Hi, I'm Hannie Kremer from the Radboud University Medical Center in Nijmegen. And I'm part of Hearing and Genes, an expertise center for hereditary hearing loss and Usher syndrome. And today, I will talk about genetic testing of the Usher 2A gene in the series, Usher talks.

Why genetic testing? In general, genetic testing is performed to provide a clear diagnosis, and a genetic diagnosis is important for genetic counseling of the affected individuals but also for family members, and it is important for prognosis, as well as for eligibility for future therapies when they are developed.

The Usher 2A gene is a very large gene. It has 73 exons. I will explain later what exactly this is. And defects, or we call them also mutations, in the Usher 2A gene are responsible for 60% to 90% of the cases with Usher syndrome type 2. But also, for 5% to 10% of the cases with nonsyndromic retinitis pigmentosa.

The User 2A gene encodes at least two different Usher 2A proteins. And these proteins are also called ushering, but I will call them Usher 2A proteins. There are two different Usher 2A proteins, two different isoforms-- isoform a, which is the smallest isoform. And this protein only occurs outside of the cells, so it's exported out of the cell after it's made inside the cell. And it consists of recognizable protein domains that are depicted here as colored disks or colored cubes.

Isoform b is much longer, and it's where the small part depicted on the right side inside of the cell. Then it goes through the membrane. That's depicted with these large disks. And then we have, again, a large part that's outside of the cell with the same type of protein domains as in the isoform a, but then many more.

But before I tell you more about DNA diagnostics, I will show you something about the basics of how the code in the DNA is translated into a protein. So the DNA, or the DNA of genes, consist of exons and introns. Exons are the parts that contain the code for the protein, so for the order of the amino acids. The introns contain DNA sequences, part of which we do know a function, but it also regulates the splicing, as I will show immediately after this to make a good messenger RNA.

So how is a messenger RNA made that can be exactly translated into protein? So first DNA is copied into a pre-messenger RNA. And this pre-messenger RNA contains both exons and introns, and these introns have to splice out. And that has to be done exactly because otherwise, the code of the mRNA, the code for the protein will be disrupted.

So then this messenger RNA will be translated into a protein. And the protein consists of amino acids, the building blocks. And the codes in the messenger RNA determines exactly which amino acids are built in and the exact

order of them. And I depicted the different amino acids with differently colored circles. In current DNA diagnostics, what is mostly done is sequence of the exons, with the code of the protein, and the short part of the introns that contain the most important signals for the splicing. And those are the sequences that we can interpret in the introns.

So DNA diagnostics step one. So if we have a normal DNA sequence in the exons, then the normal protein is encoded. But if there is, for example, a change in an exon, like depicted here we see a red star, an early stop in protein synthesis can occur. So based on the change in the DNA, we mostly can predict what happens to the protein. So in this case, there is an early stop in protein synthesis and shorter or no protein is made.

Then there can be a code shift, so either by a splice defect, or a small part of DNA is missing, or some additional DNA is in the exon. And then what you see is that the protein can be partly normal and party abnormal. And the abnormal part I have depicted with larger circles with different colors. And the so-called 2299delG mutation is such a mutation. So the code for the normal protein is interrupted.

There are also changes in the DNA where larger parts are missing. And then either the code is disturbed or the reading frame, the code is not shifted, or it can be shifted. So when it's shifted, I explain this above. And when the code is not shifted it can be that a larger part of the protein is missing, and that the first part and the large part are normal. So the part that is missing, I have depicted here with the white bar.

But there is one side of mutation that occurs most often, and that is a change in the DNA that leads to the change of a code of only one amino acid. So I have this depicted here as the white larger circle in the scheme of the protein. So only one amino acid is changing protein. And such changes are common in the DNA, common in Usher syndrome cases, but also common in the general population. And because the Usher 2A gene is large, we can see in the general population, and also, of course, in the Usher syndrome cases, several of these changes. And when these are very rare it's very difficult to predict whether this could be a disease causing mutation or not.

And we have several things that we look at to help us to predict whether such a change would be deleterious for Usher 2A protein function or not. So the first thing we look at, of course, is the change rare? Because when it would be common in the population then it could not be the cause of Usher syndrome. First thing we look at after that is whether there is a big change in the chemical characteristics of this amino acid in the new one and the one that should be there. So that's one thing.

