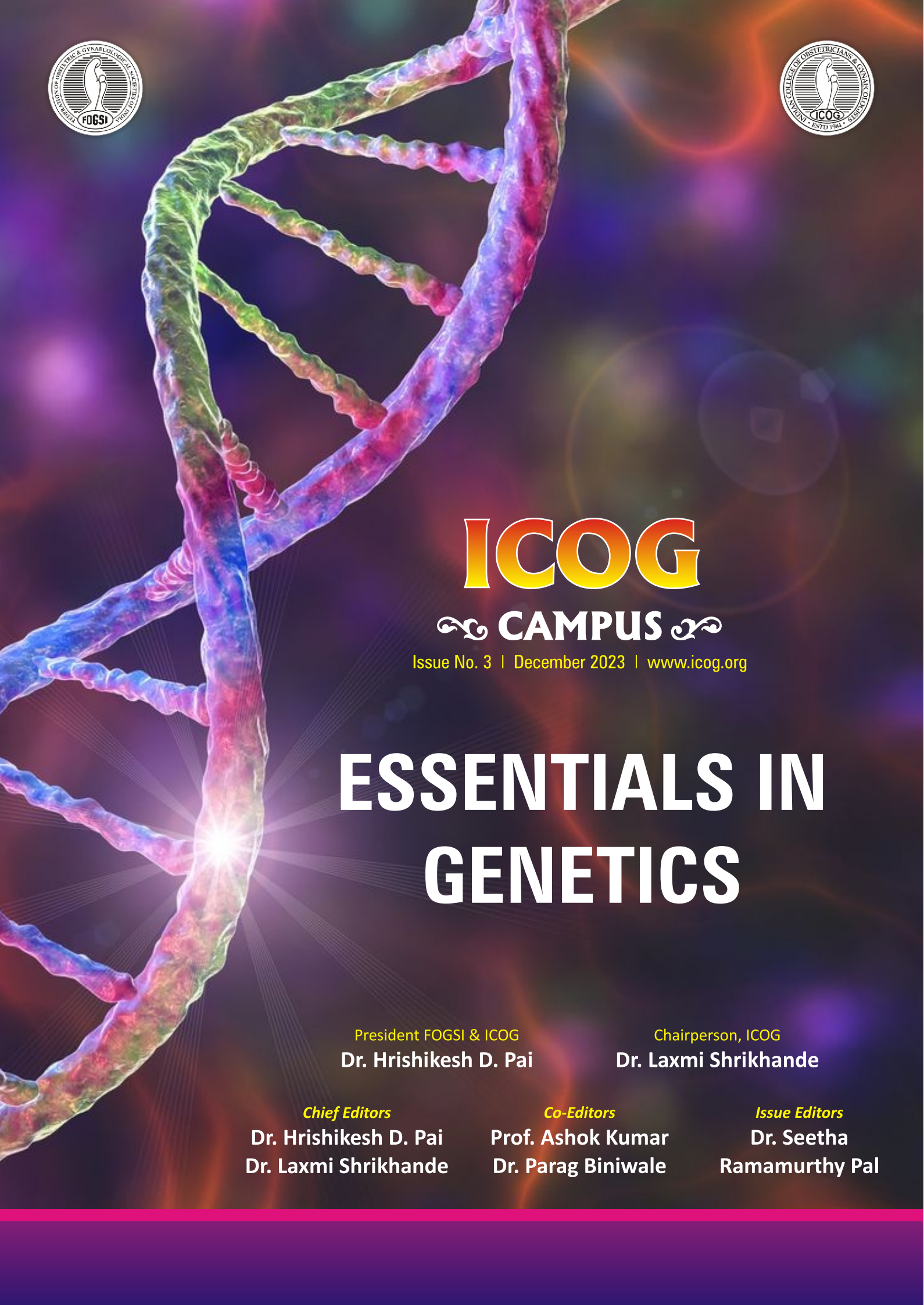

ICOG CAMPUS
Issue No. 3 | December 2023 | www.icog.org

ESSENTIALS IN GENETICS

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Dr. Parag Biniwale

Issue Editors
Dr. Seetha
Ramamurthy Pal

PRESIDENT'S MESSAGE

Dear Friends,

Warm Greetings!!

The FOGSI ICOG online certificate course on "Essentials in Genetics" was held between 22nd to 24th August 2023. The field of genetics has increased dramatically in last few decades, and we as obstetrician and gynecologists are increasingly called on to incorporate genetics and genetic testing in our day to day practice. The program spread over three days covered the important aspects of genetics and its application in the field of obstetrics, gynecology and infertility. The program was a huge success and I congratulate Dr. Seetha Ramamurthy Pal for designing and coordinating and conducting this flawlessly.

With immense pleasure, we now bring to you this ICOG campus on "Essentials on Genetics". The ICOG campus is a compilation of few important topics from the program which are relevant in day to day practice. The chapters are in the form of simple algorithms with stress on main key points thereby making it easier for readers to understand and apply the same in routine practice.

FOGSI has always played a vital role in spreading the knowledge both among doctors and patients. This year my FOGSI slogan is *Swasthya Nari, Sukhi Nari.* My CSR activity is defined as *Badlaav* (Change) including three arms - *Ekikaran* (integration of thought and action), *Samanta* (equality of treatment irrespective of economic status) and *Takniki* (technology to achieve these objectives). These academic publications are a step towards my goal of improving womens health in our country, by providing updated information about the relevant topics in women care.

It will be a ready reckoner for both the students and clinicians to update their knowledge on evidence-based management. I congratulate Dr. Seetha Ramamurthy Pal and all the editors and co-editorsfor their sincere efforts to write, collate, edit and publish this campus.

Wish u all a happy reading.

Dr. Hrishikesh D. Pai
President
FOGSI 2022-2023

CHAIRPERSON'S MESSAGE

Dear Fellows and Members,

It is indeed my pleasure to present to you yet another ICOG Campus on the issue of 'Essentials in Genetics'. Continuing my motto of the activities and newsletters have helped our clinicians to improve their understanding of situations that we may face in our practice.

Genetics is one such area, where significant advances have been made in the recent years from screening to diagnosis, both in Obstetrics and Gynaecology. Medicine is undergoing a paradigm shift with genetics being incorporated in every aspect of clinical medicine and patient management. Hence a working understanding of the underlying concepts of genetic diseases, tests used and their significance is important for all practicing clinicians.

Though its not possible to cover everything of genetics, through this campus, we have tried to cover some important topics of Genetics in terms of the basics, screening, tests undertaken and its role in Gynaecology.

I extend my gratitude to the issue editor, Dr. Seetha Ramamurthy Pal for her efforts in coming out with this campus. I hope the readers find this issue useful and looking forward to more such publications and activities in the following months.

Dr. Laxmi Shrikhande
Chairperson, ICOG

VICE CHAIRPERSON'S MESSAGE

Greetings from ICOG!

Obstetric practice has changed over times with newer advances and understanding of subject. Knowledge of human genetics has increased dramatically, and obstetrician-gynecologists and other health care providers need to incorporate genetics and genetic testing into medical practice. Advances in our understanding of the molecular basis of inherited disorders have led to the development of DNA-based tests that can be used for prenatal and postnatal diagnosis, carrier testing, and aneuploidy screening. These techniques have allowed for diagnosis of a wide variety of genetic diseases ranging from aneuploidies to single-gene disorders. Once a specific pathogenic variant in a gene has been identified, direct testing for that specific variant is possible; this is the most accurate molecular diagnostic method. Counseling patients about genetic testing and results can be challenging and obstetrician-gynecologists and women's health care providers are encouraged to make use of and refer to genetics professionals when necessary.

This issue of ICOG Camus tells us about All about genetics pertinent to Ob-Gyns. I must complements all contributors who have simplified these concepts of better understanding. A special word of appreciation to Dr. Seetha Ramamurthy Pal for putting all these chapters together under the guidance of President Dr. Hrishikesh Pai & ICOG Chairperson Dr. Laxmi Shrikhande. I am sure this will a great ready reckoner to all clinicians.

Happy reading !

Dr. Parag Biniwale
Vice Chairperson, ICOG

SECRETARY'S MESSAGE

Dear Friends,

Namaskar!

Greetings from Indian College of Obstetricians & Gynecologists,

Human genetics has evolved over several decades leading to understanding of molecular basis and DNA based testing for screening and diagnosis in Obstetrics & Gynaecology practice. Various basic tests like karyotyping and rapid aneuploidy and advanced technologies like chromosomal microarray and next-generation sequencing are being utilized in day-to-dayclinical practice and becoming an integral part of routine practice. Common clinical conditions needing genetic investigations are discussed at length in this issue of Campus. It will definitely made the readers aware of advances in the understanding of genetic disease and the fundamental principles of genetic screening and molecular testing.

FOGSI ICOG has conducted a three-day online certificate course on 'Essentials in Genetics' and Campus has followed it to update the ICOGians.

It gives me immense pleasure to introduce this edition of ICOG Campus on "Essentials in Genetics", highlighting several very interesting articles to lay foundation of the subject. ICOG would like to thank the authors for their precious scientific contributions.

I congratulate the editor and her team for bringing out this issue of Campus.

Dr. Ashok Kumar
Secretary, ICOG
Director Professor & Head
Department of Obstetrics & Gynaecology,
Atal Bihari Vajpayee Institute of Medical Sciences &
Dr. Ram Manohar Lohia Hospital, New Delhi

FROM THE EDITORS DESK

It gives me immense pleasure to present to you the ICOG Campus on 'Essentials in Genetics'. I extend my gratitude to our dynamic and visionary FOGSI President Dr. Hrishikesh Pai, Secretary General, Dr. Madhuri Patel and ICOG Chairperson, Dr. Laxmi Shrikhande for entrusting me with this Campus and for their constant encouragement and guidance.

On behalf of the editorial team, I would like to extend my heartfelt thanks to all the authors for their valuable contributions in covering important aspects of Genetics.

We are in the era of genomic revolution and genetics has become part and parcel of our everyday practice. Technological advances in the field of genetics has made us imperative to be familiar with the fundamental principles of genetic screening and testing and be aware of the advances too. In this campus, we have tried to incorporate important and basic topics of Genetics. This campus features articles from the Basics of Genetics, genetic history taking, a brief sneak-peek inside a genetic lab, Interpretation of the most common HPLC report, How to approach a positive serum screening test, Invasive prenatal testing and Genetics in Gynaecology with a brief overview of Preimplantation genetic testing. We sincerely hope that this campus will be useful and help you to incorporate genetics in your daily practice.

Happy reading!

Dr. Seetha Ramamurthy Pal
Issue Editor

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1.	Late Dr. C. L. Jhaveri, Mumbai	1989-96
2.	Late Dr. C. S. Dawn, Kolkata	1997-99
3.	Late Dr. Mahendra N. Parikh, Mumbai	2000-02
4.	Dr. Rohit V. Bhatt, Gujarat	2003-05
5.	Dr. Usha B. Saraiya, Mumbai	2006-08
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15.	Prof. Tushar Kar, Cuttack	2019
16.	Dr. Mandakini Megh, Mumbai	2020-21
17.	Dr. Uday Thanawala, Mumbai	2021-22

PAST SECRETARIES FOR THE PERIOD OF 10 YEARS

SN	SECRETARY NAME	TENURE
1.	Dr. Jaideep Malhotra, Agra	2012-14
2.	Dr. S. Shantha Kumari, Hyderabad	2015-17
3.	Dr. Parag Biniwale, Pune	2018-21

BASICS OF MEDICAL GENETICS FOR OBSTETRICIAN

Dr. Atanu Kumar Dutta
Additional Professor,
Department of Biochemistry, AIIMS, Kalyani
E-mail : atanu.biochem@aiimskalyani.edu.in

In the world of medicine, understanding the fundamentals of medical genetics is crucial for healthcare professionals, especially for obstetricians. With advancements in genomics, our ability to assess and manage genetic conditions in obstetric care has grown significantly. In this newsletter item, we will delve into the basics of medical genetics, with a focus on topics that are particularly relevant to obstetric practice. We will explore the understanding of human genome structure, medical genetics terminologies, and several complex genetic concepts, including incomplete penetrance, variable expressivity, pleiotropy, anticipation, loss of heterozygosity, dominant negative effect, germline mosaicism, somatic mosaicism, locus heterogeneity, allelic heterogeneity, and heteroplasmy. Let's begin our journey into the fascinating world of medical genetics.

