Comprehensive Molecular Diagnostics and Advanced Gene Expression Analysis

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Lecture 46 : Genetic Testing and Inherited Disorders (Part 1)

Hello students, Namaskar and welcome back to our lecture series on comprehensive molecular diagnostics and advanced gene expression analysis. We are finally in module 10 where we will be discussing the molecular diagnostics in genetics and inherited disorders, alright. So, our discussion will be focusing on these topics will be going into the overview of genetic and inherited diseases. We will be discussing what is genetic testing, what are the various types of genetic testing, what are the techniques that we are using in genetic techniques, then what are the implications I mean benefit and limitation as well as the future directions of genetic techniques. So, in this module we will be first thing about all these things, right. So, I mean there is a nice pedigree a chart to start with we can see the annotations of male and female and we are specifically tracking the orange color if you pay attention to that we can see the chromosomes.

This is the common knowledge, right. The chromosomes are coming from both father and mother nothing special in it, but if we select one chromosome that is the this one which is actually labeled I mean highlighted. So, we are seeing that this chromosome is passing from the father to the second generation to the third generation. So, if there is something problem in this chromosome then this disease as we know it will be passed on to the progeny and there will be symptoms, right.

There might be so, that some of the parents did not have any symptom they just had the problem inside hidden in their genes and some or the other members of this ancestry had problems, right. So, basically what we need to know is the inherited disease and genetic disease they are often used interchangeably, but the thing is there is a difference. So, the difference between two terms is inherited disease or heredity the thing the phenomena which passes from father to son grandfather to father, father to son, son from his next progeny, right that is basically having the potential to be carried from one generation to another, right. It is hidden in the genes. However, not all genetic diseases are hereditary in nature, right.

So, a genetic disease can be hereditary if there is a mutation in gene, but that mutation may or may not pass on to future generations. So, if it passes it becomes a genetic

disorder which is hereditary in nature then we call it an inherited disease. So, all inherited disorders are genetic disorders, but all genetic disorders are not inherited. So, please I hope you are clear on this. So, this is very important the concept to grasp before we go forward.

Now with all these problems, right, if someone is having a genetic disease or he or she might be having a fear of having a genetic disease, right, what should he do? Should he go to the physician, right? And physician may also refer him or her to genetic counselor. So, who actually plays the role in this? Well, both of them both plays a role, definitive role, right. There are issues. So, what to be done? So, whether to test the family member. So, this decision needs to be taken by the physician as well as genetic counselor whether the family members also needed to be tested for disease symptoms or we should only limit our test to the patient.

So, we require knowledge about the disease to take all these decisions, right. So, knowledge which will help in both treatment, prevention of treatment of the disease, the prevention of the disease as well as I mean in prevention of the disease in the human being, right, chance of prevention, early detection or prevention of the human I mean future generation. So, also help in reproductive decision making, right. So, this is the strong role a physician and a genetic counselor plays in order to balance the side I mean balance the dilemma or the queries of the patient, right, address the qualities of the patient. So, basically what are the reasons of genetic testing? If you just look if we just discuss few scenarios, right.

So, number one to learn whether someone I mean you and I may have a genetic condition the trans in a family before one has got some symptoms, right. Even before the symptoms the disease the presence of the abnormality can be detected. This is one good reason of genetic testing if you already know what we are dealing with. Again to learn whether there is a chance of current or future pregnancy to have a genetic condition, reproductive angle very important where genetic testing plays a major role in today's date. Next to diagnose a genetic condition if someone or someone else's child has got symptom, right.

There are many genetic diseases many genetic tests which are available we will discuss very soon. So, this is another situation where genetic testing is very very very important. And next there are many cancers which are which have got definite genetic component, right. There are many genes housekeeping genes, oncogenes those their mutation play very important role in cancer development, right. And very early detection of such genes will definitely play a role in prevention or cancer or to understand the treatment plan, right, take informed decisions.

So, with all these things what is genetic testing? Genetic testing actually refers to analysis of DNA, ok, mainly DNA to identify changes in gene sequence. So, what can be the changes? It can be deletion, addition, misspellings there can be many type of mutation basically we need to detect them all or the expression levels the gene expression levels. If we study the gene expression level that is also genetic testing, right. Also not only I mean confined to DNA it also refers to analysis of RNA definitely if we are studying gene expression basically we are studying mRNA expression, right. Biochemical test not only DNA and RNA biochemical test to analyze the presence of the gene product that is proteins, right or even microscopic analysis of chromosomes everything all of this consists of genetic testing.

