Scientific Symposium | Day 2 | July 20, 2018

Good morning! @ClutchScience is at the helm once again to tweet out the scientific content at the International Symposium on Usher Syndrome. Day 2 of #USH2018 will be underway shortly.

This scientific session is part II of the cell and molecular biology topics. The first presenter is Aziz El-Amraoui from the Pasteur Institute to give us a review of USH proteins in hair cell structure and function

AE-A: cellular structure and function of the inner ear. Hair cells reside within the cochlea, are responsible for receiving sound frequency detection & amplification. The hair bundle protruding from each hair cell is made of multiple stereocilia, linked together by USH proteins.

AE-A: structural features of the USH proteins help us deduce their function. Scaffold proteins organize other proteins to build the physical links that connect adjacent stereocilia. Deafness & balance defects result when these links are disrupted.

AE-A: The finding that patients with mutations in any of the USH1 genes have basically the same degree of hearing & balance problems provides further evidence that these proteins work together in complexes

AE-A: studying the localizations of these proteins in mouse hair cells show us that the complex acts during fetal development, when the hair cells are developing and organizing their stereocilia.

AE-A: in addition to being important members of the tip link complex, some USH1 proteins are also required for transporting other USH1 proteins to the correct location in stereocilia, before the full complex is formed

AE-A: USH1 proteins are necessary for hair bundle development, but also required for function in mature hair cells. Tip links (made of USH1 proteins) must be maintained throughout the life of the hair cell
AE-A: Tip links provide tension between the membranes of adjacent stereocilia. Sound waves cause the tethered structures to bend, & the tension causes small gaps in the membrane to open, allowing ions to move into the cell & send a signal that sound was perceived.

AE-A: USH2 proteins have similar roles in hair cells. They form a complex later and for a more limited period during development. They tethers stereocilia together at the base--termed 'ankle links'. They reinforce stereocilia structure as they develop & refine their organization.

AE-A: What is the role of USH3 (Clarin1) in hair cells? Clrn1 also has a role in hair cell development in mice. but also act later in the hearing process, helping the cell to communicate the perceived signal to adjacent neurons.

AE-A: test of gene replacement therapy for Clrn1 w/ a viral (AAV) delivery system in the mouse mutant. An improvement in hearing was detected, but developmental disruption of stereocilia was not rescued. Helps us understand the difference between developmental & functional roles.

The next speaker is Marisa Zallocchi of Boystown Nat'l Research Hospital in Omaha. She'll tell us about the role of PCDH15 (USH1F) in hair cell maturation.

MZ: Finding interacting partners that work with known USH proteins is an important part of understanding what goes wrong in USH. Using zebrafish model to investigate relationship between Pcdh15 & a protein called Itga8. Both localize to neuromasts (analogous cells to hair cells.

MZ: Biochemistry experiments showing that Pcdh15 and Itga8 interact and form a complex. Disrupting the complex in fish results in defective development of the kinocilium, a structure required for organizing the stereocilia.

MZ: new data from zebrafish motivated investigation in mouse mutants. Generated a new Itga8 knockout mouse for this study. Itga8 is localized to the stereocilia and kinocilia of mouse hair cells. When it is absent due to mutation, Pcdh15 localization is affected.

MZ: the reciprocal is also true--Itga8 is absent when Pcdh15 is mutated. Mouse and zebrafish data are consistent. The hair bundles are defective in
the direction of their growth, or polarity, a process influenced by the kinocilia.

MZ: Kinocilia position is normal in ltga8 mutants, but kinocilium maturation is abnormal in both ltga8 and Pcdh15 mouse mutants. The exact molecular reason for the polarity defect is still being investigated.

MZ: when both proteins are depleted in cell culture, defects in actin (a protein of many functions, including transport) are noted. Hypothesis is that the Pcdh15-ltga8 complex helps transport other proteins that set up cell polarity.

Next speaker: Fred Schwaller from the Max Delbruck Center in Berlin is talking about Ush2a as a vibration sensor involved in touch & hearing

FS: how do we feel? Our skin is full of touch receptor neurons that relay the sensation of touch to our nervous system. This mechanism is not well understood. Looking at the hair cells can help reveal how the touch receptors in the skin work.

