Chromosome analysis: what and when to request

F H Sharkey, E Maher, D R FitzPatrick

Arch Dis Child 2005;90:1264–1269. doi: 10.1136/adc.2004.068668

Chromosome abnormalities have long been recognised as an important cause of learning disability and multiple malformation syndromes; 0.8% of live born infants have numerical or structural chromosomal anomalies resulting in an abnormal phenotype. The identification of such anomalies is important, both clinically and for accurate genetic counselling. Recently, the human genome sequence has enabled higher resolution screens for chromosome anomalies using both molecular cytogenetic and array based techniques. This review suggests a simple algorithm for the targeted use of diagnostic cytogenetic tools in specific patient groups commonly seen in paediatric practice.

Clinical geneticists are notorious jargon users and clinic letters or consult notes often conclude with a statement to the effect that no specific syndrome diagnosis can be made but the child looks very “chromosomal”. This unusual adjective refers to a collection of clinical features that predict an increased likelihood of finding a cytogenetic abnormality on routine testing. These include:

- Congenital, global delay in neurocognitive development
- One or more major malformations
- Prenatal onset abnormal growth pattern
- Craniofacial dysmorphisms
- Unusual behavioural phenotypes, often in the autistic spectrum
- Abnormal dermatoglyphic (finger and palm epidermal ridges) or trichoglyphic (scalp hair boundaries and hair growth trajectory) pattern
- A family history of multiple miscarriages, learning disability, or malformations.

Unfortunately, no positive predictive value exists for any aspect of the “chromosomal” impression, although attempts to formalise this are beginning to appear.¹

About 0.8% of live born children have chromosomal anomalies (table 1) and half of these individuals have an abnormal phenotype.² The best predictor of deleterious phenotypic consequence is the gain or loss of chromosomal material, which is termed aneuploidy. Chromosomal anomalies are categorised as:

- Numerical (gain or loss of whole chromosomes), e.g. Down's syndrome, caused by trisomy of chromosome 21, with a birth incidence of about 1 in 800–1000

- Structural (large scale abnormalities in genomic sequence of individual chromosomes). These may be balanced (where genomic material is rearranged but not gained or lost) or unbalanced (results in aneuploidy) (fig 1).

Conventional cytogenetic analysis uses light microscopy to examine metaphase or prometaphase chromosomes that have been stained to produce a distinct banding pattern for each chromosome. This approach has a maximum resolution of 3–5 megabases (Mb) for structural anomalies^{3–4} and requires dividing cells, usually peripheral blood leucocytes, bone marrow, or fibroblasts, for analysis. Fluorescent *in situ* hybridisation (FISH) analysis uses labelled DNA probes to identify submicroscopic (or cryptic) structural chromosome anomalies—microdeletions and microduplications.

Comparative genomic hybridisation microarray technology provides a diagnostic method for systematic and comprehensive analysis of human aneuploidy (fig 2). Array-CGH provides much higher resolution (currently up to 100–200 kb) and has great potential for automation. Array-CGH is now being applied to human malformation syndromes and cohorts with learning disabilities,^{5–7} providing higher resolution definition of known structural aberrations and detecting aneuploidy that was undetected by FISH or chromosome banding techniques.^{8–9}

This review will discuss the clinical utility of each of the techniques mentioned above as diagnostic and research tools in paediatrics, and their applicability to specific patient groups.

POPULATION BASED STUDIES

In the 1960s and 1970s cytogenetic screens of unselected newborns in the “pre-banding” era showed and overall birth prevalence of 0.56% (1:178)¹⁰ with numerical and structural chromosomal abnormalities contributing 0.33% and 0.22% respectively (table 1). A later study using moderate level banding (400–500) found the respective frequencies of balanced and unbalanced structural abnormalities to be 0.522% and 0.061%. Such moderate banding increases the overall frequency of abnormalities detectable in the newborn to 0.917%.¹¹ Large scale newborn screens are not available using molecular cytogenetic or array-CGH techniques and the ethical complexities and cost implications of such studies make it unlikely that these will be attempted in the future.

