Comprehensive Molecular Diagnostics and Advanced Gene Expression Analysis

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Lecture 54: Molecular diagnostics in Metabolic,Cardiovascular and Gastrointestinal disorders

Namaskar. Hello students, welcome back to our lecture series on comprehensive molecular diagnostics and advanced gene expression analysis. We are in module 11 where we are discussing molecular diagnostics in medicine and as you can see from the title today we will be discussing the molecular diagnostic techniques I mean using them how we can diagnose various disorders. So, I will be discussing metabolic disorder, cardiovascular, respiratory and gastrointestinal disorder in this lecture and there are few more disorders that will be discussed in the next lecture alright. Now, just to get things clear in this lecture we will not be discussing the techniques because the techniques and the methods you have learnt it all right everything has been discussed across various modules what are the methods by which we do genetic testing. So, here we will be looking at the abnormal genes that are to be diagnosed you should know the names of the genes for various diseases you should know what we need to find out in order to detect or predict certain diseases right.

So, there are mainly two categories mainly for metabolic disorders actually. Number one detection of mutations, see detection of mutations is common for all. However specially for metabolic disorders some proteomic experiments are also very important specially mass spectrometry that helps us to detect the abnormal metabolites in the body alright. And you already know for the mutation detection there also can be multitude of methods for example, traditional PCR, real time PCR, DNA sequencing, microarray, next generation sequencing and you can answer it much more if I stop my list over here right.

So, we will directly go into the disorders we will go into the systems. So, we will start with the metabolic disorders I will be discussing each and every disease it is not possible for me to discuss all the diseases right because metabolomics is a very vast field and there are n number of diseases then the list is endless right, but I will be discussing the most important ones that are actually very common that appears commonly in multiple exams you can expect them to find in multiple competitive exams your theory exams

multiple diagnostic clinics. So, you that can set up and have some idea about these methods right. So, I will be discussing the most common ones, but mind it if you are interested I have the references you can go through the endless list of the diseases the principle remaining the same you just need to know the name of the gene and then you will be able to detect is the same methods right. So, conceptually nothing will change.

So, we will start with in metabolic disorder we will start with phenylketonuria the disorder of amino acid metabolism phenylalanine metabolism specially. So, what happens here there is a mutation in the gene phenylalanine hydroxylase right PAH gene this enzyme is absent. So, phenylalanine does not become tyrosine. So, what happens it leads to many problems the main thing we are concerned in with intellectual disability right mental retardation. So, how do we diagnose that once we know what enzyme is defective we with the help of genetic testing we can identify whether there is any mutation in the PAH gene.

So, for phenylketonuria PAH gene. Now there are multiple variants of phenylketonuria as well. So, for example, tetrahedral I mean if you are aware with phenylalanine metabolism again I will digress from it I will let you know that the same instructors have also got a course known as overview and integration of cellular metabolism over there you can find the details about all metabolic diseases also available in NPTEL platform you if you are interested you can visit there. So, over there you will find there is a variant of phenylketonuria that happens if there are modifications or changes in genetic or genetic mutation in other enzymes the those phenylketonuria bit milder all right. So, any BH4 or PTS gene mutation will also lead to phenylketonuria.

So, these are the genes that needs to be targeted if we are to find out phenylketonuria or if we need to design any research study on phenylketonuria. Gauchos disease very important lysosomal storage disorder what happens hepatomegaly that is enlargement of liver, spleen, bone pain and anemia. So, there are multitude of symptoms of Gauchos disease and it is caused by a mutation in glucosaribosidase gene this is a lysosomal enzyme glucosaribosidase the gene is defective the enzyme is defective disease problem. So, if we need to detect Gauchos disease we need to target GBA gene detect GBA gene mutation. So, at this point of time I would suggest you make your own note you make your own chart in the left side you can categorize the name of these diseases.

So, you can write the name of the disease on the left side and you can simply write the name of the gene on the right side. So, in that way to be much helpful for you specially for last minute revision all right. Teychac's disease again very important a variety of gangliosidos, GM2 gangliosidosis here the problem is again neurological manifestation it leads to seizure, blindness, motor disorder what is the problem hexosamine it is A enzyme is faulty due to its gene that codes for the enzyme right. So, hexagene so,

Teychac's disease we need to perform various molecular diagnostic tests. So, that we can detect mutation in the hexa or hexagene.

