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Chapter 3

Array-CGH detection of micro rearrangements in mentally retarded individuals: clinical significance of imbalances present both in affected children and normal parents.

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Array-CGH detection of micro rearrangements in mentally retarded individuals: clinical significance of imbalances present both in affected children and normal parents

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ABSTRACT

Background: The underlying causes of mental retardation remain unknown in about half the cases. Recent array-CGH studies demonstrated cryptic imbalances in about 25% of patients previously thought to be chromosomally normal.
Objective and methods: Array-CGH with approximately 3500 large insert clones spaced at ~1 Mb intervals was used to investigate DNA copy number changes in 81 mentally impaired individuals.
Results: Imbalances never observed in control chromosomes were detected in 20 patients (25%): seven were de novo, nine were inherited, and four could not have their origin determined. Six other alterations detected by array were disregarded because they were shown by FISH either to hybridise to both homologues similarly in a presumptive deletion (one case) or to involve clones that hybridised to multiple sites (five cases). All de novo imbalances were assumed to be causally related to the abnormal phenotypes. Among the others, a causal relation between the rearrangements and an aberrant phenotype could be inferred in six cases, including two imbalances of the X chromosome, where the associated clinical features segregated as X linked recessive traits.
Conclusions: In all, 13 of 81 patients (16%) were found to have chromosomal imbalances probably related to their clinical features. The clinical significance of the seven remaining imbalances remains unclear. The limited ability to differentiate between inherited copy number variations which cause abnormal phenotypes and rare variants unrelated to clinical alterations currently constitutes a limitation in the use of CGH-microarray for guiding genetic counselling.

Since karyotyping became a routine technique in clinical genetics, mental impairment, with or without other abnormalities, has often been found to be associated with chromosome rearrangements. However, in the majority of the patients, the G banded karyotype is normal, and in about half of them no obvious cause for the impairment is found (reviewed by Flint and Knight[1]). More recently, cryptic chromosome rearrangements have been reported in patients with an apparently normal karyotype and an unexplained abnormal phenotype. The best characterised rearrangements are the recurrent microdeletion syndromes, such as the Miller-Dieker lissencephaly (MIM 247200) and DiGeorge syndrome (MIM 188400). In addition, subtelomeric imbalances of variable sizes cause mental retardation in 5–7% of these cytogenetically "normal" cases.[1-3] In the last few years, genomic array (array-CGH) analysis has become available,[4,5] and appears to be a robust tool for detecting genomic imbalances in patients, with a much higher resolution than permitted by cytogenetic analyses based on chromosome banding (4–10 Mb).

Two recent studies using array-CGH with markers spaced on average at 1 Mb intervals across the genome have shown that about 25% of the patients with mental impairment associated with dysmorphisms and an apparently normal karyotype carried deletions or duplications below the level of resolution of classical cytogenetics.[6,7] About half the reported cases were de novo, and it is a reasonable assumption that the abnormal phenotype is causally associated with these imbalances. In the inherited cases, however, neither study succeeded in distinguishing between a true pathological or a chance association between copy number changes and abnormal phenotypes.

The precise contribution of microrearrangements to abnormal phenotypes has not been yet established, and in the ~1 Mb arrays used in these studies, imbalances smaller than 1 Mb would often be missed. Whatever the precise figure is, it appears large enough (>5%) to affect genetic counselling. The identification of imbalances in such families can lead to the detection of carriers and to prenatal diagnosis being offered.

Here, we report an array-CGH investigation of 81 patients with mental impairment accompanied by facial dysmorphisms and other congenital abnormalities. The significance of these findings and implication for genetic counselling are discussed.

METHODS

Patients

We studied 81 patients with mild to severe mental retardation associated with cranial/facial dysmorphisms and at least one additional dysmorphic feature, suggestive of the presence of a chromosomal abnormality. The karyotypes of all patients were considered normal after routine G-banding (~550 bands) and the

cause of the abnormal phenotypes could not be determined. Family history and consanguinity were not taken into account as exclusion criteria.
The patients were ascertained in two genetic centres: (1) 61 patients from the Department of Human and Clinical Genetics, Leiden University Medical Centre, the Netherlands (KGCL-LUMC); (2) 20 patients from the Department of Genetics and Evolutionary Biology, Institute of Biosciences, University of São Paulo, Brazil (LGH-USP). This service is the largest reference centre for fragile-X syndrome diagnosis in Brazil, and receives an overrepresentation of male patients. As a result, 18 of the 20 patients were male. In all these patients, fragile-X syndrome had been ruled out on molecular and clinical grounds.

