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Genetics and Latest Findings

Introduction: My name is Jane Houghton. I'm a principal clinical scientist and the lead clinical scientist for routine genetic testing based in Exeter.

Exeter is an international referral centre, and we provide patients from across the world with routine diagnostic testing. Most importantly we also provide them with access to world-leading research.

This presentation is a joint presentation with my colleague Professor Sarah Flanagan who leads the research into genetics of Hyperinsulinism.

The Exeter Hyperinsulinism Team is a multi-disciplinary team made up of many individuals, who are trying to find the diagnosis for patients.

What do we do in Exeter?

Provide fast, accurate, and comprehensive genetic testing and diagnosis for every patient with Congenital Hyperinsulinism. It's important because a genetic diagnosis cannot only guide treatment, but it also helps define the risk of Hyperinsulinism for siblings and for future offspring.

Routine genetic testing - we start by testing for about 26 different genetic causes of Hyperinsulinism. Now for around eight different causes these genes are important in the beta cell, and they cause isolated Hyperinsulinism and that's because these genes specifically affect the insulin-secreting beta cell which is present in the pancreas.

Hyperinsulinism can also be part of a syndrome and that's when you've got a gene that not only impacts the insulin-secreting cell in the pancreas, but when it goes wrong, it also goes wrong in other tissues and that's when you have the extra presenting features of a syndrome. Now there's over 20 different syndromes not all of these that we test in Exeter because sometimes Hyperinsulinism isn't the main presenting feature, so they may go on for genetic testing done elsewhere.

I wanted to give you a quick example by providing you with three examples of why we do genetic testing in Exeter. This is three little children Harry, Amber, and William these babies presented with Hyperinsulinism. Now one will have diffuse disease that is very unlikely to respond to treatment. One will be unresponsive to treatment but will have a focal lesion, which if resected could be curative. One will have a form of Hyperinsulinism that is likely to respond to treatment of diazoxide. But at presentation, we don't know which one is which, so what we do is to provide a genetic diagnosis that can help guide that treatment.

Exeter Laboratory: To take you through a little bit of what happens in the laboratory when the sample is received. In hospital, you'll be asked to provide a blood sample from the baby and often we also ask that blood samples are also sent at the same time from the parents. This is very important because in genetics the parental samples often enable us to help determine the cause of the disease and the mechanism of the disease as well.

When the blood samples arrive, the DNA is extracted, and this is done by an automated extraction process. All samples when they come into the laboratory are given a unique barcode, and a unique laboratory number and this number and barcode follows the sample,

from the moment it arrives in the laboratory until the moment that report is released back to the clinician.

Now the initial strategy is to do what we call Rapid Sanger sequencing, so it's a type of sequencing to identify genetic change in the potassium channel genes. The reason why we prioritise this testing is because we know how it can impact treatment decisions for the patient, but at the same time we're also testing for all of the other known causes of monogenic Hyperinsulinism. So, these are the other 24 known genes and because it's 24 genes it does take a little bit longer but again it's done at the same time.

To give you an idea of how we prioritise and how we try to get this testing turned around very quickly, the NHS guidelines are that we would have an expected turnaround time of around 80 days for that testing of all of the known genes, but we're typically presenting the data and the report and the results back within 3 to 4 weeks.

So, genetics is complex and one of the difficulties that we have is interpreting the genetic changes that we find. Everybody has a genome that is 99.9% identical, but still, we all differ by at least 3 to 4 million variants so small changes in the DNA. So, what we've got to do is find that one change that explains what has happened that is causing the Hyperinsulinism.

Now we've got a good head start with the diagnostics we know the genes that we're specifically looking for, but in those genes that we're testing we'll still find changes that we might not have seen before and so we're trying to help find and gather all of the evidence, in order to determine whether or not we think it's actually causing the disease, or it's not and that's where the parental samples come in to play because if it's inherited sometimes from a parent who's unaffected for certain types of Hyperinsulinism we know that that's unlikely to be the cause.

Equally parental samples are very important especially with the potassium channels because paternally inherited variance in a potassium channel when you have another, a change in the DNA that happens within the pancreas, that can indicate whether or not your child is likely to have focal disease and whether a PET scan would be recommended.

I take you back to Harry, Amber, and William. As I said they all presented with Hyperinsulinism, but Harry was shown to have a Homozygous ABCC8 mutation, so he has diffuse disease and often it means that a subtotal pancreatectomy would be an option if he's not responding to treatment.

Amber has a ABCC8 variant she's Heterozygous for one which has been inherited from Dad. So again, we think that a focal lesion is possibly likely, so she'll go on for a PET scan and have keyhole lesionectomy.

Then we've got William who again was diagnosed at the age of one day, but we know that from the type of change that he's got, so he's got a change in the GLUD1 gene, that he's more likely to respond to diazoxide and he also may benefit from some manipulation to his diet.

