**Overview and Integration of Cellular Metabolism**

**Prof. Arindam Ghosh**

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

**Indian Institute of Technology Kharagpur**

**Week 10**

**Lecture 47: Heme Metabolism – II (Disorders of Heme Synthesis - Porphyrias)**

Hello everyone, welcome back to our lecture series on Overview and Integration of Cellular Metabolism. We are studying heme metabolism and this is the second class where we will be discussing the disorders of heme synthesis or porphyrias all right. So, the concepts that we will be covering today are the pathway of heme synthesis that you already know will be revising that the defects in the enzymes that are there in pathway of heme synthesis that leads to these diseases known as porphyrias, what are the types and varieties of porphyrias, what are the symptoms, how we can diagnose and how we can treat them everything will be covering in this lecture. Now, you all remember right from the last class of heme synthesis how the initial two products succinyl coenzyme A and glycine was combining to form delta amino levalonic acid ok, del alla ok, delta amino levalonic acid this is the sign of delta right. And in the next step we discussed how two molecules of delta amino levalonic acid were combining to form one molecule of porphyryl inogen right, but in reality we discussed that four of these porphyryl inogen will be required. So, in total eight alla are needed.

So, first it forms a porphyryl inogen which forms a linear tetra pyrrole this is not yet circular I mean this is not the ring is not closed which is also known as hydroxymethyl bilane all right. Thereafter it transforms into euro porphyrynogen 3 all right which again transforms into copro porphyrynogen 3, then it converts to protoporphyrinogen 9, then it converts to protoporphyrin 9 and finally, with the help of iron it converts to heme all right. So, most of you who have already gone through that class I hope you have easily remembered the steps if not this is a very good way to remember it ok. If you are having struggling in remembering I have got a very good mnemonic for you the mnemonic states that some good doctors prefer hugs under cover produces perfect healing all right.

So, I have given illustration a diagram and this concept is actually very important for a doctor hugs his patient it has been depicted in many Bollywood movies for example, but

the thing is if you can just relate this mnemonic and the initials you can easily remember that the products that are coming in succession in one another right. So, succinyl-CoA, glycine, delta-amino-levalinic acid or ALA, then porphyrynogen, first linear tetrapyrite or hydroxymethyl bilane, euro porphyrynogen 3, copro porphyrynogen 3, protoporphyrinogen 9, then protoporphyrin 9 followed by heme ok. It is a very good way to remember if you are struggling ok, but if you have been able to memorize and remember I applaud you right. Not only the products you have to remember, but you also have to remember the enzymes that were catalyzing the reactions. The first enzyme is alla synthase that helps in combination of succinyl-CoA and glycine right.

Thereafter alla dehydratase, hydroxymethyl bilane, synthesis also known as porphyrynogen deaminase this enzyme has got multiple or even euro porphyrynogen 1 synthase right. Thereafter euro porphyrynogen synthase is also known as co-synthase right, then decarboxylase 2 oxidase enzyme and last enzyme is ferrochilates. So, is there any way to remember this as well right there is right. So, I give you another mnemonic aunt Alice has unusually useful crafting powers friend right. So, remember magician aunt Alice who can craft many thing by waving a magic wand right.

So, you can again see the initials of this mnemonic corresponds to the enzymes right. So, why we are stressing so much in remembering all of these products in sequence and all of the enzymes, because this very important I told you in any metabolic disorder what we will be studying today is the disorder of these enzymes right. So, any enzyme if it gets deficient or it has got low activity what I told you the product before that will get accumulated in the product after that will be deficient this is the basic rule of any metabolism. So, unless you know what comes after another in sequence it will be very difficult for you to conceptually write the answers right. If you can just memorize, but then again there are so many things to memorize so many cycles it will be very difficult challenging right nothing is impossible with a lot of hard work and writing habit anyway.

So, the disorder coming to the disorders of heme synthesis this was all about last classes revision right I hope you have gone through them very well. So, coming to the disorders of heme synthesis they are collectively known as porphyrias alright. So, disorders associated with biosynthesis of heme what is the reason basically we all know the enzymes are proteins that are coded by genes that are designed for those proteins right. And if there is some mutation in those genes the enzyme activity will be improper it will be reduced and that will lead to these diseases. So, all of the enzyme of heme synthesis leads to some or the other porphyrias ok and the problem is in mutation.

