Chromosome microarray analysis in a clinical environment: new perspective and new challenge

A. GEORGE*, R. MARQUIS-NICHOLSON*, L T ZHANG*, J. M. LOVE*, F. ASHTON*, S. AFTIMOS†, I. HAYES†, L. C. WILLIAMS‡ and D. R. LOVE*‡

Diagnostic Genetics, LabPLUS and †Northern Regional Genetic Service, Auckland City Hospital; and ‡School of Biological Sciences, University of Auckland, Auckland, New Zealand

Accepted: 13 December 2010

ABSTRACT

The analysis of the human genome has largely been undertaken in a research environment, but recent developments in technology and associated workflow have allowed diagnostic laboratories to interrogate DNA at significantly improved levels of resolution. Principally, whole genome-based analysis of copy number changes using microarrays has led to this method replacing conventional karyotyping as a routine diagnostic workhorse. The resolution offered by microarrays is an improvement of at least an order of magnitude compared to karyotyping, but it comes at a cost in terms of the time spent in data interpretation. Overall, however, the die has been cast and cytogeneticists need to become familiar with the tools used by molecular geneticists and bioinformaticists. The following review provides a brief background to array technology, but uses a series of case studies to illustrate the usefulness and challenges of interpreting array data.

KEY WORDS: Chromosomes.
Cytogenetics.
Karyotyping.
Microarray analysis.

Introduction

Conventional cytogenetics has been based on the premise of phenotype first. In other words, the clinician identifies the suspected genetic abnormality and the laboratory will either confirm or refute the suspicion. Down's syndrome is the classical example of this premise. John Langdon Down collected information on individuals displaying the characteristic 'Mongoloid' features with intellectual deficiency. It was not until the advent of conventional cytogenetics in which chromosomes could be analysed microscopically that LeJeune *et al*.[1] defined the genotype as having an additional chromosome 21 (trisomy 21).

Improvements in identifying chromosome substructure in the 1970s by trypsin pretreatment and staining with Giemsa (termed G-banding) allowed further characterisation of genotypes associated with specific phenotypes,[2] such as the association of Cri du Chat syndrome with a microscopically detectable deletion of the short arm of chromosome 5.[3] As chromosome preparations became more sophisticated, the detection of deletions and duplications became more complex. This led to the delineation of several microdeletion syndromes[4] (e.g., Miller-Dieker syndrome associated with a microdeletion of chromosome 17p13.3). The microscopic analysis of chromosomes reached its limits with high-resolution banded analysis, which allowed the detection of anomalies in the region of 3–5 megabases (Mb) in size compared with an average 5–10 Mb detected by conventional G-banding analysis.[4]

In the 1990s fluorescence *in situ* hybridisation (FISH) was developed, which is a technique that uses fluorescently labelled probes comprising DNA of varying lengths that are hybridised to a patient's chromosomes either in metaphase or interphase.[2] The detection of the fluorescent signal indicates the presence and copy number of the probed region in the patient's genome. This technique has allowed the identification of many single-gene disorders and of rearrangements that are either constitutional (inherited) or acquired. For example, submicroscopic deletions of chromosome 15 and 22 define Prader-Willi and Di George syndromes, respectively, and the ETV6/RUNX1 cryptic rearrangement involving chromosomes 12 and 21 characterises a subtype of acute lymphoblastic leukaemia. Fluorescence *in situ* hybridisation can be used to investigate single or multiple loci;[4,5] however, it can only examine a limited number of regions per hybridisation event. In keeping with other conventional cytogenetic techniques, it requires clinical suspicion of the genes involved.[6,7] For all of the above methods and disorders, the clinician or scientist first has to know what question to ask, reinforcing the concept of 'phenotype first'.[8]

Critically, the principal challenge for the clinician lies with the early childhood referrals of developmental delay (DD), autistic spectrum disorder (ASD) and multiple congenital anomalies (MCA). Parents are concerned about the cause of these disorders and the likelihood of it recurring in any subsequent children. Conventional cytogenetic techniques can only identify a chromosomal anomaly in approximately 3% of these referrals.[9] It is against this background that recent molecular techniques have played a large part in revealing the genetic complexity of the human genome and providing much-needed information for the clinician. These developments can be thought of as falling under the general title of molecular karyotyping.

Correspondence to: Dr Donald R. Love
LabPlus, Auckland City Hospital, Auckland, New Zealand
Email: donaldl@adhb.govt.nz

Molecular karyotyping

The recent development of molecular cytogenetic techniques has introduced the possibility of 'genotype first' genetics, and may answer some of the questions surrounding the less well-defined anomalies. It is no longer necessary for the clinician or scientist to know the genes of interest, although it helps when analysing the data, as will be demonstrated in the cases described below. The first of the molecular techniques, termed comparative genomic hybridisation (CGH), involves the hybridisation of differentially labelled total human DNAs (control and patient) to a metaphase spread of normal human chromosomes. This approach involves the analysis of signal intensities to identify copy number changes between control and patient DNAs, but critically it allows analysis of the whole genome as opposed to the simultaneous interrogation of one or a limited number of genomic regions (loci).

Chromosome microarray analysis (CMA) has largely superseded CGH by using the same hybridisation but against an immobilised array of defined DNA probes as either cloned genomic fragments or, more recently, as short single-stranded DNAs of known unique sequence. The development of appropriate analytical software has led to the high-resolution identification of loss or gain of fluorescence signal, which indicates deletion or duplication in the patient, respectively. At present, array technology will not detect balanced rearrangements, nor identify the location (as opposed to the extent) of a copy number gain.

Deletions and duplications are derived from chromosome recombination. Within the genome there are multiple regions of DNA, often of thousands of base pairs in length, which have sequence similarity throughout the genome.[10] This similarity occasionally leads to the misalignment of chromosome regions and pairing where the homology is either identical or near enough to allow chromosome breakage and reunion. This is referred to as non-allelic homologous recombination (NAHR), and the regions that are involved in this event are referred to as 'duplicons'.[11] These duplicons are highly homologous (>95%) sequences and can flank a relatively small region (usually <5 Mb).[12–14] If the mismatch is not repaired then the recombinant products will be reciprocally imbalanced, one being deleted, the other duplicated. Deletions can arise from intrachromatid rearrangements, as well as sister chromatid or homologous/non-homologous chromosome exchange, whereas duplications are not caused by intrachromatid rearrangements (Fig. 1).[15–17] Duplications may be arranged in a tandem fashion within the same chromosome region, different regions on the same chromosome, or may be on an entirely different chromosome. The last two cases may occur as a result of an unbalanced product from a parental reciprocal translocation, or recombination between two homologous chromosomes, one of which has a pericentric inversion (Fig. 2).