Furthermore, we look whether the amino acid is changed, whether this is conserved in evolution. This means that in other species, such as in mouse or in chicken, is the same amino acid at this place or is that position more variable and could another amino acid at this place function as well? There are also tools, bioinformatic tools, that help us to predict whether the change in the amino acid really changes the structure and thus, the function of the

protein.

An important point, also, is whether the change is previously found in the person with Usher syndrome. And very rare in the population, I already indicated that. And then there are guidelines by the American College of Medical Genetics and also expert evaluation teams that also are now busy to evaluate all changes that have been described in the Usher 2A gene. For example, to evaluate again whether all characteristics are there to call it deleterious.

But a very important tool to evaluate whether a change in amino acids also can be called deleterious is testing of family members. So here we have a pedigree with squares of males and circles of females, and a father and a mother, and four children. And the boy the white fields symbol, is, in this case, an Usher syndrome case.

So the DNA diagnostics was performed, and two of the amino acid changes were found in [? one ?] codes M1 and M2. So DNA samples of the families were collected via blood samples. And as you can see, the M1 is inherited from the father and M2 from the mother, so that would already fit with the two changes causing Usher syndrome, as both copies of the male patient are affected by a change.

So then we look in the sibs, the oldest sister on the left side has M1 and a normal copy of the Usher 2A gene-- the second sister, two normal copies. And the brother on the right has a changed M2 and also, a normal copy. So the segregation of these changes in the DNA would fit with being disease causing.

Now, I show you a second segregation model of the two variants, same pedigree, but now we see that the sister on the left side, the older sister, has the same two changes as the boy with Usher syndrome. But she has no Usher syndrome, so this indicates that the two changes cannot explain the Usher syndrome in this boy. So they can be excluded for being the cause for Usher syndrome. So this now explains why your family doctor asks for samples, your geneticist asks for blood samples of family members to confirm the changes or exclude the found changes causative for the syndrome.

So then there is one important type of variants in the Usher 2A gene left. There still are cases where one, with usher syndrome type 2, where one change is found in the DNA only that could be causative for the disease, and the second change in the other copy of the gene is not found, or no changes are found at all although there is a clear Usher type 2. And also, in the Usher 2 genes, no change was found.

So then there still can be variants in the non-coding DNA that have an effect on the synthesis of the protein. But this is not so easy to confirm because this intron DNA, for example, or sequences outside of the gene, they are much more variable than the protein coding sequences. So tests are needed, then, to prove whether a change that is found in the family and rare, or not present, in the general population, really is deleterious for the function

of the Usher 2A gene.

So what could such a change in an intron do? So as we have already seen, the pre mRNA contains both the exons and the introns, and then splicing has to occur in a very exact way. And there are mutations known that lead to the presence of part of an intron-- we call this a pseudo-exon-- in the messenger RNA between the normal exons. One such mutation is found already several times that is the pseudo-exon 40, which was found in France, but also in several of the Dutch cases.

And the presence of such a pseudo-exon leads to a code shift and code changes. And as you can see here, depicted by partly of a normal protein and then, again, a protein that is much shorter but has several aberrant amino acids built in depicted by the larger circles. But to perform these test that are needed to prove the deleterious effect, we need cells of an affected individual cells with a sufficient activity of the Usher 2A gene. And the Usher 2A gene is mainly active in the hair cells of the inner ear, and in photoreceptor cells of the retina, and poorly in many other cells of the body that we could more easily have access to.

There is our nasal cells that express the gene, but we could also use skin cells and reprogram them and differentiate them into photoreceptor-like cells. But these are very time consuming and more difficult experiments, so they are not generally performed on a larger scale. And also sequencing of the non-coding DNA is, so far, not generally performed in genetic testing. But whole genome sequencing, which can be used to analyze these sequences, is more and more being performed.

So in summary, I have shown you that protein coding regions of the Usher 2A gene in flanking regions are analyzed first in DNA diagnostics. DNA changes that only exchange one amino acid are often difficult to interpret, and I have indicated how this is done in the diagnostic process. And I have shown you that the next step in DNA diagnostics is the analysis and interpretation of variance in non-coding DNA.

So this was my presentation. I hope it was clear to you and has shown you the way we form DNA diagnostics and the difficulties that come with DNA diagnostics of the Usher 2A gene.