So, what is the generalized characteristic basically increase production and excretion of porphyrins or their precursors. For example, if we are calling the porphyrin we are we

already know what are porphyrins and the precursors mean alla and porphobilinogen I already told you before porphobilinogen is formed right. So, delta amino levalinic acid and porphobilinogen are the precursors right. How are they inherited most of the porphyrias are inherited as autosomal dominant AD very important alright. So, can we classify the porphyrias? Yes depending on the symptoms depending on the bio I mean metabolic pattern they can be classified right.

They can be broadly divided into hepatic porphyrias, erythropoietic porphyrias and a mixed variety basically some porphyria which has got both erythropoietic and hepatic abnormality ok. There are some other ways the porphyria can be classified congenital or acquired, acute or chronic ok. Even some porphyrias are also called cutaneous porphyrias and non cutaneous porphyria. So, depending on your text book it can be or depending on the presentation of the patient it can be classified in many ways we will be discussing everything. So, this is the pathway, but now you already know the compounds that are appearing sequentially and you already know the name of the enzymes with the help of two beautiful mnemonics right.

But then again you need to remember the diseases that are caused by deficiency of these enzymes ok. So, the first important name that we need to remember is acute intermittent porphyria. It is caused due to the deficiency of the enzyme porphobilinogen deaminase or hydroxymethyl bilane synthase HMB synthase same enzyme HMB synthase the same enzyme deficiency leads to acute intermittent porphyria followed by congenital erythropoietic porphyria due to the deficiency of euro porphyrinogen 3 cosynthase. The next name that we need to remember due to the deficiency of euro porphyrinogen decarboxylases porphyria cutania tarda abbreviated as PCT all of these have got abbreviation this is AIP this is CEP ok hereditary copro porphyria due to the deficiency of copro porphyrinogen oxidase variegate porphyria or porphyria variegata ok due to the deficiency of protoporphyrinogen oxidase and finally, the deficiency of heme synthase or ferrochilates. Ferrochilates deficiency leads to hereditary protoporphyria or erythropoietic protoporphyria the name protoporphyria is very important alright.

So, again there are a lot of names that you need to remember well you can remember them by memorizing this phrase all congenital porphyria have variable presentations ok presentations right. So, what how does it help you to remember acute intermittent porphyria congenital erythropoietic porphyria porphyria cutania tarda hereditary copro porphyria variegate and protoporphyria alright. So, all congenital porphyria have variable presentation this is a phrase a mnemonic by which again you can remember the names is a lot of memorization that you need to do and these are the keys by which you can take the help of these keys to memorize them in sequence ok. The first two enzymes that I did not mention are not that much important these are the ones that are most

common the six varieties of porphyria. However, there are two enzymes the enzyme before hydroxymethylgolane synthase that is the rate I mean the this enzyme alladehydratase the deficiency of this enzyme leads to alladehydratase porphyria or DOS porphyria.

It is very rare till date less than 10 cases in the whole world has been reported. So, you can upon billions and billions of population only less than 10 cases have been reported it is not 10 in 100 or 10 in a million it is total 10 till date in world population right. So, it is very rare. So, we do not consider it as a thing to be worried about while diagnosing the disease why we are studying the whole course because we are making ourselves proficient enough so that we can diagnose the most common disorders right. One goal of this course is definitely that and the first enzyme that is allosynthase right the deficiency of that enzyme does not lead to porphyria at all it leads to a disease that is known as X linked cedroblastic anemia alright.

So, we are calling about I mean talking about deficiency of all the enzymes the deficiency of all of these enzymes produces some form or the other of porphyria this is a very rare alladehydratase therefore, we do not consider it at all and allosynthase deficiency does not produce porphyria. However, one very important thing to note and you also probably remember what I told you in the last class that once heme is synthesized it actually inhibits its own production by inhibiting the feedback I mean by means of feedback inhibition it influences the enzyme allosynthase this is the rate limiting enzyme right. So, if somehow there is a situation where so once we have heme the production of more and more heme when the required amount is reached it is not I mean the excess heme is not produced therefore, excess degradation product is not a thing to worry about normally, but if due to some reason the inhibition of heme on the rate limiting enzyme is lost right or if there is a situation where the activity of this enzyme is actually increased due to some reason then there will be a problem then there will be a problem that all of this products will be produced more and more and that is exactly the problem when there is a specific disease that is known as X linked dominant protoporphyria mind it X linked dominant protoporphyria where we are not dealing with any deficiency, but we are dealing with a gain of function mutation a mutation happens due to which this have been reported very recently right. So, in older edition text books this disease might not even be documented right. So, a gain of mutation function leads to an excess production of all of this products right.