Comparative genomic hybridisation microarrays
The array-CGH procedures were carried out as previously described.[8] Briefly, slides containing triplicates of ~3500 large insert clones spaced at ~1 Mb density over the full genome were produced in the Leiden University Medical Centre. The large insert clones set used to produce these arrays was provided by the Wellcome Trust Sanger Institute (UK), and information regarding the full set is available at the Sanger Institute mapping database site, Ensembl (http://www.ensembl.org/). Insert clones were isolated from the bacteria, using the Wizard SV 96 plasmid DNA purification system (Promega, Leiden, Netherlands) in combination with the Biomek 2000 laboratory automation workstation (Leiden Genomic Technology Centre (LGTC), Netherlands). DNA amplification, spotting on the slides, and hybridisation procedures were based on protocols previously described.[9,10] We used commercially available male and female genomic DNAs (Promega), which represent pools derived from at least seven same-sex individuals as reference samples. Test and reference DNA samples were labelled with Cy3- and Cy5-dCTPs (Amersham Bioscience, Roosendaal, Netherlands), respectively. After hybridisation, the slides were scanned with a GenePix Personal 4100A scanner, and the spot intensities measured using GenePix Pro 4.1 software (Axon Instruments, Westburg BV, Leusden, Netherlands). Further analyses were carried out using Microsoft Excel 2000. Spots outside the 20% confidence interval of the average of the replicates were excluded from the analyses. Target imbalances were determined of the basis of log2 ratios of the average of their replicates, and sequences were considered as amplified or deleted when outside the ±0.33 range.
We defined as abnormal a copy number change that we had not previously detected in around 100 normal control observations for each chromosome pair. The control data compiled chromosome information from DNA hybridisations of the following:

a. Normal to normal individuals.
b. Normal individuals to individuals previously diagnosed by G-band karyotype to carry partial or complete monosomies or trisomies.

c. Normal individuals to individuals having a clear chromosome alteration detected by array (a minimum of five altered consecutive clones).
d. Normal individual to the parents of patients in item c.

We excluded from the analyses the abnormal chromosomes detected by G-banding or CGH arrays in patients, as well as the corresponding chromosomes in their parents, irrespective of their carrier status. The imbalances detected by G-banding were also used to verify the ability of the arrays to ascertain copy number changes and to detect clones mapped to wrong chromosomes. On the basis of this set of normal chromosomes, we determined the average hybridisation ratio for each clone, and excluded from the analyses the 3% clones with an SD >0.073, totalling 110 clones. The remaining ones had an average SD of 0.039. In the hybridisations between normal to normal individuals, or normal to individuals carrying chromosomal alterations (items a and b above), we did not observe changes in the profiles using dye swap (inverted combination of fluorochromes for test and reference DNAs). Based on these results, we only undertook more than one hybridisation per individual in those experiments in which more than 3% of the clones were excluded owing to low intensities of the spots or a noisy background. The threshold of 0.33 for duplications and deletions was empirically chosen because it represented the lowest combined false positive and negative rates in control hybridisation testing of normal DNA and DNA from autosomal trisomies/monosomies.

Fluorescence in situ hybridisation

Fluorescence in situ hybridisation (FISH) experiments were carried out by standard techniques to validate the presence of deletions and duplications identified by microarray analyses. When an alteration was confirmed by FISH, hybridisations using the same probes were done to investigate whether the parents carried the rearrangement present in the child, either in balanced or unbalanced form. Aliquots of the same amplified DNAs used to spot the arrays were employed as probes for the FISH experiments. Clones mapping to the unbalanced chromosome regions were hybridised to metaphases derived from patients' blood lymphocytes. In cases of duplication, interphase nuclei were also analysed. At least 25 cells were analysed per hybridisation. A region was considered as duplicated when, in interphase nuclei, the corresponding clone produced three FISH signals accompanied by two signals in a different colour from a non-duplicated adjacent clone, used as a control. We considered a chromosome region to be partially deleted when the FISH signal from the corresponding clone on one of the chromosomes was consistently less intense (<=25% intensity) than on its homologue.

