

ORIGINAL ARTICLE

Microarray based comparative genomic hybridisation (array-CGH) detects submicroscopic chromosomal deletions and duplications in patients with learning disability/mental retardation and dysmorphic features

C Shaw-Smith*, R Redon*, L Rickman*, M Rio, L Willatt, H Fiegler, H Firth, D Sanlaville, R Winter, L Colleaux, M Bobrow, N P Carter

See end of article for authors' affiliations

J Med Genet 2004;41:241–248. doi: 10.1136/jmg.2003.017731

Correspondence to:
Dr N P Carter, The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SA, UK; npc@sanger.ac.uk

Revised version received 8 January 2004
Accepted for publication 18 January 2004

The underlying causes of learning disability and dysmorphic features in many patients remain unidentified despite extensive investigation. Routine karyotype analysis is not sensitive enough to detect subtle chromosome rearrangements (less than 5 Mb). The presence of subtle DNA copy number changes was investigated by array-CGH in 50 patients with learning disability and dysmorphism, employing a DNA microarray constructed from large insert clones spaced at approximately 1 Mb intervals across the genome. Twelve copy number abnormalities were identified in 12 patients (24% of the total): seven deletions (six apparently de novo and one inherited from a phenotypically normal parent) and five duplications (one de novo and four inherited from phenotypically normal parents). Altered segments ranged in size from those involving a single clone to regions as large as 1.4 Mb. No recurrent deletion or duplication was identified within this cohort of patients. On the basis of these results, we anticipate that array-CGH will become a routine method of genome-wide screening for imbalanced rearrangements in children with learning disability.

Constitutional chromosomal imbalance is often associated with learning disability, dysmorphism, congenital anomalies, and abnormalities of growth. Examples of recurrent chromosomal imbalances include well characterised conditions such as the common trisomies, which can be studied by classical cytogenetic techniques. However, these methods have limited resolution and are unreliable for subtle copy number changes involving chromosome segments of 5 Mb in length or less.

Recurrent chromosomal microdeletion or duplication syndromes, such as for example DiGeorge syndrome (22q11 deletion syndrome), may be recognisable clinically because of the combination of clinical features or because of a characteristic facial appearance ("gestalt"). In patients with normal karyotypes, the clinical features may still suggest appropriate regions to be screened for microscopic imbalance by fluorescent in situ hybridisation (FISH) analysis using locus specific probes. Often, however, the combination of clinical features is not diagnostic of a particular syndrome but may nevertheless still suggest that a chromosomal imbalance is the likely underlying cause of the abnormality. FISH probes designed to detect subtelomeric rearrangements have given a significant diagnostic yield of around 6% in patients with unexplained learning disability and dysmorphic features,^{1–3} but interstitial chromosomal deletions and duplications are not detectable using this method.

More recently, the technique of comparative genomic hybridisation (CGH) using metaphase chromosomes has been applied to this clinical problem.^{4–5} Its sensitivity has been refined to permit detection of chromosomal deletions as small as 3 Mb. In a study of patients with learning difficulties and dysmorphism, 10% showed small deletions or duplications which were detectable by this method but which had not been identified on the G banded karyotype.⁴

*These three authors made an equal contribution to the work.

Microarray based comparative genomic hybridisation (array-CGH)⁶ has many potential advantages over other methods currently available for the investigation of children with learning disability and dysmorphism. It offers rapid genome-wide analysis at high resolution and the information it provides is directly linked to the physical and genetic maps of the human genome.

Array-CGH has been used successfully for analysis of tumour samples and cell lines^{7–12} and for high resolution analysis of constitutional abnormalities consisting of single copy gains and losses in specific chromosomal regions,^{13–15} telomeres,¹⁶ and an entire chromosome.¹⁷ A study using genome-wide array-CGH to investigate patients with dysmorphism and learning disability has been reported recently.¹⁸ In that study, five copy number changes—of which only two were de novo and one involved a rearrangement within a telomeric region—were found in 20 patients, giving a potential diagnostic yield of 15%.

We have previously reported the design and use of DNA microarrays for the detection of copy number changes¹⁰ with application to cytogenetic abnormality,¹⁹ and have used a modified version of the technique to identify breakpoints in individuals with balanced reciprocal translocations.²⁰ Here we report the application of genome-wide array-CGH at 1 Mb resolution to the study of 50 patients with learning disability and dysmorphism for whom, in the majority of cases, telomeric rearrangements had been excluded.

METHODS

Patients

We recruited patients born to unrelated parents and presenting with moderate to severe learning difficulties of unknown cause, 24 from France and 26 from the United Kingdom. Appropriate ethical approval was obtained. The patients were included in the study on the basis of the presence of at least one of the following additional criteria:

- A family history of learning disability;
- overgrowth or growth failure;
- behavioural problems;
- seizures;
- facial dysmorphism or clinical or radiological evidence of brain, trunk, or limb anomalies.

In addition, all patients had a karyotype interpreted as normal using GTG banding analysis at ISCN 400–500. The presence of a subtelomeric abnormality was excluded by FISH or genotyping in the majority of these patients (41/50; 17 by FISH, 24 by genotyping). Genomic DNA from each patient was isolated from blood lymphocytes using a Nucleon kit (Amersham, UK) according to the manufacturer's instructions. Pooled genomic DNA extracted from blood of 20 male or 20 female individuals was used as the reference in array hybridisations.

Array-CGH

The 1 Mb resolution arrays used in this study are as described previously.¹⁰ The clone set used for the construction of these arrays was verified by end sequencing and is available from the Sanger Institute. Array-CGH was undertaken generally as described but with modification.¹⁰ DNA was labelled using the Bioprime labelling kit (Invitrogen, Carlsbad, California, USA) with modifications of the nucleotide mix. Briefly, a 130.5 µl reaction was set up containing 450 ng of DNA and 60 µl 2.5× random primer solution. After denaturing the DNA for 10 minutes at 100°C, 15 µl 10×dNTP mix (1 mM dCTP, 2 mM dATP, 2 mM dGTP, and 2 mM dTTP in TE buffer), 1.5 µl 1 mM Cy5-dCTP or Cy3-dCTP (NEN Life Science Products, Boston, Massachusetts, USA), and 3 µl Klenow fragment were added on ice to a final reaction volume of 150 µl. The reaction was incubated at 37°C overnight and stopped by adding 15 µl stop buffer supplied in the kit. Unincorporated nucleotides were removed by use of microspin G50 columns (Pharmacia Biotech, Piscataway, New Jersey, USA) according to the suppliers' instructions.

