Comprehensive Molecular Diagnostics and Advanced Gene Expression Analysis

Prof. Arindam Ghosh

Dr. B.C. Roy Multi-Speciality Medical Research Centre

Indian Institute of Technology Kharagpur

Lecture 47 : Genetic Testing and Inherited Disorders (Part 2)

Namaskar. Hello everyone, welcome back to our lecture series on Comprehensive Molecular Diagnostics and Advanced Gene Expression Analysis. We are in module 10, where we are discussing the role of molecular diagnostics in genetics and inherited disorders. We already started it in last class and we are continuing with our discussion and today we will be discussing the genetic testing in primary care. We will be covering this discussion under these following concepts. We will discuss the outline and plan of how genetic testing and primary care should go hand in hand.

And while discussing we will discuss the concept of preconception genetic risk screening and assessment, how during pregnancy preconception means before pregnancy, how it is screened during pregnancy. We will be discussing various aspects of carrier testing, we will also discussing about how we are doing it one based of ethnicity right. Again maternal serum screening and referral strategies related to genetic testing right. So, we have got a lot to discuss.

Now the thing is genetic diagnosis or genetic testing is new compared to our traditional medicine or diagnostic tests. Why did it happen? Because of the human genome project right. There were if you look into the history of genetic disorders we will find there were many disorders as early as before Christ that were thought to be inherited, but the answer to the puzzle came in forefront after human genome project was completed. It led to many discoveries of many such genes or causative factors right. So, that actually triggered assured in the era of genetic testing right.

Now genetic risk assessment is becoming more and more those both screening testing is becoming more and more prevalent in primary medical care. The very first step of medical care is I mean the goal the final goal is to incorporate them in primary care not at the level of genetic specialist. We are still not there, but we can be there if clinical geneticist genetic specialist and primary care they work together to offer the appropriate services. So, we need a policy formulation, but this class I mean this lecture will be suited to the professionals who are posted in primary health care or the paramedical

professionals who are in the primary setup and it will help you to at least take first step regarding genetic counseling and testing screening assessment patient education everything right. So, what is the plan of genetic testing? Well the first step is risk assessment for common genetic condition.

The most prevalent genetic condition their risk should be first assessed and where they should be assessed definitely at primary care or prenatal setting. The most common genetic condition that should be screened first. So, screen screening means out of a many patient pool population pool we are designing a high sensitive test which will rule out the negative ones right at the first test. Then for those who are being subjected to a confirmative diagnosis they can be sent to specialists alright. Mind it for common genetic condition we are not targeting rare genetic disorders these first are the domain of primary and prenatal care.

Whereas, if it so happens the patient comes with more rare genetic disorder multiple genetic disorder complex genetic disorder or with the complex family history that is not possible to be evaluated properly in primary or prenatal health care they can be referred to specialists or clinical genetic specialist geneticists or genetic super specialist dedicated genetic clinic there are multiple portals they can be referred to. But remember primary what is the primary care means it is the first contact when where patient goes to if they are referred to another hospital that is higher that is known as secondary care. If they are referred to any higher further higher suppose a medical college that becomes a tertiary care center we are talking about primary health care setup and they should be equipped with proper risk assessment tool as well as at least a basic screening test. So, this is the plan ideally this should happen and primary care setups are gradually being equipped with tools. So, that they can screen the most common genetic conditions to start with.

So, the outline being so I mean considering the prenatal diagnosis the primary care diagnosis mainly the outline is there are three phases multiple there can be multiple phases, but we will be discussing in these steps number one preconception phase or prenatal phase where the baby the product has not been conceived right the patient is not pregnant they are planning a baby right that is known as preconception screening. So, how can that be done mind it please keep in mind we are talking about primary care the patient has come to primary setup general physician any outdoor any primary health center there he or she is being evaluated is being questioned. So, family personal history questionnaire and ethnicity based carrier screening for genetic disorders right. Next maternal serum screening and ultrasound that can be done and lastly when and where and how to refer the patient. So, a primary care physician should have idea about all these three steps ok.

So, preconception, conception screen during when the baby I mean when the patient is

pregnant and the cases which suits which are beyond the scope of evaluation or assessment or treatment in primary care they should be referred and what are those case we will be discussing them all. So, regarding the family history questionnaire basically we are screening for reproductive genetic risks right. So, number one those are appropriate for patient considering pregnancy or already pregnant it may so happen that the a couple or a young mother has turned up in clinic not before pregnancy, but after the first pregnancy right. So, a physician may have missed their chance of preconception screening well the principle the questionnaires they apply the similarly even if the pregnant patient has reported to the primary care center. Now this questionnaire has got tools and these are the tools of the questionnaire.

