USH2018 Live Tweet Thread @UsherCoalition (Usher Syndrome Coalition)
International Symposium on Usher Syndrome
July 19-20, 2018, Mainz, Germany
Tweets from Dr. Jennifer Phillips (@ClutchScience)

Scientific Symposium | Day 1 | July 19, 2018:

Hello #USHFamily! @ClutchScience has the keys to the Coalition account and will be live tweeting the Science Sessions from #USH2018. The meeting will be underway momentarily.

Mark Dunning is kicking things off now--this is the third international Symposium on Usher syndrome and the 10th Usher Family conference! #USH2018

Mark is talking about ways that hearing & sighted people can understand what it's like to have USH-- uses the analogy of living in a country where you don't speak the native tongue. Requires extra attention, concentration, contains many moments of confusion and exhaustion

Mark: Lack of a common form of communication limits self-expression and identity.

Now we're hearing welcoming remarks from Scott Dorfman, USH parent and co-founder of Odylia therapeutics

Scott is stressing the importance of the USH Symposium for bringing together researchers, clinicians, and USH families.

Uwe Wolfrum, USH researcher and #USH2018 organizer, is now introducing Bill Kimberling to the podium to give a retrospective and prospective view of USH research Kimberling: Flow of USH research: clinical information-->gene discovery-->functional analysis-->therapies

Kimberling: shows a chart of annual number of USH research publications from 1930 to present day--a steady upward trend

Kimberling: background in population genetics, connected with clinician Claas Möller to collaborate on genetic analysis of families clinically diagnosed with USH

Kimberling: Once USH genes began to be identified, animal models for each gene could be found and, later, created, to further understanding of how these factors work in vision and hearing

Kimberling: discovery of multiple causative genes associated with USH influenced clinical diagnosis and documentation, furthering understanding of phenotype-genotype correlation

Kimberling: diagnostics moved forward with the ability to screen hearing or vision impaired people with a panel of USH genes at the same time. Molecular studies of how USH proteins work together in complexes added to understanding of clinical symptoms

Kimberling: review of potential treatment targets for retinal symptoms based on progression of disease--gene augmentation within living cells-->photoreceptor cell replacement-->multi retinal cell replacement

Kimberling: in the US, Federal funding and a variety of private foundations are supporting USH research

Kimberling: speaking to the researchers in the room, says 'the people with USH want you to be their miracle makers'

The first Scientific Session: Genetics & Diagnosis of Usher Syndrome, is being chaired by Jose Milan. Anne-Françoise Roux will give the first talk on the Genetics of USH

Roux: reviews the three clinical subtypes of USH, categorized by severity and onset of auditory and visual symptoms, as well as the presence of balance problems

Roux: key dates of USH gene identification: 1st gene id'd in 1995--MYO7A, 1998-2004 was the discovery of part, then all of the USH2A gene. Pace picked up as methods became more advanced, and we now know of 14 genes associated with USH

Roux: most of the USH1 genes, along with the USH2D gene, can also cause nonsyndromic hearing loss. USH2A is a common cause of nonsyndromic RP

Roux: multiple genes, variable effects of various mutations, subtypes ranging from common to rare, make diagnosis and treatment especially complex

Roux: tiered system of genetic analysis is an effective, although laborious, method of generating a genetic diagnosis

Roux: so many different mutations in a long list of genes requires good annotation! The Leiden Open Variation Database contains over 20K variants in 9 different USH genes.

Roux: Different types of USH are more prevalent in certain populations. Globally USH1B is the most common form of USH1, USH2A is the most common form of all USH

Roux: pathogenic changes in USH genes vary from one gene to the next; there is also variability in inheritance patterns of pathogenic variants for different USH genes. tl;dr: it's complicated!