Array hybridisation using the 1 Mb array was undertaken as described previously.¹⁰ Briefly, Cy3 and Cy5 labelled test and control DNAs were combined, precipitated together with 135 µg of human Cot1 DNA (Roche, Mannheim, Germany), and resuspended in 60 µl of hybridisation buffer (50% formamide, 10% dextran sulphate, 0.1% Tween 20, 2×SSC, and 10 mM Tris-HCl, pH 7.4) and 6 µl of yeast tRNA (100 µg/µl, Invitrogen). Prehybridisation was carried out for one hour at 37°C as described. To prehybridise the arrays, 80 µl of herring sperm DNA (10 mg/ml, Sigma Aldrich, Poole, Dorset, UK) and 135 µg of human Cot1 DNA (Roche, Mannheim, Germany) were co-precipitated and resuspended in 160 µl of hybridisation buffer. Open-well hybridisation was done as described previously.¹⁰

Image and data analysis

Arrays were scanned using an Agilent scanner (Agilent Technologies). Fluorescent intensities were extracted after subtraction of local background using SPOT software.²¹ The data were normalised by dividing the mean ratio of each clone duplicate by the median ratio of all autosomal clones. Clones were excluded where the duplicate values differed from each other by more than 20%. In addition, clones that we have identified in previous control hybridisations and other studies as being polymorphic in normal individuals or consistently generating irreproducible ratios were excluded from the analysis. Mean and standard deviation values of hybridisation ratios for all clones in each experiment were calculated, and experiments where the standard deviation (SD_h) exceeded 0.1 were excluded from analysis (but were successfully repeated subsequently).

We investigated those clones where the hybridisation ratio exceeded a value of the mean plus or minus four times the standard deviation (SD_h) for the particular hybridisation experiment.

FISH

FISH experiments were done to confirm deletions and duplications identified by microarray analysis, using standard techniques.²² Metaphase spreads were prepared from patient derived lymphocytes using standard procedures. The human genomic clones from the RPCI BAC and PAC libraries were obtained from BACPAC resources (<http://www.chorio.org/bacpac/>) or locally from the Wellcome Trust Sanger Institute. Clones were grown on plates containing LB and 25 µg/ml kanamycin (PACs) or 20 µg/ml chloramphenicol (BACs), and incubated overnight at 37°C. Single colonies were picked and inoculated into 5 ml cultures of LB, supplemented with the appropriate antibiotic and grown overnight at 37°C at 215 rpm. DNA from clones was isolated using a QIAprep spin miniprep kit (Qiagen), and 500 ng of miniprep DNA was labelled with Spectrum Orange dUTP by nick translation (Vysis), following the manufacturer's instructions. Slides were analysed using a fluorescence microscope (Leica DMRB), and images recorded using SmartCapture 2 software (Digital Scientific).

Quantitative FISH analysis was used to confirm the duplications identified in cases 2 and 8 by array-CGH, essentially as previously described,²³ but with modifications. The fluorescent images captured in the conventional manner with the SmartCapture software were transferred into and visualised in the IP lab software package (Signal Analytics Inc). For each metaphase, the probe signals were isolated by splitting the colour coordinates so only the red channel containing the probe signals was displayed. Regions of interest of identical size were then drawn around each of the test and control probe signals on each chromosome homologue. The mean fluorescence for each probe signal within the defined region of interest was then measured using the IP Lab software. This procedure was carried out for 10 cells in each probe experiment. Statistical analysis was done using a Student *t* test. Log ratios of the fluorescent intensities for the test and control probe on the normal chromosome homologue were subtracted from the log ratios of the fluorescent intensities of the test and control probe on the abnormal homologue for each of the 10 cells. A fluorescence intensity ratio estimate (and 95% confidence intervals) was obtained between the test probe on the putative duplicated chromosome and the test probe on the other homologue.

Genotyping

Genotyping was carried out as described.²⁴ Briefly, the number, location, and heterozygosity scores of the microsatellites were obtained from either the GENETHON database (<http://www.genethon.fr/>) or the GENOME DATABASE (<http://www.gdb.org/>). Primers labelled with a 6-FAM or HEX fluorochromes were ordered from MWG BIOTECH (Courtabeuf, France). DNA was extracted from peripheral blood lymphocytes according to standard techniques. Polymerase chain reaction (PCR) amplification of genomic DNA from parents and patients (100 ng) was undertaken separately. PCR products were then pooled according to their size and labelling, and analysed on an automatic sequencer (ABI 3100, PE Biosystem). Gel lane tracking and sizing of the size standard peaks were checked manually for all lanes. GENESCANTM and GenotyperTM software (Applied Biosystems) was used to size the PCR products and to analyse the data.

RESULTS

We used a DNA microarray with a resolution of approximately one clone per megabase to study 50 patients with normal or apparently balanced karyotypes in whom a chromosomal abnormality was suspected because of the combination of clinical features. All patients had developmental delay or learning disability/mental retardation, together with dysmorphic facial features and other dysmorphic features involving the limbs or internal organs. All patients showed normal G banded karyotypes (with one exception, case 10), and in the majority of cases (41/50) telomere rearrangements had been excluded by FISH or genotyping.

A loss or gain of chromosomal material was detected in 12 patients, giving a rearrangement detection rate of 24%. In addition, copy number changes involving the Y chromosome were identified in two patients, but in regions largely

consisting of palindromic repeats. As these regions were unlikely to be linked to the phenotype, we discounted them from further analysis. Seven of the abnormalities were deletions, of which six were not present in either parent. Five were duplications, of which one was de novo and four were inherited from a phenotypically normal parent. The copy number changes identified ranged in size from those involving only a single clone to approximately 14 Mb. All the deletions and duplications identified within the set of 12 abnormal results were in distinct non-overlapping chromosomal regions.