So, the questionnaire has got multiple question that will elicit the risk the reproductive risk of the patient right they will also I mean cater to multiple areas when the patient is pregnant or even they are considering pregnancy right and there are also guidelines to various referral centers in that questionnaire. So, it is easier for the primary care provider they may not be a doctor they may be a nurse or any other attendant trained by anybody who knows who has been trained about the question the health care provider he or she may well participate in this family history questionnaire for genetic screening. So, what does this questionnaire assesses? So, these are the tools of the questionnaire right if you are designing a questionnaire at your setup for genetic screening please keep in mind these points should be included. Number one maternal age family medical history of both the sides both maternal and paternal pregnancy history present as well as previous right ethnic background of both side family medical history means only where the family members sake ever or whether be whether they were treated whether they are taking in the medicine regular basis ethnic background is plays a very important role in genetic disorder. So, that should also be considered right.

Now, regarding maternal age one thing should be noted why do we focus on maternal age? So, a questionnaire can be designed in such a way not the exact age whether the mother is below 35 or above 35 during first pregnancy the generally we consider the first pregnancy when the maternal age is above 35 it increases the risk of chromosomal abnormality. So, very important age maternal age 35 or older at the time of delivery we are generally considering first pregnancy right often in medical term it is known as primigravida ok first pregnancy. So, next going for options for prenatal testing and screening right. So, chorionic villus sampling amniocentesis and multiple marker screening. So, you see when we are considering a maternal age who is more than 35 then these are the options that should be explained to the mother that these are the tests that need to be done whether you are ready to do it all right.

We will be again discussing prenatal test all these parameters when we are discussing in details regarding noninvasive prenatal test right NIPT that is a major emerging thing we

will be discussing them. So, now we are not going into details, but generally these are the most common way of invasive live apart ultrasound ultrasound is definitely noninvasive. So, regarding testing when we are drawing any sample and subjecting it to genetic screening. So, chorionic villus so, either we are taking chorionic villus or we are taking amniotic fluid out from the amniotic sac or again if we are. So, for all these we can apply the multitude of molecular test that we already discussed in last class for example, PCR, FISH, microarray so on and so forth.

Next regarding multiple markers screening first or second trimester markers combined we will discuss right, but these are all the options that are provided in the with relation to maternal age even ultrasound these are mandatory right, but it they become more important when the maternal age crosses 35. What about family medical history? Generally for a family history these are important if the there is some diagnosed genetic condition right already diagnosed or a birth defect and a patient who is currently pregnant then they should be ideally referred to prenatal diagnostic clinic or prenatal genetic clinic whichever is appropriate. We are talking please bear in mind we are always talking about primary care we are sitting in a primary health set up and we are judging what to do right. So, example of this will I mean include suppose a nephew we already know names of the diseases right we discussed the names of various genetic diseases in our last class. So, a nephew with Duchenne muscular dystrophy, a brother sibling with fragile leg syndrome right, a previous child with spina bifida etcetera right.

For all these cases who are already diagnosed then family history becomes positive and that is very important that is one major consideration that this patient should be highly considered for genetic screening right. For non specific, but concerning history so in earlier case the diagnosis was absolutely confirmed they were stamped to have this disease. So, it becomes very easy to refer them right. Now in a situation where there is history present, but it is not specific for example, a close family member with mental retardation, but the etiology is unknown the cause have not been found out it may be highly probable he or she is having any rare genetic disorder due to which that mental retardation has taken place. Multiple members with kidney disease again very common right there must be some mutation that is leading to this kidney disease in multiple members right.

So, these are all the suspicious areas suspicious areas which should trigger the curiosity of the healthcare provider to suggest a genetic screening right. Again previous child with seizure disorder and or developmental delay very important. So, we should always take into consideration whether about the entire history of pregnancy may be in the previous case the seizure disorder could be due to trauma at the brain. So, everything should be considered, but again we cannot rule out a genetic cause therefore, it is always better as a primary care health primary healthcare provider to refer these cases for further molecular

diagnostics. History of pregnancy so, very important.

So, what should we consider what would be the checkpoints in history of pregnancy number one exposure to any teratogenic drug drugs which are known teratogen means drugs which are known to be harmful in pregnancy. You must be knowing that they are the drugs which are labeled to be safe during pregnancy there are drugs which are labeled not to be taken during pregnancy and there are drugs which are often you will find out safety not verified. So, again better to avoid, but for those drugs which are actually labeled bad for example, isotretinone very commonly used in acne in young females right pimples. If the young mother or the lady is exposed to isotretinone during pregnancy it can lead to genetic disorder right it can lead to defect it can lead to problem in the fetus and that can be diagnosed using genetic testing right. Again seizure medication, medication bipolar disorder, lithium, multiple viral infection, maternal diabetes all these things are contributors to genetic disorder to predispose the baby to make the baby prone to genetic disorder and should be considered to be asked or to be taken care of during asking question during prenatal or primary checkup right.