Understanding Human Genome Structure :

The human genome is the complete set of genetic information carried by an individual. It is composed of DNA, which is organized into 23 pairs of chromosomes (22 pairs of autosomes and one pair of sex chromosomes, XX for females and XY for males). Each chromosome is a long, linear molecule of DNA wound around proteins called histones. The DNA molecule contains regions known as genes, which are segments of DNA that code for specific proteins or RNA molecules. Understanding the organization and structure of the human genome is fundamental to comprehending the inheritance of genetic traits.

Understanding Medical Genetics Terminologies :

Before we delve into the more complex concepts, let’s ensure we’re on the same page with some fundamental genetic terminologies :

Genotype : This refers to the genetic makeup of an individual, which includes all the alleles (gene variants) present in their genome.

Phenotype : The observable physical or functional traits resulting from an individual's genotype. For instance, the genotype might determine a person's hair color, while the phenotype is the actual hair color.

Allele : Different versions of a gene that can exist at a specific locus (location) on a chromosome. Alleles can be dominant or recessive.

Homozygous : When an individual has two identical alleles at a specific gene locus.

Heterozygous : When an individual has two different alleles at a specific gene locus.

Incomplete Penetrance, Variable Expressivity, and Pleiotropy :

Incomplete Penetrance : This concept refers to a situation where individuals who inherit a disease-causing gene do not necessarily express the associated phenotype. In other words, the gene doesn’t always result in the disease. For example, some individuals with the BRCA1 gene mutation, associated with an increased risk of breast cancer, may not develop breast cancer.

Variable Expressivity : Even when a genetic disorder is fully penetrant, the way it is expressed can vary among affected individuals. For example, in Marfan syndrome, caused by mutations in the FBN1 gene, some patients may have severe cardiovascular problems, while others may exhibit milder symptoms, such as tall stature and joint hypermobility.

Pleiotropy : This refers to a single gene influencing multiple, seemingly unrelated traits. An example of pleiotropy is seen in the gene responsible for sickle cell anemia. This gene not only causes the characteristic blood disorder but can also provide resistance to malaria in heterozygous carriers.

Anticipation, Loss of Heterozygosity, and Dominant Negative Effect :

Anticipation : Anticipation is the phenomenon where the severity or age of onset of a genetic disorder appears to increase in successive generations. This is often observed in disorders caused by the expansion of repetitive DNA sequences. Huntington's disease, for example, exhibits anticipation, with symptoms appearing at a younger age in successive generations.

Loss of Heterozygosity : This occurs when a heterozygous individual loses one copy of a specific gene in a cell, typically due to deletion or mutation, leading to the expression of a recessive disease-causing allele. This loss of heterozygosity can contribute to the development of certain cancers.

Dominant Negative Effect : In some genetic disorders, a mutant allele not only loses its normal function but also actively interferes with the function of the normal allele. This is known as a dominant negative effect. For instance, osteogenesis imperfecta, a condition causing brittle bones, can result from mutations that interfere with the normal collagen production in bone tissue.

Germline Mosaicism and Somatic Mosaicism :

Germline Mosaicism : Mosaicism occurs when an individual has two or more genetically distinct cell populations in their body. Germline mosaicism specifically involves having distinct genetic lineages in the germline cells (sperm or egg cells). This can lead to the transmission of different genetic information to offspring. A well-known example is the condition neurofibromatosis, which can be caused by de novo mutations in the germline, leading to mosaic inheritance in the offspring.

Somatic Mosaicism : In contrast to germline mosaicism, somatic mosaicism involves distinct genetic lineages in non-reproductive cells. This can result in varying clinical presentations within the same individual. Somatic mosaicism is commonly observed in disorders such as segmental neurofibromatosis, where skin lesions are restricted to specific body regions due to mosaic mutations in skin cells.

Locus Heterogeneity, Allelic Heterogeneity, and Heteroplasmy :

Locus Heterogeneity : Locus heterogeneity occurs when mutations at different genetic loci (locations) can cause the same or similar clinical phenotypes. For example, hereditary hearing loss can be caused by mutations in various genes, such as GJB2 and SLC26A4, leading to the same auditory symptoms.

Allelic Heterogeneity : Allelic heterogeneity is a phenomenon where different mutations within the same gene can result in a similar clinical phenotype. Cystic fibrosis, for instance, is caused by a range of mutations in the CFTR gene, leading to diverse clinical manifestations.

Heteroplasmy : Heteroplasmy is a term used in the context of mitochondrial genetics. It describes the presence of a mixture of normal and mutant mitochondrial DNA (mtDNA) within an individual's cells. The proportion of mutant mtDNA can vary from cell to cell, affecting the severity and presentation of mitochondrial diseases.

In conclusion, these are the basic principles of medical genetics that are vital for obstetricians to grasp. Understanding the human genome structure, medical genetics terminology, and complex genetic concepts is essential in providing effective prenatal counselling, genetic testing, and personalized care for expectant parents and their children. As the field of genetics continues to evolve, staying up-to-date with these fundamentals is crucial in delivering the best care to your patients.

GENETIC HISTORY TAKING

Dr. Sujatha Jagdeesh
Head, Department of Clinical Genetics
Mediscan, Chennai

GENETIC HISTORY TAKING :

Genetic history taking is a systemic process of collecting detailed information about an individual's family members, including their health conditions, genetic disorders, and other pertinent medical information. This practice aims to identify patterns of inheritance, hereditary diseases, and potential genetic risks within a family.

One should consider genetic counseling if they have an anomaly, unexplained IUGR, multiple minor anomalies in the ongoing pregnancy and in case of previous child with developmental delay, structural problem in any system, parent with a problem, person with multiple problems, consanguineous marriage, different birth defects in the family. It can be considered if they have a family history of issues like deafness, night blindness, muscular dystrophy, short stature, structural defects either in close family members or found among many family members. It is also indicated in cases of recurrent pregnancy losses and infertility.

The appointment should be set up beforehand. For this the couple should come with all their medical records and in case of an index case, they should bring them. An informal discussion should be started first, introduce yourself, ask for concerns and explain about the referral. It is also good to find out who all have come for the consultation. We should explain to them that we will be taking a detailed history prior to genetic consultation.

The couple should be present for the session and with the couples consent the family members who can give information may be allowed to be present during the session. The session should be done in a private room with the phones off hook. Explain that we need information about family members' health. We will be collecting information about 3 generation members. Collecting accurate information is very essential as this information will help in genetic diagnosis and giving risk information.

It is important that we understand the mindset of family. They will be filled with anxiety and guilt. Sometimes they may not want to hear anything negative. They may or may not want to divulge information. They might also start the blame game. Some may be in fear and frustration. In these kinds of situations, our work is to establish rapport and gain their confidence.

The person who has approached us for knowing genetic risks is known as the consultant. The person who gives the family details is known as the historian, and the person with a genetic problem in the family is known as a proband / index. We need to collect details like the name of the couple / child, their age, blood group, qualification, contact no, occupation, place and ethnicity.

In case of an ongoing pregnancy fetus with anomaly we need to collect a detailed maternal history like the method of conception, paternal and maternal age, their blood group, any medical illness / surgeries in couples and any history of drug intake, alcoholism and smoking. We also need to get the information about their previous pregnancy details like the mode of delivery and the outcome.

When it comes to the history pertaining to system involvement like in case of cardiac, we need to know the parental cardiac status. We also need to collect the family history for any findings related to heart murmur, heart surgery, heart attack, high BP etc. In case of skeletal issues we need to note the parental height and any family history of fractures, bony deformities, curved spine, short stature, unusual shaped limbs, hands, and feet, also note any issues with joint mobility. As for renal issues, we should find the renal status of the parents, their family history for any dialysis, surgery USG etc.

If the previous child is affected, we should collect the previous pregnancy details like the Gravida / para, the mode of conception, course and outcomes of all previous pregnancies, medical illness in the mother, and the medications taken by her. After this, we need the pregnancy information of the affected child, like the mode of conception, medications, maternal illness, teratogen exposure, USG / Screening reports and the delivery details. At last we should collect the clinical course of the affected child. In this part we need to know the anthropometry at birth, neonatal course, feeding, milestones, any medical concerns, time of identification of the problem, tests done and the clinical course / trt given so far.

In case of infertile couples, for the wife, we need to note down their age, age of menarche, menstrual history, medical history, imaging studies, their hormonal profile, karyotyping and molecular results. As for the husband, their age, sperm count, hormonal profile, imaging studies, karyotyping and molecular results. We also need to take a 3 generation pedigree.

History taking plays a very important role when it comes to genetic counseling. Reviewing all medical records, examining family members, reviewing old photographs, documenting all the findings and getting details specific to the anomaly are all very vital.

PEDIGREE :

A pedigree is a visual representation or chart that displays the genetic relationships within a family or a specific group of individuals. It is often used in genetics and medical fields to illustrate the inheritance patterns of traits, diseases, or genetic conditions across generations. It is a representation of a complicated 3 generation family history. At one glance, we can see the concerned person in question. It can also be used to find the risk for other family members. The pedigree serves as a tool to explain families about their genetic risk as the pattern of inheritance can be seen clearly. But it has to be updated at every visit.

Before starting, introduce yourselves and explain why pedigree is necessary. “We will be asking health related questions for members over 3 generations, this is needed to provide you with appropriate medical care”. A big family representation will help to gather more information. Confidentiality and privacy should be strictly maintained. It is good to get an informed consent from the individual before revealing the medical or personal information to other family members. Necessary care must be taken not to end up breaking the families.

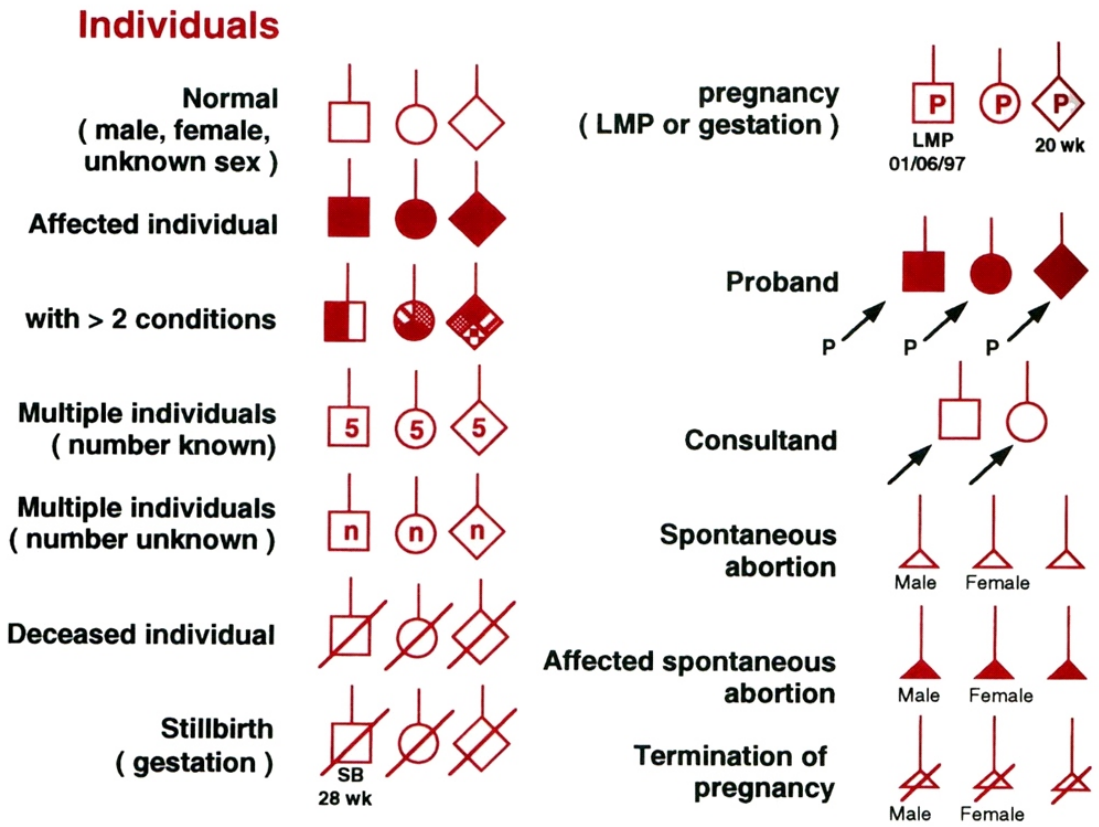
The pedigree should be taken for 3 generations. Couples and 1st degree relatives are children, siblings and parents. 2nd degree relatives are half siblings, uncle - niece, aunt - nephew. First cousins are 3rd degree relatives. If any significant history is present, it can be extended further and questions can be repeated to ensure that the answers are correct.