The software used by many diagnostic laboratories that

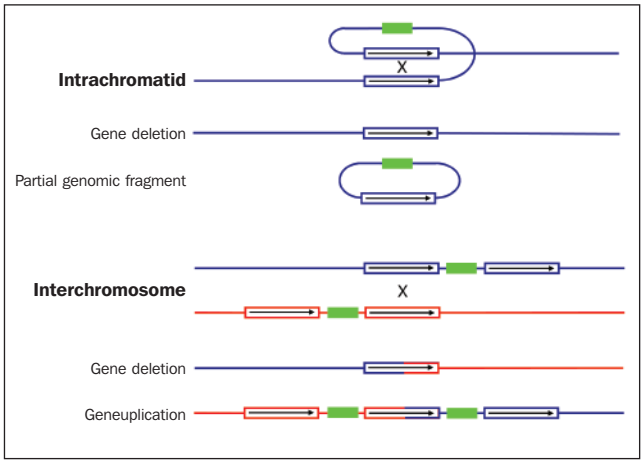

Fig. 1. Chromosome exchanges that give rise to deletion and duplication events. Intrachromatid and interchromosome (sister chromatid or homologous chromosomes) exchanges are shown with a gene (or group of genes) represented in green and flanking duplicons shown as open blue boxes. In the case of an intrachromatid exchange, the partial genomic fragment is not retained. The reciprocal events of an interchromosome exchange result in deletion and duplication outcomes.

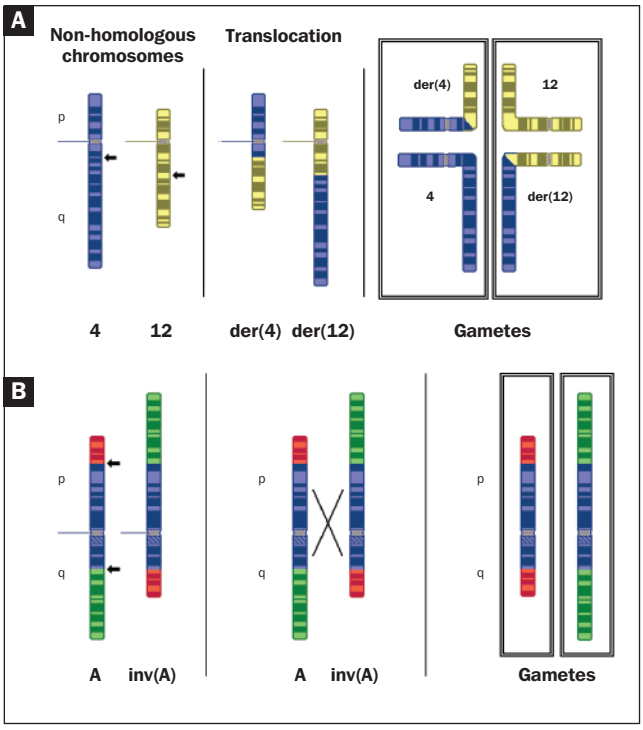

Fig. 2. Chromosome exchanges that give rise to large deletion and duplication events. Panel A shows a translocation event between two non-homologous chromosomes that give rise to two derivative chromosomes. The tetravalent at meiosis results in several segregation events, two of which are boxed that comprise unbalanced gametes, one carrying only a single copy of the telomeric end of the long arm of chromosome 4, and the other carrying two copies of most of chromosome 12.
Panel B shows an exchange between a normal chromosome (designated A) and a pericentric inversion of the same chromosome (designated inv[A]). The telomeric ends of the short and long arms of chromosome A are shown in red and green, respectively. An exchange between these chromosomes results in unbalanced gametes, one of which carries two copies of the telomeric end of the short arm of chromosome A (and no copies of the telomeric end of the long arm), and the other carrying two copies of the telomeric end of the long arm of chromosome A (and no copies of the telomeric end of the short arm).

use CMA is usually set to detect deletions >200 kilobases (kb) and duplications greater than 500 kb across the entire genome; the more dense the number of DNA probes on the array, the greater the level of sensitivity, but with the added complication of more spurious 'calls'. In the event of a 'real' abnormality, many copy number changes will be detected that are of unknown significance, as well as those known to be a benign normal copy number variant (CNV). Benign CNVs reflect the vast amount of variation that exists in the normal population;[8,18] each individual will have a varying number of copies of particular DNA sequences with no phenotypic effect.[19–21] Copy number variants are defined as chromosomal segments of more than 1 kb in length whose copy number varies, due to tandem arrays of repeats, deletions or duplications, between individuals in the population.[22,23] The term CNV is used to describe all quantitative variation occurring in the genome. The majority of CNVs are benign, but occasionally they are linked to single gene/region disorders with variable penetrance.[24] The use of CMA, coupled with the recurrence of either microdeletions or microduplications, has allowed the aetiology of some intellectual and/or dysmorphic phenotypes to be characterised at the genetic level.[8]

The following case studies provide examples of the advantages, as well as the limitations, of CMA technology. The data have been derived exclusively from our laboratory's use of the Affymetrix Genome-wide Human SNP Array 6.0 or Affymetrix Cytogenetics Whole-Genome 2.7M Array that comprise 1.8 million and 2.7 million probes, respectively. It should be noted that although these data sets

