Comprehensive Molecular Diagnostics and Advanced Gene Expression Analysis

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Lecture 55 : Molecular diagnostics in Endocrine, , Neurodegenerative, and Transplantation disorders.

Hello, Namaskar. Welcome back to the module 11. This is the last lecture of module 11 on Comprehensive Molecular Diagnostics and Advanced Gene Expression Analysis. And today we will be discussing various molecular diagnostics for autoimmune disorders, endocrine disorders, neurodegenerative disorders as well as few transplantation disorders. Now, as I told in the last lectures, we will be discussing only few very common or relevant disorders which do appear a lot either in form of patient cases in real world scenario or in form of very common questions in various levels of competitive exams right.

So, needless to say the total list is very exhaustive and you are free to read from the references section. However, I will be highlighting the most important ones. So, like last class I will always encourage you to make your own sheet where you will write the name of the disease on the left side and the genetic markers and the parameters on the right side alright. So, we will start with autoimmune disorders first.

And like always let us discuss these common methods by which which are actually employed in detection of various autoimmune disorders. Number one is definitely various types of genetics testing, you already know various methods of genetics testing for various genetic methods. Then antibody detection where various proteomic methods immunophenotyping, cytochrine profiling, gene expression profiling and next generation sequencing. By genetic method generally I mean to say about various types of polymerase chain reaction or microarray all those things right traditional PCR and RT-PCR. So, we can apply all these sub headings to the various diseases.

So, here we will be getting hold of the diseases and we will be discussing what are the various markers for various testing modalities right. For genetic test so, human leukocyte antigen HLA DRB 1. So, we are discussing RA rheumatoid arthritis very common autoimmune disorder right. And regarding antibody detection rheumatoid factor right RF and anti CCP antibody which is anti cyclic citruinated peptide very

important anti CCP antibody is a very important multiple choice question that comes in relation to rheumatoid arthritis and that is that is actually a molecular diagnosing technique. Apart from that cytokine profiling so, since any autoimmune disorder the cyto pro inflammatory cytokine plays a very important role.

So, these two that is TNF Tumor Necrosis Factor Alpha Interleukin 6 or IL 6 are done. You recently there was a COVID 19 pandemic and you saw there was a phenomenon of cytokine storm. So, these two parameters also become a very common molecular diagnostic modality to test the prognosis of COVID patients right. And lastly gene expression profiling serial analysis of gene expression is always done to understand or to categorize any type of autoimmune disorder and mostly the disorders analysis of gene expression profiling have got a role. In RA it since it is a disorder of arthritis joints it is done in sinovial tissue fluid right to identify various molecular pathways that are actually driving the inflammatory pathology.

Another very common autoimmune disease systemic Leupus erythematosus or SLE they are problem in complement system. So, complement pathway genes that is C 1 q, C 2, C 4 are targeted by various genetic testing while we are doing antibody detection anti nuclear antibody first choice ANA and anti double stranded DNA antibody. So, here antibodies against the double stranded DNA develops specially nuclear. So, anti nuclear antibody. So, anti double stranded DNA and anti Smith antibodies.

So, ANA anti DSDN and anti SM are very characteristic diagnostic markers for SLE or system systemic Leupus erythematosus. The immunophenotyping is done in this case where we need to characterize the abnormal T cell and B cell using flow cytometric technique alright. And also cytokine profiling is done mainly to measure the level of interferons and various other cytokines that are involved in the pathogenesis of the disease fine. We move on to another very common autoimmune disorder type 1 diabetes. So, the diabetes as most of you might be knowing is broadly divided according to the pathogenicity in type 1 and type 2.

Type 1 is the autoimmune cause and type 2 is the multifactorial disorder that often happens to adult population. However type 1 disorder is the autoimmune variety which has got multiple genetic component. However the most common are screening for some human leukocyte anti HLA mainly HLA DQ and DR these are associated with increased risk of type 1. Recently anti GAD antibody that is glutamic acid decarboxylase. So, GAD anti GAD antibody in serinoma associated antigen 2 that is IA 2 alright.