Multiplex amplifiable probe hybridisation

A study of genomic imbalances by multiplex amplifiable probe hybridisation (MAPH) technique[11] in 188 patients with mental retardation has recently been reported[12]: 162 loci were screened, comprising chromosome regions known to be involved in mental retardation (subtelomeric/pericentromeric regions and the genes involved in microdeletion syndromes), as well as interstitial genes randomly spaced throughout the genome. Although the MAPH patient sample partially overlapped the patients reported here (48 of the Dutch patients), importantly, the two studies were carried out independently and in parallel, and the MAPH results were unknown to those performing array-CGH. Patients 1, 2, 8, 18, and 20 listed in table 2 were part of the overlapping sample.

Table 1. Distribution of patients according to type and inheritance of imbalance

Imbalances	*De novo*	*Inherited*	*Unknown*	*Number of patients*
Interstitial	4	7	3	14
Deletions	3	5	1	9
Duplications	1	2	1	4
Deletion/duplication	0	0	1	1
Terminal	3	2	1	6
Deletions	2	0	1	3
Duplications	1	1	0	1
Deletion/duplication	0	1	0	1
Total number of patients	7	9	4	20

RESULTS

In this array-CGH analysis, imbalances not previously observed in our control samples were detected in 20 patients: six had duplications, 12 had deletions, and two carried both deleted and duplicated chromosome segments. Six other imbalances detected on arrays were not included among the imbalances because either (a) a presumptively deleted clone yielded two FISH signals of apparently similar intensities at the expected location on both homologues (one case), or (b) a BAC, supposedly in altered copy number, yielded multiple sites of FISH hybridisation in normal individual metaphases (five cases). It is unclear why we obtained disagreement between array-CGH and FISH results in the deletion case (a), but it emphasises that false positive results may occur in our test. On the other hand, clones that are known to hybridise to multiple sites are excluded from our array.

Table 2. Summary of copy number changes, associated clinical findings, and inheritance

