



Constitutional cyto- and molecular genetics: Karyotyping, FISH and CGH array

Cytogenetics is the study of genetic material at the cellular level; molecular genetics studies the structure and function of genes at a molecular level (DNA). The various techniques used vary in their clinical application. This article is a brief summary of the indications for the most commonly-used techniques. It is vital that the correct technique is used for a given clinical presentation or suspected condition, as to use the incorrect technique risks significant diagnostic errors.

Karyotyping

With this technique, lymphocytes from peripheral blood are cultured, using mitogens to stimulate the transformation of the lymphocytes into mitotically active cells. The timing of harvesting of the cells is engineered such that a maximum number of cells are in metaphase. The cells are then fixed, and spread onto a slide. The chromosomes are stained using a number of stains, usually Giemsa (G-banding and R-banding), which produces banding patterns on the chromosomes with a band resolution of 400 – 650 bands per haploid chromosome set. The chromosomes with their bands are then examined microscopically for abnormalities such as loss or gain of entire chromosomes, translocations of all or part of an arm of one chromosome to another, or more subtle changes in banding patterns associated with various genetic syndromes. The

chromosomes are photographed and rearranged into pairs for examination (a karyogram).

The benefits of karyotyping are:

1. It can view the entire genome.
2. It can visualize individual cells and individual chromosomes.

The limits of karyotyping are:

1. Resolution limited to around 5 Mb.
2. An actively growing source of cells is required.

It is important to note that classic karyotyping is timeconsuming, with the preparation of cells for examination taking several days. In addition, live lymphocytes are required so blood samples need to arrive at the laboratory within a maximum of 48 hours after sampling, preferably sooner, to avoid failure of cell growth in culture.



Fig. 1: A normal human karyogram (both male and female shown, which would of course not appear in practice).



Fig. 2: Karyogram of a female patient with Trisomy 21.

Fluorescence in situ hybridisation (FISH)

Conventional karyotyping is limited to the detection of rearrangements involving more than 5 Mb of DNA. The resolution of the FISH technique, using fluorescent probes, is about 100kb-1Mb in size. This technique involves the hybridisation of fluorescently labelled specific DNA sequence probes with patient DNA, and the subsequent microscopic detection of the presence, absence, abnormal copy number or pathological location of a given fluorescence signal. The availability of specific locus probes for many known genetic defects has greatly increased the accuracy of detection of microdeletion and duplication syndromes. This very specificity of the probes is however the main limitation of FISH: it can detect only the specific DNA sequences to which it is complementary and to which it can hybridise.

Benefits of FISH:

1. It can turn almost any DNA into a probe.
2. A much higher resolution compared to G-banding for identifying deletions, insertions, and translocation breakpoints.
3. It can use cells in any stage of the cell cycle as well as archived tissue.
4. It can analyse results on a cell-by-cell basis.
5. Shorter turnaround times, as tissue does not need to be cultured for metaphase cells.

Limits of FISH:

1. One can see only the region of the genome complementary to the probe used.

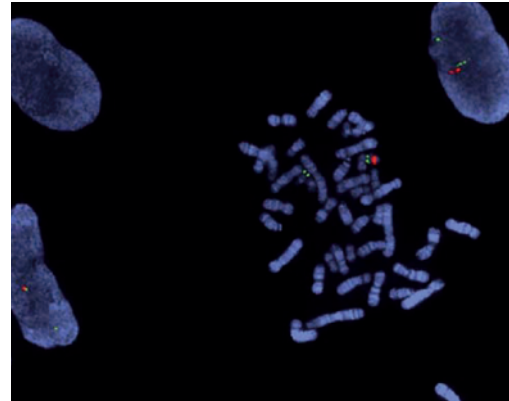


Fig.3: FISH on a metaphase from a Di George carrier. The green probe identifies the two chromosomes 22 (standard probe). The red probe is specific to the critical Di George region and is present on only 1 of the chromosomes 22, indicating a deletion on the other chromosome.

Array comparative genomic hybridisation: array CGH and SNP array

Array CGH compares the patient's genome against a reference genome (normal control or standard) and identifies differences between the two genomes and hence locates regions of genomic imbalance (copy number variations (CNVs) in the patient. A CNV is defined as a segment of DNA of 1000 bases or more which is present in a variable number of copies in comparison to standard DNA. To aid analysis, the whole genome is fragmented into many small regions and the array is arranged so the exact location of each fragment within the whole genome can be identified. From this, the gene content of any imbalance can be established and the genes can be evaluated against the patient's phenotype.

In principle, to determine how copy numbers differ from a reference (control) sample:

1. The sample and reference DNA

are labelled with different coloured fluorescent probes (green and red).

2. The two samples are applied to immobilised DNA on an array, and complementary sequences bind. Colour/Intensity information is collected by a scanner.

3. Where there is no change in the sequence copy number in the test (patient) sample, there will be equal binding of test and reference sample DNA, and equal amounts of each coloured fluorescence will produce a net emission colour (yellow).

4. For sequences where there has been a duplication in the test sample, there will be more green than red fluorescence and an overall green emission; conversely, deletions will result in a reduced level of green fluorescence relative to the red fluorescence from the reference sample, and a net emission of red light.