It's like digging out skeletons from the closet, many may want to keep the information private. It is important to choose appropriate words instead of saying positive or negative family history. It’s good to replace it with contributory or non-contributory. It is good to avoid leading questions, for example,

instead of asking “are your sisters / brothers healthy?” You could ask “Do they have any health problems?”. It is very important to be sensitive to cultural issues, do not rely on a family member for interpretation as facts may be changed.

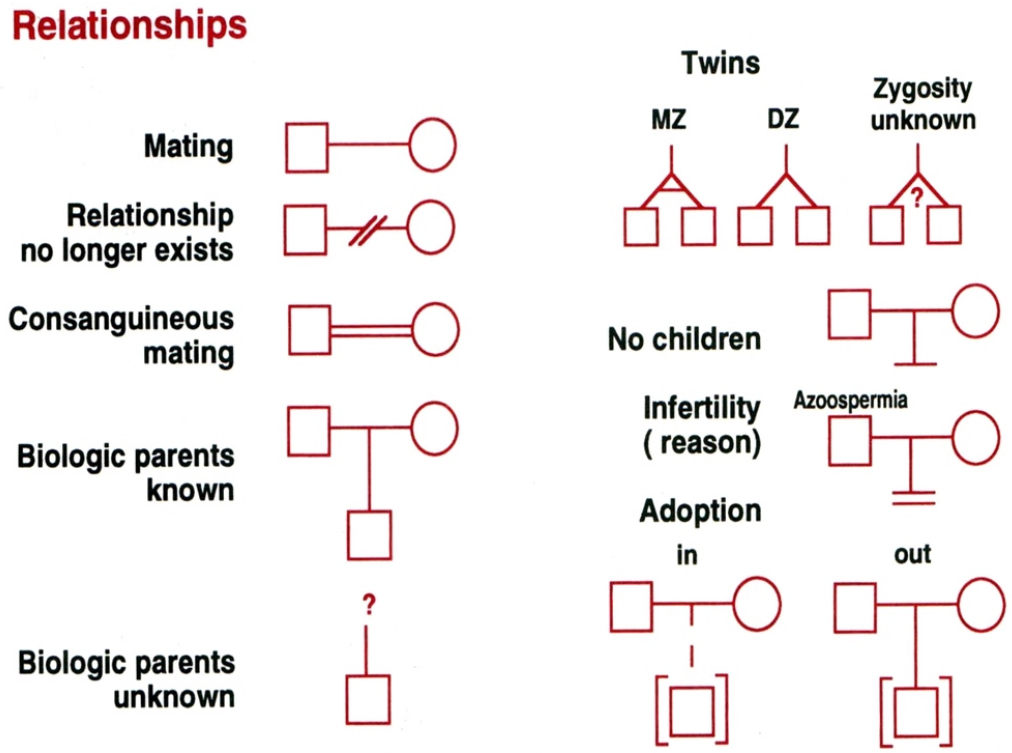
First degree relations like parent-child and siblings share 50% of genes. Second degree relations like half siblings, uncle-niece, aunt-nephew share 25% of genes. Third degree relations like first cousins, half uncle-niece, half aunt-nephew share 12.5% genes. As for the fourth degree relations like first cousins once removed, half first cousins share 6.25% genes.

Key features of a pedigree include symbols. Normal male is represented by a square, a normal female is represented by a circle and a normal unknown sex is represented by a diamond. If the individual is affected they can be shaded and if they are affected by more than 2 conditions they can be shaded in different ways. If there are multiple known individuals, the number can be written within the diagram and if they are unknown, it can be represented as ‘n’. If the individual is deceased a line should be drawn across the symbol. For stillbirth, mention the weeks below the symbol. For pregnancy, mention ‘P’ within the symbol and write LMP or gestation below the symbol. In case of spontaneous abortion, the symbol is a triangle, and if affected, shade it.



In a pedigree, lines are drawn to connect these symbols, indicating family relationships. Vertical lines represent parent-child relationships, and horizontal lines signify a marital connection. For a relationship that no longer exists, draw a horizontal line connecting 2 symbols with 2 diagonal hash marks. In case of a consanguineous marriage two parallel horizontal lines are drawn between the male and female. Twins are represented by two diagonal vertical lines originating from the same point. If a couple doesn't have children, a vertical line extends downwards and ends with a horizontal line, and in case of infertility draw two horizontal lines. If the child is adopted within the family, it is represented by a vertical dash line downwards and the child symbol is drawn within a square bracket whereas in case

of the child being adopted from the out, it is represented by a vertical line downwards and the symbol is drawn within a square bracket. For a carrier of sex linked recessive, the symbol is a circle with a dot inside it. A vertical line leading from parents to offspring is called a line of descent. A horizontal line connecting brothers and sisters is called a sibship line.



In the pedigree chart that we use, the male partners are to the left of female partners. Start in the middle of the page when drawing a pedigree. Siblings are drawn from oldest to youngest with the oldest listed on the left and the youngest on the right.

In the pedigree chart, it is good to include the age or date of birth, date of death. As for those with medical problems, write down things like if they smoked, diet or exercise habits, etc. For those with medical conditions, write down how old the person was when they were diagnosed / deceased. Ethnicity wherever required, mention key to shading of symbols, adoption status, consanguinity, race and ethnicity, date the pedigree was obtained, birth defects, heart disease, diabetes, asthma, high blood pressure, stroke, kidney disease, learning problems or mental retardation, vision or hearing loss at a young age, known genetic conditions.

There are some myths that should be broken. These myths are like for example, mother has done something like not eating well or fighting. If there is nobody with a problem in the family it's not genetic. All birth defects are inherited. If 2 boys are affected it is certainly a disease affecting male lineage only and vice versa. Facial resemblance to a diseased person does not mean disease. It's a curse or black magic cast upon the family.

In conclusion, good history taking is an art. Empathy and patience are required. Equal care should be put for eliciting details in dead/live babies. All the information should be communicated well to the family. Three generation pedigree is a useful tool in understanding inheritance patterns. We can also have an idea about co morbid conditions in the family.

INSIDE THE LAB : TECHNIQUES FOR GENETIC DIAGNOSIS

Dr. Kausik Mandal

MD (Pediatrics), DM (Medical Genetics)

Additional Professor, Department of Medical Genetics

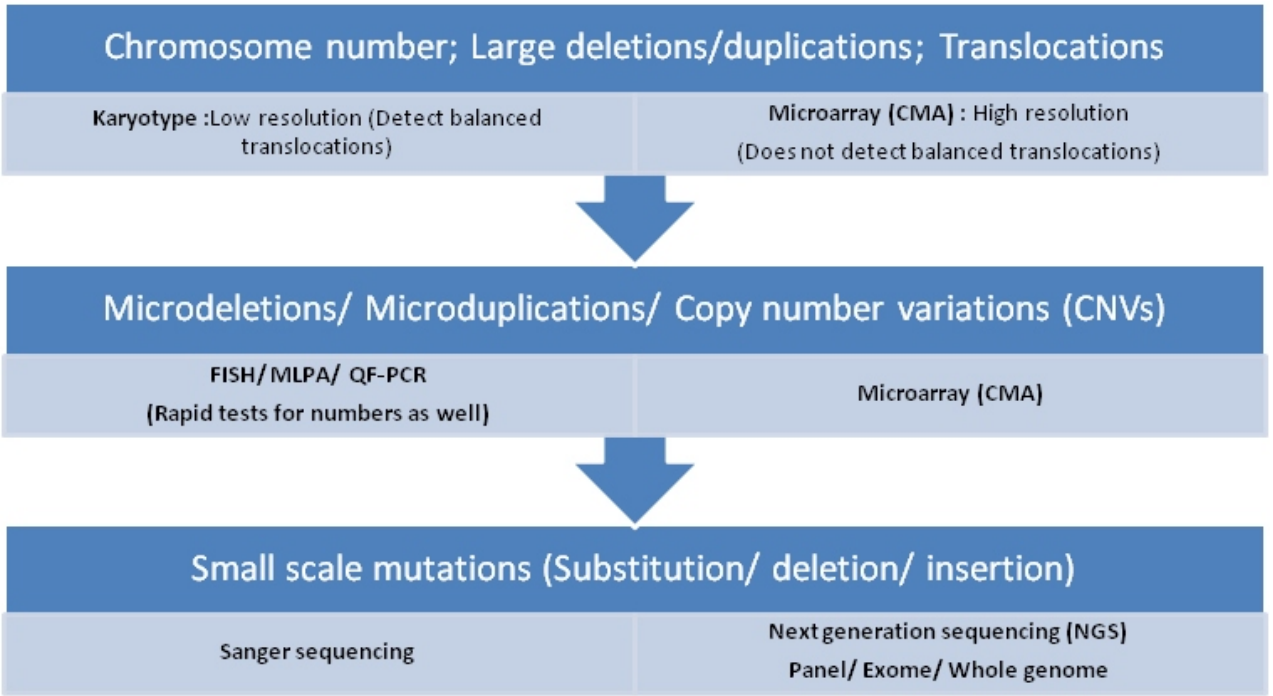
Sanjay Gandhi Post-Graduate Institute of Medical Sciences, Lucknow

+91-522-2494327 I mandal.kausik@gmail.com; kausik@sgpgi.ac.in

Introduction :

Genetic testing has become necessary for inherited (germline) and somatic variations giving rise to various genetic disorders and cancers in humans. There is no single genetic test which can be offered to an individual to identify the underlying genetic defect. The various techniques and the resolution of each method have been described below :

Resolution of different genetic techniques



Karyotype requires living cells :

Karyotype is an age old technique used to detect chromosome numbers and gross chromosomal structural defect. As there is need for cell culture and arrest of cell cycle at metaphase, we require living cells to start with. The different steps are as below :

Sample

- Blood in heparin
- Amniotic fluid
- Tissue / villi

Culture initiation :

- Sterile culture tubes / flasks
- Sterility- sterile environment under laminar flow/UV light
- Culture medium

Incubation for 72 hours : Temperature : 37°C

CO_2 incubator

Harvesting

- Colchicine metaphase - arrest
  - incubator 37°C
- Hypotonic solution
  - centrifuge
- Fixative

Slide preparation

- Fixative
- Drop to the cool, wet, good slide by drop by drop from 2 feet height
- Blow, dry on 56°C
- Keep at room temp for 3 days or overnight for 56°C
- Banding
- 0.3% trypsin solution keep at room temp (for routine GTG banding)

Staining

- Giemsa (for routine GTG banding)

Reporting :

- Low power to look for metaphases
- Oil immersion - at least 20 metaphases

Nomenclature : ISCN

- Landmark band
- Region
- Band
- Subband

ISCN Symbols

- cen centromere
- del deletion
- dup duplication
- fra fragile site
- i isochromosome
- inv inversion
- r ring chromo
- t translocation
- +7 gain of chr 7
- -7 loss of chr 7
- 7q+ extra material on 7q
- 7p- del of part of 7p
- mos mosaic
- der derivative chro
- ter terminal

Examples :

- 46,XX,t(X;14)(q27;q11) : Balanced translocation
- 46,inv (9)(q11q22) : Paracentric inversion
- 47,XX,+21 : Trisomy 21
- 46,XY,del(5)(p21): Deletion of 5p

Normal variants

- Yq+
- Inv 9
- 16 qh+

What do you expect from a karyotype report :

- Reporting takes 2 to 4 weeks
- Most karyotype may be normal
- Consider culture failure rate
- Look at the chromosomes
- See the band level/ resolution

Mosaicism : Detection by karyotype

- Mosaicism of two or more cell lines can be present with cut off levels for low-grade mosaicism yet to be defined
    - some report 5%
    - while others report 20% diagnostic levels

