REVIEW ARTICLE

Rational selection of Genetic tests in Clinical Practice Part 1 - Cytogenetics, FISH and Microarray

Sankar V H[a], Santhi Saraojam[b]

a. Department of Paediatrics, SAT hospital, & Child Development Center (CDC), Government Medical College, Thiruvananthapuram;
b. Genetic Laboratory, CDC, Thiruvananthapuram

ARTICLE INFO

KEYWORDS

Karyotype, Cytogenetic Abnormalities, Microdeletion Syndrome, Copy number variants, Comparative Genomic Hybridization (CGH)

CORRESPONDING AUTHOR

Dr Sankar V H, MD, DNB, MNAMS (Paed) DM (Medical Genetics) Professor & Geneticist,
Department of Paediatrics, SAT Hospital
In Charge Genetic Clinic & Genetic laboratory, CDC
Government Medical College, Thiruvananthapuram, Kerala
Mobile: 9349930828
Email: sankarvh@gmail.com

PUBLISHED ON

9th May 2021

DOI

https://doi.org/10.52314/fnb.2021.v1i1.5

CITE THIS ARTICLE AS

Sankar VH, Saraojam S. Rational selection of Genetic tests in Clinical Practice Part 1 - Cytogenetics, FISH and Microarray. FNB. 2021 May 7;1(1):14–8.

AUTHOR INFORMATION

1. Dr Sankar V H MD DM
   Professor of Paediatrics & Geneticist
   Department of Paediatrics, SAT hospital
   & Child Development Center (CDC)
   Government Medical College, Thiruvananthapuram
2. Dr Santhi Saraojam PhD
   Research Associate & Cytogeneticist
   Genetic Laboratory, CDC, Thiruvananthapuram

CONFLICT OF INTEREST

None

FINANCIAL SUPPORT

Nil

ABSTRACT

Advances in the fields of molecular medicine and genetic engineering have found applications in clinical practice in the form of diagnosis, treatment and prevention of genetic disorders. Genetic disorders can be broadly classified into three categories: Chromosomal disorders, Single gene disorders and multifactorial disorders. During evaluation of a suspected genetic disorder, apart from hematological, biochemical and radiological investigations we have to use some specialized investigations. Cytogenetics refers to the description of chromosome structure and the identification of genomic aberrations that cause diseases..Fluorescence In Situ Hybridization (FISH) is a process whereby chromosomes or portions of chromosomes are vividly painted with fluorescent molecules that anneal to specific regions. Chromosomal microarray analysis (CMA) is a high resolution, whole-genome screening technique that can identify most of the chromosomal imbalances detected by conventional cytogenetic analysis, as well as smaller sub microscopic deletions and duplications that are referred to as copy-number variants (CNVs) that may miss in the conventional karyotyping.

Genetic disorders are traditionally categorized into three main groups: single-gene, chromosomal, and multifactorial disorders. Chromosomal disorders are due to chromosomal aberrations including numerical and structural damages. Single gene disorders are due to mutation in a single gene causing qualitative or quantitative defects in protein. In multifactorial disease, multiple genes are involved along with environmental factors that contribute to the onset of the disease. Various molecular and cytogenetic techniques have been applied to identify variations leading to genetic diseases.[1] In this review we are giving an account of various genetic techniques used for the diagnosis of genetic disorders.

Cytogenetics- Karyotype

Chromosome disorder is one of the major reason for various genetic diseases which may affect childhood growth and development resulting in congenital malformations, mental retardation, miscarriage, stillbirths, fertility problems and/or malignancy. Cytogenetics deals with the study of chromosomes, their structure, function, variations and