Patient (institution) and inheritance	*Array-CGH imbalances*	*Confirmation*	*Clinical summary*	*Maximum size of imbalances*
De novo				
1 (LUMC)	Deletion of 5 clones at 1pter-1p36.31 (GS-232-B23 to RP11-49J3)	Confirmed by FISH	Female, MR, epilepsy, facial dysmorphism, shortening of metacarpals and metatarsals, hirsutism	7.2 Mb
2 (LUMC)	Deletion of 18 clones at 18q22.3qter (RP11-45A1 to GS- 75-F20)	Confirmed by FISH	Female, MR, short stature, hearing loss, congenital heart defect (total anomalous pulmonary venous return), mild facial dysmorphisms, narrow and long fingers	8.3 Mb
3 (LUMC)	Deletion of 12 clones at 3p24.3-p24.1 (RP11-27J5 to RP11-103N21)	Confirmed by FISH	Female, mild MR, facial dysmorphism, club feet, triphalangeal thumbs, mild anaemia	10.7 Mb
4 (LUMC)	Deletion of 2 clones at 13q32.3 (RP11-118F16 to RP11-564N10)	Confirmed by FISH	Male, MR, tall stature, corpus callosum agenesis, hearing loss, facial dysmorphism	3.2 Mb
5 (USP)	Deletion of 7 clones at 17p11.2 (RP11-524F11 to RP11-121A13)	Confirmed by FISH	Female, MR, behavioural problems including self aggression, hyperactivity, sleep disturbances, decreased pain sensitivity; midface hypoplasia, upward slanting of palpebral fissures (diagnosis: Smith-Magenis syndrome, OMIN 1822900)	5.7 Mb
6 (LUMC)	Duplication of a single clone at 17pter-p13.3 (GS-68-F18)	Confirmed by interphase FISH	Female, mild MR, facial dysmorphisms	0.8 Mb
7 (LUMC)	Duplication of a single clone at 6p12.3 (RP3-442L6)	Confirmed by interphase FISH	Female, mild MR, hypotonia, joint hyperlaxity, facial dysmorphism, strabismus	1.7 Mb
Inherited				
8 (LUMC)	Deletion of 4 clones at 6q27qter (RP11-351J23 to GS-57-H24). Duplication of 13 clones at 20q13.31qter (RP11-46O6 to bB152O15)	Confirmed by FISH [t(6,20)(q27;q13.31)mat]	Male, MR, hypotonia, microcephaly, brain anomalies, mild facial dysmorphisms	4.7 Mb deleted; 7.5 Mb duplicated
9 (LUMC)	Duplication of 2 clones at 3q29-qter (GS- 196F4 and GS -56H22)	Confirmed by interphase FISH; paternal	Female, moderate MR, facial dysmorphism, ataxia	0.4 Mb
10 (LUMC)	Partial deletion of a single clone at 15q15.3 (RP11-263I19)	Confirmed by FISH; paternal	Female, mild MR, hypotonia, facial dysmorphism, cloudy cornea	0.9 Mb
11 (LUMC)	Deletion of a single clone at 15q13.1 (RP11-408F10)	Confirmed by FISH; maternal Female, mild MR, short stature, microcephaly, minor facial dysmorphism, premature breast development	2.2 Mb	
12 (USP)	Deletion of a single clone at 10q21.1 (RP11-430K23)	Confirmed by FISH; maternal (mother with learning difficulties and similar dysmorphisms as the patient)	Male, mild MR, hyperactivity, facial dysmorphism, prominent ears, long digits, hyperextensibility of joints	2.5 Mb
13 (USP)	Duplication of a single clone at Xq28 (RP5-1087L19)	Confirmed by MAPH; maternal; two affected first cousins born to maternal aunts; duplication also present in the investigated cousin	Male, MR, hypoplasia of cerebellar vermis, Dandy-Walker anomaly, large prominent ears, high-arched palate, abdominal obesity, flat feet.	1.3 Mb
14 (USP)	Partial deletion of a single clone at Xp11.23 (RP1-54B20)	Confirmed by FISH; maternal; similarly affected males referred in the maternal family	Male, severe MR, short stature, microcephaly, prominent ears, deep set eyes, short filtrum, early onset puberty	1.8 Mb
15 (LUMC)	Partial homozygous deletion of a single clone at 2p12 (RP11-89C12)	Confirmed by FISH; first cousin; heterozygous father and homozygous mother	Female, MR, microcephaly, cleft palate, congenital cataract, microphthalmia; equally affected sibling carries same homozygous deletion	1.0 M b
16 (LUMC)	Duplication of a single clone at 8p11.1 (CTD-2115H11)	Confirmed by FISH; maternal	Male, mild MR, short stature, facial dysmorphisms	1.3 Mb
Unknown				
17 (LUMC)	Deletion of 6 clones at 6pter-p25.2 (GS-62-L11 to RP11-15N12)	Confirmed by FISH; mother not a carrier*	Male, mild MR, hearing loss, iris dysplasia, eccentric pupil, hypertelorism	5 Mb
18 (USP)	Deletion of a single clone at 16p11.2 (RP11-74E23)	Confirmed by FISH; mother not a carrier*	Female, mild MR, severe speech delay, facial dysmorphism	1 Mb
19 (USP)	Duplication of a single clone at 22q11.21 (XX-91c)	Confirmed by MAPH and array-CGH tile-path of chromosome 22; mother not a carrier*	Male, mild MR, turricephaly, convergent strabismus, myopia, high and narrow palate, large upper first incisors	3.9 Mb
20 (LUMC)	Deletion of a single clone at 22q11.21 (XX-p273a17); duplication of a single clone at 22q11.21 (XX-91c); deletion of 5 clones at 22q12.1 (CTA-390B3 to RP11-329J17)	Confirmed by MAPH and array- CGH tile path of chromosome 22**	Male, mild MR, hearing loss, microcephaly, cataract, cleft palate, double set of teeth	1.1 Mb deleted; 3.9 Mb duplicated; 3.9 Mb deleted

*Father deceased or unavailable.
**Parents deceased.
LUMC, Leiden University Medical Centre; MR, mental retardation; USP, University of Saõ Paulo.