Roux: evaluating pathogenic alleles of USH2A--most discovered mutations are novel or rare; the most common disease causing variant is found in 13% of USH2A patients

Roux: genetic material for diagnosis is commonly obtained from nasal cells. For USH2A diagnosis, a series of nested RT-PCR reactions revealed a hitherto unknown mutation causing a splicing error in several patients

Roux: Any new variant discovery requires validation that it is indeed pathogenic. Splicing errors lend themselves to treatment by antisense oligonucleotides (ASOs or AONs). We'll hear more about these later in the meeting

Roux: cutting edge genetic diagnostic practices indicate that the original subtype classification is usually accurate. There is still a lot of phenotypic variability depending on the nature of individual mutations and the particular combination of USH mutations in patients

Roux: new techniques allow us to screen USH genes in nonsyndromic HL patients. So far 16% of NSHL patients in the current (French) study have been found to carry mutations in one USH gene

Roux: This technique also offers possibility to screen other genes (beyond the USH candidates) in clinically diagnosed USH patients = new gene discovery & adding to the USH list

Next speaker: Carla Fuster Garcia from Valencia will talk about USH2A gene editing using the CRISPR system

Garcia: focus on developing treatment for vision loss. Genetic variability makes targeting hard, but choice to focus on most common form: USH2A, and the most common mutation within USH2A known as c.2299delG

Garcia: reviews method of CRISPR: seek specified region in the gene, cut DNA, trigger DNA repair mechanism of choice. Therapeutic approaches for CRISPR usually rely on homologydirected repair to rewrite the native DNA code

Garcia: using a cultured cell line to test CRISPR targeting & cutting efficiency

Garcia: next, the validated CRISPR system was co-introduced with a repair template into the cultured cells

Garcia: finally, patient-derived c.2299delG cells were treated with CRISPR system and repair template. Mutation repair was achieved in these cell cultures with low incidence of 'off target' effects. Next: testing in animal models or cultured retina

Next up: Isabelle Audo, from INSERM in Paris, talking about Clinical Diagnosis of Usher Syndrome

Audo: reviews clinical variability among subtypes, differential diagnosis with other (rarer) disorders that also have hearing & vision deficits, often alongside other symptoms

Audo: early diagnosis crucial for interventions for hearing loss, for family planning, and for consideration in emerging therapies (or clinical trials)

Audo: in the retina, rods are the cells that first show clinical defects in USH--necessary for night and dim light vision, hence the initial symptoms of night blindness in USH and other forms of RP. Rods outnumber cones and are important for cone survival

Audo: vision loss due to rod-cone degeneration is slow and progressive and the timeline can be variable even within the same family

Audo: neonatal hearing tests can identify congenital problems early and facilitate USH diagnosis. Other routes to an USH diagnosis include routine exams, evaluation of symptoms after problems begin, family studies or genetic testing for other reasons

Audo: important to test hearing in patients with retinal degeneration--hearing loss might have been overlooked & undiagnosed

Audo: Visual function analysis important beyond diagnosis--consideration of functional abilities in activities of daily living (driving, independence, etc)

Audo: shows visual field analysis, often showing a 'ring' of impaired vision in the periphery of the retina before progressing to tunnel vision. Electrophysiology via the full-field electroretinogram (ERG) is a critical, especially for children

Audo: ERGs test light and dark adapted responses, give a comprehensive picture of rod and cone function throughout the eye. Responses are recorded to different types of light flashes and light intensities

Audo: retinal imaging is also an important part of diagnosis--fundus photography usually only shows changes in more advanced stages. Retinal imaging after introduction of fluorescent dyes like lipofuscin can give information about photoreceptor health

Audo: another type of retinal imaging measures the presence and location of an autofluorescent ring that correlates with the useable visual field. Easy and quick, great noninvasive test that yields a lot of information.