So, all of this products will be excluded in urine and it will lead to a common symptom that will be discussing very soon and more over this has been found due to a gain of mutation of allosynthesis 2 right which is actually present in erythrocytes erythroid variety which has no effect of heme as a feedback inhibition. So, all of this phenomena

leads to a peculiar disease which is also very rare, but gain of mutation function and the name of the disease is X linked dominant protoporphyria and as you have guessed by now the inheritance is X linked dominant right sex chromosome not autosomal unlike the other porphyrias. So, let us discuss the first important porphyria that is acute intermittent porphyria this is the stage where this enzyme is inhibited. So, there will be no synthesis of uroporphyrinogen 3 right. So, porphobilinogen so, after porphobilinogen there was synthesis of hydroxymethyl bilane right.

So, basically hydroxymethyl bilane synthesis will be hampered right not porphobilinogen not uropilinogen. So, this will not be synthesized. So, deficiency of the enzyme hydroxymethyl bilane synthesis or porphobilinogen deaminase which is coded by the HMBS gene remember hydroxymethyl bilane synthesis the newer name and uroporphyrinogen 1 synthesis was actually the older name porphobilinogen deaminase uroporphyrinogen 1 synthesis these are all older names hydroxymethyl bilane synthesis is the newer name. The linear tetrapyrrole was named hydroxymethyl bilane much later compared to when the whole synthesis was discovered right. So, what happens the naturally the previous products that is deltamino levelonic acid and porphobilinogen level will rise alright and since heme synthesis is not happening ultimately since there is a if there is a block here the heme synthesis will not happen right and therefore, the inhibition of the previous enzymes due to feedback inhibition will not happen therefore, a secondary increase in activity of allosynthase is happening because the end product inhibition is not effective right.

So, this is the basic pathology of the disease which is known as acute intermittent porphyria AIP. So, why it is named acute intermittent right? Acute because of the symptoms intermittent also because of the symptoms. The patients present with vague symptoms which are intermittent it happens time after time ok it would not happen continuously right and what is the symptoms? Acute abdominal pain that is the most common symptom of acute intermittent porphyria. Patient will present with excruciating pain abdomen right and this is one of the reason of medical reason of pain abdomen mind it in medical science there are multiple reasons of pain abdomen. For example, even now you can probably enlist multiple reason of pain abdomen for example, problem in gallbladder appendix, stomach, pancreas so on.

So, there are multiple organs where there might be some defect and that can lead to pain abdomen, but this is a medical reason where there is a problem in metabolism and presence with acute pain abdomen. So, there is no defect in any organ most common investigation ultrasonography whole abdomen that is prescribed to any patient with pain abdomen will be absolutely normal extra abdomen absolutely normal. So, there it will rise the raise the suspicion for uncommon diagnosis like acute intermittent porphyria. Of

course, there is need to be other family history genetic predisposition etcetera. So, apart from abdominal pain there might be another group of patient that will present with neurological manifestation.

For example, motor disturbances, confusion, agitation you already know what are the neurological signs right from other previous amino acid metabolism disorders, but generally since these are not strictly speaking amino acid ureas that help in brain development right mental retardation is not a phenomena in these type of inherited disorders. Mind it these deficiencies are also enzyme deficiency, gene deficiency it may it is actually present in your gene. So, it may or manifest even since birth right, but there is no problem in mental retardation. Other neurological phenomena that leads to excess triggering of nerves and cardiovascular system visomotor symptoms like tachycardia, vomiting and hypertension are very common. So, abdominal pain neurological manifestation, vomiting, tachycardia, high blood pressure very common right.