So, what happens in type 1 diabetes there is autoimmune destruction of pancreas. So, we there are antibodies against the pancreatic antigens that actually destroys or attacks the pancreas. So, these type of diabetes are in which the pancreatic function is totally

lost. So, we need to give artificial I mean we need to give external insulin. So, in earlier days this was also known as IDDM or insulin dependent.

However that classification is not used nowadays. Now we use the pathology dependent classification that is type 1 and type 2. I mean type 1 diabetes mellitus that is autoimmune we tend to look for all these genetic markers. And as always genetic expression profiling is done to identify the signature in multiple pancreatic islet beta cell to understand the beta cell dysfunction and immune mediated destruction process right. So, to identify various genetic signatures.

Again very common I would not say very common, but very devastating autoimmune disorder is multiple sclerosis. Again here there are multiple genetic variants in human leukocyte antigen which leads to this immunogenic condition. So, the markers that we are interested to probe are CD40 and IL2RA right. This is very important myelin oligo dendrocyte glycoprotein on MOG and other myelin protein. This is typical of multiple sclerosis and if this is I mean these are the diagnostic modalities that is done if we are going for other than symptomatic diagnosis if we are targeting this autoimmune disorder along with that TH1, TH17 and I mean abnormal characterization of abnormal T lymphocyte and B lymphocyte in blood and cerebrospinal fluid is done.

So, since it is a immunogenic disease generally in all immunogenic disease the blood cells that are responsible for immune function are the lymphocyte mainly T and B lymphocytes they are characterized and often some markers are found and these are the markers. So, TH1 helper cell and TH7, TH17 helper cells or TH17 subset of T cell are abnormal over there and not only that various other cytokines such as IL17 and interleukin 12 they are specifically involved in inflammation of the nerves. So, neuroinflammation and demyelination that happens extensively in multiple sclerosis. So, those are also target for detection via molecular diagnosis. Next Hashimoto's thyroiditis autoimmune thyroiditis is very common.

So, one of the most common cause of Hashimoto's thyroiditis one of the most common cause of immunogenic thyroiditis and the how do we screen those patients that is done by looking for HLA-DR3 and HLA-DR5 gene variants. So, these I mean if there is polymorphism or if there is variation in these genes or mutation in these genes there are high risk of developing autoimmune thyroid disease and once the disease has developed if the patient has already presented with the symptom if you are suspecting we can look for anti thyroglobulin antibody or anti thyroid peroxidase that is anti TPO antibody. And again whenever there is a pathology in any type of cell if you are writing descriptive type answer as you have understood from the pattern gene expression profiling of that concern tissue to look for any abnormal pattern to understand the immune related destruction will be a common point. So, here as the problem is in the thyroid gland. So,

gene expression profiling or patterns of thyroid tissue is done.

So, we take tissue from the affected thyroid gland we subject them to various types of I mean analysis of very multiple gene expression which you have already been taught very recently and then we use that technique to understand the total picture of the tissue from in different individuals. So, that brings us to the end of certain autoimmune disorders of interest now we move on to endocrine disorder. So, again we will be discussing few common endocrine disorders and their associated marker. Mind it we already discussed one endocrine disorder that is thyroiditis and diabetes can also be considered as an endocrine disorder. So, that might be common anyway.

So, number one thyroid disorder since we already discussed about autoimmune variety of thyroiditis apart from that there are also certain variants which leads to familial hyperthyroidism or familial thyroid cancer right. Because main 1 and main 2 gene mutation, I mean you already read that I mean discussed we discussed yesterday. However one very important gene that we should know about is detection of thyroid hormone receptor mutation. Why? Because these cases are resistant to thyroid hormones. So, if any so thyroid hormone will not act on I mean endogenous thyroid hormone will not act any exogenous thyroid hormone will not act on the patient and we have to manage the symptoms individually.

