USHER SYNDROME COALITION

International Symposium on Usher Syndrome

Program and Abstracts

International Symposium: July 10-11, 2014
Family Conference: July 12, 2014

USHER SYNDROME
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#USH2014

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3rd International Symposium on Usher Syndrome and
6th Annual Usher Syndrome Family Conference

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Department of Otolaryngology & Communication Enhancement

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This conference was supported by the National Institute On Deafness And Other Communication Disorders, the National Eye Institute and the National Center for Advancing Translational Sciences of the National Institutes of Health under Award Number R13DC013968. The content is solely the responsibility of the presenters and does not necessarily represent the official views of the National Institutes of Health.

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Special thanks to Barney J. Skladany, Jr. of Akin Gump Strauss Hauer & Feld, for helping us increase awareness of Usher syndrome on Capitol Hill.

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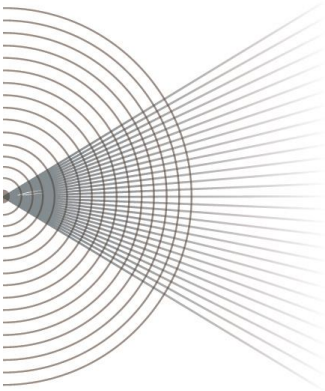

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- Mabat Latid: Vision for a Cure
- New England Consortium for Deafblind Technical Assistance and Training
- Usher 2020 Foundation

Family Conference:

- CaptionCall
- Cochlear Americas
- Deaf and Hard of Hearing Program, Boston Children's Hospital
- Deaf Blind Contact Center
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MINUTEMAN IMPLANT CLUB

Welcome

Welcome to the International Symposium on Usher Syndrome. Welcome to all the international leaders in Usher syndrome research, to all the major funding organizations, and to all the Usher syndrome families in attendance. Welcome, everyone, to the largest gathering of the Usher syndrome community in history.

Over the course of the conference, ideas will be exchanged, friendships will be built, and our Usher syndrome community will grow stronger. This is a moment of opportunity. The knowledge exchanged between researchers and families will help to accelerate the pace of Usher syndrome research and will give hope to the thousands of people with the disease.

For those in attendance, I strongly urge you to take this opportunity to foster new relationships. Families with Usher syndrome often struggle with isolation. It does not have to be that way. Families, take the opportunity to make new friends, both with families and researchers. Professionals, take the opportunity to be inspired by the people your work impacts. For these few days the future is not about fear, but about hope. We are here, together, one community. This is a time to celebrate.

Thank you again for joining us. I look forward to meeting you all.

Mark Dunning
Chairman, Usher Syndrome Coalition

Overview

The Usher Syndrome Coalition presents the third International Symposium on Usher Syndrome in conjunction with the sixth annual Usher Syndrome Family Conference.

The two-day Symposium will be a scientific forum where the world's leading experts come together in an environment that encourages collaboration and the exchange of ideas in order to advance Usher Syndrome research. One of the goals of this symposium is the creation of a roadmap. This roadmap will identify our knowledge gaps on the disease, and it will guide future research efforts for years to come.

The Family Conference is an opportunity to learn about the latest research and news and to connect with researchers, other Usher individuals and families. Breakout sessions will be held for Family Conference attendees, providing an opportunity to discuss various issues related to living with Usher syndrome. Genetic counseling sessions are also available, giving attendees the chance to have their genetics questions answered during one-on-one conversations with counselors.

Conference Location

The Joseph B. Martin Conference Center
at Harvard Medical School
77 Avenue Louis Pasteur
Boston, MA 02115

WiFi Access at the Conference

Free WiFi in all meeting rooms and lobby areas ("HMS Public" No password required)

Download the #USH2014 Mobile App

Symposium details can be found on the #USH2014 Mobile App. Download the app on your iPhone, iPad or Android. Click https://crowd.cc/s/1MuM from your mobile device (OR search Usher 2014 in the app store).

Childcare

Childcare is provided during the Family Conference on Saturday, July 12th. Located in Room 214 of the Martin Center.

Usher Syndrome Coalition

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President, The Decibels Foundation
Director of Information Technology, L.E.K. Consulting

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Family Nurse Practitioner, Family Medicine Associates

Interns

Anjana Gupta
Aashika Nagarajan
Nina Yamagata
Camelia Zheng

Staff

Krista Vasi

Working to find a cure for the most common cause of combined deafness and blindness.

The Usher Syndrome Coalition's mission is to raise awareness and accelerate research for the most common cause of combined deafness and blindness. The Coalition also provides information and support to individuals and families affected by Usher syndrome.

www.Usher-Syndrome.org

Schedule at a Glance

THURSDAY, JULY 10 Faces of Usher Syndrome: Moira Shea, Lorne Marin, Vicki Cox	
7:00 - 8:00 am	**CONTINENTAL BREAKFAST	REGISTRATION** Ground Floor Lobby
SCIENTIFIC SYMPOSIUM Amphitheater	
8:00 - 8:30 am	**WELCOME:** Mark Dunning, Usher Syndrome Coalition **INTRODUCTION: Funding for Usher Syndrome Research** *8:10-8:30* Stephen Rose, PhD, Foundation Fighting Blindness
8:30 - 10:00 am	**SYMPOSIUM SESSION A** *Moderators: Margaret Kenna, MD, MPH and Claes Möller, MD, PhD* **Diagnostics, Epidemiology and Population Genetics** *8:30-9:10* ***Keynote:*** Edwin Stone, MD, PhD - Discovering causes of and developing treatments for inherited eye diseases *9:10-9:35* Ilene Miner, LCSW - Family and Personal Responses to the Diagnosis of Usher Syndrome *9:35-10:00* Anne Fulton, MD - Photoreceptors in Pediatric Patients with Usher Syndrome
10:00 - 10:30 am	**COFFEE BREAK	POSTERS	EXHIBITS** Ground Floor	1st Floor Balcony & Catwalk
10:30 - 12:30 pm	**SYMPOSIUM SESSION A (Continued)** *Moderators: Heidi Rehm, PhD and Ilene Miner, LCSW* **Diagnostics, Epidemiology and Population Genetics** 10:30-10:55 Eric Pierce, MD, PhD and Kinga Bujakowska, PhD - Genetic Diagnostic testing for Usher Syndrome 10:55-11:20 Anne-Françoise Roux, PhD - Detection of unconventional Usher syndrome mutations and further outcomes of the LOVD-USHbases 11:20-11:45 Maria Bitner-Glindzicz, MD - Unsuspected phenotypic heterogeneity in Usher syndrome revealed by Massive Parallel Sequencing 11:45-12:05 Hanno Bolz, MD - NGS for Usher Syndrome: Benefits, Pitfalls and Unexpected Findings 12:05-12:30 Heidi Rehm, PhD - Data Sharing to Support Genetic Test Interpretation
12:30 - 1:30 pm	**LUNCH	POSTER SESSION	EXHIBITS** Tote lunch pickup in Ground Floor Lobby, Posters on 1st Floor Balcony & Catwalk, Lunch seating on 2nd floor in the Lounge and Rooms 214, 216, 217
1:30 - 3:35 pm	**SYMPOSIUM SESSION B** *Moderators: Gwenaëlle Géléoc, PhD and Hanno Bolz, MD* **Functional Genetics** *1:30-1:55* José Millán, PhD - Study of splicing variants in the USH genes through minigene assay and transcript analysis from epithelial nasal cells *1:55-2:20* Jun Yang, PhD - The Usher syndrome type 2 protein complex in photoreceptors and hair cells *2:20-2:45* Uwe Wolfrum, PhD - Decoding of Usher syndrome protein networks reveals insights in the molecular basis of the disease

	2:45-3:10 Monte Westerfield, PhD - Defective protein complex assembly produces ER stress that causes cell death in Usher syndrome *3:10-3:35* Dominic Cosgrove, PhD - Role for Usher proteins in regulated protein trafficking in photoreceptors: mechanism for light-induced retinal degeneration
3:35 - 4:00 pm	**BREAK|POSTERS|EXHIBITS** Ground Floor | 1st Floor Balcony & Catwalk
4:00 - 5:30 pm	**SYMPOSIUM SESSION B (continued)** *Moderator: José M. Millán, PhD* **Functional Genetics** *4:00-4:40:* ***Keynote*** Christine Petit, MD, PhD - Usher syndrome: gathering basic knowledge towards the development of therapeutic approaches *4:40-5:05:* Zubair Ahmed, PhD - The quest for molecular mechanisms of Usher syndrome *(Schedule Change)* *5:05-5:30:* Kumar Alagramam, PhD - Hair Cell Specific Expression of Clarin-1 is Sufficient to Prevent Auditory and Vestibular Dysfunction in the Mouse Model for Ear Disease in Usher Syndrome III
6:00 - 8:00 pm	**Foresight Award Presentation, Barney J. Skladany, Jr.** **COCKTAIL RECEPTION and POSTERS** 1st Floor Balcony & Catwalk

FRIDAY, JULY 11

Faces of Usher Syndrome: Randal DeWitt, Rachel Chaikof, Marisa Postlewate

7:00 - 8:30 am	**CONTINENTAL BREAKFAST|REGISTRATION** Ground Floor Lobby
SCIENTIFIC SYMPOSIUM Amphitheater	
8:30 - 8:35 am	**WELCOME: Bella Dunning**
8:35 -10:00 am *(*Selected Presentation)*	**SYMPOSIUM SESSION C** *Moderators: Ronald Pennings, MD, PhD and Anne-Françoise Roux, PhD* **Phenotypes and Natural History** *8:35-9:10* Margaret Kenna, MD, MPH - Usher Syndrome: When to suspect it and how to find it *9:10-9:40* Claes Möller, MD, PhD - Usher syndrome: Do audiological, vestibular and visual geno-phenotype correlations exist? *9:40-10:00* *Ronald Pennings, MD, PhD - Genotype-phenotype correlations on 161 USH2A patients – some mutation combinations cause a more severe audiometric phenotype
10:00 - 10:30am	**COFFEE BREAK|POSTERS|EXHIBITS** Ground Floor |1st Floor Balcony & Catwalk
10:30 - 12:30 pm	**SYMPOSIUM SESSION D** *Moderators: Aziz El-Amraoui, PhD and Luk Vandenberghe, PhD* **Preclinical studies** *10:30-10:55* Jennifer Lentz, PhD - Antisense oligonucleotides effectively treat Usher syndrome in mice *10:55-11:20* Jeffrey Holt, PhD - TMC Gene therapy in mouse models of human deafness *11:20-11:45* Constance Cepko, PhD - Strategies to prolong survival of cone photoreceptors

*(*Selected Presentations)*	*11:45-12:05* *Maggie Yoder, PhD Candidate - Determining the mechanism of vision and hearing loss associated with usher type 2A *12:05-12:25* *Alix Trouillet, PhD - Characterization of Usher mouse model retinal degeneration and assessment of potential therapy
12:30 - 1:30 pm	**LUNCH | POSTER SESSION | EXHIBITS** Tote lunch pickup in Ground Floor Lobby, Poster Viewing on 1st Floor Balcony & Catwalk, Lunch seating on 2nd floor in the Lounge and Rooms 214, 216, 217
1:30 - 3:30 pm	**SYMPOSIUM SESSION E** *Moderators: Zheng-Yi Chen, PhD and Isabelle Audo, MD, PhD* **Therapy and Clinical Trials** *1:30-1:55* Luk Vandenberghe, PhD - Vector discovery and design for improved delivery to the retina and cochlea *1:55-2:20* Uwe Wolfrum, PhD - Ignore the stop: translation read-through of nonsense mutations in Usher syndrome genes *2:20-2:45* Xue Liu, MD, PhD - Cochlear implantation in individuals with Usher syndrome *2:45-3:05* Charles Della Santina MD, PhD - Progress toward a vestibular implant for restoring sensation of head movement *3:05-3:30* Mark Pennesi, MD, PhD - Updates on a Gene Therapy Trial for Usher Syndrome Type IB
3:30 - 4:00 pm	**BREAK | POSTERS | EXHIBITS** Ground Floor | 1st Floor Balcony & Catwalk
4:00 - 4:45 pm	**SYMPOSIUM SESSION E** *Moderator: Jennifer Lentz, PhD* **Therapy and Clinical Trials** *4:00-4:*25 Patricia Zilliox, PhD - Getting Vision-Saving Therapies Out to the People *4:25-4:45* Isabelle Audo, MD, PhD - Consideration on Usher Syndrome to prepare future therapies
4:45 - 5:15 pm	**Living with Usher Syndrome: Words for the scientists** René Pellerin
5:15 - 6:15 pm	**PANEL DISCUSSION** *Moderator: Mark Dunning* **Funding Usher Syndrome Research** *Usher Syndrome Coalition: Mark Dunning, Hear See Hope: Lane McKittrick, Usher 2020 Foundation: Scott Dorfman, The Megan Foundation: Megan Kennedy, Usher III Initiative: Lindsey Whyte*
6:30 - 9:00 pm	**BANQUET** (Ticketed Event) Elements Café

SATURDAY, JULY 12

7:00 - 8:30 am	**CONTINENTAL BREAKFAST | REGISTRATION** Ground Floor Lobby
FAMILY DAY Amphitheater	Genetic Counseling: (All Day) Room 217 Breakout Sessions: (Afternoon) Elements Café Childcare: (All Day) Room 214
8:30 - 8:35 am	WELCOME: Elise Faucheaux

8:35 - 9:05 am	**SESSION I** *Moderators: Catherine Blanchet, MD and Susanne Morrow* **Diagnosis** *8:35–9:05* Margaret Kenna, MD - Usher Syndrome: Why a definite diagnosis matters
9:05 – 10:00 am	**SESSION II** *Moderators: Catherine Blanchet, MD and Susanne Morrow* **Psychological Aspects - Words from the professionals** *9:05 - 9:30* Ilene Miner, LCSW - Family and Personal Responses to the Diagnosis of Usher Syndrome *9:30 - 10:00* Claes Möller, MD, PhD – Physical and Psychological Aspects of Usher Syndrome
10:00 - 10:30 am	**COFFEE BREAK** Ground Floor
10:30 - 11:00 am	**SESSION III** *Moderator: Jennifer Phillips, PhD* **Gene Therapy 101** Luk Vandenberghe, PhD
11:00 - 12:00 pm	**SESSION IV** *Moderator: Jennifer Phillips, PhD* **Update to Families** *11:00-11:30* Gwenaëlle Géléoc, PhD - Usher Syndrome Research *11:30-12:00* Margaret Kenna, MD, MPH- Translational Research and the Usher Syndrome Registry
12:00 - 12:30 pm	**Question and Answers:** Margaret Kenna, MD, MPH; Mark Dunning; Claes Möller, MD, PhD; Ilene Miner, LCSW; Luk Vandenberghe, PhD
12:30 - 1:30 pm	**LUNCH** Tote lunch pickup in Ground Floor Lobby, Lunch seating in Elements Café
1:30 - 2:30 pm *(*Selected Presentations)*	**SESSION V** *Moderator: Martha Steele* **Patient care and rehabilitation** *1:30-1:50* *Nadja Högner, PhD - Stress in individuals with Usher syndrome type II *1:50-2:10* *Catherine Blanchet, MD - Usher type 2 syndrome: hearing, educational, socio-economics and vocational impacts *2:10-2:30* *Susanne Morrow - Focusing on Now for Tomorrow: A well rounded Curriculum to strengthen students with Usher Syndrome.
2:30 - 3:15 pm	**Panel: Psychological Aspects- Patient Journeys** Family Panel: Elaine Ducharme, Chloe Joyner, Ryan Thomason, Mike Walsh, Molly Watt
3:15 - 4:45 pm	**Professional Brainstorming Session (Amphitheater) | Family Breakout Sessions (Elements Café)** ***Roadmap for Usher Syndrome*:** Identify and prioritize the most pressing questions that relate to Usher syndrome diagnostics, Usher syndrome research and the development of therapeutic tools.
4:45 - 5:00 pm	**Concluding remarks** Mark Dunning
5:30 - 8:00 pm	**FAMILY BARBECUE – SIMMONS COLLEGE ACADEMIC QUAD (RAIN LOCATION: FENS CAFETERIA)**

Poster Listings

Session 1 Posters (Posters #1-16): Presenting authors are encouraged to be at their poster board during the coffee and lunch breaks on Thursday, July 10th.

Session 2 Posters (Posters #17-35): Presenting authors are encouraged to be at their poster board during the coffee and lunch breaks on Friday, July 11th.

Group 1- Diagnostics: Epidemiology and Population Genetics

1. **Targeted next generation sequencing as a comprehensive molecular diagnosis for Usher syndrome**

Aller, E.[1,2], Aparisi, M.J.[1,2], Fuster-Garcia, C.[1], Ayuso, C., Blanco-Kelly, F., Martínez Fernandez de la Cámara, C., Rodrigo RJaijo, T.[1,2] and Millán, J.M.[1,2,3]

[1]Grupo de Investigación en Enfermedades Neurosensoriales, Instituto de Investigación Sanitaria La Fe (IIS-La Fe), Valencia, Spain
[2]Unidad 755 del CIBER de Enfermedades Raras (CIBERER), Madrid, Spain
[3]Unidad de Genética y Diagnóstico Prenatal, Hospital Universitario y Politécnico La Fe, Valencia, Spain

2. **Early Diagnosis of Usher Syndrome using Next-Generation Sequencing Panel Testing**

Muirhead, A. [1], Abou Tayoun, A. [1,2], Lafferty, K. [1], Hernandez, A.L. [1], Shen, J. [1,3], Rehm, H. [1,3] and Amr, S. [1,3]

[1]Laboratory for Molecular Medicine, Partners Personalized Medicine, Cambridge, MA, USA
[2]Harvard Medical School Genetics Training Program, Boston, MA, USA
[3]Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

3. **Automated pathogenicity assignment methods for missense variants: application to usherin**

Baux, D.[1], Mireille, C.[1,2,3] and Roux, A-F.[1,3]

[1] CHU Montpellier, Laboratoire de Génétique Moléculaire, Montpellier, F-34000, France
[2] Université Montpellier 1, UFR Médecine, Laboratoire de Génétique Moléculaire, Montpellier, F-34000, France
[3] Inserm, U827, Montpellier, F-34000, France

4 **2014 update of LOVD-*USH2A* defines an extensive mutational spectrum and highlights missense hotspots**

Baux, D.[1], Blanchet, C.[2,3], Hamel, C.[3], Meunier, I.[3], Larrieu, L.[1], Faugère, V.[1], Vaché, C.[1], Claustres, M.[1,4,5] and Roux, A-F.[1,5]

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[2] CHU Montpellier, Service ORL, F-34000, France
[3] CHU Montpellier, Centre National de Référence Maladies Rares "Affections Sensorielles Génétiques", Montpellier, F-34000, France
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[5] Inserm, U827, Montpellier, F-34000, France
[5] Inserm, U827, Montpellier, F-34000, France

5. **Towards a comprehensive diagnostic test for Usher Syndrome and Non-Syndromic Deafness**

Cullup, T.[1], Drury, S.[1], Boustred, C.[1], Jenkins, L.[1], Lench, N.[1] and Bitner-Glindzicz, M.[1,2]

[1]Regional Genetics Service, Great Ormond Street Hospital NHS Foundation Trust, London, United Kingdom
[2]University College London Institute of Child Health, London, United Kingdom

6. **Comprehensive Usher syndrome molecular diagnostic strategy**

Faugère, V.[1], Moclyn, M. [1], Garcıa-Garcıa, G. [2], Besnard, T. [1], Baux, D. [1], Vaché, C. [1], Larrieu, L. [1], Claustres, M. [1, 2, 3] and Roux, A-F. [1,2]

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[2] Inserm, U827, Montpellier, France
[3] Univ, Montpellier I, Montpellier, France

7. **Extending the gene panel size to provide a positive diagnosis for non-Usher patients presenting with hearing loss and retinitis pigmentosa**

García-García, G.[1], Faugère, V.[2], Moclyn, M.[2], Constantinides, S.[2], Baux, D.[2], Vaché, C., Koenig, M.[1,2,3], Claustres, M.[1,2,3] and Roux, A-F.[1,2]

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[3] Univ, Montpellier I, Montpellier, F-34000, France

8. **Rapid identification of new pseudoexon insertions in *USH2A***

Liquori, A.[1,2], Baux, D.[3], Vaché, C.[3], García-García, G.[2],Claustres, M.[1,2,3] and Roux, A-F.[2,3].

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[2] Inserm, U827, Montpellier, F-34000, France
[3] CHU Montpellier, Laboratoire de Génétique Moléculaire, Montpellier, F-34000, France

9. **Estimation of Usher prevalence in Portugal**

Ribeiro, J.C.[1,2,3], Silva, F.[1], Paiva, A.[1,3] and Silva E[1,2,4]

[1]Faculty of Medicine, University of Coimbra, Portugal
[2]Centre of Ophthalmology and Vision Sciences, IBILI, Faculty of Medicine, University of Coimbra, Portugal
[3]Otorhinolaryngology Department, Centro Hospitalar e Universitário de Coimbra E.P.E., Portugal
[4]Center for Hereditary Eye Diseases, Centro Hospitalar e Universitário de Coimbra E.P.E., Portugal

10. **Marker of oxidative stress and inflammation in the aqueous humor of patient with retinitis pigmentosa**

Martínez-Fernández de la Cámara, C.[1], Salom, D.[2], Sequedo, M.D.[1], Hervás, D.[3], Aller, E.[1,4], Jaijo, T.[1,4], Millán, J.M.[1,4,5] and Rodrigo, R.[1]

[1]Sensorineural Disorders, Health Research Institute-La Fe, Valencia, Spain
[2] Department of Ophthalmology, La Fe University Hospital, Valencia, Spain
[3] Biostatistics Unit, Health Research Institute-La Fe, Valencia, Spain
[4] Centre for Biomedical Network Research on Rare Diseases (CIBERER), Valencia, Spain
[5] Genetics Unit, La Fe University Hospital, Valencia, Spain

11. **The prevalence and severity of cystic macula lesions in genetically confirmed Usher syndrome patients**

Sliesoraityte, I.[1], Mohand-Said, S.[1,2], Peto, T.[3] and Sahel, J.A.[1,2]

[1] Inserm -DHOS Centre d'Investigation Clinique CIC1243, Centre Hospitalier National d'Ophtalmologie des Quinze-Vingts, Paris, France
[2] Institut de la Vision, Univ Pierre et Marie Curie Paris 6, Inserm UMR_968, CNRS UMR_7210, Paris, France
[3] NIHR Biomedical Research Centre at Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology, Reading Centre, London, UK

12. **Vestibular function in patients with Usher Syndrome**

Stultiens, J.J.A., Edwards, J., Brodsky J.R. and Kenna, M.A.

Department of Otolaryngology and Communication Enhancement, Boston Children's Hospital, Boston, Massachusetts, USA

13. **Scotopic and photopic ERG responses in pediatric patients with Usher Syndrome**

Tavormina, J.[1], Hansen, R.[1], Moskowitz, A.[1], Rehm, H.L.[3], Kenna, M.[2] and Fulton, A.[1]

[1]Boston Children's Hospital, Department of Ophthalmology, Boston, MA, USA
[2]Boston Children's Hospital, Department of Otolaryngology, Boston, MA USA
[3]Brigham and Women's Hospital and Harvard Medical School, Cambridge, MA 02138, USA

Group 2- Functional Genetics: Preclinical Studies and Therapy

14. **Developing *in silico* reconstructed ancestral AAV vectors for retinal gene therapy**

Carvalho, L.S., Zinn, E., Shah, S., Xiao, R., Sarkar, D., Khaychuk, V. and Vandenberghe, L.H.

Ocular Genomics Institute, Massachusetts Eye and Ear Infirmary, Boston, MA, USA

15. **NINL^{isoB} and DZANK1 cooperate in assembling the cytoplasmic dynein 1 motor complex, a process essential for photoreceptor outer segment formation in zebrafish**

Dona, M.[1], Hetterschijt, L.[2], Tonnaer, E.[1], Peters, T.[1], Van Beersum, S.[2], Bergboer, J.[2], Van Reeuwijk, J.[2], Texier, Y.[3], Toedt, G.[4], Gibson, T.[4], Boldt, K.[3], Ueffing, M.[3], Roepman, R.[2], Kremer, H.[1,2] and Van Wijk, E.[1]

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16. **Gene augmentation therapy to treat Usher Syndrome Type IC**

Charles Askew[1], Selena Heman-Ackah[1], Yukako Asai[1], Bifeng Pan[1], Jennifer J. Lentz[2] and Gwenaëlle S. G. Géléoc[1].