Also some patients may have psychiatric problems ok and they need to be treated accordingly. It has been seen that women have less severe manifestation before menarche right before the menstruation starts they do not present with any symptom or after menopause, but during active menstrual phase of the life the disease presents right. So, there have been research articles that have tried to document and I mean gained inference that female 6 hormone have a stimulatory effect on the rate limiting enzyme that is allosynthase right. It is because we are having activity of allosynthase therefore, when the ultimately the block is there the products will accumulate ok. So, what are the mechanism of these symptoms? How a simple block in the enzyme hydroxymethyl bilane synthase leading to all these symptoms? So, let us understand first of all the exact mechanism of acute attack is not clear yet to date it still is a matter of research interest.

However, scientists have been able to pin point several reasons number one being the building up of those intermediate toxic intermediates like porphyryl nojan leads to a effect of neurons that leads to nerve conduction defect and more specifically the autonomic and peripheral nervous systems are more vulnerable because the brain is protected by the blood brain barrier right. And these porphyryl regions actually cannot cross the blood brain barrier and therefore, they have much less central CNS effect. The more effects are peripheral nervous system autonomic nervous system. So, pain abdomen the nerves that are in the outside the brain right same tachycardia also lead to same triggering of nerve in the periphery right vagus nerve mind it all the nerves that are outside brain are known as peripheral nerves and the brain and the spinal cord are central nervous system among them the brain is actually protected by the blood brain barrier this is the basic physiology. So, explanation of the findings such as abdominal pain and tachycardia are mainly attributed to the nerve conduction defect caused by the

accumulation of toxic porphyrins right.

And the toxicity may be so progressed in some cases that it may also lead to paralysis ok. How it is inherited I already told you autosomal dominant right. However, there is something known as gene penetration right mind it in autosomal dominant variety the enzyme is actually not fully absent generally there is 50 percent defect enzyme activity that is hampered, but even then everyone who has got HMBS gene mutation does not have the disease even if it is autosomal dominant mind it autosomal dominant means it is it will get transmitted generation after generation, but there is something called penetration in which even if the gene is present only 10 percent of the cases have been found to have the disease. Penetration means the genotype actually expressing as a phenotype that is the probability in rough words right there is a gist of it. So, therefore, not only the role of the gene, but there are other modifying factors for example, may be environmental factors that have been speculated to be a mechanism behind this acute attacks right.

Very important thing urine is actually colorless when it is voided right. However, urine on standing urine has got porphobilinogen right, but the porphobilinogen is colorless upon standing it will form reddish color due to conversion into porphobilin right, but porphobilin is not present in blood right porphobilin will come much later in the pathway porphobilinogen. So, urine is colorless to during voiding it will become dark colored upon standing right. So, urine samples for porphobilinogen estimation should be freshly collected and transported in dark bottle because light helps in transformation of porphobilinogen to porphobilin. So, how it can be diagnosed Arlicks aldehyde test is used to confirm the diagnosis of acute intermittent porphyria.

So, this is very important the name of this test ok, we will be again discussing the mechanism of this test later. So, distinguishing feature of acute intermittent porphyria how it is differentiated from other varieties of porphyria is there is no I mean there is absence of any photosensitive cutaneous symptoms right in addition to acute attack. There is only acute abdominal pain tachycardia vomiting, but there is no photosensitivity will be discussed in detail what is photosensitivity why you are saying there is no photosensitivity it means there will be other varieties of porphyria that do have photosensitivity alright. So, drugs like barbiturate which are known to induce allosynthase can precipitate the attack definitely allosynthase is a is an enzyme that helps in more and more synthesis of hydroxy methyl bile is a very important. So, that is the reason why glucose infusion is actually a mode of treatment to alleviate the acute attack.

Moreover other inhibitors of allosynthase like hematine and hematine are also treatment of choice during an acute attack right. So, we already discussed the reason of X linked

cedroblastic anemia that is deficiency gain of mutation in allosynthase 2 leads to protoporphyria which is X linked dominant right this is very rare and this we have discussed before hydroxy methyl bilane ok. Now, after hydroxy methyl bilane all the products ok, all of the diseases that comes after hydroxy methyl bilane have got a feature of photosensitivity why because the products formed due to block of the corresponding enzymes they get converted to toxins right and they have got a specific mechanism of action. So, the next disease that we need to study is which is happening due to the deficiency of europerphyrinogen 3 cosynthase is congenital erythropoietic porphyria. So, what is happening in that defect in europerphyrinogen 3 cosynthase inherited in autosomal recessive very important MCQ exception ok.

