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Biochemical genetics

- Biochemical genetics, also known as **inborn errors of metabolism** (IEM) include many types of diseases that we are familiar with, such as PKU, glycogen and lipids storage diseases, and diabetes. Diabetes is the most common IEM, but it is a multifactorial disease, as there are many genes and environmental influences that affect it, so it'll be explained later.

- IEM are mostly AR because if you have an enzyme you typically have to lose activity in both alleles and lose that enzyme all together to get a different phenotype, but if one enzyme is present and you have one allele that will be enough for you to remain phenotypically normal (unless you are haploinsufficient), and that is why enzymatic diseases are mainly autosomal recessive.

- Diagnosing these inborn errors of metabolism is unfortunately challenged by the vast amount of information we have to know because every IEM is presented by different set of enzymes, genes and other underlying abnormalities. However, newer genetic testing technics made the job easier as there are now panels that test all at once from the same sample numbers of IEM that are common in that region.

- What are the consequences of having a missing enzyme, or in other words; the effects of IEMs?
  1- **Substrate accumulation**: this will lead to a toxic effect.
  2- **Lack of product**: the lack of the enzyme can be presented as non-production of the usual metabolites, which will lead to a deficiency of some kind.
  3- **Over activation of an alternative pathway**: The substrate that's no longer metabolized could over activate another pathway that is normally active and is producing its normal product, and thus making an excess of that product.
  4- **Starting a pathway that is never active**: the substrate could also activate alternative metabolism altogether and link enzymes with each other in a pathway that is not normally active in the human body, except in diseased conditions, which will produce a toxic metabolite.

- But what are the biomolecules that are involved in this kind of errors? Basically, all of them, whether it's nucleic acids, carbohydrates, proteins, or lipids.

❖ ***Carbohydrates Inborn Errors of Metabolism:***

The most common of these is diabetes (we are not going to talk about it because it's a multifactorial disease you can't pin point only one responsible gene)

- Galactose:

  - Galactose is part of lactose (recall that lactose is made up of glucose and galactose).

  - Glucose metabolism is easy, however we have to deal with galactose.

  - We have an enzyme called (Galactose-1-phosphate uridylyltransferase) GALT which is needed to convert galactose to glucose so you can metabolize it. So if there's a deficiency in GALT then this will lead to *Galactosemia*.

  - New-borns with deficiency in this enzyme, GALT, will show signs and symptoms the second they start eating. Because milk has lactose.

  - So people with Galactosemia will accumulate a toxic metabolite in their bodies. So what's going to happen is that Galactose will rise to toxic levels, then it'll go and accumulate in the kidneys, liver, eyes (causing cataract), brain damage…

  - And those patients will present with vomiting, diarrhea, jaundice, sepsis, electrolyte abnormalities. And if not treated it might lead to death.

  - Treatment is; remove galactose from diet at least for the first two years of life, because you will end up accumulating toxic material all over your body as you grow.

  - Classical Galactosemia is associated with missing the enzyme GALT, however, other enzymes deficiencies like Galactosidase and UDP-glucose 4-epimerase can cause Galactosemia.

- Fructose:

  - Also autosomal receive.

- Where can we find fructose?
  Fruits, table sugar, corn syrup, soda

- Patients with fructose Inborn Error of Metabolism usually present in 6 months after birth. When they start eating Cerelac, fruits, baby food, vegetables…

- Abnormalities in fructose metabolism can involve a deficiency in one of three enzymes:

  1- *Fructokinase:*
  Missing this enzyme has no symptoms, meaning that patients are asymptomatic. You only find fructose in the urine with no other symptoms. So they're diagnosed incidentally.

  2- *Fructose 1,6-bisphosph**ate** aldolase (HFI):*
  - HFI stands for Hereditary Fructose Intolerance.
  - Patients will begin with poor feeding, failure to thrive, then liver/renal insufficiency, and it can lead to death.

  3- *Fructose 1,6-biphosph**atase**:*
  - These patients present with hypoglycaemia, and metabolic academia.
  - Why hypoglycaemia? It is because this enzyme is involved in gluconeogenesis, it converts fructose into glucose. Because when you ran out of glucose you're going to start scavenging all other biomolecules and turning them into glucose, your fuel.