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17. **Translational read-through of non-sense mutations leading to Usher syndrome and related ciliopathies**

Möller, F.[1], Penner, I.[1], Samanta, A.[1], Baasov, T.[2], Wolfrum, U.[1] and Nagel-Wolfrum, K.[1]

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18. **Generation of precise zebrafish models of Usher gene mutations**

Phillips, J.B., Wegner, J., Lerner, K.M., Banks, A.M. and Westerfield, M.

Institute of Neuroscience, University of Oregon, Eugene, OR, USA

19. **Exon-skipping as a promising therapeutic approach for treatment of retina degeneration in *USH2A* pseudoexon 40 patients**

Slijkerman R.W.N.[1a], Vaché C.[2a], Dona M.D.[1], García-García G.[2], Claustres M.[2,3,4], Hetterschijt L.[5], Peters T.A.[1], Collin R.W.[5], Kremer H.[1,5], Van Wijk E.[1b] and Roux AF.[2,3b]

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[a] Slijkerman R.W.N. and Vaché C. are co-first authors

[b] Roux A-F. and Van Wijk E. are co-last authors

20. **Direct interaction of the Usher syndrome proteins SANS (USH1g) and USH2a**

Sorusch, N., Bauß, K. and Wolfrum, U.

Cell and Matrix Biology, Inst. of Zoology, Johannes Gutenberg-University of Mainz, Germany

21. **Optimization of AAV gene delivery vectors to minimize toxicity in the mouse retina for the treatment of Usher Syndrome 3A**

Stupay, R.M.[1, 2], Zhu, P.[1], Deng, W.[1], Chiodo, V.[1], Boye, S.L.[1], Li, Q.[1], Hauswirth, W.W.[1] and Dinculescu, A.[1]

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22. **An Usher-like protein complex regulates intestinal brush border assembly**

Crawley, S.W.[1], Shifrin, D.A.[1], Grega-Larson, N.E.[1], McConnell, R.E.[1], Benesh, A.E.[1], Mao, S.[1], Zheng, Y.[2], Zheng, Q.Y.[2], Nam, K.T.[3], Millis, B.A.[4], Kachar, B.[4], and Tyska, M.J.[1]

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23. **The kinesin kif2a interacts with the Usher syndrome 1g protein SANS and kif2a is involved in the ciliogenesis of primary cilia**

Tebbe, L., Sorusch, N. and Wolfrum, U.

Cell and Matrix Biology, Institute of Zoology, Johannes Gutenberg-University of Mainz, Germany

24. **Comparison of dual AAV vector approaches for large gene delivery to the retina**

Turunen, H.T.[1], Carvalho, L.S.[1], Velez, M.V.L.[2], Bennett, J.[3] and Vandenberghe, L.H.[1]

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[3]Department of Ophthalmology, University of Pennsylvania School of Medicine, F.M. Kirby Center for Molecular Ophthalmology, Scheie Eye Institute, Philadelphia, PA, USA

25. ***De novo* mutations in *MYO7A* and *USH2A* genes**

Vaché, C.[1], Baux, D.[1], Faugère, V.[1], Larrieu, L.[1], Bitner-Glindzicz, M.[2], Hamel, C.[3], Claustres, M.[1, 4, 5] and Roux, A-F.[1, 4]

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26. **Gene Transfer Vector Core: Providing Robust and High Capacity AAV Vector Production**

Xiao, R., Shelke, R., Plovie, E., Lin, Y. and Vandenberghe, L.H.

Gene Transfer Vector Core, Dr. Luk Vandenberghe Lab, Schepens Eye Research Institute, Harvard Medical School, Boston, MA

27. **Deletion of PDZD7 disrupts the USH2 protein complex in cochlear hair cells and causes hearing loss in mice**

Zou, J.[1], Zheng, T.[1], Ren, C.[2], Askew, C.[3], Liu, X-P.[3], Pan, B.[3], Holt, J.R.[3], Wang, Y.[2] and Yang, J.[1,2]

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Group 3- Phenotypes-Natural History-Psychological Aspects

28. **Multidisciplinary team management of dual deafness and low vision disability**
Blanchet, C.[1], Dupeyron, G.[2], Remond, B.[2], Verlet, K.[3], Gilbert, M.[3], Jabouin, A.[3], Petit, P.[4], Hamel, C.[1] and Mondain, M.[1]
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[4] FAF LR, Fédération des Aveugles et Amblyopes de France, Languedoc-Roussillon, Montpellier, France, Languedoc-Roussillon, Montpellier, France

29. **Occupational activity and psychological health in persons with Usher II**
Ehn, M., Danermark, B., Möller, K. and Möller, C.
Audiological research center, University Hospital Örebro, Örebrocounty , Sweden

30. **Stress in individuals with Usher syndrome type II**
Högner, N.
Institute for Rehabilitation Sciences, Department of Education and Rehabilitation of the Blind and Low Vision Individuals, Humboldt University of Berlin, Germany

31. **Rehabilitation of Individuals with Usher Syndrome: a Qualitative Study**
Miles, C.
University of Arizona Department of Disability and Psychoeducational Studies, Tucson, Arizona
PhD Candidate and Rehabilitation Scholar US Rehabilitation Services Administration

32. **Focusing on Now for Tomorrow: Using A Well-Rounded Curriculum to Strengthen Students with Ushers Syndrome**
Morrow, S.[1] and Labeck, K.[2]
[1] New York Deaf-Blind Collaborative, Project Coordinator, Queens College, Queens, NY, USA
[2] Teacher for Children with Visual Impairments & Deaf-Blindness, Queens College, Queens, NY, USA

33. **European young investigators network for Usher syndrome**
Nagel-Wolfrum, K.[1], da Silva, S.[2], José Duarte, E.[3], Sliesoraityte, I. [4], Vaché, C.[5] and van Wijk, E.[6]
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[5] CHU (University Hospital), INSERM, Montpellier, France
[6] Radboudumc University Nijmegen Medical Centre, The Netherlands

34. **Considerations for Managing Hearing Loss for Children with Usher Syndrome**
Fredriksen, J., Kwilinksi, A. and Sands, T.
MED-EL Corporation, Durham, NC 27713, USA

35. **Blurring the Boundaries: Unmasking the genotype-phenotype overlap across Usher syndrome subtypes**
Sutti, S.[1], Lafferty, K.A.[2], Toledo, D.M.[2], Huang, Y.[1], Muirhead, A.[2], Abou Tayoun, A.N.[2], Shen, J.[2,3], Hernandez, A.L.[2], Campion, M.W.[1], Rehm, H.L.[2,3] and Amr, S.S.[2,3]
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Poster Abstracts

Session 1 Posters (Posters #1-16): Presenting authors are encouraged to be at their poster board during the coffee and lunch breaks on Thursday, July 10th.

Session 2 Posters (Posters #17-35): Presenting authors are encouraged to be at their poster board during the coffee and lunch breaks on Friday, July 11th.

Group 1- Diagnostics: Epidemiology and Population Genetics

1. **Targeted next generation sequencing as a comprehensive molecular diagnosis for Usher syndrome**

Aller, E.[1,2], Aparisi, M.J.[1,2], Fuster-Garcia, C.[1], Ayuso, C., Blanco-Kelly, F., Martínez Fernandez de la Cámara, C., Rodrigo RJaijo, T.[1,2] and Millán, J.M.[1,2,3]

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Abstract:

Background: Usher syndrome is an autosomal recessive disease that associates sensorineural hearing loss, retinitis pigmentosa and, in some cases, vestibular dysfunction. It is clinically and genetically heterogeneous. To date, 10 genes have been associated with the disease, making its molecular diagnosis based on Sanger sequencing, expensive and time-consuming. Consequently, the aim of the present study was the development of a comprehensive molecular diagnosis method for Usher syndrome, based on targeted NGS.

Methods: A custom HaloPlex panel for Illumina platforms was designed to capture all exons of the 10 causative Usher syndrome genes (*MYO7A*, *USH1C*, *CDH23*, *PCDH15*, *USH1G*, *CIB2*, *USH2A*, *GPR98*, *DFNB31* and *CLRN1*), two related USH genes (*HARS* and *PDZD7*) and the two candidate USH genes *VEZT* and *MYO15A.* A cohort of 44 patients suffering from Usher syndrome was selected for this study.

Results: We could detect 80% of expected mutated alleles, identifying 51 different mutations. These included 20 missense, 7 nonsense, 9 frameshifts, 9 intronic mutations and 6 large rearrangements.

Conclusions: Our developed targeted NGS method allowed us to detect both point mutations and large rearrangements in a unique experiment, minimizing the economic cost of the study, increasing the detection ratio of the genetic cause of the disease and improving the genetic diagnosis of Usher syndrome patients.

Acknowledgements: This work was supported by grant PI13/00638 from *Fondo de Investigaciones Sanitarias* (FIS) from the Spanish Government.

2. **Early Diagnosis of Usher Syndrome using Next-Generation Sequencing Panel Testing**

Muirhead, A. [1], Abou Tayoun, A. [1,2], Lafferty, K. [1], Hernandez, A.L. [1], Shen, J. [1,3], Rehm, H. [1,3] and Amr, S. [1,3]

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Abstract:

The OtoGenome Test is a 70 gene nonsyndromic hearing loss panel performed by next-generation sequencing (NGS) and is capable of detecting sequence and copy number variants (CNVs). It includes genes that are known to cause nonsyndromic hearing loss and syndromes that may initially present as nonsyndromic, such as Usher syndrome (USH). The onset of sensorineural hearing loss (SNHL) in USH patients is predominantly congenital; however retinitis pigmentosa (RP) presents later in life delaying a clinical diagnosis in affected individuals. Early diagnosis of USH through genetic testing provides anticipatory guidance and can change clinical management. Alternatively, a 9 gene USH panel run on the same platform can be ordered for individuals with a clinical diagnosis or suspicion of USH. Since the launch of the OtoGenome and the USH panel in September 2012, diagnostic testing was performed for 361 probands on the OtoGenome Test and for 39 probands on the USH Panel. For the OtoGenome cohort, 4% (14/361) were positive for a molecular diagnosis of USH and approximately 3% (13/361) had an inconclusive test result with one heterozygous pathogenic variant in a gene associated with USH. Taken together, genetic testing on the OtoGenome identified a clear or suspected diagnosis of USH in 7% of apparently nonsyndromic cases. For the USH panel cohort, 43% (17/39) of probands were positive, and 31% (6/39) were inconclusive with a suspected molecular diagnosis of USH. The average age at testing was earlier for the OtoGenome compared with the USH panel (7.3 years and 28.6 years respectively) for individuals whose results confirmed or suggested a diagnosis of USH. Of the 14 individuals whose OtoGenome results indicated a diagnosis of USH, 13 did not report retinal disease. In contrast, of the 39 completed Usher panels, 29 indicated RP or features suggestive of RP. Clinically significant variants in 7 out of 9 USH genes were detected, and the spectrum included SNVs (n=40), indels (n=22), and CNVs (n=7). CNVs, which accounts for 10% of all clinically significant variants identified in these two cohorts, cannot be detected by other sequencing platforms previously run by our laboratory (Sanger sequencing and oligo-hybridization array based sequencing) and a secondary assay was necessary to detect this class of variants. In summary, these results demonstrate the utility of comprehensive hearing loss testing using the OtoGenome Test, which is capable of detecting a broad range of genetic variation across a large number of hearing loss genes, for early diagnosis of Usher syndrome.

3. **Automated pathogenicity assignment methods for missense variants: application to usherin**

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Abstract:

USH2A is the most frequently mutated gene in the most common type of Usher syndrome. Its unusually large size (a cDNA of 18kb encodes usherin, a protein of 5,202 amino acids) has been yielding a high proportion of different variants predicting missense substitutions at the protein level. Assigning pathogenicity to such variants in this gene has always been challenging.
Automated classification methods for missense variants are numerous and varied, but none is able to exceed the 80-85% threshold of correct prediction rate. Following the release of a massive amount of data generated by second generation sequencing techniques, consensus methods based on the combination of the results obtained with several predictors have appeared. In addition, both single and consensus methods are now able to treat a high number of variants at the same time (batch mode).
In this study we have assessed 258 manually annotated missense variants (reference set) extracted from LOVD-*USH2A* (http://www.LOVD.nl/USH2A) using seven prediction tools, including 4 popular tools and 3 consensus methods. Sensitivity, specificity and correct prediction rates were computed and compared in order to define the most reliable method to assess missense variants in *USH2A*.
The selected method was then used to assign a pathogenic effect to a set of 466 missense variants identified in *USH2A* in the NHLBI GO Exome Sequencing Project (ESP, http://evs.gs.washington.edu/EVS/). Of these 466 variants, only 85, including the most common ones, overlapped with LOVD-*USH2A*, meaning that the majority were new.
Surprisingly, even after basic statistical correction, more than a hundred remained as potentially pathogenic, leading to a *USH2A* pathogenic missense carrier rate of about 1/130. These findings will be discussed and compared to other carrier rates computed using different sources.

	SIFT	Polyphen 2 (HumVar)	Polyphen 2 (HumDiv)	Mutation Assessor	SNPs&GO	CoVEC (wv)	CoVEC (svm linear)	CoVEC (svm radial)	Condel	PON-P2
%predicted	100.00	100.00	100.00	37.60	100.00	100.00	100.00	100.00	100.00	43.80
sensitivity	0.76	0.93	0.98	0.73	0.32	0.76	0.87	0.92	0.84	0.95
specificity	0.80	0.64	0.51	0.94	0.93	0.84	0.74	0.70	0.66	0.98
correct rate (all set)	0.78	0.79	0.76	0.33	0.61	0.79	0.81	0.81	0.76	0.42
correct rate (predicted)	0.78	0.79	0.76	0.89	0.61	0.79	0.81	0.81	0.76	0.96

Table: Assessing several computational methods for predicting missense pathogenicity in *USH2A*.
The reference dataset consisted of 258 missense variants annotated in LOVD-*USH2A* (135 defined as pathogenic, 123 as neutral). Polyphen 2 comes with two different models trained on different datasets HumDiv and HumVar. CoVEC is supplied with two main modes of calculations, a weighted vote (wv) and a Support Vector Machine with two models, linear and radial

4. **2014 update of LOVD-*USH2A* defines an extensive mutational spectrum and highlights missense hotspots**

Baux, D.[1], Blanchet, C.[2,3], Hamel, C.[3], Meunier, I.[3], Larrieu, L.[1], Faugère, V.[1], Vaché, C.[1], Claustres, M.[1,4,5] and Roux, A-F.[1,5]

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Abstract:

Alterations of *USH2A*, encoding usherin, are responsible for more than 70% of cases of Usher syndrome type II (USH2), a recessive disorder that combines moderate to severe hearing loss and retinal degeneration. The longest *USH2A* transcript encodes usherin isoform b, a 5,202-amino-acid transmembrane protein with an exceptionally large extracellular domain consisting notably of a Laminin N-terminal domain and numerous Laminin EGF-like (LE) and Fibronectin type III repeats (FN3). Mutations of *USH2A* are scattered throughout the gene and mostly private. Annotating these variants is therefore of major importance to correctly assign pathogenicity. Recently, we have added more than 150 new *USH2A* complete genotypes (representing 2,489 cumulated variations including 93 new mutations). Pooling this new data with the existing pathogenic variants already incorporated in USHbases from previous studies reveals several previously unappreciated features of the mutational spectrum. We show that parts of the protein are more likely to tolerate single amino-acid variations whereas others constitute pathogenic missense hotspots. We have found, in repeated LE and FN3 domains, a non equal distribution of the missense mutations which highlights some crucial positions in usherin with possible consequences for the assessment of the pathogenicity of the numerous missense variants identified in *USH2A*.

5. **Towards a comprehensive diagnostic test for Usher Syndrome and Non-Syndromic Deafness**

Cullup, T.[1], Drury, S.[1], Boustred, C.[1], Jenkins, L.[1], Lench, N.[1] and Bitner-Glindzicz, M.[1,2]

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Abstract:

We have developed a Next Generation Sequencing panel which provides comprehensive analysis of genes implicated in Usher Syndrome, as well as genes causative of a number of other syndromic deafness phenotypes and non-syndromic deafness. By using custom SureSelect enrichment followed by sequencing on the Illumina MiSeq, we have developed a laboratory protocol which provides data quality and turn-around time appropriate for use in a diagnostic setting. By combining high quality data with local clinical expertise, we are able to offer a valuable diagnostic service to patients and their families.

Our bioinformatics pipeline allows phenotype-specific analysis of gene subgroups and, together with post-pipeline automated filtering, time spent on manual analysis of likely-benign variants is minimised.

By using ExomeDepth[1] we have demonstrated the ability to call copy number variation in our data, including heterozygous deletions and duplications.

To date we have sequenced 40 Usher syndrome patients. Of these, mutations have been detected in 36, giving a detection rate of 90%.

We also present a comparison of techniques used for enrichment of target loci and modifications used to achieve high coverage across these regions, with 99% of target bases achieving the benchmark 30x coverage. In-house validation for our latest diagnostic panel calculates a minimum sensitivity of 97.6%, with 95% confidence, for detecting point mutations.

1. Plagnol, V. et al, A robust model for read count data in exome sequencing experiments and implications for copy number variant calling *Bioinformatics first published online August 31, 2012 doi:10.1093/bioinformatics/bts526*

Acknowledgements: This work has been supported by the Great Ormond Street Children's Charity

6. **Comprehensive Usher syndrome molecular diagnostic strategy**

Faugère, V.[1], Moclyn, M. [1], Garcıa-Garcıa, G. [2], Besnard, T. [1], Baux, D. [1], Vaché, C. [1], Larrieu, L. [1], Claustres, M. [1, 2, 3] and Roux, A-F. [1,2]

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Abstract:

Usher syndrome (USH) is a rare autosomal recessive disorder characterized by the association of sensorineural hearing loss (HL) and retinitis pigmentosa (RP). Both clinical and genetic heterogeneity are recognized. For long, molecular diagnosis has been challenging as at least eleven genes are known to be implicated and the 3 most frequently involved contain are large genes. *MYO7A* and *CDH23*, both responsible for USH1 contain 49 and 69 exons respectively, and USH2A, responsible for 80 % of the USH2 cases, contains 72 exons.

Over the last 10 years, we have developed an exhaustive approach, coupling halotype analyses at the different USH loci, cascade sequencing of the genes (including all exons and their boundaries) and array-CGH to identify large rearrangements.

As this approach remained laborious in terms of time and costs and is inefficient for atypic USH patients, we have designed a Next Generation Sequencing (NGS)-based workflow using a custom solution-based sequence capture method manufactured by Roche Nimblegen with the GS junior system (Roche 454). We have established criteria and thresholds for accurate generation and filtering

of the data, as well as prioritization and annotation of the variants. This is now automated in a publicly available tool named GSdot. The pathogenicity of candidate variants is investigated using tools dedicated to Usher syndrome (USHbases, USHVaM and USMA). All likely pathogenic variants are confirmed by Sanger sequencing and familial segregation when possible.

Because NGS is not yet efficient for detection of large rearrangements and as MLPA is only available for 2 Usher genes (*USH2A* and *PCDH15*), array-CGH is still the method of choice to complement sequencing. Indeed, we have shown that nearly 10% of the mutated alleles consist in copy number variations. A custom Sequence Capture Array focused on the Usher genes was designed and synthesized by Nimblegen, which allows the detection of pathogenic copy number variants as small as single exon deletions or duplications that would otherwise remain undetectable with NGS. This strategy, leads to a mutation detection rate higher than 90% of USH cases.

7. **Extending the gene panel size to provide a positive diagnosis for non-Usher patients presenting with hearing loss and retinitis pigmentosa**

García-García, G.[1], Faugère, V.[2], Moclyn, M.[2], Constantinides, S.[2], Baux, D.[2], Vaché, C., Koenig, M.[1,2,3], Claustres, M.[1,2,3] and Roux, A-F.[1,2]

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Abstract:

Our group has recently validated next generation sequencing (NGS) of targeted Usher exome as a reliable strategy for molecular diagnosis of Usher syndrome (USH). Indeed, we designed a NGS-based workflow using a solution-based capture method, which we applied to forty-seven Usher patients, either negative for USH gene mutations or carrying a single mutation after Sanger sequencing and array-CGH analyses. This test sample was used to define a pipeline to select the variants of interest. We observed a mutation detection rate of 90% and an analytical sensitivity of 98%. However, coverage was not sufficient in several regions, misalignments occurred in homopolymeric stretches and some patients, although clinically diagnosed with Usher syndrome, displayed no mutation after the analysis.

We now have access to an Illumina platform (MiSeq instruments). The higher throughput leads to a greater global coverage of the targeted regions and reduces time and costs as samples can be pooled. Moreover, a better variant detection is expected within the homopolymeric stretches or in regions with particular sequence complexity. In this study we are comparing two different methods for the targeted capture: Nimblegen SeqCap Choice (28 genes that include all USH genes and a selection of non syndromic hearing loss (NSHL) genes) and a new approach using Nextera Rapid Capture Custom Enrichment (Illumina). In this new panel we have increased the number of genes to 112: the genes tested in the first design, 36 genes responsible for arRP and 48 additional genes associated to NSHL. The data obtained from the Usher genes (common in both designs) will be used to compare the efficacy and accuracy of the two methods. Furthermore, with the new gene panel we extend the

analysis to other genes involved in Usher syndrome and associated diseases. This design will be applied to a new cohort of patients presenting either with non syndromic forms of arRP or HL. In addition, we expect to elucidate the molecular basis of patients, clinically diagnosed as Usher patients so far, but presenting with non syndromic RP and hearing loss.

8. **Rapid identification of new pseudoexon insertions in *USH2A***

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Abstract :

Usher syndrome (USH) is an autosomal recessive disorder affecting hearing and vision, characterized by clinical and genetic heterogeneity. *USH2A* is implicated in 80% of the USH2 cases. Approximately 10% of USH cases remain genetically unsolved after extensive molecular analysis of the different genes, that includes sequencing of the exons and their intronic boundaries combined to large rearrangements screening by aCGH. These unsolved cases represent patients who do not carry any mutation in any of the known USH genes and patients who carry a single mutation in one of the USH genes. For the latter group, the second mutation is likely to lie in unscreened regions such as in introns or promoters. This hypothesis is supported by the recent identification in USH2 patient of a pseudoexon in *USH2A* mRNA resulting from a point mutation in intron 40 (Vaché *et al.* 2012).
Deep intronic mutation identification in USH is challenging, as it requires mRNA analysis from patients' nasal cells biopsies. In addition, patients are not always easily reachable and biopsies often yield insufficient quantities of material to scan the transcript in full.
In order to circumvent this problem, we have developed a new approach to identify deep intronic variants in USH genes and evaluate their consequences on splicing.
We designed a solution-based capture of the entire *USH2A* gene that spans 800-kb followed by NGS. We investigated DNAs from 7 USH2 patients carrying a monoallelic mutation. We set up a bioinformatics pipeline to prioritize candidate variants. When possible segregation analysis was also used to filter out variants in cis to the monoallelic known mutation. The remaining variants were assessed for potential splicing effect using the MaxEntScan and Human Splicing Finder programs.

Thus, we identified three distinct novel deep intronic mutations in four unrelated patients. All were predicted to create a cryptic splice donor site leading to an out-of-frame pseudoexon insertion. We validated the abnormal splicing effect by minigene assays.
The effect of these mutations will be further confirmed on nasal epithelial cell RNAs and proteins from affected individuals.
Our approach using Next Generation Sequencing to analyse the entire genomic DNA sequence is not only applicable to other USH genes but also to any disease gene. Moreover, this study supports the hypothesis of pseudoexon insertions as a common class of mutations and establishes exon skipping as a suitable therapy to treat Usher syndrome in these patients.

Acknowledgments: This work was supported by UNADEV and SOS Retinite Association

9. **Estimation of Usher prevalence in Portugal**

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Abstract:

In rare conditions, a diagnosis can often take many years. Rare diseases face a problem common diseases doesn´t: lack of sufficient number of patients to achieve solid evidence on disease diagnosis, prognosis and therapeutics. Therefore the knowledge about them is quite scarce. Special combined efforts are needed to address them. That´s why specialized referral centres, interdisciplinary consultation and international networks are so important.

Usher syndrome (USH) is an autosomal recessive disease characterized by hearing loss, retinitis pigmentosa (RP), and, in some cases, vestibular dysfunction. It is clinically and genetically heterogeneous.

Clinically, USH is divided into three types. Usher type I (USH1) is the most severe form and is characterized by severe to profound congenital deafness, vestibular areflexia, and prepubertal onset of progressive RP. Type II (USH2) displays moderate to severe hearing loss, absence of vestibular dysfunction, and later onset of retinal degeneration. Type III (USH3) shows progressive postlingual hearing loss, variable onset of RP, and variable vestibular response.