Array-CGH profiles for aberrant chromosomes are shown in fig 1. The imbalances in each of these cases were confirmed either by FISH (fig 2) or by microsatellite analysis of samples from the proband and both parents (table 1). For duplications, quantitative FISH analysis was carried out. In one case (patient 9), microarray analysis at tiling path resolution was

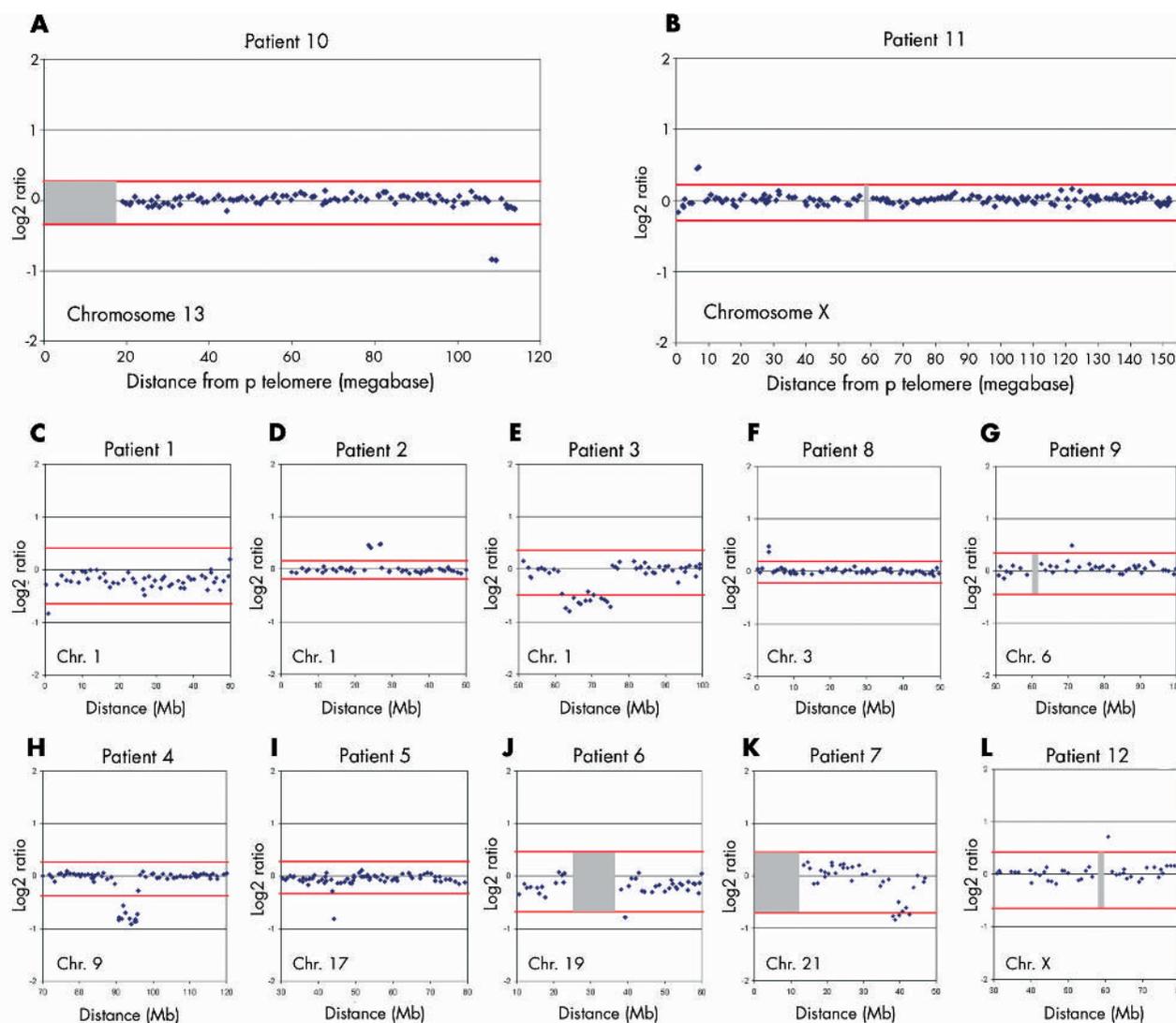


Figure 1 Summary of the array-CGH ratio profiles showing chromosomal abnormalities. Hybridisation results are shown for each of the 12 study patients in whom an abnormality was identified by array-CGH. For each panel, the X axis marks the distance, in megabases, along the chromosome from the p telomere. The Y axis marks the hybridisation ratio plotted on a log₂ scale. Red lines indicate thresholds for clone deletion or duplication (mean \pm 4 SD). For each subject, the result for the whole chromosome (panel 1–2) or for the 50 Mb length chromosomal segment (panels 3–12) that contains the abnormality is shown. Further details for each patient are given in table 2. Centromeres, which are not covered on this array, appear in grey. (A) Patient 10 shows a deletion involving two clones in 13q. (B) Patient 11 shows a duplication involving two clones in Xp. (C) Patient 1 shows a deletion involving a single clone in 1p. (D) Patient 2 shows a duplication involving five clones in 1p. (E) Patient 3 shows a deletion involving 14 clones in 1p. (F) Patient 8 shows a duplication involving two clones in 3p. (G) Patient 9 shows a duplication involving a single clone in 6q. (H) Patient 4 shows a deletion involving 11 clones at 9q22.3. (I) Patient 5 shows a deletion involving a single clone at 17q. (J) Patient 6 shows a deletion involving a single clone at 19q. (K) Patient 7 shows a deletion involving seven clones in 21q. (L) Patient 12 shows a duplication involving a single clone in Xq.