FS: ush2a depletion in mice causes structural deficits in the hair cells. Q: are there common sensory relay systems between the hair cells and the touch sensory cells in the skin? Finger vibration test given to USH2A patients

FS: Patients with truncating mutations in USH2A have reduced ability to detect vibrations on their skin. Next Q: is Ush2a a tether protein involved in touch perception in the skin?

FS: Ush2a protein is found in touch receptor fibers in the skin that respond to vibration. It does not appear to be expressed in the sensory neurons themselves, but in adjacent supporting cells.

FS: recording response to touch stimulus (vibration) from touch sensors in the skin in normal and Ush2a mutant mice. Response to vibration is impaired in the Ush2a mouse.
Next speaker is Mingjie Zhang, Hong Kong University of Science and Technology, to talk about structures of the USH1 complexes and their homologs in other tissues.

MiZh: stereocilia in hair cells and microvilli in hair cells share structural and molecular properties.

MiZh: How do proteins choose their partners from an array of similar types of proteins? Similar multi-protein structures can form from different components in different body tissues.

MiZh: as we heard yesterday, the USH1C protein harmonin is the common denominator in the protein complexes that provide structural organization to the stereocilia and to the microvilli. The other partners in intestinal cells are not USH proteins, but have similar shapes & domains.

MiZh: Investigating the alternate role of harmonin in microvilli shows some novel and interesting protein binding behavior. The new (to us) binding relationship is necessary to form the right kind of links between microvilli.

MiZh: Mutations that cause USH1C in eyes & ears also inhibit the binding behavior of harmonin in microvilli.

MiZh: now turning attention to a dense collection of proteins located at the interface (synapse) between neurons.

MiZh: this is called the postsynaptic density. Proteins in this region complex with each other to form very dynamic, concentrated packets. Usher 1 proteins (and microvilli-specific homologs) can form in this way in cell culture.

MiZh: hypothesis is that USH proteins that make up the tip link complex use a similar 'condensing' technique to achieve the right shape and spatial orientation to perform their function there.

MiZh: conclusion: looking at how proteins structurally similar to USH proteins behave in other tissues can give us valuable insight into what USH proteins are doing. Cool stuff!

Coffee Break! Back in 30 minutes or so.
Ok, we're back to finish up the Cell & MolBio session with Daniele Dell'Orco from the University of Verona, talking about function of the USH1J gene, CIB2

DD'O: involvement of CIB2 in USH1J involves a particular mutation that seems to be quite mild on paper. CIB2 is expressed in many human tissues, so function of the protein could be of broader interest to more than just the USH community.

DD'O: To date the protein structure has not been resolved, but looking at homologous proteins can give some clues. The protein has functional domains that are capable of binding positively charged ions, but additional interaction domains as well

DD'O: we want to understand how an apparently mild mutation in CIB2 can cause severe dysfunction in eyes & ears. Used many different approaches to analyze the effects on protein structure and function.

DD'O: CIB2 forms homodimers, meaning to CIB2 proteins can bind each other to perform their cellular function. The 'mild' mutation, p.E64D, affects formation of homodimers and also impacts protein folding (folding gives proteins a characteristic shape that affects function

DD'O: CIB2 is known as a positive ion binding protein (calcium and magnesium) when these ions are bound to the protein, the amino acid E64 is involved due to the folded protein shape. Ion binding is affected when this residue is mutated to D

DD'O: Interestingly, under physiological conditions, CIB2 doesn't bind Calcium. Interesting because the 'C' in CIB2 stands for 'calcium'. Shows that homologous proteins can have important differences!

Next session beginning: Usher models and Therapy. First talk is by Alberto Auricchio from Naples, to talk about dual AAV vectors for gene therapy of USH1B retinitis pigmentosa

AA: developing ways to target photoreceptors and RPE cells to treat a number of different retinal diseases. Adeno-Associated Viral vectors have a
lot of advantages for delivering genetic cargo to human cells. Main disadvantage is limited cargo capacity

AA: Viral vectors are best delivered by subretinal injection, inserting a needle behind the retinal cell layers to give virus particles access to the RPE and photoreceptors.