In developed countries such as the United States, about four babies in every 100,000 births have Usher syndrome. With a relatively recent group studying common deaf blind patients we present a new cohort of 83 Usher patients in Portugal (8.3/100,000 inhabitants). We present an estimated prevalence of 3.6 USH1, 4.6.5USH2 and 0.1 USH3/ 100,000 births in Portugal.

Given the known phenotypic heterogeneity observed among Usher patients, a rather complete report on the phenotype is presented. Clinical observation showed an excess of males, some variability in audiograms, and a wide ophthalmologic variation.

We expect this work to improve the acquaintance of the Portuguese Usher patients' characteristics as well as add useful information for genotype-phenotype correlation.

10. **Marker of oxidative stress and inflammation in the aqueous humor of patient with retinitis pigmentosa**

Martínez-Fernández de la Cámara, C.[1], Salom, D.[2], Sequedo, M.D.[1], Hervás, D.[3], Aller, E.[1,4], Jaijo, T.[1,4], Millán, J.M.[1,4,5] and Rodrigo, R.[1]

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Abstract:

Retinitis Pigmentosa (RP) is a common form of hereditary retinal degeneration constituting the largest single cause of blindness in the developed world. It has been suggested that oxidative stress and inflammation probably play an important role in its pathogenesis. We measured the levels of total antioxidant capacity, free nitrotyrosine, thiobarbituric acid reactive substances (TBARS) formation, superoxide dismutase activity, protein, metabolites of the nitric oxide/cyclic GMP pathway, heme oxygenase-I, inducible nitric oxide synthase expression, TNF- alpha, IL-1 beta, IL-10 and IL-6 in aqueous humor or/and peripheral blood from 41 patients with non-syndromic RP, 15 patients with Usher syndrome and 60 subjects without systemic or ocular oxidative stress-related disease.

Multivariate analysis of covariance revealed that retinitis pigmentosa alters ocular antioxidant defence machinery and the redox status in blood. Patients with non-syndromic RP or Usher syndrome present low total antioxidant capacity including reduced superoxide dismutase activity and protein concentration in aqueous humor. Patients also show reduced superoxide dismutase activity, increased TBARS formation and upregulation of the nitric oxide/cyclic GMP pathway in peripheral blood. Inflammatory mediators IL-6 and TNF-alpha were also altered in the aqueous humor of RP patients.

Together these findings confirmed the hypothesis that non-syndromic RP or syndromic RP due to Usher syndrome are associated with reduced ocular antioxidant status, oxidative-nitrosative damage in the peripheral blood and ocular inflammation of these patients.

Acknowledgements: Authors are very grateful to the patients participating in the study and to their relatives, to ONCE and to RETINA COMUNIDAD VALENCIANA. This work was supported by the European Regional Development Fund, Institute of Health Carlos III, PI10/01825 and PI12/0481 from the Spanish Ministry of Economy and Competitiveness (MEC). CIBERER is an initiative of the Institute of Health Carlos III from the MEC. Regina Rodrigo has a research-contract SNS Miguel Servet (CP09/118) from Institute of Health Carlos III.

11. **The prevalence and severity of cystic macula lesions in genetically confirmed Usher syndrome patients**

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Abstract:

Purpose: Cystic lesions in macula are common feature of retinitis pigmentosa, although there is little information about cystic lesions in syndromic retinitis pigmentosa cases. The purpose of this study was to investigate the prevalence and severity of cystic macula lesions in a large cohort of genetically confirmed Usher syndrome affected patients.

Methods: Prospective study enrolled 62 eyes of 76 patients (mean 42±14 years) with Usher syndrome (USH). All patients were found to carry at least one mutation and 67 (88%) were found to carry two mutations in USH associated genes. All patients underwent in-depth phenotypic examination, including visual acuity, color vision, visual field testing, full-field electroretinography, multifocal electroretinography and retina imaging. Only high quality cross-sectional images through the fovea from the right eye were evaluated by the means of optical coherence tomography (*Spectralis HRA+OCT, Heidelberg Engineering, Dossenheim, Germany*). The cystic lesions severity was graded as mild, moderate and severe assuming the width of the total cystic lesion area and the total quantity of detectable cystic lesions at fovea scan. Intra- and inter-grader reproducibility was evaluated.

Results: Cystic macula lesions were observed in 23(37%) of 62 USH eyes; while distribution by USH types was: 6(40%) of 15 USH1 eyes, 14(32%) of 44 USH2; 3(100%) of 3 USH3. Monolayer lesions were observed in 16(70%) and multilayer 7(30%) cases. Retinal layers affected by cystic lesions were: 14(47%) outer nuclear layer; 12(40%) inner nuclear layer; 3(10%) retinal ganglion cell layer and 1(3%) inner plexiform layer. The prevalence of cystic macula lesions severity was as following: 12(52%) mild, 5(22%) moderate, 6(26%) severe, without significant difference between USH types. Intra-grader reproducibility was of 98%, while inter-grader reproducibility was of 96%.

Conclusions: Cystic macula lesions are a common complication in patients with genetically confirmed Usher syndrome, while cystic lesions severity might vary from mild to severe. Proposed cystic lesions grading system seems to be efficient in a complicated Usher cases and eventually could be used for macular pathology objective tracing in a clinical therapy trials.
Acknowledgments: *ERAREI N°58: Eur-USH, http://eur-ush.eu, http://www.e-rare.eu*

Registration: *http://www.clinicaltrials.gov N°: NCT01954953*

12. **Vestibular function in patients with Usher Syndrome**

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Abstract:

Usher syndrome (USH) is a clinically heterogeneous condition characterized by sensorineural hearing loss (SNHL), progressive retinal degeneration, and vestibular dysfunction. Although it is commonly defined as the leading cause of deaf-blindness, USH type 1 and 3 can also cause vestibular dysfunction.

The focus is often on hearing loss and retinitis pigmentosa (RP), but little is known about vestibular function in these patients, even though USH patients can have considerable vestibular dysfunction. The presence of vestibular dysfunction in a child with SNHL, manifested by late walking or clumsiness, could suggest USH. However, signs of vestibular dysfunction in children can be underappreciated and are highly variable.

Minimal clinical research has been reported on vestibular function in infants and children with genetically documented USH, even though the early and persistent balance dysfunction in these children adds considerably to their disability, especially as their vision impairment worsens. Moreover, the heterogeneous nature of the USH genotype-phenotype is increasingly apparent, suggesting that patients with any of the USH types may be at risk for vestibular dysfunction, not just those with USH type 1. If vestibular function in infants and young children with genetically confirmed USH was better defined, these findings could be used prospectively to make an earlier diagnosis of USH and develop effective management strategies for this syndrome. Also, vestibular rehabilitation has shown efficacy in improving motor development and balance in children with SNHL.

This study aims to characterize vestibular function in patients with Usher syndrome. Subjects with a genetically confirmed diagnosis of USH undergo a series of vestibular tests and this data will be correlated with genetic diagnosis and audiometric and ophthalmologic results.

Characterization of vestibular function in this population could benefit USH patients in multiple ways. First, our results could facilitate earlier diagnosis of USH. Secondly, this research could further define the relationship between specific genetic mutations and clinical findings of hearing and balance. Furthermore, it may help to predict if many children with USH would benefit from vestibular-specific therapies, such as vestibular rehabilitation.

13. **Scotopic and photopic ERG responses in pediatric patients with Usher Syndrome**

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Abstract:

Purpose: To evaluate scotopic and photopic ERG responses in pediatric patients with a genetic diagnosis of Usher Syndrome, a recessively inherited ciliopathy characterized by hearing loss and retinal degeneration affecting both rods and cones.

Methods: Twenty-two patients (age 2 months to 23 years) with USH2A (n=13) or MYO7A (n=9) disease were studied. ERG responses to a range of full-field scotopic and photopic single flash and 30 Hz flicker stimuli (including the ISCEV standard conditions) were recorded and compared to responses in healthy controls (n=72). A model of the activation of phototransduction was used to estimate rod photoreceptor sensitivity (S_{ROD}) and saturated amplitude (R_{ROD}). Post-receptor b-wave sensitivity was characterized by the stimulus that produced a half maximum response (log σ) and saturated b-wave amplitude (V_{MAX}). Dark adapted thresholds were estimated using a two-alternative, forced choice method in all patients.

Results: Responses to 30 Hz flickering stimuli were detected in all USH2A patients (range 7 to 169, median 99 µV) and all MYO7A patients (range 1 to 18, median 10 µV). Photopic b-wave amplitude was within the normal range in 9/13 of the USH2A patients and 0/9 of the MYO7A patients. In the seven MYO7A patients whose photopic b-wave was detectable, the responses were less than 20% of the normal mean. Scotopic b-waves were detectable in all USH2A patients but only six MYO7A patients; only four USH2A patients had normal scotopic b-wave amplitudes. Responses were sufficient for estimation of both photoreceptor and post-receptor response parameters in nine USH2A and only two MYO7A patients. For these 11 patients, deficits in scotopic post-receptor b-wave sensitivity were greater than deficits in rod photoreceptor sensitivity. For the 22 patients, the median threshold elevation was 0.9 (range 0.13-2.77) log units, and did not differ between USH2A and MYO7A patients. For 14/22 patients, threshold elevation was outside the 99% prediction interval for normal, whereas 21/22 had log σ values outside the 99% prediction interval for normal.

Conclusion: The greater deficit in post-receptor than photoreceptor sensitivity is consistent with a ciliopathy. In this sample, ERG log σ was more often abnormal than the dark adapted threshold. Therefore, the ERG may be a more sensitive detector of retinal dysfunction than the dark adapted threshold in children at risk for Usher Syndrome.

Acknowledgements: Supported by NIH EY 010597

Group 2- Functional Genetics: Preclinical Studies and Therapy

14. **Developing *in silico* reconstructed ancestral AAV vectors for retinal gene therapy**

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Abstract:

Replication-deficient adeno-associated virus (AAV) has been the preferred delivery system for most clinical trials studies involving gene delivery and supplementation to the eye. These clinical trials and innumerous pre-clinical studies have provided us with concrete evidence that AAV vectors are a safe and effective tool to treat inherited retinal disorders. However, AAV is endemic in humans and the pre-existing immunity raised from AAV natural infections are known to block gene transfer and give rise to adverse immunotoxicity. This has severe implications such as restricting the pool of putative gene therapy patients and therefore limiting the broader application gene supplementation-based therapies can offer.

We hypothesized that ancestral AAVs would be less susceptible to the immunity raised by contemporary AAVs. Using maximum likelihood methods, we therefore reconstructed a probabilistic sequence space around an ancestral node of the AAV capsid gene phylogeny. This space was comprised in a synthetic DNA library and evaluated for viral production and gene transfer functionality. Members from this library were shown to yield high titer infectious particles, which we next evaluated for retinal gene transfer.

A gene expression cassette of eGFP under a ubiquitous CMV promoter was packaged into three selected ancestral virus capsids. Alongside the best control vectors currently used for retinal studies (AAV2/2, AAV2/8 and AAV2/9), the ancestral capsids were administered at matching titers both into the subretinal space and the vitreous of wildtype adult mice. The tropism of these ancestral AAV particles in the different retinal tissues was analyzed by fundus imaging at 1 and 4 weeks post-injections and immunohistochemistry of eGFP expression through retinal sections.

Fundus imaging of eGFP fluorescence at 1 week post-injection showed significant differences amongst the transduction efficiencies of the ancestral vectors compared to the controls and expression was maintained and stable up to at least 15 weeks post-injection. Histological analysis shows that the ancestral AAV viruses are extremely efficient at targeting photoreceptors and RPE cells. To date, our results show that all three ancestral AAV particles are fully capable of transducing retinal tissues *in vivo*, with both RPE and photoreceptors showing strong eGFP expression. These results, coupled with further studies about the seroprevalence of these ancestral particles in the eye, could help reduce current patient exclusion criteria based on current AAVs seroprevalence therefore provide an alternative system for future use in human gene therapy approaches.

15. **NINL^{isoB} and DZANK1 cooperate in assembling the cytoplasmic dynein 1 motor complex, a process essential for photoreceptor outer segment formation in zebrafish**

Dona, M.[1], Hetterschijt, L.[2], Tonnaer, E.[1], Peters, T.[1], Van Beersum, S.[2], Bergboer, J.[2], Van Reeuwijk, J.[2], Texier, Y.[3], Toedt, G.[4], Gibson, T.[4], Boldt, K.[3], Ueffing, M.[3], Roepman, R.[2], Kremer, H.[1,2] and Van Wijk, E.[1]

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Abstract:

The most common cause of Usher syndrome type 2 are mutations in the gene encoding *usherin* (USH2A). In 2009, the centrosomal ninein-like protein NINL^{isoB} was identified as a novel interaction partner of USH2A^{isoB}. In fact, after a detailed study of the interaction repertoire of NINL^{isoB} it appeared to be a key connector of three large retinal ciliopathies: Leber Congenital Amaurosis, Bardet-Biedl syndrome and Usher syndrome. In order to gain insight into the pathogenic mechanisms underlying these three retinal degenerative disorders, the role of NINL^{isoB} was scrutinized using a combination of (affinity) proteomics and morpholino-induced knockdown studies in zebrafish. A bovine retinal cDNA library screen identified DZANK1 as an important novel interaction partner of NINL^{isoB}. Subsequent morpholino-induced knockdown in zebrafish embryos of either ninl, dzank1, or both ninl and dzank1 resulted in defective photoreceptor outer segment formation, accumulation of trans Golgi-derived vesicles, and rhodopsin mislocalization. In addition, retrograde melanosome transport was impaired in these morphant zebrafish. Affinity proteomics revealed that NINL^{isoB} and DZANK1 associate with complementary subunits of the cytoplasmic dynein 1 complex. These subunits together are essential for the assembly and proper functioning of this motor unit. Our results support the hypothesis that the NINL^{isoB}-DZANK1 protein module is essential for the proper assembly of the cytoplasmic dynein 1 motor complex in photoreceptor cells of the zebrafish retina. Absence of either NINL^{isoB} or DZANK1 will result in a defective transport of molecules necessary for photoreceptor outer segment formation, maintenance and/or functioning (e.g. rhodopsin and USH2A) towards the base of the connecting cilium.

Acknowledgements: Stichting Nederlands Oogheelkundig Onderzoek', 'Stichting Blindenhulp', 'Stichting Researchfonds Nijmegen', 'Foundation Fighting Blindness', 'Landelijke Stichting voor Blinden en Slechtzienden', NWO-ZonMW

16. **Gene augmentation therapy to treat Usher Syndrome Type IC**

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Abstract:

Numerous mouse models of Usher Syndrome (USH) have been identified or engineered over the past decade. Interestingly from over seven models that target harmonin (USH1C), only one reproduces both auditory and retinal deficits. The Ush1c.216G>A (Lentz et al. 2007, 2009) is a knock-in model with the cryptic splice site mutation found in French-Acadian USH1C patients. The mutation results in abnormal or absent ABR responses in homozygous mutant mice at one month of age indicating the mice are profoundly deaf. Cochlear histology at P30 shows disorganized hair cell rows, abnormal bundles, and loss of both inner and outer hair cells along the organ (Lentz et al. 2013).

To determine if gene therapy may be a viable technology to treat USH1C patients, we assessed the development of hair cells in the Ush1c.216G>A mutant mouse. We analyzed hair bundle morphology, the developmental expression of voltage dependent channels as well as the presence of functional mechanosensitive ion channels in wild type, heterozygous and homozygous littermates during the first postnatal week. While disorganized hair bundles were evident in homozygous mice all along the organ of Corti for both inner (IHCs) and outer hair cells (OHCs), normal voltage-dependent currents and resting potentials were observed for all genotypes. Direct measurement of transduction currents evoked by stiff-probe deflections of OHC bundles mice revealed similar properties in Ush1c.216G>A heterozygous and wild-type littermates with maximal currents (Imax) of 475 ± 54pA (n=9, ±S.D., Apical half P4-P6) and 456 ± 74pA (n=5, ±S.D., Apical half P4-P7) respectively and at a holding potential of -64mV. In contrast OHCs from homozygous mutants had significantly smaller currents with a mean Imax of 170 ± 80pA (n=24 ±S.D., range: 31pA to 293pA, Apical half, P3-P6). Adaptation was not always present in homozygous mutants and was typically slower with decreased extent of adaptation, similar to that described for other harmonin mutants. More complete adaptation was also occasionally observed in heterozygous mutants. This work therefore demonstrates that hair cells of Ush1c.216AA mutant mice remain functional during the first postnatal week.

To assess gene therapy approaches to treat Ush1c.216AA, utricles and organ of Corti from neonatal Ush1c.216AA were transfected with adeno-associated viral vectors (AAV2) expressing different isoform of USH1C labeled with a fluorescent dye. Interestingly differential targeting of each isoform was evident by immunohistolabeling. We are now assessing mutant mice exposed to these vectors to determine if gene augmentation improves hair bundle morphology and hair cell physiology both *in vitro* and after round window injection of neonatal mutant mice.

Acknowledgements: This work has been supported by the Manton Center for Orphan Disease Pilot Award 2011 to GSGG (Boston Children's Hospital), a generous donation from Kids B Kids as well as a faculty fellowship from Harvard Medical School to SHA.

17. **Translational read-through of non-sense mutations leading to Usher syndrome and related ciliopathies**

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Abstract:

Purpose: The human Usher syndrome (USH) is the most frequent cause of inherited combined deaf-blindness, distinguished into three clinically and genetically heterogeneous subtypes. So far, no effective treatment for the retinal degeneration of USH exists. The majority of USH genes are large and frequently spliced, therefore gene augmentation therapies are difficult to apply. Approximately 20% of all disease-causing mutations in USH genes are in-frame nonsense mutations. The skipping of nonsense mutations by translational read-through inducing drugs (TRIDs) has become a promising pharmacogenetic strategy for degenerative diseases. Here we evaluate TRID efficacy on different USH causing nonsense mutations in *USH1C*, *USH2A* and *CLRN1* (USH3A). Furthermore, we included nonsense mutations in other ciliopathy genes associated with retinitis pigmentosa, namely the nephronophthisis gene *NPHP4* and Bardet Biedl syndrome genes *BBS1* and *BBS10*.

Methods: We generated reporter constructs coding for disease-related nonsense mutations in *USH1C* (p.R155X), *USH2A* (p.W3955X, p.Y4318X, p.R4608X), *CLRN1* (p.Y63X, p.Y176X), *NPHP4* (p.L104X) as well as *BBS1* (p.G73X, p.Q291X) and *BBS10* (p.E19X, p.Y321X). We quantified TRIDs induced read-through in transient transfected HEK293T cells by quantitative immunofluorescence microscopy and Western blot analyses. We evaluated the retinal toxicity by applying TUNEL staining on TRID treated organotypic retina cultures.

Results: Our work focused on the 3rd and 4th generation designer aminoglycosides NB84 and NB124, as well as the small organic compound PTC124. We demonstrated the recovery of protein expression after TRIDs application for all analyzed nonsense mutations. Efficacies in cell culture experiments varied for the genes analyzed and for the different TRIDs applied. TUNEL assays of the analyzed TRIDs revealed an enhanced biocompatibility of NB84, NB124 and PTC124 compared to clinical approved aminoglycosides like gentamicin, with PTC124 having the most favorable safety profile.

Conclusion: Our data indicate that the improved biocompatibility of NB84, NB124 and PTC124 combined with the excellent read-through efficacies emphasizes the potential of TRIDs as a personalized treatment option for USH and other retinal disorders caused by in-frame nonsense mutations.

Acknowledgements: German Ministry of Education and Research (E-Rare-2, the ERA-Net for Research on Rare Diseases) “EUR-USH”, FAUN-Stiftung, Nuremberg, Foundation Fighting Blindness (FFB), EU FP7 “SYSCILIA”

18. **Generation of precise zebrafish models of Usher gene mutations**

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Abstract:

The genetic complexity of Usher syndrome creates unique challenges in developing gene-specific treatments for USH patients. In addition to the large number of genes that are implicated in the disease, mutations in different regions of the same gene contribute additional heterogeneity. These variables

underscore the importance of reproducing human USH lesions in model systems as precisely as possible to study the specific molecular and cellular consequences of a given mutation. To augment mouse and cell culture models, we have used new gene editing technologies to generate targeted, heritable mutations in zebrafish Usher genes.

Although zebrafish mutants have been previously reported for a number of USH genes, we now have the ability to direct the position of the lesion with great precision. Transcription activator-like effector nuclease (TALEN) and clustered regularly interspaced short palindromic repeats (CRISPR) gene editing tecnologies allow us to induce double stranded breaks within a specified region of just a few nucleotides. To date, we have successfully generated mutant lines for four zebrafish Usher genes: *ush1c, cdh23, ush1g* and *ush2a*.

We injected RNA constructs containing the target sites and encoded endonucleases into one-cell stage zebrafish embryos to generate indels at selected exonic locations. We assayed the presence and frequency of deleterious induced lesions by sequencing genomic DNA from the founder generation, and confirmed germline transmission by sequencing DNA from offspring. Mutations in founders are mosaic, so we selected individuals who passed on frameshift indels to at least 60% of their offspring. We observed such high efficiency with USH1 targets that a phenotype can be observed in a subset of the injected founders. Roughly 40% of the embryos injected with one of the USH1 constructs had swimming and balance behavior consistent with mechanosensory hair cell dysfunction. Hair bundles in injected animals or their progeny were disrupted, consistent with previously published zebrafish USH1 mutants.

We are building a collection of functional null mutations to serve as genetic backgrounds for pathogenicity and interaction tests as well as for use in traditional loss of function studies. We are also generating mutations that precisely match particular patient genotypes to assess pathogenicity of specific gene variants. These targeted mutations will provide new insights into Usher protein function and an outstanding system in which to validate emerging therapies.

Acknowledgment: This work was supported by NIH Grants DC004186 DC010447 and HD22486, and generous contributions from Vision for a Cure and The Megan Foundation.

19. **Exon-skipping as a promising therapeutic approach for treatment of retina degeneration in *USH2A* pseudoexon 40 patients**

Slijkerman, R.W.N., Vaché, C., Dona, M.D., García-García, G., Claustres, M., Hetterschijt, L., Peters, T.A., Collin, R.W., Kremer, H., Van Wijk, E. and Roux, A-F.

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Abstract:

Usher syndrome (USH) is the most common cause of combined deaf-blindness in man. The hearing loss can be partly compensated by providing patients with hearing aids or cochlear implants but for the loss of vision currently no treatment is available. Patients are categorized into three types (USH1, USH2, and USH3) based on the presence, progression and the age of onset of their clinical symptoms. In general, mutations in the *USH2A* gene are the most frequent cause of USH explaining up to 50% of all patients worldwide. The *USH2A* transcript is built up by 72 exons that together encode Usherin, a large transmembrane protein consisting of 5,202 amino acids. In the retina, Usherin is found to be expressed in the region of the photoreceptor connecting cilium and in the synapse, whereas in the inner ear this protein is present in the synaptic region and hair bundles of hair cells. Recently, we reported the identification of the first deep intronic mutation in the *USH2A* gene leading to the inclusion of a pseudoexon (PE40). Insertion of PE40 in the *USH2A* transcript subsequently results in a truncated protein lacking the C-terminal half including the transmembrane and intracellular region, if translated. In this study we explored the therapeutic potential of antisense oligonucleotides (AONs) to restore the native *USH2A* transcript. Our strategy is to induce skipping of PE40 by interfering with the splicing machinery in the PE40 region. For this purpose we used engineered AONs with complementary chemical backbones (phosphorothioate and morpholino) directed against intron-exon bounderies and exonic splice enhancer (ESE) regions of PE40. In this way we were able to induce the skipping of PE40 from the majority of the mature mRNA transcripts, predicting the translation of fully functional wild-type Usherin. Following this approach we expect to be able to stop the progression of this devastating blinding disorder and provide *USH2A* PE40 patients a prospect on vision in the future.

Acknowledgments:
This work is financially supported by the Dutch Organisation for Scientific research (NWO Veni grant 016.136.091), the Dutch Organisation for Health Research and Development (ZonMW E-rare grant 40-42900-98-1006) and the French national union of blind and visually impaired people (UNADEV).

20. **Direct interaction of the Usher syndrome proteins SANS (USH1g) and USH2a**

Sorusch, N., Bauß, K. and Wolfrum, U.

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Abstract:

The human Usher syndrome (USH), mainly affecting the ciliated sensory cells of the inner ear and the retina, is a complex ciliopathy. USH is a genetically and phenotypically heterogenous disorder with tree clinical types (USH1-3) and additional atypical cases with overlapping phenotypes. While USH1 is the most severe phenotype, USH2 is the most common form of USH. We and others have previously elucidated an USH1-USH2 protein network at the periciliary membrane of photoreceptor cells. This network is organized by whirlin (USH2d) and SANS (scaffold protein containing ankyrin repeats and SAM domain; USH1g) through multiple direct interactions to several other USH1 and USH2 proteins. There is growing evidence that it serves in protein transport from the Golgi apparatus to the photoreceptor outer segment. To gain further insights into the function of periciliary USH1-USH2 protein network, we analysed the putative binding ability of SANS to the USH2 transmembrane protein USH2a.