- <u>Lactose</u>:

  - Lactase is found in the brush border of the intestine. It is present in everybody when born, then it's lost in about six months after birth (after weaning).

  - Now for Lactase to persist beyond 6 months of age, that is Autosomal Recessive. Meaning that in order to still have the Lactase enzyme even after 6 months of age you must be homozygous for the recessive allele.

- How did that happen? Natural selection.
  In populations that had been historically known for continue ingesting dairy products (milk, etc.) after weaning, there has been a natural selective advantage for people who maintain their Lactase because these people will receive adequate calcium intake, vitamin D, so they won't have rickets. So there was a selection pressure for people who are autosomal recessive to become the dominant population.

- If you lose one of those alleles then you become Lactose deficient. So Lactase persistent is what autosomal recessive.

- There's also a congenital Lactase deficiency, this is autosomal recessive, but it's very rare. Meaning that in order to have a congenital Lactase deficiency you have to lose both alleles.

- Most people who are lactose intolerant are <u>not</u> of this congenital type. It's just that lactase persistent is not of their genotype.

- You can also lose Lactase after gastroenteritis due to the massive damage to the brush border that's why patients are advised a lactose free diet.

- Now what happens to all that lactose (if there's no lactase)? Lactose will go through fermentation giving CO_2 as a by-product. It will also lead to diarrhea.

❖ To summarize lactase deficiency:
  - There are two causes of lactose intolerance, when we're talking genetics:
    - The vast majority of people who are lactase intolerant just don't have both alleles that allow lactase to persist beyond weaning.
    - There is also a very small percentage of people who actually have a mutation in the Lactase enzyme. And for that to make them lactose intolerant they have to receive two mutations on both alleles. Those patients are autosomal recessive but they're particularly rare.
      Those patients will present from birth, rather than after weaning.
  - Other than from genetics; you can lose the lactase enzyme from gastroenteritis.

- Glycogen:
  Can be divided into two major types: Hepatic, and Muscular.

  - Hepatic:

    - What are you going to expect? Hepatomegaly & Hypoglycaemia.

    - But why?
      Your liver is responsible for making sure that blood glucose level maintain normal. So if the liver can't convert glycogen into glucose, or propel the store properly, then glycogen is not going to be able to maintain a normal glucose level. Which means these patients will present with hypoglycaemia and hepatomegaly.

  - Muscular (Myopathic):

    - These patients have an enzyme deficiency in their muscles.

    - Normally, when you first start exercising your muscles begin to use glucose from the blood, then glycogen. After that, and when your muscles don't have enough oxygen, they start producing lactic acid.

    - But what if you don't have glucose in the first place, are you going to produce lactic acid? Obviously not.

    - So these patients will have a very low exercise tolerant, they will cramp up quickly, not because of lactic acid (which is also a common cause of cramping), but because they ran out of glucose before they ran out of oxygen.

- Now these groups of diseases are 1 through 7 (in the table).

- *The doctor does not want you to know every single information in table, just recognise that some of them are unique*

- For example, Pompe disease is a glycogen storage disease, and a lysosomal storage disease. Because the glycogen degradative

TYPE	DEFECT	MAJOR AFFECTED TISSUES
Ia (Von Gierke)	Glucose-6-phosphatase	Liver, kidney, intestine
Ib	Microsomal glucose-6-phosphate transport	Liver, kidney, intestine, neutrophils
II (Pompe)	Lysosomal acid β-glucosidase	Muscle, heart
IIIa (Cori)	Glycogen debranching enzyme	Liver, muscle
IIIb	Glycogen debranching enzyme	Liver
IV (Anderson)	Branching enzyme	Liver, muscle
V (McArdle)	Muscle phosphorylase	Muscle
VI (Hers)	Liver phosphorylase	Liver
VII (Tarui)	Muscle phosphofructokinase	Muscle

enzyme for this particular disease is present in the lysosomes.

- What's special about Pompe disease too that it's the only disease that can present in cardiomyopathy.

❖ ***Amino Acids Inborn Errors of Metabolism:***

- Phenylalanine (PHE):

    - Phenylalanine is present everywhere in your diet. And it's an essential amino acid, meaning that you cannot synthesize it.