RECOGNISABLE CHROMOSOMAL SYNDROMES

Since the advent of clinical cytogenetic analysis it has been recognised that chromosomal deletions

See end of article for authors' affiliations

Correspondence to:
Dr D R FitzPatrick, MRC
Human Genetics Unit,
Edinburgh EH4 2XU, UK;
david.fitzpatrick@hgu.mrc.
ac.uk

Accepted 26 August 2005

Table 1 Chromosome anomalies detected using conventional cytogenetics in newborn screens

Reference	Sample no.	Rearrangements		Detection rate (%)
		Structural	Numerical	
Jacobs <i>et al</i> , 1992 ¹¹	14677	112	NC	0.7
Hansteen <i>et al</i> , 1982 ²³	1830	25	11	1.9
Hamerton <i>et al</i> , 1975 ¹⁰	46150*	103	155	0.56

NC, not considered in this study.
*Data from six studies reviewed by authors in addition to their own data using conventional staining methods.

and duplications, like autosomal trisomies, can produce recognisable syndromes. There is an increasing number of such syndromes, but two of the best known are deletion (del) of the distal short arm of chromosome 4 (del(4)(p16), a.k.a. 4p-), which results in Wolf-Hirschhorn syndrome, and del(5)(p13→p15) (a.k.a. 5p-), which causes Cri du Chat. A puzzling group of children who had convincing clinical features of these syndromes but normal chromosomes using conventional analysis were explained with the ability to identify microdeletions using molecular cytogenetics. Many different microdeletion syndromes are now recognised and most, like Williams syndrome (del(7)(q11.2)ish), are truly cryptic and are only detectable by FISH and such technologies. The speedy diagnosis of such conditions requires the referring clinician to request specific FISH analysis (tables 3 and 4). This is now a common role for the paediatric geneticist or dysmorphologist.

LEARNING DISABILITY/MENTAL RETARDATION (LD/MR)

The reported detection rates for different cytogenetic approaches to learning disability are summarised in table 2. These studies are plagued with inconsistencies in definition, severity, patient selection criteria (for example, outpatient clinic, institutional population), sample size, presence/absence of congenital anomalies, rigour of syndromic assessment, and assay specifications. In a review of nine screening studies by van Karnebeek and colleagues,¹² chromosome banding techniques were shown to have a detection rate of 13.3% (3.8% structural/7.8% numerical rearrangements) in moderate-severe LD/MR cases (IQ score <50) and 4.1% for mild-moderate cases (IQ 50-70). The equivalent detection rate for subtelomere FISH screens in four studies reviewed was 6.7% for moderate-severe LD cases and 0.5% for mild-moderate LD. The recommendation of this

Figure 1 Common structural anomalies in clinical cytogenetics: various categories of structural anomalies are reported in clinical cytogenetics. Among the most common are translocations (top panel, left side) which may be reciprocal (a swap of chromosome material between non-homologous chromosomes) or Robertsonian (a fusion of two acrocentric chromosomes). Translocations are further classified into whether they are balanced (no apparent gain or loss of chromosomal material) or unbalanced (rearrangement results in aneuploidy). Inversions (top panel, right side) are where the normal order of genomic material within a chromosome is altered by the abnormal repair of chromosomal breakpoints (block arrows and dotted lines). If the inversions include the centromere of the chromosome they are called pericentric, if not, paracentric. Deletions (bottom panel, far left) and duplications (bottom panel, second left) are structural rearrangements causing loss or gain, respectively, of part of a chromosome. Ring chromosomes (bottom panel, second right) are caused by fusion of the ends of chromosomes. Fragile sites (bottom panel, right hand side) are apparent gaps in chromosomes. These may be due to expansion of small repeats in DNA. The best known pathological example of this is a marker of the fragile X syndrome at Xq27.3.