The direct interaction of USH2a with the central domain of SANS was identified via GST-pulldown analysis and was confirmed in co-transfection assays in Hek293T cells. We pinpointed the binding of the C-terminus of USH2a to the central of SANS. Phosphorylation of SANS by treatment of cell lysates with the phosphatase inhibitor ocadaic acid, revealed the phosphorylation dependency of the SANS-USH2a interaction. We showed, by means of co-precipitation experiments with SANS deletion constructs, that SANS and USH2a form a ternary complex together with whirlin. Immunofluorescence and immunoelectron microscopy revealed that these protein network components were localized in the periciliary region of photoreceptor cells. The periciliary localization of the protein complex was additionally confirmed by proximity ligation assays.

In conclusion, our data further elucidate the periciliary membrane USH1-USH2 protein complex. The scaffold proteins SANS (USH1g) and whirlin (USH2d) may play in concert, organizing this complex by differential binding the transmembrane protein USH2a. Furthermore we provide evidence that the molecular composition of the periciliary membrane complex and its function is controlled by phosphorylation state of SANS, regulating its interaction with USH2a. This may also contribute to cargo sorting in the periciliary compartment. Together, the direct interaction between the USH1g protein SANS and the USH2 proteins USH2a and whirlin (USH2d) underlines the close molecular alliance of USH type 1 and type 2, with overlapping retinal phenotypes in USH patients as a logical consequence.

Supports: German Research Council (DFG, GRK-1044); EC FP7/2009/241955 (SYSCILIA); FAUN-Stiftung; Pro Retina Germany.

21. **Optimization of AAV gene delivery vectors to minimize toxicity in the mouse retina for the treatment of Usher Syndrome 3A**

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Abstract:

Purpose: Usher syndrome type III (USH3A) is an autosomal recessively inherited disorder as a result of mutations in the Clarin-1 (*CLRN1*) gene. The patient phenotype includes sensorineural hearing loss as well as progressive retinal degeneration. Previously, our lab have shown that overexpression of AAV-clarin can be toxic for the retina. This study utilizes various rAAV vectors containing wild-type human *CLRN1* in order to assess for a safe optimal delivery method, titer, and promoter for USH3A gene therapy.

Methods: AAV quadruple capsid mutant (Y272F+Y444F+ Y500F+Y730F) vectors were generated with a human *CLRN1* cDNA with a hemagglutinin (HA) tag on the C-terminus driven by either a ubiquitous

CBA promoter or GRK photoreceptor specific promoter. These constructs were injected either subretinally or intravitreally into wild-type C57BL/6 mice. A full strength virus (viral titer of 8.43 $x10^{12}$ vg/ml) as well as serial dilutions of sc-smCBA-h*CLRN1*-HA were tested out to 1:1000 (viral titer of 8.43 $x10^{9}$ vg/ml). The sc-GRK-h*CLRN1*-HA vector was used at a viral titer of 2.1 $x10^{12}$ vg/ml. Retinal function and morphology were analyzed up to 10 months post-injection by electroretinography (ERG) and histology using an anti-HA antibody.

Results: The CBA driven *CLRN1* construct was toxic upon subretinal injection with a full strength viral titer. Scotopic ERG response amplitudes were significantly reduced in treated eyes, and histological analysis revealed that there was significant photoreceptor loss at 6 weeks post-injection and was more severe near the site of injection. Strong human Clrn1 expression was seen in both RPE and photoreceptor cells. This retinal toxicity was not seen following an intravitreal injection approach which targets ganglion, Muller, inner retina, and photoreceptor cells. There appeared to be less photoreceptor cell death using the GRK1 construct which targets Clrn1 specifically to photoreceptor cells. This could be due to the lower titer of the GRK1 vector compared to the CBA vector, or to the fact that Clrn1 expression was restricted to photoreceptor cells thus avoiding potential toxicity from expression in the RPE.

Conclusions: The vector delivery dose is critical when using a CBA promoter to drive *CLRN1* expression through a subretinal approach. It is believed that endogenous *CLRN1* is expressed at low levels within the retina, and it is therefore crucial to employ therapeutic vectors expressing *CLRN1* at similar levels. This can be achieved by either using a photoreceptor specific promoter, an intravitreal method of delivery, or by careful vector dosing.

Support Detail: Usher III Initiative, Inc.; EY021721; RPB, Inc.

22. **An Usher-like protein complex regulates intestinal brush border assembly**

Crawley, S.W.[1], Shifrin, D.A.[1], Grega-Larson, N.E.[1], McConnell, R.E.[1], Benesh, A.E.[1], Mao, S.[1], Zheng, Y.[2], Zheng, Q.Y.[2], Nam, K.T.[3], Millis, B.A.[4], Kachar, B.[4], and Tyska, M.J.[1]

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Abstract:

Transporting epithelial cells build apical microvilli to increase membrane surface area and enhance absorptive capacity. The intestinal brush border provides an elaborate example with tightly packed microvilli that function in nutrient absorption and host defense. Although the brush border is essential for physiological homeostasis, its assembly is poorly understood. We found that brush border assembly

is driven by the formation of Ca(2+)-dependent adhesion links between adjacent microvilli. Intermicrovillar links are composed of protocadherin-24 and mucin-like protocadherin, which target to microvillar tips and interact to form a trans-heterophilic complex. The cytoplasmic domains of the microvillar protocadherins interact with the scaffolding protein, harmonin, and myosin-7b, which promote localization to microvillar tips. Finally, a mouse model of Usher syndrome lacking harmonin exhibits microvillar protocadherin mislocalization and severe defects in brush border morphology. These data reveal an adhesion-based mechanism for brush border assembly and illuminate the basis of intestinal pathology in patients with Usher syndrome.

23. **The kinesin kif2a interacts with the Usher syndrome 1g protein SANS and kif2a is involved in the ciliogenesis of primary cilia**

Tebbe, L., Sorusch, N. and Wolfrum, U.

Cell and Matrix Biology, Institute of Zoology, Johannes Gutenberg-University of Mainz, Germany

Abstract:

The human Usher syndrome (USH) is the most common form of inherited combined deaf-blindness. It is genetically heterogeneous and can be clinically divided into three subtypes (USH1-3). Mutations in USH genes lead to profound inner ear defects and degeneration of the retina. The ten so far identified USH genes encode proteins of diverse protein families. Previous studies revealed that all known USH1 and USH2 molecules are integrated in protein networks. To get more insights into the USH protein networks and their cellular functions we performed yeast-two hybrid (Y2H) screens.
We identified the kinesin superfamily protein 2a (kif2a) as a putative binding partner for SANS (scaffold protein containing ankyrin repeats and SAM domain) (USH1g) by an Y2H screen of a retinal cDNA-library. Kif2a belongs to the kinesin subfamily 13 which is characterized by a motor domain localized in the central position. It is known that kif2a's motor activity leads to the depolymerization of microtubules making kif2a essential for the correct formation of the mitotic spindle. Furthermore depletion of kif2a leads to an aberrant development of the brain *in vivo* characterized by an increase of axonal collateral branching.

We validated the interaction between SANS and kif2a via reciprocal Y2H-assays, GST-pull downs and by assays in cell culture, namely by GFP-Trap®-A analyses, and membrane targeting assays. In addition, we detected a partial co-localization of kif2a and SANS in the photoreceptor cilium of the mouse retina by high resolution indirect immunofluorescence. In starved IMCD3 cells, kif2a was also present in the primary cilium. Furthermore, we depleted kif2a by siRNA in starved IMCD3 cells. The depletion of kif2a led to a significant increase of the length of primary cilia, demonstrating a regulative role of kif2a in ciliogenesis of primary cilia.

We conclude that the USH1g protein SANS and kif2a may play an important role in the regulation of primary cilia ciliogenesis. This gives rise to the hypothesis that the SANS/kif2a-complex may participate to the dynamic of microtubules in vertebrate photoreceptor cilia. Defects in the complex components

may lead to deregulation of the ciliary microtubule system and the dysfunction of photoreceptor cells, which might be a cause for the retinal degeneration characteristic for the pathogenesis in USH patients.

Supports: German Research Council (DFG, GRK-1044); EC FP7/2009/241955 (SYSCILIA); FAUN-Stiftung; Pro Retina Germany

24. **Comparison of dual AAV vector approaches for large gene delivery to the retina**

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Abstract:

Adeno-associated virus (AAV) has established itself as one of the most important gene therapy vectors. It is not known to cause any human disease, it transfects several cell types efficiently, and it is relatively easy and cost-effective to produce in large quantities. However, a major hindrance in its applicability is its small size, allowing transportation of only ~5 kbp of genetic material. This limitation requires the use of minimal regulatory elements, compromising optimal transgene expression parameters, but more importantly it straight out prevents AAV mediated gene transfer of larger genes, including major retinal disease genes, including several Usher genes. However, due to the numerous desired properties of the AAV, researchers have sought for novel methods to overcome its size limitation to enable its use as a carrier of larger transgenes for clinical gene therapy purposes. One such method is using a dual vector system, where the transgene construct is divided into two parts and delivered into the cells by two separate AAV vectors. Due to proper construct design and properties of the virus itself, the two genomes can come together and reconstitute the full length transgene.

In this study we have generated a reporter transgenic construct mimicking a transgene too large for the AAV to transport. We divided the construct into two vectors by previously validated dual vector strategies. The correct reconstitution of our construct results in production of β-galactosidase, an enzyme which amount in a tissue is easy to quantify. Thus, by using this construct we have been able to accurately compare *in vitro* and *in vivo* in the mouse retina the relative efficiencies of different dual vector strategies and how well they function compared to a single vector transgenic construct. Our results show that the best dual vector approaches provide gene expression levels of over 10% from single vector at matching titers. However, importantly, signal from dual vectors increases roughly linearly with increasing vector doses, suggesting that clinically relevant transgene expression levels can be achieved by using higher amounts of dual vectors. Further, the most efficient approach utilizes the use of an artificial intron in the construct, indicating that similar efficiencies can be obtained regardless of the actual transgene sequence. Thus, our data adds to previously published results suggesting that

dual AAV vector strategies may be a valid option in clinical gene therapies requiring transport of large genes.

25. ***De novo*** **mutations in *MYO7A* and *USH2A* genes**

Vaché, C.[1], Baux, D.[1], Faugère, V.[1], Larrieu, L.[1], Bitner-Glindzicz, M.[2], Hamel, C.[3], Claustres, M.[1, 4, 5] and Roux, A-F.[1, 4]

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Abstract:

Purpose: The purpose of this study is to report two *de novo* causative mutations in *MYO7A* and *USH2A* genes, the two major genes implicated in Usher syndrome. *USH2A* is also frequently implicated in nonsyndromic autosomal recessive retinitis pigmentosa.

Methods: Clinical characterizations and genetic studies were performed on two unrelated patients and their respective family members.
DNA samples from the probands were sequenced to screen for the causative mutations of their diseases. Variations detected were further analyzed by sequencing in family members and microsatellite marker studies at the implicated locus were also performed. Pathogenicity of each novel variant was investigated and a classification was proposed in accordance with CMGS guidelines and following our classification prediction tool USMA.

Results: The first proband presented with clinical symptoms of Usher type 1. *MYO7A* sequencing identified the p.(Arg1240Gln) mutation and the novel missense p.(Gly1191Asp) variant. The p.(Arg1240Gln) mutation was carried by the paternal allele while the p.(Gly1191Asp) variant was absent in both parents. Results obtained from *in silico* studies suggested a pathogenic effect of this *de novo* alteration. Eventually, allele-specific amplifications have established the trans position of the two mutations.

The proband of the second family suffered from nonsyndromic retinitis pigmentosa. Sequencing of the coding region and intron-exon boundaries of *USH2A* identified the presence of the p.(Cys759Phe) mutation. The other alteration identified in this study was the novel missense variant p. (Leu2451Pro) classified as likely to be pathogenic by our *in silico* analysis. The p.(Cys759Phe) mutation was carried by the maternal allele and the p. (Leu2451Pro) variant had arisen *de novo* in the patient.

Conclusion: This study is another example of the pertinence of family studies to follow the transmission and onset of mutations. *De novo* mutations are rare and these findings have major implication for further genetic counseling of the affected families.

26. **Gene Transfer Vector Core: Providing Robust and High Capacity AAV Vector Production**

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Abstract:

For the past 20 years, recombinant Adeno-Associated Viral (AAV) vectors have been proven to be a powerful and attractive tool in the gene therapy field. In addition, the promising results from the recent Leber's Congenital Amaurosis (LCA) clinical trials have pushed the exploration of gene therapy with AAV vectors into many new disease applications. This increasing interest has led to a demand for efficient, high quality vector production.

Most of the conventional methods used to produce high titers of serotype specific AAV need unique techniques for purification. Our Gene Transfer Vector Core has developed a universal protocol to produce high yield AAV vectors independent of serotypes in 10 days. In the past eighteen months, the core has produced more than 250 preps with final titers ranging from $1x10^{11}$ to $1x10^{14}$ Genome-Containing particles per milliliter (GC/ml), with 90% of the preps above $1x10^{12}$ GC/ml. These vectors have been proven to be highly efficient in *in vivo* and *in vitro* assays. For convenience, the core built up an inventory of aliquots with the vectors, which are in 100 ul with over $1x10^{12}$ GC/ml, carrying the most commonly used promoters and reporter transgenes in a variety of AAV serotypes. At present, the core can produce 8 preps per 10-day production cycle, which is adequate to satisfy the demand for large quantities of high quality AAV vectors in a short time.

To meet the growing demand for good quality viral vectors that are suitable for fast *in vitro* screening assays at a lower cost, the core has developed a mini prep protocol. The preps are prepared with a few simple purification steps and produce 20 ml of total volume, with titers ranging from $5x10^{9}$ to $2x10^{11}$ GC/mLin an approximate turnaround time of 6 days. The mini prep, in comparison to the regular prep protocol, takes half the time for production and purification, and reduces the cost to 1/7 the original. The core also assists investigators with the design and construction of AAV vectors, providing consultation and cloning services for specific applications.

With an inventory of standard vectors and the capacity to produce customized vectors in different scales, our Gene Transfer Vector Core can provide high quality AAV vectors for a variety of individual needs.

27. **Deletion of PDZD7 disrupts the USH2 protein complex in cochlear hair cells and causes hearing loss in mice**

Zou, J.[1], Zheng, T.[1], Ren, C.[2], Askew, C.[3], Liu, X-P.[3], Pan, B.[3], Holt, J.R.[3], Wang, Y.[2] and Yang, J.[1,2]

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Abstract:

Background: Usher syndrome (USH) is the leading genetic cause of combined deafness and blindness. Among its three clinical types, type 1 (USH1) and type 2 (USH2) are the most severe and predominant form, respectively. *PDZD7* was recently reported to be implicated in USH2 and nonsyndromic deafness. It is a paralog of the *USH1C* and *USH2D* (*WHRN*) genes and encodes a protein with multiple PDZ domains. The biological function of *PDZD7* and the pathogenic mechanism caused by*PDZD7* mutations are currently unknown.

Methods: A *Pdzd7* knockout mouse model was generated and thoroughly characterized. In the *Pdzd7* knockout mice, the auditory function was tested by ABR, DPOAE and CM tests; the mechanotransduction in cochlear and vestibular hair cells was assessed by single cell recording; the morphology of inner ear hair bundles was examined by scanning electron microscopy; the integrity of the ankle link complex in hair cells and the periciliary membrane complex in photoreceptors was studied by immunofluorescence; and the vision function was evaluated by ERG test.

Results: The *Pdzd7* knockout mice exhibit congenital profound deafness, as assessed by ABR, DPOAE and CM tests, and normal vestibular function, as assessed by their behaviors. Lack of PDZD7 leads to disorganization of stereocilia bundles and reduction in mechanotransduction currents and sensitivity in cochlear outer hair cells. At the molecular level, PDZD7 determines the localization of the USH2 protein complex, composed of USH2A, GPR98, and WHRN, to ankle links in developing cochlear hair cells, likely through its direct interactions with these three proteins. The localization of PDZD7 to the ankle links of cochlear hair bundles also relies on the USH2 proteins. In photoreceptors of *Pdzd7* knockout mice, the USH2 proteins largely remain unchanged at the periciliary membrane complex. The electroretinogram responses of both rod and cone photoreceptors are normal in knockout mice at one month of age.

Conclusion: This study presents novel evidence suggesting the difference between the organization of the USH2 complex in hair cells and photoreceptors. More importantly, this study reveals that PDZD7 is a new component of the USH2 complex and plays an essential role in organizing this complex in cochlear hair cells. Our findings provide valuable insights into the pathogenic mechanism underlying USH and deafness as well as the cell biology of hair cells and photoreceptors.

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Group 3- Phenotypes-Natural History-Psychological Aspects

28. **Multidisciplinary team management of dual deafness and low vision disability**

Blanchet, C.[1], Dupeyron, G.[2], Remond, B.[2], Verlet, K.[3], Gilbert, M.[3], Jabouin, A.[3], Petit, P.[4], Hamel, C.[1] and Mondain, M.[1]

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Abstract:

Objective: To describe how two separated resources centers, one specialized in deaf children rehabilitation and one specialized in adult low vision rehabilitation adjusted their rehabilitation program in order to take in charge patients with multisensory disabilities.

Study design: in 2005, a network called "RUSH" was created in South of France's area (Languedoc-Roussillon) including services providing rehabilitation for nonsyndromic deafness or low vision.
In 2005, a low vision rehabilitation centre instigated a teaching program of sign language for its professionals during 3 years in order to receive Usher patients whatever their communication mode. Rehabilitation program was adapted to Usher syndrome.

In parallel, since 2011, an ambulatory deaf children service made a partnership with an ambulatory low vision rehabilitation service. Optometry, occupational therapy, mobility training and psychomotility could be added if necessary to deafness rehabilitation.
The rehabilitation adjustments and the characteristics of Usher patients enrolled in both projects are described.

Results: The low vision rehabilitation center took care of 25 Usher syndrome patients since 2005 (11 Usher type 2 patients, 14 Usher type 1 patients). Mean age was 44 (27-69) and mean duration of the stay in the centre was 170 days (44-199). All Usher type 2 patients carried hearing aids and used oral communication mode. The main difference in their rehabilitation program was the collaboration between locomotion therapists, audiologists and speech therapists. Sign language Usher type 1 patients were received by pairs to avoid isolation during the stay. Communication mode was oral for 1 patient (who received a cochlear implant), sign for 11, total for 1 and tactile for 1. Psychomotility and mobility training programs had to be adapted because of the bilateral vestibular areflexia (balance and muscular tone control training).

Six children were enrolled in the dual sensory rehabilitation program since 2011. Four had Usher type 1 syndrome, two had other dual disabilities. Entrance median age in deaf program was 9 (3-16) and entrance median age in dual sensory rehabilitation program was 15 (5-17). One had bilateral conventional hearing aid, all others had cochlear implant. Communication mode was oral for 3, total for one and sign for 2. Rehabilitation included optometry for 2, locomotion training for 2, and adaptation of computer use for 2. Two children requested no specific low vision rehabilitation yet.

Conclusion: Dual sensory disabilitiy request specific adaptations to optimize rehabilitation program with a multidisciplinary collaboration. Actions to provide funding for such programs are essential.

29. **Occupational activity and psychological health in persons with Usher II**

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Abstract:

Introduction: Resent research from our group in Sweden have demonstrated that persons with Usher syndrome type II has significantly lower physical and psychological health compared to a reference group. (Wahlqvist et al 2013)

Aims: To investigate how occupational, individual and environmental factors correlate with physical and psychological health in people with Usher syndrome type II

Material: Participants were recruited from the Swedish Usher database where 122 persons received a questionnaire and 93 persons aged 18-84 y agreed to participate where 34 were working, 33 had 100% disability pension and 26 persons where retired (>65 year!). The sample included 50 women and 43 men.

Methods: The Swedish Health on Equal Terms questionnaire was used, which among others covered items as health, living conditions, occupational activity and social relationships. Two groups were formed; A) subjects who were working N=34 B) subjects who had disability pension n=33.

Results: Preliminary data show that the 2 groups did not differ in age, sex ? degree of hearing loss, visual acuity or visual field loss. Subjects who were working (50-100%) had statistically significant better physical and mental health compared to those on 100% disability benefit. Those with 100% disability benefits were significantly more stressed, and reported significantly higher degrees of feeling tense. They also had lost significantly more faith in their environment and they also reported suicide thoughts and suicidal attempts significantly more often compared to those who had an occupation. Factors as age, family status, level of hearing and vision impairment could not explain the results.

Conclusions: To get good rehabilitation, vocational training and possibilities to maintain work seems to be extremely important in keeping as good physical and psychological health as possible.

30. **Stress in individuals with Usher syndrome type II**

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Abstract:

Due to their dual sensory impairment people with Usher syndrome are assumed to have a high risk of stress experience. The purpose of this study was the development and evaluation of a questionnaire (SQ) to assess frequency and intensity of stress by external stressors in people with usher syndrome type II (USH2).

The construction of the questionnaire is based on the domains of the component “Activities and Participation” of the WHO’s ICF concept, which have been modified. These modifications lead to the domains “Communication”, “Orientation and Mobility”, “Activities of Daily Living”, “Interpersonal Interactions and Relationships”, “Recreation and Leisure” and “Work and Employment”, which were used to postulate external stress factors. The questionnaire was administered to 262 adults with USH2 (ages 17-79, mean age = 51; 53 % female; 32 % employed) and evaluated through item and factor analyses. The evaluation shows good indices in terms of item and factor analysis. The a priori postulated structure was well reflected in five factors (after exclusion of the items belonging to the domain “Work and Employment” since most adults were unemployed).

In addition to the SQ, the standardized stress questionnaire “Trierer Inventory of Chronic Stress (TICS)” was administered to compare stress frequency between the USH2-sample group and a German reference group (n = 604). The investigation concluded that people with USH2 experience stress more often in the TICS scales, which indicate a lack of social-emotional need fulfillment (“Chronic Worry”, “Social Isolation”, “Being Overwhelmed with Work” and “Social Tensions”). Less stress was experienced in scales which include high expectations (“Work Overload”, “Social Overload” and “Success Pressure”).

As a result of the examination of differences between the single factors’ and the TICS scales’ mean values, it turned out that the biggest stress in SQ (related to frequency and intensity) was seen in the factor “Orientation and Mobility” and in TICS (with regard to frequency) in the scales “Chronic Worry” and “Social Isolation”; these obviously are the central problem areas of the participants of the study. In the SQ as well as in TICS stress frequency and stress burden were dependent on person specific variables (age, gender, partner and work).

The results give indications for rehabilitation arrangements to avoid and reduce stress in people with USH2 especially in the areas of Orientation and Mobility, Chronic Worry and Social Isolation.

31. **Rehabilitation of Individuals with Usher Syndrome: a Qualitative Study**

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Abstract:

Abstract to be published at a later date

32. **Focusing on Now for Tomorrow: Using A Well-Rounded Curriculum to Strengthen Students with Ushers Syndrome**

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[2]*Teacher for Children with Visual Impairments & Deaf-Blindness, Queens College, Queens, NY, USA*

Abstract:

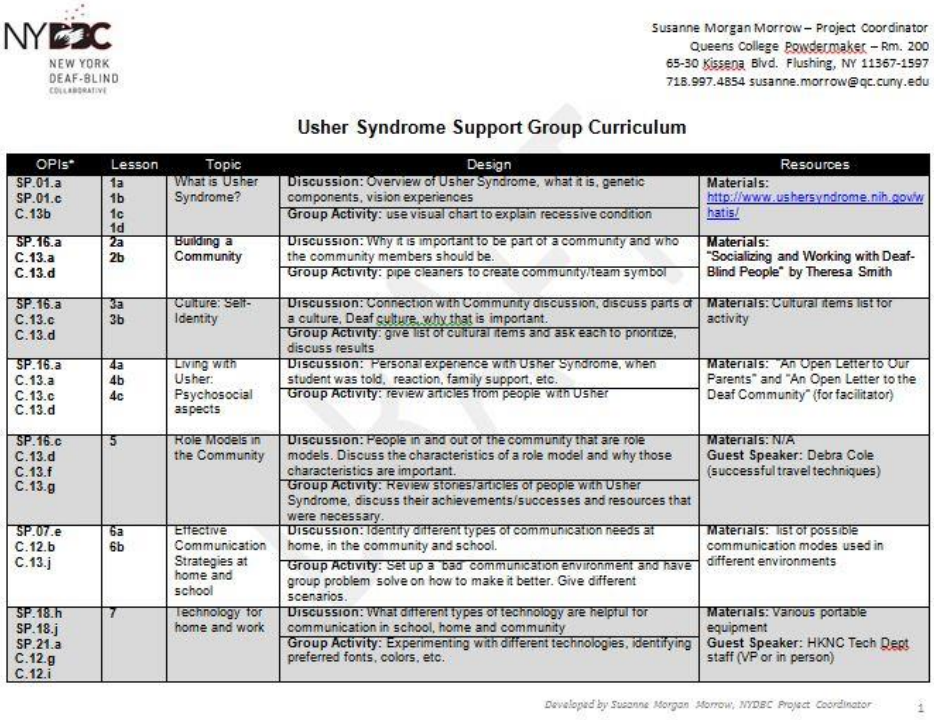
Schools for the deaf are prepared to address issues regarding the educational needs for students with hearing loss but they are ill-prepared to address the compounding issue of progressive vision loss. Students with Usher Syndrome, particularly during the transition age years, are in critical need of support from knowledgeable professionals who can address the many questions and uncertainty that lingers. While there are singular resources available in the form of articles and journal publications on the topic, there is no comprehensive curriculum designed to address the myriad needs of students with Usher Syndrome. To address this issue a multi-year Usher Syndrome Support Group (USSG) curriculum was designed and piloted and is adaptable for students in the mainstream.