Photosensitivity is a major manifestation ok due to presence of porphyrins the intermediates gets converted to porphyrins in the capillaries. This porphyrins actually absorbed ultraviolet light and they get excited and they generate free radicals or reactive oxygen species which actually destroys lysosomes, lysosomes contain lytic enzymes digestive enzymes. So, once these lysosomes are damaged these are I mean released into the system and mainly the reason where there is most UV lathes the skin it leads to destruction of the skin in some cases also hemolysis. So, cutaneous destruction that is the reason where UV ray if it strike the skin it leads to damage of the skin. And repeated attack of this dermatitis healing again getting lesion it leads to scarring and since face is one of the area where it is most exposed the face becomes deformed and it is known as monkey skin.

You can see a dreaded face of congenital erythropoietic porphyria very tough to cope up with these sort of patient and the patients who have got this disease has got a very difficult life to live with right. Lipid lipid ulceration and scarring may cause mutilation of nose ear cartilage that may mimic leprosy right. And in some cases UV light right when reflect on to teeth red fluorescence is seen due to presence of this porphyria it is also known as erythrodontia ok. So, you can see red teeth right the next disease that we are studying is porphyria cutanea tarda. So, now, you can just remember the names now it is very easy you can just remember what are the things that are getting blocked what are the stuffs that are getting accumulated ok.

So, I will be just breezing through the slides and you will be able to easily relate on your own. So, hepatic autosomal dominant and most common porphyria ok porphyria cutanea tarda and MCQ question caused by partial deficiency of euro porphyrinogen decarboxylase ok which is involved in the conversion of euro porphyrinogen 3 to copro porphyrinogen 3 right. Next what happens euro porphyrinogen 1 and 3 and porphyrinogen are excreted in urine that leads to a production of a already dark coloured urine port wine stain ok and urine becomes pinkish to brown it appears pinkish to brown.

Clinical symptoms are photosensitivity a common thing and appears during 4 to 6 decades of life through in elder patient 40 to 60. And hepatocellular damage and alcoholism is characteristic of this condition right.

Hepatocellular damage already liver is getting assaulted due to alcohol and in presence of porphyria cutanea tarda, hepatocellular damage is again triggered and that leads to ineffective erythropoiesis which is a symptom of this disease. So, this is the cutaneous symptom of porphyria cutanea tarda right due to photosensitivity again excess scarring. Next hereditary copro porphyria due to efficiency of copro porphyrinogen 3 oxidase the corresponding products that are excreted in urine is porphyribilinogen and copro porphyrinogen 3 right. It is also excreted in feces urine again red colour due to same presence of pigment and fecal excretion of urine copro porphyrinogen is more clinical symptom photosensitivity right. And sometimes acute intermittent porphyria this may mimic acute intermittent porphyria right, but the basic thing is same.

Again in variegate enzyme deficiency, proto porphyrinogen, oxidase the corresponding products are accumulated and excreted in urine, urine is again coloured, feces is coloured, clinical symptom, photosensitivity. Then this porphyria is actually very rare right and the other symptoms actually may vary therefore, this porphyria was named variegate that is differential symptoms. Again alcohol and other drugs can aggravate the condition. And lastly erythropoietic proto porphyria due to definition of ferrochilates enzyme there is excess proto porphyri 9 and again excretion of proto porphyri 9 in feces and other precursors in the urine are normal right. Clinical symptoms again photosensitivity major symptom liver cirrhosis anemia right.

So, this chart basically shows what is the inheritance how it is what are the enzyme that are deficient. So, this is basically the same thing that has been repeated in the previous slides, but in a tabulated manner you can actually pause and take a snapshot. So, that will help you to remember the diseases easily alright. So, all the porphyrias have been summarized here and same the laboratory findings that is what are the intermediates that are getting accumulated in specific type of porphyrias. It is already very easy for you to actually write it on your own just note what are the steps where it was blocked and you can easily get the inference what are the things that are raised in plasma and what will be excreted in urine right.