And all since thyroid is such a gland or thyroid function is such a vital function that deals with entire global body function. So, multiple patient can report multiple complaints and we need to trace it back to the thyroid disorder right. And once we find there is a abnormal thyroid profile we need to look for the answers in the genes and see THRB thyroid hormone receptor beta there are other variations. So, THRB is one such gene which may be mutated that is the reason of this thyroid hormone resistance disorder resistant disorders familial disorders. Again we come back to diabetes mellitus now we are discussing other than autoimmune we have already discussed autoimmune.

So, what is the monogenic diabetes mellitus? What is monogenic diabetes mellitus? The term monogenic means problem in one single gene is leading to diabetes mellitus. And diabetes mellitus is actually a very common example of a monogenic disorder. In fact, in diabetes mellitus the two main form of monogenic diabetes are number one NDM that is neonatal diabetes mellitus that happens in small babies and maturity onset diabetes of the young or MODY these are the two main forms of monogenic diabetes. So, so we need to know some factors or some genes for both number one is in I mean gene coding for insulin or the insulin receptor may be mutated in MODY glucokinase receptor defect or glucokinase gene right might be defective. And other genes related to insulin secretion those may have some variety of mutation or polymorphism suppose KCNJ 11 ABCC 8 this is very important ABCC 8 is very important in to insulin

secretion in neonatal diabetes mellitus.

And these type of genes that is IRS insulin receptor substrate 1 or PPAR gamma PPAR gene paroxysmal proliferating activator receptor gamma PPARG all these genes these two mainly are responsible in I mean variation in these genes or polymorphism or mutation these genes are responsible for detection in type 2 diabetes mellitus. So, mind it I already told you we are discussing various molecular diagnostic markers of these diseases. So, please do not think that in order to diagnose diabetes mellitus you need to undergo an excision sequencing for glucokinase receptor mutation or expression no diabetes is traditionally diagnosed by the level of blood glucose right. So, as I told you these molecular diagnostic modalities may not be the diagnostic modality of choice for certain disorders. However, we are discussing only the molecular diagnostic part as the disease is concerned.

So, few of them may be relevant for diagnostics and few of them are of course, relevant for research and development right which will help in establishment of any new predictive marker or new score to improve the health for all right. So, again adrenal disorders CYP 21A2 this is basically 21 alpha hydroxylase enzyme and melanocortin 2 receptor MC 2 R right. So, these are associated with congenital adrenal hyperplasia or CAH and multiple endocrine neoplasia this is men 1 right this is also associated this men 1 mutation is also associated with adrenal tumor formation mind it you might get a question the what are the tumor or what are the cancers or neoplastic condition related to MEN1 mutation all right. And then you may have to select the odd one out here I have given the example of men 1 in adrenal cancers, but I leave it up to you to do some homework and end list the list of malignancies that are possible in men 1 mutation. So, make it your own note attached to your own class note of this course.

So, that you might answer that if it appears in any exam right. Again TP 53 that is tumor protein 53 mutation can also happen in adrenocortical cancer. So, these are the few markers that we are important in relation to adrenal or supra adrenal gland tumor. Pituitary again we have men 1 cyclin dependent kinase inhibitor 1B, CDKN 1B right AIP aryl hydrocarbon receptor interacting protein. So, these are associated with pituitary adenoma rather familial pituitary tumor syndrome.

So, again from pituitary there can be you know multiple 6 hormones related from anterior pituitary some are also some other hormones related from posterior pituitary. So, in pituitary disorders one example is growth hormone. So, in case of growth hormone growth hormone the genes that are regulating growth hormone secretion that can be mutated and that will lead to growth hormone deficiency or excess either dwarfism or gigantism. What are those growth hormone releasing hormone receptor gene might be mutated again growth hormone secreta of receptor. So, these are two

GHRHR and GHSR are the ones that are mainly studied whenever we are looking into any disorder relate to growth hormone and again that comes under pituitary disorders.