Again preconception discussion should always include a discussion of folic acid very very very important when the mother has come to the primary care clinic with the I mean and they are planning pregnancy two things are very important as I told you maternal diabetes two things just remember. Number one the mother should ideally be euglycemic means the blood glucose level should be normal, normal blood glucose level. Euglycemic means normal hypoglycemia means low blood glucose hyperglycemia means high blood glucose. Number one the mother should be euglycemic and number two the mother should be on folic acid very important why folic acid helps in decreasing the risk of neural tube defects by 50 to 70 percent pardon me if it is not visible let me tell you folic acid decreases the risk of neural tube defects by 50 to 70 percent which is one of the most common disorder that can happen or that can be diagnosed using markers in prenatal testing alright. Next ethnicity based genetic carrier screening right.

So, what is the purpose? The purpose is to identify or suspect the risk of diseases that are having a high prevalence with respect to some ethnicity right. So, to get couples at risk for prenatally diagnosable genetic diseases right we are targeting common disorders which have got diagnostic tests ok. So, tests are offered based on ethnic background not all patients with the one ethnic background will be offered all the tests because we will be seeing very soon why should be offered to patients who are seeking preconception counselling definitely they are eligible or they are seeking infertility care may be due to some reason the pregnancy is failing or the couple is trying they are not conceiving there might be. So, that there is a genetic disorder which is actually leading to the failure of implantation very important should be found out right and if the pregnancy is already happened if the mother is has reported in the first and second trimester of early

pregnancy or early second trimester of pregnancy definitely they should be offered these tests. So, what are these? See we have we are discussing the most common or the most prevalent carriers in various ethnic populations right.

So, whether you are a student who is watching the discourse from India or whether you are taking for overseas in the NPTEL platform you should be knowing about what countries you are not even then if you are associated with any world health organization project or any project that is leading I mean associated in NGO that warrants you to go to any foreign country and work on them you should be always aware that these are the most important public health agendas or the diseases that we should focus on because these based on the frequency for example, in African American population the carrier frequency of sickle cell anemia very high cystic fibrosis beta thalassemia right Asian population alpha thalassemia and beta thalassemia very high European American cystic fibrosis very high French Canadian, Cajun population, Tayshax disease right. So, you see the based on the carrier frequency and how do we know all this multiple research multiple tests have been done by public health researchers and then we have we have been able to compile this data and this also keeps on changing as the medical treatment advances. However, for genetic diseases we can treat, but often the genetic prevalence remains because the it is a population based disorder the gene the parcel lies in the genes right. So, the carrier frequency remains more or less static, but depending on the country they should be offered these tests irrespective of I mean irrespective of their status whether they are in preconception stage or they are conceptual stage or they are seeking infertility care. So, for example, in case of India Asian country generally we offer thalassemia screening right thalassemia screening to both the father and mother to see what to see if they both are carriers then the baby might be affected with thalassemia then we can warn them right it is not good to continue the pregnancy or you must be cautious like that.

Cystic fibrosis test is not done routinely over here because it is not common. However, in case of European population very important cystic fibrosis test CFTR gene I am not going to details of the genes right we will be discussing again this specific disorders and rare genetic disorders there we will be discussing further what are genes and what needs to be tested, but the point being we should always be aware what is the prevalence based on that we should design our carrier counselling tests. So, what is the principle of carrier counselling there are few principles right number one we should explain the voluntary nature we should explain the purpose of the screening right this is basically to prevent the future generation from any suffering and the test should be voluntary in nature right we should also explain the patients about the range of symptom and severity of each disease. So, that if the baby is born what can happen right again risk of carrier status and unaffected of string everything should be explained the baby might not be affected, but they might be a carrier of the disease right and they also may transmit the disease in

future right. So, all of these should be mentioned what is the probability what is the meaning of positive and negative results I told you in the last class what are the implications of positive and negative screening test right here what is the importance of you being a positive carrier or if you are not a carrier right mind it if someone is a tested for a carrier positive he or she has nothing to worry about if he or she marries a person who is not a carrier right.