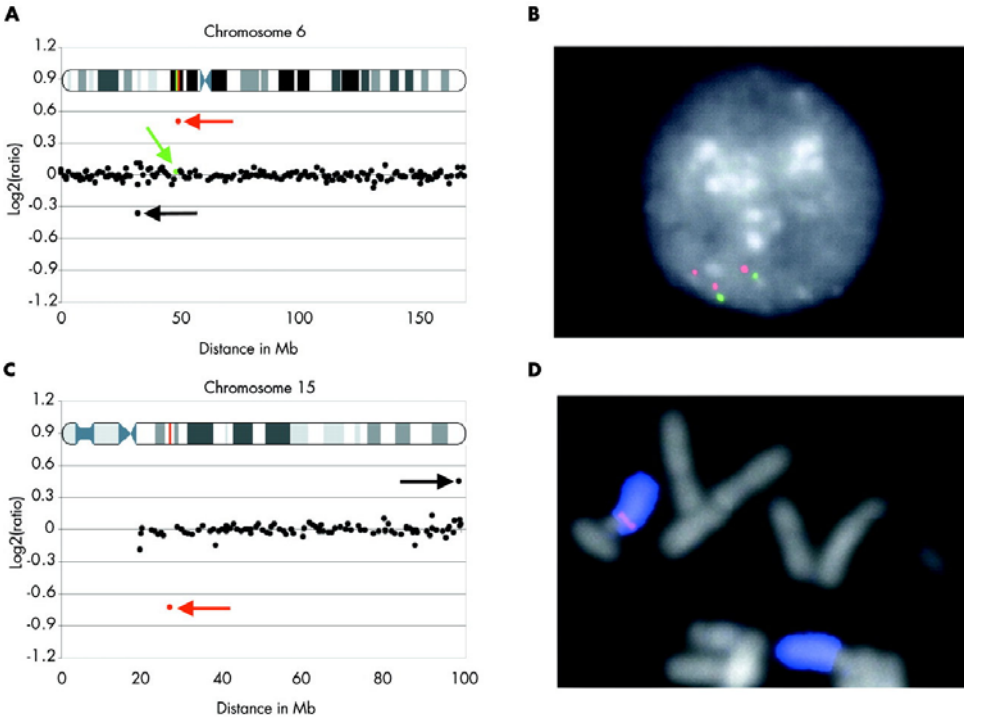

Figure 1 Examples of duplications and deletions ascertained by array-CGH and confirmed by FISH. Unbalanced and control insert clones are represented in red and green colours, respectively, in the array profiles and corresponding ideograms. The black arrows show large insert clones which are also found altered in normal controls. (A) Chromosome 6 array-CGH profile from patient 7, showing a duplication of a single clone at 6p12.3 (RP3-442L6). (B) Three interphase-FISH signals of the duplicated PAC RP3-442L6 (red) and two signals of the adjacent non-duplicated BAC RP11-334H12 (green) confirm this duplication. (C) Deletion of a single clone at 15q13.1 (RP11-408F10) in patient 11. (D) FISH to a metaphase showing the presence of two chromosomes 15 (whole-chromosome 15 library in blue), but a single signal for BAC RP11-408F10 (red). BAC, bacterial artificial chromosome; CGH, comparative genomic hybridisation; FISH, fluorescence in situ hybridisation.

Table 1 shows the distribution of the copy number changes according to the type and inheritance of the imbalances, and table 2 presents the copy number changes, clinical data, and family analysis.

Fourteen of the imbalances were interstitial and six were terminal. Deletions and duplications were confirmed by FISH; case 13 was confirmed by MAPH, and for chromosome 22 (cases 19 and 20) an array of overlapping sequencing tile path clones[13] allowed us to delineate the duplication and deletions (Kriek et al, unpublished data). Figure 1 shows examples of array-CGH deletions and duplications, and FISH confirmation. Among the 16 patients whose parents were available for examination, seven carried de novo imbalances and nine had inherited rearrangements. Among the rearrangements, one patient (case 15) had a homozygous deletion inherited from heterozygous father and homozygous mother (fig 2).