The other important point of having a genetic diagnosis is because it helps to find the risk of Hyperinsulinism for the future siblings and future offspring. So, in the case of Harry, we know that he's got diffuse disease, we know that both parents have the change in the potassium

channel, so the risk to his siblings is around 25% so one in four other children will likely have the disease. In the case of Amber, the recurrence risk for focal disease is very low. Then for William, the change that we identified in William was not present in either of his parents so the risk for future siblings again is extremely low, but the risk for his future offspring is 50%.

I've just touched on a few of the different types of genetic changes that we see, but we know that there's a whole host of different genetic causes of Hyperinsulinism, all of which can benefit from guided treatment once you know that genetic cause.

So, we know the NHS has really embraced and harboured the power of genetic testing in recent years to improve prediction of disease, prevention, diagnosis, and also to target precision medicine. Genetic testing in the UK now is centrally funded, so we know that all patients diagnosed in the UK with Hyperinsulinism have equitable access to genetic testing.

That's not the case for all children in the world diagnosed with Hyperinsulinism so in 2018 a partnership was formed between the University of Exeter, Congenital Hyperinsulinism Charity and the NHS laboratory to ensure that every child wherever they live in the world regardless of geography, income, or economic status would all have access to genetic testing at the same high level as every child in the UK as well. To give you a little bit of background since 2018 we've received referrals from over 685 children through this pathway, so that's 510 family members as well, that's 59 different countries from across 5 different continents. We've been able to provide a genetic diagnosis in over 350 of those patients.

Since we started the service, we've tested over 4500 individuals with Hyperinsulinism, and screening the known causes we found a genetic cause in around 46%. But that means that there's over half of children that don't have a genetic diagnosis and one of the key missions in Exeter is that a no diagnosis is turned around to be a no diagnosis yet. Because all of these children we ensure have access to the research pipeline and research opportunities that are undertaken. This is where I'm going to hand over to my colleague Professor Flanagan who will talk you through the research which she leads in Exeter.

Research: So as Jayne has showed we have a large number of children who are referred to us in Exeter for genetic testing. We screen for the known genes but then we don't find a disease-causing variant in one of those genes, so those children fall into this box of genetically unsolved.

As Jayne said that's not where we stop that's where my research starts. We have really good evidence that a large number of these children actually do have a genetic form of Congenital Hyperinsulinism, and this comes from for example pedigrees, so if we look at families there's often multiple individuals within a family who've got Hyperinsulinism, siblings, parents which is highly suggestive of a genetic form of the disease. When we look at the severity of the Hyperinsulinism we also see that the children with genetically unsolved HI and those with a variant in a known gene, there's often very little difference in the severity of the disease, again suggesting that this could be a genetic condition.

For all children who get referred to Exeter, there's a possibility of them enrolling into research studies, so genetic research studies and individuals are asked to sign consent to enrol in the Genetic Beta Cell Research Bank. Once you're in the Genetic Beta Cell

Research Bank we can then perform genome sequencing which allows us to sequence the entire DNA region, so the entire genome of individuals and so far, we've done this on over 200 children with genetically unsolved HI.

Our approach is that we sequence a small number of children to start with, we then prioritise changes in the DNA within these children, and then we go back to our much larger cohort to see if we can find similar changes in the other children who also don't have a genetic diagnosis. We've had quite a lot of success using this approach.

So this is an unpublished study this is work done by Tom Laver and our team and he's recently shown that large deletions affecting Chromosome 20 can cause Congenital Hyperinsulinism and these are all de novo changes. So these are changes which are present in the child and had occurred during foetal development, they're not present in either parent. What we can see is that these deletions take out quite a large number of genes on the Chromosone 20 itself and these children have Hyperinsulinism plus extra pancreatic features, so they have a syndromic form of Hyperinsulinism, and this is work that we're hoping to publish in the next few weeks.

We've had other successes, we've shown that PMM2 variants can cause polycystic kidney disease and Hyperinsulinism, and other groups have also found novel causes of Hyperinsulinism. But what we can see is that most of these cause syndromic HI and actually they are only present in very few numbers of patients, so you'll see that with the Chromosome 20 we've actually only found these variants in five individuals so far within our cohorts. But when we look at our genetically unsolved cohorts, 1600 patients, we can see that actually the largest amount of, or the greatest percentage of individuals actually have non-syndromic Hyperinsulinism, and this is the group where we've been making less progress up until now in terms of the genetics of the condition.

So we've been really trying to focus on understanding what causes non-syndromic Congenital Hyperinsulinism and this is one patient who I'd like to introduce you to. This is a little girl who was diagnosed with Hyperinsulinism at birth, and she didn't respond to diazoxide and had a near total pancreatectomy at the age of 23 months. At the time of diagnosis, she was sent to us in Exeter, DNA was sent to us in Exeter for genetic testing and no disease-causing variants were identified in the known genes.

It was at one of these conferences a few years ago that the family approached me and said that their daughter was sort of now entering teenage years and still had ongoing Hyperinsulinism and could they enrol in research studies. So, we got consent from the family to enter the research, we took further blood samples, and we performed genome sequencing on the child and both parents. We prioritised variants which we knew were only present in the child but not present in the unaffected parents, so these are the de novo changes. This is work done by Matthew Wakeling in Exeter and what we found when we sequenced the genome, we found this deletion affecting a gene called HK1.

