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Medi Quest BRS Hospital

A monthly News letter from BRS Hospital

GENETIC TESTING FROM BENCH TO ~~BEDSIDE~~ CLINIC Part - II

CYTOGENETICS

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CYTO GENETICS

Cytogenetics is a branch of biology focused on the study of chromosomes and their inheritance, especially as applied to medical genetics

CHROMOSOMAL DISORDERS

There are many types of chromosome abnormalities. However, they can be organized into two basic groups: numerical abnormalities and structural abnormalities.

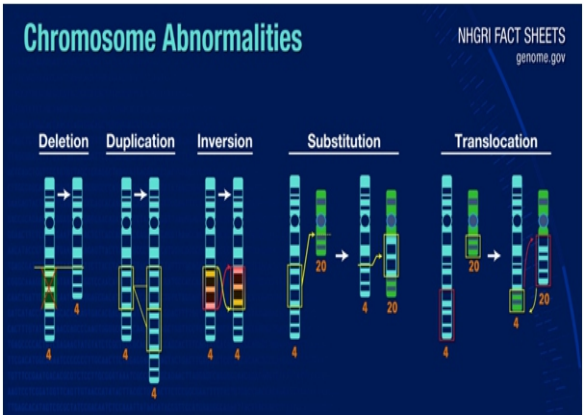
NUMERICAL ABNORMALITIES:

When an individual is missing one of the chromosomes from a pair, the condition is called monosomy. We are familiar with Down's syndrome which is one chromosome extra and Turner's syndrome which is one chromosome less. This is also referred in genetic parlance as aneuploidy which is gain or loss of chromosome . Contrast it with polyploidy where there is a extra set or sets of chromosomes instead of 23 pairs , there is 46 pairs , however polyploidy is not seen in humans. Polyploidy is more common in plants .

STRUCTURAL ABNORMALITIES:

A chromosome's structure can be altered in several ways.

- **Deletions**: A portion of the chromosome is missing or deleted.
- **Duplications**: A portion of the chromosome is duplicated, resulting in extra genetic material.
- **Translocations**: A portion of one chromosome is transferred to another chromosome. There are two main types of translocation. In a reciprocal translocation, segments from two different chromosomes have been exchanged. In a Robertsonian translocation, an entire chromosome has attached to another at the centromere.
- **Inversions**: A portion of the chromosome has broken off, turned upside down, and reattached. As a result, the genetic material is inverted.



Most chromosome abnormalities occur as an accident in the egg or sperm. In these cases, the abnormality is present in every cell of the body. Some abnormalities, however, happen after conception; then some cells have the abnormality and some do not.

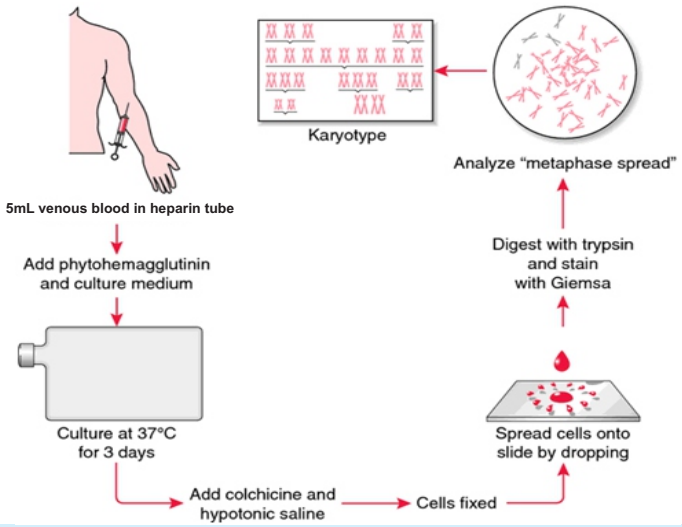
Chromosome abnormalities can be inherited from a parent (such as a translocation) or be "*de novo*" Hence, when a child is found to have an abnormality, chromosome studies should also be performed on the parents

The following investigations in cytogenetics will be discused.

