1st INTERNATIONAL SYMPOSIUM ON INNER EAR THERAPIES

NOVEMBER 01-03 2017 Marrakech

ABSTRACT BOOK

Institut Pasteur

FONDATION POUR L'AUDITION
A L'ÉCOUTE DE LA VIE

MARRAKECH
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كلية الطب والصيدلة - مراكش
FACULTÉ DE MÉDECINE ET DE PHARMACIE - MARRAKECH

SOCIETE MARROCAINE
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الجمعية المغربية لدراسة الدوار و التوازن

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FONDATION POUR L'AUDITION
À L'ÉCOUTE DE LA VIE

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جامعة القاضي عياض
UNIVERSITÉ CADI AYYAD

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Welcome

The organizing committee is delighted and honored to welcome you to the first International symposium on Inner Ear Therapies. We have chosen a place that, we think, best guarantees a successful symposium amid the culture and landscape of one of Morocco's most hospitable cities. The symposium is organized with the goal of providing everyone the best opportunity to interact and to share visions regarding the future and trends of inner ear therapies, especially how the current basic research can be bridged to clinical applications.

The program of the conference, rich and varied, includes several keynote speakers, and invited talks and about 60 posters. We are about 240 attendees coming from 12 countries, which far exceeds our best initial hopes for a first symposium.

We hope that this unique opportunity for ENT, scientists, engineers, graduate students and technology providers to share ideas will promote the growth of a strong and complementary community committed to address patient expectations and devoted to the development of inner ear therapies, and to fostering the translation of new promising approaches into preclinical and clinical trials.

The success of this conference largely rests on the great help we received from many people who have worked with us in planning and organizing both the program and social events. We wish to thank in particular those who helped to define the program for their thorough and timely reviewing of the papers; our sponsors who made it possible for us to keep the costs down for all participants; the members of the local organizing committee who have worked hard to solve the many practical issues regarding the conference program, logistics and social activities. We are particularly grateful to the members of the Fondation Pour l'Audition for their involvement and for the wise and often brilliant suggestions they gave us throughout the process of the preparation of this symposium.

Welcome to Marrakech!

Christine PETIT
Collège de France, Institut Pasteur, Paris, France

Said SAFIEDDINE
CNRS, Institut Pasteur, Paris, France

Abdelaziz RAJI
Mohammed VI University Hospital, Marrakech, Morocco

November 1st, 2017

WELCOME

8:30 am

Christine PETIT, Collège de France, Institut Pasteur, Paris, France
Said SAFIEDDINE, CNRS, Institut Pasteur, Paris, France
Abdelaziz RAJI, Mohammed VI University Hospital, Marrakech, Morocco

NEXT GENERATION OF IMPLANTS

Chairs : Paul AVAN, *University Clermont-Auvergne, Clermont-Ferrand, France*
Naima DEGGOUJ, *Saint-Luc University Hospital, Brussels, Belgium*

8:45 am **KEYNOTE LECTURE**

Cochlear implantation: An opportunity for targeted drug delivery
I. Hochmair
MED EL, Innsbruck, Austria

9:35 am **COFFEE BREAK**

1 10:00 am **Cochlear implantation outcome in children with syndromic deafness**
H. Ardhaoui
Department of Otolaryngology–Head and Neck Surgery, the King Hassan II University of Casablanca, Casablanca, Morocco

2 10:15 am **Cochlear implants: from a sound present to a better future**
Y. Raphael
Department of Otolaryngology - Head and Neck Surgery, Michigan Medicine, Kresge Hearing Research Institute, Ann Arbor, United States

3 10:45 am **Towards the optical cochlear implant: optogenetic stimulation of the auditory pathway**
T. Dombrowski
Max Planck Institute, Göttingen, Germany

4 11:15 am **Programming cochlear implants with artificial intelligence**
N. Deggouj
Saint-Luc University Hospital, Brussels, Belgium

5 11:30 am **The Vestibular Implant**
N. Guinand
Oto-rhino-laryngology and Head and Neck Surgery, University hospital of Geneva, Geneva, Switzerland

12:00 pm **LUNCH BUFFET ON SITE**

II INNER EAR GENE THERAPY: WHERE ARE WE NOW?

Chairs : Saaid SAFIEDDINE, *CNRS, Institut Pasteur, Paris, France*
Wade CHIEN, *Johns Hopkins School of Medicine, USA*

6 1:25 pm **Gene therapies and animal models for neurodegenerative diseases**
D. Dalkara
Institut de la Vision, France

7 1:50 pm **Local gene therapy durably restores vestibular function in a mouse model of Usher syndrome type 1G**
S. Safieddine
Génétique et Physiologie de l'Audition, CNRS, Inserm UMRS1120, Sorbonnes Universités UPMC, Institut Pasteur, Paris, France

8 2:15 pm **Gene therapy restores balance and auditory functions in the whirler mouse**
W. Chien
Johns Hopkins School of Medicine, Baltimore, USA

9 2:40 pm **Dual AAV gene therapy restores hearing in a mouse model for human genetic Deafness**
O. Akil
Department of Otolaryngology-HNS, UCSF, San Francisco, USA

10 3:05 pm **Next Generation Gene Therapies for DFNB7/11 and DFNA36**
J. Holt
Otolaryngology, Harvard Medical School / Boston Children's Hospital, Boston, USA

OFFICIAL WELCOME

3:30 pm

- **Prof. Abdellatif MIRAOUI**, *President of the Cadi Ayyad University*
- **Prof. Mohammed Bouskraoui**, *Dean of the Cadi Ayyad University School of Medicine*
- **Prof. Abdelaziz RAJI**, *Mohammed VI University Hospital, Marrakech, Morocco*
- **Prof. Christine PETIT**, *Institut Pasteur, Paris, France.*

3:45 pm **COFFEE BREAK**

III STEM CELL THERAPIES OF THE INNER EAR: CHALLENGES & PITFALLS

Chairs : Marcelo RIVOLTA, *Sheffield University, Western Bank, UK*
Jeffrey HOLT, *Harvard Medical School, Boston, MA, USA*

11 4:15 pm **Combining stem cells and electrode arrays: Cochlear implants meet regenerative biology**

M. Rivolta
Centre for Stem Cell Biology, Department of Biomedical Science, Sheffield University, Western Bank, United Kingdom

12 4:40 pm **Approaches to the conversion of human fibroblasts to hair cell-like cells**

M.B. Durán Alonso
Molecular genetics of Disease, Institute of Biology and Molecular Genetics, Valladolid, Spain

13 4:55 pm **Derivation of Otic Sensory Progenitors from Human Induced Pluripotent Stem Cells and *in Vivo* Approaches for Transplantation into the Cochlea of a Guinea Pig Model of Ototoxicity**

A. Zine
CNRS UMR 7260, Marseille & University of Montpellier, Montpellier, France

14 5:10 pm **Characterization of Molecular and Stereociliary Structural Changes in Hair-Cell-like-Cells from Human Induced Pluripotent Stem Cells of Patients with Mitochondrial DNA A8344G Mutation**

Y.C. Hsu
Institute of Biomedical Sciences, Mackay Medical College, New Taipei City, Taiwan

IV EXPERIMENTAL MODELS OF DEAFNESS

Chairs : Karen STEEL, *King's College London, UK*
Christine PETIT, *Institut Pasteur, Paris, France*

15 5:25 pm **Impact of extreme heterogeneity in genetics and pathology of deafness on treatment strategies**

K. Steel
Wolfson Centre for Age-Related Diseases, King's College London, London, United Kingdom

16 5:50 pm **Questions raised by the diversity of animal models with impaired hearing in noise**

P. Avan
Neurosensory Biophysics, INSERM 1107, University Clermont Auvergne, Clermont-Ferrand, France

DINER ON YOUR OWN

November 2nd, 2017

V GENETICS, PATHOGENESIS AND DIAGNOSIS OF INHERITED DEAFNESS

Chairs : Lawrence R. LUSTIG, *University of Columbia, NY, USA*
Abdelaziz RAJI, *Mohammed VI University hospital, Marrakech, Morocco*

17 8:00 am
Oculo-auditory syndrome
Screening of ophthalmologic impairment in deaf children population
A. Raji
Mohamed VI University Hospital Marrakech, Morocco

18 8:15 am
Genetic of non syndromic deafness, The experience of the oto-rhino-laryngology department, Hassan II university hospital, Fes, Morocco (About 1070 cases)
S. Sensou
Otorhino-laryngology department, Hassan II university hospital, Fes, Morocco

19 8:30 am
The Gap Junction B2 mutations spectrum in 152 Moroccan families with nonsyndromic hearing loss
A. Bousfiha
Human Molecular Genetics Laboratory, Institut Pasteur of Morocco, Casablanca, Morocco

20 8:45 am
Epidemiology of Genetic hearing loss in infants receiving cochlear implantation
I. Nakkabi
ENT department, Military Teaching Hospital Mohamed V, Rabat, Morocco

21 9:00 am
Cellular And Molecular Mechanisms Of Hearing Loss In Type III Usher Syndrome
A. El-Amraoui
Génétique et Physiologie de l'Audition, Inserm UMRS1120, Sorbonnes Universités UPMC, Institut Pasteur, Paris, France

22 9:15 am
Epigenomics of the Inner Ear: Defining Potential Entry Points for Therapeutics
K.B. Avraham
University of Washington, Seattle, USA; Fondation Pour l'Audition, Paris, France

POSTER SESSION & COFFEE BREAK

9:40 am **Poster exhibition**
Rooms ASNI & IMLIL

12:00 pm **LUNCH BUFFET ON SITE**

VI COCHLEAR IMPLANTS OUTCOMES

Chairs : Paul AVAN, *University Clermont Auvergne, Clermont-Ferrand, France*
Naima DEGGOUJ, *Saint-Luc University Hospital, Brussels, Belgium*

23 1:30 pm **How can we improve the outcomes of cochlear implants? A surgeon's perspective**
B. Fraysse
CHU Toulouse, Toulouse, France

24 1:55 pm **Benefit of cochlear implantation in adults**
I. Mosnier
Otolaryngology Department, Unit of Otology, Auditory Implants and skull Base Surgery, APHP, GH Pitié Salpêtrière, Paris, France

VII PHARMACOLOGICAL THERAPIES: ON THE MOVE!