FISH s a better method to detect mosaicism

Chromosomal constitution in Turner syndrome : Example

Chromosomal constitution	Remarks	Percentage of Turner syndrome
45,X	Monosomy of X in all cells	40 - 60%
46,X,i(Xq)	Isochromosome of long arm of X (duplication of long arm with loss of short arm)	5- 10%
Other structural defects involving X chromosome	Ring X, Partial deletion of p or q arms, Isochromosome of p arm, X-autosome or X-Y translocations	Rest
45,X/46XX; 45,X/47,XXX; rarely 45,X/46,XY	Mosaicism of 45,X with various other cell lines	Rest

Chromosome Translocations

- Exchange of material between chromosomes
- 2 types
    - Reciprocal
    - Robertsonian
- Incidence at birth : 0.27%(0.17 reciprocal & 0.1 robertsonian)
- Inherited: 80%, De novo: 20%

Balanced Translocations and Gamete Production

- Individuals carrying balanced translocations have a greater risk of producing gametes with unbalanced combinations of chromosomes
    - This depends on the segregation pattern during meiosis I

- During meiosis I, homologous chromosomes synapse with each other
    - For the translocated chromosome to synapse properly, a translocation cross / quadrivalent must form
- Behaviour at meiosis
    - Alternate segregation
    - Adjacent - 1 segregation
    - Adjacent-2 segregation

Fluorescence in situ hybridization (abbreviated FISH) is a rapid technique

In this technique, the chromosomes affixed to a glass slide and then exposed to a "probe" - a small piece of purified DNA tagged with a fluorescent dye. The fluorescently labeled probe finds and then binds to its matching (complimentary) sequence within the target area in the set of chromosomes. FISH involves unwinding of the double helix structure and binding of the DNA of all probes attached to a fluorescent molecule with a specific sequence of sample DNA, which can be visualized under the fluorescent microscope. It s used to detect certain chromosome numbers or microdeletions. It can be used in interphase or after preparation of metaphase as per requirement.

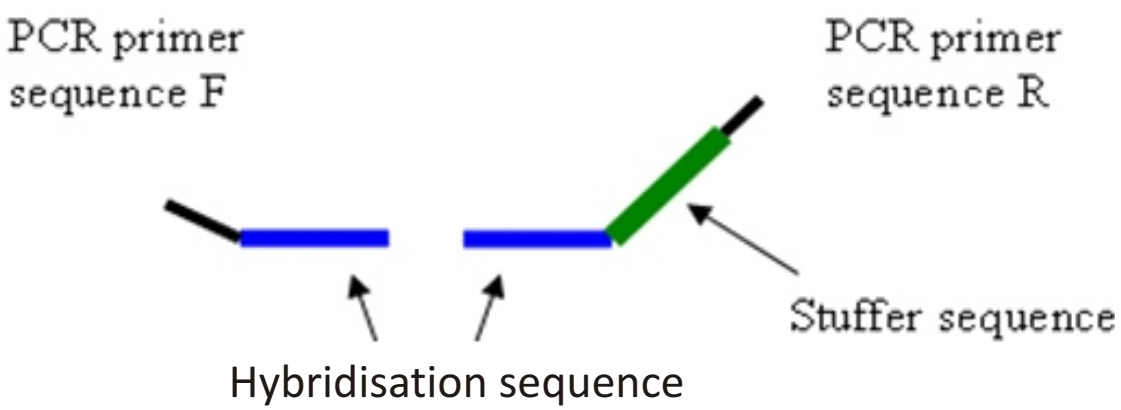
Multiplex Ligation Probe Amplification (MLPA) :

In various single gene disorders, the variation might be deletions or duplications which are fairly large, involving whole or large part of an exon (many nucleotides). Sequencing techniques, as described above are unable to pick them up, since, in heterozygous condition, the normal allele will amplify and show normal peaks in Sanger and in homozygous condition, the area of deletion would be uncovered in NGS. In such situations, the technique used is MLPA, which has the capability of dosage analysis. The principles are :

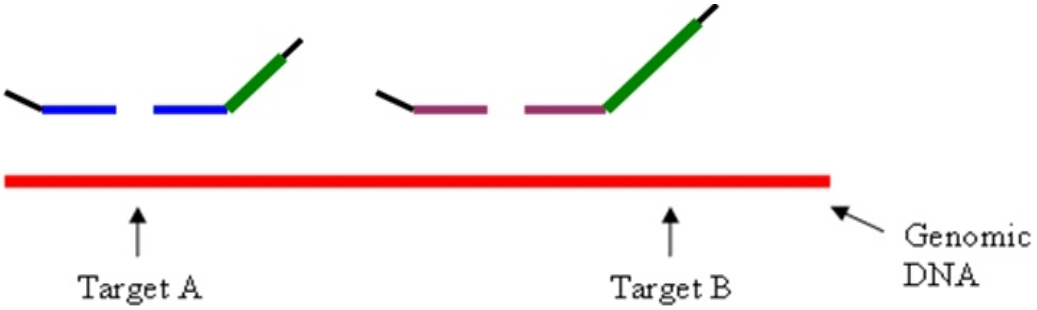
- Here there is amplification of probes and not the target DNA
- 40-45 targets can be analysed, simultaneously, in a single tube
- Deletions / duplications are the main targets, can also detect single nucleotide change
- Various probe sets available for different applications
- Machines required are :
    - Thermocycler (PCR machine)
    - Sequencer for fragment size analysis

Technique of MLPA :

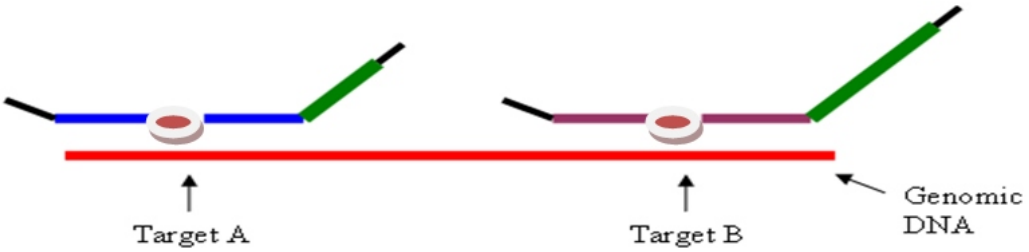
A probe pair for a target



1. Denaturation and Hybridization

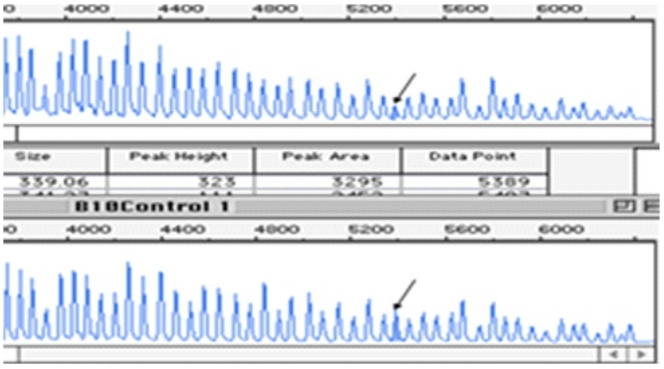


2. Ligation

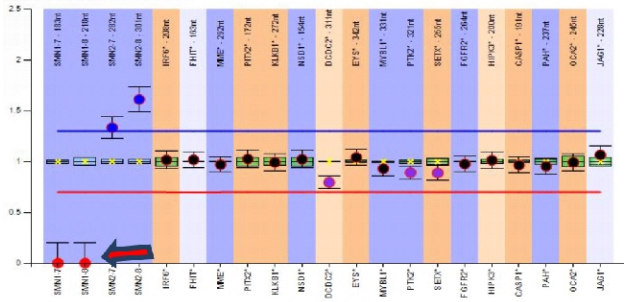


3. PCR of ligated probes

4. Capillary electrophoresis- Fragment analysis



5. Result analysis by software



Chromosomal microarray / Cytogenetic microarray (CMA)

Different types of array are used.

- Recent ones like 750K Assay Kit (CytoScan® 750K Assay Kit, Affymetrix, USA) use both ligos and SNPs

Lab cut-offs for deletion and duplication are largely uniform

Softwares are required for analysis and interpretation

Overview of different cytogenetic and molecular cytogenetic techniques :

- **Conventional karyotype** can scan the whole genome
    - Look into gross numerical and structural variations
    - It is time consuming but gives lot of information in certain situations
- **FISH** can detect smaller deletions - interstitial or terminal
    - FISH is a rapid test
    - Targeted FISH cannot scan the genome
    - Prior knowledge of target is necessary (understanding the disease)
    - FISH cannot detect tandem duplications no dosage analysis
- **Multiplex Ligation-dependent Probe Amplification (MLPA)**
    - Detect small deletions (less than around 4 Mb)
    - Can also detect duplications
    - Does not require living cells
    - Shorter reporting time
    - Limitations
        - Does not detect balanced translocations
        - Does not detect polyploidy
        - Prior knowledge of target
- **Cytogenetic microarray (CMA)** can detect small deletions and duplications undetectable by routine karyotype
    - CMA does not detect translocations
    - Sometimes parents need to be screened for copy number variations
    - Many gains or losses detected in chromosomal micro-array might have minimal effect
    - Need for bioinformatics and databases

DNA sequencing :

Sequencing essentially targets to find out the sequence of nucleotides in a given segment of the genome. We assume that a change of a nucleotide/ base (A, T, G or C) by another (substitution) or omission (deletion) or introduction (insertion / duplication) of one or few nucleotides in an exon of a gene or in nearby areas is likely to affect the protein encoded by the gene. There can be a change in amino acid (missense mutation) in the sequence or introduction of a stop codon (Nonsense mutation), shift in the coding sequence (frameshift mutation) or aberrant splicing defect (splice-site mutation). The technique has evolved with time and can be divided into generations as below :

The Generations of DNA Sequencing

- **1st Generation**
    - **Sanger Sequencing**
    - Capillary based (96 capillaries * 4 hours) = 512 Seq / day
    - Di-deoxy sequencing reaction (prior to machine run)

- Variable length reads; typically 700-1000 nucleotides long

- **2nd Generation**
  - **Illumina / Ion Torrent Sequencing (NGS)**
  - Chip or slide based sequencing of 'clusters' - 10s of millions of reads
  - Sequencing-by-synthesis sequence is generated with cycles of incorporation
  - Typically fixed length short reads; 100nt 250nt (depends on application / platform)
- **3rd Generation**
  - **"Long read sequencing" or SMRT-sequencing**
  - Continuous native process (ZMW + phospholinked nucleotides)
  - Variable length reads; 100 nt to 75,000 nt in length

The techniques and chemistry involved in 1st generation sequencing are as below :

Methods of sequencing : Base by base

A. **Sanger dideoxy (primer extension / chain-termination) method :**
- most popular protocol
- very adaptable
- scalable to large sequencing projects

B. **Maxam-Gilbert chemical cleavage method : (2 years after Sanger) 1976-1977**
- DNA is labelled and then chemically cleaved in a sequence-dependent manner
- Became very popular to start with (Non-purified DNA)
- This method is not easily scaled
- Tedious
- Uses radioactive methods

Because of various above reasons, Sanger sequencing has become the standard sequencing method for many years now.