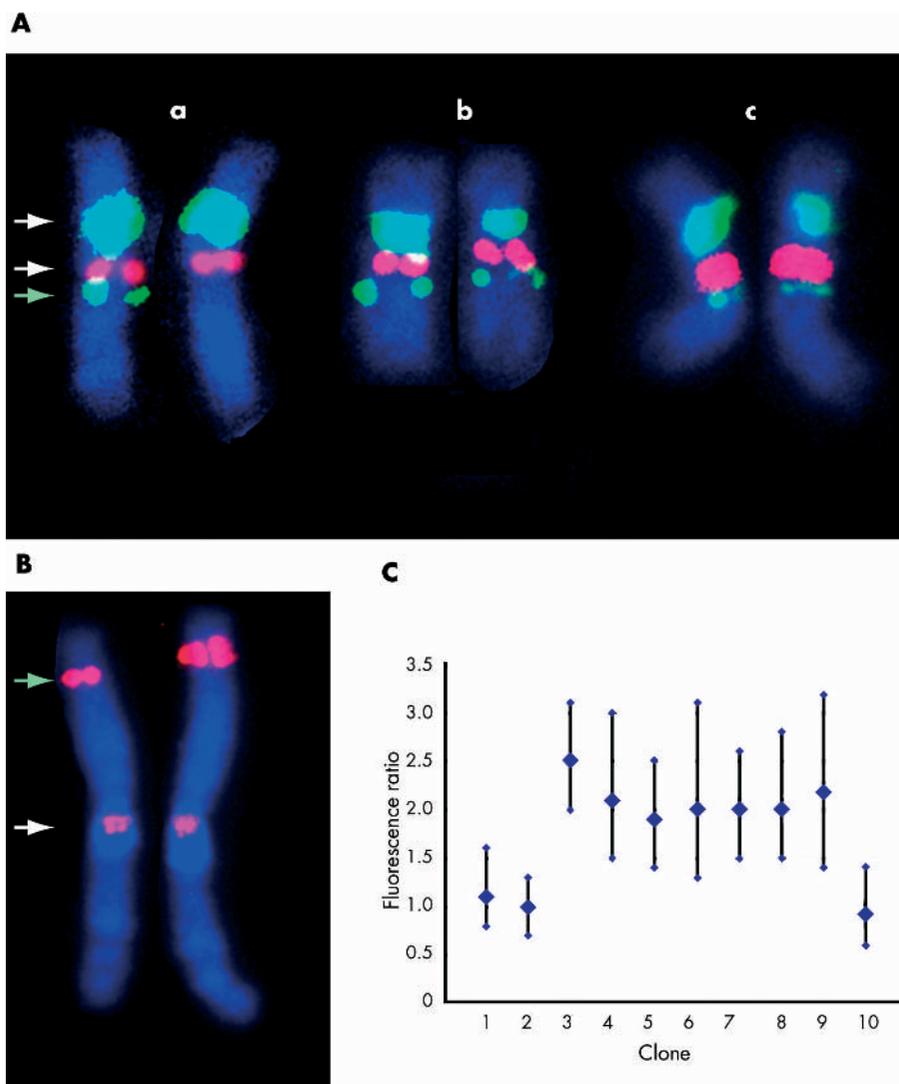


Figure 2 Confirmation of array-CGH results by fluorescent in situ hybridisation: two examples. (A) Confirmation of array-CGH finding in patient 5 (see table 2). A deletion involving a single clone was identified in this patient by array-CGH. Fluorescent in situ hybridisation (FISH) was undertaken on the proband (panel a), mother (panel b), and father (panel c), using three different probes. The white arrows indicate control probes (D17Z1, centromere probe (green) and RP11-94L15, Her2 locus (red)). Both these probes give a similar signal on each chromosome in panels a, b, and c. The green arrow indicates the signal obtained with BAC probe RP5-843B9, the same clone that gave an abnormal result by array-CGH. Signal is observed on both chromosomes in panels b and c, but on one chromosome only in panel a, indicating a de novo deletion. (B) Confirmation of array-CGH finding in patient 2 (see table 2) by quantitative FISH analysis. A duplication involving five clones was identified by array-CGH in this patient. FISH was carried out on the proband using a clone (RP11-187B23) from within the putative duplicated region (green arrow) and a control centromeric probe (white arrow). Results for chromosome 1 are displayed. A difference in signal size and intensity between the putative normal chromosome 1 (left of figure) and the chromosome 1 containing the duplication (right of figure) is observable by inspection. The signal was quantified for clones both from within and adjacent to the putative duplicated region. (C) The results of quantitative FISH analysis. A fluorescence intensity ratio of 2 is consistent with a duplication, and of 1 is consistent with a 1:1 signal ratio (no duplication). Clones from within the putative duplicated region gave a fluorescence ratio consistent with a duplication, while clones outside the region gave a 1:1 intensity ratio. The vertical bars indicate 95% confidence limits. Analysis of parental samples indicated that this duplication was a de novo event. Clones used in the experiment were: (1) RP1-184J9, (2) RP5-1057J7, (3) RP11-10N16, (4) RP11-373M8, (5) RP11-799D16, (6) RP3-469D22, (7) RP1-187B23, (8) RP11-492M19, (9) RP11-40H20, (10) RP1-159A19.

Table 1 Genotype analysis using chromosome 9 markers confirms the 9q22.3 deletion for patient 4 and demonstrates paternal inheritance

	Distance	Father	Patient 4	Mother
D9S1815	89.74 Mb	223/225	223/225	223/233
D9S287*	93.6 Mb	168	170	168/170
D9S1809*	93.93 Mb	124/138	122	122/124
D9S1690	99.48 Mb	223/225	223/225	223/233
D9S1677	107.31 Mb	259/265	259	259/269
D9S1675	108.4 Mb	228	226/228	226

*Markers in bold lie within the putatively deleted region identified by array-CGH analysis in this patient.

Table 2 Summary of copy number changes detected by array-CGH, associated clinical data and parental analysis

Case	Clinical details	Karyotype‡	Result of microarray-CGH	Confirmation, parent analysis	Flanking clones
1*	Microcephaly, short stature, facial dysmorphism, developmental delay, seizures	46, XX	Deletion of single clone at 1p36.33	Yes§, apparently de novo§	RP11-465B22
2†	Microcephaly, developmental delay, severe learning difficulties	46, XX	Duplication of five clones at 1p36.11–12	Yes¶, de novo¶	RP11-509F14 RP1-144C9
3†	Microcephaly, severe learning difficulties, cleft palate	46, XX	Deletion of 14 clones at 1p31.1–31.3	Yes¶, de novo¶	RP4-662P1 RP11-25F16
4*	Overtgrowth, severe mental retardation, craniosynostosis, macrocephaly	46, XY	Deletion of 11 clones at 9q22.3	Yes§, apparently de novo§	RP11-333I7 RP11-547C13
5†	Severe learning difficulties, short stature, mild contractures and patchy skin pigmentation	46, XY	Deletion of single clone at 17q21.31	Yes¶, de novo¶	RP5-843B9
6†	Intrauterine growth retardation, developmental delay, cutis aplasia	46, XY	Deletion of single clone at 19q13.11	Yes¶, de novo¶	RP11-413M10
7*	Mental retardation, seizures, microcephaly, constipation, brachydactyly and syndactyly	46, XX	Deletion of 7 clones at 21q22	Yes§, apparently de novo§	RP11-98O13 RP11-113F1
8†	Microcephaly, short stature, Dandy-Walker malformation, developmental delay	46, XY	Duplication of two clones at 3p26.2–3	Yes¶, inherited (paternal) ¶	RP11-95E11 RP11-10H6
9*	Severe learning difficulties, postnatal growth retardation and facial dysmorphism	46, XY	Duplication of single clone at 6q13	Yes**, inherited (maternal)††	RP11-462G2
10†	Microcephaly, developmental delay, kyphoscoliosis	46, XY t(1;5) de novo	Deletion of two clones at 13q33.3–q34	Yes¶, inherited (maternal)¶	RP11-141M24 RP11-40E6
11*	Seizures, ataxia, severe mental retardation, hyperphosphatasia, facial dysmorphism	46, XX	Duplication of two clones at Xp22.3	Not done, inherited (maternal)††	RP11-483M24 RP11-323F16
12*	Developmental delay, cryptorchidism, supernumerary nipple, facial dysmorphism	46, XY	Duplication of single clone at Xq11.2	Not done, inherited (maternal)††	RP13-34C21

*Cases ascertained from France.