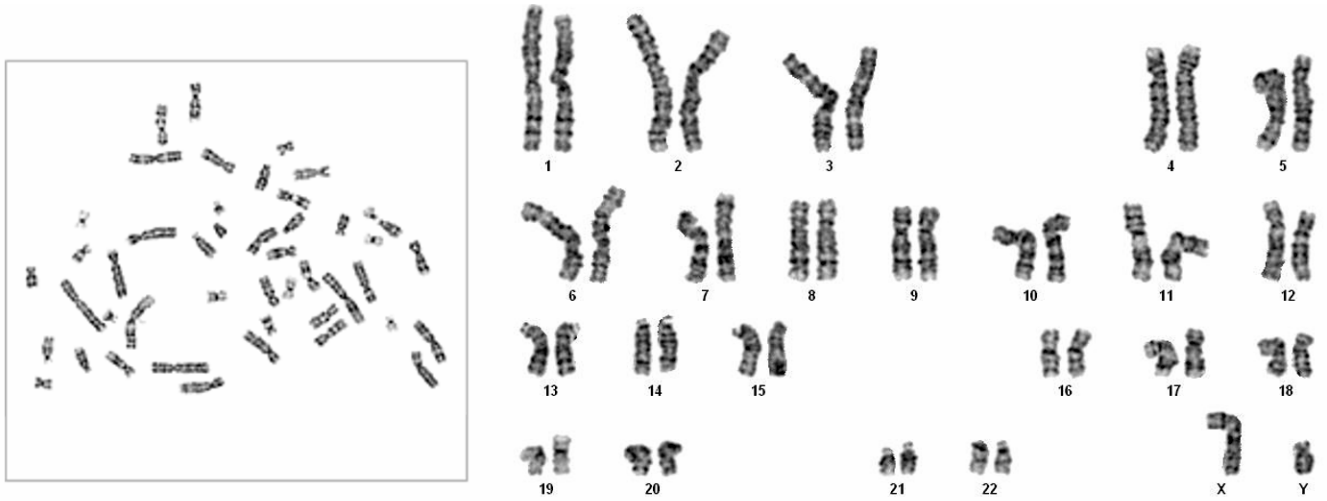

Figure 1. Metaphase spread under microscope and a normal male karyotype

their role in genetic diseases. Cytogenetic analysis plays an important role in investigation of a suspected genetic disorder which provides significant diagnostic and prognostic information. By doing karyotype the structurally visible chromosomal abnormalities like gains and losses of portions of the genome, as well as rearrangements within and among the chromosomes can be visualized under a microscope.

Cytogenetic consists of two branches: Constitutional cytogenetics and Cancer cytogenetics.[2]

Constitutional cytogenetics includes the diagnosis of heritable genetic abnormalities which may be inherited or de novo. The indications for doing karyotyping includes congenital malformation, developmental disorders, intellectual disability, primary or secondary amenorrhea, infertility, recurrent spontaneous abortions and previous child with a chromosomal abnormality.

Cancer cytogenetics includes the detection of chromosomal alterations that get acquired during the course of the disease. Cancer cytogenetics detects the genetic abnormalities for the diagnosis, prognosis, therapy, and/or monitoring of many types of cancer, esp. hematologic malignancy

Karyotype refers to the ordered display of chromosomes starting from the largest chromosome to the smallest followed by the sex chromosomes based on the size, shape, centromere position and banding pattern **(Figure 1).** Chromosomes are visible only during the time of cell division. The most common sample used for cytogenetic analysis is peripheral blood collected in heparinized vacutainer. Other samples like bone marrow aspirate, skin fibroblast and fetal cells by amniocentesis or CVS can also be used for cytogenetic testing. The lymphocytes in the peripheral blood samples are cultured in a culture bottle and are stimulated to undergo division by phytohemagglutinin [PHA] which is a mitogen that induce mitosis in lymphocytes. The dividing cells are arrested in the metaphase stage by adding colchicine, a mitotic inhibitor. These cells are treated with hypotonic solution and fixed with fixative [methanol:acetic acid]. The cells are then dropped on to a clean glass slide from 2 feet height, so that the cell breaks and the chromosome get spread on to the slide. Usually the metaphase from a single cell are found in groups. The slides are then dried, banded using trypsin solution and stained with Giemsa stain [GTG or G Banding]. G Banding is an important technique done before staining, to produce dark and light bands (G bands) in the chromosome which makes the chromosome unique otherwise it will be difficult for recognizing each and every chromosome only based on the size, shape and centromere position. The dark bands correspond to the heterochromatin region [non coding] and the light bands corresponds to the euchromatin region [coding region] of the chromsome. The slides are then viewed under the microscope and well spread G-banded metaphases were imaged, individual chromosomes were identified based on their shape, size, band pattern and arranged to get the karyotype with the assistance of computer software.