AA: First in-human trial using viral vectors was in 2008 to treat Leber Congenital Amaurosis. This is the treatment that was approved by the FDA late last year.

AA: Now a long list of clinical trials underway using AAV and other viral vectors to treat different forms of inherited retinal disease. Challenge remains the limitations on cargo size. This is an esp. important limitation for the large USH genes

AA: strategy is to use dual AAV hybrid vectors, breaking the gene into two parts putting them into separate virus particles and designing the paired viral vectors to promote assembly of the full length gene once they are both introduced into the cell.

AA: The amount of final gene product delivered to the cell is reduced in this strategy, but the in-cell assembly scheme is successful.

AA: Dual AAV vector delivery for the ABCA4 gene (Stargardt's) has been shown to be successful in mouse and pig models. Same strategy is employed for USH1B gene MYO7A.

AA: delivery to mice & pigs was successful, retinal defects in mouse model of USH1B were improved after dual AAV treatment.

AA: Clinical trial with this USH1B gene therapy was recently approved and is just getting started at several EU institutions.

AA: some USH proteins, like Usherin (USH2A) are too large to fit into the dual AAV system. The system is being expanded to triple and quad vectors to accommodate larger proteins. Triple AAV appear to be more efficient in RPE than in photoreceptors
AA: better success of triple AAV in pig retinas. A triple AAV vector containing CDH23 in three parts has been created, but the mouse model doesn't have a retinal phenotype so rescue can't be tested.

AA: levels of protein can be tested in the mouse, even if rescue isn't measurable. Full length protein is restored, but levels are very low, probably too low to have therapeutic value. This tells us that more optimization is needed to deliver large gene cargo.

Next speaker is Jeffrey Holt from Boston Children's Hospital presenting "next generation gene therapy restores hearing, balance and quality of life in mouse models of genetic inner ear disorders"

JH: not speaking about an USH protein, but a protein called TMC1 associated with stereocilia that forms the membrane channels that are opened by tension on the (USH protein based) tip links.

JH: a number of recessive and dominant human mutations cause hearing loss in humans. Viral vectors targeted to the hair cells have been developed to treat genetic hearing disorders.

JH: AAV vectors also target the vestibular cells. Tmc1 delivered by AAV promotes hair cell survival in the mouse mutant of Tmc1 when delivered early in life.

JH: Sensory transduction--successful ion influx through the membrane channels-- and sound perception are restored in animals treated with this Tmc1 AAV.

JH: Tmc1 mutant mice don't breed very well and sometimes fail to thrive. Treatment with the AAV vector improves breeding success and growth rate of the offspring.

JH: all data so far was on a recessive mutation. What about dominant ones? Dominant mutations usually involve a mutated protein acting inappropriately, so strategies to treat include overexpressing a normal copy or inhibiting the mutated protein. Neither worked in this case.

JH: third strategy was used using CRISPR/Cas9 to target the dominant mutation at the source--changing the DNA code. System optimized to cut
only the mutant DNA, not the wild type copy. This approach preserved hearing in the mutant mice.

JH: hair cell survival was also improved in animals with dominant mutation of Tmc1 after this CRISPR/Cas9 treatment.

**Up next is Alaa Koleilat from the @MayoClinic**, presenting the development of the first pharmacotherapy for the treatment of USH1B.

AK: using zebrafish model of USH1B because zebrafish are awesome, basically.

AK: zebrafish model of USH1B have profound deafness and balance problems. Affected fish swim in circles and don't respond to sound stimulus.

In zebrafish myo7a mutants, the hair cell synapses appear disorganized. The number of vesicles—packets of neurotransmitters that are released when the cell is activated by sound—are decreased in these animal models.

AK: Behavior analysis of mutants can now be used to test efficacy in a drug screen. FDA approved drugs were chosen that affect cellular behavior that results in neurotransmitter release.

AK: three different compounds created measurable improvements of swimming behavior. One of the three resulted in an improved response to sound stimulus.

AK: improvement was also seen in the number of vesicles positioned near the synapse with one of the drugs. Project is now moving to mouse model of USH1B.