    - We produce from PHE: Tyrosine and Melanin.
      So if you have a problem metabolising PHE then you're not producing Tyrosine and Melanin and you will look particularly pale in color.

    - Phenylketonuria (PKU) is an autosomal recessive disorder that results in an impaired metabolism of PHE due to a deficiency in an enzyme called phenylalanine hydroxylase. And this enzyme usually converts PHE to Tyrosine. And because it's not there then we will basically have increased levels of PHE.

    - The problem that follows this high level of PHE is that it impairs brain development.

    - So these patients should be on a restricted PHE diet. Meaning that you can't take PHE out completely from the diet because it's an essential amino acid.

    - There's a fine line between enough PHE to age and development, and much PHE to cause intellectual disabilities.

    - Now let's say you grew up, do you still have to continue on your diet? No, not necessarily. Unless you are a female and you got pregnant, because there is a whole human being developing inside you, so if your PHE levels are high, then this child is going to be born with severe developmental abnormalities because of accumulated PHE.

- Now PHE restricted diet is not the only treatment. You can supplement them with tetrahydrobiopterin (BH4), and that is important as a cofactor to convert PHE into Tyrosine.
  So if the patient still have some residual enzymatic activity of phenylalanine hydroxylase then you can give that patient BH4.

- However, some patients do not respond to BH4 supplement. And these patients stay on the restricted diet.

- Also in treatment, some patients have received a bone marrow transplantation, and some also have received recombinant enzymes to aid in PHE metabolism.

❖ ***Lipid Inborn Errors of Metabolism:***

- Medium chain fatty acids:

  - A deficiency in Medium Chain Acyl CoA Dehydrogenase (MCAD) make you unable to utilize the medium chain fatty acids.

  - When do you start using fatty acid oxidation in your metabolism? During fasting. So in our societies Ramadan might very well be a time when these patients present. But these patients probably don't present as children, which may be because children don't fast, and also because they get fed all the time by their parents.

  - However, children do not eat when they are ill. Because whenever they have a disease that makes them lose their appetite, they start becoming very fussy, their glucose levels start dropping, and their glycogen stores are gone. So then they start using medium chain fatty acids, but they cannot utilize it.

  - You should be aware that the symptoms a young child would have are very similar to the symptoms of Reye's Syndrome.

  - What is Reye's Syndrome?
    When small children get a viral infection you can't treat them with Aspirin, because Aspirin is contraindicated in children with viral infections because it can cause an idiopathic reaction and cause damage to the liver.

- So the only difference between Reye's Syndrome patients and MCAD deficiency is that the former had Aspirin, while the latter didn't.

- MCAD patients usually get treated by avoiding fasting, adequate calorie intake, and supportive care when needed.

- Long chain fatty acid:

  - Long chain fatty acid can also be a problem if you have an LCHAD deficiency

  - These patients are characterized by several clinical manifestations like:
    - Severe liver diseases
    - Cardiomyopathy
    - Skeletalmyopathy
    - Retinal disease
    - Peripheral myopathy. Also sudden death.

  - Treatment is also avoiding fasting, low fat diet, and supplementation with medium chain fatty acid because they are easier to metabolise than the long chain fatty acid. Carnitine is also given as a treatment.

  - Unfortunately, if you have a child that with LCHAD deficiency. During pregnancy this can result in an acute fatty liver in pregnancy and the HELLP Syndrome (H- hemolysis, EL- elevated liver enzymes, and LP- low platelets counts).

  - This HELLP Syndrome results from toxic metabolites from the fetus and affecting the mother, where these toxic metabolites pass through the placenta to the mother, and the first stop is the liver.

  - Note: In PKU; the mother affected the fetus. Here it's the other way around.

  - In countries where the disease is common we do diagnosis to see if we can help the mother and the fetus in antenatal clinic.

- Cholesterol:

    - Here we're not talking about hypercholesterolemia, but we're talking about DHCR7 deficiency, which is one of the enzymes in the cholesterol pathway.

    - These patients present with congenital abnormalities in their brain, heart, genitalia, and hands.

    - So patients with deficiency in that enzyme have a syndrome called Smith-Lemli-Opitz Syndrome.

    - Cholesterol supplementation is being currently tried out to see if these patients will respond to it.