Audo: the OCT (optical coherence tomography) scan can show areas of the retina that are degenerating as well as revealing cysts beneath the area

of central vision. Together these tests can give a comprehensive picture of how retinal symptoms are progressing and affecting vision

Next up is Margaret Kenna, pediatric otolaryngologist, from Harvard Medical School Center for Hereditary Deafness, with more about clinical diagnosis

Kenna: with early diagnosis of HL in the US, pediatric otolaryngology patients are getting younger. Early screening is picking up more and more hearing problems and providing opportunity for early intervention to facilitate communication, education and social skills

Kenna: early HL diagnosis requires further testing to see if other organs or tissue are affected. USH1 audiograms look identical to nonsyndromic genetic HL of other types, so diagnosis is important to get right!

Kenna: ruling out causes of syndromic symptoms is just as important in confirming them, both for peace of mind and clinical trial eligibility

Kenna: several other conditions can present with a similar clinical phenotype to USH, but aren't. Genetic testing and as much clinical information as possible matters a lot

Coffee Break! Stay with us for more beginning at 4:30 Mainz time

Science Session is starting again, with a welcome address from the DE's State Minister of Social Affairs Sabine Bätzing-Lichtenthäler

SB-L: extolling the virtues of these symposiums geared toward patient information and inclusion, building bridges between scientists and people affected by USH. Describes EU definition of 'rare disease'

SB-L: although the incidence of these diseases is rare, collectively millions of people are affected by rare diseases--considering as a group adds recognition, increases advocacy and funding for efforts toward finding cures

SB-L: Germany is ahead of the curve in forging national and international efforts toward diagnosis & treatments for rare diseases (great federal organization that other countries can/should model!)

SB-L: important contributions of this structure include guiding patients & families to appropriate resources, including but not limited to early & correct diagnosis. Research studies aimed at optimizing diagnoses are well supported

SB-L: Software and web development for collating diagnostic outcomes as well as patient information is another broad and ongoing effort

Back to the scientific program, Adam Dubis from Moorfield Eye Hospital in London will talk about the clinical analysis of USH2A

AD: plans for therapeutic trials are growing, so more studies on normal disease progression are critical for measuring whether treatments are effective. Age & condition dictate the types of eye tests that are possible or useful.

AD: study of 57 USH2A patients between from teens to seniors. collected data with OCT scans, eye chart tests, visual field tests, to observe trends in disease progression over 2+ years

AD: visual acuity did not change much during the 2 yr time frame, so limited use as an endpoint for therapy trials. OCT scans were used to measure retinal thickness/structure. Ability to get consistent results in these measurements was challenging. Change correlated with pt. age

AD: the pattern of autofluorescence (AF) rings also used as marker of retinal symptoms. Diameter of AF rings measured consistently, showed correlated change over time in each patient

AD: diagnostic goal for therapy follow up is to predict degree and rate of future changes in vision. Using ratio derived from OCT and AF test results combined has some predictive value

AD: advanced retinal imaging system to visualize the number of photoreceptors that are intact in the retinas of USH patients--gives information on how any cells are present (and therefore could be saved/functionally restored by therapy)

AD: These images can be mapped onto functional data from each patient for a more robust readout of retinal condition & impact of treatment during and after a clinical trial

Next session starting: Usher models & cell and molecular biology (part I), chaired by @ggeleoc

First speaker is Nikolai Klymiuk from LMU in Munich, speaking on generation and preliminary analysis of the USH1C transgenic pig model

NK: genetically modified pigs have been in use for a few years to develop resources for human organ transplants. Pig model of USH was desired that would recapitulate human disease under study for diagnostic and treatment testing

The USH1C R31X mutation was introduced into the pig USH1C gene to generate a 'humanized' disease model. The mutation was generated in pig cell lines and then transferred to the nucleus of a pig oocyte, implanted and gestated

USH1C pigs have severe auditory and vestibular defects, and an apparent partially decreased ERG at an early age (preliminary data, needs to be repeated & expanded). Retinal morphology appeared disorganized and molecules that are markers of degeneration were detected.

At 4 months, visual behavior is severely impaired compared to normal pigs--pigs were tested by navigating an obstacle course.