The next speaker is Kerstin Nagel-Wolfrum from Johannes Gutenberg University in Mainz, presenting her work on translational read-through therapy for USH caused by nonsense mutations.

KN-W: One complication in treating USH is that many of the USH genes encode multiple isoforms—proteins that mix and match the genetic code to create different functional molecules. Problematic because we don't which isoform(s) we need to deliver by gene therapy.
KN-W: this motivated choice to look at read-through drugs that can override certain mutations that cause the protein building process to arrest before it’s complete. Certain drugs can trick the protein building machinery into skipping over the premature stop signal.

KN-W: Translational readthrough drugs have been around for a while, some of the early ones have some toxic effects on the body, so search for new compounds that are well tolerated is ongoing.

KN-W: Using several pharmacological compounds from PTC therapeutics to test how well they read-through and restore the full length protein in USH1C mutant cells in culture.

KN-W: treatment with the drug Ataluren restores full length protein in the USH1C mutant cells. It also restores the ability for the USH1C protein, harmonin, to bind to other USH proteins and other functional proteins in these cells

KN-W: next looking at USH2A mutant introduced into cultured cells. Ataluren treatment can also induce read-through of this mutation. Next step is to move into patient derived cell cultures from USH2A patients with this mutation. USH2A protein levels were increased w/treatment

KN-W: cultured patient cells can grow cilia under the right conditions, giving us a model of the connecting cilium of the photoreceptor. USH2A mutant cells had reduced cilia inducement, but this was improved with Ataluren treatment.

KN-W: Moving from cell culture to animal models, a mouse with a nonsense mutation in the gene Nphp4, which causes retinal degeneration. Mice were treated with Ataluren eye drops, which delayed the retinal degeneration.

KN-W: future animal tests will move to the pig model, and various delivery modes will be tested (eye drops vs. ingested compound). Information about the time window for treatment will also be gained by these experiments.

The last talk of this session is by Muna Naash from the University of Houston, speaking about and USH2A knock-in mouse.
MN: to review, Usherin is localized at the connecting cilium of photoreceptors and complex with other USH proteins. It's thought to provide physical support and facilitate protein transport there. In hair cells it's in the ankle links of the stereocilia

MN: knock-in model of one of the most common USH2A mutations, the 2299delG allele. This mutation is present in 45% of all USH2A cases.

MN: an engineered protein with a green fluorescent protein label was introduced into mice in order to track the place and time of Usherin protein localization in various cells.

The knock in mice started to show reduced vision at about 1 year of age. Rod and cone functions were both diminished.

The knock-in also affected hearing in the mice—a louder sound intensity was required to get a response. There were also changes to the retinal cell organization, and to the morphology of the connecting cilium.

MN: Some proteins travel to one compartment of the photoreceptor to another when the environment changes from light to dark, or vice versa. The knock-in animals had a delay in this movement, indicating a problem with protein transport.

Breaking for lunch, back in a bit.

---

**Scientific Symposium | Day 2 | July 20, 2018:**

We're getting ready to start the final scientific session here at #USH2018. These talks will cover USH models & therapy

The first talk of the session is by Erwin van Wijk from the Radboud Medical Center in Nijmegen. His talk is on antisense oligonucleotides for the treatment of Usher syndrome caused by splice site mutations

EvW: nearly half a million people around world have vision loss due to mutations in USH2A. These mutations cause either Usher syndrome or non-syndromic RP.
EvW: USH2A associated RP is a slow progressive disease—'legal' blindness is often not reached until the sixth decade of life. That gives us a large window in which to introduce therapeutic interventions.

EvW: canonical route to therapies is to test gene replacement therapies in mouse. Mouse models of USH are often significantly different to humans, and USH2A is too large for our current gene replacement options.

EvW: zebrafish are a great model for studying USH2A, with a strong retinal phenotype that is detectable in the first week of life. Using CRISPR/Cas9, a frameshift mutation was introduced in zebrafish ush2a.

EvW: treatment options other than gene augmentation? Gene editing is not ready for clinical application. Treating the RNA transcript—the template for protein production—does not require fiddling with the source DNA, and gene size is not a factor.