Table 2 Application of subtelomere FISH, conventional cytogenetic techniques, and array-CGH for the detection of chromosomal abnormalities involved in LD/MR						
Level of LD/MR	Method	Sample no.	Rearrangements		Detection rate (%)	Reference
			Structural	Numerical		
Mild-moderate	Subtelomere FISH					
	Multiprobe FISH	182	1		0.5	Knight <i>et al</i> ¹⁵
	Multiprobe FISH	103	1		1.0	Van Karnebeek <i>et al</i> ¹⁴
	Multiprobe FISH	40	4		10.0	Dawson <i>et al</i> ¹³
	Various	4 studies	NR	NR	0.5	Van Karnebeek <i>et al</i> ¹²
		44	1%	3%		
	Conventional cytogenetics					
	Various	5 studies			0.0	Rossi <i>et al</i> ²⁵
	400–550 chromosome banding	200			4.1	Van Karnebeek <i>et al</i> ¹²
	Array-CGH	N/A			4.1	Rossi <i>et al</i> ²⁵
Moderate-severe	Subtelomere FISH					
	Multiprobe FISH	284	21		7.4	Knight <i>et al</i> ¹⁵
	Multiprobe FISH	254	13		5.1	Riegel <i>et al</i> ²⁶
	Multiprobe FISH	81	0		0.0	Van Karnebeek <i>et al</i> ¹⁴
	Multiprobe FISH	117	12		10.2	Rossi <i>et al</i> ²⁵
	Various	4 studies	NR	NR	6.7	Van Karnebeek <i>et al</i> ¹²
	Conventional cytogenetics					
	Various	9 studies	3.8%	7.8%	13.3	Van Karnebeek <i>et al</i> ¹²
	Array-CGH					
	1 Mb BAC/PAC array	50	12		24	Shaw-Smith <i>et al</i> ⁶
	1 Mb BAC/PAC array	20	4		20	Visser <i>et al</i> ⁷

NR, not reported; N/A, no available studies for comparison.

review was to use standard karyotyping as a first line test. High resolution chromosome banding has been reported to have an overall detection rate of 7.5% for anomalies in patients with mild-moderate and moderate-severe LD/MR,^{12–13} although there are various reports documenting much lower detection/prevalence rates.^{14–15}

The specialised regions at the ends of all chromosomes are called telomeres. The adjacent subtelomeric regions of the genome are gene-rich and are susceptible to abnormal chromosomal rearrangements during meiosis. This has led to various FISH based diagnostic strategies being developed to detect such rearrangements. PCR quantitation and more

Figure 2 Array-CGH analysis: a test and a normal control DNA are labelled with different fluorochromes (in the figure, test is green, normal is red) and co-hybridised to a microarray containing DNA clones whose position in the human genome is known. The resolution of the analysis is dictated both by the size of the DNA clones and the size of the gap between the clones. The most commonly used arrays contain 3000–4000 clones of 200 000 base pairs in size spaced at 1 000 000 base pair intervals throughout the genome. Digitised intensity differences in the hybridisation patterns between the test/normal samples onto these cloned fragments can be interpreted as copy number change(s) at that position(s). For example, if the hybridisation is equal between tester and control DNA the red/green fluorochrome signal mix will give a yellow colour. If the test DNA contains a deletion, then red > green and an orange colour will result. If the test DNA contains a duplication, the green fluorochrome will be more abundant than the red.

Table 3 Microdeletion syndromes

Locus	Syndrome	Incidence	Major gene	Clinical features
22q11.2	Di George, velocardiofacial, conotruncal facial anomaly	1 in 4000	TBX1 (cardiac defects and cleft palate)	Cardiac outflow tract defects, distinctive facial appearance, thymic hypoplasia, cleft palate, and hypocalcaemia
15q11-q13	Prader-Willi	1:15 000	Imprinted region, deletions on paternal allele in 70% cases	Distinctive facial appearance, hyperphagia, small hands and feet, distinct behavioural phenotype
15q11-q13	Angelman	1:15 000	Imprinted region, deletions on maternal allele in UBE3A	Distinctive facial appearance, absent speech, EEG abnormality, characteristic gait
7q11.23	Williams	1:10 000	Elastin (supravalvular aortic stenosis)	Distinctive facial appearance, supravalvular aortic stenosis, learning disability, and infantile hypercalcaemia
17p11.2	Smith-Magenis	1 in 25 000	RAI1	Distinctive facial appearance and behavioural phenotype, self-injury, and REM sleep abnormalities
17p11.3	Miller-Dieker	Rare	UIS1 and 14-3-3	Distinctive facial appearance, lissencephaly, severe learning disability, heart defects, growth retardation, and seizures
5p15	Cri du Chat	Rare	Unknown	Microcephaly, micrognathia, epicanthic folds, and high pitched cry
13q14	Retinoblastoma	Rare	RB1	Retinoblastoma, learning disability
16p13.3	Rubinstein-Taybi	Rare	CREB deletions account for <10% of cases	Distinctive facial appearance, short stature, broad thumbs
4p16.3	Wolf-Hirschhorn	Rare	WHSC1	Distinctive facial appearance, seizures, short stature, cleft lip, coloboma
11p13	WAGR	Rare	PAX6, WT1	Aniridia, Wilms' tumour, male genital tract malformations, learning disability