Principles of Sanger sequencing (DNA sequencing)

- Random incorporation of a dideoxynucleoside triphosphate into a growing strand of DNA.
- Incorporation of di-deoxynucleotides into growing strand terminates synthesis.
- The 3'-OH group necessary for formation of the phosphodiester bond is missing in ddNTPs.
- **Fluorescent** dyes are attached to either the primer (I° generation) or the**ddNTP**(II° generation).
- With addition of enzyme (DNA polymerase), the primer is extended until a ddNTP is encountered.
- The chain will end with the incorporation of the ddNTP.
- The collection of fragments is a sequencing ladder.
- The resulting terminated chains are separated by gel or capillary electrophoresis.
- The DNA ladder is read on an electropherogram

Overview of Sanger sequencing technique

- Know the gene : OMIM and other databases
- Count the number of Exons:
  - FASTA
  - CDS
- Design primers for each exon (more primer pairs may be required if the exon is large)
- Steps (Pre-sequencing)
  - DNA extraction
  - PCR

- Product purification
- Sequencing PCR (Separately Forward and Reverse)
- PCR purification

- -Sequencing by capillary electrophoresis
- Data analysis

Nomenclatures that need to be understood for depicting sequence change

- NM_ID / ENST_ID
- c. Number (Starting from first nucleotide of coding sequence)
- cDNA Number
- g. Number (genomic)
- c. Number + or c. Number (Distal to last or proximal to the first nucleotide of the neighbouring exon) for intronic variations
- - c. Number for 5 prime UTR

Applications of Sanger sequencing

- Targeted analysis for known sequence variations
  - Achondroplasia
  - Apert
- Small genes with fewer than 15 to 20 exons
  - Beta-thalassemia (3 exons)
  - VHL (3 exons)
- Few small genes / certain mutation causing the same phenotype
- Along with NGS
  - Validation of NGS result
  - Sequencing gaps
  - Targeted testing in family members for segregation
  - Targeted testing for prenatal

Sequencing Workflow for Next generation sequencing (NGS)

1. Obtain tissue/ cells
2. Extract DNA/RNA
3. Sample preparation
4. Sequencing (above steps)
5. Bioinformatics analysis

Check QC (Tool: FASTQC)

Trimming (TRIMMOMATIC)

Reference mapping (STAMPY)

Data analysis

- ➢ Remove variants frequently present in population
- ➢ Select variants which are primarily present in exons and splicing junction
- ➢ Remove synonymous variants
- ➢ Select zygosity

Understanding terms :

- ➢ Coverage : It denotes how much of the sample is covered by sequencing. It can be reported as the percentage of the bases covered by sequencing reads, e.g. 95%

coverage indicates that 95% of the bases in the sample have been sequenced (at depth n)

- Depth : It indicates how many reads detected a specific nucleotide. This can be a strong indicator of the reliability of a base call. Low read depth can also indicate that a specific region is poorly represented in the sample. For example, if at a region, base call has 30 reads mapping onto it, then it has a 30x read depth.
- VOUS / Pathogenic / Likely pathogenic variations : ACMG criteria to classify variants

Applications of NGS :

NGS is done for

- Large gene where sequence variations are cause of disease
- Multiple causative genes for a phenotype
- Difficult clinical diagnosis
- Novel disease gene discovery

Panels : Exons and known mutations of selected genes responsible for similar phenotypes e.g. Nephrology panel, Hereditary cancer panel

Exome sequencing : Exons (comprises of 1% of genome) harbouring more than 80% of mutations

Whole genome sequencing : produces huge amount of data

Yield of NGS is variable

- 30% (unknown aetiology / poorly understood phenotype)
- 60% (well characterised phenotype)
- 90% in very well characterized single gene disorders
- **PHENOTYPING IS THE KEY** (HPO terms)

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INTERPRETATION OF HPLC REPORT FOR THALASSEMIA SYNDROMES / HEMOGLOBINOPATHIES : AN ART TO UNDERSTAND AND DECIDE A TIMED MANAGEMENT

Dr. Kushagradhi Ghosh*, Dr. Ankur Biswas**

*MBBS, MD, DNB, FICOG, FRCOG, RCR / RCOG PG Dip in Advanced Fetal USG

Professor : OBGYN, RSMSP/ VIMS, Kolkata University

Being the Thalassemia capital of the entire world, one in eight thalassemia patients in the world resides in India with an estimated 42 million beta-thalassemia carriers and 10,000 new cases are reported each year. High-performance liquid chromatography (HPLC) is a novel, easy and cost-effective technique with excellent resolution, reproducibility & quantification of several normal & abnormal hemoglobin resulting in accurate screening of thalassemia syndromes and hemoglobinopathies.

Interpretation of HPLC report in correlation with clinico-hematological profile is required for appropriate diagnosis and timed management and intervention. First and foremost step is to look for complete hemogram picture including hemoglobin level, RBC indices, peripheral smear, reticulocyte count, white cell and platelet count. Hb level below 11 gm% indicates anemia whereas microcytosis (MCV<80 fL), hypochromia (MCH<27 pg) may be found in both thalassemia and iron deficiency anemia. Presence of abnormal cells like target cells, sideroblasts, inclusion bodies or any immature white cells in PBS as well as platelet counts have to be looked for. Another important parameter which is increased in thalassemia group of diseases unlike iron deficiency anemia is red blood cell count due to increased production of more and more RBCs by bone marrow and microcytic phagocytic system (ineffective erythropoiesis).

Thalassemia trait (minor) can masquerade as iron deficiency anemia as Hb% and RBC indices remain almost same. Therefore, CBC alone is not sufficient to diagnose and distinguish thalassemia and iron deficiency anemia especially India where burden of both the aforesaid diseases are quite high. Recommendation to do routine HPLC in all pregnant women to rule out thalassemia before confirming iron deficiency anemia with costly iron studies is justified from cost effective point of view.

Being a screening tool, HPLC has pitfalls of both false positivity and negativity. Clinically measured hemoglobin in human circulation is heterogenous --- it's made up of different fractions. HPLC detects relative proportions of those normal various hemoglobin fractions as well as presence of any abnormal hemoglobins. Two hematologically distinct entity is there called thalassemia and hemoglobinopathy. Thalassemia is a group of disorder where there is reduced production of normal Hb chain either in alpha or in beta. Instead, in hemoglobinopathy, structurally abnormal Hb chains are produced. Both the cases result in hemolytic anemia owing to low functional hemoglobin formation.

Normal Hb composition in our body :

HbA_0 : made up of $\square_2$ $_2$ --- usually >95%

HbA_2 : made up of $\square_2$ $_2$ --- < 3.5%

HbF : made up of $\square_2\square_2$ --- <1%

HbA_1 (abc) : trace

No abnormal Hb

HPLC of a normal human adult has 3 peaks --- HbA1 which is closely related to and co-elutes with HbF, HbA0 and HbA2.

Interpretation of HPLC :

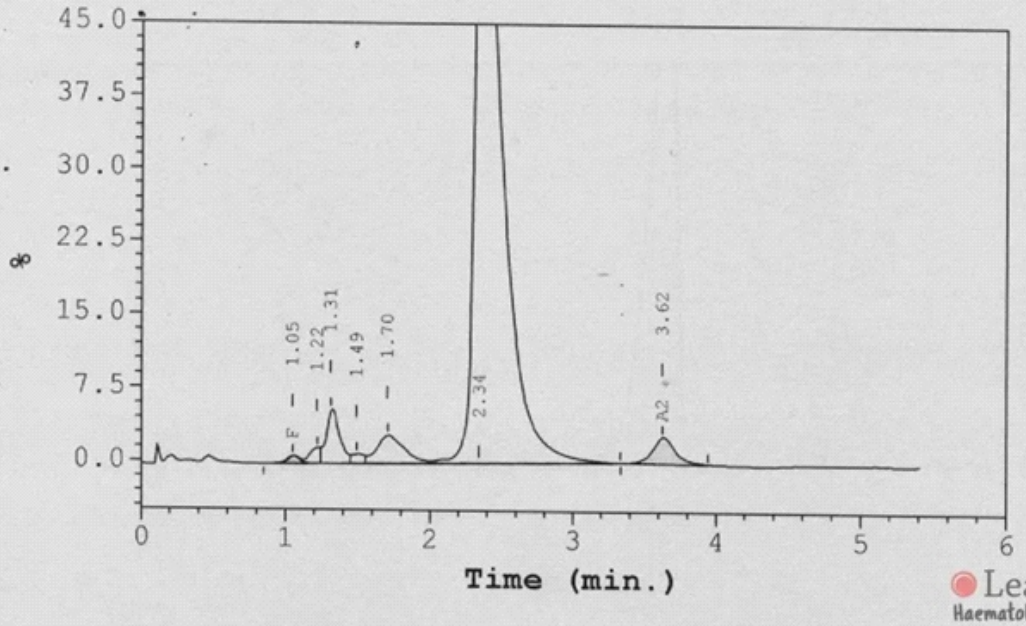
Normal HPLC:First look at HbA2 whether it's in normal range or not. Then look upon other variants. For example, in pic 1, HbA2 is 2.9% which is less than 3.5%, HbF is 0.5% which is less than 1.5% no abnormal spike. So, it's a normal HPLC report.

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
F	0.5	---	1.05	9772
Unknown	---	0.9	1.22	17939
P2	---	3.4	1.31	69912
Unknown	---	0.6	1.49	11829
P3	---	3.7	1.70	75191
Ao	---	87.9	2.34	1787232
A2	2.8	---	3.62	62277

Total Area: 2,034,152

F Concentration = 0.5 %
A2 Concentration = 2.8 %

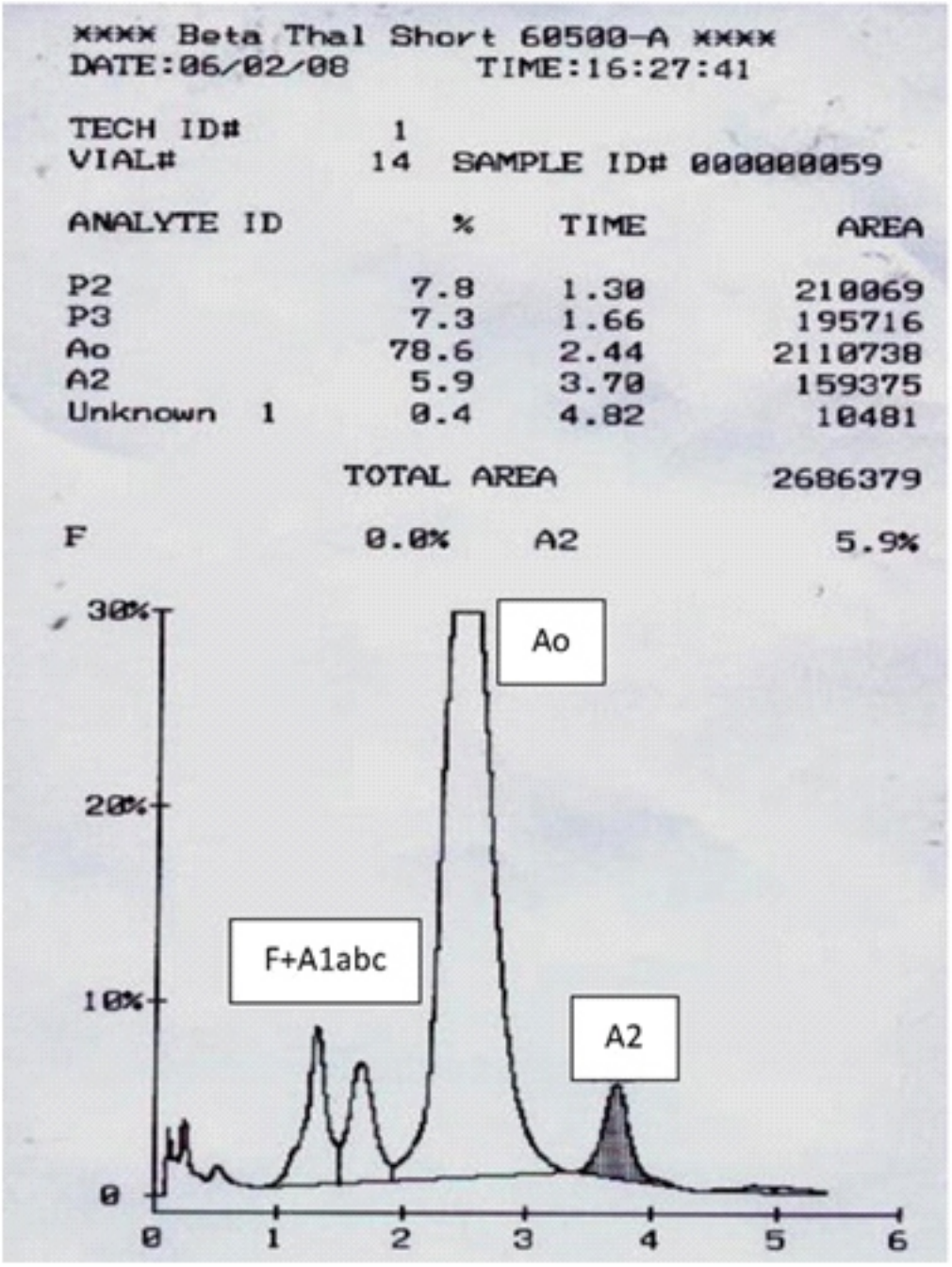
Analysis comments:



Pic 1

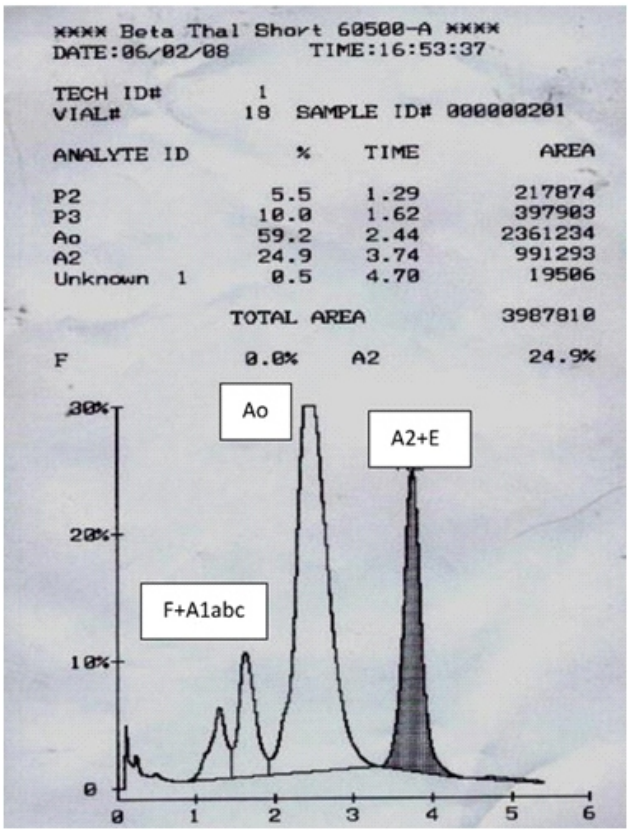
Abnormal HPLC :

In beta thalassemia trait, reduced beta chain synthesis results in excess unattached alpha chains which subsequently attach with delta chain mainly to form HbA2 and also with gamma chain slightly to form HbF. Therefore, HbA0 is reduced, HbA2 is increased moderately (between 3.5%-10%) and HbF is mildly elevated or normal. Now, for example, in pic 2, HbA2 in 5.9% which is between 3.5% and 10%, HbF is 0.8% which is normal and HbA0 is 78.6% which is reduced. So, it's showing beta thalassemia trait.

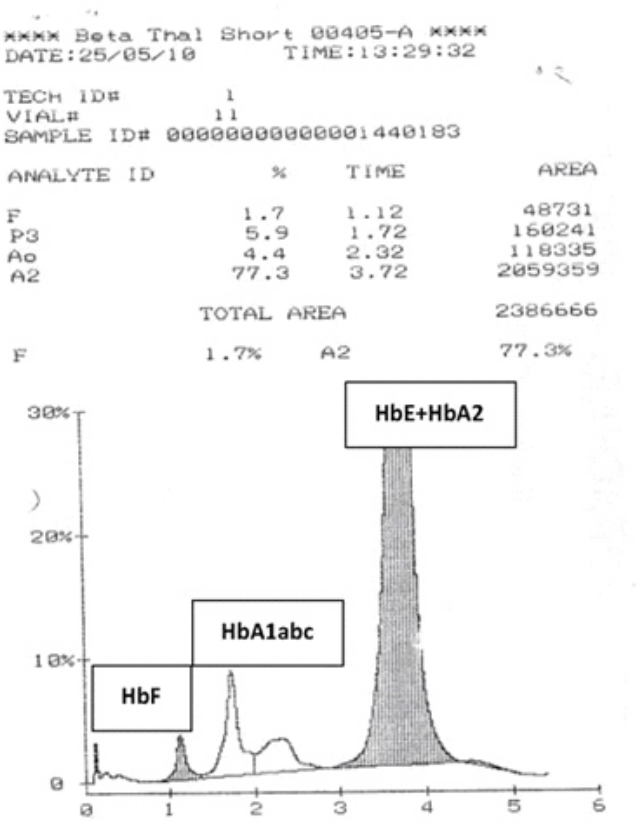


Pic 2

In HbE trait / disease, spike of abnormal Hb E is seen alongwith HbA2 as they co-elute. That means, abnormal spike of Hb E rides over the normal peak of Hb A2 leading to abnormally high combined peak. So, here is no way of differentiating whether it's of Hb A2 or Hb E. Generally, if HbA2 spike is abnormally high above 10%, it's considered to have the spike of Hb E. In that case, it's called Hb E trait. But when this HbA2 peak is tall enough to reach the value of around 70%, it's considered homozygous form of Hb E, that is Hb E disease. For example, in pic 3, HbA2 spike is 24.9% which is more than 10% but not tall enough to call it as homozygous form. It's a HPLC report of Hb E trait. In another example of pic 4, HbA2 reached 77.4% denoting it clearly as Hb E disease.



Pic 3



Pic 4

HbF is increased in beta thalassemia major, intermedia and hereditary persistence of fetal hemoglobin.

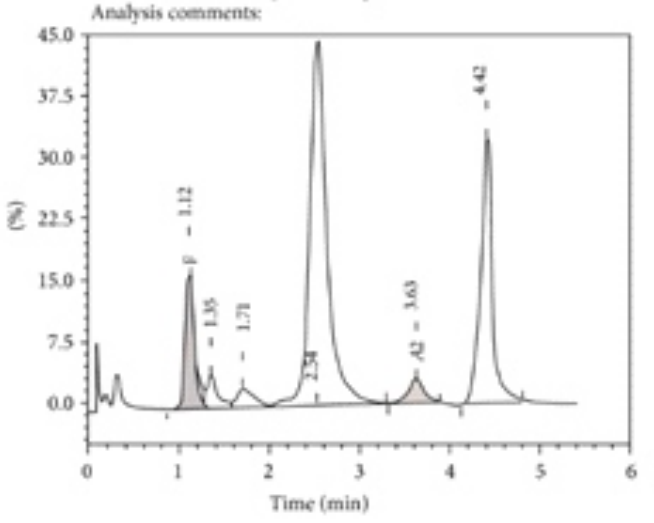
In Sickle cell disease, separate peak of Hb S is found (pic 5).

Peak name	Calibrated area (%)	Area (%)	Retention time (min)	Peak area
F	10.8*	—	1.12	99893
P2	—	3.2	1.35	29854
P3	—	2.7	1.71	25154
A0	—	54.5	2.54	513793
A2	3.0	—	3.63	30726
S window	—	25.9	4.42	244100

Total area: 943,519

F concentration = 10.8*%
A2 concentration = 3.0%

*Values outside of expected ranges
Analysis comments:



Pic 5

Pitfalls of HPLC :

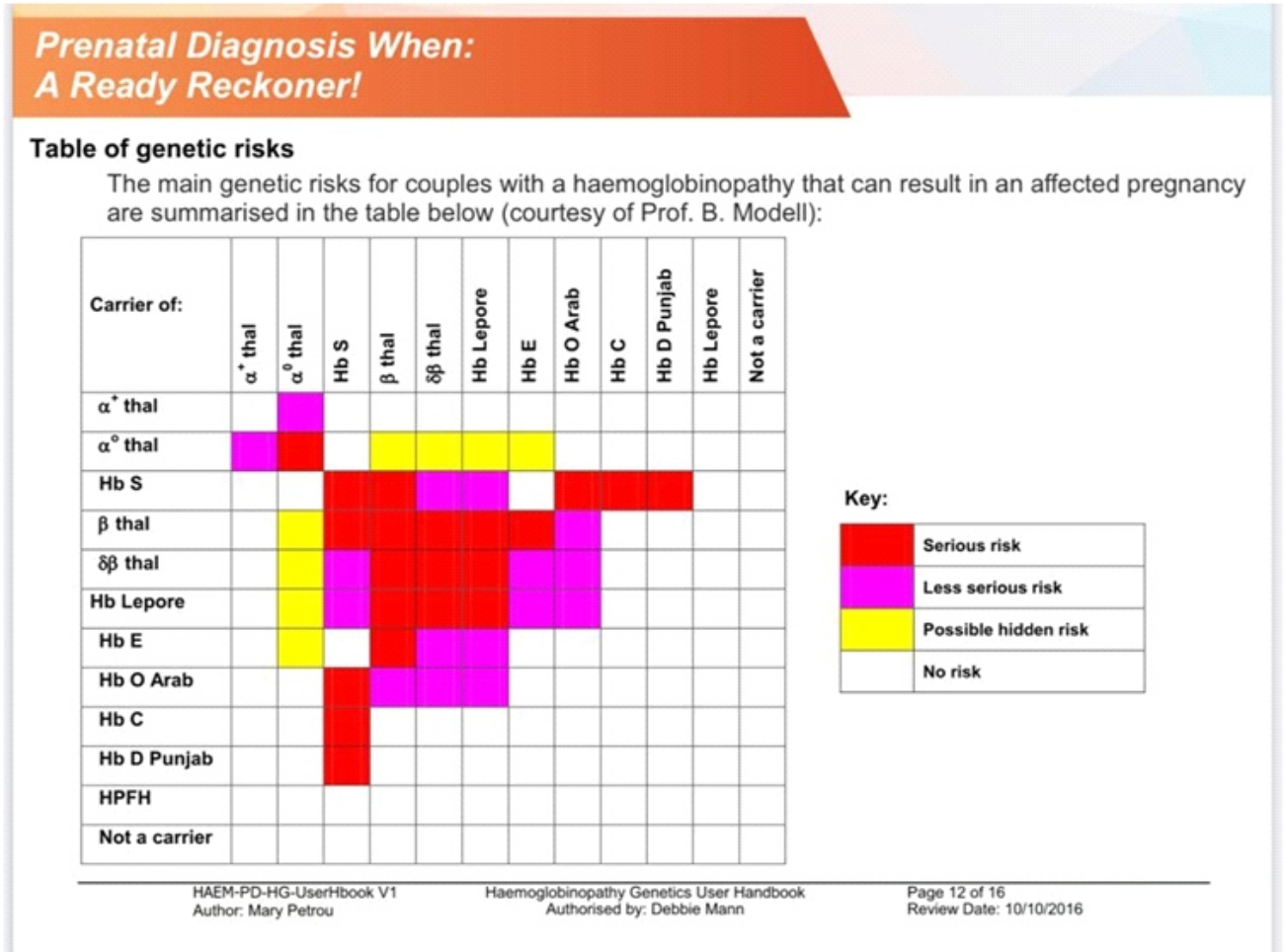
1. RBC indices may be normal even in the presence of beta thalassemia resulting in confusing picture, it's usually seen in silent beta thalassemia.
2. HbA2 may be normal in concurrent iron deficiency anemia, alpha thalassemia (due to no excess alpha chain as alpha chain is also reduced along with beta chain) and delta-beta variant.
3. Recent blood transfusion may cause normal HPLC report as it shows the HPLC pattern of donor blood. In this case it's recommended to do HPLC at least 3 weeks after blood transfusion.

Counselling :

If both wife and husband is found to have beta thalassemia trait in HPLC report, confirmation has to be done by genetic study. After that, risk calculation is done it's explained that there is 25% chance of having baby affected with beta thalassemia major. In that case, Invasive test (Chorionic Villus Sampling or Amniocentesis) is advised for confirming the fetal disease status along with all its pros and cons. If fetus is found affected, lifelong prognosis along with genotype / phenotype correlation is explained followed by option is given for medical termination of pregnancy.

If both partners are found having Hb E trait, there is no need of invasive testing to confirm fetal disease status as Hb E disease is usually mild phenotypically. But, proper counselling and documentation are required as continuation of pregnancy is advised.

In other hemoglobinopathies, risk for severe disease and need for invasive testing to confirm fetal disease status can be ascertained from the following table ***(Table 1)***



Prenatal Diagnosis When:
A Ready Reckoner!

