

(Uwe Wolfrum) Thank you very much for the introduction. I'd like to welcome you here in Mainz, and we are hosting now the scientists first, we have hosted them for two days, and now you as patients come and join and can get the discussion going on on Usher syndrome with other goals.

I would like to summarize a little bit what we have heard about the cell and molecular biology of Usher syndrome. When we look at the Usher syndrome, we have 10 different genes, and I guess it was introduced to you now from Margaret, and we have 10 different Usher genes, and they are all expressed in these two cell types, hair cells and photoreceptor cells.

Therefore, when we have to keep the touch to the cellular mechanisms, we have to look at these cells, maybe to other cells, maybe also to intestine cells sitting in the gut, and maybe also to the skin. And we would like to understand how these molecules are functioning. And I think it's very important to stress that we have to decipher the molecular and cellular function of the disease, of the Usher disease, the disease molecules related to the Ush-

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er syndrome, to develop an effective therapy. This is all about what we do. The goal is the therapy for you, but we have to understand the molecules to get an effective therapy developed.

The first thing we and others show is that all these different Usher genes are interconnected in the cell and they are functioning together. Like we are functioning as a society, they are functioning together in the photoreceptor cells or in the hair cells. Let me go to the hair cell system. The hair cells are sitting in the inner ear, all of you know that. And here we have an image where the hair cells are sitting. These are the hair cells in the cochlea. And when we look from above on this cochlea, we see that there are some hair bundles of these cells. The name is coming from these hair bundles. And they are important, these hair bundles, we call them also stereocilia or stereovilli, because they have a cytoskeleton, ecto-cytoskeleton inside. Okay, okay. You may hear me now better? Okay.

But it's important to understand that these hairs are important for the stimuli of these cells. A stimulus is a bending of these hairs. And then there is a depolarization of these neurons occurring, and then hearing can occur. Still problems? Okay. We and others, and there was also a big contribution from the French research team around Christine Petit, to figure out where these molecules are sitting in the hair cells which are affected by the disease. And the localization of these Usher molecules is shown here in this cartoon. There are different molecules sitting at the base of these hairs during the development.

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The so-called anchor links, they form some fibers linking these little hairs, these stereocilia. And in addition, these are the molecules related to Usher type II sitting at the base and forming anchor links.

On the other hand you see up here that during the development also some other molecules which are related to Usher type I, they are present at some membrane links between the stereocilia and the tips of the stereocilia and forming also some connections between these stereocilia. And this is during development. And when we have defects in Usher molecules as a developmental effect leading to deafness from birth or during the early years of life. When we look now to the mature or the adult cell, then these molecules related to Usher syndrome have a different function. One function is to get an interconnection the so-called tip-link structure. And this is important for the signal transduction, because this tip-link is related to the mechanosensitive channel. And this channel has to be open to get a depolarized cell. And this tip-link is the important structure leading to the depolarization of the hair cells and hearing.

Now we have two different problems, when we have defective hair cells our molecules related to Usher syndrome. We have a problem during differentiation. We see that these hair bundles in wild type, in the normal mouse cochlea, for instance, are well structured and really nicely orientated. On the other hand this is a MYO7A Usher2B deficient hair bundle. And you see that this is not really organized. These contacts between the stereocilia are not

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functioning. Then we have a defect in usher molecules. But in addition also defects occur in the mature hair cell, because we have not this system of the tip-link formed.

We heard yesterday also that it's also important to have molecules related to the Usher syndrome at the synapses of the hair cells. Aziz was presenting data, very new data, that Clarin-1 is necessary for the contact to the neurons, which are projecting towards the brain for hearing. And also this we have to take into consideration.

Now it was already mentioned today that these molecules related to Usher syndrome are not only present in our ear and the eye, but they are expressed all over the body. And you see for instance some molecules also present in the gut. And the two guys here, Matt and Mingjie, they are studying these molecules and the proteins forming network complexes in these different organs, predominantly here the brush border and microvilli of these cells in the gut.

And they see that there are parallels between the stereocilia tip-link complexes which I introduced to you, at the tip of the stereocilia, and the molecules sitting in the intestine cells. And if they are defective, then we have also problems when this complex is not effectively working.

We have the problem which we can see here, we have not any longer the number of the extensions of these intestinal cells present, these microvilli, and we have these mice are suffering. There is a defect in this intra-microvillar

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adhesion complex in the intestine. They are smaller, and there is a decrease in growth, probably due to the nutritional deficiency of these mice. And we may observe this also in patients of Usher syndrome that there is a problem with the nutritional deficiency.