recently array based comparative genomic hybridisation (array-CGH) can also detect the same anomalies (fig 2). Cryptic subtelomeric chromosomal anomalies may cause either specific (table 4) or non-specific phenotypes such as "idiopathic" learning disability/mental retardation (LD/MR) with or without craniofacial dysmorphism.¹³

Subtelomeric FISH can detect cryptic deletions and balanced or unbalanced translocations. Probes must be chosen carefully as subtelomeric regions are complex and can cross-hybridise (for example, 8p with 1p and 3q) and show polymorphic deletions (for example, 2q).^{14 15} Family studies are critical for interpretation of positive FISH results. There is extreme variability in the reported prevalence of subtelomere rearrangements in patients with LD/MR. However, subtelomeric rearrangements may be the second most common cause of LD/MR in children after Down's syndrome, with an overall detection rate of 5.1% LD/MR.¹⁶ The reported detection rates using subtelomere FISH in moderate-severe LD/MR patients are, as would be expected, higher than those in mild-moderate groups (table 2). A five point checklist has been developed to facilitate preselection for subtelomeric screening and increase diagnostic detection rates in patients with LD/MR. The checklist includes family history of LD/MR, prenatal onset growth retardation, post-natal growth abnormalities, ≥ 2 facial dysmorphic features, and one or more non-facial dysmorphic feature(s) and/or with congenital abnormalities.¹

Array-CGH (fig 2) holds great promise for the detection of chromosomal anomalies in children with LD/MR. Using an array where the clones were targeted to the subtelomeric region, a blinded study of 20 patients with unclassified MR and/or with multiple congenital anomalies, all subtelomeric

abnormalities detected by FISH were correctly identified by array-CGH; this technique also identified rearrangements in an additional three patients missed by FISH.⁹ A whole genome based DNA microarray with a resolution of approximately one clone per megabase has been used to investigate 50 patients presenting with moderate to severe learning difficulties of unknown cause.⁶ All patients showed normal G banded karyotypes, with one exception, and in 82% of patients, subtelomeric rearrangements were excluded by FISH and genotyping. A loss or gain of genetic material was detected in 12 patients (seven deletions and five duplications), giving a rearrangement detection rate of 24%. Another recent study has shown a similar rate of cryptic aneuploidy in patients with moderate-severe LD/MR.⁷ The patients in these studies were selected on the basis of a phenotype suggestive of a chromosome anomaly and thus may not be representative of all patients within the MR category. Two of the genomic abnormalities were also found in a normal parent, suggesting that these may be copy number polymorphisms⁷ and emphasising the need for sufficient normal control data before this technique can be used in routine clinical analysis. Array-CGH has also been successfully used to analyse patients, with an abnormal phenotype and LD/MR, but carrying apparently balanced de novo translocations.²⁷ Abnormal phenotypes in patients with apparently balanced de novo translocations are thought to result from gene dosage alterations at the breakpoints or by microdeletions/duplications. Additional rearrangement complexity at the breakpoints in balanced cases were detected using high resolution microarrays, with unsuspected cryptic imbalances detected in one third of the patients involving chromosomes that were not directly related to their de novo translocations.