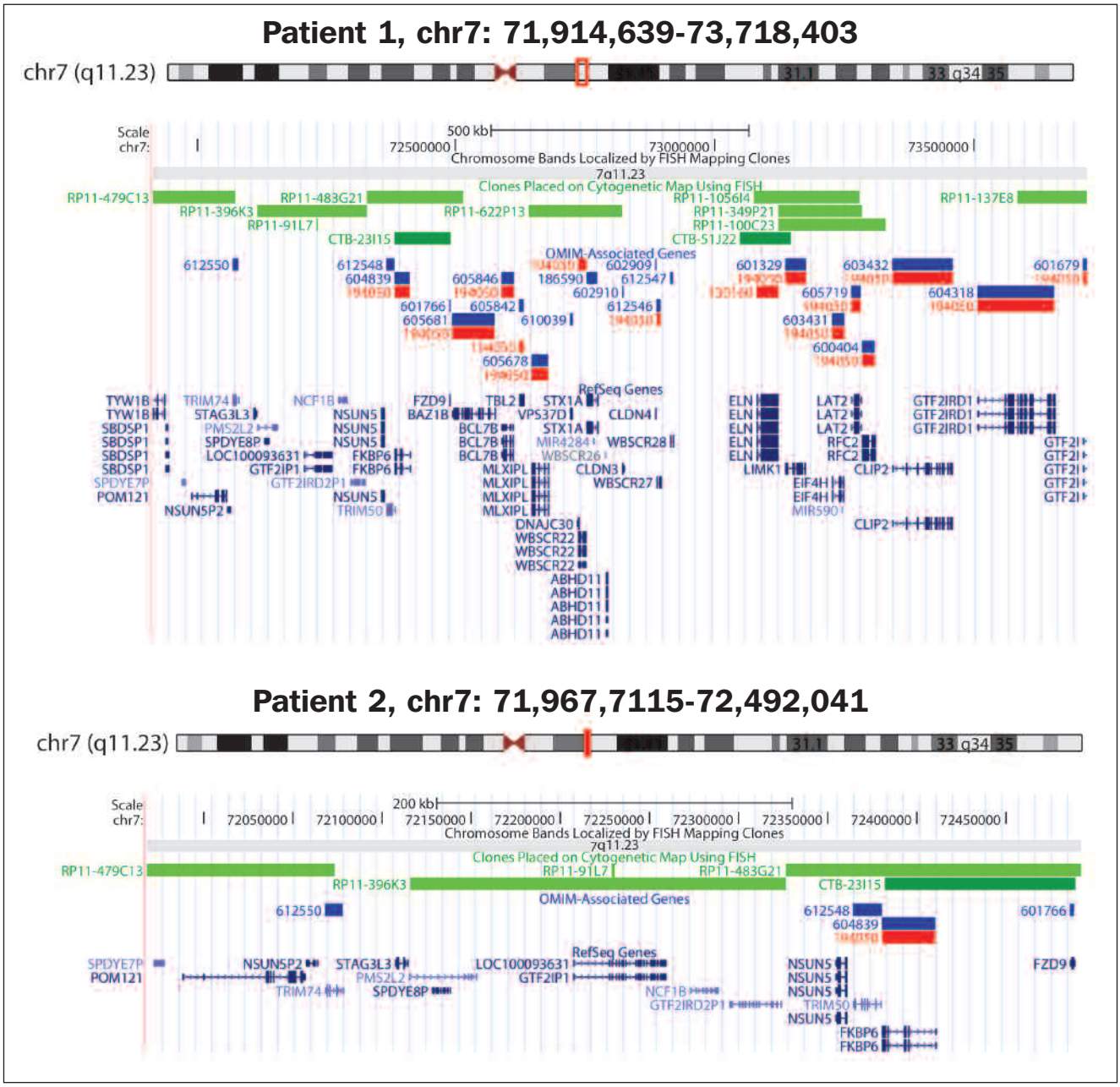

Fig. 3. Location and extent of interstitial duplications in patients 1 and 2. The upper panel shows an ideogram of the relevant chromosomal regions carrying the proposed duplication events, and the FISH probes and genes that are localised to these regions (taken from the UCSC genome browser http://genome.ucsc.edu).

enable the identification of regions of homozygosity, for the purposes of this discussion only copy number changes will be discussed (Box 1). Regions of copy number change were calculated using Affymetrix Genome Console v.3.0.2 or Chromosome Analysis Suite (ChAS) v.1.0.1 software, and interpreted with the aid of the UCSC genome browser (http://genome.ucsc.edu/; Human Mar. 2006 [hg18] assembly).

Case studies: duplication events

The following cases describe both the variation in phenotype and the various techniques used for the resolution of a relatively rare microduplication involving the proximal region of the long arm of chromosome 7.

Patient 1

A female child was referred to the medical geneticist at age four years with mild language delay and the minor dysmorphic feature of a slightly short philtrum but little else of note. The standard G-banding karyotype result was normal. The CMA analysis (Affymetrix Genome-wide Human SNP Array 6.0 chip) revealed a molecular karyotype of arr 7q11.23 (71914639–73718403)x3. The data suggested three copies of approximately 1.8 Mb of a defined region of chromosome 7 (Fig. 3). This result was confirmed using a complementary dosage assay termed multiplex ligation-dependent probe amplification (MLPA).[31] Neither CMA nor MLPA could identify whether the duplication was segmental or occurred within an entirely different region of the genome. Fluorescence *in situ* hybridisation using BAC clone RP11-396K3 specific for the 7q11.23 region confirmed that a tandem duplication event on one chromosome was more likely as only two fluorescent signals were detected as opposed to three (Fig. 4).

Patient 2

A 38-year-old primigravida mother was referred to clinic due to a fetal nuchal translucency measurement of 5.6 mm. The fetus had a normal female karyotype at 15 weeks' gestation by conventional G-banding analysis. An ultrasound scan at 32+3 weeks revealed IUGR and microcephaly. Detailed examination revealed an enlarged fetal heart and significant brachycardia. The mother elected to terminate the pregnancy. Post-mortem revealed broad, prominent nasal root, microcephaly, low-set posteriorly rotated ears and low hairline. Campodactyly of the fourth and fifth fingers of both hands was found, together with an abnormally long toe on the left side. The brain had a notably thick dura; histopathology revealed evidence of white matter gliosis and apparent loss of cortical levels 3 and 5. The heart had an atrial septal defect and endocardial thickening. Chromosome microarray analysis was performed on DNA extracted from post-mortem spleen tissue (Affymetrix Genome-wide Human SNP Array 6.0 chip) and revealed a molecular karyotype of arr 7q11.23 (71967715–72492041)x3; a duplication of approximately 524 kb (Fig. 3).

Duplications in chromosome 7q have been reported in about 30 cases since 2005.[17,32–35] There is no distinct phenotype; however, speech delay is frequently mentioned. Cognitive abilities range from normal to moderate mental retardation, dysmorphic features include short philtrum, thin lips and straight eyebrows. Congenital abnormalities encompass heart defects and non-specific brain abnormalities including gliosis.[32,35]

Patients 1 and 2 share some of these phenotypic features and so they add to the phenotypic spectrum associated with this relatively new dup7q syndrome.