So, there is also variety that is known as acquired porphyria which mimics enzyme deficiency that happens mainly due to lead poisoning. Lead actually inhibits the enzyme ferrochilates and allard dehydrates and the corresponding symptoms that are actually belong to those porphyrias, but that is not inborn not gene deficient, but due to poisoning that is lead poisoning are known as acquired porphyrias. So, acute intermittent porphyria

is variety of acute porphyria because the patient presents acutely sudden yesterday the patient was normal today there is acute abdominal pain right. So, not only acute intermittent porphyria the three varieties of porphyria that are considered acute are actually acute intermittent variegate and hereditary coprophoraphora as well as allard efficient porphyria. So, these four are actually comprising of the similar features.

So, they are known as acute porphyria whereas, chronic means symptoms develops very slowly. So, what are the chronic varieties? X-ling dominant, congenital erythropoietic, porphyric cutaneous, tarda and erythropoietic portoporphyria. Along with the along with these the variegate and hepatic cutaneous porphyria or hereditary coprophorphyria sorry are also having manifestation of skin. So, they are also known as cutaneous porphyria. So, as I told you there can be multiple such classification and you can actually place some of these in multiple classes.

So, depending on the multiple choice question there may be an all exit type of question from all these varieties of porphyria. So, we can see this is again the chart of porphyria we have added a new table where all the skin lesion porphyria that are skin lesion or acute attack have been shown here. So, how we can diagnose porphyria? Already told during diagnosis of acute intermittent porphyria that is we need to diagnose the intermediates in urine ok. So, for acute attack what samples we need to collect porphyrin and if these are abnormal then these are actually also tested in 24 hour urine sample. And to distinguish variegate and hereditary coprophorphyria porphyrin are tested in feces also right.

And for cutaneous porphyria urine I mean whole blood and urine are also tested. And enzyme testing such as porphyrinogen deaminase may be done to defect latent porphyria ok, because that is the most common porphyria. How we can diagnose? Diagnosis is very easy by UV not only by laboratory estimation if we detect UV fluorescence I mean if we transmit UV light we can get a specific peak we will be discussing with very soon. Next erlichs aldehyde reagent test it is very easy to see basically we mix the urine with an aldehyde and depending on the presence of porphyriogen or urobilinogen a colored pink color urine positive if it is formed it gives rise to the suspicion of porphyria, acute intermittent porphyria and that is also insoluble in chloroform right. So, when urine is absorbed in ultraviolet light if porphyrin is present it will emit strong red fluorescence.

So, we are almost at the end of our lecture. So, I discussed that is UV fluorescence. So, what is UV fluorescence? There is a term known as saurite band that is actually formed when we expose the urine or the sample to UV range of light. So, what happens specific in specific peaks specific porphyrin have got their absorption maxima. So, they give this characteristic band that are known as saurite band after the discoverer Jacques Louis

saurite ok.

He does not possess this property. So, only the intermediate should possess this property. Now, you know there have been mythical stories regarding vampires that have got very common features like porphyria. So, sunlight sensitivity you know vampire skin burns with sunlight, they have got pale skin due to anemic, they have protruding and red teeth, they drink blood due to erythrodontia and due to some unknown reason patients of porphyria specially congenital erythropoietic porphyria have got garlic allergy which is again a symptom of vampires right. So, basically congenital erythropoietic porphyria since it is autosomal recessive it was not found everywhere only few members of the family had this vampirism disorder right. And lastly to treat basically symptomatic treatment with IV fluid glucone injection, hemine injection that have already been discussed to alleviate the acute symptoms.

And the definitive therapy is gene therapy by which you can replace the dysfunctional gene to coat the proper enzymes, but that is still under trial in non primate human. So, this is the final slide where you can see the pathway that is of heme synthesis that partly occurs in the mitochondria and partly occurs in the cytosol all the enzymes in abbreviated form and their corresponding porphyria have been shown. So, it is very important that you draw this pathway on your own and by now you should already know what are the abbreviations and what are the specific porphyria. So, to conclude the we have discussed the pathway of heme synthesis what are the defects of heme synthesis pathway specific enzyme that leads to these porphyria how they are inherited, what are the symptoms, what are the mechanism of porphyria, how we can diagnose and how we can manage the porphyria. So, these are my references and I thank you for your kind attention.