normal findings on G-banding and HR-CGH (**b**): the 99.5% confidence interval HR-CGH ratio profiles together with the array-CGH profiles of case 72 (Table 2) and case 79 (Table 3) are shown. The imbalances mapped by HR-CGH and array-CGH analyses are shown as green (gains) or red (losses) bars below an 850-band ideogram. (**a**) HR-CGH of case 72 indicated a non-terminal 19q duplication from 19q13.2 to 19q13.3, whereas array-CGH showed a terminal duplication (dup(19)(q13.41qter)). (**b**) Normal findings by HR-CGH of case 79, whereas array-CGH detected a 14q deletion (del(14)(q32.2q32.32)).

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