In the case of patient 1, the duplication of 7q11.23 is the reciprocal of the deletion event that is associated with Williams-Beuren syndrome (WBS), which is a neurodevelopmental disorder occurring in approximately one newborn in 7500.[36] The typical WBS deletion involves between 25 and 30 genes,[37–40] with haploinsufficiency accounting for aspects of the overall phenotype. A decrease in gene dosage for the elastin (*ELN*) gene is thought to explain some of the clinical phenotype, but additional genes such as *LIMK1, CYLN2* and *GTF21RD1* are linked to craniofacial and cognitive pathology.[41–44] The gene overdose found in patient 1 may account for the expressive language delay, sparing of visuospatial cognition and relative behavioural withdrawal, which is in direct contrast to the outgoing personality profile observed in WBS patients.[32]

The WBS deletion is mediated by NAHR between large flanking low-copy repeats (LCRs)[45] and facilitated by a

BOX 1. SNP ARRAYS AND COPY NUMBER ANALYSIS

A robust method of measuring copy number changes across the genome involves the use of arrays originally designed to detect single nucleotide polymorphisms (SNPs).[25] More than 11.5 million SNPs have been found to exist within the human genome.[26] They are known to contribute not only to population diversity and phenotypic differences between individuals, but also to cause predisposition to certain diseases, such as inflammatory bowel disease, age-related macular degeneration and type II diabetes mellitus.[27] As the importance of copy number variation became increasingly apparent, the manufacturers of SNP arrays (predominantly Illumina and Affymetrix) adapted these microarrays to allow them to offer both SNP and copy number analysis.[25] Together, array CGH and SNP-array copy number detection can be classified as array–based copy number analysis or chromosomal microarray analysis.[28]

An additional feature that SNP arrays offer over traditional CGH arrays is the ability to identify loss of heterozygosity and uniparental disomy. The SNP arrays do not require reference DNA to be hybridised with the test DNA as, instead, they obtain copy number by analysing hybridisation intensities using probes designed to detect individual alleles. Thus, through measurement of allelic ratios and intensity differences the profiling of both DNA copy number and copy neutral loss of heterozygosity is permitted.[5] Loss of heterozygosity can be acquired either as a result of deletion or mitotic recombination events (including uniparental disomy). It is very important in many malignant conditions,[27] including AML and various solid tumours.[30] Uniparental disomy is the causative factor in a range of genomic disorders, including Prader-Willi syndrome in which there is loss of the paternally derived copy of 15q11-13.

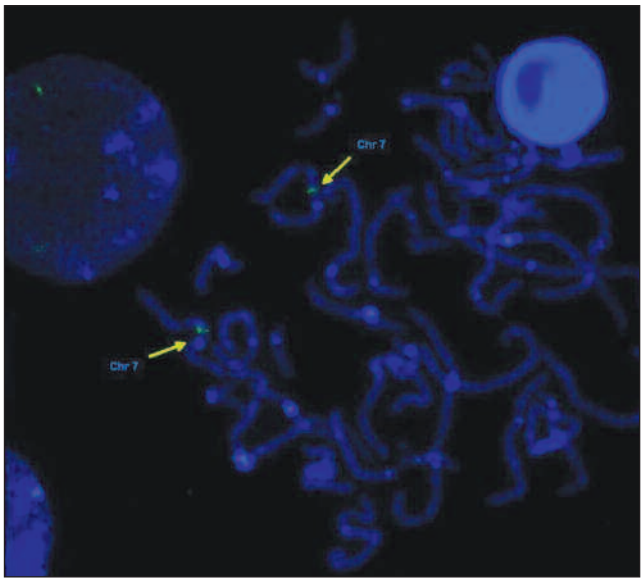

Fig. 4. Fluorescence *in situ* hybridisation analysis of patient 1.

structural variant in this region: an approximately 2 Mb paracentric inversion present in 20–25% of WBS-transmitting parents. It has been noted that there is a significantly higher frequency of a deletion-type CNV in WBS-transmitting parents which facilitate chromosome misalignment and recombination in meiosis.[45] The estimated frequency of the 7q11.23 duplication of one in 13,000–20,000 is lower than that of WBS.[35,46] This discrepancy agrees with expectations, given the molecular mechanisms underpinning each outcome.

Case studies: deletion events

A deletion may dictate a more severe phenotype than the corresponding duplication due to the haploinsufficiency of multiple contiguous genes; this was noted above in the relatively mild phenotype of the 7q duplication (patient 1) compared to the more severe WBS deletion syndrome. The following cases illustrate the detection of deletion events using CMA technology, but also underscore the pitfalls associated with the biological interpretation of the deletions.

Patient 3

A male seen at three years of age was referred due to ASD with generalised developmental delay. He was a premature baby born at 31 weeks' gestation with low Apgar scores of 2 and 3. At the time of examination he was hypotonic and small, with height below the 3rd centile for his age group. Prader-Willi syndrome was considered but molecular studies excluded this diagnosis.

There were no obvious dysmorphic features, and a conventional cytogenetic analysis showed a normal male karyotype. Chromosome microarray analysis (Affymetrix Cytogenetics Whole-Genome Array) revealed the molecular karyotype arr 16p11.2 (29522477–30107306)x1; a deletion of approximately 585 kb (Fig. 5). The analysis also revealed a deletion of 120 kb on chromosome 2, arr 2p21 (44,305,631–44,425,668)x1.

The interpretation of the above findings is not clear. The short arm of chromosome 16 is rich in intrachromosomal segmental duplications which may facilitate the NAHR required to produce either a duplication or a deletion.[10] There have been numerous reports of both deletions and duplications within this region.[47–52] All cases appear to