So, it is a blanket term given to multitude of procedures done at many levels. We will be discussing what are the levels and what needs to be done. So, mainly broadly if we do consider why do we do genetic test or what are the types there are three types there are many other tests we will be discussing again. So, pre symptomatic or predictive testing. So, what does it do? Can inform a person if he or she carries a mutation change in gene that will cause or that will put him or her under risk to have a disease later in life that is not present now, but it can happen later detecting it now predictive testing, right pre symptomatic even before the symptom happens.

Next one part of genetic testing is newborn screening a baby is born there might be common disorders which may be undetected or which if remains undetected may lead to problems for example, mental retardation, ok. So, very important we need to detect these disorders via genetic testing and it will also help to formulate special diet or take special care to ready be ready to tackle the disease process or the complication or prevent the complication in future, ok and another thing is carriers. So, the person may or may not have the disease, but they may have a consistent deformity in the gene. So, a situation where there is a problem in gene, but the disease is not expressed the gene is dormant, right this sort of situation the person is known as carrier this you probably know. So, we can detect carrier.

So, carrier can transmit the disease to another person his offspring if they marries a carrier person another carrier person they do have a child, right. So, another preventive testing basically. So, we are detecting carriers we are preventing disease by predictive testing where this I mean doing newborn testing well if you look at the detailed list. So, what are the various types these are the various types if you list it one by one the broadly it was those three. So, number one suppose I am symptomatic I need to confirm my diagnosis definitely I can do it by genetic testing.

Pre symptomatic disease as I told you estimating the risk of developing the disease right again pre symptomatic diagnosing for disease that will manifest later, alright. So, these

are predictive testing these two, ok. Next prenatal screening even before the baby is born in utero we can do it newborn screening after the baby is born we can do carrier screening already mentioned these two in the previous slide forensic testing there are multiple genetic tests that are done in forensic medicine in order to diagnose unsolved case study patternity etcetera the paternal testing pre implantation testing even before conception we can test whether the mother is fit to have the baby, right. So, even before conception those are known as pre implantation testing pharmacogenetics is a very important emerging test panel which tells us if someone is specifically sensitive or immune to a specific drug action, right. This is basically evolving more into pharmacogenomics which will be detect I mean discussing in very soon in this module on the future modules itself.

So, these are the various types of genetic testing that are done types purpose whatever you say these are prevalent now there are many more roles that will be unveiled as you go because this is one thing that is constantly evolving we are we have not reached at the end game right, ok. So, what happens basically you know the central dogma there is a DNA. So, genetic testing actually involves as I told you molecular techniques, molecular techniques to diagnose all these things. So, from DNA the mRNA is expressed from the coding region, right then from the mRNA translation is happening protein is being synthesized and then the protein is exerting its function. So, at every level there might be problem and genetic testing the job is to detect them, ok.

Mainly it focuses on these two and the protein products the gene product that is detection of proteins, ok. So, looking at some changes in the DNA. So, you might be knowing, but let us visualize right what can happen even with some minor changes and if the changes may just this is a normal gene this is how it looks like comma less the genetic codon, right you already know the genetic code how it looks like ATGC combination, right. A small change you see it was G it became A a single base pair mutation in this case is leading to sickle cell anemia, right.

This is one example. What if a part of the gene is deleted altogether very much possible example cystic fibrosis. You say small part of the gene has been deleted what if the deletion is large very much possible one example is Dushin's muscular dystrophy, ok. I am not going into the disease I mean the nature of the disease itself, right you can read up that is very important for you to the names actually, right because explaining all these diseases we can have courses on each of these disease it is so huge, right. Dushin's muscular dystrophy in case of deletion insertion extra pair of genes nucleotides are being added very important genetic Huntington disease inherited. Multiple mutations can happen, right.

So, multifactorial diseases autoimmune diabetes, right susceptibility to breast cancer

there are many fold diseases, right that can have in genetic components, right we are still looking into it, ok. So, our job is to analyze all those things, right. So, analysis of the whole chromosome look at the entire thing, right. Next there are tests. So, these are the how the genetic testing is actually broadly divided into categories, right one analysis of the whole chromosome it analyze the entire chromosome.

So, very large deletion insertion can be detected using this test. Sequence small changes small insertion deletion analysis of DNA sequence will give us the information. Again analysis of protein shape and protein function we already discussed all of these in much details in our previous modules of high throughput proteomics. So, I will not be going into details of any of these any new method definitely I will be touching, right. So, when it comes to analysis of whole chromosomes fluorescence in situ hybridization next generation sequencing real time PCR karyotyping.