†Cases ascertained from the UK.

‡Karyotype does not include information arising from array-CGH analysis.

§Performed by genotyping.

¶Performed by FISH.

**Performed by tiling path array-CGH.

††Performed by array-CGH.

CGH, comparative genomic hybridisation.

done in order to confirm and refine the abnormal finding (data not shown).

The clinical features of each patient in whom a chromosomal imbalance was detected are summarised in table 2. The band position and the number of clones giving an abnormal result are shown, together with the results of the analysis of parental samples. The names of the two flanking abnormal clones (or of the single clone where appropriate) are also shown.

Comments on aspects of the individual cases are given below.

Case 1

Case 1 was a 21 year old woman who presented with developmental delay, microcephaly, short stature, seizures, and facial dysmorphism. The clinical phenotype of the patient was strongly suggestive of 1pter deletion syndrome.²⁵ By karyotyping and genotyping, no deletion was detected in this region. However, a deletion involving a single clone at 1p36.33 was detected by array-CGH which was apparently de novo, and was less than 2 Mb in size. Interestingly, the most telomeric clone (CTB-14E10), which is also often used in the FISH based telomeric rearrangement screen, failed to show a deleted ratio. This might explain why this patient was screened negative for telomeric rearrangement using other methods. Further work is in process to fully characterise this microdeletion and to delineate a small subset of candidate genes for this particular recurrent syndrome.

Case 2

Case 2 was a 12 year old girl with microcephaly, severe learning disability, and mildly dysmorphic facial features. A duplication spanning five clones in 1p36.11–12 was identified by array-CGH. This duplication was confirmed by quantitative FISH analysis, and similar studies in the parents indicated that this finding was apparently a de novo event and therefore was the likely underlying cause of the patient's

phenotype. A duplication spanning 1p36.11–12 has previously been reported in conjunction with a deletion of 2q33–qter²⁶—the child, who died at three months, had multiple congenital malformations.

Case 3

Case 3 was a 13 year old girl with severe learning disability, microcephaly, and cleft palate. A deletion of approximately 14 Mb involving 14 clones at 1p31.1–31.3 was identified by array-CGH and confirmed by FISH. FISH studies in the parents indicated that this finding was an apparently de novo event and therefore was the likely underlying cause of the patient's phenotype. Reanalysis of the G banded karyotype revealed that this deletion, though subtle, could in retrospect be detected. Cytogenetically visible deletions encompassing this region have been reported previously,^{27–29} though without a clear phenotypic resemblance between individual cases.

Case 4

Case 4 was a three year old boy presenting with severe learning disability, craniosynostosis, microcephaly, and overgrowth. A deletion of 6.5 Mb involving 11 clones at 9q22.3 was identified by array-CGH. Genotyping of parental DNA showed that this deletion was apparently de novo. A cytogenetically visible substantially larger deletion encompassing this region has been reported previously.³⁰ However, the clinical phenotype was quite different, although overgrowth was a common feature.

Case 5

Case 5 was a 17 year old boy with severe learning disability, short stature, mild contractures, and patchy skin pigmentation. An interstitial deletion involving a single clone at 17q21.31 was identified by array-CGH and confirmed by FISH analysis. FISH studies in the parents indicated that this finding was a de novo event and therefore was the likely underlying cause of the patient's phenotype. There are two

previous case reports of visible deletions spanning this region.^{31,32} Phenotypes and breakpoints are different in all three cases, although all had dysmorphic features and learning disability.

Case 6

Case 6 was a four year old boy who presented with intrauterine growth retardation, developmental delay, and cutis aplasia. A deletion involving a single clone at 19q13.11 was identified by array-CGH and confirmed by FISH. FISH studies in the parents indicated that this finding was a de novo event and therefore it is reasonable to assume that this is likely to be the underlying cause of the patient's phenotype. No published reports of a similar deletion were identified.

Case 7

Case 7 was a 22 year old girl presenting with learning disability, seizures, microcephaly, and synbrachydactyly. Array-CGH identified a deletion at 21q22, including the minimal critical region duplicated in Down's syndrome.³³ This 7 Mb deletion was not visible cytogenetically and was shown by genotyping to be apparently de novo.

Case 8

Case 8 was a four year old boy presenting with microcephaly, Dandy-Walker malformation, agenesis of the corpus callosum, short stature, and moderate learning disability. A duplication involving two clones at 3p26.2–26.3 was identified by array-CGH. The duplication was confirmed by quantitative FISH analysis, and shown by the same method to be inherited from his phenotypically normal father.

Case 9

Case 9 was a 19 year old boy presenting with severe learning disability, postnatal growth retardation, and facial dysmorphism. A duplication involving one single clone was found at 6q13. We confirmed the presence of this rearrangement and showed that it is about 600 kb in length by using a tiling path BAC microarray covering the whole of chromosome 6 (data not shown). However, the duplication was also detected in the phenotypically normal mother, thus suggesting that its occurrence may be independent of the observed phenotype.

Case 10

Case 10 was a 13 year old boy with microcephaly, learning disability, dysmorphic facial features, and a kyphoscoliosis. Although this patient showed a de novo, apparently balanced, translocation (46, XY, t(1;5)(p32q15)), he was accepted into the study because his phenotype seemed too severe to be explained by the translocation alone. We suspected that a loss or gain of chromosomal material at one or other of the translocation breakpoints might be detected by array-CGH. However, no copy number changes were identified on the array at the breakpoints. A deletion of two clones at 13q33.3–34 was identified and confirmed by FISH analysis. This deletion was inherited from the boy's phenotypically normal mother. Whether one or both of these chromosomal rearrangements is related to the phenotype is difficult to determine.