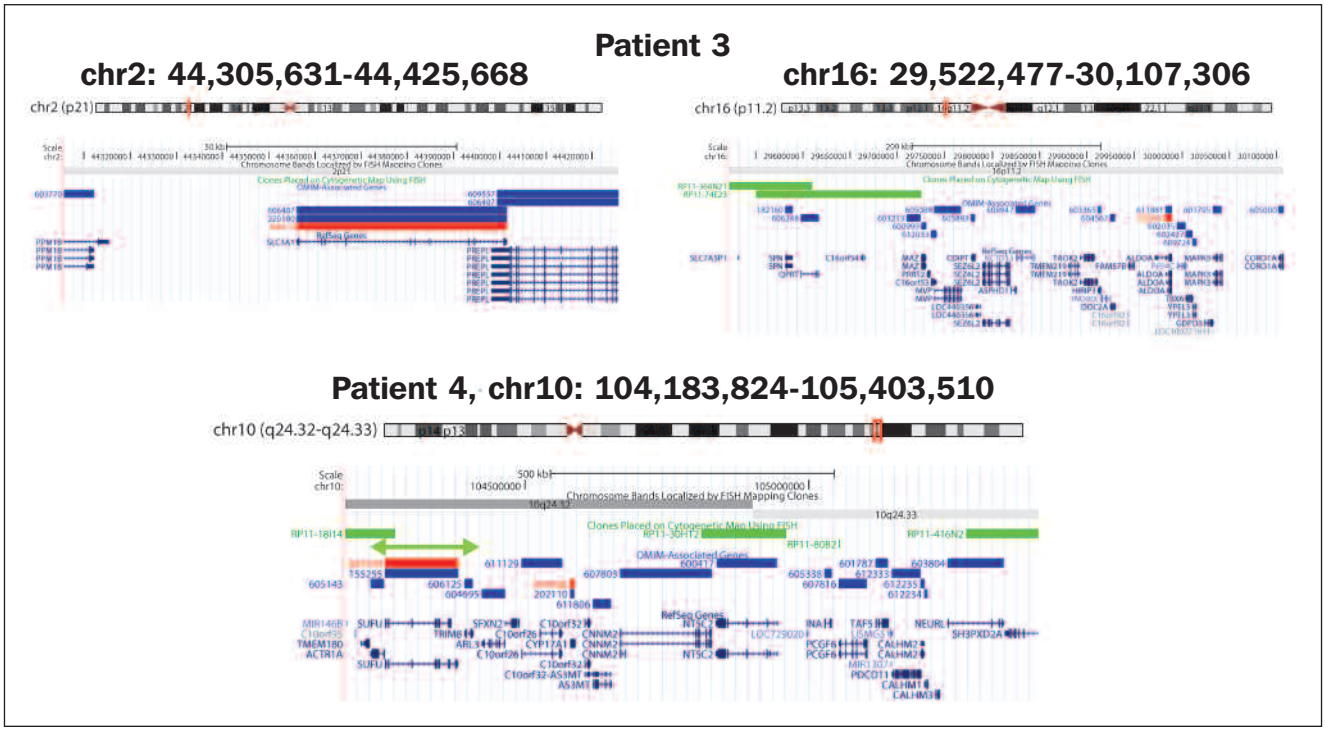

Fig. 5. Location and extent of interstitial duplications in patients 3 and 4. The upper panel shows an ideogram of the relevant chromosomal regions carrying the proposed deletion events, and the FISH probes and genes that are localised to these regions (taken from the UCSC genome browser http://genome.ucsc.edu). The green double arrowed line in the lower panel (patient 4) represents the location and extent of the region covered by the BAC clone RP11-2F13.

predispose the individual to mental retardation and/or autism. Indeed, the deletion 16p11.2 has been identified in up to 1% of autistic individuals.[50–52] The deletion can arise *de novo* or can be inherited from one parent. In the case of the latter, the parents have either a normal or milder phenotype. The variation in phenotype from the carrier parent to the affected child may be an example of a 'two hit' process.[53]

The deletion event might be a risk factor that acts in concert with a second factor to give rise to variation in the severity of neurodevelopmental disease The 'second hit' could be another CNV, a small disrupting mutation in a related gene or an environmental event; 70% of individuals presenting with autism also have a learning disability.[54] This two-hit hypothesis may also explain the co-morbidity that exists between cognitive impairment, autism and schizophrenia in addition to the previously mentioned variation associated with microdeletion/duplication syndromes.[55–57] In terms of patient 3, it is tempting to speculate that the 120 kb microdeletion of chromosome 2 may be the 'second hit' required for display of the clinical phenotype associated with a deletion of 16p11.2.

The chromosome 2p deletion harbours the *SLC3A* and *PREPL* genes. Loss of both genes is associated with a recessive contiguous gene deletion syndrome called hypotonia-cystinuria syndrome (HCS). This syndrome is characterised by neonatal and infantile hypotonia, growth retardation, mild facial dysmorphism and cystinuria type I.[58,59] Patient 3 showed both hypotonia and short stature, which may or may not be coincidental to the genotype.

Patient 4

A male referred at 16 months of age due to developmental delay, macrocephaly (head circumference >97th centile) and dysmorphic features including frontal bossing, hypertelorism, bilateral epicanthic folds, synophrys with prominent eyebrows and bilateral single palmar creases. Conventional G-banding cytogenetic analysis showed a normal male karyotype. Subsequent CMA (Affymetrix Genome-wide Human SNP Array 6.0 chip) revealed a microdeletion within the long arm of chromosome 10 with the molecular karyotype arr 10q24.3 (104183824-105403510)x1; a deletion of approximately 1.2 Mb (Fig. 5). This deletion was confirmed by FISH using the BAC clone RP11-2F13 encompassing the *SUFU* gene (Fig. 6).

A previously reported case with a 10q24.3 deletion revealed some phenotypic features of nevoid basal cell carcinoma syndrome, including frontal bossing, prominent jaw and hypertelorism in addition to severe developmental delay.[60] Loss of heterozygosity (LOH) and homozygous deletions of various regions of the genome are frequently found in tumours;[61] multiple tumour suppressor genes (TSGs) have been postulated to occur in the 10q24.3 region.

The *LAPSER1* gene is a candidate TSG located within 10q24.3 near the *PTEN* locus which has been implicated in various cancers.[62] The *SUFU* gene, located distal to *PTEN*, and within our region of interest, encodes a component of the sonic hedgehog signalling pathway and may also be implicated in having a TSG function; reports of individuals with early-onset medulloblastoma and germline mutations in this gene have been reported.[63,64] Mutations of the *SUFU* gene are found in both germline and somatic forms of medulloblastoma.[65] The *SUFU* gene acts as a TSG in a subset of desmoplastic medulloblastomas and, when involved in a deletion of contiguous genes, leads to a phenotype which may include psychomotor retardation, hypertelorism, broad nasal bridge, nevoid basal cell carcinoma syndrome and developmental delay.[62] This patient had not developed medulloblastoma by the age of seven years; however, his maternal aunt developed the disorder at the age of 10 with subsequent herpes encephalitis and significant delay as a result.