Chromosome abnormalities comprise of two main categories: (1) numerical abnormalities (aneuploidy and polyploidy); (2) structural abnormalities (structural rearrangement). Aneuploidy is the most common clinically significant abnormality leads to deviation of the normal chromosome number causing loss or gain of one or several individual chromosomes from the diploid set, which include monosomy (only one copy of a chromosome) eg; Turner syndrome, trisomy (three copies of a chromsome) eg: Down syndrome and tetrasomy (four copies of a chromosome) eg; super female etc. In case of a mosaic, two or more cell lines with different chromosomal constitution can be observed. Aneuploidy commonly results from nondisjunction during meiosis

Structural chromosomal anomaly can be identified by band pattern in the chromosomes. In deletion there will be loss of a part or segment of the chromosome and in duplication there will be an extra copy of a genomic region resulting in partial trisomy. Translocation/ rearrangements involves transfer of

segment of a chromosome to another chromosome. This can be either reciprocal translocation or Robertsonian translocation. In reciprocal translocation there is rearrangement in the position of chromosomal regions between non-homologous chromosomes. In robertsonian translocation, two acrocentric chromosomes join together at the centromeric region to form a metacentric or submetacentric chromosome. In inversion there is reverse orientation of chromosomal regions ie the chromosomal segment is flipped 180 degrees caused by a break at two different sites on one chromosome, which can be pericentric (involving centromere) or paracentric (without centromeric involvement). Other structural rearrangements include isochromosome, an inverted duplication of one arm of chromosome with loss of the other arm, ring chromosome, dicentric chromosome etc. Microdeletion is another structural abnormality where small regions are deleted which usually escape detection during routine karyotyping due to the small size of the deletion.

In **balanced translocation there is no loss or gain of chromosome material,** whereas in case of unbalanced translocation the exchange of chromosome material is unequal resulting in extra or missing genes. Balanced translocations are usually harmless but the carriers of balanced reciprocal translocations have increased risks of creating gametes with unbalanced chromosome translocations, leading to infertility, miscarriages or children with abnormalities. Likewise carriers of Robertsonian translocations are also not associated with any phenotypic abnormalities, but there is a risk of unbalanced gametes that lead to miscarriages or abnormal offspring.

Numerical aberrations and structural anomalies can be detected by cytogenetic analysis. **The major limitation of karyotyping** is that the chromosomal aberration should be large enough at least 5-10Mb size to be detected microscopically. Small sub microscopic alterations below 5Mb cannot be picked up by this method. Moreover, banding analysis is considered to be time- consuming and labour-intensive. Limited chromosome-specific banding resolution makes the characterization and correct interpretation of complex and cryptic chromosome alterations difficult to ascertain. Many of the genetic disorders have mutations involving only one or very few nucleotides which are not analysable by karyotyping. Some special cytogenetic tests can overcome this limitation.

Molecular Cytogenetics

In order to overcome the limitations of conventional cytogenetic analysis, various molecular cytogenetic techniques such as fluorescence in situ hybridization (FISH), spectral karyotyping (SKY), M-Fish and comparative genomic hybridization (CGH)/ Microarray have emerged as successful diagnostic tools and are widely used as accompaniment to classical cytogenetics for identifying chromosomal aberrations. Various molecular cytogenetic techniques have been recognized as valuable additions or even alternatives to conventional cytogenetics as they enhance thorough interpretation of numerical and complex chromosome aberrations by bridging the gap between conventional banding analysis and molecular genetic studies.

Fluorescence *In Situ* Hybridization (FISH)

FISH is a molecular cytogenetic technique that uses fluorescent labelled probes (small fragments of DNA) that bind to specific regions of DNA with a high degree of sequence complementarity. This technique can detect chromosomal abnormalities that cannot be appreciated by standard chromosomal analysis (e.g. microdeletion