So, we have discussed in details you have been taught in this course about all of this, right. Now karyotyping let us briefly discuss these are the method in which a stained chromosomes are stained and they are arranged based on size and banding patterns and the centromere location you know acrocentric, telocentric you know what are those chromosomal different types of chromosome, right metacentric, submetacentric. So, basically this karyotyping detects abnormal numerical abnormalities for example, trisomy 21 extra chromosomal 21, right Turner syndrome on a absent X chromosome, right X 0. So, all of these can be detected by karyotyping. Then comparative genomic hybridization what does it do it compares the fluorescence intensity of DNA from a test sample to a reference sample.

So, genomic hybridization of two different samples is compared by comparing the fluorescence intensity, right and it helps to detect any chromosomal imbalance deletion mind it we are analyzing whole chromosome we are looking at the big picture over here, right duplication amplification very important, right. So, CGH comparative genomic hybridization a very similar technique which uses the same principle, but in multiple small I mean in a large scale in large number of sample in a small setups you know the concept of microarray. So, array of comparative genomic hybridization very important. So, it helps us to detect the copy number variation identify the chromosomal imbalances. Next multiplex ligation dependent probe amplification MLPA what does it do? It uses as you can understand by the name of the test it uses multiple probes, all right.

Pre known probes are sent I mean exposed to a DNA and we look where it is analyzing and hybridizing we already know what are the normal abnormal DNA sequence. So, based on the hybridization patterns we can gather information about if there is any problem, right. So, it also identifies any copy number variation deletion duplication all those things are very much detected, right and chromosomal microarray analysis

basically we are using microarray to analyze DNA for chromosomal imbalances. So, all these techniques, all right. So, all of these they can give us information about the big picture numerical abnormality or deletion or insertion if it is in a large scale.

So, you need to remember all these names, all right. So, these are techniques of analysis of the whole chromosomes. Next analysis of the sequence this you already know many methods have been discussed Sanger sequencing, polymerase chain reaction, allele specific polymerase chain reaction, real time PCR, qPCR, high resolution melting, melt curve analysis you know it was one technique in real time PCR where you are using cyber green to know how two different DNAs are I mean different from each other, right. RFLP, pyro sequencing and NGS, ok. So, we already know these techniques have been discussed in detail.

So, you can look back and if you do remember all of them now you know what was their importance, what was the clinical importance, ok. So, we are moving forward. So, there are variety of genetic tests, right. So, genetic test can be single gene testing in which we know one single problem for example, Leuschenmuscular dystrophy or sickle cell disease generally these genes I mean these diseases are caused by mutation of one single gene unifactorial, right.

So, we look for change in only one gene. So, a single gene testing will be done for this syndrome we already know it has got very characteristic syndrome and generally these type of mutation that have got susceptibility to propagate across the family, right in familiar mutation they are mainly a single gene type mutation there can be multiple, but mainly they are single. So, whenever we identify disorder in which a family member might be having a single gene mutation we always go for a single gene testing. What can be the other one? Not single there can be multiple such I mean a disease may have a presentation a vague presentation which may be caused by multiple genetic factors then we go for panel testing, right. So, changes in many genes in a test, right. So, they are usually I mean grouped basically on categorize based on different kinds of medical concern.

For example, a generalized presentation low muscle tone it can be Duschen, it can be Baker many type of muscular dystrophy we need to look. Short stature can have many genetic reason there may be multiple symptoms due to syndromes due to which patient might be a short stature epilepsy again very important. So, since there can be multifactorial cause for all of them, right we generally look for panels. Again certain kinds of cancer for example, breast cancer, colon cancer they have got multifactorial origin multiple genes might be problematic. So, we for these cases we go for panel testing.

So, basically genetic test might be a single test might be a panel of tests depending on the disorder we are investigating into. So, for in today's date I have given few examples there are multiple more examples we will be discussing as we go forward with the module in today's class let us learn some cases where generic tests are available which will help us to diagnose. For example, cystic fibrosis, Tay Shacks disease metabolic disorder, Down syndrome, Huntington disease neural degenerative disorder, albinism, Duchenne muscular dystrophy a name which is coming very much very repeatedly right. Spina bifida an anatomical condition, but the spine is bifid in nature. Von Willebrand syndrome coagulation disorder right.