Adults with Usher Syndrome anecdotally report great frustration and despair having gone through their educational experience without adequate or any information about their diagnosis, prognosis and resources to cope. These feelings of anger and outrage are expressed clearly in “An Open Letter to Our Parents” (http://bit.ly/1q0sIed). Research suggests that early preparation builds a foundation that supports the various aspects of the student’s life thus allowing for psychological reassurance and preparedness for adulthood. This multi-year curriculum addresses the following: etiology, cultural identity, peer-to-peer connections, safety travel, independent living, self-advocacy, self-determination, eye health, environmental modifications, effective communication strategies, technology at school, home and community, laws and citizen rights, transition planning, community resources, and social groups. To maximize the learning style of students, the curriculum was designed in a multidimensional format, which includes live presentation, demonstration and group activity. It also incorporates the use of distance technology to bring guest lecturers who have Usher Syndrome virtually into the sessions.

Another critical thread to the success of students is the early development of vision skills (http://bit.ly/1jKq506). Under the guidance of a Teacher for the Visually Impaired & Orientation & Mobility instructor, it is important for the student to begin to understand how the eye functions, use of trailing skills, use of light as a tool for access, text modification software, etc.

The USSG Curriculum was designed in partnership with the New York State deaf-blind project, a mental health counselor at a state school for the deaf, and reviewed by a deaf-blind family specialist and staff from the national deaf-blind project. This pilot later evolved into an Usher Syndrome Social Group, a safe environment for students and family members to learn and share in a casual, fun environment.

Figure: Snap shot of Usher Syndrome Support Group Curriculum



NYDBC NEW YORK DEAF-BLIND COLLABORATIVE

Susanne Morgan Morrow – Project Coordinator
Queens College Powdermaker – Rm. 200
65-30 Kissena Blvd. Flushing, NY 11367-1597
718.997.4854 susanne.morrow@qc.cuny.edu

Usher Syndrome Support Group Curriculum

OPIs*	Lesson	Topic	Design	Resources
SP.01.a SP.01.c C.13b	1a 1b 1c 1d	What is Usher Syndrome?	**Discussion:** Overview of Usher Syndrome, what it is, genetic components, vision experiences **Group Activity:** use visual chart to explain recessive condition	**Materials:** http://www.ushersyndrome.nih.gov/whatis/
SP.16.a C.13.a C.13.d	2a 2b	Building a Community	**Discussion:** Why it is important to be part of a community and who the community members should be. **Group Activity:** pipe cleaners to create community/team symbol	**Materials:** "Socializing and Working with Deaf-Blind People" by Theresa Smith
SP.16.a C.13.c C.13.d	3a 3b	Culture: Self-Identity	**Discussion:** Connection with Community discussion, discuss parts of a culture, Deaf culture, why that is important. **Group Activity:** give list of cultural items and ask each to prioritize, discuss results	**Materials:** Cultural items list for activity
SP.16.a C.13.a C.13.c C.13.d	4a 4b 4c	Living with Usher: Psychosocial aspects	**Discussion:** Personal experience with Usher Syndrome, when student was told, reaction, family support, etc. **Group Activity:** review articles from people with Usher	**Materials:** "An Open Letter to Our Parents" and "An Open Letter to the Deaf Community" (for facilitator)
SP.16.c C.13.d C.13.f C.13.g	5	Role Models in the Community	**Discussion:** People in and out of the community that are role models. Discuss the characteristics of a role model and why those characteristics are important. **Group Activity:** Review stories/articles of people with Usher Syndrome, discuss their achievements/successes and resources that were necessary.	**Materials:** N/A **Guest Speaker:** Debra Cole (successful travel techniques)
SP.07.e C.12.b C.13.j	6a 6b	Effective Communication Strategies at home and school	**Discussion:** Identify different types of communication needs at home, in the community and school. **Group Activity:** Set up a 'bad' communication environment and have group problem solve on how to make it better. Give different scenarios.	**Materials:** list of possible communication modes used in different environments
SP.18.h SP.18.j SP.21.a C.12.g C.12.i	7	Technology for home and work	**Discussion:** What different types of technology are helpful for communication in school, home and community **Group Activity:** Experimenting with different technologies, identifying preferred fonts, colors, etc.	**Materials:** Various portable equipment **Guest Speaker:** HKNC Tech Dept staff (VP or in person)

Developed by Susanne Morgan Morrow, NYDBC Project Coordinator 1

Acknowledgments: The New York Deaf-Blind Collaborative is a five-year federally funded grant through the Office of Special Education Programs. The current grant cycle runs from 10/1/13 – 9/30/18 and focuses on the provision of technical assistance throughout New York State on behalf of children and young adults who are deaf-blind, their families, educators and service providers. www.nydbc.org

33. **European young investigators network for Usher syndrome**

Nagel-Wolfrum, K.[1], da Silva, S.[2], José Duarte, E.[3], Sliesoraityte, I. [4], Vaché, C.[5] and van Wijk, E.[6]

[1]*Cell and Matrix Biology, Institute of Zoology, Johannes Gutenberg University of Mainz, 55099, Germany*

[2]*AIBILI, Coimbra Coordinating Centre for Clinical Research, Portugal*
[3]*IBILI - University of Coimbra, Portugal*
[4]*Institut de la Vision, Paris, France*
[5]*CHU (University Hospital), INSERM, Montpellier, France*
[6]*Radboudumc University Nijmegen Medical Centre, The Netherlands*

Abstract:

The Usher syndrome (USH) is the most common form of inherited deaf-blindness. USH is a complex disorder divided into three clinical types, which are genetically heterogeneous, making diagnosis and treatment challenging. So far, ten causative genes and one genetic modifier have been identified. Molecular analyses revealed that all USH1 and 2 proteins are organized in protein networks in the eye and inner ear. Although, this has provided insights into the function of USH proteins and explains why defects in proteins of different families result in USH, the exact pathomechanisms in the retina remain unclear.

The European young investigators network for Usher syndrome, shortly EUR-USH, is composed of three overlapping components. In component A we aim to improve diagnosis. We have compiled a multinational clinical protocol for a prospective observational longitudinal Usher cohort study. The data of the clinical examinations and molecular analyses will be uploaded in an USH database. Combination of data will improve diagnosis and provide more details to genotype/phenotype correlations. In component B we will gain more insight into the molecular pathogenesis. To achieve this we adopted proteomics analyses in transgenic zebrafishes to extend to USH protein networks and analyzed USH protein expression and interaction of USH proteins with different imaging methods in human donor retinas. In component C we evaluate gene-based therapy options for the retinal degeneration. For this, we generated minigenes for gene replacement in USH2A. Furthermore, we analyze the read-through efficacy of different USH causing nonsense mutations and compared the retinal biocompatibility of different read-through inducing drugs.

In EUR-USH young scientists with different backgrounds in medicine, genetics, cellular and molecular biology aim to synergize their expertise. Our efforts will bring new insights towards the understanding of USH and possible cures with the ultimate goal to improve the life quality of USH patients.

Acknowledgements: German Ministry of Education and Research (E-Rare-2, the ERA-Net for Research on Rare Diseases) “EUR-USH”, FAUN-Stiftung, Nuremberg, the Foundation Fighting Blindness (FFB), EU FP7 “SYSCILIA”

34. **Considerations for Managing Hearing Loss for Children with Usher Syndrome**

Fredriksen, J., Kwilinksi, A. and Sands, T.

MED-EL Corporation, Durham, NC 27713, USA

Abstract:

It is well documented that early diagnosis of Usher syndrome is important, in order to begin managing the loss of hearing and vision for young children. This is also critical so that parents can begin considering communication options, educational placements for their children, and when to begin receiving early intervention and therapeutic services.

During this session, the presenters will discuss cochlear implant technology as a possible treatment option for young children's hearing loss. Specific details including what a cochlear implant is, how cochlear implants work, cochlear implant candidacy criteria, and surgical indications will be discussed. Further considerations, including the benefits of unilateral vs. bilateral cochlear implantation, the timing of receiving cochlear implants, communication choices and educational options will be explored.

Co-presenter, Amy Kwilinski, is a mother of three children with Usher syndrome. All of her children received cochlear implants for management of their hearing loss. She will share her family's journey in regards to managing her children's hearing loss, communication choices, special education programs, and other personal experiences.

35. **Blurring the Boundaries: Unmasking the genotype-phenotype overlap across Usher syndrome subtypes**

Sutti, S.[1], Lafferty, K.A.[2], Toledo, D.M.[2], Huang, Y.[1], Muirhead, A.[2], Abou Tayoun, A.N.[2], Shen, J.[2,3], Hernandez, A.L.[2], Campion, M.W.[1], Rehm, H.L.[2,3] and Amr, S.S.[2,3]

[1]*Boston University School of Medicine, Boston, MA, USA*
[2]*Partners Healthcare Personalized Medicine, Laboratory of Molecular Medicine, Cambridge, MA, USA*
[3]*Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA*

Abstract:

Historically, Usher syndrome's presentation and clinical diagnosis has been narrowly-defined into three types with distinct clinical presentations. With genetic diagnoses now driving classification of these three subtypes, clinical variability has been noted both anecdotally and in the literature. This study describes the phenotypic spectrum in patients with pathogenic variants in *MYO7A* and *USH2A*, which represent the greatest contribution to Usher syndrome Type I (USH1) and Type II (USH2) respectively. Our patient cohort consisted of 140 individuals who were reported to present with hearing loss or hearing loss and additional clinical features, and carried clinically significant variants in either *MYO7A* (n=73) or *USH2A* (n=67). Clinical features associated with Usher syndrome were compiled for each patient using information reported in genetic testing requisition forms by the ordering genetic counselor or physician. Overall, our cohort was largely consistent with classical clinical presentations associated with USH1 and USH2 with congenital onset and greater severity of the hearing loss reported more frequently for patients with *MYO7A* variants compared with patients in *USH2A* variants. In addition, the presence of vestibular problems and earlier onset (<10 yrs) of retinitis pigmentosa (RP) was more likely to be observed in patients with *MYO7A*. However, marked variation was noted in several patients in both the *MYO7A* and the *USH2A* cohorts from the classical clinical definitions, namely in the onset of RP, which is traditionally thought to be detectable within the 1stdecadeof life in USH1 individuals and expected to present in the 2nd or 3rd decade in USH2 individuals. Eye findings reported for our cohort reveals that nearly 21% of the USH1 subtype (n=15) did not have RP findings prior to the age 10 years. In addition, notable variation was also seen in the presence of vestibular problems, typically associated with USH1 but not with USH2. Vestibular issues (balance problems and delayed walking) were not indicated for 16% of individuals from the *MYO7A* cohort and were reported in 22% of individuals of the

USH2A cohort. These findings highlight the overlapping phenotypic variability across the USH1 and USH2 subtypes beyond clinical classification criteria and cautions against the use of these criteria in guiding the diagnostic odyssey. In addition, the clinical heterogeneity of these subtypes reiterates the need for comprehensive genetic testing panels in achieving a molecular diagnosis.

Speaker Listings

Thursday, July 10, 2014

Symposium Session A : *Diagnostics, Epidemiology and Population Genetics*

1. **Discovering causes of and developing treatments for inherited eye diseases**

Stone, E.

University of Iowa, Iowa City, IA, USA

2. **Family and personal responses to the diagnosis of Usher Syndrome**

Miner, I.D.

Licensed clinical social worker and consultant, Los Angeles, CA, USA

3. **Photoreceptors in Pediatric Patients with Usher Syndrome**

Fulton, A.[1], Hansen, R.[1], Tavormina, J.[1], Moskowitz, A.[1], Kenna, M.[2], and Rehm, H.[3]

[1]Department of Ophthalmology, Boston Children's Hospital, Boston, MA, USA

[2]Department of Otolaryngology, Boston Children's Hospital, Boston, MA, USA

[3]Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

4. **Genetic Diagnostic Testing for Usher Syndrome**

Pierce, E. and Bujakowska, K.

Massachusetts Eye and Ear, Boston, MA, USA

5. **Detection of unconventional Usher syndrome mutations and further outcomes of the LOVD-USHbases**

Roux, A-F.[1,2], Baux, D.[1], Vaché, C.[1], Faugère, V.[1], Moclyn, M.[1], Constantinides, S.[1], Liquori, A.[2,3], Garcia-Garcia, G.[2] and Claustres, M.[1,2,3]

[1] CHU Montpellier, Laboratoire de Génétique Moléculaire, Montpellier, F-34000, France

[2] Inserm, U827, Montpellier, F-34000, France

[3]Université Montpellier 1, UFR Médecine, Laboratoire de Génétique Moléculaire, Montpellier, F-34000, France

6. **Unsuspected phenotypic heterogeneity in Usher syndrome revealed by Massive Parallel Sequencing**

Bitner-Glindzicz, M.[1], Cullup, T.[2], Steele-Stallard, H.[1], Lenassi, E.[3], Vache, C.[4], Roux, A-F.[4], Luxon, L.[5], Webster, A.[3] and Lench, N.[2]

[1] UCL Institute of Child Health, 30 Guilford St, London, UK

[2] North East Thames Regional Clinical Molecular Genetics Lab, Great Ormond Street Hospital, London, UK

[3] UCL Institute of Ophthalmology, London, UK

[4] Laboratoire de Génétique Moléculaire, CHU Montpellier Montpellier, F-34000, France

[5] Department of Neuro-otology, The National Hospital for Neurology and Neurosurgery, London, UK

7. **NGS for Usher syndrome: Benefits, pitfalls and unexpected findings**

Bolz, H.J.[1,2]

[1]Center for Human Genetics, Bioscientia, Ingelheim, Germany

[2]Institute of Human Genetics, University Hospital of Cologne, Cologne, Germany

8. **Data Sharing to Support Genetic Test Interpretation**

Heidi L. Rehm[1,2], Ahmad Abou Tayoun[1], Andrea Muirhead[1], Katherine A. Lafferty[1], Amy L. Hernandez[1], Jun Shen[1, 2], Sami S. Amr[1, 2]

[1]Laboratory for Molecular Medicine, Partners Healthcare Personalized Medicine, Cambridge, MA, USA

[2]Department of Pathology, Brigham & Women's Hospital and Harvard Medical School, Boston, MA, USA;

Symposium Session B: *Functional Genetics*

9. **Study of splicing variants in the USH genes through minigene assay and transcript analysis from epithelial nasal cells**

Millan, J.

Unidad de Genética, Hospital Universitario La Fe, Fundación para la Investigación del Hospital La Fe, Valencia, Spain

10. **The Usher syndrome type 2 protein complex in photoreceptors and hair cells**

Yang, J.

Department of Ophthalmology and Visual Sciences, Moran Eye Center, University of Utah, Salt Lake City, UT, USA

11. **Decoding of Usher syndrome protein networks reveals insights in the molecular basis of the Usher disease**

Wolfrum, U.

Cell & Matrix Biology, Institute of Zoology, Johannes Gutenberg University of Mainz, Germany

12. **Defective protein complex assembly produces ER stress that causes cell death in Usher syndrome**

Blanco-Sànchez, B., Clément, A. and Westerfield, M.

Institute of Neuroscience, University of Oregon, Eugene, OR, USA

13. **Role for Usher proteins in regulated protein trafficking in photoreceptors: potential mechanism for light-induced retinal degeneration in Usher syndrome**

Cosgrove, D., Zallocchi, M., Cheung, L., Peng, YW. and Delimont, D.

Boys Town National Research Hospital, Omaha, NE, USA

14. **Usher syndromes: gathering basic knowledge towards the development of therapeutic approaches**

Pepermans, E.[1,2,3], Michel, V.[1,2,3], Bonnet, C.[2,3,4], Sahly, I.[,3,4], Bahloul, A[1,2,3], Avan, P.[5], El-Amraoui, A.[1,2,3,4] and Petit, C.[1,2,3,4,6]

[1] Institut Pasteur, Unité de Génétique et Physiologie de l'Audition, Paris, France

[2] INSERM UMRS 1120, Paris, France

[3] Sorbonne Universités, UPMC Univ Paris06, Paris, France

[4] Institut de la vision, Syndrome de Usher et autres Atteintes Rétino-Cochléaires, Paris, France

[5] Laboratoire de Biophysique Sensorielle, INSERM UMR 1107, Faculté de Médecine, Université d'Auvergne; 63000 Clermont-Ferrand, France

[6] Collège de France, Paris, France

15. **The quest for molecular mechanisms of Usher syndrome**

Ahmed, Z.M.[1]

[1]Divisions of Pediatric Ophthalmology, Otolaryngology Head & Neck Surgery, Cincinnati Children's Hospital Medical Center, University of Cincinnati, OH

16. **Hair cell specific expression of Clarin-1 is sufficient to prevent auditory and vestibular dysfunction in the mouse model for ear disease in Usher Syndrome III**

Geng, R.[1*], Gopal, S.R.[1], Chen, D.[1], Furness, D.N.[2] and Alagramam, K.N.[1]

[1]Department of Otolaryngology, University Hospitals Case Medical Center, Case Western Reserve University, Cleveland, OH, USA

[2]Keele University, Keele, UK

**Current address: Department of Otolaryngology, University of Toronto, Toronto, Canada*

Friday, July 11, 2014

Symposium Session C: *Phenotypes and Natural History*

17. **Usher Syndrome: When to suspect it and how to find it**

Kenna, M.[1,2], Lafferty, K.[3], Rehm, H.[2,3], and Fulton, A[1,2]

[1]Boston Children's Hospital, Boston, MA, USA

[2]Harvard Medical School, Boston, MA, USA

[3]*Partner's Healthcare/Personalized Genetic Medicine, Cambridge, MA, USA*

18. **Usher syndrome: Do audiological, vestibular and visual geno-phenotype correlations exist?**

Möller, C.

Audiological Research Centre, University Hospital, Örebro, Sweden

19. **Genotype-phenotype correlations on 161 USH2A patients – some mutation combinations cause a more severe audiometric phenotype**

Pennings, R.J.E.[1], Löfgren, M.[2], Huygen, P.L.M.[1], Sadeghi, A.M.[3], Tranebjaerg, L.[4], Kremer, H. [1], Kimberling, W.J.[5], Cremers, C.W.R.J.[1] and Möller C.[2]

[1] *Radboud university medical centre, Department of Otorhinolaryngology, Nijmegen, the Netherlands*

[2] *University Hospital Örebro, Audiological Research Centre, Örebro, Sweden*

[3] *University of Gothenburg, Department of Audiology, Gothenburg, Sweden*

[4] *University of Copenhagen, Department of Cellular and Molecular Medicine, Copenhagen, Denmark*

[5] *Boys Town National Research Hospital, Genetics Center, Omaha, NE, USA*

Symposium Session D: *Preclinical Studies*

20. **Antisense oligonucleotides effectively treat Usher syndrome in mice**

Lentz, J.J.[1], Jodelka, F.M.[2], J. Hinrich, A.J.[2], Ponnath, A.[1], Amato, R.[1], Flaat. A.[1], McCaffrey, K.E.[2], Bazan, N.G.[1], Duelli, D.M.[2], Rigo, F.[3] and Hastings, M.L.[2]

[1]*Neuroscience Center, LSUHSC, New Orleans, LA, USA*

[2]*Rosalind Franklin University of Medicine and Science, North Chicago, IL, USA*

[3]*Isis Pharmaceuticals, Carlsbad, CA, USA*

21. **TMC gene therapy in mouse models of human deafness**

Askew, C.[1], Rochat, C.[2], Ahmed, H.[1], Pan, B.[1], Asai, Y.[1], Child, E.[1], Schneider, B.[2], Aebischer, P.[2] and Holt, J.R.[1]

[1]*Boston Children's Hospital, Harvard Medical School, Boston, MA, USA*

[2]*Ecole Polytecnique Federale de Lausanne, Switzerland*

22. **Strategies to prolong survival of cone photoreceptors**

Cepko, C.

Department of Genetics, Harvard Medical School, Boston, MA, USA

23. **Determining the mechanism of vision and hearing loss associated with usher type 2A**

Yoder, M., Adijanto, J. and Naash, M.I.

University of Oklahoma Health Sciences Center, Cell Biology, Oklahoma City, OK, USA

24. **Characterization of Usher mouse model retinal degeneration and assessment of potential therapy**

Trouillet, A.[1], Dubus, E.[1], Moutsimilli, L.[1] ,Degardin, J.[1], Estivalet, A.[1], Simmonutti, M.[1], Sahly, I. [1], Sahel, J.[1,3,4,5], Petit, C.[1,2] and Picaud, S.[1]

[1]*Institut de la Vision-17 rue Moreau,Paris, F-75012, France INSERM, U968-UPMC, UMRS968 -CNRS, UMR 7210 Paris, France*

[2]*Institut Pasteur Paris, Paris, France*

[3] *Centre Hospitalier National d'Ophtalmologie des Quinze-Vingts, Paris, France*

[4] *Institute of Ophthalmology, University College of London, London, UK*

[5] *Fondation Ophtalmologique Adolphe de Rothschild, Paris, France*

Symposium Session E: *Therapy and Clinical Trials*

25. **Vector discovery and design for improved delivery to the retina and cochlea**

Vandenberghe, L.H.