Parathyroid one common MEN 1 right associated with primary hyper parathyroidism calcium sensing receptors CASR right. So, molecular testing for all these genes are done and there are also some genes that are involved in parathyroid tumor formation such as CDC 73 right specially in hyper parathyroidism jaw tumor syndrome important question for any competitive exam alright. So, note this make your own note as I told you these classes it is very difficult to just memorize the whole thing just by looking at the slide know you have to make your own note you have to read and reread them over and over again discuss right and then only to and also in spite of all that you will need to revise them in the last moment. So, that you can remember it all on the day of exam when if you are applying this knowledge in your research laboratory or in a specialized diagnostic clinic you have the freedom of looking into your notes right, but since the exam proctor exam for this course is not an open book exam for that day you have to memorize it in some way right. So, lastly we will discuss in endocrine disorders reproductive endocrine disorders and follicular stimulating hormone there are two important hormones that are governing the entire reproductive system that is FSH as well as follicle stimulating hormone receptor as well as the genes that is coding for the luteinizing hormone alright.

You know there are multiple such multiple genetic parameters that play a role in governing the signaling pathway that leads to polycystic ovarian syndrome PCOS they may not be directly causing PCOS, but if you just go into any medical index I mean journal portal be it medline be it PubMed be it ghoul scholar be it m base be it scopus web of science anywhere you just types PCOS comma and genetic marker and you will see there are lots and lots and lots of genetic marker every group of team globally are working on multiple research markers and showing their correlation in development of polycystic ovarian syndrome. However some are closely related some are not closely related. So, if you are to mention any marker of polycystic ovarian syndrome I would suggest you to stick to the markers that are related to insulin resistance alright. So, directly PCOS marker it is very laborious to remember all those markers just for exam purpose. However, just know this just know that in spite of other hormone factors there are multiple genetic factors that lead to PCOS.

So, that being said let us now look in details regarding those markers. So, I will just name them and you can pause the slide and take a note. So, number 1 follicle stimulating hormone receptor. So, you know what is their function they help in stimulation ovulation of ovarian follicle utilizing hormone and utilizing hormone receptor chorionic gonadotropin receptor right, utilizing hormone chorionic gonadotropin receptor. So, mutation in these can disrupt the ovulation and contribute to PCOS right.

As I told you insulin receptor again insulin resistance is a very common feature of PCOS. So, any insulin resistance I mean problem in insulin resistance in insulin receptor will ultimately lead to the PCOS. So, not only insulin receptor, insulin receptor substrate 1 IRS 1 again a big contributor of PCOS. In PCOS there is an androgenic hormone compound androgen receptor compound and genetic variation in androgen receptor again can increase the risk of sensitivity I mean can affect the androgen sensitivity lead to hyper androgenism which is a hallmark and diagnostic finding of polycystic ovarian syndrome. CYP 19A1, CYP 19A1 that is that codes for the aromatase enzyme AMH antimalarian hormone sex hormone binding globulin glucokinase tumor necrosis factor.

The list is endless right, but some way these are the main 10 to 12 markers that have been proven across multiple population based studies that they have got a very high causal correlation in to development of polycystic ovarian syndrome pathology. So, this is the main reproductive endocrine disorder for which multiple diagnostic molecular diagnostic modalities are implemented right and this is one of the growing concern because in our country in Indian population majority of a of the women of child bearing age have some degree of polycystic ovarian syndrome which may be symptomatic or which may be latent. So, we now move to neurodegenerative disorders alright. So, neurodegenerative disorders molecular diagnostics are very important and the first disease that I would like to discuss will be Alzheimer's disease. In Alzheimer's disease Alzheimer's disease again are of two types.