We move on to another disorder of amino acid metabolism this time, maple syrup urine disease this is caused by defect defect in branched chain amino acid metabolism. We will find out very similar features in all amino acid metabolism mostly they lead to mental disorder neurological problems right here you can see its poor feeding vomiting seizures and developmental delays right. So, why it is caused it is caused because of the problem in the defect in the enzymes that are involved in the catabolism of these branched chain amino acid. So, if we need to do or need to perform genetic test we need to target mainly three genes B C K D H A, B C K D H B and D V T all right. So, these are the full forms if you are interested.

So, mainly all are the variants of dehydrogenase enzyme specially branched chain keto acid dehydrogenase enzyme complex where there are E 1 and E 2 there are multiple polypeptides. So, there are two genes which actually codes for this enzyme branched chain keto acid dehydrogenase all right there is an alpha and beta polypeptide both can be mutated as well as dihedral per branched chain transacylase. So, this is another enzyme E 2 that is also part of the complex. So, any of it can be mutated, but mainly the main culprit is branched chain keto acid dehydrogenase. So, B C K D H A or B C K D H B mutation are the ones that we need to focus on if we are focusing on genetic studies to detect maple syrup urine diseases.

Again you might be thinking that all right these disorders are generally not diagnosed by genetic testing right you are right we will be getting there these disorders are generally diagnosed by analyzing the higher abnormal metabolites in the body fluids right and the gold standard is you will get your answer very soon. So, again Wilson's disease, Wilson's disease is a disorder of copper metabolism that leads to neurological functions in advanced psychiatric problems, but it starts with jaundice all right abnormal pain tremor solve neurological problem culprit ATP 7 B gene. So, again serum seruloplasmin assay seruloplasmin serum seruloplasmin assay serum copper assay all those will help us in diagnosing Wilson's disease also there are multiple clinical features, but mind it here in this lecture we are discussing genetic tests or molecular diagnostics for diseases. So, this might be so that this is not the diagnosis of choice for all these diseases, but if we want to diagnose all these diseases with the type of genetic test or simply if we want to know at a glance what genes are responsible for all diseases then this lecture is very very valuable for you. So, mind it ATP 7 B gene for Wilson's disease.

Homocystinuria very important what homocystinuria does it cause eye problem subluxation of lens inward and downward subluxation. So, lens gets displaced apart from there are multiple systemic disabilities or abnormalities from all bony abnormalities

increase of blood clot multiple incidence of heart disorder the main problem over here is the metabolism of homocystin and methionine those enzymes are at fault. So, naturally the genes that are responsible for coding those enzymes specially cystathionine beta synthase or CVS or methylalanine or methylenetetrahydrofolate reductase MTHFR. So, any using any method by traditional PCR by sequencing by using any mutation detection technique if we can target cystathionine beta synthase gene or methylenetetrahydrofolate reductase gene that will also help us to pin point the diagnosis of homocystinuria. Mind it homocystinuria just like phenylketonuria also has got some variants right they are milder forms of homocystinuria over there even the enzyme methionine synthase or even methionine synthase reductase the genes coding for those enzymes.

So, MTR or MTR are mind it MTR the students make mistakes over there. So, they remember MTR, but when they are asked the full form they they might wrongly recall it as a methionine reductase no it is methionine synthase that stands for MTR and MTRR stands for methionine synthase reductase all right. So, be careful over here when you are answering. So, MCQ question might be tricky, but they might ask you methionine reductase as an option as MTR it will be very tempting to answer that, but you should be careful. Alketonuria again disorder very closely related to phenylketonuria, I mean disorder of phenylalanine and tyrosine metabolism symptoms are dark urine right urine becomes very dark in color, but the problem is those Alkapton bodies get deposited in multiple joints and connective tissue leading to various problems.

The enzyme is homogeniticet oxidase or homogeniticet 1, 2 dioxygenation. So, that is the gene HGD gene that needs to be targeted for Alketonuria. If you feel at any point I am rushing through all the diseases that is because at your level you just need to remember the names of the diseases and the corresponding genes you already know what methods we should use in order to detect the mutation all right. So, I could have jolly well given you a chart or a table with that consisted of only the name of the diseases and the genes, but that would have appeared too much daunting. So, I decided to make it a bit intuitive.