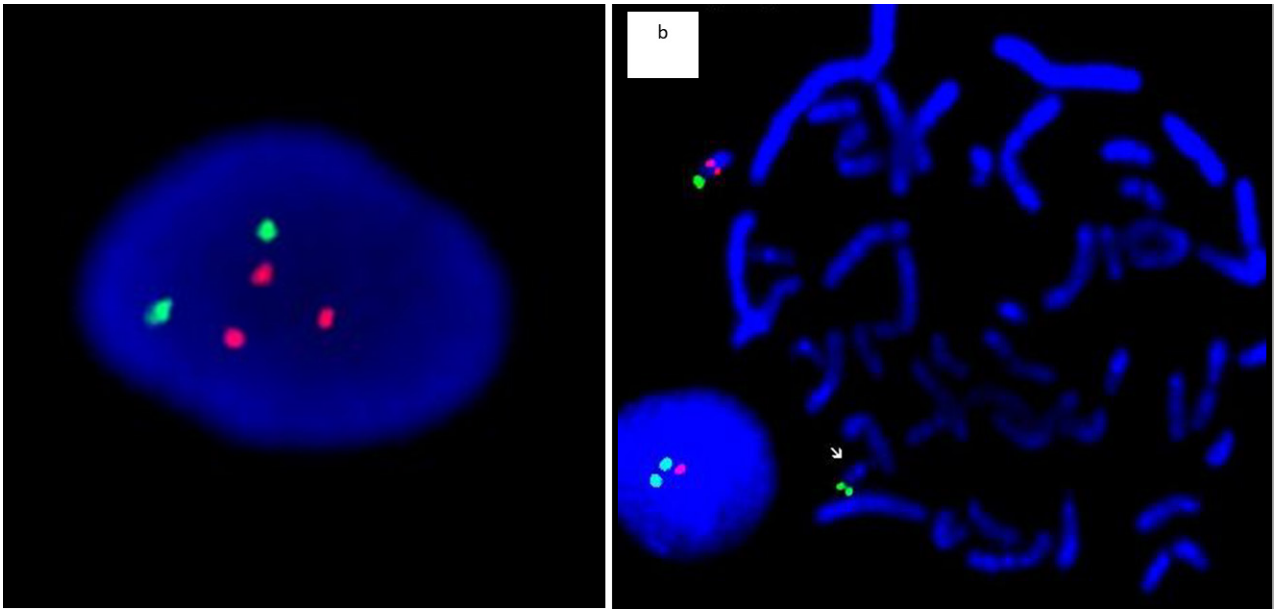

Figure 2 (a). Fluorescent insitu hybridization showing three copies of chromosome 21 [trisomy 21 (3 red signal)] and two copies of chromosome 13 (2 green signals); **Figure 2 (b).** Interphase and metaphase cell showing microdeletion in one of the chromosome [1 red signal], and two green signals as control.

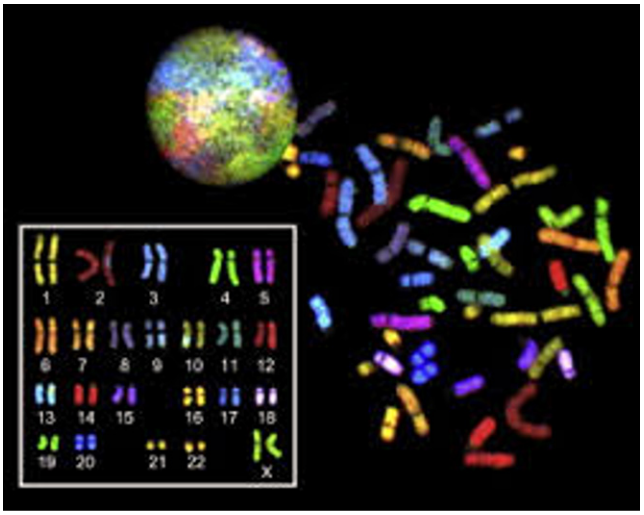

Figure 3. SKY image showing metaphase spread and karyotype with chromosomes specifically coloured with different fluorochromes.

syndromes).[3] FISH is widely used for the localization of genes and specific genomic regions on target chromosomes, both in metaphase and interphase cells. The binding of these fluorescent labeled DNA probes produces fluorescent signals at the chromosomal location. This technique allows to visualize the location of a particular gene or DNA sequence within the chromosome through a fluorescent microscope.

There are different type of fluorescent probes, they are (a) Gene/locus specific probes that target specific nucleic acid sequences on a chromosome. (b) Centromeric probes bind to repetitive sequences that are specific to the centromeric regions. (c) Telomeric probes recognize the repetitive sequence TTAGGG, and can be used to visualize all telomeres simultaneously. Chromosome-specific telomeric probes hybridize to sub telomeric, chromosome-specific repeats. (d) Chromosome-painting probes consist of pools of chromosome-specific probes.