Table of genetic risks

The main genetic risks for couples with a haemoglobinopathy that can result in an affected pregnancy are summarised in the table below (courtesy of Prof. B. Modell):

Carrier of:	α^+ thal	α^0 thal	Hb S	β thal	δβ thal	Hb Lepore	Hb E	Hb O Arab	Hb C	Hb D Punjab	Hb Lepore	Not a carrier
α^+ thal												
α^0 thal												
Hb S												
β thal												
δβ thal												
Hb Lepore												
Hb E												
Hb O Arab												
Hb C												
Hb D Punjab												
HPFH												
Not a carrier												

Key:

	Serious risk
	Less serious risk
	Possible hidden risk
	No risk

Table 1

It's important to know the indications to involve medical geneticist or genetic counsellor while dealing with the current scenario :

1. Clear mismatch between CBC and HPLC picture
2. HPLC report is not conclusive
3. Genes are not identified in the conventional genetic studies. In that case, further mutation analysis like MLPA, PCR have to be done

Conclusion :

- Routine HPLC has to be done in all antenatal women.
- Common thalassemia syndromes and hemoglobinopathies prevalent in India are beta, E trait, Sickle cell trait, E-beta thalassemia, D-Punjab, Lepore, Deletions.
- While interpreting HPLC report, always look for HbA2 peak first followed by HbF and other abnormal peaks.
- 90% cases can be diagnosed with the aforesaid technique. Pitfalls and equivocal results occur in only 10% cases.

POSITIVE SERUM SCREENING TEST - WHAT NEXT

Dr. Seetha Ramamurthy Pal
MBBS, DGO, MD from Mumbai
FICOG, FRCOG, RCOG / RCR Diploma in Advanced Obstetric USG
Consultant Fetal Medicine & Obstetrics
Apollo Multi Speciality Hospital and Institute of Fetal Medicine, Kolkata

Screening in pregnancy for any condition is mainly done to identify a group of women who are at risk of developing a certain condition. This is done with an intention to reduce unncecessary interventions and to help the couple and the treating physician to triage and plan the pregnancy and reduce health costs also.

Certain parameters characterize screening and a few of the important ones are -

Sensitivity : Defined as the ability of the test to detect all those with disease in the screened population. This is expressed as the proportion of those with disease correctly identified by a positive screening test result.

Specificity : defined as the ability of the test to identify correctly those free of disease in the screened population. This is expressed as the proportion of those without disease correctly identified by a negative screening test result

False positive test : Number of individuals without disease but have a positive screening test

Median : The middle value in a set of numbers (observations) listed in ascending order

Multiple of Median : The observed patient value divided by the median value for normal pregnancies *at the same gestational age.* This allows allow data from different labs and assays to be used in the same statistical calculation

'A' priori risk : The background risk of any pregnant woman for a particular condition, usually aneuploidy. The a priori risk depends on the maternal age and gestational age. It is important to remember that every time a test is carried out the a priori risk is multiplied by the likelihood ratio of the test to calculate a new risk, which then becomes the a priori risk for the next test.

Positive Predictive value : Also known as the OAPR - Odds of being affected with a positive result -This gives the probability that subjects with a positive screening test truly have the disease. It is calculated as the Ratio of True positives to false positives.

Screening in pregnancy can be done by biophysical methods like Ultrasound and Biochemical methods that use different serum markers. Conditions that can be screened are
Aneuploidy
FGR
Preterm births
Open NTD
Preecclampsia

Different serum markers are used for screening in pregnancy and they include :

First Trimester markers : Free B HCG, PAPP-A. PlGF

Second trimester : MSAFP, Unconjugated Estriol, Alpha Fetopreotein, Inhibin A.

Before interpreting a result, it is necessary to know the various alterations these serum markers have in different conditions.

Table A :

Placental products	Fetal products
Free B HcG - Inc in Down	MSAFP - Inc in Open NTD and FGR and dec in Aneuploidy
PAPP-A - Red in Down and FGR	U Estriol - Dec in Aneuploidy
Inhibin A - Inc in Down	
PLGF - Dec in FGR	

All the markers are calculated as MoM and a value of 1 is considered normal. One abnormal variable can make the screening test positive

Table B :

Typical FTS marker profile of euploid and aneuploid fetuses [10]

Karyotype	Median nuchal translucency (mm)	Median-free beta-hCG (MoM)	Median PAPP-A (MoM)
Normal	2.0	1.0	1.0
Trisomy 21	3.4	2.0	0.5
Trisomy 18	5.5	0.2	0.2
Trisomy 13	4.0	0.5	0.3

Any test to be considered effective, it should have a high detection rate and a Low False positive rate .

Various screening options in pregnancy are

1. First trimester
   Double marker - PAPP-A + Free HCG
   FTS (Combined test) - NT+ PAPP-A+ Free B HCG +NB/DV/TR
   Enhanced FTS - PAPP-A, B hCG, AFP, PLGF
   Penta screen : PAPP-A + Hyperglycosylated hCG + PLGF + InhA + AFP
2. 2nd Trimester - Quadruple test - AFP + HCG + u E3 + Inh A
3. NIPS - can be done in both first and second trimesters

They can be used in different models like Combined, Sequential, Integrated or Contingent

For Aneuploidy, the efficiency of different screening methods are :

Table 3 :

Maternal age	30-50 %
Age + PAPPA + f BHCG	60-63%
Age + NT	74-80%
Age + Combined test	86-90%
Age + Combined test + Additional markers	95%
Quadruple test	75-80%
Cell free fetal DNA in maternal plasma	> 99%

All tests that have a detection rate of 70% and above are used for a false positive rate of 5%. Hence the Triple test should no longer be recommended as the sensitivity is less than 70%.

Before interpreting a result, it is also important to consider certain factors that might affect the biochemical markers like

- Diabetes Mellitus: PAPP-A reduced, B hCG - no change
- IVF : PAPP-A lower, B hCG higher
- Vanishing twin - may alter
- Fetal reduction - May alter
- Early Preg bleeding - No change

No pregnancy should be terminated just on the basis of a positive screening test and they should be confirmed by other methods or invasive techniques.

Once a screening test comes as positive, it is important to counsel the couple and the family that it does not actually mean that the fetus has the problem and it needs further evaluation and confirmation.

Individual markers have their own significance and the points that need to be considered when a screening test becomes positive :

1. Check gestational age - All parameters in the screening test are gestational age dependent and hence correct dating of the pregnancy is required
2. It is important to check which variable has made the test positive.

First Trimester :

- If it is positive only due to the biochemical markers, they do not have any significance.
- If the Combined FTS has come positive due to an increased NT (> 95th centile), then an invasive testing is strongly recommended as it is a structural anomaly with risk of Chromosomal defects very high.

  The Nuchal transluscency is always considered with respect to the CRL and hence ant NT> 95th centile for that getational age should be considered abnormal. Apart frfom Aneuploidy, Increased NT is associated with Cardiovascular and pulmonary defects, skeletal dyplasias, infections and metabolic disorders in the fetus.

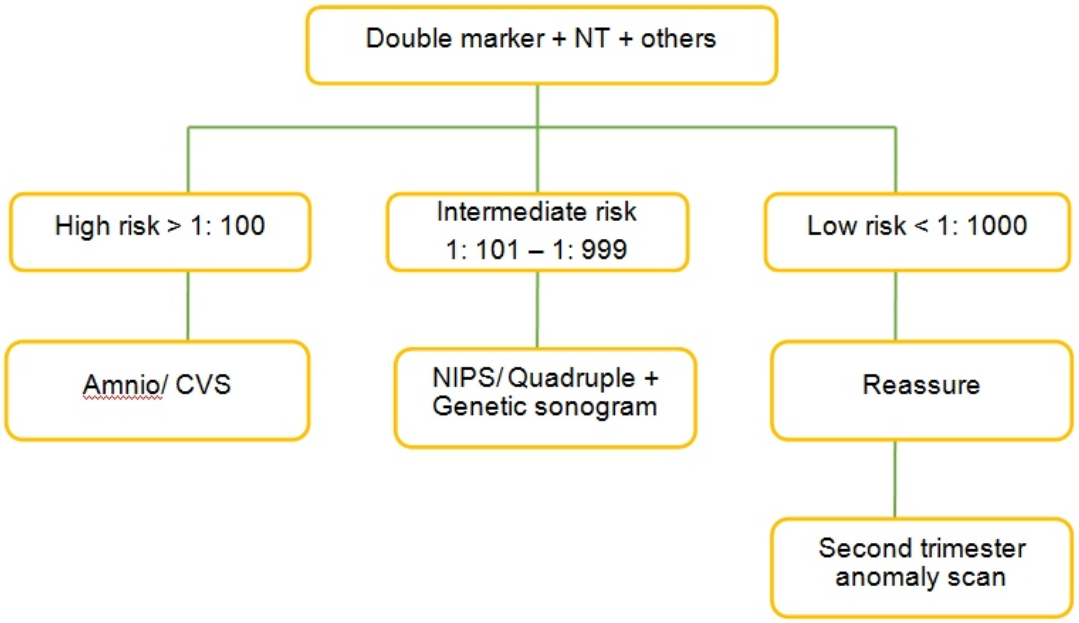
- PAPP-A which is a large glycosylated protein produced by the trophoblasts are implicated in placental function. Hence low levels (<0.4 MoM) are associated with higher incidence of PE and FGR and the pregnancies need to be followed up accordingly.
- Placental Growth factor (PlGF) : Member of the Vascular endothelial growth factor family, levels decrease in pregnancies with aneuploidies and impaired placentation.
- BHCG : Serum BHCG levels also predict outcomes and HCG MoM < 0.2 or > 5 can be regarded as risk factors for adverse maternal or fetal outcomes irrespective of the presence of other abnormalities in the FTS results.
- Prediction of PE can be done with first trimester markers like PlGF and other factors like Mean Arterial Pressure, Uterine artery PI and a risk of > 1 in 100 is highly predictive of early PE (90%). Aspirin prophylaxis of 150 mg started before 16 weeks can reduce the development of early PE by 82%

Second Trimester : The markers used in Quadruple test also help in screening for conditions. Increased AFP levels usually suggest open NTD and fetal growth disroders, but as open Neural tube defects can be picked up efficiently on ultrasound, pregnancies with increased AFP should be followed up with serial growth scans.

Quadruple test can be used as a secondary test where final risk of FTS used as "A Priori risk' and the test integrated from the same lab. But this has a lower sensitivity and it is always pereferable to avoid two biochemistries.

Overall positive FTS can be categorised as Low risk (<1 in 1000), Intermediate risk (1in 250 - 1 in 1000) and high risk (> 1 in 250). The antenatal screening protocol adopted by FOGSI is the contingent approach.

Table 4 :



Chorion villus sampling (after 11 weeks) and Amniocenetsis (after 16 weeeks) should be considered if the test is high risk or if there is presence of an anomaly and the sample should be sent for Rapid aneuploidy testing or Chromosomal microarray depending on the indication.

Conclusion : Positive serum screening depend on the variable that has made it positive and it is our responsibility to provide the couple an accurate assessment of risk. Adequate pre and post test counseling is required regarding the risk of abnormal child or abnormal outcome following a positive screening test. Individual expectations of parents need to be considered and Informed decision made regarding the further steps to be taken.

Suggested Reading :

1. Accuracy of first-trimester combined test in screening for trisomies 21, 18 and 13.M. Santorum, D. Wright, A. Syngelaki, N. Karagioti, K. H. Nicolaides Ultrasound in Obstetrics &Gynecology, Volume 49, Issue 6 p. 714-720.
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4. The International Federation of Gynecology and Obstetrics (FIGO) initiative on pre-eclampsia: A pragmatic guide for first-trimester screening and prevention. Liona Poon et al; International Journal of Gynaecology and Obstetrics, Volume 145, Issue S1, pg1-33.

INVASIVE PRENATAL TESTING - WHICH TEST FOR WHAT?

Dr. Chinmayee Ratha
MS (ObGy), FRCOG (UK), FIMSA, FICOG

Prenatal invasive testing is done for diagnosis of fetal chromosomal or genetic disorders as well as infective pathology. Invasive tests carry a risk of miscarriage due to "invasion" of the pregnancy sac by the needle but the huge advantage of getting fatal cell directly for testing makes such a risk justified in some situations.