Case 11

Case 11 was an eight year old girl presenting with severe learning disability, facial dysmorphism, seizures, ataxia, and hyperphosphatasia. A duplication spanning two clones in Xp22.3 was identified by array-CGH; analysis of parental DNA by the same method indicated that this was inherited from her phenotypically normal father. One brother of this patient, who has the same clinical traits, did not show the Xp22.3 duplication by array-CGH, indicating that this

duplication is unlikely to be the cause of the observed phenotype. Interestingly, the locus was previously shown to be deleted in a subset of patients with learning disability.³⁴ The investigators concluded that one member of a gene family, *VCX-A*, was specifically deleted in patients with learning disability. Our finding suggests that the involvement of the Xp22.3 locus in syndromic learning disability needs to be further investigated.

Case 12

Case 12 was a nine year old boy presenting with developmental delay, facial dysmorphism, cryptorchidism, and a supernumerary nipple. Two distinct rearrangements were identified by array-CGH—a small maternally inherited duplication involving a single clone at Xq11.2, and a deletion of the long arm of the Y chromosome which was apparently de novo. As previously mentioned, the region involved in the Y chromosome deletion is mainly composed of palindromic repeats³⁵; we therefore postulate that the learning disability is more likely to be linked to the Xq11.2 duplication. In support of this hypothesis, the mother showed a bias in chromosome X inactivation (80%/20%; data not shown), although the duplication itself could possibly drive this bias.

DISCUSSION

We have used array-CGH to investigate patients with learning disability and dysmorphic features. We used a whole genome array with an average resolution of 1 Mb (approximately 3500 clones in total) to analyse 50 previously undiagnosed patients with learning disability and dysmorphism. We identified abnormalities of copy number in 12 patients (24% of the total), discounting two Y chromosome rearrangements. Each deletion or duplication was detected in a single experiment without previous knowledge of the genomic region involved. Seven of the abnormalities identified (14% of patients) were autosomal deletions or duplications that had not been inherited, and so were thought likely to account for the phenotype of the patient. In the majority (41/50) of the patients included in the study, a subtelomeric abnormality had been excluded before enrolment; in keeping with this, no subtelomeric abnormalities were identified by array-CGH in the study group.

The diagnostic yield of microdeletions or microduplications we found in our 50 patients is comparable with that obtained in a similar recently published study of 20 patients.¹⁸ The proportions of de novo and inherited rearrangements are comparable, and interestingly, there are no imbalances that are common to the two groups of patients.

The identification of non-inherited microdeletions or microduplications in patients with severe phenotypes is presumptive evidence that the rearrangement and the phenotype are causally related. For de novo rearrangements visible by chromosome banding, the causal relation between karyotype and phenotype is usually accepted, even where the abnormality has not been reported previously. However, the abnormalities that we and others¹⁸ have identified by array-CGH represent a new class of genomic rearrangement, differing significantly in size from those usually detected by karyotypic analysis. Currently, there is insufficient information available to determine how many of these copy number differences are of pathological significance. Additional data, gained both from the study of patients and from normal individuals, will be needed in order to address this question.

Five of the observed copy number changes were inherited from phenotypically normal parents. In this situation it is probably more likely that the copy number change is an incidental finding, and not the cause of the diagnosed phenotype. It is possible that these rearrangements represent genomic polymorphisms of no pathological significance. This

underlines the need for a systematic study of copy number polymorphisms in a large population of normal controls. However, the existence of inherited cytogenetically visible rearrangements involving euchromatic chromosomal regions is well recognised. The following examples have been documented as present in both phenotypically abnormal children and in their phenotypically normal parents: del (5)(p14.3-p14.3); del (7)(q14.3-q14.3); del (11)(q14.3-q14.3); del (13)(q14-q14); dup (1)(q42.11-q42.12); dup (3)(q25-q25); dup (4)(q31.3-q33); dup (6)(q24.2-q24.2); and dup (14)(q24.3-q31) (<http://www.som.soton.ac.uk/research/geneticsdiv/anomaly%20register/index.html>). Other euchromatic deletions and duplications have been reported in phenotypically normal individuals. Examples include del (2)(q13-q14.1), del (8)(p23.1/2-pter), del (11)(p12); dup(1)(p21-p31), dup (8)(p23.1-p23.3), and dup (18)(p11.2-pter). None of the rearrangements reported here coincides with any of these previously reported examples. Alternatively, it is also possible that the inherited deletions and duplications described are pathogenic but show non-penetrance in the parent or may be imprinted. Non-penetrance has been described previously, for example in the DiGeorge syndrome, where apparently phenotypically normal deletion carriers have been reported.³⁶

The detection rate for abnormalities in this series is 24%. After exclusion of those abnormalities that were inherited, the detection rate is 14%. Although patients with previously detected telomeric rearrangements were excluded from our study, such patients (6%) who would most probably have been detected as telomeric regions are represented on our array. In general use, one may therefore expect array-CGH with a 1 Mb resolution array to detect de novo copy number abnormalities in about 20% of patients. It is likely that the detection rate could be further improved by the introduction of arrays with higher resolution, although diminishing returns with increasing resolution are probable. In our study, the size of the region involved in the copy number abnormality was less than 10 Mb in the majority of cases. It is unlikely that these abnormalities would have been detected by conventional metaphase CGH. Eight of our patients showed rearrangements that were less than 3 Mb in size, which would most probably have remained undetected even using high resolution metaphase CGH.⁴ Our study shows that array-CGH is a powerful tool for the investigation of chromosomal changes associated with mental disability and dysmorphism.

ACKNOWLEDGEMENTS

We are grateful to Arnold Munnich, Michel Vekemans, Stanislas Lyonnet, Jeanne Amiel, Valérie Cormier-Daire, and Martine le Merrer for clinical support. We would like to thank Andrew Mungall and Koichi Ichimura for constructing the chromosome 6 tiling path array and the Wellcome Trust Sanger Institute microarray facility for printing arrays. We are grateful to clinicians at Addenbrooke's and Great Ormond Street hospitals for referring patients. We thank Leeanne Sparnon, James Cox, and Lucy Raymond for expert technical assistance, and Christine Hall, Clare Brady, and Sandra Wombwell for administrative help. This work was supported by the Wellcome Trust and the Programme Hospitalier de Recherche Clinique No AOM 02 122.