Massachusetts Eye and Ear, Boston, USA

26. **Ignore the stop: translational read-through of nonsense mutations in Usher syndrome genes**
Nagel-Wolfrum, K.
Cell and Matrix Biology, Inst. of Zoology, Johannes Gutenberg University of Mainz, Germany

27. **Cochlear implantation in individuals with Usher syndrome**
Liu, XZ
Department of Otolaryngology, University of Miami Ear Institute, Miami, FL

28. **Progress toward a vestibular implant for restoring sensation of head movement**
Della Santina, C.C, Ward, B.K, Sun, D.Q, Semenov, Y.R, Dai, D., Fridman, G.Y, Hayden, R, Davidovics, N.S, Rahman, M.A, Valentin, N.S, Ahn, J, Hageman, K, Migliaccio, A.A, Kalayjian, Z, Andreou, A, Tejada, F, Mitchell, D, Cullen, K.E, Carey, J.P, Hedjoudje, A, Boutros, P and Treviño, C.
Vestibular NeuroEngineering Lab, Johns Hopkins School of Medicine, Baltimore, MD, USA

29. **Updates on a Gene Therapy Trial for Usher Syndrome Type IB**
Pennesi, M.E., Yang, P., McBride, M., Lauer, A.K., Stout, J.T., and Weleber, R.G.
Oregon Health and Science University, Porland, OR, USA

30. **Getting Vision-Saving Therapies Out to the People**
Zilliox, P.
Foundation Fighting Blindness, Columbia, MD, USA

31. **Consideration on Usher Syndrome to prepare future therapies**
Audo, I., Mohand-Said, S., Zeitz, C., Bonnet, C., Petit, C. and Sahel, J.A.
Centre de Recherche Institut de la Vision, UMR S 968 Inserm / Université Pierre et Marie Curie/CHNO des Quinze-Vingts, Paris, France

32. **Living with Usher Syndrome: Words for the Scientists**
Pellerin, R.
The Unstoppable, Waterbury Center, VT, USA

Saturday, July 12, 2014

Family Conference Session I: Diagnosis

33. **Usher Syndrome: Why a definite diagnosis matters**
Kenna, M.[1,2], Lafferty, K.[3], Rehm, H.[2,3], and Fulton, A[1,2]
[1]Boston Children's Hospital, Boston, MA, USA
[2]Harvard Medical School, Boston, MA, USA
[3]Partner's Healthcare/Personalized Genetic Medicine, Cambridge, MA, USA

Family Conference Session II: Psychological Aspects – Words from the Professionals

34. **Family and Personal Responses to the Diagnosis of Usher Syndrome**
Miner, I.D.
Licensed clinical social worker and consultant, Los Angeles, CA, USA

35. **Physical and Psychological Aspects of Usher Syndrome**
Möller, C.
Audiological Research Centre, University Hospital, Örebro, Sweden

Family Conference Session III: Gene Therapy 101

36. **Gene Therapy 101**
Vandenberghe, L.H.
Mass Eye and Ear and Harvard Medical School, Boston, USA

Family Conference Session IV: Updates to the Families

37. **Usher Syndrome Research**
Géléoc, G.
Boston Children's Hospital, Department of Otolaryngology, Boston, MA, USA

38. **Translational Research and the Usher Syndrome Registry**

Kenna, M.A.
Department of Otolaryngology and Communication Enhancement, Boston Children's Hospital, Boston, MA, USA

Family Conference Session V: Patient Care and Rehabilitation (Selected Presentations)

39. **Stress in individuals with Usher syndrome type II**

Högner, N.
Institute for Rehabilitation Sciences, Department of Education and Rehabilitation of the Blind and Low Vision Individuals, Humboldt University of Berlin, Germany

40. **Usher type 2 syndrome: hearing, educational, socio-economic and vocational impacts**

Blanchet, C.[1], Vache, C.[2], Baud, D.[2], Hamel, H.[1], Meunier, I.[1], Uziel, A.[1], Roux, A-F.[2] and Mondain, M.[1]
[1]*Sensory Genetic Disease National Centre, Montpellier University Hospital, Montpellier, France*
[2] *Molecular Genetic Laboratory, Montpellier University Hospital, Montpellier, France*

41. **Focusing on Now for Tomorrow: Using A Well-Rounded Curriculum to Strengthen Students with Ushers Syndrome**

Morrow, S.[1] and Labeck, K.[2]
[1] *New York Deaf-Blind Collaborative, Project Coordinator, Queens College, Queens, NY, USA*
[2]*Teacher for Children with Visual Impairments & Deaf-Blindness, Queens College, Queens, NY, US*

Speaker Abstracts

Thursday, July 10, 2014

Symposium Session A: *Diagnostics Epidemiology and Population Genetics*

1. **Discovering causes of and developing treatments for inherited eye diseases**

Stone, E.

University of Iowa, Iowa City, IA, USA

Abstract:

This talk will cover the following topics:
1) The inherited eye disease landscape
    a) the treatments that will be useful for a given patient depend in part on that patient's stage of disease — we should have a strategy that "leaves no one behind" regardless of their stage of their disease
    b) the strategy for developing and deploying treatments depends to some degree on whether the disease is above or below the "commercial viability threshold"
2) Tiered genetic testing for genetically heterogeneous diseases — our current strategy and results
3) Strategic choices for gene therapy — including genome editing (TALEN and CRISPR) for correction of disease-causing mutations in vivo and in vitro
4) Strategic choices for cell-based therapy — including: patient derived stem cells as a means to:
    a) investigate pathogenic mechanisms
    b) evaluate therapies designed to prevent, arrest or slow the disease
    c) replace photoreceptors to restore vision

2. **Family and personal responses to the diagnosis of Usher Syndrome**

Miner, I.D.

Licensed clinical social worker and consultant, Los Angeles, CA, USA

Abstract:

Introduction: There were issues raised at the last conference about what providers do and should communicate to parents and patients about Usher Syndrome.

Survey: In the interest of answering that question, a survey was undertaken with adults with Usher and parents of children with Usher. A survey was composed and sent out in two forms: on the Web, and through email. Recipients were informed that only demographic material, current age, age at diagnosis,

diagnosis and whether or not they had been referred to a geneticist were being asked. No names were asked.

Questions were asked concerning what and how parents and people with Usher were told about their diagnosis, what information was given to them, and to rate the empathy and interest shown by providers. They were also asked about referrals to support services, what information they had now that was most helpful, and concerns they have for themselves or for the future.

Literature review: No literature was found specific to informing parents of their child's diagnosis of Usher, but there is literature on informing parents of their child's diagnosis of diabetes, and vision impairment. There is literature on the life of people with Usher from the Nordic countries which includes experiences of diagnosis.

Results: The sample was small. Forty surveys were returned, 16 were from parents with children and young adults up to the age of 22. The remaining ones were from parents of adult children with Usher and adults with Usher ranging from age 24 to 66, for whom these questions felt very fresh. Two families filled them out together: the adult with Usher and their hearing sighted parent. Four families had two children with Usher.

Findings: Parental and Usher adult experiences ran the gamut from "as good as can be expected" to "disastrous." There were examples of missed diagnosis and incorrect diagnosis. Most parents felt their providers were fairly empathetic, but they had excellent suggestions about how the experience could be improved, including access to other parents, printed material so parents don't have to look for information themselves, and guidance. Parents are fearful about their children's futures; this applies to parents of adults with Usher as well. Parents referred to the Coalition for Usher Syndrome Research as having filled an important need for them. Some contributions from other countries related to support services may be useful. Issues of coping style and information will be discussed.

There was no funding for this survey.

3. **Photoreceptors in Pediatric Patients with Usher Syndrome**

Fulton, A.[1], Hansen, R.[1], Tavormina, J.[1], Moskowitz, A.[1], Kenna, M.[2], and Rehm, H.[3]

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Abstract:

Purpose: To study phototransduction and post receptor retinal processes in pediatric patients with a genetic diagnosis of MYO7A and others with a USH2A. A cilium connects the photoreceptor outer segment (the site of phototransduction) to the more proximal portion of the photoreceptor which synapses with the second order retinal neurons.

Methods: ERG responses to full field stimuli were recorded from 22 patients (MYO7A, n= 9; USH2A, n=13) in scotopic and photopic conditions. We calculated the phototransduction parameters specifying sensitivity and saturated amplitude of the rod and cone photoreceptor response (a-wave) and also the post receptor response sensitivity and saturated amplitude parameters (b-wave) were calculated. The dark adapted visual thresholds were measured in all patients.

Results: Despite attenuated ERG responses, photoreceptor and post receptor parameters could be fully assessed in 11. As anticipated in a ciliopathy, the deficits in post-receptor sensitivity were greater than deficits in photoreceptor sensitivity. The saturated amplitude for outer segment activity was reduced, consistent with the short outer segment length seen on OCT imaging. The deficits in the saturated amplitude of outer segment activity and post-receptor activity were correlated and significantly associated with deficits in dark adapted visual sensitivity.

Conclusions: The activation of phototransdution, occurring in the photoreceptor outer segment is quite normal in those in whom it can be assessed. Deficits in post receptor retinal sensitivity, set proximal to the connecting cilium in the photoreceptor, is associated with visual deficits.

4. **Genetic Diagnostic Testing for Usher Syndrome**

Pierce, E. and Bujakowska, K.

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Abstract:

Purpose: With over 200 genes associated with the inherited eye disorders, next generation sequencing (NGS) became a common tool for the genetic diagnostic testing of patients with these diseases. Usher syndrome (USH) is associated with at least ten genes, which are encoded by 368 exons. The high genetic heterogeneity and large number of exons make molecular diagnosis with traditional methods laborious and costly, therefore we developed a targeted enrichment and NGS based Genetic Eye Disease (GEDi) test. Despite the increased use of NGS approaches in clinical settings, the performance characteristics of these techniques have not been fully defined with regard to test accuracy and reproducibility. We performed detailed quality control measurements and compared it to whole exome sequencing (WES). GEDi was used for genetic screening of over 200 inherited retinal degeneration (IRD) patients including a cohort of 47 USH type 1 probands.

Method: Targeted enrichment included all known genes associated with IRDs, optic atrophy and glaucoma. For the GEDi test quality control measurements four samples were prepared and sequenced in triplicate on three separate days. GEDi results were compared to WES and a dense SNP array (Illumina Omni 2.5), the latter serving as a reference. 47 USH1 probands were analyzed with GEDi.

Results: The performance analyses of the GEDi test showed very high quality measurements with 97.9% sensitivity for single nucleotide variant detection, which was notably higher then for WES (88.3%). This was in part due to better coverage of targeted genes in the GEDi test than in commercially available exome capture sets. We detected two likely pathogenic variants in 33 of the 47 USH1 probands, revealing clinical sensitivity of 70% for USH1. Twenty-two of the identified mutations were novel. Ten patients in addition to primary disease causing mutations carried rare likely pathogenic USH1 alleles or variants in other genes associated with deaf-blindness, which may influence disease phenotype.

Conclusions: Given a high genetic heterogeneity of the inherited eye disorders and fast developing gene therapy approaches, a well-designed comprehensive genetic test is crucial for screening of patients and potential disease carriers. With almost half of the identified USH1 mutations being novel, next generation sequencing had an advantage over microarray-based techniques, which detect only known variants. The data from this study also suggests that based on quantified performance metrics, selective targeted enrichment is preferable to WES for genetic diagnostic testing.

5. **Detection of unconventional Usher syndrome mutations and further outcomes of the LOVD-USHbases**

Roux, A-F.[1,2], Baux, D.[1], Vaché, C.[1], Faugère, V.[1], Moclyn, M.[1], Constantinides, S.[1], Liquori, A.[2,3], Garcia-Garcia, G.[2] and Claustres, M.[1,2,3]

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Abstract:

Haplotype analyses combined to cascade Sanger sequencing of the different USH genes and the more recent targeted Usher exome using Massive parallel sequencing (MPS) have proven their efficiency to achieve molecular diagnosis for Usher patients. However, these routine approaches primarily target the coding exons and their intronic boundaries and thus fail to detect unconventional mutations such as large genomic rearrangements (LGRs) or deep intronic mutations.

The detection of LGRs remains quite challenging, as the estimation of depth ratio by MPS requires experience, robustness of the technology and a substantially higher depth of coverage than that usually sufficient for small events identification. Unfortunately, the MLPA kits are effective but unlikely to become available for all Usher genes. Currently, CGH-array remains the most efficient method to detect LGRs. Consequently, we have designed a custom CGH-microarray covering all Usher genes as well as other sensorineural genes to accurately detect deletions or duplications and easily identify the breakpoint junctions.

Detection of deep intronic mutations requires the analysis of Usher transcripts, extracted from the patients nasal epithelial cells. In addition, it is now also possible to sequence the entire candidate gene by MPS using a targeted design to look for mutations likely to affect splicing of the pre mRNA. Subsequently, predicted splicing alterations are validated by minigenes assays.
In addition to these customized detection tools improving our mutation detection rate we are pursuing the development of the LSDBs (formerly LOVD-USHbases) and prediction tools to assess the pathogenicity of any new variant identified in Usher genes.

Overall, we can provide mutation detections for accurate molecular diagnosis with a rate exceeding 90 % of Usher patients.

6. **Unsuspected phenotypic heterogeneity in Usher syndrome revealed by Massive Parallel Sequencing**

Bitner-Glindzicz, M.[1], Cullup, T.[2], Steele-Stallard, H.[1], Lenassi, E.[3], Vache, C.[4], Roux, A-F.[4], Luxon, L.[5], Webster, A.[3] and Lench, N.[2]

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Abstract:

In the era before massive-parallel sequencing, the clinical sub-classification of Usher syndrome into types 1 to 3, proved to be extremely useful for gene identification and mutation detection surveys. Recently, the economies of scale and availability afforded by high throughput sequencing means that we are beginning to identify individuals with mutations in the genes whose disease is highly atypical.

Although it is likely that all of the Usher genes will turn out to display significant phenotypic heterogeneity, we discuss several individuals, particularly those with mutations in *USH1C* whose disease is milder than expected. This is still the second most common gene causing *USH1C* in the UK population after *MYO7A*. We have previously published a discordant sib pair with c.2227-1G>A and p.Arg103His mutations; both had mild sector RP, one with moderate hearing loss with vestibular hypofunction and the other with severe hearing loss and normal vestibular function, both with normal language acquisition using hearing aids only (Zaihan et al 2011). We now present other cases with normal language acquisition, normal vestibular function and mild RP, but whose mutations have been previously reported with typical disease, including c.238dup p.(Arg80Profs*69) or mutations which are expected to be complete loss of function alleles. The identification and study of these unusually presenting cases is important not only because they may reveal at the molecular level why there is

such variability associated with this disease but also because they highlight that it will be important to counsel parents about hitherto unsuspected variability in clinical outcomes.

7. **NGS for Usher syndrome: Benefits, pitfalls and unexpected findings**

Bolz, H.J.[1,2]

[1]*Center for Human Genetics, Bioscientia, Ingelheim, Germany*
[2]*Institute of Human Genetics, University Hospital of Cologne, Cologne, Germany*

Abstract:

The recent advent of next-generation sequencing in routine diagnostics has transformed the field of medical genetics, making genetically heterogeneous entities accessible to routine genetic testing. Usher syndrome (USH), with 12 (mostly very large) genes known to contribute to this phenotype, represents a prime condition for the application of NGS. However, NGS approaches differ from lab to lab, e.g. in terms of NGS platforms, composition of the "gene panel" and bioinformatics. I will present results from comprehensive genetic analysis (including Sanger sequencing, MLPA and targeted NGS) of samples from ~100 patients clinically diagnosed as USH, demonstrating that mutations in the known USH genes explain the vast majority of cases, and discuss possible pitfalls.

NGS covering non-syndromic hearing loss (NSHL) genes and/or retinitis pigmentosa (RP) genes in addition to the USH genes further increases the diagnostic yield because it also detects coexistent mutations in non-syndromic deafness and RP genes that clinically mimick USH – with important implications for genetic counseling. Because particular mutations in several USH genes (*MYO7A*/USH1B, *USH1C*, *CDH23*/USH1D, *PCDH15*/USH1F, *CIB2*/USH1J) may cause non-syndromic deafness without RP, the USH genes have to be included in NGS panels aimed at identifying the causative mutations in patients with apparently isolated deafness. Thus, USH-causing mutations are unevitably being identified in deaf children by massively parallel NGS. Despite the predictive nature of such results, this will increasingly be helpful in correcting the diagnosis and thereby improve patient management. However, parents of newborns with hearing loss must be informed about this possible outcome before giving consent for testing.

We have established quantitative readout of NGS data, thereby enabling the detection of large structural rearrangements (copy number variations, CNVs). We identified several causative one- to multi-exon deletions in *MYO7A*, *PCDH15*, *USH2A* and *GPR98* which were key to the diagnosis in hitherto unsolved constellations (CNVs complemented apparently monoallelic recessive alleles).

Finally, I will discuss potentially actionable mutations and the role of whole exome sequencing (WES) in USH.

8. **Data Sharing to Support Genetic Test Interpretation**

Heidi L. Rehm[1,2], Ahmad Abou Tayoun[1], Andrea Muirhead[1], Katherine A. Lafferty[1], Amy L. Hernandez[1], Jun Shen[1, 2], Sami S. Amr[1, 2]

Laboratory for Molecular Medicine, Partners Healthcare Personalized Medicine, Cambridge, MA, USA; Department of Pathology, Brigham & Women's Hospital and Harvard Medical School, Boston, MA, USA;

Abstract:

The use of next generation sequencing (NGS) has enabled dramatic improvements in the ability to offer comprehensive diagnostic testing at affordable costs to patients. We developed the OtoGenome Test a comprehensive test for nonsyndromic hearing loss as well as many causes of syndromic hearing loss including all forms of Usher syndrome. A subpanel is offered for just Usher genes. The tests use barcoding and hybrid capture followed by NGS on the Illumina HiSeq. VisCap detects copy number variations (CNVs). Sanger sequencing fills in missing data from NGS and confirms variants. Validation showed 100% sensitivity for substitutions (335/335), 97% for indels (63/65), and 100% for CNVs (16/16). The most challenging aspect of the assay is data interpretation. Several hundred variants are identified in each sample. Although most are pre-classified as Benign or Likely Benign using population frequency data and other auto-classification rules, up to 26 novel variants per case are identified. A comprehensive evidence-based variant assessment strategy is used to support variant interpretation and our GeneInsight software auto-drafts patient reports to allow efficiency for the geneticist sign-out process and delivers them in structured form to the EHR enabling automated variant updates as classifications change over time. To date, 355 samples have been analyzed using the OtoGenome test with 42% (150) positive for at least one pathogenic or likely pathogenic variant, using our rigorous evidence-based classification system. Among patients with a possible or known diagnosis of Usher syndrome 65% were positive for one (n=11) or two (n=20) pathogenic or likely pathogenic variants. In addition, 3% of patients with apparent nonsyndromic hearing loss were positive for pathogenic or likely pathogenic variants in Usher syndrome genes. A major challenge of testing is that roughly 48% of cases are inconclusive due to variants of uncertain significance with limited available data in the literature and public databases to infer their potential impact. We recognized that community data sharing would be highly beneficial to enable better understanding of rare variants. To address this need for hearing loss and all diseases, the Clinical Genome Resource (ClinGen) program was launched in Sept 2013 and includes 3 funded NIH grants and the ClinVar database (http://www.ncbi.nlm.nih.gov/clinvar). ClinVar currently contains over 65,000 variants with clinical interpretations, including our own hearing loss clinical data collected over the last 10 years with detailed evidence-based clinical classifications. This includes 3483 variants in 71 hearing loss genes from ~3000 probands. This presentation will include a review of the current state of the ClinVar database and ClinGen program including future plans to enhance community data-sharing of case and variant level data as well as expert curation of genes and variants.

Symposium Session B: *Functional Genetics*

9. **Study of splicing variants in the USH genes through minigene assay and transcript analysis from epithelial nasal cells**

Millan, J.

Unidad de Genética, Hospital Universitario La Fe, Fundación para la Investigación del Hospital La Fe, Valencia, Spain

Abstract:

The first purpose of this study was to determine the pathologic nature of eighteen USH1 putative splicing variants found in our series and their effect in the splicing process by minigene assays. These variants were selected according to bioinformatic analysis.

The second aim was to analyze the USH1 transcripts, obtained from nasal epithelial cells samples of our patients, in order to corroborate the observed effect of mutations by minigenes in patient's tissues.

The last objective was to evaluate the nasal ciliary beat frequency in patients with USH1 and compare it with control subjects.

In silico analysis were performed using four bioinformatic programs: NNSplice, Human Splicing Finder, NetGene2 and Spliceview. Afterward, minigenes based on the pSPL3 vector were used to investigate the implication of selected changes in the mRNA processing. To observe the effect of mutations in the patient's tissues, RNA was extracted from nasal epithelial cells and RT-PCR analyses were performed.

Four MYO7A (c.470G.A, c.1342_1343delAG, c.5856G.A and c.3652G.A), three CDH23 (c.2289+1G.A, c.6049G.A and c.8722+1delG) and one PCDH15 (c.3717+2dupTT) variants were observed to affect the splicing process by minigene assays and/or transcripts analysis obtained from nasal cells. Based on our results, minigenes are a good approach to determine the implication of identified variants in the mRNA processing, and the analysis of RNA obtained from nasal epithelial cells is an alternative method to discriminate neutral Usher variants from those with a pathogenic effect on the splicing process. In addition, we could observe that the nasal ciliated epithelium of USH1 patients shows a lower ciliary beat frequency than control subjects.

10. **The Usher syndrome type 2 protein complex in photoreceptors and hair cells**

Yang, J.

Department of Ophthalmology and Visual Sciences and Division of Otolaryngology, University of Utah, Salt Lake City, UT, USA

Abstract:

Usher syndrome type 2 (USH2) is the most common clinical type of Usher syndrome. Up to now, USH2A, GPR98 and WHRN have been identified as causative genes of USH2. The proteins encoded by these genes have been localized to the periciliary membrane complex in photoreceptors and the ankle link complex in hair cells. Recently, PDZD7, a paralog of WHRN, has been reported as a modifier gene of Usher syndrome. Although the localization of the PDZD7 protein remains unclear in photoreceptors, the protein is localized at the ankle link complex in hair cells. Using biochemical assays, we thoroughly investigate the interactions among the four proteins and present evidence that they are able to form a multiprotein complex in vitro. However, our mouse genetic studies demonstrate that the Usher syndrome type 2 protein complex is not exactly the same in photoreceptors and hair cells.

11. **Decoding of Usher syndrome protein networks reveals insights in the molecular basis of the Usher disease**

Wolfrum, U

Cell & Matrix Biology, Institute of Zoology, Johannes Gutenberg University of Mainz, Germany

Abstract:

The human Usher syndrome (USH) is the most common cause of combined hereditary deaf-blindness. USH is genetically and clinically heterogeneous: 15 chromosomal loci assigned to 3 clinical types, USH1-3. All known USH1 and 2 proteins are organized into protein networks by the scaffold proteins harmonin (USH1C), whirlin (USH2D) and SANS (USH1G) (scaffold protein containing ankyrin repeats and SAM domain). This has contributed essentially to our current understanding of the USH protein function in the eye and the ear and explains why defects in proteins of different families cause very similar phenotypes.

In the inner ear interactions between USH proteins are essential for the correct organization of stereocilia/villi during differentiation of hair cells. Ongoing in depth analyses of USH protein networks in the eye revealed the participation of USH proteins in function modules of photoreceptor cells, namely structural cytoskeletal functions and the participation at endocytosis processes as well as roles in the molecular transport modules and ciliary cargo delivery. The systematic analysis of the USH protein interactome demonstrated molecular links of USH to other ciliopathies, including non-syndromic inner ear defects and isolated retinal dystrophies but also to kidney diseases and syndromes like the Bardet-Biedl syndrome. These findings provide emerging evidence that USH is a ciliopathy molecularly related to other ciliopathies, which opens an avenue for common therapy strategies to treat these diseases.

Supports: German Ministry of Education and Research (“HOPE2” and E-Rare-2, the ERA-Net for Research on Rare Diseases, “EUR-USH”), DFG (GRK 1044), ProRetina Deutschland eV, the FAUN-Stiftung, EU FP7/2009/241955 (SYSCILIA), EU FP7/2009/242013 (TREATRUSH), Foundation Fighting Blindness; FcB – Initiative Usher-Syndrom .e.V.

12. **Defective protein complex assembly produces ER stress that causes cell death in Usher syndrome**

Blanco-Sànchez, B., Clément, A. and Westerfield, M.

Institute of Neuroscience, University of Oregon, Eugene, OR, USA

Abstract:

Human Usher syndrome, the most frequent cause of deaf blindness, is a genetically heterogeneous recessive disease. Patients present with congenital deafness and progressive retinal degeneration. Fourteen loci and eleven genes have been linked to Usher syndrome to date. Surprisingly, these genes encode a wide range of different kinds of proteins including transmembrane adhesion and signaling molecules, intracellular scaffold proteins, and a myosin motor. In vitro binding studies suggest that the scaffold proteins bind the other Usher proteins into a macromolecular complex. Although this model that Usher proteins act together in a complex is an appealing explanation for how the human disease can result from mutation of any one of a number of different genes, it is still controversial. Moreover, the effects of mutations on protein complex formation, subcellular transport, and stability are completely unknown. We have developed an in situ proximity assay to identify if, where, and when Usher proteins form complexes. We find that a subset of Usher proteins preassemble into a complex in the endoplasmic reticulum (ER). In Usher mutants, transport of this complex to the Golgi is disrupted, leading to ER stress and, in some cases, apoptosis. We propose that improper assembly of the Usher complex is a proximal cause of cell death in Usher syndrome. This link between ER stress and apoptosis suggests that therapeutics being developed for the treatment of other neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases, will be useful in managing the progression of symptoms in Usher syndrome patients. Although hearing defects are typically congenital due to defects in the mechanoreceptors, hair cells ultimately die, and vision loss is progressive as photoreceptors degenerate over decades. Treatments that delay or reduce cell loss will provide time to patients, while therapies that address the defects are developed and applied to non-degenerating cell populations.
Funding: the National Institute of Child Health & Development, the National Institute of Deafness & Other Disorders, the National Eye Institute, and the Vision for a Cure and Megan Foundations.

13. **Role for Usher proteins in regulated protein trafficking in photoreceptors: potential mechanism for light-induced retinal degeneration in Usher syndrome**

Cosgrove, D., Zallocchi, M., Cheung, L., Peng, YW. and Delimont, D.