EvW: USH2A Exon 13 harbors a number of different pathogenic mutations. Exon 13 is 'in frame', meaning that you could delete it completely and leave the coding region of the rest of the gene intact.

EvW: in-frame deletion of Exon 13 would cause some protein domains to be lost—would the remaining protein be functional? Computer models of a protein lacking the information from Exon 13 showed that the loss of information would not dramatically affect protein shape or function.

EvW: skipping the exon with Antisense oligonucleotides, which bind to the splice junction on the RNA template during processing and prevent the inclusion of the proceeding exon.

EvW: lots of computer analysis was involved in designing optimized antisense oligos for splice blocking. Oligos were injected into fertilized zebrafish eggs, which were then grown to 5 days old—a point where visual function is active and testable.

EvW: Exon 13 was effectively skipped in injected zebrafish larvae. Usherin production was partially rescued in the zebrafish model with a mutation in ush2a Exon 13, and retinal function measured by ERG was improved.
EvW: This could be an effective therapy for people with USH2A caused by mutations in Exon 13. Now we need to test to see if it will work in people as well as fish.

The molecule optimized for human treatment is called QR-421a. A partial conversion to transcripts lacking USH2A Exon 13 was observed in cell culture tests.

EvW: treated mice with QR-421a & showed that the molecule goes to the photoreceptors--targeting the right tissue is important. Final pre-clinical steps are to look at function, best delivery route and test for toxicity. Phase I/II trials could be later this year!!!

EvW: Shout out to @usher_syndroom and other supporters of this work

The next speaker in this session is Jennifer Lentz of the Louisiana State University Health Sciences Center. She’ll continue the discussion of antisense therapy rescue for USH

JL: focus on the USH1C mutation in the population of Acadians in Louisiana and Canada. This particular mutation, c.216G>A, causes abnormal splicing and disruption of the normal reading frame of the template used to build Harmonin protein

JL: the c.216G>A mutation was knocked-in to mice along with surrounding sequences, and generated a mouse Ush1c gene with the same altered, disease causing transcripts. Mice have hearing & balance disorders and a slow retinal degeneration

JL: Can an ASO block the incorrect splicing and induce restoration of the properly spliced transcript? Yes. Lots of screening went in to selecting the antisense oligo that would work best.

JL: antisense oligo delivery by injection into the gut or skin, to the inner ear, or to the vitreous of the eye

JL: systemic and local Antisense oligo treatment rescues balance behavior. Lots of information obtained about the timepoints of treatment that would work for each tissue.
JL: hearing rescue is dependent of age at treatment. Systemic antisense oligo worked the best in the ear overall. In retina, different dosages and number of doses were tried. Single intravitreal injections of antisense oligos improved visual function.

JL: harmonin protein levels are recovered in c. 216G>A fish after retinal treatment with the ush1c antisense oligonucleotide.

The next speaker is Erik de Vrieze from Radboud University Medical Center in Nijmegen, talking about finding new proteins that interact with the USH protein network.

EdV: using a histological marker for autophagy, an increase in the signal is noted in the ush2a mutant zebrafish, indicating inefficient waste disposal. This effect is lightdependent, & ush2a fish also show a defect in regulating cellular waste when shifting from dark to light

EdV: What is the reason for increased need for waste disposal in ush2a mutant fish retinas? The protein rhodopsin is mislocalized in the mutant photoreceptors, which may trigger the cellular waste response and reveal a downstream defect.

EdV: it's unclear whether autophagy is a primary dysfunction in ush2a or whether that observed defect is a response to mislocalized protein that creates excess cell waste. Either way, it gives us new insight into the reasons why photoreceptors die in USH2A eyes.

Next speaker is Yoshikazu Imanishi from Case Western Reserve University in Cleveland. He’s speaking about a treatment for hearing loss in USH3.

YI: Traditional "wide net" drug discovery is slow, challenging, and requires a lot of luck. "Reverse pharmacology" starts with identifying a target protein, then selecting a drug that can specifically interact with it. Also has a high failure rate & false positives.