So, include some symptoms. So, that you can recall the slides and recall any symptoms if you are facing any such patients or cases in your laboratory patient history in the laboratory. Glycogen storage diseases we are still in the metabolic disorders all right. So, glycogen storage diseases there can be multiple glycogen storage disorder there from type 1 to type 6 multiple disorders are there. Mainly they are leading to hepatomegaly, hypoglycemia, muscle weakness there are multitude of symptoms.

The main genes or the main type of glycogen storage disorder that we are concerned with often the maximum type that are found in the clinical practice are type 1, type 6 and the genes that are concerned are glucose 6 phosphatase. So, G 6 PC in glycogen storage

is type 1 and glycogen phosphorylase the gene is named PYGL in type 6. However, I would recommend if you are interested in scoring good marks. So, you should always extend your knowledge. So, whatever is being taught here you are always entitled to incorporate some degree of self-directed learning to from the references that we provide to make your own notes.

So, that you can answer any nice to know I mean you can have some concept about any other topics that are associated with this and you can answer some questions that might not be the exact content that is taught, but using the concepts that we teach over here all right. So, my suggestion is just look at least just know the names of at least 3 or 4 glycogen storage disorders and the corresponding genes or the enzymes that are deficient in the genes that codes for the enzyme specially if you are an aspiring student of MD medicine or biochemistry all right and even MD pediatrics. So, till now we discussed what genes are targeted in various metabolic disorders. So, now we move on to what are the abnormal metabolites that can be detected through mass spectrometry because mass spectrometry. So, since most of these disorders are present in newborn, newborn screening is a very important part of any developing country developing and developed countries.

So, there are multiple panels that are done. However, the gold standard is always MS, MS or tandem mass spectrometry by which we can diagnose many metabolic disorders. So, here again I will be giving you the names of the diseases and the corresponding enzymes or the abnormal metabolites. So, for phenylketonuria it is abnormal phenylalanine blood, for Gauchos disease it is glucosylceramide in blood and tissue all right. For T-shax disease hexazaminidase A enzyme or GM2 ganglioside level it is very I mean related if you know the names of the genes you can easily relate to the products that are either accumulating due to those enzymes not being active.

So, the immediate reactant of those enzymes on which the enzymes act generally are the ones that are accumulated and excreted in the body they are the abnormal metabolites. For maple syrup urine disease branched chain amino acids and their corresponding keto acids. For Wilson's disease high level of copper, for homocysteineuria high level of homocysteine and methionine, for cystic fibrosis sweat chloride I have included cystic fibrosis or because you already know the disease you have heard this name across multiple slides. However, since we are discussing mass spectrometry I thought of putting this. However, sweat chloride can also be detected by multiple methods number one is ion selective electrode.

I will write the full form ion selective electrode or ISE by the method of via the method of ISE or ion selective electrode this is the method of choice for detecting any electrolyte for example, sodium, potassium, chloride, bicarbonate not bicarbonate lithium all right.

However, there is also other methods of detecting multiple ions for example, by using ABG what is ABG? It is arterial blood gas analyzer or ABG in which we tap out arterial blood and there is a machine which first blood needs to be I mean liquid blood it should not clots. So, heparinized blood as a heparinized syringe is put into the radial artery or the femoral artery depending on the patient's condition and then we draw out red arterial blood which is a bright red in color and then we insert them into the ABG analyzer and ABG analyzer arterial blood gas analyzer in I mean other than in addition to reporting the dissolved gases that is oxygen and carbon dioxide can also be designed to report various electrolytes like sodium, potassium, chloride, bicarbonate etcetera etcetera all right. So, but my NED 60 fibrosis will be again coming back to this disease when we are discussing the respiratory disorders molecular diagnostics in respiratory disorders. In alcaptonuria homogenic acid, glycogen storage diseases definitely abnormal accumulation of various glycogens right glycogen in various tissues they can be analyzed.

In branchen keto acid dehydrogenous deficiency abnormal this is I think I am repeating myself because you already I mean discussed maple syrup urine disease this is the same thing. Any other organic acidemia so, right for all organic acid be it lactic acid doses we can quantify that special acid or that specific acid in the body fluid using mass spectrometry. Again urea cycle disorder very important in urea cycle disorder we can measure ammonia and various amino acid levels that are products of the urea cycle in blood and urine using mass spectrometry. Medium channel acyl-coid dehydrogenous deficiency analysis of acyl carnitine profiles in blood or dried blood spot. So, all of these can be diagnosed using blood spot detection.