So, a positive result does not means the patient should be emotionally devastated no it is the job of the caregiver to explain all these things right. So, all these factors do help in consider the decision making of the couple and even if both are carriers they conceive we should always make sure counsel the patient that further testing will be necessary for prenatal diagnosis just because you are a carrier it does not mean that will transmit the disease 100 percent to the next progeny ok. Very important informed consent this is a very important ethical and legal issue this regarding this the health care providers are primary centre are always encouraged to utilise various publicly based resources from bodies like world health organisation UNICEF etcetera. So, there can be multiple such brochures and the patients should be made aware of those brochures for example, brochures about cystic fibrosis and other ethnicity based genetic screening are available right they should be given to the patients to. So, that the patient is educated why they are doing all the purpose should be well explained right and they can also be shown how the screening test is done videos the visual illustration is much more appealing what if what will happen if the disease is transmitted to the offspring right.

So, very very important informed consent means the patient is saying yes being informed ok and very important everything should be documented how the patient was explained whether he or she is informed about the testing or not what is the outcome that is the patient's decision whether he or she will undergo the test everything should be properly written without proper documentation there can be legal issues and lawsuits which might not be the best thing in the interest of the health care management health care provider managers. So, important points to be noted is carrier screening is optional number one as I told you patient education informed decision making is very very very crucial testing can be done sequentially or concurrently alright for example, if the if the pregnancy is more than 12 weeks gestation discuss concurrent testing. So, multiple markers can be done and if the patient has arrived early there can be first test for first trimester or the one type of test then when the pregnancy advances based on the first finding then there can be multiple more tests right. Again the next point as you can see cost might be a inhibiting factor for many patients in private setups right whether this genetic test is being covered by the medical insurance ok the all these things needs to be looked upon right. So, carrier screening being optional it is up to the choice of the patient whether he or she will undergo this test or not.

However, since genetic counseling is available right it is strongly advised that the primary health care givers with positive history they should strongly motivate all the carrier carrier couples to undergo or to refer them to specialized genetic clinic mind it we are talking about couples who both are carriers. Now regarding maternal screening so, maternal screening will be discussing in detail, but just to give you an overview test for maternal serum markers right here we are discussing gene products ok. Serum markers to test detect increased risk of fetal trisomyelitis down syndrome you see from chorionic villus sampling and amniocentesis we can also test for these test for example, karyotyping will easily show us trisomy 21 trisomy 18 right. We have already discussed what are the panel of tests that we need to do here we are just discussing it in clinical scenario. So, this is the test where we are testing the gene products ok.

So, Edwards syndrome trisomyelitis down syndrome open neural tube defects all have got their specific markers and that can be tested or that should be tested if there is that is a high risk pregnancy ok. So, there are multiple test for example, second time master maternal serum screening there are panels we will be discussing them right. Again the first time master maternal screening without you doubt mucal translucency measurement these are all the guidelines I am not going to details because this is not an obstetrics class, but mucal translucency is measured by ultrasound alright ultrasonography these are markers of open neural tube defects. Again integrated serum maternal screening is all the test multiple parameters are employed. So, there are triple test double test quadruple test right.

So, we will be discussing them in our class of reproductive genetics in detail. There are also multiple variations. So, multiple parameters can be combined in many ways to make sure that the proper genetic screening is done at the level of primary care center. So, what are the patient education points right how the patient should be made just like area screening in conception screening we should inform the patient that this is only a screening test means the screening test what does the screening test mean I will tell you number of the test is also optional right one may choose to do one may not we cannot force anyone to do it and negative result does not guarantee a healthy baby. So, screening test means the test is positive that should be confirmed later whether with other tests right with definitive tests.

So, that we can confirm the diagnosis of any such genetic disorder. So, screening test being positive does not necessarily mean the baby will be sick. Similarly a negative result does not guarantee a healthy baby right. So, positive result does not mean that the baby has a problem, but further testing for example, ultrasonography, CVS that is chorionic villus sampling amniocentesis should be offered alright. So, first since serum markers are easy we do serum markers first and then on positive serum markers we generally go for all those chorionic villus sampling and amniocentesis.

So, that those products can be then subjected to molecular diagnostics. And they should be offered to all patients regardless of the age why till now I was discussing more than 35 definitely more than 35 increase the risk manifold, but there is a small risk in every pregnancy alright for these genetic conditions specially that those I mentioned trisomy 21 trisomy 18 open neural tube defects. So, they ideally this maternal serum screening should be offered to all patients. Now regarding referral we have already discussed we are just recalling. So, from the primary care center who are the ones that should be referred or when should be referred them to whom to clinical geneticists genetic specialists.