Chairs : Jean-Luc PUEL, *Institut des neurosciences de Montpellier, France*
Takayuki NAKAGAWA, *Kyoto University, Japan*

25 2:10 pm **Topical IGF-1 therapy for treatment of sensorineural hearing loss: Mechanisms and clinical applications**
T. Nakagawa
Otolaryngology, Head and Neck Surgery, Kyoto University, Kyoto, Japan

26 2:35 pm **Netrin1 mediates the protection of cochlear hair cells by IGF1 through its canonical receptor, UNC5B**
N. Yamamoto
Otolaryngology Head and Neck Surgery, Kyoto University, Kyoto

27 2:50 pm **Reversible p53 inhibition prevents cisplatin ototoxicity without compromising chemotherapeutic efficacy**

J. Puel

Institut des Neurosciences de Montpellier, INSERM/Université de Montpellier, Montpellier, France

3:05 pm **COFFEE BREAK**

28 3:30 pm **Fetal Gene and Pharmacotherapy to Treat Congenital Deafness and Balance Dysfunction**

J. Brigande

Oregon Hearing Research Center OHSU, United States

29 3:55 pm **Connecting a new ear to an old auditory system: restoring hearing using a radically new approach**

B. Fritzsch

Biology & Otorhinolaryngology, University of Iowa, Iowa City, United States

30 4:20 pm **Computational repositioning and preclinical validation of mifepristone for human vestibular schwannoma**

J. Sagers

Program in Speech and Hearing Bioscience and Technology, Division of Medical Sciences, Harvard Medical School

KEYNOTE LECTURE

Chair : Karen B. AVRAHAM, *University of Washington, Seattle, USA; Fondation Pour l'Audition, Paris, France*

Sponsored by Sensorion The Inner Ear Diseases company

4:35 pm **KEYNOTE LECTURE**

Hidden cortical deficits: therapeutic strategies

C. Petit

Genetics and Physiology of Hearing, Institut Pasteur, Paris, France

POSTER AWARD CEREMONY 5:25 pm

Chairs : Jeffrey HOLT, *Harvard Medical School, Boston, MA, USA*
Said SAFIEDDINE, *CNRS Institut Pasteur, Paris, France*

Sponsored by MED EL

GALA DINNER

VIII LESSONS FROM GENE THERAPY SUCCESSES

Chairs : Jeffrey HOLT, *Harvard Medical School, Boston, MA, USA*
Hinrich STAECKER, *University of Kansas Medical Center, KS, USA*

8:30 am **KEYNOTE LECTURE**

Gene therapy: not only a matter of vector choice

M. Cavazzana
Imagine Institute, France

31 9:20 am **Design and preclinical evaluation of an inner ear gene therapy program**

H. Staecker
Department of Otolaryngology Head and Neck Surgery, University of Kansas Medical Center, United States

32 9:45 am **Cochlear gene therapy for Usher IIIa**

L. Lustig
Columbia University Medical Center, New York, United States

33 10:10 am **Delivery of Adeno-Associated Viral Vectors in Adult Mammalian Inner Ear Cell Subtypes without Auditory Dysfunction**

Y. Tao
Department of Otolaryngology-Head and Neck Surgery, Shanghai Ninth People's Hospital, Shanghai Jiaotong University School of Medicine, China

34 10:25 am **Main advances, issues and perspectives of AAV-mediated in vivo gene therapy**

P. Colella
Genethon and INSERM U951 Integrare, Evry, France

10:40 am **COFFEE BREAK**

ROUND TABLE

Chairs : Christine PETIT, *Collège de France, Institut Pasteur, Paris, France*
Marina CAVAZZANA, *Imagine Institute, France*
Anne SCHILDER, *UCL Ear Institute, London, UK*

11:10 am **From bench to bedside: Insights for a good clinical trial design**
Participants:

- **Prof. Karen B. AVRAHAM**, *University of Washington, Seattle, USA; Fondation Pour l'Audition, Paris, France*
- **Prof. Jeffrey HOLT**, *Harvard Medical School, Boston, MA, USA*
- **Prof. Lawrence R. LUSTIG**, *Columbia University Medical Center, NY, USA*
- **Prof. Karen STEEL**, *King's College London, UK*
- **Prof. Hinrich STAECKER**, *University of Kansas Medical Center, KS, USA*

CLOSING OF THE SYMPOSIUM

12:10 pm **Christine PETIT**, *Collège de France, Institut Pasteur, Paris, France*
Said SAFIEDDINE, *CNRS, Institut Pasteur, Paris, France*
Abdelaziz RAJI, *Mohammed VI University Hospital, Marrakech, Morocco*

12:30 pm **LUNCH BUFFET ON SITE**

ORAL SESSIONS

Cochlear implantation outcome in children with syndromic deafness

H. Ardhaoui, S. Naceur, K. Chaker, R. Abada, R. Rouadi, M. Roubal, M. Mahtar
Department of Otolaryngology–Head and Neck Surgery, the King Hassan II University of Casablanca, Casablanca, Morocco

Background: Severe to profound hearing loss occurs in approximately one in 1000 live births, with more than 50% of cases having a genetic cause. Hundreds of syndromes linking congenital deafness with other systemic abnormalities have been described, and the causative genes have been identified for approximately 100 of these.

Purpose of the study: To determine the effect of syndromic deafness on cochlear implant outcomes in prelingual deaf children, in terms of language development, auditory performances, speech perception and comprehension, oral expression, and speech intelligibility.

Materials and methods: Our study is a prospective, longitudinal study performed in a tertiary referral center. Seventy-four patients with profound prelingual hearing loss received cochlear implant during the study period (from January 2010 to December 2016). We examined the rate of children with syndromic deafness. All children were evaluated at 3, 6, 12, 18,24, 36 and 48 months after cochlear implantation using the APCEI scale, the Categories of Auditory Performance scale (CAP) and the Speech intelligibility Rate scale (SIR). We dived our patients into two groups and examined group differences for children with syndromic deafness (Group 1), and children with non syndromic deafness (Group 2),

Results: 74 patients were implanted during the study period, among them 6 children (8,1%) had syndromic deafness. The syndromes identified were Waardenburg syndrome (n = 4), Alport syndrome (n = 2). Mean age at the diagnosis of deafness was 8,6 months, mean age at cochlear implantation was 31,2 months. The average time of follow up was 26,6 months.

The p APCEI scores at 24 months after CI were 73,42 for Group 1 and 75,35 for group 2, this difference was not stastically significant (p=0,43), Evaluation at 24 months after implantation in terms of speech intelligibility scores and Auditory performances scores didn't show any differences between the two groups. Correlation coefficients did not reach significance for any of the outcome skills measured.

Conclusion: Additional disabilities are frequently encountered when considering children for cochlear implantation, and may be part of a recognised syndrome. Outcome is often excellent but can be variable even within the same syndrome group. Our Results

Cochlear implants: from a sound present to a better future

Y. Raphael, B.E. Pfingst
Department of Otolaryngology - Head and Neck Surgery, Michigan Medicine, Kresge Hearing Research Institute, Ann Arbor, United States

Background: Cochlear implants (CIs) provide amazing improvements in quality of life for individuals with hearing loss, but there remains a significant variability in outcomes across patients, especially in difficult listening conditions. Pathology of the implanted cochlea probably contributes to the variability across patients. Therefore, improving the biological substrate in ears that receive CIs can potentially enhance the outcomes.

Objectives: To reverse the regression of peripheral auditory fibers from the sensory epithelium and increase auditory nerve (AN) soma survival.

Methods: We have implanted deaf guinea pigs with a CI, treated the ears with viral-mediated neurotrophin over-expression and then used psychophysical and electrophysiological measures together with histology to assess the impact of the neurotrophin treatment. Histological results focused on spiral ganglion neuron (SGN) density, inner hair cell (IHC) survival and neurite regeneration into the basilar membrane area. Because the guinea pig animal model exhibited SGN loss in conjunction with deafening (eliminating IHCs), we also designed and implemented technology for placing CI electrodes in the diphtheria toxin receptor (DTR) mouse (Golub et al., 2012). These mice exhibit robust AN survival despite complete IHC depletion following diphtheria toxin (DT) injection (Tong et al., 2015; Kurioka et al., 2016).

Results: In guinea pigs that received neurotrophins, we observed re-growth of fibers into the basilar membrane area, and enhanced density of spiral ganglion neurons in Rosenthal's canal as compared with controls. These outcomes could be sustained for months, even after levels of neurotrophins had returned to baseline. Electrically-evoked compound action potential amplitude-growth functions were steeper in neurotrophin treated animals compared to deafened controls. Preliminary experiments with the DTR mice show that wild type (hearing) mice and DTR mice treated with DT (deaf) are amenable to recording electrically-evoked auditory brain stem responses (EABRs) under anesthesia. Amplitude growth functions (the magnitude of the recorded neural response vs stimulus current level) are similar between deaf and hearing mice.

Conclusions: In the guinea pig model, transient elevation of neurotrophin levels can increase neuronal survival and induce sprouting, which can be sustained in the long term. Chronically implanted DTR mice provide a promising model for investigating the effects of HC and AN survival on CI function. Data obtained with these two models begin to show how tissue-engineering procedures for enhancing the cochlear health can improve performance in CI recipients.

Acknowledgements: Supported by the Williams Professorship, MED-EL, and NIH R01 grants DC010786, DC015809, DC014456, and DC014832.

Towards the optical cochlear implant: optogenetic stimulation of the auditory pathway

T. Dombrowski
Max-Planck-Institute, Göttingen, Germany

While being the most successful neuroprosthesis, the restoration of hearing based on electrical stimulation of spiral ganglion neurons (SGNs) is technically limited in frequency resolution due to the spread of current. The Göttingen Cochlear Optogenetics Program aims to encounter this limitation of cochlear implants by replacing electrical stimulation with spatially confined optical stimulation of SGNs based on an optogenetics approach. So far, we have successfully established the optogenetic stimulation of the auditory pathway in rodents following virus-mediated transfection of SGNs with channelrhodopsins. Fast opsins enabled SGN firing at near physiological rates and activated several stages of the auditory pathway. Approximations of the spatial spread of cochlear excitation in the inferior colliculus in response to suprathreshold optical and electrical stimuli suggested a better frequency resolution for optogenetic than for electrical stimulation. We further characterized the induced percept by activation of neurons in the primary auditory cortex and analyzed a behavioral response in virus-injected gerbils based on a modified shuttle box paradigm. Behavioral thresholds of light amplitude were found to be below physiological thresholds (< 2mW, close to the threshold of the neurons in auditory cortex) and thresholds of light pulse duration were as short as 0.1ms. In summary, we demonstrated that the optogenetic stimulation of channelrhodopsin-transfected SGNs leads to both stable physiological and behavioral responses promising high potential for future application in hearing restoration.

Programming cochlear implants with artificial intelligence

J. Wathour[2], D. De Siati[2], M. Decat[2], P. Govaerts[1], N. Deggouj[2]
[1]*Eargroup, Antwerp* [2]*Saint-Luc University Hospital, Brussels, Belgium*

Introduction and aim: Currently, the fitting of cochlear implants (CI) is carried out by clinicians, including audiologists, speech therapists and physicians. The variability in the training background of those who program CI leads to very disparate programming.

Several parameters can be changed in CI fitting to improve the cortical perception of the electric field created from an acoustic sound through the MAP: the dynamics of the microphone, the compressions and the frequency at which electrical stimulations and acoustic analysis can be performed by the voice processor, among others.

In order to facilitate the CI programming through this wide spectrum of parameters, an application based on artificial intelligence has been developed by the Eargroup (Antwerp), named "Fitting to Outcome eXpert" (FOX).

To understand whether FOX improves the functional results obtained with a CI and the speed of adaptation, and whether, in patients with limited CI results, FOX improves the fitting and the quality of life hearing.

To this end, we compared functional results and the time required for programming.

Material and methods: We will compare two methods of programming cochlear implants. The manual programming method (the audiologist looks for the level of electrical stimulation producing an effective and comfortable hearing perception at each electrode) versus the programming with artificial intelligence.

Conclusions: Programming a cochlear implant is a "challenge" because various factors are playing a role in the hearing performance.

FOX is one useful tool to help audiologists in fitting CI.

Keywords: Cochlear implant; artificial intelligence

The Vestibular Implant

N. Guinand
Oto-rhino-laryngology and Head and Neck Surgery, University hospital of Geneva, Geneva, Switzerland

The primary goal of the vestibular implant is to restore the vestibular function in patients with a disabling bilateral vestibular loss for whom there is currently no available treatment. The prototype developed by our team is a hybrid system consisting of a modified cochlear implant incorporating additional vestibular electrodes. Therefore, in addition of delivering sound information it is also capable of delivering motion information to the central nervous system using electrical stimulation. To date, thirteen patients have been implanted with such vestibular implant prototypes. For ethical reasons, only deaf ears were implanted and all patients experienced a clinical benefit from the hearing rehabilitation. The recent demonstration of partial restoration of the vestibulo-ocular and the vestibulo-collic reflexes in implanted patients suggests that gaze stabilization and postural control, fundamental functions of the balance system, can be artificially restored using a vestibular implant. This allows us to glimpse a useful clinical application in a near future. In parallel, we show how the vestibular implant provides a unique opportunity to explore the integration of the vestibular sensory input into the multisensory, multimodal balance system in humans, since it is able to selectively stimulate the vestibular system.