    - Now what's cholesterol is made into in the body?
      Bile, plasma membranes, steroidal hormones; estrogen, testosterone, aldosterone (if you don't have aldosterone you're going to lose sodium and retain potassium, so you're going to be hyponatremic and hyperkalemic).

- Steroid hormones:

    - Let's start with congenital adrenal hyperplasia (congenital- means at birth, adrenal- the gland, and hyperplasia- larger so produces more hormones).

    - There's going to be excess androgens (ex. Testosterone), and there's going to be cortisol deficiency because you're producing too many androgens and all the cells have converted to that. And there's also going to be a variable aldosterone deficiency.

    - We said this is congenital (at birth), so who do you think is going to present at birth, male or females?
      Females.

Female Fetuses:

- Females when they're exposed in utero to androgens they will start developing external male genitalia. It's not going to be perfectly normal. So those patients will present with ambiguous genitalia. Internally they are females. And externally they are somewhat males.

- Note: this disease is one of the most common of ambiguous genitalia in females.

Male Fetuses:

- Whereas in males even when with more androgens, they are still males!

- So when are males going to present?

  - Either they are going to present at puberty, because they will have puberty much sooner than normal people (precocious puberty). And the reason for that is that they have been exposed to too much androgens (testosterone). So they will start having early secondary sexual characteristics; hair in their armpits, chest, genital region.

  - Or they will present with hypoaldosteronism (much before puberty). So depending on how much aldosterone is deficient they might present well before puberty.

Treatment:

- You're going to give them cortisol and aldosterone, because they don't have any. And you're going to try to stop the excess androgens, especially in females.

- And for females who are born with ambiguous genitalia you're going to perform surgery.

- Steroid hormone receptor mutations:

  - It's either CAIS (complete androgen insensitivity syndrome) or PAIS (partial androgen insensitivity syndrome)

- They are X- linked receive diseases.
- These females are genetically XY and they are not quite normal females.
- How is that possible? These patients are insensitive to androgen (they have mutations in the X-linked androgen receptor), so there's no male characteristics and they will grow up as females, develop breasts, and have female external genitalia.
- Remember we are talking about the Y chromosome, so we have on it the SRY (sex determining region) that is going to turn off female internal organs development, and turn on male internal development.
- So these patients will develop testes. But they will not develop a penis and a scrotum. So they will look on the outside as females. And on a closer examination you will find that they have a blind short vaginal vault and there are no female internal organs.
- These mutations typically prevent androgen from binding or impair binding of the androgen receptor to DNA. Remember that steroid receptors are intracellular receptors, and when the hormone binds the receptor goes to the nucleus and bind to the DNA to affect transcription.
- They are obviously infertile.

❖ ***Lysosomal storage disease:***

Lysosomes contain lots of enzymes, and they break down proteins, organelles, or anything that the cells endocytose from the outside and needs to be degraded.

- An abnormality in lysosomes would be **AR**, and will typically produce **hepatosplenomegaly** and **CNS problems**.
- Patients with lysosomal storage diseases present at an **early age**.

- **Mucopolysaccharidoses:**

  - Where are mucopolysaccharides present?
    Extracellularly. So, they are associated with the extracellular matrix, and any ECM degradation product is going to end up in the lysosomes.

  - In this conditions there is a lack in the enzymes that degrades mucopolysaccharides, and so that toxic substrate will accumulate in the lysosome, then the lysosomes are going to enlarge and get bigger, resulting is many symptoms, mainly, CNS problems and hepatosplenomegaly.

  - This kind of disease has seven types that all shares some common features:

    - Hepatosplenomegaly
    - Skeletal deformities
    - Heart valves lesions
    - Subendothelial arterial deposits
    - Brain lesions

  - Type I is called **Hurler syndrome**, and those patients have very characteristic facial features; a gap between the first two teeth, depressed nasal bridge, intellectual disabilities, and they often die due to cardiac problems, which explains their very short life span, as they live to an average of 6-10 years.
    There is no treatment for this condition, yet.
  - Type II is called **Hunter's syndrome**, and it is very similar to Hurler syndrome, but much milder, as they present no corneal opacities. And also, rather than being AR it is X-linked recessive.

Good luck