For example, those who are from the medical background they already know now till now genetics was a domain of biochemistry pathology medicine pediatrics right, but now a super specialty DM that is doctor of medicine DM are the super specialist in Indian scenario there is MBBS, MD specialist and then DM. So, now DM genetics is a course that is being under whom postdoctoral trainees are being trained in multiple premier institutes why because subject is advancing so much and we have the primary care center have got so many patients who needed to be referred. So, who are to be referred number one in with referred to special ethnic backgrounds I am now covering everyone right. So, individuals with positive history of cystic fibrosis or any other autosomal recessive disease positive history I mean family history all right if someone has got a family history refer them. Couples where both the members are known carriers for autosomal recessive disease absolutely should be referred again whose one member is a carrier and other member has got some query that cannot be answered by the primary health care giver should be referred.

Pregnant carriers who do not have results on the father of the baby right. So, carriers who have got pregnant, but the other person is not found to be normal mind it one carrier and one normal the pregnancy can be continued without referring to further without the need of referring to genetic specialist if there is no such family history. Next for those with positive family history if the patient or partners indicate a family history of birth defect or inherited condition or history of pregnancy exposure right for example, to teratogens we should always refer them. So, we should assess the level of concern right and desire for more information about risks to pregnancy. Next these are all cases of positive family history that needs to be referred right.

So, someone who is very much concerned who wants more information about the risk of pregnancy from the primary health care giver should always be referred to geneticist because geneticist will be more experienced to answer all these question right. Next and the I mean of course, with patient concerned they should be referred to genetic counseling right. Next prenatal genetic services when should we absolutely consider

referral for prenatal genetic service number one advanced maternal age more than 35 for the first pregnancy right. Request for first trimester screening with numerical translucency right. Abnormal serum marker screening result absolutely we should refer them.

Peter abnormalities on prenatal ultrasound right for example, single umbilical artery ok. There might be problem see we are in general talking about referring to prenatal genetic services. There might be multiple genetic disorders right and they might need intervention, but at the level when an ultrasound is going on it is very difficult to assess what patient ultimately we will have what genetic disorders and these are the common markers based on ultrasound gross markers which triggers the alarm that they should be further processed for genetic diagnosis. Next personal family history of known or suspected genetic disorder birth defect or chromosomal abnormality absolutely they are candidates who should be referred. Family history of mental retardation with unknown etiology see I am just repeating myself all these have been discussed already right.

So, just fit your answer if the if you are sub feel I mean facing in a descriptive type of question regarding referral of genetic services just give all those options right. Again patient with medical condition known or suspected to affect the fetal development for example, suppose someone with torch infection right toxoplasma, rubella there are multiple these are some few cytomegalovirus these are few important viruses culprit that are known to cause fetal defects right. So, just as I am giving an example. So, a medical condition which is known to cause a fetal defect should also be referred right that list does not end any known exposure I mean any exposure to known or suspected teratogen might be problematic should be referred. Again either parent or family member with chromosome rearrangement right if someone has been tested genetically not for prenatal diagnosis, but they have got a history of any chromosome rearrangement either parent or a family member very important the baby that is going to be born might have inherited the disorder right.

And most importantly a known carrier or a family of a disorder or a prenetic testing is available right whenever prenatal testing is available as I told you a small chance lies in every pregnancy to have any genetic disorders. So, if test is available if the facility is available why not referred them right. Unexplained infertility, multiple pregnancy losses, previous still bonds absolutely they must have some genetic reasons either anatomical deformity anomaly or genetic I mean genetic disorder the most common being genetic disorder. Transparent problem generally leads to loss of pregnancy, but a bit during advanced stage, but absolutely during implantation right if there is some problem there is some there is a genetic disorder that is not actually allowing the growth of the embryo that might happen that might be the case again they should be referred. So, unexplained infertility if we have got everything if we know there is anatomical disorder if we know

the reason teratogenicity if there was some physical exertion or stress or known disorder fine if something is unexplained we should always be aware.

Again there are multiple such disorder specifically absence of vast difference premature ovarian failure they are also the cases they should also be referred to genetic testing right. So, we end here we have discussed a lot we have discussed multiple areas related to genetic testing in primary care. So, to summarize we have discussed the outline and plan of genetic testing how the genetic testing is done at preconception stage as well as when the patient is pregnant, how we enquire family and personal history, what are the tools of the questionnaire, how do we screen for genetic disorder based on ethnicity right, how the maternal serum screening markers are designed mind it will be discussing them in much more detail in our future classes. And lastly how and when and where to refer the patient and what are the main cases basically cases that are positive with these screening tools should be referred all right. So, these are my references for today's class and I thank you all for your patient hearing. Thank you.