Gene therapies and animal models for neurodegenerative diseases

D. Dalkara
Institut de la Vision, France

Retinal degenerative diseases are a leading cause of irreversible blindness. Retinal cell death is the main cause of vision loss in genetic disorders such as retinitis pigmentosa, Usher Syndrome, Stargardt's disease and Leber's congenital amaurosis, as well as in complex age-related diseases such as age-related macular degeneration (AMD). For these blinding conditions, gene and cell therapy approaches offer therapeutic intervention at various stages of the disease. Classical gene replacement therapy has been effective in rare diseases where the causal mutation is known and the cells bearing the mutation are still present. To go beyond these diseases, the development of new gene and cell therapies is necessary. The focus of this talk will be the developments in gene and cell therapy technologies to prevent vision loss and restore vision in advanced stage retinal degenerations. In late stages of retinal degeneration gene therapy can be used to promote the survival of retinal neurons or to reanimate the retina through the expression of optogenetic modulators of membrane potential. Such protective and restorative gene therapies can be combined to extend therapeutic benefit. In the most advanced stages of disease, once the photoreceptor cells have completely degenerated, cell therapy can provide therapeutic benefit provided that functional photoreceptors are transplanted. A new methodology to generate light-sensitive cones from human induced pluripotent stem cells will be described. Lastly, the new avenues opened by these emerging gene and cell therapies for restoring vision will be discussed in view of their applicability in sensorineural deafness.

Local Gene Therapy Durably Restores Vestibular Function in a Mouse Model of Usher Syndrome Type 1G

S. Safieddine, A. Emptoz, V. Michel, A. Lelli, O. Akil, J. Boutet De Monvel, G. Lahlou, A. Meyer, T. Dupont, S. Nouaille, E. Ey, F. Franca De Barros, M. Beraneck, D. Dulon, J.P. Hardelin, L. Lustig, P. Avan, C. Petit
Neuroscience, UMRS 1120, Unité de Génétique et Physiologie de l'Audition, Institut Pasteur, Paris, France

Our understanding of the mechanisms underlying inherited forms of inner ear deficits has considerably improved during the last twenty years, but we are still far from curative treatments. We investigated gene replacement as a strategy for restoring inner ear functions in a mouse model of Usher syndrome type 1G (USH1G), characterized by congenital profound deafness and balance disorders. These mice lack the scaffold protein sans, which is involved both in the morphogenesis of the stereociliary bundle, the sensory antenna of inner ear hair cells, and in the mechanoelectrical transduction process. We show that a single delivery of the sans cDNA by the adeno-associated virus AAV8 to the inner ear of newborn mutant mice reestablishes the expression and targeting of the protein to the tips of stereocilia. The therapeutic gene restores the architecture and mechanosensitivity of stereociliary bundles, improves hearing thresholds, and durably rescues these mice from the balance defects. Our results open up new perspectives for efficient gene therapy of cochlear and vestibular disorders by showing that even severe dysmorphogenesis of stereociliary bundles can be corrected.

Gene therapy restores balance and auditory functions in the whirler mouse

K. Isgrig, J.W. Shteamer, I. Belyantseva, M. Drummond, T. Fitzgerald, S. Jones, T. Friedman, L. Cunningham, W. Chien
Johns Hopkins School of Medicine, Baltimore, United States

Background: Whirlin, encoded by *Whrn,* is important for stereocilia elongation. *Whn*$^{wi/wi}$ (whirler) mice are born deaf due to a failure in stereocilia elongation. In this study, we utilize whirler mice to examine the potential therapeutic effects of whirlin gene therapy.

Methods: Homozygous whirler mice and their wild-type littermates were used in this study. AAV8-whirlin was delivered into the inner ear of neonatal mice. Auditory brainstem-evoked responses (ABRs) and vestibular testing were done to assess auditory and vestibular functions, respectively. Immunohistochemistry and scanning electron microscopy were used to examine stereocilia morphology.

Results: Whirlin expression was restored at stereocilia tips in *Whn*$^{wi/wi}$ hair cells infected by AAV8-whirlin. In these cells, stereocilia length was similar to the wild type animals. Whirler mice that were treated with AAV8-whirlin showed improved ABR thresholds as well as restored vestibular function.

Conclusions: Our data indicate that AAV8-whirlin gene therapy was successful at improving auditory and vestibular functions in whirler mice. This proof-of-concept study demonstrates that inner ear gene therapy can potentially be a treatment for hearing loss and dizziness.

Dual AAV gene therapy restores hearing in a mouse model for human genetic Deafness

O. Akil[4], F. Dyka[2], A. Emptoz[1], W. Hauswirth[2], C. Petit[1], S. Safieddine[1], L. Lustig[3]
[1]Unite de Genetique et Physiologie de l'Audition, Institut Pasteur, Paris, France [2]Department of Ophthalmology, University of Florida, Florida [3]Department of Otolaryngology-HNS, University of Columbia, New York [4]Department of Otolaryngology-HNS, UCSF, San Francisco, United States

Introduction: Hearing impairment is the most common sensory deficit in humans. About 1.5 in 1000 newborns suffer from congenital hearing loss (HL), and genetic forms of sensorineural deafness account for almost half of all patients with HL. Treatments such as amplification and cochlear implantation have substantial limitations and do not restore normal hearing. As a result, cochlear gene therapy has been suggested as an alternative approach for treatment for both genetic and acquired forms of hearing loss. However, there remains a significant hurdle in targeting the numerous larger genes (>5kb) that cause deafness, because they are well over the packaging limits of AAV (<5kb). To overcome this challenge, we used viral gene therapy in a deaf mutant mouse established as model for human deafness forms to test a novel approach of using a dual vector technique. The correct cDNA of the defective gene will be delivered to this mouse model using the trans-splicing approach, which reconstitutes gene expression from two independent AAV vectors, each encoding non-overlapping halves of the therapeutic protein.

Methods: First we investigated the tropism of different AAV serotypes for cochlear hair cells. Mice were subjected to the viral cochlear delivery at postnatal day 1-3 (P1-3). The cochleae were injected with different serotypes encoding the green fluorescent protein GFP as a reporter gene. We found that the AAV2 serotype was the most efficient to transduce the targeted hair cells (about 80% of targeted hair cells where transfected). Next, we split the correct cDNA of the defective gene of interest into two different inserts. The 5' end of the gene has the promoter and an overlapping sequence and the 3' end of the gene has the overlapping sequence and the polyA signals. Each insert was subcloned into separate AAV2 vectors genome and sequenced. Dual viral particles expressing the protein of interest were produced in the AAV2 serotype. The dual-cDNA-AAV2 vectors were delivered to P1-3 and at >P10 mouse cochlea.

Results and Conclusions: The preliminary data demonstrate wild-type protein expression in the targeted hair cells and hearing restoration in the rescued mice using a dual vector approach. These findings represent a significant advance in our ability to tackle large (>5kb) genes as targets for gene therapy in general, and for hearing loss specifically, using AAV.

Research supported by generous financial support from Hearing Research Incorporation

Next Generation Gene Therapies for DFNB7/11 and DFNA36

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Recessive mutations in *TMC1/Tmc1* cause congenital deafness in humans and mice, while dominant mutations cause progressive hearing loss. To develop biological treatments for humans with recessive or dominant *TMC1* mutations, we designed several novel approaches and evaluated their effectiveness in mouse models of DFNB7/11 and DFNA36. We investigated novel viral vectors, various promoter sequences and four different therapeutic DNA sequences and assayed for their ability to restore or preserve auditory function in mice that carried dominant or recessive mutations in *Tmc1*. Assays included measurement of exogenous gene expression, evaluation of hair cell survival, imagining of hair bundle morphology, functional measurement of sensory transduction, recording of auditory brainstem responses and distortion product otoacoustic emissions, behavioral assays of acoustic startle responses and assays for balance behavior. We identified vectors, promoters, therapeutic sequences capable of restoring correct gene and protein expression, enhancing hair cell survival, preserving hair bundle morphology and restoring sensory transduction in inner and outer hair cells. ABR thresholds were as low as 35 dB in *Tmc1* mutant mice injected with optimized therapeutic sequences. Recovery of auditory function persisted for up to 24 weeks, the latest time point tested. We also explored different injection approaches and various time points for injection, ranging between postnatal days one and thirty. Here we will summarize our latest efforts to develop gene therapy approaches for treatment of DFNB7/11 and DFNA36 hearing loss in humans. We conclude that some of these next generation gene therapy approaches may be suitable for translation from proof-of-concept studies in mouse models to therapeutic application in human clinical trials.

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Combining stem cells and electrode arrays: Cochlear implants meet regenerative biology

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The performance of a cochlear implant relies on the auditory nerve working adequately in order to convey the information to the brain. Thus, for some people, particularly those with auditory neuropathies or cochlear nerve deficiencies, a cochlear implant is not a recommended therapeutic option. The overall aim of our work is to repair the auditory nerve with stem cells while functionally replacing the hair cells with a cochlear implant. This should deliver a true 'bionic' implant, combining biology and electrical arrays.

We have previously shown that we can functionally restore the cochlear nerve in a gerbil model of auditory neuropathy using stem cells. Human Embryonic Stem Cells can be driven to produce otic neuroprogenitors (hONPs) that, in turn, can differentiate into spiral ganglion neurons. hONPs were transplanted into the cochleae of ouabain-treated gerbils, showing engraftment and functional integration. More recently, we have developed a new gerbil model with a two-pronged sensorineural hearing loss - auditory neuropathy is induced with topical ouabain and subsequently the hair cells are lesioned with a kanamycin/furosemide treatment. To recapitulate cochlear implantation, we are using a fully-implantable rodent stimulator in which the electrode is activated by a magnetic field.

Initially, we implanted animals in which only the hair cells were damaged. Brainstem evoked responses were obtained after electrode stimulation, and animals showed behavioural changes compatible with auditory responses. We are now combining the cochlear implant prototypes with the rebuilding of the auditory nerve using hONPs. These progenitors were produced using a hESC line that expresses a GFP construct which reports the expression of SOX2 under the control of specific otic/nasal placode enhancers, allowing the purification of hONPs in vitro prior to transplantation. Histology suggests that the transplanted cells survive and differentiate in the implanted animals, with neural fibres tracking towards the implant.

Achiving functional integration of transplanted cells generated in vitro with a cochlear implant should expand the indication for the device, increasing the patient base that could benefit from it.

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Approaches to the conversion of human fibroblasts to hair cell-like cells

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Hearing loss is the most prevalent neurosensory disorder in humans. Its most frequent cause is the loss of hair cells (HCs), highly specialized mechanosensory cells in the cochlea; these cells do not regenerate, hearing impairment being most often a progressive disorder. Approaches are being followed to obtain HC-like cells from cell sources such as embryonic stem cells (ESC), mesenchymal stem cells and induced pluripotent stem cells (iPSCs). In addition, fully differentiated somatic adult cells, such as fibroblasts, may be converted into cell types from ontogenically different lineages. Our work aims at obtaining HC-like cells from human fibroblasts. We have adopted two different approaches: The first one consists on the overexpression of the Oct4 gene, reported to render fibroblasts responsive to differentiation signals that drive them towards other non-related phenotypes. We have applied various culture conditions to Oct4-overexpressing fibroblasts; one of these leads to the appearance of Myosin VIIA-positive cells, accompanied by an increase in the mRNA levels of Atoh1, Six1 and Myosin VIIA. Our second method aims at the direct conversion of fibroblasts into HC-like cells by overexpressing the transcription factors Atoh1, Brn3c and Gfi1, that results in the generation of HC-like cells from murine ESCs. It leads to the emergence of Myosin VIIA-positive cells in fibroblast-derived cultures; QPCR data confirm the overexpression of human Atoh1, murine Brn3c and human Gfi1, and an increase in the mRNA levels of endogenous Brn3c, Espin and Myosin VIIA. Obtaining HC-like cells from fibroblasts represents a new avenue towards possible future regenerative cell-based approaches and may also serve as a model for *in vitro* studies on the biology, survival and differentiation of HCs, and a screening platform for the identification of promising therapeutic agents.