Number one familial AD that is familial Alzheimer's disease in which there is a definite mutation that runs through the families and generation after generation siblings will be affected by this disorder. However that is only the minority majority of Alzheimer's disease are actually sporadic in nature. So, there are multi they are multifactorial any genetic mutation might not be found. However for the familial variety of AD these are the proven mutation amyloid precursor protein that is APP pre cenilin 1, pre cenilin 2, pre cenilin acni llin APP stands for amyloid precursor protein. So, APP, PCEN 1, PCEN 2 again various biomarkers suppose A beta tau protein specially in CSF and see in some of the slides I have also mentioned some diagnostic modality though they are not part of the molecular diagnostic imaging, but since we are discussing the disease I have also mentioned them in the slide for you to know.

For example, the position positron emission tomography or PET scan is in 2 deoxyglucose is very important marker for or very important diagnostic modality in case of Alzheimer's disease. And again another genetic component is apolipoprotein E4, apoE4 allele variation is very important contributor risk factor that leads to late onset AD mainly because of the alteration in oxidative stress. Like AD another very common neurodegenerative disorder PD that is parkins disease, SNCA, LRK 2, parkin all of these

gene mutation lead to familiar PD and just like Alzheimer's disease that was associated with abnormal amyloid beta A beta here the protein that these are these are the disorders of protein misfolding right. And here the alpha synuclein level in CSF and blood sample will gives some idea about the development of this neurodegenerative disorder right. So, there are nowadays there are diagnostic scoring.

So, level of alpha synuclein finding in CT or spectral PET, CSF markers find of the gene mutation all those things we get a scoring and based on that scoring we diagnose the stages of these diseases like. And just like Alzheimer's disease here again dopamine is defect in parkins disease. So, dopamine transporter imaging using single photon right emission computer tomography or SPECT is very important diagnostic modality to assess the dopamine transporter function mind it this is not a molecular diagnostic this is the preferred diagnostic modality of choice in countries where it is available right. However, the genes that we should be careful about are these. Huntington disease the abnormal mutant protein is huntingtin right.

So, HTT gene should be profiled all right and the diagnostic imaging modality of choice is either PET or MRI to assess any brain atrophy. ALS, Amyotropic Lateral Sclerosis, superoxide dismutase 1, chromosome 9, open reading frame 72, C9 ORF right TRDBP these are all associated with familial variety of Amyotropic Lateral Sclerosis. Apart from there are various biomarkers of axonal damage in CSF or blood samples and since it is a disorder of nerve and muscle ultimately muscular dysfunction happens. So, electromyography and narcoconduction velocity do helps in supporting the diagnosis. FTD frontotemporal dementia what are the genes that we should be careful about familial variety all these neurodegenerative disorders.

So, the familial variety of neurodegenerative disorders that we are discussing about say MAPT, GRN again chromosome 9, open reading frame 72, C9 ORF plays a very big role in familial frontotemporal dementia. TDP43 and tau these are the characteristic protein aggregates that ultimately are resulting in the neuronal and glial pathology right that again leads to all these neurodegenerative disorder and these two special in case of FTD. Spinocerebellar ataxia right the diseases or the genes that are associated with these spinocerebellar ataxia are ATXN1, ATXN2 and ATXN3. There are multiple repeats over here right and also apart from those genes there are other biomarkers and MRI neuroimaging that can be assessed to address the cerebellar and brain stem atrophy. Earlier it was the atrophy of the cerebrum spinocerebellar ataxia as the name suggests is the atrophy of cerebellum which deals with balance ataxia means imbalance all right.

So, now lastly we move on to molecular diagnostics in transplantation disorders. What are the transplantation disorders? So, while discussing transplantation disorders we will be looking to various processes and I mean diagnostic modalities and what are the

various types of transplantation to which these are applicable. Number one we have already discussed while we are discussing autoimmune disorders or HLA typing human leukocyte antigen typing. So, HLA compatibility is a must for kidney transplantation, liver transplantation, bone marrow as well as lung transplantation. If I want to summarize without HLA compatibility the graft will be rejected as simple as that.