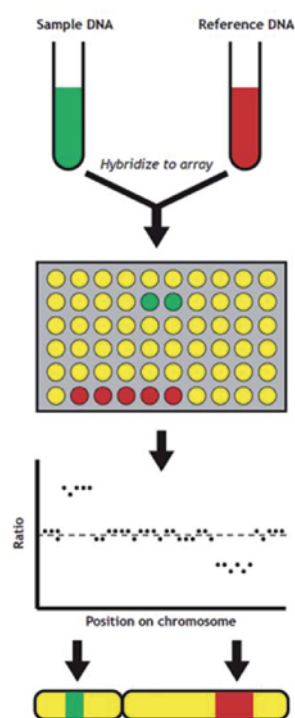


Fig. 4: Principle technique of Array CGH

Single nucleotide polymorphism array:

A single nucleotide polymorphism (SNP), a variation at a single site in DNA, is the most frequent type of variation in the genome. For example, there are around 50 million SNPs that have been identified in the human genome. Most of them are non pathological. The basic principles and techniques of SNP array are similar to those of the array CGH, but the use of SNP enables the collection of genotyping information in addition to standard intensity data.

Therefore, the chief advantages of SNP array over classical CGH array is that it can determine both CNVs and LOH (loss of heterozygosity i.e.: loss of genetic material of one of the two parents), and it can detect aneuploidies like triploidies, which represent approximately 5% of chromosomal abnormalities responsible for miscarriages.

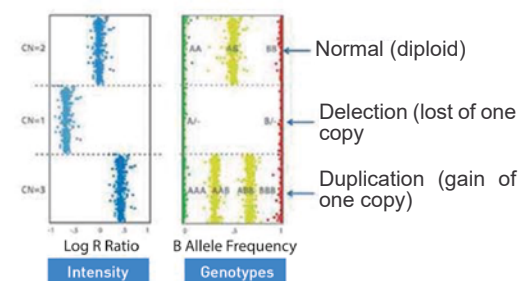


Fig. 5: Profiles obtained by SNP array: Intensity and genotyping analysis

Karyotyping vs array CGH and SNP array

In principle, both karyotyping and arrays are genome-wide technologies which can be used to assess the presence of genomic imbalance such as copy number variations (CNVs). Although they may look like very different technologies, the primary difference between them is in the resolution, which is a measure of the level of magnification of the genome. A standard G-banded karyotype usually has a resolution of around 5 Mb (i.e. it

can detect changes of greater than a five million base pairs). Modern arrays act like a more powerful microscope. Depending upon the particular array and how many DNA probes it uses, it is possible to detect changes greater than 1 Mb (one million base pairs) at low resolution or changes as small as 10 kb (10 thousand base pairs) at high resolution.

Much smaller CNVs can be detected by using higher resolution technologies, which means that more pathogenic CNVs may be detected using modern arrays than through karyotyping.

As CNVs are relatively common throughout the genome, numerous benign CNVs will also be detected, so careful interpretation and follow-up testing is needed.

Clinical uses of karyotyping, FISH and array CGH/SNP

Because of the differences in resolution and the various benefits and limitations of each technique, great care must be taken when deciding on which test(s) to request. The appropriate test will depend on the clinical condition or syndrome suspected and a carefully taken family history of genetic disease (pedigree). Consultation with a clinical geneticist is advised.

In general, karyotyping is indicated as first-line testing for:

1. Common aneuploidy assessment, e.g. trisomies 21, 18 or a sex chromosome aneuploidy.
2. Ambiguous genitalia/indeterminate gender.
3. Delayed puberty/inappropriate secondary sexual development.
4. Short stature or amenorrhea in females.
5. Isolated clinical features, e.g. cleft lip, heart disease.
6. Chromosome breakage syndromes.
7. Infertility.

FISH with a single probe is useful to confirm a suspected diagnosis of a well-described syndrome, such as Williams' syndrome, for example.

Array CGH/SNP is indicated as first-line testing for:

1. Unexplained learning difficulties
2. Intellectual disabilities/cognitive impairment
3. Developmental delay.
4. Behavioural problems including autism spectrum disorders.
5. Dysmorphism/multiple congenital abnormalities suggestive of a chromosome abnormality.
6. Miscarriages (SNP array)
7. For prenatal cases with abnormalities detected during ultrasound

In terms of the diagnostic yield in the diagnosis of mental retardation:

METHOD	METHOD DIAGNOSTIC YIELD
Karyotyping and FISH	5 – 10%
CGH arrays (BAC arrays)	+ 16.7 % of cases unexplained by karyotyping/FISH
SNP arrays	+ 22.7 % of cases unexplained by karyotyping/FISH

It is important to note that the above are only general recommendations. In several cases more than one test will be needed to make a diagnosis, with follow-up testing sometimes required depending on the results of the first-line test used. Nous contacter

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References:

1. ACMG Practice Guidelines: Array-based technology and recommendations for utilization in medical genetics practice for detection of chromosomal abnormalities. Manning, M et Hudgins, L. Genetics in Medicine Volume 12, Numéro 11, 742 – 745, Novembre 2010.
2. Human Cytogenetics: constitutional analysis. 3e édition. E. Rooney éd. Oxford University Press 2001
3. Levy, B. et al. Genomic Imbalance in Products of Conception: Single-Nucleotide Polymorphism Chromosomal Microarray Analysis. Obstetrics & Gynecology 124, 202–209 (2014).