Boys Town National Research Hospital, Omaha, NE, USA

Abstract:

Usher mouse models show elevated light thresholds, following dark adaptation, required to activate the movement of α-transducin and arrestin between photoreceptor outer and inner segments. While the

threshold shifts vary somewhat, we have found this to be true for all Usher models tested to date, including shaker-1,whirler, waltzer, Aims waltzer, Usherin hypomorph, VLGR1 carboxy terminal deletion mutant, and clarin-1 knockout. Thus this appears to constitute a common functional defect in protein trafficking. Recently we demonstrated that RGS9-1 and Gβ5L, components of the GTPase-accelerating complex which determines photoresponse duration, also move between photoreceptor segments in response to light, albeit at much lower light thresholds. This threshold is also shifted to a higher light level in Usher mouse models tested to date. These observations predict that photoreceptors in Usher mouse models would be susceptible to light induced degeneration under conditions that are insufficient to damage wild type retinas. Several different light induced degeneration protocols have proven this to be the case. We used a lentiviral gene therapy vector to deliver wild type human Myosin VIIa to Shaker -1 photoreceptors. We were able to rescue both the light threshold for α-transducin translocation and the retinal degeneration phenotype, proving that these phenotypes are due to the defective myosin VIIa function. Thus we predict humans with Usher syndrome may be more susceptible to light –induced damage due to the inability of the retina to rapidly quench phototransduction when exposed to bright light. This is predictably a direct consequence of defective light-induced trafficking of phototransduction proteins.

14. **Usher syndromes: gathering basic knowledge towards the development of therapeutic approaches**

Pepermans, E.[1,2,3], Michel, V.[1,2,3], Bonnet, C.[2,3,4], Sahly, I.[,3,4], Bahloul, A[1,2,3], Avan, P.[5], El-Amraoui, A.[1,2,3,4] and Petit, C. [1,2,3,4,6]

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Abstract:

Usher syndrome (USH) is the most frequent cause of inherited dual hearing and visual sensory impairment. Three types of USH (USH1, USH2, and USH3), caused by different gene defects, have been distinguished clinically. USH1 is the most severe form in terms of both the extent of the sensorineural hearing impairment and the precocity of retinitis pigmentosa onset. Since the identification of the first causative gene, encoding myosin VIIa (Weil, D, …, Petit,C 1995) a wealth of data enlightening the roles of the USH1 proteins— myosin VIIa (USH1B), harmonin (USH1C), cadherin-23 (USH1D), protocadherin-15 (USH1F) and sans (USH1G) — in the auditory sensory cells (hair cells) has been obtained, mostly through studies of mice carrying mutations in the five orthologous genes. These mutant mice (Ush1 mice) indeed faithfully mimic the human hearing impairment. We will illustrate how multidisciplinary approaches, including a constant back and forth between these models

(with the development of postnatal hair cell-specific conditional knockout mice) and patients (with the development of molecular diagnosis detecting more than 95% of the mutations in the patients), provide key information for the identification and characterization of components of the immature and mature auditory mechano-electrical transduction machinery and the molecular network they form. In contrast, the understanding of the mechanisms underlying retinal dystrophy in USH1 remained totally obscure up to recently, mainly because mutant mice lacking any of the USH1 proteins do not display retinal degeneration. By studying primate photoreceptor cells, we showed that all USH1 proteins also form a protein complex in these cells, where they colocalize at membrane interfaces between the inner and outer segments in rods and between the calyceal processes and the outer segment basolateral region in rods and cones, forming an adhesion belt around the basolateral region of the photoreceptor outer segment. This has led to the conclusion that a defect in the USH1 protein complex-mediated connections likely causes the USH1 retinal dystrophy in humans. Based on this information, alternative animal models for the retinitis pigmentosa of Usher syndrome type 1 are under development. The already gathered knowledge will serve as a basis for the development of gene therapy for both the retinal dystrophy and deafness of Usher syndrome type 1.

Acknowledgments: This work was supported by European Union Seventh Framework Programme, under grant agreement HEALTH-F2-2010-242013 (TREATRUSH), ERC-Hair bundle (ERC-2011-ADG_20110310), LHW-Stiftung, BNP-Paribas, Fondation Raymonde & Guy Strittmatter, FAUN Stiftung (Suchert Foundation), the French State program "Investissements d'Avenir" managed by the Agence Nationale de la Recherche [ANR-10-LABX-65], and the Fondation Voir et Entendre.

15. **The quest for molecular mechanisms of Usher syndrome**

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Abstract:

Usher Syndrome (USH) is a neurosensory disorder affecting both hearing and vision in humans. Linkage studies of families of USH patients, studies in animals, and characterization of purified proteins have provided insight into the molecular mechanisms of hearing. To date, eleven USH proteins have been identified, and evidence suggests that all of them are crucial for the function of the mechanosensory cells of the inner ear, the hair cells. Most USH proteins are localized to the stereocilia of the hair cells, where mechano-electrical transduction (MET) of sound-induced vibrations occurs. Therefore, elucidation of the functions of USH proteins in the stereocilia is a prerequisite to understanding the exact mechanisms of MET. Recently, using linkage analysis and positional cloning strategies, we have mapped *USH1M* locus on chromosome 1q and identified an in-frame eighteen base pair deletion in a *USH1M* gene. This mutation results in deletion of six highly positively charged amino acids from the actin-binding domain of the encoded protein. Currently, we are performing the functional studies to characterize the effect of this mutation on the actin binding and bundling properties of USH1M protein. Results of these studies will be presented at the meeting.

16. **Hair cell specific expression of Clarin-1 is sufficient to prevent auditory and vestibular dysfunction in the mouse model for ear disease in Usher Syndrome III**

Geng, R.[1*], Gopal, S.R.[1], Chen, D.[1], Furness, D.N.[2] and Alagramam, K.N.[1]

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Abstract:

Background: Usher syndrome III (USHIII) is an autosomal recessive disorder caused by mutation in the human Clarin-1 gene and characterized by progressive loss of hearing and vision. Previously, we showed that, in the mouse, 1) Clarin-1 mRNA is expressed as early as embryonic day 17 (e17), and its expression is restricted to ganglion and hair cells of the inner ear, 2) Clarin-1 mRNA is expressed in the inner ear during embryonic, neonatal and postnatal periods, 3) mutation in Clarin-1 affects hair cell function and bundle morphology as early as postnatal day 2 (P2), and 4) mutation in Clarin-1 results in profound hearing loss and variable degree of vestibular dysfunction by P21. In this project, we tried to answer two important questions: Is the expression of Clarin-1 in ganglion cells and hair cells necessary for hearing and balance? Is the ear dependent on postnatal expression of Clarin-1, or is Clarin-1 required only during ear development? Answers to these questions are important to understand the role of Clarin-1 in the inner ear and guide our efforts to develop therapies for USHIII patients.

Method: A transgenic line was generated to limit expression of mouse Clarin-1 to hair cells between e12.5 and P0-3. The transgene construct, TgAC1, is composed of Atoh1 enhancer::beta-globin basal promoter fused to mouse Clarin-1 cDNA. We then generated mice carrying TgAC1 in the Clarin-1 knockout background. Offspring of this cross were designated 'KO-TgAC1.' Hearing (ABR), balance (VsEP) and hair cell morphology were evaluated in KO-TgAC1 mice at various time points.

Results: KO-TgAC1 mice displayed wild-type hearing and balance function at weaning age (P21). However, longitudinal evaluation showed progressive loss of function, starting around P25-30. At a young age (<P15), hair cells from the KO-TgAC1 mice are comparable to wild-type hair cells. However, stereocilia begin to show signs of degeneration by P21. Examination of older mice (>P21) showed that hair cells fail to maintain their stereocilia, and this is consistent with the phenotype observed in KO-TgAC1 mice. Our investigation also revealed potentially critical 5'and 3' untranslated sequence associated with mouse Clarin-1 mRNA.

Conclusion: The inner ear is dependent on prenatal and postnatal expression of Clarin-1. Specifically, maintenance of stereocilia in the mature ear depends on Clarin-1. Expression of Clarin-1 in the ganglion cells is not necessary for the development of hearing or balance in the mouse.

Funding: This work was supported by NIDCD R01 and Maniglia Chair Funds to K.N.A.

Friday, July 11, 2014

Symposium Session C: *Phenotypes and Natural History*

17. **Usher Syndrome: When to suspect it and how to find it**

Kenna, M.[1,2], Lafferty, K.[3], Rehm, H.[2,3], and Fulton, A[1,2]

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[2]*Harvard Medical School, Boston, MA, USA*
[3]*Partner's Healthcare/Personalized Genetic Medicine, Cambridge, MA, USA*

Abstract:

Until very recently, Usher Syndrome was diagnosed in late childhood or adulthood, generally by an ophthalmologist, when a patient with hearing loss developed symptoms of vision impairment. However, with the recent introduction of two technologies, the timing and means of the diagnosis have changed. Newborn hearing screening, the first new technology now available in all 50 states and in many countries, means that babies with hearing loss are often identified in the first weeks or months of life, and often by an otolaryngologist. Additionally, because Usher Syndrome initially presents as a non-syndromic form of hearing loss in newborns and young children, clinical genetic testing, the second new technology, makes it possible to identify a definitive diagnosis in these young children long before routine ophthalmological examinations can.

The early identification of Usher Syndrome allows planning for both the hearing loss and the visual impairment, and will allow access to therapeutic interventions as they become available.

This presentation will outline the early identification of hearing loss, the process of genetic testing and counseling, phenotype-genotype correlations including balance testing, and how this leads to the development of potential interventions and treatment.

18. **Usher syndrome: Do audiological, vestibular and visual geno-phenotype correlations exist?**

Möller, C.

Audiological Research Centre, University Hospital, Örebro, Sweden

Abstract:

In order to make early, good and reliable diagnosis and find possible geno-phenotype correlations, state of the art clinic and genetic tests have to be applied. Diagnosis can in most cases be made as

early as in the first year of life. The advantages of correct early genetic and clinical diagnosis are manifold, giving opportunities to give a prognosis and correct habilitation. This presentation is based on some 350 subjects with US. US type 1 (150), type 2 (170), type 3 (30). The majority of these subjects have been genetically confirmed. The hearing loss/deafness is first recognized by universal newborn screening, to be confirmed around 1 month of age. Audiological geno/phenotypes will be described with examples of possible mutation specific differences. The balance organ and the vestibular function can be determined at age 1-2 months. A bilateral vestibular areflexia together with a profound deafness is indicative for US type I with few exceptions. Visual examination has to be electrophysiological by the use of electroretinography (ERG). Possible visual geno/phenotype differences will be demonstrated.

19. **Genotype-phenotype correlations on 161 USH2A patients – some mutation combinations cause a more severe audiometric phenotype**

Pennings, R.J.E.[1], Löfgren, M.[2], Huygen, P.L.M.[1], Sadeghi, A.M.[3], Tranebjaerg, L.[4], Kremer, H. [1], Kimberling, W.J.[5], Cremers, C.W.R.J.[1] and Möller C.[2]

[1] *Radboud university medical centre, Department of Otorhinolaryngology, Nijmegen, the Netherlands*
[2] *University Hospital Örebro, Audiological Research Centre, Örebro, Sweden*
[3] *University of Gothenburg, Department of Audiology, Gothenburg, Sweden*
[4] *University of Copenhagen, Department of Cellular and Molecular Medicine, Copenhagen, Denmark*
[5] *Boys Town National Research Hospital, Genetics Center, Omaha, NE, USA*

Abstract:

Usher syndrome type 2a is caused by mutations in the *USH2A* gene. Previously, several studies evaluated hearing impairment in Usher syndrome type 2a, but in order to evaluate the effect of mutations on the audiometric phenotype, larger data samples are necessary. It was, therefore, decided to combine the audiometric data from two large data sets of Usher syndrome patients from the Netherlands and Sweden.

This study presents the results of a retrospective analysis of hearing impairment in 161 (78 Dutch and 83 Swedish) patients with Usher type 2a. A total of 976 audiograms were included in the study and in these patients at least one mutation in the *USH2A* gene was identified by sequencing of the entire or several exons of the *USH2A* gene. Cross-sectional nonlinear regression analysis of last-visit audiograms in patients with two confirmed USH2A mutations demonstrates a gradual decline in hearing over decades. The annual threshold deterioration was about 0.4, 0.3, 0.2, 0.3, 0.5 and 0.5 dB/y at 0.25, 0.5, 1, 2, 4 and 8 kHz, respectively and can not be attributed to presbyacusis.

A thorough analysis of the thresholds demonstrated a lot of variability of the thresholds per frequency when compared to the linear regression line. Based on these findings, it was decided to perform longitudinal evaluations of those patients that demonstrated exceptionally severe hearing impairment for their age and those with exceptionally progressive hearing impairment. After the identification of these patients, the underlying mutation combinations were evaluated in order to identify those

mutations that cause a more severe phenotype. Several homozygous and compound heterozygous mutation combinations were identified to lead to a more unfavourable audiometric phenotype, including the homozygous c.2299delG mutation. It can from this study, however, also be concluded that other factors like modifying genes and environmental factors definitely play a role in the progression of hearing impairment in Usher type 2a. Further multi-centre studies are necessary to collect epidemiological data to enable the identification of those other contributing factors.

Symposium Session D: *Preclinical Studies*

20. **Antisense oligonucleotides effectively treat Usher syndrome in mice**

Lentz, J.J.[1], Jodelka, F.M.[2], J. Hinrich, A.J.[2], Ponnath, A.[1], Amato, R.[1], Flaat. A.[1], McCaffrey, K.E.[2], Bazan, N.G.[1], Duelli, D.M.[2], Rigo, F.[3] and Hastings, M.L.[2]

[1]*Neuroscience Center, LSUHSC, New Orleans, LA, USA*
[2]*Rosalind Franklin University of Medicine and Science, North Chicago, IL, USA*
[3]*Isis Pharmaceuticals, Carlsbad, CA, USA*

Abstract:

Usher syndrome (Usher) is the leading genetic cause of combined deafness and blindness. Type 1 Usher (Usher 1) is the most severe form and characterized by profound hearing impairment and vestibular dysfunction at birth, and adolescent-onset of retinitis pigmentosa (RP). Approximately 6-8% of Usher type 1 cases are caused by mutations in the *USH1C* gene. The c.216G>A (216A) mutation in *USH1C,* which accounts for nearly all Usher 1 cases in Acadian populations of South Louisiana in the United States and Quebec, Canada, creates a cryptic splice site resulting in a truncated harmonin protein. We created a mouse model for Usher 1C by knocking in the 216A mutation to test the feasibility of correcting the disease-associated genetic defect. Mice homozygous for the *Ush1c*.216G>A mutation (216AA) exhibit circling behavior indicative of severe vestibular dysfunction and deafness, have retinal dysfunction by 1 month of age and begin to lose photoreceptors between 6 and 12 months of age. Antisense oligonucleotides (ASOs) were designed to target the 216A mutation and test the hypothesis that blocking the mutant splice site will rescue protein expression and be therapeutic for mice and ultimately Usher patients. 216A-targeted ASOs were used in vitro in cell-free and cellular assays and in vivo by systemic or local injection. Correction of splicing and protein expression was evaluated by RT-PCR and western blot. Immunofluorescence was used to analyze Harmonin expression and ASO localization. Hearing and vestibular function were evaluated by auditory-evoked brain stem response (ABR) and open-field chamber analysis, respectively. Visual function was evaluated by electroretinogram (ERG) analyses and optical coherence tomography (OCT) imaging was used to examine ocular structures. ASOs effectively target 216A RNA in an Usher patient cell line, and in the cochleae and retinas of 216AA mice when delivered systemically or locally. Treatment with 216A-targeted ASOs to neonate mice corrected protein expression with an improvement in harmonin localization in the cochlea and retina; rescued vestibular and hearing function, and demonstrated an improvement in visual function. Antisense oligonucleotides (ASOs) are a powerful tool that can be used

to correct aberrant expression. Our results show that the 216A mutation in *Ush1c* can effectively be targeted both in vitro and in Ush1c.216AA mice and suggest the therapeutic potential of ASOs in Usher syndrome and other diseases of the ear and eye.

Acknowledgements:
FFB WG-Early TRAP: TA-NMT-0613-0609-LSU-WG
NIH/NIDCD: 1R01DC012596-01A1
Eye on Jacob Foundation

21. **TMC gene therapy in mouse models of human deafness**

Askew, C.[1], Rochat, C.[2], Ahmed, H.[1], Pan, B.[1], Asai, Y.[1], Child, E.[1], Schneider, B.[2], Aebischer, P.[2] and Holt, J.R.[1]

[1]*Boston Children's Hospital, Harvard Medical School, Boston, MA, USA*
[2]*Ecole Polytecnique Federale de Lausanne, Switzerland*

Abstract:

Viral gene transfer in the cochlea has been investigated in animal models over the past 15 years. One recent study demonstrated recovery of auditory function *in vivo* in a mouse model of human deafness (Akil et al. 2012). While encouraging, many challenges remain including translation to other genetic models of progressive hearing loss. In the current study, we investigated rescue of hair cell mechanotransduction *in vitro* and restoration of auditory function *in vivo* using adeno-associated viral (AAV) vectors to deliver the wild-type sequence for Transmembrane Channel-like genes 1 or 2 (*Tmc1* or *Tmc2).* TMC1 and TMC2 are putative components of the hair cell transduction complex (Pan et al. 2013) and are likely interaction partners with Usher I proteins in hair cells. Mutations in human TMC1 cause both recessive (DFNB7/11) and dominant progress hearing loss (DFNA36).

AAV1 was selected based on initial screens of five AAV serotypes (AAV1, 2, 6, 8, and 9) for high hair cell transfection rates in P0 cochlear cultures: ~50-70% after 7 days incubation. Next we delivered an AAV1-GFP construct *in vivo* into the scala tympani of wild-type mice via round window membrane (RWM) injection with micropipettes. We found GFP expression in inner hair cells along the length of the cochlea and in a few outer hair cells at the base. Neither AAV1 transfection nor GFP expression altered hair cell mechanotransduction, Auditory Brainstem Responses (ABR) or Distortion Product Otoacoustic Emission (DPOAE) relative to sham injected control mice.

Next we generated AAV1 vectors that carried a chicken beta-actin promoter (CBA) followed by the coding sequence for either *Tmc1* or *Tmc2.* We found that AAV1-*Tmc1* and AAV1-*Tmc2* constructs rescued hair cell mechanotransduction when measured in transfected *Tmc1*/*Tmc2* doubly-deficient hair cells *in vitro* and *in vivo*. We discovered that *in vivo* delivery of AAV1-*Tmc1* via the RWM of P1 *Tmc1* KO mice resulted in modest ABR threshold recovery at P25-P30 in 6 of 11 mice tested. Histological

assessment of injected cochleas indicated outer hair cell degeneration persisted, consistent with the lack of DPOAE recovery and AAV1-GFP preference for inner hair cells.

The data reveal functional recovery at the cellular and system levels and demonstrate important proof-of-concept progress toward development of gene therapy strategies to restore auditory function in deaf patients. Further work will be required to refine AAV vector serotypes, promoters and injection strategies that optimize the number of hair cells transfected *in vivo* and maximize recovery of auditory function.

Funding: Bertarelli Foundation

22. **Strategies to prolong survival of cone photoreceptors**

Cepko, C.

Department of Genetics, Harvard Medical School, Boston, MA, USA

Abstract:

In retinitis pigmentosa (RP), there is a progressive loss of vision. The cells that mediate the first step in vision, photoreceptors, are the cells that are the primary target of the genetic lesions that lead this disease. Rods, the photoreceptor type that mediates vision in dim light, are often the first cell type affected in RP, leading to night blindness. However, subsequent to rod dysfunction and death, the cone photoreceptors, which mediate color and daylight vision, also lose function and die. We have suggested a model wherein cones are affected due to dysregulated metabolism, which occurs after rods die. As rods are the major cell type in the area of the retina where cones reside, cones experience a greatly altered environment following rod death. The cone outer segments collapse, they lose their intimate association with the retinal pigmented epithelium, and they are exposed to a hyperoxic environment. They show greater oxidation of their nucleic acids, proteins, and lipids. They also begin to digest some of their own proteins, a sign of metabolic dysregulation, perhaps following a reduction in their uptake of nutrients, or another type of imbalance in their metabolism. Because of these problems, we have begun to develop AAV-mediated gene therapy to combat metabolic stress in the cones. Our progress along these lines will be discussed.

23. **Determining the mechanism of vision and hearing loss associated with usher type 2A**

Yoder, M., Adijanto, J. and Naash, M.I.

University of Oklahoma Health Sciences Center, Cell Biology, Oklahoma City, OK, USA

Abstract:

Purpose: Usher syndrome (USH) is the leading cause of combined deaf/blindness. The study of USH is increasingly important, as there are no curative therapies. There are three types of USH, the most common being USH type II (USH2). And, although three USH2 genes have been identified, mutations in USH2A account for ~80% of all USH2 cases. The USH2A gene codes for the protein, usherin, which is located at the connecting cilium of photoreceptors and the ankle links of developing hair cells. Usherin is extremely large (cDNA ~15 kb), making it difficult to study therefore the USH2A-associated disease mechanisms remain elusive. The c.2299delG mutation accounts for 45% of USH2A. In order to more accurately study the role of usherin, we have generated an USH2A mouse model expressing the c.2299delG mutation. Here we undertake retinal and cochlear characterization of this newly developed USH2A model with the ultimate goal of developing novel therapeutics for treatment of this debilitating disease.

Methods: The c.2299delG knock-in model has a mutation that results in a frameshift with the deletion of a guanine at position 2299, resulting in a premature stop codon. The knock-in construct also contains an IRES (internal ribosomal entry site) followed by GFP producing a dicistronic message **(Fig. 1).** Using RT-PCR full length and truncated usherin expression in WT and KI animals was evaluated. Cochlear phenotype was evaluated at postnatal day 21 (P21) and P60 using immunohistochemistry (IHC) staining of phalloidin. The retinal phenotype was evaluated using IHC at P30, funduscopy and fluorescein angiography at P70, and electroretinography (ERG) up to P90.

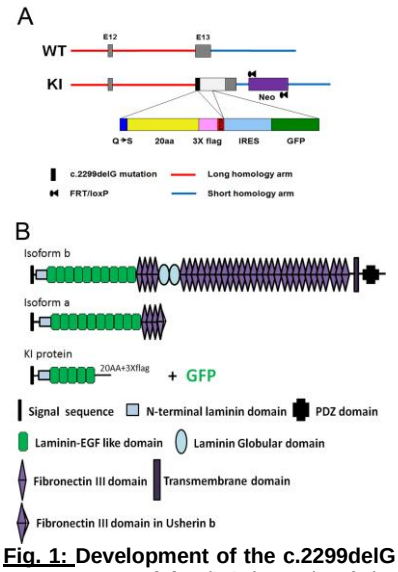

Fig. 1: Development of the c.2299delG KI mouse model. A) Schematic of the generation of the usherin KI construct. **B**) Protein Isoforms of usherin and predicted truncated protein produced from the KI message, along with GFP expression.

Results: The KI mutation is present in the endogenous locus, truncated message is stable, and the mutant protein is expressed. GFP expression identified new tissues expressing usherin that have not been previously reported. Cochlear IHC evaluations at P60 show inner hair cell dismorphology consistent with hearing loss and retinal data show normal morphology via IHC and normal ERG up to P90 under low light conditions.

Conclusion: Based on the data we are ageing mice to determine retinal phenotype onset and severity. GFP expression will allow for further study of possible systemic effects usherin may have in conjunction with deaf/blindness. Data thus far is consistent with USH patients having a later onset of visual impairments compared to hearing loss. Full characterization of this model will lead to a better understanding of the human disease and a valuable resource to develop targeted therapies for treatment.

Funding: This project was supported by Foundation Fighting blindness and NEI (EY10609, EY18656, EY022778).

24. **Characterization of Usher mouse model retinal degeneration and assessment of potential therapy**

Trouillet, A.[1], Dubus, E.[1], Moutsimilli, L.[1] ,Degardin, J.[1], Estivalet, A.[1], Simmonutti, M.[1], Sahly, I. [1], Sahel, J.[1,3,4,5], Petit, C.[1,2] and Picaud, S.[1]

[1]*Institut de la Vision-17 rue Moreau,Paris, F-75012, France INSERM, U968-UPMC, UMRS968 -CNRS, UMR 7210 Paris, France*
[2]*Institut Pasteur Paris, Paris, France*
[3] *Centre Hospitalier National d'Ophtalmologie des Quinze-Vingts, Paris, France*
[4] *Institute of Ophthalmology, University College of London, London, UK*
[5] *Fondation Ophtalmologique Adolphe de Rothschild, Paris, France*

Abstract:

Usher syndrome is the most frequent cause of monogenic inherited sensory disorder associating both deafness and blindness with a prevalence estimated to 1/25.000. Nine different genes have already been associated with the disease including Ush1c/harmonin gene and Ush1g/sans gene. Although the mouse models mutated for these genes generally reproduce well the hearing phenotype of the disease, very few visual symptoms have ever been reported. We here present result showing photoreceptor dysfunction in animal models under specific experimental conditions.
We have designed experimental conditions generating visually impaired phenotypes in two Usher animal models, an harmonin mutant mouse and a SANS Knockout mouse. These retinal phenotypes included a significant reduction of electroretinograms (ERGs) amplitude. The reduction was greater in homozygote animals than in heterozygote mice for both SANS KO mice and Harmonin mutant mice. These functional changes were consistent with photoreceptor alterations on histological sections. These phenotypes enabled us to investigate therapeutic treatments in these animal models of the Usher syndrome type 1. A first pharmacological treatment was found to rescue the visual phenotypes at both the functional and morphological levels.