Authors' affiliations

C Shaw-Smith, L Rickman, H Firth, M Bobrow, University of Cambridge Department of Medical Genetics, Addenbrooke's Hospital, Hills Road, Cambridge, UK

R Redon, H Fiegler, N P Carter, The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK

M Rio, D Sanlaville, L Colleaux, Département de Génétique and INSERM U393, Hôpital Necker-Enfants Malades, rue de Sèvres, Paris, France

L Willatt, Regional Cytogenetics Laboratory, Addenbrooke's Hospital, Cambridge

R Winter, Department of Clinical and Molecular Genetics, Institute of Child Health, London WC1, UK

Conflicts of interest: none declared

REFERENCES

- 1 **Flint J**, Wilkie AO, Buckle VJ, Winter RM, Holland AJ, McDermid HE. The detection of subtelomeric chromosomal rearrangements in idiopathic mental retardation. *Nat Genet* 1995;**9**:132-40.
- 2 **De Vries BB**, Winter R, Schinzel A, van Ravenswaaij-Arts C. Telomeres: a diagnosis at the end of the chromosomes. *J Med Genet* 2003;**40**:385-98.
- 3 **Biesecker LG**. The end of the beginning of chromosome ends. *Am J Med Genet* 2002;**107**:263-6.
- 4 **Kirchhoff M**, Rose H, Lundsteen C. High resolution comparative genomic hybridisation in clinical cytogenetics. *J Med Genet* 2001;**38**:740-4.
- 5 **Ness GO**, Lybaek H, Houge G. Usefulness of high-resolution comparative genomic hybridization (CGH) for detecting and characterizing constitutional chromosome abnormalities. *Am J Med Genet* 2002;**113**:125-36.
- 6 **Albertson DG**, Pinkel D. Genomic microarrays in human genetic disease and cancer. *Hum Mol Genet* 2003;**12**(spec No 2):R145-52.
- 7 **Hodgson G**, Hager JH, Volik S, Hariono S, Wernick M, Moore D, Nowak N, Albertson DG, Pinkel D, Collins C, Hanahan D, Gray JW. Genome scanning with array-CGH delineates regional alterations in mouse islet carcinomas. *Nat Genet* 2001;**29**:459-64.
- 8 **Cai WW**, Mao JH, Chow CW, Damani S, Balmain A, Bradley A. Genome-wide detection of chromosomal imbalances in tumours using BAC microarrays. *Nat Biotechnol* 2002;**20**:393-6.
- 9 **Wilhelm M**, Veltman JA, Olshen AB, Jain AN, Moore DH, Presti JC, Kovacs G, Waldman FM. Array-based comparative genomic hybridization for the differential diagnosis of renal cell cancer. *Cancer Res* 2002;**62**:957-60.
- 10 **Fiegler H**, Carr P, Douglas EJ, Burford DC, Hunt S, Scott CE, Smith J, Vetrie D, Gorman P, Tomlinson IP, Carter NP. DNA microarrays for comparative genomic hybridization based on DOP-PCR amplification of BAC and PAC clones. *Genes Chromosomes Cancer* 2003;**36**:361-74.
- 11 **Kraus J**, Pantel K, Pinkel D, Albertson DG, Speicher MR. High-resolution genomic profiling of occult micrometastatic tumour cells. *Genes Chromosomes Cancer* 2003;**36**:159-66.
- 12 **Paris PL**, Albertson DG, Alers JC, Andaya A, Carroll P, Fridlyand J, Jain AN, Kamkar S, Kowbel D, Krijtenburg PJ, Pinkel D, Schroder FH, Vissers KJ, Watson VJ, Wildhagen MF, Collins C, Van Dekken H. High-resolution analysis of paraffin-embedded and formalin-fixed prostate tumours using comparative genomic hybridization to genomic microarrays. *Am J Pathol* 2003;**162**:763-70.
- 13 **Bruder CE**, Hirvela C, Tapia-Paez I, Fransson I, Segraves R, Hamilton G, Zhang XX, Evans DG, Wallace AJ, Baser ME, Zucman-Rossi J, Hergersberg M, Boltschauser E, Papi L, Rouleau GA, Poptodorov G, Jordanova A, Rask-Andersen H, Kluwe L, Mautner V, Sainio M, Hung G, Mathiesen T, Moller C, Pulst SM, Harder H, Heiberg A, Honda M, Niimura M, Sahlen S, Blennow E, Albertson DG, Pinkel D, Dumanski JP. High resolution deletion analysis of constitutional DNA from neurofibromatosis type 2 (NF2) patients using microarray-CGH. *Hum Mol Genet* 2001;**10**:271-82.
- 14 **Yu W**, Ballif BC, Kashork CD, Heilstedt HA, Howard LA, Cai WW, White LD, Liu W, Beaudet AL, Bejjani BA, Shaw CA, Shaffer LG. Development of a comparative genomic hybridization microarray and demonstration of its utility with 25 well-characterized 1p36 deletions. *Hum Mol Genet* 2003;**12**:2145-52.
- 15 **Veltman JA**, Jonkers Y, Nuijten I, Janssen I, van der Vliet W, Huys E, Vermeesch J, Van Buggenhout G, Fryns JP, Admiraal R, Terhal P, Lacombe D, van Kessel AG, Smeets D, Schoenmakers EF, van Ravenswaaij-Arts CM. Definition of a critical region on chromosome 18 for congenital aural atresia by arrayCGH. *Am J Hum Genet* 2003;**72**:1578-84.
- 16 **Veltman JA**, Schoenmakers EF, Eussen BH, Janssen I, Merckx G, van Cleef B, van Ravenswaaij CM, Brunner HG, Smeets D, van Kessel AG. High-throughput analysis of subtelomeric chromosome rearrangements by use of array-based comparative genomic hybridization. *Am J Hum Genet* 2002;**70**:1269-76.
- 17 **Buckley PG**, Mantripragada KK, Benetkiewicz M, Tapia-Paez I, Diaz De Stahl T, Rosenquist M, Ali H, Jarbo C, De Bustos C, Hirvela C, Sinder Wilen B, Fransson I, Thyrc C, Johnsson BI, Bruder CE, Menzel U, Hergersberg M, Mandahl N, Blennow E, Wedell A, Beare DM, Collins JE, Dunham I, Albertson D, Pinkel D, Bastian BC, Faruqi AF, Lasken RS, Ichimura K, Collins VP, Dumanski JP. A full-coverage, high-resolution human chromosome 22 genomic microarray for clinical and research applications. *Hum Mol Genet* 2002;**11**:3221-9.
- 18 **Vissers LE**, De Vries BB, Osoegawa K, Janssen IM, Feuth T, Choy CO, Straatman H, Van Der Vliet W, Huys EH, Van Rijk A, Smeets D, Van Ravenswaaij-Arts CM, Knoers NV, Van Der Burgt I, De Jong PJ, Brunner HG, Van Kessel AG, Schoenmakers EF, Veltman JA. Array-based comparative genomic hybridization for the genomewide detection of submicroscopic chromosomal abnormalities. *Am J Hum Genet* 2002;**73**:1261-70.
- 19 **Rost I**, Fiegler H, Fauth C, Carr P, Bettecken T, Kraus J, Meyer C, Enders A, Wirtz A, Meifinger T, Carter NP, Speicher MR. Tetrasomy 21pter-q21.2 in a male infant without typical Down syndrome dysmorphic features but moderate mental retardation. *J Med Genet*.
- 20 **Fiegler H**, Gribble SM, Burford DC, Carr P, Prigmore E, Porter KM, Clegg S, Crolla JA, Dennis NR, Jacobs P, Carter NP. Array painting: a method for the