So, why do we why did I specify this in blood or dried blood spot? It is actually to the fact that all of these tests are done in babies that are just born being born newborn. So, we undo a heel prick with a very small stilett or lancet or needle we do a very small prick in the heels and since we do not have so much blood in adult patients we take 2, 3 or even 5 ml of blood. However in babies one drop is all we have. So, we take one drop of blood from the heel we soak it in a blotting paper or a what man number one what man filter paper and with that spot we can do so many new model screening and MCA DDD is one of the disorder. We move on to cardiovascular disorder.

So, mind it metabolic disorders are the major chunk of knowledge that you need to know. However, there are also other disorders which I mean genetic tests might not be the diagnostic modality of choice because you already know for cardiovascular disorders there are multiple ways of evaluating the heart there are electrical ways there are imaging ways, but here we will be discussing the genetic testing aspect of diagnosing cardiovascular disorders. So, we will start with familiar hypercholesterolemia and you know familiar hypercholesterolemia is a disorder where there is excess cholesterol in

blood. However specially LDL cholesterol and that actually is due to the defect in it can be multiple. So, low density lipoprotein receptor or apolipoprotein B right or even pro protein convert is sub link x in 9.

So, PCSK 9 alright. So, any of these, but specially LDLR and APOB are the ones who are generally faulty in familial hypercholesterolemia again just like glycogen storage disorder familial hypercholesterolemia are also various types. So, it can be chylomicronemia it can be due to deficient it there can be multiple right and in standard biochemistry text books you will find various categories of hypercholesterolemia and what are the genes that are responsible. So, I would like to suggest you here also make your own note to have those diseases and enzymes at a glance alright. So, familiar hypercholesterolemia is one in which there can be multiple gene mutation leading to the abnormality in LDL receptor. Hypertrophic cardiomyopathy disorder where the heart muscle is thickened it can lead to sudden cardiac death or it can to multiple.

So, the problem is over here any heart disorder since it is a very vital organ will lead to sudden cardiac death or arrhythmia right and ultimately that may lead to cardiac arrest. So, it is very important that any anyway how you can diagnose it should be done. So, if we diagnose syntromatically or electrophysiologically it is always supportive for the clinician to have genetic information to pinpoint or delineate the diagnosis or trace any family history. So, MYH7, MYBPC3, TNNT2 these are all the genes that are responsible I mean in which mutations can lead to HOCM, hypertrophic HCM, hypertrophic cardiomyopathy in some text book in older text book it was named hypertrophic obstructive cardiomyopath or HOCM right.

Next LQTS. So, long QT syndrome or prolonged QT syndrome it is again a genetic disorder in which the hearts electrical activity is affected or hampered that can lead to arrhythmia that is abnormal rhythm. So, molecular diagnostics involved identifying mutation in KCNQ1, KCNH2 and SCN5A yes it is lot to remember, but there is no way out if you want to score good marks if you want to do good in competitive exam if you want to increase your knowledge you should have at these information at a glance all right especially for exams you need to memorize because it is not an open book exam the proctor exam for this course all right. Arrhythmogenic right ventricular dysplasia genetic disorder again it is involving heart muscle all right. So, the heart muscle development is at a problem which will lead to abnormal rhythm abnormal pumping mechanism and sudden cardiac death. What are the genes that we should be investigating gene mutation PKP2, DSP and DSG2 these are the genes for arrhythmogenic right ventricular dysplasia.

DCM dilated cardiomyopathy very dangerous condition which is not that uncommon and death of multiple young athletes it is to reduce cardiac function and many young

athletes or sports person often face with casualties due to presence of this undiagnosed dilated cardiomyopathy. So, identifying mutations in TTN, LMNA or MYH7 these genes give us will give us some clue about the will give us the definitive diagnosis of dilated cardiomyopathy. If even if we suspect dilated cardiomyopathy we can look into identifying this gene mutations. Marfan's syndrome it is not a specific cardiogenic disorder it can lead to multiple problems because it is affects all the connective tissue specially the aorta the elastic tunica media of the aorta Marfan's syndrome can lead to problem in eye even just like homocysteine urea right. What gene we need to know about Marfan's syndrome? FBN1 gene fine.