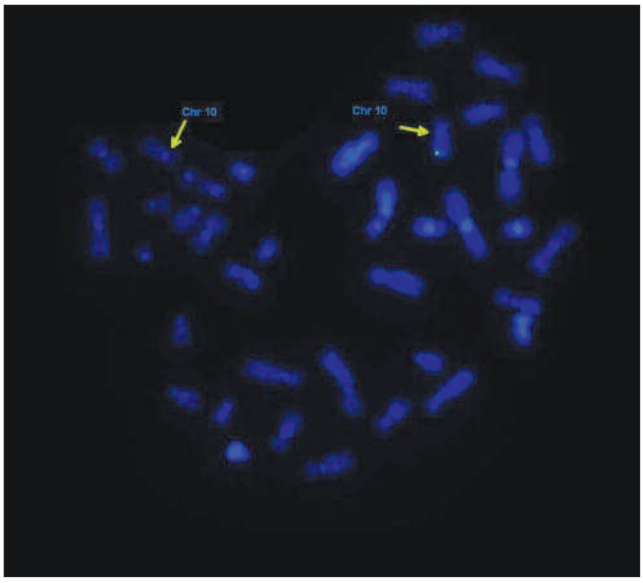

Fig. 6. Fluorescence *in situ* hybridisation analysis of patient 4.

The desmoplastic/nodular subtype of medulloblastoma is characterised by a double peak of age of onset: the first in early childhood and the second in adolescence and adulthood. This type of tumour has been directly associated with *SUFU* gene mutations with an estimated 30% penetrance.[65] The incomplete penetrance could be due to modifier genes, environmental factors or parental imprinting, although none of these have been proven to date. There was no family history of macrocephaly but the occurrence of medulloblastoma may suggest the deletion could be familial with variable penetrance.

Conclusions

Chromosome microarray analysis provides an additional tool for the investigation of multiple disorders and may allow a definitive diagnosis to be made where previous techniques have proved limiting (Table 1). The diagnosis may provide information about the clinical course of the disorder and long-term prognosis.[66] A diagnosis allows accurate advice for families of the affected individual, and carrier testing and recurrence risks can be calculated with the potential for prenatal diagnosis of future pregnancies.[66,67] Where necessary, surgical or behavioural intervention can prevent the manifestation of complications associated with the syndrome.[67] The delineation of new syndromes and the expansion of previously recognised phenotypes add to the clinical picture. There will be many occasions when the rearrangement has not been reported in the literature; in these cases interrogation of the affected genes may add value to patient management.[4]

Table 1. Summary of potential benefits and limitations of CMA in clinical practice.

	Benefits	Limitations
Laboratory	•Simplified workflow due to automation •More rapid than conventional techniques •Enhanced detection of mosaicism •Fewer confirmatory tests required	•Large initial cost •Detection of results of unknown significance and need to make recommendations to clinical team based on these findings
Clinical team	•Smaller sample volume required •Definitive diagnosis •Recognition of atypical presentations of known disorders •Shorter turnaround time of test •Enhanced ability to anticipate potential physical and functional limitations/complications •Increased enrollment in appropriate clinical trials	•Correlating clinical findings with results of unknown significance
Affected individual(s) and their family	•Smaller sample volume required •Definitive diagnosis •Fewer additional diagnostic tests (including imaging and invasive procedures) •More accurate prenatal and carrier testing •Access to support groups •Access to appropriate clinical trials if desired	•Uncertainty due to results of unknown clinical significance

The prediction of a phenotype associated with the identified genotype can allow pre-emptive treatment for the individual. Speech therapy, for example, may prevent the exacerbation of the predicted language impairment associated with the duplication 7q syndrome.[67] In future, new therapeutic treatments may become available for the delineated disorders, with currently diagnosed but untreatable individuals being grouped for future potential treatment.[68] The CMA diagnosis can provide families and individuals with answers to their doubts as to whether or not they 'did something wrong' to cause the disorder. They can now give a name to the condition[67] and have information about the associated (if any) recurrence risks.

A note of caution surrounds the numerous CNVs of unknown clinical significance which will inevitably be detected in the course of CMA. The interaction and possible 'second hit' also adds confusion when counselling families. This confusion is compounded in the case of prenatal diagnosis, especially if consideration is being given to termination of pregnancy.[20] There will be significant concerns surrounding the identification of CNVs of unknown clinical relevance, which may lead to additional stress and anxiety for the individual, especially when making difficult decisions during prenatal diagnosis.[69,70]

The cases examined in this review have highlighted some of the considerable variation in phenotype and potential pitfalls associated with this new technology. Chromosome microarray analysis will not completely replace conventional cytogenetic or molecular analysis as it does not identify balanced translocations or point mutations. Currently, referrals for recurrent miscarriage and infertility will not be candidates for CMA, nor will balanced rearrangements associated with acquired disorders such as leukaemia. It will, however, become the test of choice for many clinicians facing an uncertainty of diagnosis.[70] The use of CMA has been shown to detect clinically significant chromosomal rearrangements in 15–20% of patients referred for assessment of unexplained DD, ASD or MCA. This level of detection provides a much higher diagnostic value than conventional G-banding cytogenetic analysis (3%), excluding those recognisable chromosomal syndromes which do not necessitate the need for CMA.[9] For the unexplained cases, the referring clinician does not need to have a suspicion of the particular chromosomal abnormality involved.[4] □

References

1 Lejeune J, Gautier M, Turpin R. Study of somatic chromosomes from 9 mongoloid children (in French). *C R Hebd Seances Acad Sci* 1959; **248**: 1721–22.

2 Baldwin EL, Lee JY, Blake DM *et al.* Enhanced detection of clinically relevant genomic imbalances using a targeted plus whole genome oligonucleotide microarray. *Genet Med* 2008; **10** (6): 415–29.

3 Lejeune J, Lafourcade J, Berger R *et al*. Three cases of partial deletion of the short arm of chromosome 5 (in French). *C R Hebd Seances Acad Sci* 1963; **257**: 3098–102.

4 Beijani BA, Shaffer LG. Clinical utility of contemporary molecular cytogenetics. *Annu Rev Genomics Hum Genet* 2008; **9**: 71–86.

5 Speicher MR, Carter NP. The new cytogenetics: blurring the boundaries with molecular biology. *Nat Rev Genet* 2005; **6** (10): 782–92.