- rapid analysis of aberrant chromosomes using DNA microarrays. *J Med Genet* 2003;**40**:664–70.
- 21 **Jain AN**, Tokuyasu TA, Snijders AM, Seagraves R, Albertson DG, Pinkel D. Fully automatic quantification of microarray image data. *Genome Res* 2002;**12**:325–32.
 - 22 **Rooney DE**. Human Cytogenetics: constitutional analysis, a practical approach, 3rd ed. Oxford: IRL Press at Oxford University Press, 2001.
 - 23 **Barber JC**, Cross IE, Douglas F, Nicholson JC, Moore KJ, Browne CE. Neurofibromatosis pseudogene amplification underlies euchromatic cytogenetic duplications and triplications of proximal 15q. *Hum Genet* 1998;**103**:600–7.
 - 24 **Colleaux L**, Heuertz S, Molinari F, Rio M. Fluorescence genotyping for screening cryptic telomeric rearrangements. *Methods Mol Biol* 2002;**204**:181–9.
 - 25 **Heilstedt HA**, Ballif BC, Howard LA, Lewis RA, Stal S, Kashork CD, Bacino CA, Shapira SK, Shaffer LG. Physical map of 1p36, placement of breakpoints in monosomy 1p36, and clinical characterization of the syndrome. *Am J Hum Genet* 2003;**72**:1200–12.
 - 26 **Halal F**, Vekemans M, Der Kaloustian VM. A presumptive translocation 1p;2q resulting in duplication 1p and deletion 2q. *Am J Med Genet* 1989;**32**:376–9.
 - 27 **Bene M**, Duca-Marinescu A, Ioan D, Maximilian C. De novo interstitial deletion del(1)(p21p32). *J Med Genet* 1979;**16**:323–7.
 - 28 **Lai M**, Robards M, Berry A, Fear C, Hart C. Two cases of interstitial deletion 1p. *J Med Genet* 1991;**28**:128–130.
 - 29 **Petersen MB**, Warburg M. Interstitial deletion 1p in a 30 year old woman. *J Med Genet* 1987;**24**:229–31.
 - 30 **Olivieri C**, Maraschio P, Caselli D, Martini C, Beluffi G, Maserati E, Danesino C. Interstitial deletion of chromosome 9, int del(9)(9q22.31–q31.2), including the genes causing multiple basal cell nevus syndrome and Robinow/brachydactyly 1 syndrome. *Eur J Pediatr* 2003;**162**:100–3.
 - 31 **Khalifa MM**, MacLeod PM, Duncan AM. Additional case of de novo interstitial deletion del(17)(q21.3q23) and expansion of the phenotype. *Clin Genet* 1993;**44**:258–61.
 - 32 **Park JP**, Moeschler JB, Berg SZ, Bauer RM, Wurster-Hill DH. A unique de novo interstitial deletion del(17)(q21.3q23) in a phenotypically abnormal infant. *Clin Genet* 1992;**41**:54–6.
 - 33 **Rahmani Z**, Blouin JL, Creau-Goldberg N, Watkins PC, Mattei JF, Poissonnier M, Prieur M, Chettouh Z, Nicole A, Aurias A, et al. Critical role of the D21S55 region on chromosome 21 in the pathogenesis of Down syndrome. *Proc Natl Acad Sci USA* 1989;**86**:5958–62.
 - 34 **Fukami M**, Kirsch S, Schiller S, Richter A, Benes V, Franco B, Muroya K, Rao E, Merker S, Niesler B, Ballabio A, Ansong W, Ogata T, Rappold GA. A member of a gene family on Xp22.3, VCX-A, is deleted in patients with X-linked nonspecific mental retardation. *Am J Hum Genet* 2000;**67**:563–73.
 - 35 **Skaletsky H**, Kuroda-Kawaguchi T, Minx PJ, Cordum HS, Hillier L, Brown LG, Repping S, Pyntikova T, Ali J, Bieri T, Chinwalla A, Delehaunty A, Delehaunty K, Du H, Fewell G, Fulton L, Fulton R, Graves T, Hou SF, Latrielle P, Leonard S, Mardis E, Maupin R, McPherson J, Miner T, Nash W, Nguyen C, Ozersky P, Pepin K, Rock S, Rohlfing T, Scott K, Schultz B, Strong C, Tin-Wollam A, Yang SP, Waterston RH, Wilson RK, Rozen S, Page DC. The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes. *Nature* 2003;**423**:825–37.
 - 36 **Wilson DI**, Goodship JA, Burn J, Cross IE, Scambler PJ. Deletions within chromosome 22q11 in familial congenital heart disease. *Lancet* 1992;**340**:573–5.

ECHO

DJ-1 mutations in Parkinson's disease

D G Healy, P M Abou-Sleiman, E M Valente, W P Gilks, K Bhatia, N Quinn, A J Lees, N W Wood



Please visit the *Journal of Medical Genetics* website [www.jmedgenet.com] for a link to the full text of this article.

Mutations in the DJ-1 gene have recently been shown to cause autosomal recessive Parkinson's disease. To estimate the prevalence of this mutation, an analysis was undertaken of 39 index cases of Parkinson's disease in whom a family history suggested autosomal recessive inheritance. No DJ-1 mutations were found in these patients, indicating that this gene is unlikely to be of numerical significance in clinical practice. The hypothesis was also tested that young onset Parkinson's disease patients in whom, despite extensive analysis, only a single heterozygous parkin mutation was found, might harbour a second mutation in the DJ-1 gene—that is, digenic inheritance. No patient was found with a single mutation in both DJ-1 and parkin genes, making this mode of inheritance unlikely. Finally it was confirmed that PARK6 and PARK7 (DJ-1), despite being phenotypically similar and mapping to the same small chromosomal region of 1p36, are caused by mutations in separate genes.

▲ *Journal of Neurology, Neurosurgery, and Psychiatry* 2004;**75**:144–145.