We move on to molecular diagnostics in respiratory disorders. Again I have highlighted few respiratory disorders the number one is cystic fibrosis, cystic fibrosis trans membrane conductance regulator gene all right. So, any mutation involving CFTR gene will lead to cystic fibrosis there are multiple I mean specially western countries America and Europe it this is one of the essential diagnostic panel for which a patient will need genetic testing, they will need genetic counseling that we have already discussed right. So, navigation sequencing to identify any mutation any as a phenotype genotype correlation are very important to ascertain any familial relationship of CF or cystic fibrosis. Again very important disease or very common that is alpha 1 antitrypsin deficiency that can lead to multiple problems specially lung and liver.

So, it can lead to M5 sigma liver cirrhosis. So, the gene that is codes for this enzyme that is alpha 1 antitrypsin is a SIRPINA1 or SIRPINA1 and any we can ultimately if we want to diagnose or if you want to target this mutation, we can do allele specific PCR or ASPCR or arms PCR to detect any wild type of allele or any mutant type of allele in the population that will lead to diagnosis of this type of disorder. Because alpha 1 antitrypsin deficiency will again lead to multiple different types of lung disorders right. So, this is the fundamental reason of lung disorder. Bronchial asthma very common the one that is prevalent in almost all categories of ages right. So, is there any genetic reason? Yes polymorphisms in gene such as ADRB to basically this is the beta 2 adrenergic receptor and it is the beta 2 adrenergic receptor that is that controls the bronchoconstriction and bronchodilation and to treat asthma we target this beta 2 receptors.

So, we give beta 2 receptor agonist in the form of inhaler that helps in bronchodilation. However, if there is a mutation in or polymorphism in those genes specially adrenergic receptor or interleukin 13, they will are associated with increased asthma susceptibility that may be resistant to traditional medication. Very close to asthma is another restrictive lung disorder that is the chronic obstructive pulmonary disease cause may be alpha 1 antitrypsin deficiency. So, polymorphism in Sarpina 1 may lead to COPD. Another reason of COPD acquired is smoking all right make smoking or any

occupational lung hazard for example, in silicosis all those inhalation of smoke leads to chronic obstructive pulmonary diseases.

However, there are various genetic components because not all smokers land up in COPD right. There are multiple studies, but smoking has very good I mean very definite correlation causal correlation with development of COPD. However, there are other genetic factors like alpha 1 antitrypsin deficiency, transforming oat factor beta, metal matrix, metal opportunase 12. So, MMP 12, TGF B 1, Sarpina 1 all of these are associated with risk of COPD development.

Again idiopathic pulmonary fibrosis. So, we cannot spin point any specific gene, but this is one of the lung diseases in which gene expression profiling are gaining more and more interest, gene expression profiling and excision sequencing to find a sequence of whole genome or whole exome to find answers in those patients who are actually susceptible to IPF or idiopathic pulmonary fibrosis. Actually viral infections we do not need to tell you right because you have just witnessed a few years back COVID pandemic COVID 19 pandemic and you already know by now the diagnosis of diagnostic modality of choice was RT-PCR reverse transcript is PCR right. So, any lung disorder. So, it may be respiratory syncytial virus H1N1 influenza COVID 19 or SARS-CoV-2 diagnostic modality of choices PCR based or nucleic acid amplification based rapid testing right. So, talking of nucleic acid amplification it is also implemented in diagnosis of other infectious respiratory disorder like tuberculosis where we can go for this nucleic acid amplification test or even DNA of a TB-PCR all right.

So, micro bacterium tubercular complex DNA can be amplified using PCR and that is also a very important diagnostic modality of choice confirmative diagnostic modality of choice for diagnosing mycobacterium tuberculosis. And lastly we are in the last phase of our discussion we will be touching few gastrointestinal disorder so GI disorders that also has some molecular components where we can apply this molecular diagnostics. So, IBD or inflammatory bowel disease NOD 2 ATG 16L1 interleukin 23 it is all these are disease genes that may be variant or abnormal in some individual and that have got a strong that may lead to strong susceptibility to inflammatory bowel disease like Crohn's disease or ulcerative colitis right. So, any detection these genes any mutation or polymorphism detection these genes greatly helps us to know about the causality or the susceptibility of or predict the susceptibility of the patient to develop inflammatory bowel disorders right. Celiac disease very common gastrointestinal disorder human leukocyte antigen HLA-DQ2 HLA-DQ2 HLA-DQ8 right via genetic testing also detection of antibodies right serum and tissue transglutaminase or anti TTG or anti endomysial antibodies.