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Derivation of Otic Sensory Progenitors from Human Induced Pluripotent Stem Cells and *in Vivo* Approaches for Transplantation into the Cochlea of a Guinea Pig Model of Ototoxicity

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The cochlear sensory epithelium in the adult mammalian ear do not regenerate following trauma or ototoxicity. Stem cell approach for inner ear damage is attractive for substitution cell therapy that has received considerable attention over the past decade. However, major challenges remain to be addressed for potential use of stem cells for hearing loss i.e., to have an unlimited source of human otic progenitor cells (hOPC) *in vitro* and to promote their survival and migration within damaged cochleae *in vivo*. In this study, we first generated a large number of hOPC from induced pluripotent stem cells (hiPSC) in a monolayer culture. These hOPC expressed a comprehensive panel of otic/placodal markers as determined by RNA-Seq, Fluidigm qPCR and immunohistochemistry analyses. For transplantation, the hOPC were pre-labeled with Vybrant™ dye to monitor their migration and cell fate in a guinea pig model of ototoxicity induced hearing loss. After injection into the basal turn of the scala tympani via cochleostomy, the Vybrant-labelled hOPC migrated in other scalae (vestibuli and media), in addition to the scala tympani within two weeks post-transplantation. Histological analysis showed clusters of surviving hOPC that migrated between different compartments along the cochlear duct of ototoxic-deafened guinea pigs. Moreover, by combining cell fate analysis with 2- and 3-dimensional (2D-3D) confocal imaging, it was possible to observe a subset of Vybrant-labelled hOPC that incorporated at the base of the organ of Corti. Interestingly, some of these hOPC localized to the supporting cell layer and expressed the hair cell marker MyoVIIa in ototoxin-damaged cochleae four weeks post-transplantation. Our data provide a useful *in vitro* assay to derive large numbers of hOPC from guided differentiation of hiPSC. In addition, our results indicate that injection of these hOPC into the scala tympani following ototoxicity resulted in their migration throughout the structures of the cochlea, including the organ of Corti. These hOPC were competent to survive, integrate and differentiate in the guinea pig model of ototoxicity. This study shows that iPSC can provide a source of human otic progenitors for stem cell-based cell therapy to promote cell replacement in patients with sensorineural hearing loss.

Characterization of Molecular and Stereociliary Structural Changes in Hair-Cell-like-Cells from Human Induced Pluripotent Stem Cells of Patients with Mitochondrial DNA A8344G Mutation

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Background: Myoclonus epilepsy associated with ragged-red fibres (MERRF) syndrome is primarily caused by an A–G point mutation at mitochondrial DNA (mtDNA) 8344 that disrupts $tRNA^{Lys}$ and causes protein synthesis dysfunction. MERRF is a mitochondrial disease characterised by myoclonus epilepsy, ataxia, and sensorineural hearing loss.

Objectives: In this study, we differentiated the inner ear hair cell (HC)-like cells from human induced pluripotent stem cells (hiPSCs) and investigated the effects of the mtDNA A8344G mutation on HC differentiation.

Methods: Both MERRF-iPSCs and MERRF-HC-like cells were characterised using flow cytometry, immunocytochemical staining, and scanning electron microscopy, and qRT-PCR were used to evaluate the expression levels of the HC markers and antioxidant genes.

Results: Compared with isogenic iPSCs ($M1^{ctrl}$-iPSCs), MERRF-iPSCs (M1-iPSCs and M2-iPSCs) exhibited elevated reactive oxygen species (ROS) production, *CAT* gene expression, and differentiated iPSCs colonies. Furthermore, MERRF-HC-like cells (M1 and M2) exhibited significantly elevated ROS levels and *MnSOD* and *CAT* gene expression. SEM results revealed that MERRF-HC-like cells exhibited more single cilia with a shorter length, but fewer stereocilia bundle-like protrusions than $M1^{ctrl}$-HC-like cells.

Conclusion: Significantly higher ROS levels, impaired ROS scavenging capacity, and less stereociliary hair bundles, suggesting molecular and stereociliary structural changes in MERRF-HC-like cells harbouring the mtDNA A8344G genetic mutation.

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Impact of extreme heterogeneity in genetics and pathology of deafness on treatment strategies

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Adult-onset, progressive hearing loss is very common in the human population but we still know little about the underlying pathology. Over 360 genes are known to be involved in deafness in human or mouse, but the majority of these have early developmental effects, giving few clues to the genetic contribution to adult-onset hearing loss. We set out to identify further genes underlying deafness including those with mild effects using a physiological screen, the Auditory Brainstem Response (ABR), of a large cohort of newly-generated targeted mouse mutants. From the unbiased sample of 1211 genes tested, we found 38 unexpected genes to be involved in hearing impairment. This indicates that around 600 additional genes remain to be found, making deafness an extremely heterogeneous condition with around 1000 genes that may contribute. The 38 genes represent a range of functions from transcription factors and a microRNA to enzymes involved in lipid metabolism. The impairment we found ranged from mild to profound, with a wide range of underlying pathological mechanisms. Several of the mutant lines which we have studied in more detail show normal early development of hearing followed by progressive loss of responses. New molecular pathways have been identified, some of which should be suitable for manipulation by drug treatments

Questions raised by the diversity of animal models with impaired hearing in noise

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Background: When therapies will become available for attempting cures of sensorineural hearing impairment, the precise determination of the underlying disorder will be required for the proper patients to be targeted. Up until now, standard audiological check-ups scarcely go beyond identifying functional disorders, e.g., impaired hearing in noise. The implicit assumption that all patients who share this common disorder also share a common pathophysiological framework (e.g., impaired hearing in noise out of proportion with the pure tone audiogram would be *the* signature of auditory neuropathy) is misleading, however, and prone to leading to a likely inefficient choice of therapeutic strategy.

Objectives: Here we present a diversity of conditions in animal models in which noise processing is abnormal, due to different, well-documented pathophysiological frameworks.

Methods: a combination of otoacoustic emissions, auditory evoked potentials at threshold, then at higher levels and in the presence of interfering sounds, complemented with histological and molecular analysis, was used for deciphering the profile of hearing disorder.

Results: All patterns led to a similar disorder characterized by exaggerated sensitivity to noise, suggesting that the same functional disorder in a patient would lead to impaired intelligibility in noise out of proportion with the pure-tone audiogram. Yet the disorder was attributed to damage to outer hair cells in one case; distorted frequency mapping along the tonotopic axis in two cases; neural disorder either with disrupted timing on different time scales (short-term dyssynchrony; excessive fatigability) or with loss of high-threshold neurons.

Conclusions: Hearing-in-noise disorders may have widely different causes, that require widely different interventions. Every one of them generates a distinctive functional pattern provided non-conventional audiological tests are used in appropriate combinations.

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Oculo-auditory syndrome
Screening of ophthalmologic impairment in deaf children population

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Introduction: The association of visual disturbances with deafness is frequent. These disorders range from a simple anomaly of refraction to blindness which may constitute a hard handicap.

The objective of our study is to show the importance of ophtamological disorders screening and multidisciplinary collaboration in deaf children.

Materiel and methods: Our study is prospective and mono-centric involving 200 children followed for congenital hearing loss from January 2014 to December 2015.. All patients with mental retardation were excluded from the study: cerebral palsy (30 cases) and trisomy (15).

All children have had a complete ophthalmologic examination; ENT examination; and a general review.

Results: Only One hundred and fifty-five files were retained. Ophtalmologic impairment was observed in 24 children (18.7%). the average age has been 7 years (1 month-15 years) with 10 boys and 14 girls. We have diagnosed an Usher Syndrome in 4 cases, Waardenburg Syndrome in 5 cases, Alport Syndrome in 1 case, Wolfram Syndrome in 2 cases, Goldenar syndrome in 3 cases, Cogan Syndrome in 1 case, Fracheschetti Syndrome in 1 case, Charge Syndrome in 1 case, Otomandibular Syndrome in 2 case, Stickler Syndrome 1 case, Alstrom Syndrome in 1 case, Refsum Syndrome in 1 cas and KID Syndrome in 1 case. Management was multidisciplinary. the deafness was rehabilitated either by auditory prosthesis or by cochlear implantation depending on the degree of deafness. the visual disturbance was taken care by the ophthalmologist. A genetic survey was done.

Discussion and conclusion: Oculo-auditory Syndrome in a disabling situation and must to be screened because of the embryological and cellular similarity of these two organs, in particular the retina and the inner ear. The diagnosis of these disorders is facilitated by the existence of a facial dysmorphism, but it remains difficult when the visual and auditory impairments are isolated.

The earliest diagnosis of oculo-auditory disorders allows for better psychomotor development and optimal social integration. Therefore, early multidisciplinary care is necessary in order to allow the best psychomotor, speech and visual rehabilitation.

Genetic of non syndromic deafness, The experience of the oto-rhino-laryngology department, Hassan II university hospital, Fes, Morocco (About 1070 cases)

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Introduction: Deafness is a frequent sensory deficit; one child over 1000 suffers from this ailment. The repercussions on the lingual acquisitions are severe when the affliction precedes them.

Genetic deafness can be either syndromic or non-syndromic, the most frequent inheritance mode is the recessive autosomic one, other forms have been cited, the dominant inheritance mode represents 20%, while theX-linked inheritance mode represents (1%) and the mitochondrial inheritance mode represents (1%)

Genetic deafness are mostly monogenic pathologies, 67 genes implicated in non-syndromic deafness were cited up to this day, particularly GJB2 located on 13q11, which expresses the connexin26: Which is a transmembrane protein which intervenes in the intercellular protein transport in the inner ear.

Material and Method: This is a retrospective study, carried out between April 2003 and December 2016, involving 1070 patients, of whom 368 control cases, with isolated deafness, recruited in consultation, associationsand schools for deaf children. We have excluded all patients that have a syndromic and environmental deafness. A detailed interrogation, an ear nose and throat clinical exam, and a complete physical exam were conducted concerning all patients and their families. The extraction of DNA was then carried out from a peripheral blood sample.

Direct sequencing of the coding region of the GJB2 gene was performed. Systematic screening of the 35DelG mutation was performed by PCR and fluorescent automatic sequencing. The study was supplemented by the search for other mutations in the absence of 35DelG mutation.

Results: We recorded 634 cases of non-syndromic deafness, including 364 familial cases and 270 sporadic cases.

The average age of our patients was 9 years, with a sex ratio of 1.2. The degree of deafness was variable, most often profound deafness (90% of cases).

The frequency of GJB2 gene mutation in the study population was 25%.The 35delG mutation was the most frequent with 88% compared to other mutations in the same gene. Similarly, four other mutations of connexin 26 have been identified.

Conclusion: Through this study, we confirm the undeniable and frequent involvement of mutations in the GJB2 gene in autosomal recessive non syndromic deafness in Morocco.Thus, the type of deafness represents a substantial health problem, given its high frequency among the Moroccan population.

Molecular diagnosis greatly improves the quality of genetic counselling requested by parents and accurately answersmany questions about the hereditary nature of deafness, the risks for the children to come, and the development of deafness.

The Gap Junction B2 mutations spectrum in 152 Moroccan families with nonsyndromic hearing loss.

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Background: Deafness is one of the most common genetic diseases in humans and is subject to important genetic heterogeneity. The most common cause of nonsyndromic hearing loss (NSHL) is mutations in the Gap Junction B2 (*GJB2*) gene.