Locus specific or centromeric probes are mainly used for detection of aneuploidies. Each specific probe will be labelled with a different fluorophore. Normally two fluorescent signals are visible for a particular gene locus [eg: 2 red signal for chromosome 21, 2 green signal for chromosome 13 etc], one for each of the homologous chromosomes, are visualized in the interphase and metaphase cells, while trisomy is revealed by the presence of an extra signal and monosomies by the absence of one signal **(Figure 2).**

FISH allows a higher level of resolution than standard GTG-banding allowing the detection of small genomic alterations of 50 Kb to 100 Kb. FISH can be used for aneuploidy screening in prenatal specimens, microdeletion syndromes, evaluation of gene rearrangements in hematological malignancies, rearrangements of subtelomeric regions, and to identify genes with increased copy number. When compared to cytogenetics which require banded and well spread metaphases for analysis, FISH can be performed in both metaphase and interphase cells. FISH is highly sensitive and specific and can be performed rapidly. At the same time the disadvantage is that only one or a few abnormalities can be assessed simultaneously based on the number of FISH probes used for the detection. Moreover, it is possible to detect only the known aberrations eg: microdeletion of a particular gene, translocation, sub telomeric deletion, detection of aneuploidy etc involved with specific disease condition depending on the probes designed.

Spectral Karyotyping (SKY) and Multiplex-FISH (M-FISH)

SKY is a powerful advanced technique and the most significant development in molecular cytogenetics that improves karyotype analysis by identifying chromosomal aberration not previously detected by G-banding alone.[4] Traditional karyotyping, although generally informative, is limited in its ability to detect cryptic translocations, marker chromosomes and to resolve complex karyotypes. While FISH provide accurate means to locate specific aberrations, not possible to visualize all the chromosome simultaneously. Spectral karyotyping on the other hand, which allows the simultaneous visualization of all human chromosomes in different colours using mixture of 24 chromosome-specific painting probes, is a powerful tool to identify subtle chromosomal rearrangements, marker chromosomes, complex karyotypes etc. The detection of these chromosomal aberrations is of great importance for precise diagnosis, risk assessment and genetic counselling both in clinical cytogenetics and in cancer analysis.

Chromosome-specific probes are made by labelling the DNA fragments complementary to each individual chromosome with a different coloured fluorescent dye. The technique make use of 24 chromosome-specific painting probes; each one being labelled with a different combination of five fluorochromes. The labelled whole chromosome DNA probes are then pooled and used to hybridization experiments with metaphase chromosome spreads. The labelled DNA probe sets bind to their complementary chromosomes, allowing each individual chromosome to be labelled with a specific fluorescent colour along its entire length, so that the homologous chromosome will be labelled with the same colours **(Figure 3).**

Each chromosome set (homologous) has its own colour, chromosomal translocations are easily detected when a chromosome shows a region with a different colour; moreover, the second colour reports the identity of the other chromosome involved in the translocation. SKY help to detect subtle chromosomal rearrangements in individuals who appears to have a normal karyotype and more precisely detects the cytogenetic aberrations in individuals with complex aberrant karyotypes.

The resolution of SKY for the detection of inter-chromosomal rearrangements has been shown to be between 500-2,000 Kb. SKY provides the advantage of easy visual interpretation when analysing results, but it is not possible to evaluate

structural abnormalities, such as inversion, deletion, insertion, and duplication in the same chromosome, because these are shown with the same colour. The resolution limit of detection is approximately 1–2 Mb, minor structural abnormalities of less than 1 Mb cannot be detected.

Comparative Genomic Hybridization [CGH]

Comparative genomic hybridization (CGH) is a technique that allows the detection of copy number variation, deletions and duplications of small DNA segments.[5] For this a control DNA and an experimental DNA were isolated, fragmented and labelled with green and red fluorescence respectively for easy detection. The two DNA (green and red) samples are pooled and used together as DNA probes in hybridization experiments with normal metaphase spread. For unaltered chromosomal regions, the green and red probes should bind equally, resulting in an orange/yellow colour. In case of deletion of a chromosomal region in the experimental group, that region will appear green under the microscope. Similarly, if a chromosomal region was amplified in the experimental group, the corresponding chromosomal region will appear red under the microscope.