NK conclusions: early success evident in generating the humanized stock of animal models that show early and easily detectable auditory, vestibular and visual dysfunction. Future plans for more phenotypic characterization and beginning to evaluate therapy options.

next speaker Aziz El Amraoui from the Pasteur Institute in Paris, reviewing animal models of the retinal phenotype in USH.

AEA: even though the proteins affected by mutations in USH genes are constructed very differently, the symptoms from disrupting any one of them

are quite similar. Animal models help us figure out the common denominators that translate molecular events to clinical symptoms

AEA: focus on USH1 proteins, examining cells affected by USH and looking for similarities. Hair cells of the inner ear and photoreceptor cells of the retina share structural and functional properties

AEA: more than 30 different USH1 mouse mutants are available, but few have retinal abnormalities, and those are quite mild compared to human symptoms

AEA: generating strains of USH1 mutant mice who are also albino and eliminating some nutritional factors necessary for healthy retinas result in an enhanced retinal phenotype. Defects are reversible when mice are raised in the dark and given nutritional supplements

AEA: Studying USH1 proteins in primate eyes rather than mice enable us to see a situation very similar to that in humans--shows us the protein localization and cell structures that rely on USH1 proteins.

AEA: Mice lack a structure in the photoreceptor that is present in primates (including humans). USH1 proteins localize to this structure and vision loss is likely due to dysfunction at this location. Mice don't have it ---> Ush1 mice don't show retinal symptoms

Primate models of USH are not available, but frogs also have these special structure--the calyceal processes, and USH1 frog models show disrupted formation of the calyceal processes. Dysfunction in these structures results in changes to the architecture of photoreceptors.

AEA: mammalian models (primate and pig) are now available to study USH1 retinal symptoms and use for therapy testing. USH1G was selected for the first models. Photoreceptor analysis shows defects consistent with human disease symptoms. Small sample size & some variability.

Next speaker is Adam Yildirim from the JGU in Mainz, to tell us about a new function for the USH1G protein Sans

Sans is a member of the important USH1 protein network in hair cells and photoreceptors. Sans also has a novel localization in the nucleus of these cells. Looking for nuclear proteins that might interact with Sans...

Several candidates were identified that participate in RNA processing, including splicing-- the process of editing the genetic code into a concise molecule that serves as a template for protein translation.

AY: Splicing factor 3b subunit 1 was confirmed as a Sans interacting partner. SF3B1 and Sans colocalize in the nucleus, along with two other RNA processing proteins called PRPF6 and PRPF31. Is Sans a splice regulator?

AY: cell culture was used to test splice regulation potential--Sans was shown to be a splice regulator of various RNAs, including the USH1C and USH1B genes.

AY: when SANS is depleted in these cultured cells, formation of the protein complex that facilitates RNA splicing is impaired.

AY: These results add new info to the picture of what molecular processes are affected in the retinal phenotype of USH1G

Next up is Jun Yang from the @MoranEyeCenter in Salt Lake City. She'll be talking about the USH2 protein complex in photoreceptors and hair cells.

JY: The USH2 proteins have functional domains that physically interact with eachother to form a complex. Two (Usherin & Adgrv1) are large proteins with extensive extracellular domains, two others (Whrn and Pdzd7) are small scaffolding molecules.

JY: previous studies show that protein interactions are mediated between PDZ binding domains at the ends of the large proteins and PDZ domains on the small ones

JY: new work also shows that the extracellular regions of Usherin and Adgrv1 interact. PDZ domain binding is relatively weak, but may be a feature of flexibility--quick binding and disassembly can be an asset

In the mouse ear, USH2 proteins and Pdzd7 form links at the base of stereocilia--the actinbased projections from hair cells that are required for the cell to receive sound. USH2 and Pdzd7 proteins rely on each other for their localization in these structures.