Objectives: This study aims to update and evaluate the spectrum of *GJB2* allele variants in 152 Moroccan multiplex families with nonsyndromic hearing loss.

Methods: The *GJB2* coding exon was amplified by Polymerase Chain Reaction (PCR) and all PCR products were directly sequenced using the ABI Big-Dye Terminator v 1.1 sequencing standard Kit and run on an ABI 3130 Genetic Analyzer.

Results: Seven different mutations were detected: c.35delG, p.V37I, p.E47X, p.G200R, p.Del120E, p.R75Q, the last three mutations were described for the first time in Moroccan deaf patients, in addition to a novel non sense mutation, the c.385G>T which is not referenced in any database. Sixty six families (43.42%) have mutations in the coding region of *GJB2*, while the homozygous c.35delG mutation still to date the most represented 51/152 (33.55%). The analysis of the geographical distribution of mutations located in *GJB2* gene showed more allelic heterogeneity in the north and center compared to the south of Morocco.

Conclusions: Our results showed that the *GJB2* gene is a major contributor to nonsyndromic hearing loss in Morocco. Thus, this report of the *GJB2* mutations spectrum all over Morocco has an important implication for establishing a suitable molecular diagnosis.

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Epidemiology of Genetic hearing loss in infants receiving cochlear implantation

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Background: Cochlear implantation is an effective habilitation modality for severe to profound sensorineural hearing loss. This loss can be genetic or non-genetic in origin.

Objective: The aim of this study was to focus on genetic deafness in a retrospective case series of patients with cochlear implantation.

Methods: Series of Moroccan hearing loss patients who received cochlear implants, since March 2010 to June 2016, in the ENT department of Military Teaching Hospital Mohamed V of Rabat. The diagnosis was accomplished by family history (similar cases and parent's consanguinity), physical examination (performed by pediatrician), audiologic, otologic and other ancillary tests such as temporal bone CT scan, electrocardiography (ECG) and electroretinigraphy (ERG). Diagnostic molecular genetic testing, such us mutation in the connexin 26 gene, is very helpful. Unfortunately it was not always available.

Results: total number of patients was 195. Genetic hearing loss represents 40% of all implanted patients. Syndromic deafnesses were 2 cases of PENDRED syndrome, 3 cases of Usher syndrome, 1 case of Waardenberg syndrome and 1 case of CHARGE syndrome. Non-syndromic genetic deafness was suspected on above criteria. Consanguinity was the main factor and represented 34% of all implanted patients.

Conclusions: In the present study, genetic etiology is a major cause of hearing loss in CI patients. Syndromic deafness can involve other life threatening conditions requiring a multidisciplinary approach.

Source of funding: none

Cellular And Molecular Mechanisms Of Hearing Loss In Type III Usher Syndrome

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Introduction: Usher syndrome (USH) is the major cause of hereditary deaf-blindness in humans. Mutations in the clarin-1 gene cause USH3A, characterized by postlingual progressive deafness and blindness. Clarin-1 is a four-transmembrane glycoprotein that is predicted to be targeted to the plasma membrane. The precise distribution pattern of clarin-1 in the cochlea is still unknown, but *Clrn1* transcripts have been detected, from embryonic day 16 (E16) to adult stages, in both sensory hair cell types (inner and outer hair cells) and in the primary auditory neurons. The role of clarin-1 in these cells is still elusive.

Methods: To elucidate the USH3A pathogenesis, we generated two distinct clarin 1-deficient mouse mutants; one, referred to as $Clrn1^{-/-}$, displays a ubiquitous and early gene inactivation of clarin-1, whereas the second, referred to as $Clrn1^{fl/fl}Myo15\text{-}cre^{+/-}$, exhibits a delayed, hair cell-specific loss of clarin-1. Audiometric tests (ABR, DPOAEs, CAPs) were used to study the auditory function of $Clrn1^{-/-}$ and $Clrn1^{fl/fl}Myo15\text{-}cre^{+/-}$ mice. The structural defects in the cochlea of these mice were analyzed by scanning and transmission electron microscopy, and immunohistochemistry and confocal microscopy were used to detect molecular changes. Clarin 1-mediated interactions were analyzed by pull-down and co-immunoprecipitation experiments. Viral injections of *Clrn1* containing adeno-associated viruses were used to rescue hearing abilities in the two mutant mice.

Results and Conclusions: We found that ubiquitous absence of clarin-1 at embryonic stages in $Clrn1^{-/-}$ mice causes severe hearing loss, which was attributed mainly to the disruption of hair bundle stereocilia. By contrast, we show that post-natal and hair cell-specific loss of clarin-1 in $Clrn1^{fl/fl}Myo15\text{-}cre^{+/-}$ mice leads to a late appearing and progressive hearing loss that mimics the hearing phenotype in USH3A patients. Detailed physiological analyses, along with quantitative molecular and structural analyses, in the hair cells of both inborn and conditional clarin-1-deficient mice provides new insights into the mechanisms by which clarin-1 acts in specific compartments of the auditory hair cells. We show that the morpho-functional defects observed in the conditional $Clrn1^{fl/fl}Myo15\text{-}cre^{+/-}$ mice could be prevented by viral-mediated transfer of a *Clrn1* cDNA to the cochlea. The potential impact of our findings on the management of USH3A patients will be discussed.

Epigenomics of the Inner Ear: Defining Potential Entry Points for Therapeutics

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Background: Gene expression can determine cell fate and function and the form of multicellular organisms. Changes in gene expression are regulated by agents including non-coding RNAs, DNA methylation, chromatin structure, and histone modification. The non-coding regulatory regions of mouse and human genomes display significant conservation of functionality and regulatory interactions, rendering the mouse inner ear a suitable model to study regulation of human hearing and balance.

Objectives: Our overall goal is to decipher the regulation of gene and protein expression in the mammalian inner ear, and understand how dysregulation leads to deafness. To this end, we are mapping the epigenomic landscape of the auditory and vestibular systems of the mouse inner ear to reveal critical regulatory elements that control cell patterning, planar cell polarity, synaptogenesis, and mechanotransduction, as well as those that define differences between the auditory and vestibular systems.

Methods and Results: Mouse cochlear and vestibular sensory epithelium non-coding RNAs and epigenetic regulatory elements were profiled during development and maturation between E16.5 and P22, integrating RNA and DNA library construction and high-throughput sequencing. MicroRNA, long non-coding RNA (lncRNA), and methylation profiles were created using a combination of experimental and bioinformatics tools.

Conclusions: Epigenetic intervention has been proposed for several diseases, although its significance in auditory and vestibular systems has largely been overlooked. Our findings implicate microRNAs in regulating auditory and vestibular differentiation. lncRNAs may regulate auditory genes, especially when in *cis* to a deafness gene. Given that mCH accumulates specifically in neurons, the increase of mCH in whole genome methylomes observed upon maturation of the sensory epithelium might be related to the acquisition of the ability to transduce auditory signals into nerve impulses. We believe that understanding inner ear regulation may lead to epigenetic interventions that can alter the downstream events leading to inner ear damage and deafness.

How can we improve the outcomes of cochlear implants ? A surgeon's perspective

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Objective: The goal of the study is to investigate factors that may influence cochlear implantation (CI) outcomes in adult patients. Two types of factors have been considered, including biographic (age, etiology duration of hearing loss) and electrode position factors (insertion depth of apical electrodes and scala location).

Method: 118 adults with unilateral CI over a period of 4 years with at least one year follow up. In addition spiral computerized tomography (CT) scan for evaluation of the position of the electrode was also obtained for a subgroup of 82 subjects. The evaluation was based on sentence recognition in quiet and in the presence of noise at 10 dB SNR when heard using CI alone. Predictive factors were analyzed for covariance and linear models constructed by stepwise linear regression.

Results: Individual longitudinal data were well fitted by logarithmic functions. More than 60% of CI users had initial scores > 50 in quiet, and 25% scored > 70 at 10 dB SNR. Certain etiologies such as chronic otitis, congenital hearing loss and meningitis were associated with significantly lower initial scores ($r=0.52, p<0.001$). Brand and design of device ($r=0.38, p<0.01$) and duration of hearing loss ($r=0.30, p<0.01$, -0 .6 pts per year) also significantly influenced initial scores. In the subgroup with CT data, insertion depth ($r=0.58$, $p<0.01$) and the proportion of the electrode array in scala vestibuli ($r=0.37, p<0.05$) was associated with a reduction in intercept scores of 10-20 points. The rate of growth in scores as a function of time varied widely across subjects, such that the upper quartile advanced three to five times more rapidly than the lower quartile.

Conclusion: Considerable variability exists in auditory outcomes. According to this predictive factors the author will provides suggestions regarding: counseling patient, surgical technique and personalized extra rehabilitation.

Benefit of cochlear implantation in adults

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In 1984, the FDA approved the first cochlear implantation (CI) in adult. There are now about 600 000 cochlear implant users in the world and more than 45000 CIs are sold worldwide each year. In France, 60% of cochlear recipients are adults. Studies on long-term effect of cochlear implantation showed that by restoring communication capacity in quiet and in noise, CI improves sustainably quality of life, depression and may be a preventive strategy against cognitive decline in the elderly. Recent studies showed that new sound processor technology significantly improved the perception of speech in noise, even in patients implanted for more than 20 years. It is now obvious that bilateral auditory stimulation (by cochlear implant or hearing aid in the opposite ear) improves speech benefit in noise. We also observed that bilateral implantation improved the poorest performance after 1 year, which was usually not reported in unilaterally implanted patients, probably by improvement of brain processing after reactivation of the bilateral auditory pathways.

Advances in electrode design and soft surgery minimize the deleterious effect of electrode array insertion, and we can now achieve more than 90% of hearing preservation in selected candidates, who can benefit from electro-acoustic stimulation. A robot, Robotol, was built in our research laboratory to assist cochlear implantation as well as middle ear surgery, and a mechatronic insertion tool was constructed allowing a constant slow insertion with an embarked force sensor. Robotol has now the EC mark and is entering into operation room which will permit to achieve an atraumatic electrode array insertion. All these results suggest that CI should be performed earlier when hearing in noise becomes difficult, even in case of residual hearing on low frequencies or in case of benefit of hearing aids in quiet, especially when hearing loss is asymmetrical between the two ears.

Topical IGF-1 therapy for treatment of sensorineural hearing loss: Mechanisms and clinical applications

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Backgrounds: Insulin-like growth factor-1 (IGF-1) is a peptide that mediates actions of growth hormone. IGF-1 plays crucial roles in the development and maintenance of the auditory system. We have demonstrated the potential of IGF-1 for the treatment of acute SNHL in animals and humans. Here we report recent findings on mechanisms of IGF-1 therapeutic effects on sensorineural hearing loss (SNHL).

Objectives: To reveal mechanisms for hearing recovery after topical IGF-1 treatment, we examined IGF-1 effects on hair cell protection and synapse regeneration using explant cultures of mouse cochleae.

Methods: To examine mechanisms for hair cell protection, explant cultures of mouse cochleae damaged with neomycin, an aminoglycoside, were used. To identify up-regulated mRNA following IGF-1 treatment, cDNA microarray analysis and qRT-PCR was performed in explants cultured with or without IGF-1. We examined protective effects of a candidate of IGF-1 downstream against neomycin. To examine IGF-1 effects on synaptic contacts between inner hair cells and spiral ganglion neurons, we used NMDA and kainic acid, excitatory amino acids (EEA), to damage synaptic contacts, and assessed synapse regeneration by IGF-1 application to culture media.