Table 4 Recognised syndromes associated with subtelomeric deletions

Locus	Clinical features
1p36	Distinctive facies, seizure, sensorineural hearing loss, cardiomyopathy, learning disability
2q37	Partial phenocopy of Albright hereditary osteodystrophy, learning disability
9q34	Midface hypoplasia, learning disability, conotruncal defects, hypotonia, obesity
22q13	Severe speech and language delay and autistic-like behaviour, learning disability

Figure 3 Facial photographs of patients with clinically recognisable chromosome abnormalities. (A) 1.5 year old girl with typical facial appearance of 1p36 deletion: note straight eyebrows, deep-set eyes, small mouth with short philtrum. A hearing aid is visible as hearing loss is common in this condition. This girl also has a severe seizure disorder, prenatal onset growth failure, and learning disability. (B) 13 year old boy with del(22)(q11.2) microdeletion syndrome. He has upslanting palpebral fissures, a broad nasal bridge, and a small mouth. (C) Hands of the same boy showing characteristic long slim digits. He also had tetralogy of Fallot and speech delay. He is now doing well at normal school with learning support. Parental consent was obtained for publication of the children in this figure.

CONGENITAL MALFORMATIONS

Unfortunately, there are few systematic studies of chromosome anomalies in large cohorts with specific congenital malformations. Much of the available diagnostic information on malformations comes from fetal chromosome analysis following abnormal prenatal ultrasound findings.¹⁷ Interpretation of the available data in paediatric populations is difficult as many of malformation cases investigated also have LD/MR. Array-CGH has particular promise in diagnosis of malformations and syndrome entities. For example, in a pilot study of 22 patients with iris hypoplasia (6p25 segmental duplication) and Axenfeld-Rieger syndrome (6p25 segmental deletion),⁵ all samples was correctly identified by array-CGH (10 duplications, two deletions, and seven normals). Another study using CGH arrays has

established a critical candidate region of 5 Mb exists on 18q22.3–18q23 for patients with congenital aural atresia (CAA), and narrowing or absence of the middle ear.⁹ Further studies will inevitably narrow this region more specifically. Campomelic dysplasia (CD) syndrome is a rare, sporadic, autosomal dominant skeletal dysplasia marked by bowed long bones in the limbs, severe respiratory distress, and reversed sex in some male patients. Most CD cases have heterozygous de novo mutations in the coding region of *SOX9*. Array-CGH detected two deletions in 11 CD patients, the first being a de novo deletion of approximately 4 Mb that included the *SOX9* locus. The other deletion knocked out two evolutionary conserved regulatory elements 5' to *SOX9*.¹⁸ CGH has also recently been used to determine gene copy number in other well known genomic disorders including

Figure 4 Summary of the main reasons for referral for specific cytogenetic investigations.

Smith Magenis, Charcot-Marie-Tooth disease type IA, hereditary neuropathy and dup(17)(p11.2p11.2),¹⁹ Wolf-Hirschhorn syndrome,²⁰ and cardio-facio-cutaneous syndrome.²¹

FUTURE PERSPECTIVES AND RECOMMENDATIONS

Array-CGH is a sensitive, flexible, and automatable technique that is likely to revolutionise paediatric genetic investigations over the next five years. It is already in routine use in cancer diagnostics, prognosis, and therapeutic choices.²² Array technologies are not without inherent limitations; they do not provide information on the precise location of the rearranged sequences responsible for the copy change(s). The currently available whole genome slides are currently too expensive to apply to routine clinical referrals. For now conventional cytogenetics +/- FISH remains the mainstay of clinical cytogenetic investigation. A simple summary of appropriate referrals for clinical cytogenetic analysis is given in fig 4. This is likely to change, and it is important that paediatricians maintain close contact with their colleagues in clinical genetics to ensure that appropriate genetic investigations are carried out in common clinical situations.

Authors' affiliations

F H Sharkey, D R FitzPatrick, MRC Human Genetics Unit, Edinburgh, UK
E Maher, Regional Cytogenetics Service, Western General Hospital, Edinburgh, UK

Competing interests: none declared

Parental consent was obtained for publication of the children in figure 3

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