6 Shinawi M, Cheung SW. The array CGH and its clinical applications. *Drug Discov Today* 2008; **13** (17–18): 760–70.

7 Martin CL, Nawaz Z, Baldwin EL *et al.* The evolution of molecular ruler analysis for characterizing telomere imbalances: from fluorescence *in situ* hybridization to array comparative genomic hybridization. *Genet Med* 2007; **9** (9): 566–73.

8 Shaffer LG, Theisen A, Bejjani BA *et al*. The discovery of microdeletion syndromes in the post-genomic era: review of the methodology and characterization of a new 1q41q42 microdeletion syndrome. *Genet Med* 2007; **9** (9): 607–16.

9 Miller DT, Adam MP, Aradhya S *et al*. Consensus statement: chromosomal microarray is a first tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. *Am J Hum Genet* 2010; **86**: 749–64.

10 Conrad DF, Pinto D, Redon R *et al.* Origins and functional

impact of copy number variation in the human genome. *Nature* 2010; **464**: 704–12.
11 Ji Y, Eichler EE, Schwartz S, Nicholls RD. Structure of chromosomal duplicons and their role in mediating human genomic disorders. *Genome Res* 2000; **10**: 597–610.
12 Thomas NS, Durkie M, Potts G *et al*. Parental and chromosomal origins of microdeletion and duplication syndromes involving 7q11.23, 15q11-13 and 22q11. *Eur J Hum Genet* 2006; **14**: 831–7.
13 Potocki L, Chen KS, Park SS *et al*. Molecular mechanism for duplication 17p11.2 – the homologous recombination reciprocal of the Smith-Magenis microdeletion. *Nat Genet* 2000; **24**: 84–7.
14 Baumer A, Dutly F, Balmer D *et al*. High level of unequal meiotic crossovers at the origin of the 22q11.2 and 7q11.23 deletions. *Hum Mol Genet* 1998; **7**: 887–94.
15 Peng HH, Ven den Veyver IB. Clinical application of microarray based comparative genomic hybridization in prenatal diagnosis. *Expert Rev Obstet Gynecol* 2009; **4** (1): 81–92.
16 Turner DJ, Miretti M, Rajan D *et al*. The rates of *de novo* meiotic deletions and duplications causing several genomic disorders in the male germline. *Nat Genet* **40** (1): 90–5.
17 Torniero C, Bernardina BD, Novara F *et al*. Dysmorphic features, simplified gyral pattern and 7q11.23 duplication reciprocal to the Williams-Beuren deletion. *Eur J Hum Genet* 2008; **16**: 880–7.
18 Sebat J, Lakshmi B, Troge J *et al.* Large-scale copy number polymorphism in the human genome. *Science* 2004; **305** (5683): 525–8.
19 Iafrate J, Feuk L, Rivera MN *et al*. Detection of large-scale variation in the human genome. *Nat Genet* 2004; **36**: 949–51.
20 Lee C, Iafrate AJ, Brothman AR. Copy number variations and clinical cytogenetic diagnosis of constitutional disorders. *Nat Genet* 2007; **39** (7 Suppl): S48–54.
21 Conrad DF, Pinto D, Redon R *et al*. Origins and functional impact of copy number variation in the human genome. *Nature* 2010; **464**: 704–12.
22 Komura D, Shen F, Ishikawa S *et al.* Genome-wide detection of human copy number variations using high-density DNA oligonucleotide arrays. *Genome Res* 2006; **16** (12): 1575–84.
23 Macconaill LE, Aldred MA, Lu X, Laframboise T. Toward accurate high-throughput SNP genotyping in the presence of inherited copy number variation. *BMC Genomics* 2007; **8**: 211.
24 Conrad DF, Hurles ME. The population genetics of structural variation. *Nat Genet* 2007; **39** (7 Suppl): S30–36.
25 Carter NP. Methods and strategies for analyzing copy number variation using DNA microarrays. *Nat Genet* 2007; **39** (7 Suppl): S16–21.
26 Madsen BE, Villesen P, Wiuf C. A periodic pattern of SNPs in the human genome. *Genome Res* 2007; **17** (10): 1414–9.
27 Li M, Li C, Guan W. Evaluation of coverage variation of SNP chips for genome wide association studies. *Eur J Hum Genet* 2008; **16** (5): 635–43.
28 Beaudet AL, Belmont JW. Array-based DNA diagnostics: let the revolution begin. *Annu Rev Med* 2008; **59**: 113–29.
29 Van der Vegt B, de Bock GH, Hollema H, Wesseling J. Microarray methods to identify factors determining breast cancer progression: potentials, limitations, and challenges. *Crit Rev Oncol Hematol* 2009; **70** (1): 1–11.
30 Gondek LP, Dunbar AJ, Szpurka H, McDevitt MA, Maciejewski JP. SNP array karyotyping allows for the detection of uniparental disomy and cryptic chromosomal abnormalities in MDS/MPD-U and MPD. *PLoS One* 2007; **2** (11): e1225.
31 Schouten JP, McElgunn CJ, Waaijer R, Zwijnenburg D, Diepvens F, Pals G. Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res* 2002; **30** (12): e57.
32 Somerville MU, Mervis CB, Young EJ *et al*. Severe expressive-language delay related to duplication of the Williams-Beuren locus. *N Engl J Med* 2005; **353**: 1694–701.
33 Berg JS, Brunetti-Pierri N, Peter SU *et al* Speech delay and autism spectrum behaviours are frequently associated with duplication of the 7q11.23 Williams-Beuren syndrome region. *Genet Med* 2007; **9**: 427–41.
34 Depienne C, Heron D, Batancur C *et al*. A. autism, language delay and mental retardation in a patient with 7q11 duplication. *J Med Genet* 2007; **44**: 452–8.
35 Van der Aa N, Rooms L, Vandeweyer G *et al*. Fourteen new cases contribute to the characterization of the 7q11.23 microduplication syndrome. *Eur J Med Genet* 2009; **52**: 94–100.
36 Stromme P, Bjornsta PG, Ramstad K. Prevalence estimation of Williams syndrome. *J Child Neurol* 2002; **17**: 269–71.
37 Francke U. Williams-Beuren syndrome: genes and mechanisms. *Hum Mol Genet* 1999; **8**: 1947–54.
38 Peoples R, Franke Y, Wang YK *et al*. A physical map, including a BAC/PAC clone contig. of the Williams-Beuren syndrome-deletion region at 7q11.23. *Am J Hum Genet* 2000; **66**: 47–68.
39 De Silva U, Elnitski L, Idol JR *et al*. Generation and comparative analysis of approximately 3.3Mb of mouse genomic sequence orthologous to the region of human chromosome 7q11.23 implicated in Williams syndrome. *Genome Res* 2002; **12**: 3–15.
40 Merla G, Ucla C, Guipponi M, Reymond A. Identification of additional transcripts in the Williams-Beuren syndrome critical region. *Hum Genet* 2002; **110**: 429–38.
41 Hoogenraad CC, Eussen BHJ, Langeveld A *et al*. The murine CYLN2 gene: genomic organization, chromosome localization, and comparison to the human gene that is located within the 7q11.23 Williams syndrome critical region. *Genomics* 1998; **53**: 348-358.
42 Meng Y, Zhang Y, Tregoubov V *et al.* Abnormal spine morphology and enhanced LTP in LIMK-1 knockout mice. *Neuron* 2002; **35**: 121–33.
43 Tassabehji M. Williams-Beuren syndrome: a challenge for genotype-phenotype correlations. *Hum Mol Genet* 2003; **15**: 229–37.
44 Tassabehji M, Hammon P, Karmiloff-Smith A *et al*. GTF21RD1 in craniofacial development of humans and mice. *Science* 2005; **310**: 1184–7.
45 Cusco I, Corominas R, Bayes M *et al.* Copy number variation at the 7q1.23 segmental duplications is a susceptibility factor for the Williams-Beuren syndrome deletion. *Genome Res* 2008; **18** (5): 683–94.
46 Donnai D, Karmiloff-Smith A. Williams syndrome: from genotype through to the cognitive phenotype. *Am J Med Genet* 2000; **97**: 164–71.
47 Ballif BC, Hornor SA, Jenkin E *et al*. Discovery of a previously unrecognized microdeletion syndrome 16p11.2-p12.2. *Nat Genet* 2007; **39**: 1071–3.
48 Ullman R, Turner G, Kirchhoff M *et al.* Array CGH identifies reciprocal 16p13.1 duplications and deletions that predispose to autism and/or mental retardation. *Hum Mutat* 2007; **28**: 674–82.
49 Hannes FD, Sharp AJ, Mefford HC *et al*. Recurrent reciprocal deletions and duplications of 16p13.11: the deletion is a risk factor for MR/MCA while the duplication may be a rare benign variant. *J Med Genet* 2009; **46** (4): 223–32.
50 Kumar RA, KaraMohamed, Sudi J *et al.* Recurrent 16p11.2 microdeletion in autism. *Hum Mol Genet* 2008; **17**: 628–38.
51 Marshall CR, Noor A, Vincent JB *et al*. Structural variation of chromosomes in autism spectrum disorder. *Am J Hum Genet* 2008; **82**: 477–88.