Results: cDNA microarray analysis and qRT-PCR demonstrated that netrin-1 can be a candidate of downstream of IGF-1 signaling. Netrin 1 showed protective effects on cochlear hair cells, suggesting that neterin-1 is a mediator of IGF-1 actions for hair cell survival against neomycin toxicity. After degeneration of synapses between inner hair cells and spiral ganglion neurons by EEA, IGF-1 promoted synapse regeneration, which was inhibited by IGF-1 antagonists.

Conclusions: Present findings indicate that IGF-1 has effects for hair cell protection and regeneration of synaptic contacts in cochlear sensory epithelia, which could be involved in mechanisms for hearing recovery that had been observed in patients with acute SNHL.

Netrin1 mediates the protection of cochlear hair cells by IGF1 through its canonical receptor, UNC5B

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Sensorineural hearing loss (SNHL) is mainly caused by the damage of cochlear hair cells (HCs). As HCs and supporting cells (SCs) that exist around HCs do not proliferate in postnatal mammals, the loss of HCs and SCs is irreversible, emphasizing the importance of preserving their numbers to prevent SNHL. It is shown that insulin-like growth factor 1 (IGF1) is instrumental in the treatment of SNHL. Our previous study indicates that IGF1 protects HCs against aminoglycoside by activating the IGF1 receptor and its major downstream pathways in SCs, which results in the upregulation of the expression of the Netrin1-encoding gene (Ntn1).

To determine if NTN1 acts as a downstream molecule of IGF1 to protect cochlear HCs against inner ear damage, we performed several experiments. First, we demonstrated that NTN1, similar to IGF1, promoted HC survival. Secondly, we found that NTN1 blocking antibodies attenuated IGF1-induced HC protection from aminoglycoside, indicating that NTN1 is the effector molecule of IGF1 signaling during HC protection.

To elucidate the responsible receptors among six canonical NTN1 receptors that mediated the effect of cochlear HC protection, the localization of canonical NTN1 receptors were tested using in situ hybridization. UNC5B is the only receptor that was expressed in the organ of Corti through whole cochleae. Addition of blocking antibodies of UNC5B attenuated the cochlear HC protection by IGF1 or NTN1, indicating that UNC5B is the NTN1 receptor involved in the HC protection.

Apoptosis was significantly suppressed by NTN1 when HCs were protected from aminoglycoside although the proliferation of SCs was not affected by NTN1.

These results provide new insights into the mechanisms underlying IGF1 protection of cochlear HCs, suggesting a possibility of using NTN1 as a new treatment for SNHL.

Reversible p53 inhibition prevents cisplatin ototoxicity without compromising chemotherapeutic efficacy

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Background: Cisplatin (cis-diamine-dichloroplatinum II; CDDP) is a widely used chemotherapy drug, despite its significant ototoxic side effects. In tumors and cancer cells, CDDP-induced DNA damage has been recognized as the major cause of cell injury and death during chemotherapy.

Objective: In the cochlea, it is unclear whether the DNA damage response pathways are triggered by CDDP and how they are involved in cochlear cell apoptosis. Our objective was thus to decipher the CDDP-toxicity pathway in the organ of Corti at both cellular and systemic levels in order to develop therapeutic strategies to prevent cochlear toxicity without diminishing the chemotherapeutic effect of CDDP.

Methods: CDDP-induced DNA damage and prevention cell death were investigated on cochlear explants from postnatal day 3 mice. To evaluate a therapeutic strategy that would be suitable for clinical use, we perform experiments in mice bearing patient-derived triple negative breast cancer.

Results: Our results showed that DNA damage can lead to ATM activation, resulting in phosphorylation of Chk2 and p53. Genetic or pharmacological ablation of p53 substantially attenuated cochlear cell apoptosis, thus preserving hearing. Importantly, systemic administration of a p53 inhibitor in mice bearing patient-derived breast cancer protected auditory function, without compromising the anti-tumor efficacy of cisplatin.

Conclusions: Altogether, these results highlight pharmacological inhibition of p53 as an attractive approach towards improving the safety of CDDP-based chemotherapy.

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Fetal Gene and Pharmacotherapy to Treat Congenital Deafness and Balance Dysfunction

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Recently defined therapeutic strategies that rescue hearing and balance in mouse models of human inner ear disease deploy, at early neonatal stages, virus-mediated gene transfer to the cochlea and vestibule, or systemic delivery of a pharmacological agent that crosses the blood-labyrinth barrier. The mouse inner ear is functionally immature at these early stages, with audition first emerging during the second postnatal week. Gene and pharmacotherapies deployed closer to or after the onset of hearing are dramatically less effective or fail entirely. A corollary window of therapeutic efficacy in humans would be present prior to hearing onset at 16-19 weeks gestation. Our objective is to assess the feasibility of fetal gene therapies using mouse experimental embryology, a palette of surgical, virological, and microinjection techniques that permits reliable manipulation of the developing inner ear *in utero*. We devised strategies to treat the Ush1c mouse mutant harboring the human *Ush1c* c.216G>A mutation (*Ush1c*^{216AA}) and the VGLUT3 knockout mouse. Hearing loss and vestibular dysfunction in the *Ush1c*^{216AA} mutant is caused by splicing at a cryptic splice site introduced by the point mutation leading to truncation of harmonin protein. Antisense oligonucleotide-29 (ASO-Ush) was microinjected into the embryonic day 12.5 (E12.5) otic vesicle and fetuses were born naturally. Treated mutant mice at postnatal day 30 (P30) demonstrated harmonin splicing correction, protein expression in hair cells, improved stereociliary bundle morphology, and enhanced hair cell survival. Auditory brainstem response (ABR) thresholds were significantly improved at 8, 16, and 24 kHz, and distortion product otoacoustic emission (DPOAE) thresholds were improved at 8, 12, and 16 kHz. The ABR threshold recovery persisted in the top quartile of responding mutants for over 240 days. Treated mutants also displayed an intermediate startle response and Rota-Rod, open field, reaching, and swimming behaviors were consistent with controls. The VGLUT3 knockout mouse fails to load glutamate into synaptic vesicles of inner hair cells and is born deaf. A Cre recombinase-responsive adeno-associated viral vector (AAV1) encoding the chick beta actin promoter driving VGLUT3 was microinjected in the E12.5 otic vesicle of VGLUT3 knockout mice carrying a VGLUT3^{Cre} allele. VGLUT3 expression was constrained to inner hair cells and ABR thresholds at 8, 16, 24, and 32 kHz were significantly improved for over 540 days. Our data suggest that the fetal inner ear harboring a single gene mutation may possess unique cellular and molecular receptivity to preemptive correction by gene therapy and pharmacotherapeutics.

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Connecting a new ear to an old auditory system: restoring hearing using a radically new approach

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Background: Age related hearing loss and birth defects combined affect ~ 1 billion people worldwide. Replacement of lost sensory organs, lost neurons and reestablishing tonotopic connections of the ear with the cochlear nuclei are essential to fully reestablish hearing. Molecular cues for organ development emerge but restoring the functionally relevant details of the functionally relevant distribution of hair cells and supporting cells as well as their connection is beyond reach. Reconnecting such newly formed hair cell requires understanding currently mostly unknown guidance cues leading to proper innervation of hair cells and tonotopic projection to cochlear nucleus.

Objectives: We will review obstacles for gene and cell therapy to restore hearing followed by the introduction of a new approach: transplanting ears.

Methods: Mutant mice with defects in genes that regulate organ of Corti development, innervation of organ of Corti and cochleotopic projections will be presented. We will also introduce the concept of ear transplantation developed to analyze molecular inner ear axonal guidance cues.

Results: Three transcription factors are essential for organ of Corti formation, Pax2, Gata3 and Lmx1a/b. We will provide insights how they may interact to drive organ of Corti development. We will also provide novel insights into organ of Corti intrinsic cell fate regulation mechanisms that set up the patterning of cell distribution of the organ of Corti crucial for its normal function. Cell distribution affects innervation pattern but not the molecular guidance of spiral ganglion neuron projections to develop a cochleotopic projection to the cochlear nuclei. After having introduced obstacles of hearing restoration reflecting mostly our incomplete molecular understanding of these developmental processes we will introduce a novel approach using whole ear transplants that may be particularly useful to treat detectable congenital deafness such as Connexin26 mutations. We will show data that reveal fibers of transplanted ears can reach into the topographically correct area of the brain even when mouse ears are transplanted onto chicken. Using Xenoplastic transplantation approach we hope to establish ear replacement as an viable alternative matching the success of heart transplantations using pig otocyst to replace defect human ears.

Conclusions: Molecular developmental insights have provided us with a rudimentary understanding of critical steps in organ of Corti and innervation development. These insights do not yet suffice to restore a lost hearing organ. Using the capacity of the otocyst to develop into a functional ear after transplantation could be a potential cure for congenital hearing defects.

Computational repositioning and preclinical validation of mifepristone for human vestibular schwannoma

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Background and Objective: There are no FDA-approved drug therapies to treat vestibular schwannoma (VS), the fourth most common intracranial tumor. The computational repositioning of existing drugs represents an appealing avenue for identifying effective compounds for diseases with no FDA-approved pharmacotherapies. Using computational methods and human gene expression data, we aim to identify and preclinically validate FDA-approved drugs with high potential for repurposing in VS.

Methods: Here we present the largest meta-analysis to date of differential gene expression in human VS and use these data to inform the first application of algorithm-based drug repositioning for this tumor class**.** We apply the open-source computational drug repositioning platform ksRepo to gene expression data from 80 patient tumors, then validate promising drug candidates using primary human VS cells, primary human Schwann cells, and immortalized schwannoma and meningioma lines.

Results: We identify eight promising FDA-approved drugs with potential for repurposing in VS. Of these eight, mifepristone, a progesterone and glucocorticoid receptor antagonist, consistently and adversely affects the morphology, metabolic activity, and proliferation of primary human VS cells and HEI-193 human schwannoma cells. Mifepristone treatment reduces VS cell viability more significantly than cells derived from patient meningiomas, while healthy human Schwann cells remain unaffected.

Conclusions: The computational repositioning of FDA-approved drugs based on human gene expression data represents a valuable method by which promising therapeutics can be identified. Our data recommend an immediate Phase II clinical trial of mifepristone in VS.

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Design and preclinical evaluation of an inner ear gene therapy program

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Hearing loss and vestibular disorders are common and potentially make ideal targets for treatment with gene therapy. Proof of concept experiments demonstrate the utility of this approach in targeting both genetic and degenerative disorders. Adenoviral vectors have the ability to deliver large genes or multiple genes safely to the inner ear. Several groups have used these vectors carrying atoh1 to induce hair cell regeneration in both the auditory and vestibular systems. Moving from these experimental observations to a preclinical program involves developing multiple animal models to institute a rigorous preclinical safety program that is acceptable to the regulatory authorities. The process can be divided into manufacturing, assay development, bio-distribution, preclinical safety, scaling and delivery. We will discuss the key points in the development of a preclinical atoh1 delivery program which can serve as a model for a variety of other gene delivery programs in the inner ear.

Cochlear gene therapy for Usher IIIa

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Usher syndrome type 3 (Ush3) is caused by mutations in the clarin-1 gene (CLRN1) and results in progressive loss of hearing and vision. The delayed onset phenotype in humans makes this an ideal disorder in which to target using a gene therapy approach. A transgenic mouse model in which CLRN1 is silenced post-natally resulted in a delayed onset hearing phenotype that closely mimicked that seen in Ush3 patients. We then utilized a gene therapy approach to attempt hearing rescue in this animal model. Adeno-associated virus (AAV) containing the CLRN1 gene was delivered to P1 mice. This resulted in preservation of hearing as measured by ABR in this mouse model of hearing loss as compared to non-treated mice. Further, when examining the morphology of the organ of Corti by electron microscopy, hair cell cytoarchitecture was preserved in the AAV-CLRN1 treated mice, compared to hair cell degeneration in the non-treated mice. These data document the feasibility of treating hearing loss associated with mutations in CLRN1 in Usher3 patients and represents a promising step toward treatment of other forms of genetic deafness.