And apart from that familial susceptibility to many type of cancer for example, breast cancer, colon cancer, thyroid cancer these are the information lies in the genes. So, whether it is a single test or panel of test these are few very common tests I mean common diseases which have got genetic testing which will help us to detect and predict and prevent them their complications right and I mean take informed decisions right. So, what are the benefits as we are discussing. So, genetic test might be the outcome might be three variety ok.

Number one the genetic test might be negative. So, what is the patient's perspective the patient will feel relief right. So, even if he or she is the having a problem if the condition is not genetic then we will look for some other reason to find it right. So, basically that will warrant a fewer health checkups fewer health checkups for genetic testing right specially if the suspicion of someone something lies in a family. So, if we rule out any familial mutation. So, not all of the members of the family will have to be I mean vigilant to be tested right.

So, it is very very very important. What if it is positive? Again there is always a good side of things. So, if it is positive then as I have telling multiple times it helps us to take informed decisions. So, we know a disease is imminent we can take steps to tackle the disease emotionally as well as therapeutically right. It can help us to tackle the symptoms alright and decrease the severity of the symptoms by various lifestyle modification like diet food etcetera lifestyle modification anyway. The third result might be I mean the outcome we this is the benefit of genetic test the outcome the outcome also be positive or negative one very outcome might be inconclusive very important.

You can undergo genetic test, but the genetic test does not reveal any convincing report that can happen. So, that will warrant either a further testing or workup with other panel of investigation that will help us to understand the disease better right. So, we have to be vigilant if the thing is inconclusive we can neither be too much relieved or neither will be too much worried right. So, remember a genetic test if it happens might be positive negative or even inconclusive we need to consider the third outcome as well. So, coming

to the implications of genetic diseases what are the limitations? The limitation being mutation may not always lead to disease very important.

So, we may detect the mutation we already know this thing right a mutation. So, in present and if we found find a mutation it may not always translate to presence and absence of the disease process right. So, a genetic test being positive we cannot stamp the patient to have a diseases right we do not test the reports we test the symptoms of the patient. Next existing test look for the most common mutation there are many things which we still do not know right. We only have tools currently to look for what we know right what mind does not know eyes cannot see and this is very true for even genetic disorder.

So, for detecting unknown mutation conventional test will not be able to detect them and there are method by which we can detect unknown mutation unknown changes in sequences right. However, those are not routinely clinically implemented right. So, even in today's date if we have got a direct panel of a disease mentioned in a specific diagnostics center that might might skip some unknown mutation right. Again errors in testing procedure as I said inconclusive result may be due to error some errors that will warrant another future test, test might be done immediately or after a few duration. So, that is the decision of the whole team physician JT council, as well as patient, family members everyone right.

And lastly one limitation of genetic testing is testing is not always matched by treatment. So, what do we mean test? So, you know you can understand all these three right. So, testing is not always matched by treatment. So, basically this deals with the confidentiality issue right taboo social stigma. So, there may be many angles to this one is the angle of the person itself the patient and the family member.

So, patient might feel what if I am being tested and I become positive right. Again the familiar disease is running the family members know they might be hesitant to suggest a test to their younger family member. So, what if he tests positive right. So, there lies a very important role of the genetic council or genetic counseling which will be discussing in our future modules very very very important relation to genetic test.

So, the future so today's class was basically an overview. So, the future direction might be to resolve all these ethical legal and social issues right. There should be experts in science ethics and law and basically policy makers who are taking care of these things. So, that the access to genetic tests are much easier there is not much legality involved definitely there has to be some legality involved that should not be the burden in order to get a genetic test done right. Again in future there is always a scope of more the more the merrier the better. So, there is a chance of developing new cheap simple and effective

and accurate test.

So, that it is available to everybody right. Right now these tests are expensive not all physician do have got a liberty of genetic test getting done right. And drug therapy as I told you one angle was diagnosis is not matched with treatment, one was the taboo of getting diagnosed getting tested and another thing is what if the disease is so that even if it is diagnosed there is no such cure that will also hinder a patient to spend their hard earned money to in order to get tested. So, what if it is positive I know there is no medicine that has to change. So, we are in search of cure either by drug or cure at the grass root level by gene therapy. Since this is a diagnostics module we are not discussing gene therapy, but this is also very much goes hand in hand with genetic testing ok.

So, to summarize today's class was an overview in which we discuss the inherited diseases and genetic diseases, what are the genetic tests, what are the techniques, what are the various panels of genetic tests, what are the various varieties of genetic tests, single test panel of test, what are the implication benefits or limitation and what are the future direction by which we can make genetic testing better acceptable and available to everyone. So, these are my references for today's class and I thank you for your patient hearing.