This was really interesting to us because HK1 is a gene that has been considered a good candidate gene for Hyperinsulinism for over 10 years, but nobody's ever been able to pinpoint exactly how changes in this gene could cause disease. So, we went back to other children in our cohort who we had genome sequencing data on and we found an additional 16 families with de novo variant in the HK1 gene and these were scattered within this sort of

42 base pair region within the gene, so a very small region of the gene. So identifying variance in 17 individuals really confirmed this as a novel cause of Congenital Hyperinsulinism.

What is Hexokinase 1? So it's a glycolytic enzyme which is involved in glucose metabolism, and it's present in every cell of the body with the exception of the liver and the pancreas, and within those other tissues, it's involved in sort of metabolizing glucose and sort of providing energy for the cell. But within the pancreatic beta cell, Hexokinase 1 is replaced by an enzyme which you may be familiar with, the name Glucokinase. So Glucokinase is present in the beta cell but Hexokinase 1 is turned off. This is a difficult graph to understand, but essentially it just shows you why Glucokinase is present in the beta cell and why we don't have Hexokinase 1. It's because Hexokinase 1 has a very high affinity for glucose. So, what will happen if you have Hexokinase 1 present in your beta cell, you'll end up secreting insulin at much lower glucose blood glucose concentrations. So, if you have hypoglycaemia and Hexokinase 1 in your beta cell you will secrete insulin. Which is obviously not what we want to do, so Glucokinase comes in instead. So our hypothesis was that these variants we'd identified in these children were actually causing Hexokinase 1 to be switched on in the pancreatic beta cell.

So the baby had a pancreatectomy at the age of 23 months her pancreas had actually been stored at the local hospital, and the patient and her parents very kindly allowed us to ship the pancreatic tissue from the hospital to Exeter. We were able to look at this tissue down a microscope and what you can see here, this is the patient's tissue, and this is an individual without Hyperinsulinism. We stained for Hexokinase within the pancreas, which is a red dye and you can very clearly see Hexokinase 1 within the pancreas here of the little girl, but no Hexokinase 1 in the patient without Hyperinsulinism. We also stain for insulin and merge the two and what you can see is the Hexokinase 1 overlays the insulin which shows us that the Hexokinase 1 is present in the insulin-producing beta cells. So, this proved to us how these variants were causing disease. They were causing the gene to be switched on in the beta cell, resulting in glucose being metabolized at low glucose concentrations, and Hyperinsulinism.

To follow up on the studies Jaz Hopkins has been leading, trying to screen HK1 in our historic cohort. As you can imagine this is a huge job we have 1600 children without a genetic diagnosis, and we've also been working with the group in Paris and Germany. So far, we screened Hexokinase1 in over 1700 children with Hyperinsulinism and the current figures show variance in 97 of those individuals and 28 family members. Some of the work that we're doing in Exeter is really trying to work out how those sorts of variants are switching on and off the gene, but we have now been able to show that they are essentially acting on a switch within the beta cell, to cause the gene to be turned on and off.

When we look at those 97 patients with Hexokinase 1 HI we can see that most of the individuals have de novo variant, so a variant present in the child but not the unaffected parents, but we also have examples of where children have inherited Hexokinase 1 variance from affected parents, where there's multiple siblings with Hexokinase 1 Hyperinsulinism. Interestingly, we've also got a large number of families where there are individuals within that family who have a variant but don't have Hyperinsulinism, and this is something we refer to as variable penetrants, but this is well described in other forms of Hyperinsulinism as well.

The children have a whole range of birth weights, the majority are born appropriate for gestational age in terms of size, some have low birth weights, some have high birth weights, the average age at diagnosis of HI in these individuals is around 3 months, some are diagnosed at birth, but we've also had some individuals who are diagnosed for the first time at 8 years, 77% are treated with diazoxide and 9% have undergone a pancreatectomy.

So that's a Whistle Stop tour of some of the work that we are doing in Exeter. Showing you that we are making progress, and we are going to continue to try and make sure that everyone does get a genetic diagnosis. So far we've now shown that HK1 is the fourth most common cause of HI in the Exeter cohort, but we still have more children to screen, so it's possible that that could go up, it won't be ABCC8 but it might get a bit closer.

I think my message here is just that the patient who I showed you the picture of really was the start of this discovery, and it was because the parents came up and kept asking about the genetics, and really pushed for genetic studies and it really there is new genetic causes of HI being discovered all the time. So, if you are without a genetic diagnosis, please do keep asking about your genetics, because in the end we will, we will get a diagnosis for you.

To summarise, as I've shown you research studies are underway in Exeter, we've shown that HK1 is now a very common cause of HI, and that just leaves me to thank clinicians, funders and of course you guys, families thank you.

The Children's Hyperinsulinism Charity would like to express our gratitude to Dr Jayne Houghton and Professor Sarah Flanagan for providing their expertise and time resulting in such a valuable video and information, for the children, young adults and families we support. As well as a thank you to all the Exeter Team for their dedication and devotion to finding answers for Hyperinsulinism.