Delivery of Adeno-Associated Viral Vectors in Adult Mammalian Inner Ear Cell Subtypes without Auditory Dysfunction

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Background: Adeno-associated virus (AAV) mediated gene transfer has been shown to effectively recover auditory functions in mouse models of genetic deafness when delivered at neonatal stages. However, the mouse cochlea is still developing at those time points whereas in human the newborn inner ears are already fully mature.

Objective: Ten-week C57BL/6J male mice were randomly assigned to the different experimental groups, with at least three mice in each group.

Methods: 1 µL AAV1, 2, 6.2, 8, 9, rh.39, rh.43-CMV-EGFP or AAV2/Anc80L65.CMV.EGFP.WPRE was injected via the posterior semicircular canal. 2 weeks later, mice's auditory brainstem response and distortion product otoacoustic emission were tested. Cochleae were harvested and dissected, and MYO7A and GFP antibody were used to label hair cells and infected cells. Hair cells and GFP positive cells were quantified according to individual responsive frequency.

Results: Canalostomy delivery preserves hair cells and maintains normal hearing after injection. AAVs transduce the sensory inner hair cells efficiently, but less efficiently in transducing outer hair cells. A subset of AAVs also transduces non-sensory cochlear cell types.

Conclusions: Multiple AAV vectors can infect hair cells, as well as some supporting cells and non-sensory cells. AAV vectors efficiently transduce diverse adult inner ear cell types, which are useful tools for gene therapy to treat genetic deafness in mature mouse.

Canalostomy approach may establish an efficient and safe route for inner ear delivery in adults, without hearing impairment. Combined with the information on the cochlear cell types targeted, this represents an important step toward developing treatment for different types of hearing loss.

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Main advances, issues and perspectives of AAV-mediated in vivo gene therapy

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In recent years, the number of clinical trials in which adeno-associated virus (AAV) vectors have been used for in vivo gene transfer has increased dramatically. The excellent safety profile, together with the high efficiency of transduction of a broad range of cells, has established AAV vector as the platform of choice for in vivo gene therapy, and contributed to the successful application of the technology in the clinic for a variety of indications, including coagulation disorders, inherited blindness, and neurodegenerative diseases, among others. Additionally, the large number of proof-of-concept studies in animal models showing therapeutic efficacy following AAV vector gene transfer represents a reservoir for future potential clinical trials.

However, several limitations of the AAV-vector technology still need to be overcome to expand the number of indications in which clinical success can be achieved, including inherited deafness.

This lecture will review the recent successes of AAV in vivo gene therapy in humans and the relevant issue encountered. Recent advances in the development of AAV gene therapies for metabolic and neuromuscular diseases will be discussed including stategies we developed to overcome the AAV size limitation and proof-of-concepts of their efficacy in the retina of Usher Ib mice and large animal models.

POSTER SESSION

Topic	Page

Effect of consanguinity on Cochlear implant outcomes in prelingual deaf children

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Background: Prognostic tools to evaluate rehabilitation progress in cochlear implant patients are of great importance. Prevalence of hereditary diseases is higher in consanguine families, as most of these are caused by recessive gene defects.

Purpose of the study: To determine the effect of consanguinity on cochlear implant outcomes in prelingual deaf children, in terms of language development, auditory performances, speech perception and comprehension, oral expression, and speech intelligibility.

Materials and methods: Our study is a prospective, longitudinal study performed in a tertiary referral center. Seventy-four patients with profound prelingual hearing loss received cochlear implant during the study period (from 2010 to 2016). We examined the rate of children with consanguine parents. All children were evaluated at 3, 6, 12, 18,24, 36 and 48 months after cochlear implantation using the APCEI scale, the Categories of Auditory Performance scale (CAP) and the Speech intelligibility Rate scale (SIR). We dived our patients into two groups and examined group differences for children with parents consanguinity (Group 1), and children without parents consanguinity (Group 2),

Results: 74 patients were implanted during the study period, among them 22 children (30,8%) had consanguine parents. Mean age at the diagnosis of deafness was

20,60 ±13,34 months, mean age at cochlear implantation was 42,5+/- 17,3months.

The p APCEI scores at 24 months after CI were 70,29 for Group 1 and 71,35 for group 2, (p=0,84), and 72 for Group 1 and 74 for Group 2 (p= 0,75). Evaluation at 24 months after implantation in terms of speech intelligibility scores and Auditory performances scores didn't show any differences between the two groups. Correlation coefficients did not reach significance for any of the outcome skills measured.

Conclusion: Our Results support that parental consanguinity doesn't influence outcomes in prelingual deaf after cochlear implantation in terms of auditory performances, oral expression, and speech intelligibility.

Profound Deafness And Cochlear Implantation

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Abstract: Cochlear implantation has revolutionized the management of deep deafness, especially in children. It allows the restoration of an auditory canal of excellent quality, which, combined with a well-coded speech therapy and an oral communication mode. Allows a total suppression of the handicap

The objective of our work is to evaluate the epidemiological characteristics, the differents etiologies of the deafness, the management and the post implantation future of our patients.

Material and method: It is a descriptive retrospective study containing 20 cases of patients with profound deafness and implanted in our institution, spread over a period of 2 years from September 2015 to September 2017.

Our work consists in studying the demographic aspects of our patients, the different etiologies of their deafness and their evolution after implantation.

Data collection was carried out by farm records containing: age, sex, personal and family history of patients, surgical side and post surgical evolution.

The data were collected from the data recorded on the files filed in the archives of the service.

The data entry was carried out on the SPSS 20 .0 software, the analysis of the data was done with the same software. Quantitative variables were expressed as mean ± SD.

Results: The analysis showed a male predominance 2/3 of the cases, the middle age of our patients is 3.4 years. A family history of deafness was found in 3 patients. A history of meningoencephalitis was reported in 25% of cases

The otoscopic examination was normal in 60% with 3 cases of otitis seromucositis and one case of congenital cholesteatoma

Thus, in our 20 cases, we were dealing with hereditary congenital deafness in 3 cases, Waldenberg syndrome in 1 case and deafness after bacterial meningencephalitis in 5 cases

The evoked potentials auditory showed a bilateral cophosis in 80% of the cases

The CT scan and magnetic resonance imaging did not demonstrate any associated anatomical abnormalities.

Implantation was of interest to the right ear in the majority of cases90%

And no cases of surgical incidents were reported. One case of surgical site infection was reported

All our patients have benefited from an intense rehabilitation program and a mode of oral communication.

The benefit based on hearing improvement and language acquisition was estimated to be satisfactory in 80% of cases

Conclusion: Deafness is the most common sensory disability. Affecting one child in 1000 births, of various etiologies, the cochlear implantation, despite its high cost, carried out by experienced otologists and in association with an intense reeducation program, revolutionized the future of patients with disabling hearing loss by allowing communication with the outside environment and completes social reintegration.

Surgical features in Cochlear Implantation of labyrinthitis ossificans

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Background: Labyrinthitis ossificans is a pathologic ossification in the otic capsule due to an inflammatory or destructive process. Ossification of the cochlea may be partial or complete.

The main causes are meningitis and trauma.

Objective: describing the appropriate surgical technique for the electrode array insertion in ossified cochlea, in a retrospective case series of patients who underwent cochlear implantation.

Methods: Retrospective study of a choclear implants series. Since March 2010 to June 2016. The diagnosis of cochlear ossification was accomplished by the temporal bone CT scan. However in some cases the diagnosis was missed on imaging and made during surgery. The cochlear ossification was divided on 3 degrees: 1. Limited to the niche of the round window; 2. in the basal turn only, and 3. When the obliteration goes beyond this and reaches the middle turn. Only degrees 1 and 2 were implanted and so included in this study.

Results: 195 was the total number of patients. 16 cases had a cochlear ossification (12 cases degree 1 and 4 cases degree 2). In degree 2 etiologies were meningitis for 3 cases and trauma for 1 case. Only 3 patients had a history of meningitis in degree 1, the etiology remained unknown in other cases. Surgical management of degree 1 was cochleostomy after a wide posterior tympanotomy. Degree 2 required more complicated procedure such us drilling along the basal turn of the cochlea or insertion in the scala vestibuli through the oval window.

Conclusions: Cochlear ossification does not contraindicate cochlear implantation, however it makes the electrode array insertion much more difficult. Deafness following meningitis should be managed quickly before the constitution of an extensive cochlear ossification.

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Gene Therapy Restores Auditory and Vestibular Functions in a Mouse Model of Usher Syndrome

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Background: The various hereditary forms of hearing loss include Usher syndrome, whose most severe clinical form, User syndrome of type 1 (USH1), combines profound congenital deafness with prepubertal retinis pigmentosa leading to blindness. The specific USH1G genetic form of this syndrome syndrome is due to mutations in the gene encoding the scaffold protein sans, which is essential for the cohesion of the hair bundle of the sensory hair cells. *Ush1g*$^{-/-}$ mutant mice display profound deafness and vestibular dysfunction, characterized by circling behavior and head tossing. The hair bundles of their cochlear and vestibular hair cells undergo abnormal morphogenesis and lack functional tip-links. We explored the feasibility, reliability, and long-term efficacy of local viral gene therapy in these mice.

Methods: *Ush1g*$^{+/-}$ and *Ush1g*$^{-/-}$ were used. We chose the adeno-associated virus of serotype 8 as the vector carrier. AAV8 particles producing the protein sans were delivered to the cochlea by injection through the round window membrane of *Ush1g*$^{-/-}$ mice on postnatal day P2-P3. Immunofluorescence was used to investigate the expression and targeting of the protein sans at various stages after the injection. The hearing function of these mice was assessed by auditory-evoked brainstem response recordings at different sound frequencies. The structure and function of the transduced hair cells were probed using scanning electron microscopy and mechanoelectrical transduction current measurements, respectively. Finally, several behavioral tests were performed to evaluate the rescue of vestibular function, including circling behavior analyzed with a tracking software system and swimming tests.

Results: We show that a single delivery of the sans cDNA by the adeno-associated virus AAV8 to the inner ear of newborn mutant mice reestablishes the expression and targeting of the protein to the tips of the stereocilia. The therapeutic gene restores the architecture and mechanosensitivity of stereociliary bundles, it improves hearing thresholds, and it durably rescues these mice from the balance defects.

Conclusions: Our results open up new perspectives for developing efficient gene therapy of cochlear and vestibular disorders. Remarkably, severe dysmorphogenesis of stereociliary bundles can be corrected after the early developmental period.

Gene Therapy Of GJB2 p.V37I Mutation Knock-in Mice

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Objective: To investigate the effects of gene therapy on GJB2 p.V37I Mutation Knock-in Mice.

Methods: AAV vectors were delivered into the left cochleae of P0-P1 ICR mice through scala media or round window membrane, the other cochlea was used as self-control. ABR of both ears was tested at P30, while the cochleae were harvested at P7, P14 and P30. Whole mount and cryosection was done for immunofluorescence.

Results: Both approaches didn't affect the hearing(P>0.05), although there was lower expression of AAV in the lateralwall of cochlea through round window approach. There was no AAV expression detected at P7, few at P14, and stable expression at P30. But the transduction rate was still very low(around 10%), with a reducing trend from basal to apical turn. By using the scala media approach, exogenous Cx26 was observed in the lateral wall and supporting cell of KI mice at P30, with a normal hearing(P>0.05) and cochlea morphology.

Conclusion: Scala media approach and round window approach are both feasible to introduce gene into the inner ear of mice, and the choice should be made based on the target positions. Better vectors should be condidered.