CGH is a variation of FISH technology which allows screening of the entire genome for diagnosing the aberrations and revealing imbalances across the whole genome. Due to limited resolution (5-10 Mb) of metaphase chromosomes, very small copy number variants cannot be identified. Moreover these experiments are labor- intensive and time-consuming, especially when multiple genomic regions are interrogated. For the better resolution, a high-resolution technique like microarray-based CGH method which does not require the use of metaphase chromosomes.

Cytogenetic Microarray [CMA]

Chromosomal microarray analysis (CMA) is a high resolution, whole-genome screening technique that can identify most of the chromosomal imbalances detected by conventional cytogenetic analysis, as well as smaller sub microscopic deletions and duplications that are referred to as copy-number variants (CNVs) that may miss in the conventional karyotyping. CMA is recommended as the first-tier test in the postnatal evaluation of congenital abnormalities and neurodevelopmental disorders

CMA is based on the principle of complementary hybridization of nucleotide. This method uses arrays containing base-pair fragments corresponding to various region of whole genome adhered to a microchip. The microchip contain 3 million DNA fragments attached to it. Each individual DNA fragment, which is located in a specific position on the chip, corresponds to a known DNA sequence that has been mapped to a specific chromosomal region. The fluorescent labelled probes (green for the control group, and red for the experimental group) are then used in hybridization experiments with the CGH microarray platform, which can be scanned using an automated approach. As described for standard CGH experiments, unaltered chromosome regions show equal binding of the green and red probes and a resulting orange/yellow colour, whereas amplified and deleted chromosomal regions in the experimental group appear red and green, respectively. Microarrays can very precisely determine the chromosomal regions and genes that are amplified or missing. In fact, the information derived from a single array CGH experiment is equal to that derived from thousands of FISH experiments.

The main advantage of this technique is that it can identify cryptic imbalance at the break points which otherwise appears as an apparently balanced translocation by classical cytogenetics.[6] The extra or additional regions attached to the chromosomes can be easily delineated through this method. Likewise an extra chromosome of unknown origin (a marker chromosome) can be clearly identified. The resolution of this method is 1-5 Mb, but can be increased up to approximately 50-100Kb level. Another advantage of CMA is that this technique does not require dividing cells, in contrast to conventional karyotyping, which requires cell culture. Limitations of this technique includes the difficulty in detecting low level mosaicism, balanced translocation, inversions and whole genome ploidy.

CONCLUSION

During evaluation of a suspected genetic disorder, apart from hematological, biochemical and radiological investigations we have to use some specialized investigations. In this narrative review we have discussed the indications, procedure, advantages and limitations of various cytogenetic techniques including advanced molecular cytogenetics. These investigations will help us to detect loss or gain in chromosomal regions and structural chromosomal abnormalities. In the next part we will discuss about the application of molecular diagnostic techniques in the evaluation of single gene disorders.

REFERENCES

1. Korf, Bruce &Sathienkijkanchai, Achara. (2009). Introduction to Human Genetics.;2009
2. Gersen SL, Keagle MB, editors. The Principles of Clinical Cytogenetics [Internet]. 3rd ed. New York: Springer-Verlag; 2013 [cited 2021 May 5].
3. Goldenberg P. An Update on Common Chromosome Microdeletion and Microduplication Syndromes. Pediatr Ann. 2018 May 1;47(5):e198–203.
4. Guo B, Han X, Wu Z, Da W, Zhu H. Spectral karyotyping: an unique technique for the detection of complex genomic rearrangements in leukemia. Transl Pediatr. 2014 Apr;3(2):135–9.
5. Knutsen T. Multicolor FISH (SKY and M-FISH) and CGH. In 2017. p. 833–901.
6. Waggoner D, Wain KE, Dubuc AM, Conlin L, Hickey SE, Lamb AN, et al. Yield of additional genetic testing after chromosomal microarray for diagnosis of neurodevelopmental disability and congenital anomalies: a clinical practice resource of the American College of Medical Genetics and Genomics (ACMG). Genet Med. 2018 Oct;20(10):1105–13.