1. Karyotyping
2. FISH - Fluorescent In Situ Hybridisation
3. Comparative Genomic Hybridization also known Chromosomal Microarray testing
4. MPLA - Multiplex Ligase Dependent Probe Amplification Study

1. KARYOTYPING

Karyotype is the pairing and arranging in order all the 23 pairs of chromosomes. Clinical cytogeneticists analyze human karyotypes to detect gross genetic changes—anomalies involving several megabases or more of DNA. The preparation of a karyotype is pictorially given below .



The process of generating a karyotype begins with the short-term culture of cells derived from a specimen. After a period of cell growth and multiplication, dividing cells are arrested in metaphase by addition of colchicine, which poisons the mitotic spindle. The cells are next treated with a hypotonic solution that causes their nuclei to swell and the cells to burst. The nuclei are then treated with a chemical fixative, dropped on a glass slide, and treated with various stains that reveal structural features of the chromosomes.

Banding Patterns Reveal the Structural Details of Chromosomes

Without any treatment, structural details of chromosomes are difficult to detect under a light microscope. Thus, to make analysis more effective and efficient, cytologists have developed stains that bind with DNA and generate characteristic banding patterns for different chromosomes.

Karyotypes can reveal changes in chromosome number associated with aneuploid conditions, such as trisomy 21 (Down syndrome) Trisomy 18 Edward's syndrome and trisomy 13 (Patau Syndrome). Careful analysis of karyotypes can also reveal more subtle structural changes, such as chromosomal deletions, duplications, translocations, or inversions.

2. FISH (Fluorescent In Situ Hybridisation)

How does FISH work?

FISH is useful, for example, to help a researcher or clinician identify specific locations within an individual's chromosomes. The first step is to prepare short sequences of single-stranded DNA that match a portion of the gene the researcher is looking for. These are called probes. The next step is to label these probes by attaching one of a number ofcolors of fluorescent dye.

Sample (patient) DNA is double stranded , by the process of denaturation the single strand DNA obtained. the chromosomes to be tested are fixed on a slide and

denatured in place (in situ) the single strand DNA obtained. Since the researchers' probes are single-stranded, they are able to bind to the complementary strand of DNA, wherever it may reside on a person's chromosomes. When a probe binds to a chromosome, its fluorescent tag provides a way for researchers to see its location.

The uses of FISH technique has the following applications

1. Counting the number of chromosomes
2. Detecting duplications and deletions
3. Chromosomal abnormalities in cancer

You tube video to understand the concept

• FISH – Henrik's Lab
https://youtu.be/LiRJoTi44TA

3. CGH - Comparative Genomic Hybridisation also known as Chromosomal Microarray

Chromosomal microarray checks for deletions or duplications by comparing the chromosomes of the patient with a normal control. Microarray is a device that looks like a microscope slide. It is the slide contains millions of pieces of DNA sequences specific to the type of variation or chromosome being studied. It can span the length of every human chromosome. Alternatively, the microarray may contain only the DNA sequences targeted to detect specific genes or chromosome regions known to cause an abnormal phenotype.

Karyotyping can detect changes or imbalances in Genome if above 5-10 megabases.

CMA can detect changes genome wide imbalances of 400 kilobases or more

Note: 1 Mega base = 1 Million base pairs

1 Kilo base = 1000 base pairs

CMA has better resolution, a 10 fold higher resolution than Karyotyping

Microarray analysis is recommended for patients with:

- Autism spectrum disorders
- Developmental delay/intellectual disability
- Multiple congenital anomalies
- Neurodevelopmental disorders
- Neuromuscular disorders
- Dysmorphic features

Limitations of CMA

Balanced translocation and single gene disorders cannot be identified

You tube video to understand the concept.

• What is Micro Array Analysis - Dr Janine Cody
https://youtu.be/yfPEcXbVk2Y

4. Multiplex Ligase Dependent Probe Amplification Study

In this study the target exons are known , what the investigator looks for is a deletion or duplication .

This investigation has great use in Duchenne Muscular

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Dystrophy and Spinal Muscular Atrophy to identify carriers and for predicting treatment outcomes in patients with DMD .

You tube video to understand the concept

• MPLA - How does MPLA work - MRC Holland
https://youtu.be/gfLJxKuqleY

Next Issue : Next Generation Sequencing and when to order the right Genetic test in different clinical situations

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