This study demonstrates the possibility to generate a measurable retinal phenotype in animal models of Usher syndrome type 1. It therefore paves the way to assess different therapeutic strategies on these animal models to prevent or at least slow down the degenerative processes.

Funding: This project was supported by the European Community contract TREATRUSH (no. HEALTH-F2- 2010-242013).

Symposium Session E: *Therapy and Clinical Trials*

25. **Vector discovery and design for improved delivery to the retina and cochlea**

Vandenberghe, L.H.

Massachusetts Eye and Ear, Boston, USA

Abstract:

Gene therapy for neurosensory disorders is a powerful approach in single gene disorders such as Usher Syndrome. Our research has focused on the discovery of methods and reagents to safely and efficiently deliver genes to the relevant cell types in the retina, and more recently to the cochlea. Here, we will describe the status of the field in terms of AAV gene delivery, the progress that has been made with the use of natural AAV serotypes, and the ongoing discovery work with lab derivatives and in silico designed vectors. Specifically, we will discuss vectors that were built to unlock future therapeutic strategies such as optogenetic gene therapy and intravitreal administration for clinical translation.

26. **Ignore the stop: translational read-through of nonsense mutations in Usher syndrome genes**

Nagel-Wolfrum, K.

Cell and Matrix Biology, Inst. of Zoology, Johannes Gutenberg University of Mainz, Germany

Abstract:

The Usher syndrome (USH) is the most common form of inherited deaf-blindness with a prevalence of ~1/6,000. Three clinical subtypes (USH1-USH3) are defined according to the severity of the hearing impairment, the presence or absence of vestibular dysfunction and the age of onset of retinitis pigmentosa (RP). Currently only the amelioration of the hearing deficiency symptoms by cochlear implants is implemented, but no treatment of the senso-neuronal degeneration in the eye exists.

Patient screenings predict that ~15% of all pathogenic variants identified for USH patients are nonsense mutations. Therefore, a therapy that targets nonsense mutations could be beneficial for a substantial cohort making the approach both practical and economical. Recently, a gene based therapeutic approach for nonsense mutation-caused genetic diseases has emerged, namely the so-called read-through therapy. Nonsense mutations are caused by point mutations, which generate premature stop codons. During translation nonsense codons lead to premature translational termination of the mRNA, and subsequently to the lack of normal full-length protein expression. The read-through therapy is based on the discovery of small molecules that allow the translation machinery to recode a nonsense codon into a sense one and consequently the synthesis of functional full-length proteins. In our project, we currently focus on designer aminoglycosides and PTC124 as translational read-through inducing drugs (TRIDs).

So far, we demonstrated TRIDs induced read-through on different USH causing nonsense mutations in *USH1C*, *USH2A* and *CLRN1* (USH3A) in cell culture. In addition, we observed read-through of an USH1C causing nonsense mutation in organotypic retina culture and *in vivo* following application of PTC124 and designer aminoglycosides. Our data indicate that the read-through efficiency is not only dependent on the mutation itself, but also on their position and the sequence context in the gene.

Therefore, independent analysis of each specific USH causing nonsense mutation might be necessary before TRIDs can be applied for personalized therapy. Furthermore, our comparative analyses demonstrated the improved safety profile of designer aminoglycosides and PTC124 compared to classical aminoglycosides like gentamicin.

In conclusion, the high ocular biocompatibility combined with the sustained read-through efficacies places PTC124 and designer aminoglycosides in the spotlight for treating the progressive vision loss in USH patients carrying nonsense mutations.

Supports: German Ministry of Education and Research (E-Rare-2, the ERA-Net for Research on Rare Diseases) “EUR-USH”, EC FP7/2009/241955 (SYSCILIA); FAUN-Stiftung Nuremberg.

27. **Cochlear implantation in individuals with Usher syndrome**

Liu, XZ

Department of Otolaryngology, University of Miami Ear Institute, Miami, FL

Abstract:

Remarkable progress has been made in understanding the pathogenesis of Usher syndrome, which results in the loss of the two most vital human senses and therefore burdens patients with a severe disability. Virtually all members of the deaf community view the visual impairment that accompanies USH as a devastating handicap. Molecular genetic testing with high-throughput sequencing techniques have provided comprehensive genetic testing covering all previously identified USH genes. The earlier intervention is important to maximize the likelihood of development of useful auditory-oral communication skills prior to the onset of the visual impairment. Cochlear implantation is a safe and effective mode of rehabilitation for patients with severe to profound hearing loss. Our study shows that early intervention is critical to developing effective oral-auditory skills prior to visual loss. Benefits of early intervention and diagnosis also should include timely counseling regarding genetic issues, educational and vocation placement. The current knowledge of cochlear implants, implications of in cochlear implants in patients with USH including the auditory performance, improvement of hearing handicap, and the risk of cochlear implantation will be discussed.

Acknowledgements: The work is supported by NIH DC 05575

28. **Progress toward a vestibular implant for restoring sensation of head movement**

Della Santina, C.C, Ward, B.K, Sun, D.Q, Semenov, Y.R, Dai, D., Fridman, G.Y, Hayden, R, Davidovics, N.S, Rahman, M.A, Valentin, N.S, Ahn, J, Hageman, K, Migliaccio, A.A, Kalayjian, Z, Andreou, A, Tejada, F, Mitchell, D, Cullen, K.E, Carey, J.P, Hedjoudje, A, Boutros, P and Treviño, C.

Vestibular NeuroEngineering Lab, Johns Hopkins School of Medicine, Baltimore, MD, USA

Abstract:

While the impacts of Usher syndrome on vision and hearing are well known, many affected individuals also experience bilateral vestibular deficiency (BVD), which causes illusory drift of visual fields during head movement, chronic disequilibrium and postural instability due to failure of vestibulo-ocular and vestibulo-spinal reflexes. Recent epidemiologic data from a national survey of United States adults reveal that more than 64K US adults (28/100K) suffer a constellation of symptoms consistent with chronic, profound bilateral loss of vestibular sensation. Affected individuals report reduction or cessation of driving due to their symptoms (44%), a high incidence of reduced participation in social activity (56%), a >24-fold increase in fall risk in comparison to the nationwide average. BVD individuals also report significantly reduced quality of life, increased health care expenses, and decreased productivity due to dizziness-related workplace absenteeism.

An implantable prosthesis that continuously and accurately emulates normal sensory transduction of head rotation could significantly and cost-effectively improve quality of life for these individuals. Progress toward such a device has accelerated over the past decade, and clinical feasibility trials of similar devices designed to deliver transient stimulation of the labyrinth have yielded promising results in humans. Achieving sufficient stimulation selectivity and intensity to accurately and chronically/continuously encode the full range of natural 3-dimensional head movements remains a key challenge. This lecture will provide an update on prosthetic vestibular stimulation research at the Johns Hopkins Vestibular NeuroEngineering Lab, including the neurophysiologic and anatomic basis of current prosthesis development efforts; comparison of different stimulation and encoding strategies; impact of vestibular implantation on hearing; directional plasticity and adaptive responses to chronic multichannel stimulation; correlation of vestibular electrically-evoked compound action potentials to eye movement responses, infrared laser stimulation; novel approaches to inhibiting undesired neuronal activity; and progress made through University-industry partnership toward a system for clinical application.

Supported by: NIH R01DC009255, R01DC13536, R01DC002390, T32DC000023, T32DC000027, T32EB003383

29. **Updates on a Gene Therapy Trial for Usher Syndrome Type IB**

Pennesi, M.E., Yang, P., McBride, M., Lauer, A.K., Stout, J.T., and Weleber, R.G.

Oregon Health and Science University, Portland, OR, USA

Abstract:

Usher Syndrome Type 1 is a syndromic form of retinitis pigmentosa that results in congenital sensorineural loss, vestibular areflexia, and retinal degeneration. Type 1B Usher syndrome is due to

mutations in *MYO7A* and accounts for about 50% of cases of Type I Usher Syndrome. There remains no effective treatment for this disease, but gene replacement therapy has the potential restore a normal copy of *MYO7A* to the affected patients. UshStat[tm], developed by Oxford Biomedica (now Sanofi-Fovea), is an EIAV based lentiviral vector that carries a normal copy of the *MYO7A* gene. In collaboration with Hopital Quinze Vignt, we have undertaken a phase I/II clinical trial to evaluate the safety of UshStat[tm] in patients with Usher Syndrome Type IB. To date, four patients have been treated without any serious adverse events. The trial is ongoing with a planned enrollment of up to 18 patients.

Funding: Hear See Hope, Foundation Fighting Blindness, Research to Prevent Blindness, Oxford-Biomedica, Sanofi-Fovea

30. **Getting Vision-Saving Therapies Out to the People**

Zilliox, P.

Foundation Fighting Blindness, Clinical Research Institute, Columbia, MD

Abstract:

The Clinical Research Institute is a subsidiary of the Foundation Fighting Blindness with the focus of accelerating development of promising therapies for retinal degenerative diseases toward clinical trials and regulatory approvals.

To move a treatment into human studies requires vast capital resources, strong regulatory expertise, and clinical-trial know-how. Many of these promising therapies are emerging from academic centers or incubators with limited experience in drug development and limited financial resources.

The Clinical Research Institute's mission is to identify promising therapies (e.g., small molecules, biologics, gene therapies, stem cells therapies), and for which preclinical efficacy and safety have already been demonstrated in pre-clinical models.

Strong therapeutic candidates are evaluated by members of the Scientific Advisory Board and by drug development experts. The Clinical Research Institute prioritizes these therapies based on their chances of success, as well as gaps in resources, expertise, management and finances and will assist with clinical-development expertise and funding to get these treatments into human studies.

In parallel, the Clinical Research Institute is building close relationships with Big Pharma (e.g., Sanofi) to create visibility and awareness of these therapies, so their development can be taken over until commercialization.

One of the challenges is the absence of clinical trial endpoints that have been validated and accepted by the FDA and European regulatory agencies. These agencies want guarantees that the treatments are safe and also bring a significant clinical benefit for the patients.

The Clinical Research Institute is taking on this challenge through programs such as ProgSTAR, a natural history study of Stargardt disease. ProgSTAR has been designed in collaboration with world experts with the objective of following up to 250 genotyped Stargardt disease patients for two years, measuring disease progression using state of the art imaging and functional testing equipment. The study will enable to validate new clinical trial endpoints and greatly improve study design and potential for success.

The Clinical Research Institute is also leading an effort to validate the ellipsoid zone, as measured with spectral domain optical coherence tomography, as a possible clinical endpoint for some patients with retinitis pigmentosa. The basis of these efforts comes from work published by David Birch, Ph.D., at Retina Foundation of the Southwest, and Richard Weleber, Casey Eye Institute, Oregon Health & Science University.

Another Foundation-supported resource for better understanding disease progression and accelerating enrollment in clinical trials is My Retina Tracker, a state-of-the-art patient registry implemented in collaboration with the Office of Rare Diseases. All patients with retinal degenerative diseases are encouraged to enroll in the registry.

31. **Consideration on Usher Syndrome to prepare future therapies**

Audo, I., Mohand-Said, S., Zeitz, C., Bonnet, C., Petit, C. and Sahel, J.A.

Centre de Recherche Institut de la Vision, UMR S 968 Inserm / Université Pierre et Marie Curie/CHNO des Quinze-Vingts, Paris, France

Abstract:

Usher syndrome is a clinically and genetically heterogeneous disorder with the multisensory impairment, including cochlear and retinal impairment. The underlying genetic defects are now better understood although underlying physiopathologic mechanisms still need to be better understood. The first gene therapy trial targeting the retinal disease in patients with *MYOVIIA* mutations is ongoing. We will take this example to open the discussion toward specific challenges inherent to Usher syndrome to develop future therapies.

32. **Living with Usher Syndrome: Words for the Scientists**

Pellerin, R.

The Unstoppable, Waterbury Center, VT, USA

Saturday, July 12, 2014

Family Conference Session I: Diagnosis

33. **Usher Syndrome: Why a definite diagnosis matters**

Kenna, M.[1,2], Lafferty, K.[3], Rehm, H.[2,3], and Fulton, A[1,2]

[1]Boston Children's Hospital, Boston, MA, USA
[2]Harvard Medical School, Boston, MA, USA
[3]Partner's Healthcare/Personalized Genetic Medicine, Cambridge, MA, USA

Abstract:

To make a diagnosis of Usher Syndrome, you first have to suspect it, and since it presents in early childhood as a non-syndromic form of hearing loss, the level of suspicion must be high enough to prompt testing. However, many parents with new babies with hearing loss are overwhelmed with unfamiliar and complex information, the requirements for language services, the need for hearing aids or cochlear implants, and uncertainly about the future. Diagnostic testing to figure out the cause of the hearing loss, especially if the baby seems otherwise healthy, is an additional emotional and time-consuming burden. Insurance issues, the possibility that other family members may be affected, and heightened uncertainty about the future based on both hearing loss and now vision loss may make the idea of genetic testing difficult.

However, defining a specific cause of hearing loss is very helpful in planning for the child's future, academically, socially, and medically. This presentation will review how the diagnosis of Usher syndrome is suspected and confirmed, how to connect with Usher Syndrome resources, and how you/your child might connect with research that may apply to you and your family.

Family Conference Session II: Psychological Aspects – Words from the Professionals

34. **Family and Personal Responses to the Diagnosis of Usher Syndrome**

Miner, I.D.

Licensed clinical social worker and consultant, Los Angeles, CA, USA

Abstract:

Introduction: There were issues raised at the last conference about what providers do and should communicate to parents and patients about Usher Syndrome.

Survey: In the interest of answering that question, a survey was undertaken with adults with Usher and parents of children with Usher. A survey was composed and sent out in two forms: on the Web, and

through email. Recipients were informed that only demographic material, current age, age at diagnosis, diagnosis and whether or not they had been referred to a geneticist were being asked. No names were asked.

Questions were asked concerning what and how parents and people with Usher were told about their diagnosis, what information was given to them, and to rate the empathy and interest shown by providers. They were also asked about referrals to support services, what information they had now that was most helpful, and concerns they have for themselves or for the future.

Literature review: No literature was found specific to informing parents of their child's diagnosis of Usher, but there is literature on informing parents of their child's diagnosis of diabetes, and vision impairment. There is literature on the life of people with Usher from the Nordic countries which includes experiences of diagnosis.

Results: The sample was small. Forty surveys were returned, 16 were from parents with children and young adults up to the age of 22. The remaining ones were from parents of adult children with Usher and adults with Usher ranging from age 24 to 66, for whom these questions felt very fresh. Two families filled them out together: the adult with Usher and their hearing sighted parent. Four families had two children with Usher.

Findings: Parental and Usher adult experiences ran the gamut from "as good as can be expected" to "disastrous." There were examples of missed diagnosis and incorrect diagnosis. Most parents felt their providers were fairly empathetic, but they had excellent suggestions about how the experience could be improved, including access to other parents, printed material so parents don't have to look for information themselves, and guidance. Parents are fearful about their children's futures; this applies to parents of adults with Usher as well. Parents referred to the Coalition for Usher Syndrome Research as having filled an important need for them. Some contributions from other countries related to support services may be useful. Issues of coping style and information will be discussed.

There was no funding for this survey.

35. **Physical and Psychological Aspects of Usher Syndrome**

Möller, C.

Audiological Research Centre, University Hospital, Örebro, Sweden

Our Swedish research group on Usher syndrome are at the moment doing extensive research concerning, physical and psychological problems related to the dual sensory loss in Usher syndrome. Over 200 persons with Usher syndrome have answered an extensive national health enquiry regarding general health. Results will be presented which shows that persons with usher type II and III have severe physical and psychological problems compared to the large Swedish reference group. This includes fatigue, headache, insecurity, feeling worthless etc, but also significantly higher prevalence of suicidal thoughts. The severity of physical and psychological problems were partly correlated to age of

diagnosis. The severity of problems were however not correlated to the severity of hearing or vision problems but were statistically correlated to “having a job or not”. Studies have been performed in persons with Usher type I and their problems are similar but to some extent not so severe.
The results of these studies will be discussed.

Family Conference Session III: Gene Therapy 101

36. **Gene Therapy 101**
Vandenberghe, L.H.

Mass Eye and Ear and Harvard Medical School

The session Gene Therapy 101 is meant to provide background on what gene therapy is, what can be expected from this approach for Usher syndrome patients, and what the status of the field is currently. A question and answer session will allow people to ask their questions to the presenter.

Family Conference Session IV: Updates to the Families

37. **Usher Syndrome Research**
Géléoc, G.
Boston Children’s Hospital, Department of Otolaryngology, Boston, MA, USA

38. **Translational Research and the Usher Syndrome Registry**
Kenna, M.A.
Department of Otolaryngology and Communication Enhancement, Boston Children’s Hospital, Boston, MA, USA

Family Conference Session V: Patient Care and Rehabilitation (Selected Presentations)

39. **Stress in individuals with Usher syndrome type II**

Högner, N.

Institute for Rehabilitation Sciences, Department of Education and Rehabilitation of the Blind and Low Vision Individuals, Humboldt University of Berlin, Germany

Abstract:

Due to their dual sensory impairment people with Usher syndrome are assumed to have a high risk of stress experience. The purpose of this study was the development and evaluation of a questionnaire (SQ) to assess frequency and intensity of stress by external stressors in people with usher syndrome type II (USH2).

The construction of the questionnaire is based on the domains of the component “Activities and Participation” of the WHO’s ICF concept, which have been modified. These modifications lead to the domains “Communication”, “Orientation and Mobility”, “Activities of Daily Living”, “Interpersonal

Interactions and Relationships", "Recreation and Leisure" and "Work and Employment", which were used to postulate external stress factors. The questionnaire was administered to 262 adults with USH2 (ages 17-79, mean age = 51; 53 % female; 32 % employed) and evaluated through item and factor analyses. The evaluation shows good indices in terms of item and factor analysis. The a priori postulated structure was well reflected in five factors (after exclusion of the items belonging to the domain "Work and Employment" since most adults were unemployed).

In addition to the SQ, the standardized stress questionnaire "Trierer Inventory of Chronic Stress (TICS)" was administered to compare stress frequency between the USH2-sample group and a German reference group (n = 604). The investigation concluded that people with USH2 experience stress more often in the TICS scales, which indicate a lack of social-emotional need fulfillment ("Chronic Worry", "Social Isolation", "Being Overwhelmed with Work" and "Social Tensions"). Less stress was experienced in scales which include high expectations ("Work Overload", "Social Overload" and "Success Pressure").

As a result of the examination of differences between the single factors' and the TICS scales' mean values, it turned out that the biggest stress in SQ (related to frequency and intensity) was seen in the factor "Orientation and Mobility" and in TICS (with regard to frequency) in the scales "Chronic Worry" and "Social Isolation"; these obviously are the central problem areas of the participants of the study. In the SQ as well as in TICS stress frequency and stress burden were dependent on person specific variables (age, gender, partner and work).

The results give indications for rehabilitation arrangements to avoid and reduce stress in people with USH2 especially in the areas of Orientation and Mobility, Chronic Worry and Social Isolation.

40. **Usher type 2 syndrome: hearing, educational, socio-economic and vocational impacts**

Blanchet, C.[1], Vache, C.[2], Baud, D.[2], Hamel, H.[1], Meunier, I.[1], Uziel, A.[1], Roux, A-F.[2] and Mondain, M.[1]

[1]: Sensory Genetic Disease National Centre, Montpellier University Hospital, Montpellier, France
[2]: Molecular Genetic Laboratory, Montpellier University Hospital, Montpellier, France

Abstract:

Objectives: Study of educational, socio-economic and occupational impacts of usher syndrome type 2 and of auditory rehabilitation acceptability impacts.

Study design: Retrospective study of a French cohort composed of 73 patients (females 40; males 33) suffering from *USH2A* Usher syndrome. The median age at the first consultation in the referral center was 41 years old (18 to 76). Hearing loss degree was moderate in 49 cases and severe in 24 cases. Speech perception, lip-reading (open set word recognition test, connected discourse tracking CDT), and speech intelligibility (speech Intelligibility Rating SIR) were evaluated by a speech therapist. Educational, socio-economic and occupational statuses were analyzed following French National Institute of Statistics and Economics Studies (INSEE) criteria.

Results: Fourteen patients (19%) didn't use any hearing aid, 7 patients (10%) used unilateral hearing aid and 52 (71%) bilateral hearing aids. All patients but one used oral communication (one total communication). With hearing aid(s) alone, median open set word recognition was 80% (54-100%) and median CDT was 79 words per min (25-147). With hearing aid(s) and lip reading, median open set word recognition increased to 96% (62-100) and median CDT to 87 words per min (46-142). Patients were intelligible in 98% of cases (SIR at 4 or 5). Full-time mainstreaming schooling was the most common (88%) (part-time mainstreaming education in 2 cases, school for the deaf in 6 cases). Four patients were school students at the time of study, 39 (57%) only had achieved a junior high school degree or lower (versus 32% in the global French population), 5 (7%) were high school graduates, 19 (28%) associate or bachelor graduates, 6 (9%) master or doctoral graduates (versus 12 % in the global French population). The final academic degree was statistically lower in Usher cohort compared to the global French population (p=0.009, Mann Whitney test). However, hearing rehabilitation had no statistical positive effect on this gap (p=0.17). Thirty nine (53%) patients were working, 2 (3%) patients were unemployed, 12 (16%) stayed at home, 11 (15%) received a disability pension and 5 (7%) were retired. Five patients lived at their parents' home and 12 needed an attendant to go outside of their home.

Conclusion: As visual disability usually appears years after deafness, some patients trend to minimize their hearing disability, using visual cues to facilitate their communication, and refuse optimal hearing rehabilitation. Usher type 2 syndrome impacts everyday life, schooling, academic achievements and vocational concerns.

41. **Focusing on Now for Tomorrow: Using A Well-Rounded Curriculum to Strengthen Students with Ushers Syndrome**

Morrow, S.[1] and Labeck, K.[2]

[1] *New York Deaf-Blind Collaborative, Project Coordinator, Queens College, Queens, NY, USA*
[2]*Teacher for Children with Visual Impairments & Deaf-Blindness, Queens College, Queens, NY, USA*

Abstract:

Schools for the deaf are prepared to address issues regarding the educational needs for students with hearing loss but they are ill-prepared to address the compounding issue of progressive vision loss. Students with Usher Syndrome, particularly during the transition age years, are in critical need of support from knowledgeable professionals who can address the many questions and uncertainty that lingers. While there are singular resources available in the form of articles and journal publications on the topic, there is no comprehensive curriculum designed to address the myriad needs of students with Usher Syndrome. To address this issue a multi-year Usher Syndrome Support Group (USSG) curriculum was designed and piloted and is adaptable for students in the mainstream.

Adults with Usher Syndrome anecdotally report great frustration and despair having gone through their educational experience without adequate or any information about their diagnosis, prognosis and resources to cope. These feelings of anger and outrage are expressed clearly in "An Open Letter to Our Parents" (http://bit.ly/1q0sIed). Research suggests that early preparation builds a foundation that

supports the various aspects of the student's life thus allowing for psychological reassurance and preparedness for adulthood. This multi-year curriculum addresses the following: etiology, cultural identity, peer-to-peer connections, safety travel, independent living, self-advocacy, self-determination, eye health, environmental modifications, effective communication strategies, technology at school, home and community, laws and citizen rights, transition planning, community resources, and social groups. To maximize the learning style of students, the curriculum was designed in a multidimensional format, which includes live presentation, demonstration and group activity. It also incorporates the use of distance technology to bring guest lecturers who have Usher Syndrome virtually into the sessions.

Another critical thread to the success of students is the early development of vision skills (http://bit.ly/1jKq506). Under the guidance of a Teacher for the Visually Impaired & Orientation & Mobility instructor, it is important for the student to begin to understand how the eye functions, use of trailing skills, use of light as a tool for access, text modification software, etc.

The USSG Curriculum was designed in partnership with the New York State deaf-blind project, a mental health counselor at a state school for the deaf, and reviewed by a deaf-blind family specialist and staff from the national deaf-blind project. This pilot later evolved into an Usher Syndrome Social Group, a safe environment for students and family members to learn and share in a casual, fun environment.

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