So, mind it antibodies are diagnosed using proteomic studies for example, ELISA right.

However, they do belong to the molecular diagnostics. So, for celiac disease again molecular diagnostics very important just note down the parameters that are in red and you are good to go. IBS mind it IBD is inflammatory bowel disease IBS irritable bowel syndrome, but both are very common. However, again just like idiopathic pulmonary fibrosis we cannot pinpoint any gene.

However, there are various markers for example, calprotectin in our intestinal fatty acid binding protein. So, calprotectin run is tool alright they are indicators of intestinal inflammation. However, we need to find out the answer in the genes right. So, nowadays multiple micro RNA profiling. So, miRNA profiling or gene expression analysis that is serial analysis of gene expression SAGE those are done for these gastrointestinal diseases to note down any pattern and that will help us to create any genetic biomarker.

So, that we can identify the diseases in future if those biomarker footprint matches with those of the data that is already in our system. Again gastrointestinal disorder we cannot skip molecular diagnostic in gastrointestinal disorders unless we also discuss about gastric cancers. We have been taught about diagnosis of cancer using molecular techniques, but for gastrointestinal cancer MINDED, KRAS, BRAF and EGFR these three are very important to give us information about whether or not the case a suspect gastrointestinal cancer has got some molecular component or what is the reason or what is the oncogenic trigger for the development of various gastrointestinal cancer. So, mainly what do we do we can also assess any micro satellite instability MSI or MMR proteins because basically we have find found out that the defect in DNA repair leads to various type of gastrointestinal and colonic type of cancers.

However, the main problem lies in these gene mutations. Again gastrointestinal disorder where you helicopter pylori infection that causes hyper acidity, gastritis and even gastric carcinoma. How can we diagnose it number on either we are diagnosing the disease that is directly diagnosing I mean demonstrating helicopter pylori DNA HB, HP DNA via PCR or even we are trying to note the antibiotic resistant for example, resistant to any macro light like laryithromycin via mutation in 23S rRNA or mutation to fluoroquinolones by DNA mutation DNA zytase enzyme that is the GYRA. We can undergo conduct those type of studies to know why I mean at all if there is helicopter pylori infection or if there is any resistance to treatment I mean treatment resistant H pylori infection. Again pancreatitis very important you know cystic fibrosis, transmembrane conductance regulator gene this gene we are reading I mean we are coming across this name so many times because CFTR gene not only leads to lung problem it is a multisystemic disorder cystic fibrosis and again pancreases also affected and pancreatitis is one of the common presentation right. Serine protease 1 gene may be problem serine peptidase inhibitor casual type 1 basically SPINK 1 very important this is

also associated with hereditary pancreatitis mind it the most common reason of pancreatitis is alcohol induced pancreatitis, but here we are discussing hereditary pancreatitis.

So, the person who has great history of alcohol if you try to find out these mutations he or she might not have those mutations we are discussing hereditary pancreatitis and the genetic tests that are associated with it. So, with that here we would like to summarize just by recalling what we discuss we discuss for metabolic disorders we discuss various types of genetic mutation techniques for genetic mutation to identify various types of genetic disorders as well as mass spectrometry detect all the abnormal metabolites. For cardiac cardiovascular disorder we discuss hypercholesterolemia we discuss various conditions that lead to either muscle problem or problem in electrical activity of heart for respiratory disorders we discuss multiple enzyme deficiency like alpha 1 anti rips in deficiency genetic condition like cystic fibrosis as well as some infectious diseases like SARS-CoV viral infection or tubercular infection and for gastrointestinal disorders as we just read we discussed multiple genetic testing that can be employed to diagnose disorders like interval bile syndrome an ancillary disease or gastrointestinal cancer or H pylori infection. So, if you are interested in reading more here are the references.

So, we have provided the references according to various disorders. So, for metabolic disorder cardiovascular disorder respiratory and gastrointestinal disorder these are the various references mostly they are systematic review and meta analysis across multiple clinical studies and I thank you all for your patient hearing.