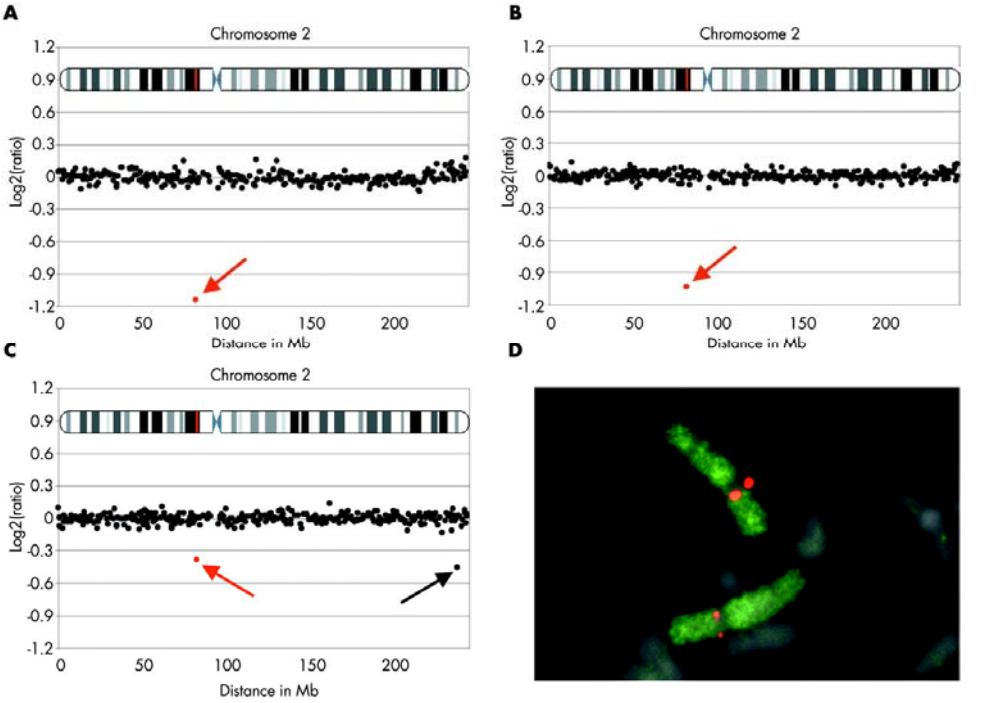

Figure 2 Chromosome 2 imbalances in the family of patient 15. Chromosome 2 array-CGH profiles of the proband (A) and his mother (B) show a deletion of BAC RP11-89C12 (red arrow) at 2p12. The log2 ratios around –1.0 indicate that the deletion is homozygous. (C) Chromosome 2 array-CGH profile of the father shows a deletion of the same BAC (red arrow) in a ratio compatible with heterozygosity. (D) FISH using BAC RP11-89C12 (red) to the father's metaphase shows that the signal on one chromosome 2 (whole chromosome library in green) is less than one quarter the size and intensity of the homologue. BAC, bacterial artificial chromosome; CGH, comparative genomic hybridisation; FISH, fluorescence in situ hybridisation.

DISCUSSION

We used array-CGH to investigate DNA copy number imbalances in 81 individuals presenting with mental retardation, dysmorphic features, and an apparently normal karyotypes, and detected unique alterations in 25% of these. In addition, 33 clones contained in our array (>0.1%) exhibited copy number alterations detected at least once in the control analysis, and were considered as normal variants. These genomic imbalances might well represent some of the large scale copy number variations of DNA segments recently described in humans and mice.[14–16]

More than half the imbalances comprised one clone or less (partial deletions). The two largest alterations (patients 2 and 3) encompassed between 8 and 11 Mb and, after CGH-array results were known, they could retrospectively be visualised on G-banded chromosomes. The frequency of imbalances detected in our study is similar to the 24–25% found in previous array-CGH studies of mentally impaired individuals.[6,7] However, the patients in ours and in the two previous

array-CGH studies were selected not to be solely representative of undiagnosed mental retardation, but rather to include patients whose dysmorphic features in conjunction with mental retardation were suggestive of chromosomal abnormalities. It is well known that the frequency of chromosomal abnormalities is increased when mental retardation is associated with congenital abnormalities or dysmorphic features. While these studies prove the value of array-CGH for uncovering cryptic rearrangements, further studies in categorised samples of mentally retarded individuals will be required to establish the frequency of imbalances which have a pathological consequence giving rise to mental retardation, and to differentiate them from rare variants picked up serendipitously in the screening. Among the 20 chromosome rearrangements that we detected, six (7.4%) were subtelomeric and 14 (17.3%) were interstitial. Similarly to the two previous studies with array-CGH, the frequency of cryptic interstitial rearrangements was two to three times greater than the frequency of terminal imbalances.