YI: USH is a complicated disease--focus on type 3 gene Clrn1 to narrow down the targets. Understanding the common mutation N48K. The substitution of an incorrect amino acid leads to degradation of the protein.
YI: A cell line with the N48K mutation was established to test ways to inhibit the degradation that normally happens in cells with this mutation.

YI: Looking for drugs that can stabilize specific mutant proteins. Created labeled proteins that would report (with fluorescent signal) when degradation was occurring---or when it was suppressed by successful drug treatment in cell culture.

YI: Out of many molecules tested, one was discovered that stabilized the N48K Clrn1 protein, but it had a fairly high toxicity. Sought chemical modification to reduce toxic effects.

YI: Interestingly, the stabilizing effect of the modified compound is indirect. It does not bind to the N48K protein, but to some intermediate proteins. Treatment is effective in cell culture. Not only does the protein not degrade, it localizes normally to the cell membrane.

YI: Next: test the drug in the mouse model of USH3. Hearing performance was improved significantly in the N48K mouse model when treated with the drug. Treatment is most effective if given before onset of the progressive hearing loss.

YI: Unfortunately the N48K mice do not have a retinal phenotype. Looked for other proteins involved in retinal disease that might be helped by the drug. A particular mutation in Rhodopsin was a good candidate, and degradation of the mutant protein was inhibited by drug treatment.

Next talk is by Anai Gonzalez Cordero from University College London, entitled "using hiPSC-derived retinal organoids to model Ush2a pathophysiology" (translation--stem cells were developed into a full retina in a dish to study cell loss mechanism in USH2A)

AGC: New technology used to develop the retinal organoids. Also trying to develop hair cell organoids in this lab.

AGC: Induced pluripotent stem cells (iPSCs) skin cells are obtained from a patient and reprogrammed into stem cells, then further programmed to develop into particular body tissues, in this case retina.
AGC: many markers of mature, functional photoreceptors are present in the cultivated patient derived retinal organoids.

AGC: retinal cells derived from USH2A 2299delG patient cells still have properly localized USH2 protein (quite a different result from the animal models we've heard about this meeting).

AGC: the organoids from another USH2A mutation show some defects consistent with animal models

Next speaker is Scott Dorfman of Odylia Therapeutics, talking about moving preclinical findings of therapeutics into clinical trials for rare retinal disorders.

SD: ultra rare diseases have limited commercial traction--how do we get those demonstrated effective treatments out of the lab and into the clinic?

SD: combined phases of clinical trials to expedite transfer to clinic. Non profit model does not select treatments based on marketability.

SD: Memberships established with pharmaceutical companies, legal and regulatory services--at a discount.

SD: also trying to create master road map so that trials will be progressively easier and faster to set up as more experience with this model is gained.

SD: Financial sponsors have lower risk buy-in and right of first refusal if the trial doesn't pan out

SD: Hope is that this system will reduce the frustration of knowing that lots of productive USH research is going on that can't get out of the lab because it's too expensive to develop in the traditional way.

SD: Three USH genes are in the pipeline so far.

Last research talk of the Science Session is Mike Cheetham of UCL, talking about retinal organoids as disease models for other types of retinal dystrophies (not USH)
MC: CEP290 is another gene that causes a range of different ciliopathies that affect the retina and other ciliated cells of the body. A common mutation that causes Leber Congenital Amaurosis causes abnormal splicing and premature truncation of the protein.

MC: organoids made from CEP290 patient cells to study what goes wrong in cells with this particular mutation.

MC: interesting finding that the mutation incorrectly splices the gene only part of the time in regular cell culture, but makes the mistake in splicing much more often in photoreceptors. Other data also suggest that photoreceptors may be more vulnerable to this type of mutation.

MC: Organoids with this mutation respond to splice blocking (antisense oligonucleotide), presenting a treatment option that could be pursued for clinical trials.

MC: working with @ProQR to test compounds that will restore normal splicing in cells with this CEP290 LCA10 mutation.

Uwe Wolfrum takes the final turn at the microphone to close the scientific portion of the #USH2018 meeting. All attendees are grateful for the organization and wonderful scientific content of this meeting.

That’s all from me--the Patient’s Symposium begins tomorrow, so stay tuned for updates from that meeting. Thanks for following!