JY: 'cross-talk' between USH2 proteins and members of the USH1 complex has also been revealed from previous studies. How does this relate to position of USH2 proteins in the stereocilia? These proteins are mislocalized in the hair cells of Myo7a (USH1B) mutant mice

JY: The mislocalization of these proteins contribute to disorganized stereocilia in USH2 mutant mice--showing us the cellular basis of hearing loss in USH2

JY: analysis of hearing in mice show some difference in severity of hearing loss depending on which USH2 gene is mutated. Adgrv1 (ush2c) is most severe, Whirlin (ush2d) is least severe.

JY: USH2 proteins localize to a restricted space in photoreceptor cells, at the base of a structure called the connecting cilium. Pdzd7 was not detected in mouse retinas.

JY: USH2 proteins rely on eachother for colocalization at this periciliary region in mouse photoreceptors

JY: in contrast to its connection to USH2 proteins in the hair cells, Myo7a is not required for localization of USH2 proteins in mouse photoreceptors.

JY: USH2 mouse mutant photoreceptors have abnormal subcellular structures in the region of the connecting cilium, suggesting that the USH2 complex plays a role in structural integrity iin photoreceptors

JY: using patient derived cells to establish these molecular relationships in human cells. The next speaker up is me, @ClutchScience, so I'm signing off on the live tweeting for the moment.

This is Mark. Taking over temporarily because our Tweeter, Dr Jennifer Phillips, University of Oregon, is the next speaker! #ushersyndrome

Analysis of the functional relationships among Usher Type 2 proteins in zebrafish models.

Jen is working on a lot of Usher genes. Only talking about Ush 2 complex today

Usher 2a is most common cause of Usher. And also causes non-syndromic hearing loss.

JP: Usherin EVD is required for stabilizing adhvr1 and whirlin b localization at the PCM. PDZ is required for whirlin A stability

JP: Research bolsters idea that relationship between Whirlin b and asgrv1.

JP: increases understanding of how Usherin and Adgrv1 interact.

JP: mutant zebrafish have more rapid vision loss when exposed to bright light.

Conclusion: Usherin stablizes all other components of USH2a interactome.

Up next Matthew Tyska. Shaping the intestinal brush border with adhesion links.

Matthew is our last talk of the day--he's mixing things up by moving completely out of eye & ear tissues and talking about USH proteins in gut cells. Common denominator: actin bundles projecting from the cells that do important stuff. In the gut they're called microvilli.

MT: In the gut, microvilli are used to increase surface area of the cell, which gives more functional capacity to absorb the nutrients coming through the gut tract.

MT: interest in how the microvilli-adorned gut cells, called the brush border cells, organize into a tightly packed array. Turns out they use molecular bridges to join microvilli together, much like stereocilia and photoreceptors do

Harmonin (USH1C) is also a component of the molecular links between neighboring intestinal microvilli. Instead of partnering with other USH proteins in this case, it complexes with similarly structured proteins.

MT: molecular links between actin projections is an evolutionarily ancient activity--even our distant cousins the sponges do it.

MT: loss of these links in between microvilli affects the shape and function of these structures. This results in a significantly reduced number of microvilli, and consequent reduction of surface area with which to absorb nutrients.

MT: Not only is surface area impaired, but digestive enzymes that are stored on these membranes are also reduced. The mice that bear these mutations are smaller, presumably because of decreased nutrient intake.

MT: In addition to providing stability to the microvilli, does the USH1C complex have a role in transporting nutrients? This question is best addressed in cell culture--no microvilli in these cells, but another actin based structure called filopodia.

MT: movies of live cells show us that the USH1C complex is moving along filopodia, suggestive of a transport role, delivering cargo to the tips of these actin projections

MT: transporting the connecting molecules to the tips of actin based structures also appears to influence the uniformity of actin projection length (this is important if you are making an organized structural projection out of many parts).

And there you have it! Day one of the Science Sessions is in the books. We'll be back on line tomorrow for round 2. Thanks for following along! @ClutchScience signing off. @UsherCoalition Thanks @ClutchScience for tweeting #USH2018

…