PREREQUISITES :

- Detailed counseling should precede (risks / benefits / technical aspects of tests)
- Indications :
  - ✓ Increased risk for fetal chromosomal abnormality
  - ✓ Hereditary, genetic cases
  - ✓ Perinatal infections.
  - ✓ Maternal Request - Extreme maternal anxiety inspite of extensive counselling

Techniques of fetal "invasive " diagnostic testing :

- Amniocentesis
- Chorionic villus sampling
- Fetal blood sampling

Table 1 gives a comparison of these techniques in terms of the timing and complications.

Table 1 : Comparison of few aspects of various invasive tests

Aspect	**Amniocentesis**	**CVS**	FBS
Timing	**After 16 weeks GA**	**11-14 weeks GA**	Usually after 18 Weeks GA
Complications Fetal loss	0.1-1% in comparision with controls, with recent studies being closer to 0.1%	Observational Trials showed ranging from 0.2 to 2% . **Transabdominal CVS vs Amniocenetsis -** No difference **Transcervical CVS vs Amniocenteses -** Pregnancy loss	Risk of fetal loss between 1 - 2%
Amniotic fluid leakage	Varies between 1-2%. ✓ Increased risk of leakage after amnio in those upto 24 weeks.	Extremely rare	

	✓ Spontaneous closure occurs ✓ Low perinatal loss compared to PVPROM		

Checklist for clinicians while planning an invasive test :

- The **Rhesus status** of the mother.
- **Strict Asepsis**
- **Ultrasound -** Viability, number of fetus, placenta location, Gestational age, Amniotic fluid should be checked before and after.
- Universal maternal screening for HIV / HBV / HCV
- **Antibiotic prophylaxis** for bacterial infections - **unit based protocol**

GENETICS IN DISORDERS OF SEXUAL DEVELOPMENT

Dr. Veronica Aurora

MBBS, MD, DNB (OBGYS), DrNB (Medical Genetics) - Gold Medalist
Consultant and Assistant Professor, Medical Genetics
Institute of Medical Genetics and Genomics, Sir Gangaram Hospital

Genetic testing for ambiguous genitalia is an essential tool in the diagnosis and management of disorders of sexual development (DSD). It aims to uncover the genetic causes underlying this condition, which is vital for making accurate diagnoses, devising appropriate treatment plans, and offering informed counseling to families[1].

Ambiguous genitalia can manifest in various DSDs, such as congenital adrenal hyperplasia, androgen insensitivity syndrome, or 5-alpha reductase deficiency. To identify the genetic basis, the testing process typically involves karyotyping to determine chromosomal patterns, along with specific gene testing for mutations in key genes like SRY, AR, and CYP21A2, which play significant roles in sexual development.

Accompanying the genetic testing, a comprehensive clinical assessment is conducted. This includes a detailed physical examination, hormonal assays, and imaging studies, all of which contribute to a full understanding of the condition. However, interpreting the results of genetic tests can be challenging[2]. Not all genetic variations identified are directly linked to the observed physical characteristics. Therefore, a multidisciplinary team approach, often involving geneticists, endocrinologists, and psychologists, is essential for accurate interpretation and management.

The process of genetic testing in cases of ambiguous genitalia also brings forth various ethical considerations. Issues concerning future fertility options, gender identity, and the overall psychosocial impact on the individual are critical. Ensuring informed consent and providing comprehensive genetic counseling are therefore integral parts of the genetic testing process[3].

The outcomes of these tests significantly influence the management of the condition. They can guide decisions regarding potential surgical interventions, the necessity and type of hormonal therapy, and the requirements for long-term follow-up and support. Additionally, genetic testing has implications for the family, particularly in terms of understanding the risk of recurrence in future pregnancies and making informed family planning decisions.

In summary, genetic testing in the context of ambiguous genitalia is a complex but crucial aspect of managing DSDs. It requires a sensitive, informed, and multidisciplinary approach to ensure that the medical, psychological, and ethical aspects of the condition are adequately addressed, ultimately aiming for the best possible care and quality of life for the affected individuals.

References :

1. Nature Reviews Genetics : Towards improved genetic diagnosis of human differences of sex development
2. PubMed : Genetics of human sexual development and related disorders
3. PubMed : Genetic control of typical and atypical sex development.

PREIMPLANTATION GENETIC TESTING (PGT) : REVOLUTIONIZING REPRODUCTIVE MEDICINE

Dr. Manjeet S. Mehta
Consultant Medical Geneticist

Introduction

Preimplantation Genetic Testing (PGT) is a groundbreaking technique in reproductive medicine that has transformed the way we approach fertility treatments and family planning. This advanced genetic testing method allows prospective parents to make informed decisions about embryo selection during in vitro fertilization (IVF) to reduce the risk of passing on genetic disorders to their children. PGT has gained significant attention and recognition for its potential to enhance the success rates of assisted reproductive technologies while ensuring the health and well-being of future generations.

Definition and Terminology

Preimplantation Genetic Testing (PGT), also known as preimplantation genetic diagnosis (PGD), refers to the genetic assessment of embryos before their transfer into the uterus during IVF. PGT involves the examination of the genetic material within embryos to identify specific genetic abnormalities or traits, enabling the selection of embryos that are free from certain diseases or conditions.

To better understand PGT, it is important to be familiar with some key terminology :

In Vitro Fertilization (IVF) : IVF is a fertility treatment where eggs and sperm are combined outside the body in a laboratory to create embryos. These embryos are then cultured for a few days before being transferred to the woman's uterus.

Embryo Biopsy : During PGT, a small number of cells are removed from the outer layer of the embryo, known as the trophectoderm, for genetic testing. This biopsy does not harm the embryo and is usually performed on the third to fifth day of development.

Genetic Analysis : Various techniques can be used for genetic analysis during PGT, including Polymerase Chain Reaction (PCR), Fluorescence In Situ Hybridization (FISH), Comparative Genomic Hybridization (CGH), and Next-Generation Sequencing (NGS).

Basics of Preimplantation Genetic Testing

PGT is carried out as a part of the IVF process, and the basic steps involved are as follows :

Ovarian Stimulation : The woman undergoes hormonal treatment to stimulate the ovaries to produce multiple eggs, which are then retrieved through a minor surgical procedure.

Fertilization : In the laboratory, retrieved eggs are fertilized with sperm to create embryos.

Embryo Culture : Embryos are cultured in a controlled environment for a few days to allow them to develop.

Embryo Biopsy : A few cells are carefully removed from each embryo, and these cells are then subjected to genetic analysis.

Genetic Screening : The genetic material is analyzed to identify genetic abnormalities, such as chromosomal abnormalities or specific genetic mutations.

Embryo Selection : Based on the genetic information obtained, only embryos without identified genetic disorders or abnormalities are selected for transfer to the uterus.

Applications of Preimplantation Genetic Testing

PGT can be employed for various genetic purposes, including :

PGT-A (Aneuploidy Screening) : PGT-A is used to assess the chromosomal health of embryos, ensuring that they have the correct number of chromosomes. This can reduce the risk of miscarriages and implantation failure.

PGT-M (Monogenic Disorder Screening) : PGT-M is utilized to detect specific genetic mutations or disorders known to be present in the family, such as cystic fibrosis, Tay-Sachs disease, or sickle cell anemia. This allows for the selection of embryos that are not affected by the targeted genetic condition.

PGT-SR (Structural Rearrangement Screening) : PGT-SR is used when one or both parents carry structural chromosomal abnormalities. It helps identify embryos with balanced chromosomal arrangements to prevent genetic abnormalities in offspring.

PGT-HLA (Human Leukocyte Antigen Typing) : This specialized form of PGT allows parents to have a child who can serve as a compatible donor for an existing sick child in need of a hematopoietic stem cell transplant.

Benefits of Preimplantation Genetic Testing

Reduced Risk of Genetic Disorders : The primary benefit of PGT is the significant reduction in the risk of having a child with a known genetic disorder. This is particularly important for couples with a family history of genetic conditions.

Increased IVF Success Rates : PGT can enhance the success rates of IVF by improving embryo selection. Higher-quality embryos are more likely to implant, reducing the likelihood of multiple pregnancies and associated complications.

Reduced Emotional Burden : PGT can alleviate the emotional burden associated with the uncertainty of genetic diseases in offspring. Knowing that embryos are free from specific disorders can provide peace of mind to prospective parents.

Ethical Considerations : PGT can also address ethical dilemmas by allowing parents to make informed choices about whether to continue with a pregnancy if the embryo carries a severe genetic disorder.

Risks and Considerations

While PGT offers numerous benefits, it's important to be aware of potential risks and considerations associated with the procedure :

False Positives and Negative s : PGT is not foolproof, and there is a small possibility of false-positive or false-negative results, which can lead to the transfer of embryos with undetected genetic abnormalities or the discarding of healthy embryos.

Invasive Procedure : Embryo biopsy is an invasive procedure that carries a slight risk of harming the embryo, although modern techniques have minimized this risk.

Financial Cost: PGT can be expensive, adding to the overall cost of IVF treatments.

Emotional Impact : The process of PGT, including embryo selection and the potential discarding of embryos, can be emotionally challenging for prospective parents.

Not Suitable for All : PGT is not necessary for all couples, and it should be considered based on individual circumstances, including family history and genetic risk factors.

Who Would Benefit from Preimplantation Genetic Testing?

PGT is a valuable tool for various groups of individuals and couples :

Couples with Known Genetic Disorders : Couples with a family history of genetic diseases can benefit greatly from PGT to reduce the risk of passing on these disorders to their children.

Women of Advanced Maternal Age : As women age, the risk of chromosomal abnormalities in embryos increases. PGT-A can be particularly beneficial for older women undergoing IVF.

Recurrent Pregnancy Loss : Couples who have experienced recurrent miscarriages can use PGT to identify embryos with chromosomal abnormalities, increasing the chances of a successful pregnancy.

Balanced Translocation Carriers : Couples with balanced chromosomal rearrangements may require PGT-SR to avoid the birth of children with unbalanced translocations, which can lead to developmental issues.

Sex linked disorders : Some couples choose PGT to avoid having children with specific gender-linked genetic disorders which is known to be present in the family.

Non-Invasive PGT

In recent years, non-invasive PGT methods have emerged as an alternative to traditional invasive embryo biopsy. These methods rely on the analysis of genetic material obtained from the culture medium in which embryos are grown, rather than directly from the embryos themselves. Non-invasive PGT can offer several advantages :

Reduced Risk : Since it doesn't require embryo biopsy, non-invasive PGT eliminates the risk of harm to the embryos.

Simplified Procedure : It simplifies the IVF process by removing the need for biopsy procedures, potentially making it more accessible to couples.

Increasing Popularity : Non-invasive PGT is gaining popularity as it offers a less invasive and potentially more ethical approach to genetic testing during IVF.

Wider Applicability : Non-invasive PGT methods can be applied to a broader range of genetic testing purposes, including aneuploidy screening and monogenic disorder screening.

However, it's important to note that non-invasive PGT is still an evolving field, and its accuracy and reliability may vary depending on the specific method used. Traditional invasive PGT methods are well-established and may be preferred in cases where absolute certainty is required.

Conclusion

Preimplantation Genetic Testing (PGT) represents a significant advancement in reproductive medicine, allowing prospective parents to make informed decisions about embryo selection during in vitro fertilization. By identifying genetic abnormalities and disorders in embryos, PGT reduces the risk of passing on these conditions to future generations. It also enhances the success rates of IVF, reduces the emotional burden on couples, and provides ethical options for family planning.

While PGT offers numerous benefits, it's not without risks and considerations, including the possibility of false-positive or false-negative results and the financial cost associated with the procedure. Therefore, PGT should be carefully considered based on individual circumstances, including family history and genetic risk factors.

In recent years, non-invasive PGT methods have emerged as a promising alternative, offering reduced risk and simplified procedures. However, these methods are still evolving, and their accuracy may vary. As technology continues to advance, it is likely that both traditional invasive PGT and non-invasive PGT will play essential roles in the future of assisted reproductive technologies.

Ultimately, the decision to pursue PGT, whether invasive or non-invasive, should be made in consultation with a qualified medical professional who can provide guidance based on individual needs and circumstances. Preimplantation Genetic Testing has undoubtedly opened up new possibilities in reproductive medicine, offering hope and assurance to couples striving to build healthy families.

ESSENTIALS IN GENETICS

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