When patients carry de novo imbalances, either interstitial or terminal, it is reasonable to assume that the copy number change is the cause of the phenotype. On the other hand, inherited subtelomeric and interstitial rearrangements have different implications for genetic counselling. Inherited subtelomeric rearrangements result from the segregation of a balanced translocation in one of the normal parents,[1] and their detection allows genetic counselling and prenatal diagnoses to be provided. In contrast, inherited interstitial rearrangements detected by array-CGH seem to be equally imbalanced in a normal parent, and no carriers of balanced interstitial rearrangements were detected in ours or in the previous array-CGH studies. Therefore, these inherited interstitial rearrangements pose a new situation in genetic counselling, because the normal parent apparently carries the same imbalance as the affected child. We observed various different situations that suggest that rare inherited copy number variations can either affect the phenotype or represent "normal" variants. The X chromosome imbalances (patients 13 and 14) were associated with clinical features showing an X linked pattern of inheritance—that is, other affected males were related to the probands by their phenotypically normal mothers. An intriguing case is that of patient 15: he and his equally affected sibling are homozygous for a partial deletion of one clone at 2p12. Their clinically normal parents are first degree cousins, and the father is heterozygous for the same deletion, while the mother is homozygous (fig 2). This deletion therefore appears as a rare variant segregating in the family, and the abnormal phenotype of the children is likely to be caused by homozygosity for another recessive mutation. Furthermore, this family is of Turkish descent, and the frequency of the deletion in this population is unknown.

In four cases (patients 17 to 20), one or both parents could not be investigated for the presence of imbalances. In patient 17, although the de novo status could

not be proved, the patient had the typical features of the deletion 6pter syndrome,[17,18] thus indicating that the deletion was causative. Among the infrequent DNA segment imbalances found in our patients, there was a single map location overlap—namely, the duplication of cosmid XX-91c on chromosome 22 which is present in patients 19 and 20 (table 2). The tilling path analysis of chromosome 22 in patient 19 showed that this duplication encompasses ~1.7 Mb, and overlaps the often deleted region in the DiGeorge/velocardiofacial syndrome (DG/VCFS). Two recent papers reported on patients with duplication of this region, suggesting that dup22q11.2 is an emerging syndrome[19,20]; the learning abilities of the carriers ranged from normal to severely impaired, and associated clinical features were extremely variable, including normal individuals ascertained through affected relatives. Our patient had only mild mental retardation and some unspecific dysmorphic features, which may be present in different syndromes, including patients with dup22q11. As the frequency of the duplication 22q11.2 is significantly increased among mentally retarded patients, we considered that the alteration in our patient was probably causative of his phenotype. Patient 20 carries two deletions on chromosome 22 in addition to this recurrent duplication. Furthermore, this patient had specific congenital abnormalities, some of which do not seem to be associated with the dup22q11.2 syndrome, and might be caused by at least one of the deletions (Kriek M et al, unpublished data). Unfortunately, further investigation could not be undertaken because not all the parents of these two patients were available.

Thus, among the 20 rearrangements detected in our patients (table 2), we were able to identify 13 as causative of the abnormal phenotypes: those which were de novo (patients 1–7), the imbalanced rearrangement inherited from a balanced parent (patient 8), the two familial X chromosome alterations (patients 13 and 14), the deletion 6pter (patient 18), the dup22q (patient 19), and the rearrangements of chromosome 22 in patient 20, which were too large and complex not to be the probable cause of the phenotype. In fact, the rearrangements involving two or more clones were all de novo (mean (SD) maximum average size, 5.4 (3.6) Mb), while the rearrangements present in normal carriers tended to be smaller (maximum average size 1.6 (0.8) Mb). It is not itself surprising that rearrangements involving large segments of DNA have a smaller probability of being present in normal carriers.

Among the 13 cases with copy number imbalances considered causative of the phenotype, five were terminal and eight were interstitial, showing a similar contribution of these rearrangements to mental retardation associated with other clinical features. However, in patients who inherited an apparently identical interstitial imbalance from their parents, we cannot disregard the possibility that imprinting, incomplete penetrance, and loss of heterozygosity for a detrimental recessive gene contribute to the different effect in parents and affected children, as has been reported for dup22q11.2.[19,20]

The frequent occurrence of rare genomic imbalances in affected children and their normal parent represents a complicating factor in the interpretation of array-CGH results. Among our patients we found inherited imbalances that were appeared clearly associated with a pathological effect, while others most probably represented genomic variants not contributing to the abnormal phenotype. Recent initiatives such as those of the Sanger Institute (www.sanger.ac.uk/PostGenomics/decipher/) and the European Cytogeneticists Association (http://www.ecaruca.net/) to create platforms for compiling molecular cytogenetic data from clinical genetic studies will hopefully provide a base for understanding the role of different DNA copy number variations in genetic diseases. Collecting and understanding larger sets of data will improve our ability to determine which copy number variations contribute to abnormal phenotypes, and eventually result in a more consistent application of CGH-microarray for genetic counselling.

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FOOTNOTES

Conflicts of interest: none declared

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