52 Weiss LA, Yipin Shen D, Korn JM *et al.* Association between microdeletion and microduplication at 16p11.2 and autism. *N Engl J Med* 2008; **358**: 667–75.

53 Girirajan S, Rosenfeld JA, Cooper GM *et al*. A recurrent 16p12.1 microdeletion support a two-hit model for severe developmental delay. *Nat Genet* 2010; **42** (3): 203–10.

54 Fombonne E. Epidermiological trends in rates of autism. *Mol Psychiatry* 2002; **7** (Suppl 2): S4–6.

55 Woodberry KA, Giuliano AJ, Seidman LJ. Premorbid IQ in schizophrenia: a meta-analytic review. *Am J Psychiatry* 2008; **165**: 579–87.

56 Sharp AJ, Mefford HC, Li K *et al*. A recurrent 15q13.3 microdeletion syndrome associated with mental retardation and seizures. *Nat Genet* 2008; **40**: 322–8.

57 Font-Llitjos M, Jimenez-Vial M, Bisceglia L *et al*. New insights into cystinuria: 40 new mutations, genotype-phenotype correlation, and digenic inheritance causing partial phenotype. *J Med Genet* 2005; **42**: 58–68.

58 Chabrol B, Martens K, Meulemans S *et al*. Deletion of C2orf34, PREPL and SLC3A1 causes atypical hypotonia-cystinuria syndrome. *J Med Genet* 2008; **45**: 314–8

59 Cabeza-Arvelaiz Y, Thompson TC, Sepulveda JL, Chinault AC. LAPSER1: a novel candidate tumor suppressor gene from 10q24.3. *Oncogene* 2001; **20**: 6707–17.

60 Taylor MD, Liu L, Raffel C *et al*. Mutations in *SUFU* predispose to medulloblastoma. *Nat Genet* 2002; **31**: 306–10.

61 Ellison D. Classifying the medulloblastoma: insights from morphology and molecular genetics. *Neuropathol Appl Neurobiol* 2002; **28**: 257–82.

62 Ng D, Stavrou T, Liu L *et al.* Retrospective family study of childhood medulloblastoma. *Am J Med Genet* A 2005; **134**: 399–403.

63 Lobo S, Cervenka J, London A, Pierpont ME. Interstitial deletion of 10q: clinical features and literature review. *Am J Med Genet* 1992; **43**: 701–3.

64 Brugieres L, Pierron G, Chompre A *et al*. Incomplete penetrance of the predisposition to medulloblastoma associated with germline *SUFU* mutations. *J Med Genet* 2010; **47**: 142–4.

65 Bejjani BA, Shaffer LG. Application of array-based comparative genomic hybridization to clinical diagnostics. *J Mol Diagn* 2006; **8** (5): 528–33.

66 Wordsworth S, Buchanan J, Regan R. Diagnosing idiopathic learning disability: a cost-effectiveness analysis of microarray technology in the National Health Service of the United Kingdom. *Genomic Med* 2007; **1** (1–2):35–45.

67 Valente EM, Ferraris A, Dallapiccola B. Genetic testing for paediatric neurological disorders. *Lancet Neurol* 2008; **7** (12): 1113–26.

68 Manning M, Hudgins L. Use of array-based technology in the practice of medical genetics. *Genet Med* 2007; **9** (9): 650–3.

69 Pergament E. Controversies and challenges of array comparative genomic hybridisation in prenatal genetic diagnosis. *Genet Med* 2007; **9** (9): 596–9.

70 Bijlsma EK, Gijsbers AC, Schuurs-Hoeijmakers JH *et al.* Extending the phenotype of recurrent rearrangements of 16p11.2: deletions in mentally retarded patients without autism and in normal individuals. *Eur J Med Genet* 2009; **52**: 77–87.