So, apart from HLA compatibility what is done? So, we need to ensure there is HLA matching in order to minimize the risk of graft rejection for all these type of transplant. Donor specific antibody testing very important. So, it detects antibodies. So, directed against the donor HLA antigen cells. Apart from HLA compatibility this is also another type of testing that is done specially for kidney transplant.

So, you will see the major organ transplants will be overlapping they will be the common. So, for any organ transplant we need to do multiple multitude of these test panels to ensure there is compatibility to reduce the graft rejection. So, kidney transplantation, liver transplantation, pancreatic transplantation very important peripheral blood stem cell transplantation and lung transplantation. So, if there is any I mean we should always test for donor specific antibodies. So, it should be actually monitored that is why even the care should be taken.

So, that the amount of anti, but donor specific antibodies are less that is why the recipient is always immuno suppress. So, that these donor specific antibodies do not develop and then the chance of graft rejection will be low. Gene expression profiling true for all transplantation diseases we have got room to do it specially in heart transplantation, lung transplantation and bone marrow transplantation. The main thing that we need to know is what will be the tissue. So, in case of heart transplantation gene expression profiling for peripheral blood from lung transplantation broncho alveolar lavage or it can be done from peripheral blood as well.

And in case of bone marrow transplantation we need a sample of bone marrow as well as peripheral blood to know to understand the profile of the patient how they are compatible to assess the immune reconstitution to assess the immune function and prevent the risk of any relapse in graft versus host rejection. Again chimerism analysis very important, chimerism analysis is done with the help of my monitoring proportion of specific donor recipient cells and it is mainly done to know or to detect the risk of engraftment or the risk of graft rejection in case of bone marrow transplant. So, mind it chimerism analysis mainly it can be done other transplant, but mainly we are concerned in case of bone marrow transplant. This is the term which we discussed in last lecture MMR gene testing mismatch repair gene testing. So, if you are wondering what is this it is basically done in lung transplantation right again to identify various genetic variants associated with sorry in liver transplantation.

If I said lung that was wrongly mispronunciation it is done in liver transplantation and what are that methods that are done. So, we do sequencing we do copy number analysis, methylation analysis analysis of various structural rearrangement with the long range PCR the after that we test for various variants of deep intronic variants are analyzed and after that we get the information whether there is mismatch repair I mean mismatch repair testing is done. So, if there is an incidence of mismatch repair properly fine if there is a mismatch repair defect then it will be incompatible and we should be cautious when going for transplantation. And cytokine gene polymorphism definitely because it is the cytokines who play a major role. So, in kidney transplantation and peripheral, but stem cell transplantation profiling of cytokines polymorphism gives us an idea about the immune reaction that can be predicted in case of graft and host.

They are also insulin specific gene polymorphism for example, in pancreatic transplant we analyze insulin gene polymorphism. So, to summarize we have discussed about genetic testing for various types of disorders we have discussed multiple biomarkers that can be analyzed for various disorder various system. We have discussed the role of immunophenotyping that also plays role specially in autoimmune disorders, alright I mean also monitoring the post transplantation we have highlighted the gene expression profiling, alright. And very important metabolomic profiling for various endocrine disorders, alright what are the role of metabolic metabolomic profiling we already discussed this earlier in metabolomics that is metabolomic profiling can be done for multiple endocrine disorders that is the name I mean the disease the hormones and the abnormal metabolites because most hormones will cause metabolic dysfunction for example, in I mean I am not going to give any specific example over here, but any hormone you can make your own chart regarding this any hormone that will lead to any metabolic dysfunction and then metabolomic profiling also becomes a part of molecular diagnostic, but this is related to it and we can use mass spectrometry for it and lastly next generation sequencing has got a role in all types of molecular diagnostic disorders. So, these are the references for today's class and I thank you all for your patient hearing.