Okay, now let me go to the eye. This is also a very very important part of my presentation here, that we have to see where these molecules are found and what problems they may cause in the eye. And we see here the retina and the photoreceptor cells, cones and rods are sitting here in the retina, and they are connected as the hair cells to other neurons. And we found out that the photoreceptor cells are containing the Usher molecules. These symbols are indicating where in the photoreceptor cell we find the Usher syndrome molecules. We have here the outer segment, where the visual transduction cascade is localized, and we have here a little link a so-called connecting cilium, and you see here that they are associated at this base of the outer segment, the molecules related to the Usher syndrome.

And we have the inner segment, and there the inner segment of the photoreceptor cells, it harbors the molecules for biosynthesis of molecules necessary for the signal transduction, for instance, the visual pigment. And we have a synapse, a contact to the other neurons. You see that there is a distribution all over the cell more or less, of the Usher molecules, but we have here a localization present at the base of the cilium.

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And this we have studied, and Jun Yang was presenting new data on this. And there we see, for instance, the Usher II molecules are sitting right at this place here at the connecting cilium, and they are forming some connections like the hair cells between membranes, right here in this little pocket. And these are molecules related to the Usher type II. Based on other findings we think that this is the function of this complex is related to the transport of the molecules.

For instance, we have to transport, as I mentioned, opsin from the place where it is synthesized in the inner segment to the outer segment, and this is the pathway here shown through the inner segment first and then through this little tiny bottleneck, the kinocilium, and to the outer segment. And there the molecules have to function, and we can detect some light with the visual signal transduction cascade. And if this transport is defective, we may have also a defective visual system. And right here at the linkage between the inner segment and the outer segment, we find the Usher molecules related to Usher type II.

But we learned also that we are not mice, when we started the Usher I molecules, because we didn't see any degeneration in these mice, or only mild degenerations which were not representing the disease. And therefore we also ask - and when I say we, the scientific community ask - what is the difference between the mouse and the human. And when we look at the photoreceptor cells, we see prominent differences. They are a little bit larger, our cells, but we have the so-called calyceal processes.

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And right there in these calyceal processes, these are extensions and projections of the inner segment. Right in these calyceal processes we find the Usher I molecules, but also some Usher II molecules were observed. And right here we have big differences between the two cell types. This inspired Aziz, for instance, Aziz El Amraoui, to test the hypothesis that these calyceal processes are important for the photoreceptor function.

Therefore he tested this in an animal model. And the animal model was the frog, *Xenopus*. You see here some data, this is a photoreceptor cell, and these are the tiny extensions. There is Protocadherin-15, Usher 1F sitting in there indicated. And when you look at the deficient frog photoreceptor cells, these are absent. Without Protocadherin-15 we don't have calyceal processes. And if we look also a little bit more closer at these photoreceptor cells, they are bent. The outer segment is bent. These calyceal processes may support mechanically the outer segment. And right here you see that this, there is another phenotype. The outer segment discs are over growing and are really not in the correct shape like in the normal condition. And these calyceal processes, they have also an actin filament sitting in there.

Again a parallel, which is the molecular parallel to the hair cell stereocilia. We also are looking for other models to see whether we can do some research on these issues. And we thought on a larger animal model. And the pig. And you see here a pig photoreceptor cell, and you see also these calyceal processes. And right now we are in the

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progress of generating an Usher pig. This is a consortium from Munich together with Mainz, and we are teaming up to get an USH1C pig model, which will be good.

We decided and have generated it, and now we are trying to get the animals around to go for gene therapy, read-through therapies, we will hear about therapeutic options later today. In the next slide I like to introduce again another strategy, which is in our lab that we look for interacting partners. We have Usher network working, I introduced this in the hair cells, but we found out that other molecules may also interact.

And when we identify these, when we have identified your friend - tell me who your friends are, and I will tell you who you are - and then we can really see what kind of function these molecules have in the cell. This is an important thing. And we go for proteomics. We found out that there are several other relations between these molecules related to the Usher syndrome. We have endocytosis or gene regulation, also related to this and with this slide. And we will have to do a lot to figure out how these molecules are participating in these cellular functions. And with this slide I would like to thank you for your attention, and you are very welcome to address questions to me also during the entire meeting. And I would like to dedicate this presentation to two families, the Suchert family and Steffen Suchert, and the Thomas Welp family, and with this I'd like to thank you. (applause)