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Viral gene transfer of short otoferlins partially restores the fast component of synaptic exocytosis in auditory hair cell from OTOF knock-out mice

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Transmitter release at auditory inner hair cells (IHCs) ribbon synapses involves sustained exocytosis of glutamatergic vesicles during voltage-dependent activation of L-type (Cav1.3) calcium channels (Glowatzki and Fuchs, 2002; Brandt et al., 2003). Remarkably, IHCs do not use the conventional two-C2 domains synaptotagmins (Syt1 and Syt2) as calcium (Ca^{2+}) sensors to trigger synaptic vesicle fusion (Beurg et al., 2010). Otoferlin, a large six-C2 domains protein (C2A-F), has been proposed to function as a high affinity Ca^{2+} sensor that controls the fast and indefatigable release of synaptic vesicles at the IHCs ribbon synapses (Roux et al., 2006; Beurg et al., 2010; Vincent et al., 2014). However, the precise molecular events by which individual otoferlin C2-domains contributes and/or regulates the synaptic vesicle cycle is still incompletely understood.

In the present study we have characterized the role of the C-terminal C2-domain of otoferlin. For that purpose, we used an *in vivo* cochlear viral gene transfer to newborn mutant mice. The Adeno-Associated Virus (AAV) mediated efficient transfer of several otoferlin short forms (otoferlin C2-EF or C2-DEF) into mouse IHCs lacking otoferlin. The expression of these various otoferlin short forms failed to restore hearing. Surprisingly, IHC patch-clamp recordings showed that the expression of these short otoferlin forms resulted in a partial restoration of the fast component of synaptic exocytosis but not of the sustained component. These results confirm that otoferlin is involved in both the fast vesicle fusion and vesicle replenishment (Pangrsic et al. 2010), and suggest that a cooperativity between the six-C2 domains structure is required for an efficient priming-mobilization of synaptic vesicles in IHCs. Interestingly, the partial exocytotic restoration of the fast component was associated with a recovery to near normal amplitude of the fast inactivating Ca^{2+} currents (Vincent et al., 2017), suggesting that Cav1.3 channel short isoforms interacts with otoferlin.

In vivo genetic manipulation of inner ear connexin expression by bovine adeno-associated viral vectors

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We have previously shown that in vitro transduction with bovine adeno–associated viral (BAAV) vectors restores connexin expression and rescues gap junction coupling in cochlear organotypic cultures from connexin–deficient mice that are models DFNB1 nonsyndromic hearing loss and deafness. The aims of this study were to manipulate inner ear connexin expression in vivo using BAAV vectors, and to identify the optimal route of vector delivery. Injection of a BAAV vector encoding a bacterial Cre recombinase via canalostomy in adult mice with floxed connexin 26 (Cx26) alleles promoted Cre/LoxP recombination, resulting in decreased Cx26 expression, decreased endocochlear potential, increased hearing thresholds, and extensive loss of outer hair cells. Injection of a BAAV vector encoding GFP-tagged Cx30 via canalostomy in P4 mice lacking connexin 30 (Cx30) promoted formation of Cx30 gap junctions at points of contacts between adjacent non-sensory cells of the cochlear sensory epithelium. Levels of exogenous Cx30 decayed over time, but were still detectable four weeks after canalostomy. Our results suggest that persistence of BAAV-mediated gene replacement in the cochlea is limited by the extensive remodeling of the organ of Corti throughout postnatal development and associated loss of non-sensory cells.

Creating Mice using CRISPR/Cas9 to Carry Candidate Alleles for Human Hearing Loss

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Background: CRISPR/Cas9 is an advanced and precise gene editing tool and can be used to generate mouse models for human deafness. A family under study, T263, is characterized by congenital, autosomal dominant, moderate to severe hearing loss in four generations. *ATOH1,* essential for development of cerebellar neurons and generation of inner ear hair cells, was included in the HEar-Seq panel used to identify mutations for hearing loss.

Objectives: To create a mouse model for ATOH1 c.1030delC in order to prove whether this variant leads to deafness in humans and to determine the mechanism leading to hearing loss.

Methods: We developed a targeted hybridization and multiplexed sequencing panel, HEar-seq, of 375 genes for hearing loss. HEK293 cells were transfected with N-Flag-Atoh1-WT or N-Flag-ATOH1-Mut in the presence of cyclohexamide. We replicated the ATOH1 mutation in mice using CRISPR/Cas9 genome editing. We designed sgRNAs directing the Cas9 to cut at both mutation sites, and oligonucleotides containing the mutation, in order to facilitate homologous direct repair. These were injected into mouse zygotes, along with Cas9 RNA.

Results: Sequencing indicated the proband to be heterozygous for *ATOH1* c.1030delC, which segregated with hearing loss in the family. *ATOH1* c.1030delC leads to loss of the C-terminal peptide FSPHSHYSDS, which includes the last four serines of the ATOH1 protein and their loss may alter stability of the protein. Atoh1-Mut protein degraded significantly more slowly than Atoh1-WT protein, suggesting that accumulation of the protein might exacerbate cell death. Four CRISPR/Cas9 founder mice with the ATOH1 variant were generated.

Conclusions: To date, no human mutations for ATOH1 have been detected, most likely since most variants would lead to lethality. If the hearing loss found in the family will be mirrored in the CRISPR/Cas9 mouse, it will verify the ATOH1 variant as the cause of deafness.

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Examination of *In Vitro* Model for Functional Analysis of TRIOBP

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Background: TRIOBP is identified from human hereditary deafness. Stereocilia are composed of actin bundles which have rootlets extending into the cytoplasm and TRIOBP is localized in stereocilia rootlets. *Triobp* KO mice fail to form rootlets of stereocilia and show progressive deafness. It is considered that bundling of actin filament by TRIOBP is required for maintenance of stereocilia morphology. Until now, cochlea explant cultures of *Triobp* KO mice were used for functional analysis. However, cochlea explants culture is inefficient in gene transfer. Therefore, new model should be developed. In this study, we attempt to establish TRIOBP KO *in vitro* model using iPS cells.

Objectives: To establish *in vitro* model for the analysis of TRIOBP function by cell biological approach with gene transfer, using iPS cells.

Methods: We generated *Triobp* KO mouse iPS cells from embryonic fibroblasts (MEFs) using retrovirus vector and selected ES cell-like cell. Then we performed characterization of these cells by examination of marker expression, differentiation potency into three germ layer cells, genotyping and transgene silencing. Additionaly, we examined the hair cell induction of *Triobp* KO mouse-derived iPS cells using methods reported by some groups.

Results: We obtained several lines of ES cell-like cells. We identified these cells as *Triobp* KO mouse MEF-derived iPS cells (TRIOBP KO iPS cells) by marker expression, differentiation potency, transgene silencing and *Triobp* KO mouse genotype. Then, we obtained otic progenitor-like cells in hair cell induction experiment using TRIOBP KO iPS cells.

Conclusion: We generated TRIOBP KO iPS cells and confirmed differentiation potency into otic progenitor of these iPS cells. These results indicate the possibility that TRIOBP KO iPS cells has differentiation potency into hair cell.

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c.1978C>T mutation of the TCOF1 gene in a Moroccan family case of Treacher Collins

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Treacher Collins Syndrome or Franceschetti-Klein syndrome is a genetic disorder with autosomal dominant expression. It is estimated that Treacher Collins Syndrome occurs in 1 out of 50,000 births. Clinically, this syndrome includes characteristic craniofacial dysmorphism associated with conductive hearing loss secondary to external ear abnormalities. The syndrome is caused by mutations in the TCOF1gene (5q32) encoding the nucleolar phosphoprotein Treacle or in the POLR1C(6p21.1) or POLR1D (13q12.2) genes, coding for RNA polymerase I and III subunits. We report in this work an observation of Treacher Collins collected in the Medical Genetics Department of university hospital center mohammed VI of Marrakech.

It is a family form with autosomal dominant transmission, concerning a female newborn presenting a typical craniofacial dysmorphism associated with anomalies of the external ear while her mother presents only one moderate dysmorphism associated with hypoacousia, which illustrates the variable expression of this pathology. In this family. there is a risk of recurrence of 50% in subsequent pregnancies. Therefore, a molecular study of the TCOF1 gene was carried out in the mother and her daughter and demonstrated the c.1978C> T mutation at exon 13 of the TCOF1 gene. Through this observation, we emphasize the interest of the geneticist, to establish the diagnosis, the development of an adequate genetic counseling as well as the proposal of an antenatal diagnosis

Key words: Treacher Collins, Franceschetti-Klein, TCOF1 gene, Molecular study, Genetic counseling

Genetic deafness

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Genetic deafness is deafness caused by genetic mutations during fetal development. is the most common birth defects in humans, with a rate of 3/1000 live births. About 1/1000 children exhibit hearing loss before school age. Over 60% of pre-lingual.

Genetic factors account for at least half of all cases of profound congenital deafness, and can be classified by the mode of inheritance and the presence or absence of characteristic clinical features that may permit the diagnosis of a specific form of syndromic deafness. Although a large number of genes can clearly cause deafness, recessive mutations at a single locus, GJB2 or Connexin 26, account for more than half of all genetic cases in some, but not all populations. The high frequency may well be related to the greatly improved social, educational, and economic circumstances of the deaf that began with the introduction of sign language 300-400 years ago, along with a high frequency of marriages among the deaf in many countries. Similar mechanisms may account for the rapid fixation of genes for speech after the first mutations appeared 50,000-100,000 years ago. Molecular studies have shown that mutations involving several different loci may be the cause for the same form of syndromic deafness. Even within a single locus, different mutations can have profoundly different effects, leading to a different pattern of inheritance in some cases, or isolated hearing loss without the characteristic syndromic features in others. Most cases of genetic deafness result from mutations at a single locus, but an increasing number of examples are being recognized in which recessive mutations at two loci are involved. For example, digenic interactions are now known to be an important cause of deafness in individuals who carry a single mutation at the Connexin 26 locus along with a deletion involving the functionally related Connexin 30 locus.

The discovery of deafness in a child or adult requires the use of audio-visual skills and quality technique to define the importance of deafness, the frequencies reached, the foreseeable repercussions on the language, need and prognosis of hearing aids. However, next to this diagnostic phase necessary for the best choice of auditory rehabilitation (conventional prosthesis, implant of middle ear, cochlear implant...), it is important not to neglect the etiological research that is now possible in a large number of cases given the progress of genetics molecular deafness. Moreover, the molecular genetics of deafness better understanding of pathophysiological mechanisms different types of deafness, which is a preliminary step development of non-prosthetic therapies.

Keywords: Genetic hearing loss- Syndromic hearing loss.

Mutation analysis of GJB2 (connexin 26) and GJB6 (connexin 30) genes in patients with congenital non-syndromic hearing loss around Denizli

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Hearing loss is the most common sensory disorder and is classified according to its various characteristics. The GJB2 and GJB6 genes are often associated with autosomal recessive type of hearing loss, and autosomal dominant inherited mutations are also described. The most common GJB2 mutation in European countries and in our country is 35delG. Other GJB2 mutations that are detected in the studies in our country are W24X, delE120, 233delG, Q80R, 310del14, 167delT, P184R, 236-239delTGCAinsAGATCCG, L90P, R127H, Q80K. There is only one study performed with Sanger sequencing in our country about GJB6 but no mutation was detected. In our study; the coding exons and exon-intron boundaries of GJB2 and GJB6 genes were analyzed by Sanger sequence in 36 patients with non-syndromic bilateral sensorineural hearing loss. Allele frequency for 35delG which is the most frequent mutation in the GJB2 gene was 16.6% (12/72) and the rate of biallelic 35delG mutation rate was found to be 11.1% (4/36). Allele frequency for the other pathological variants detected in GJB2 gene, 167delT and p.Leu90Pro is 1.38% (1/72). The allele frequency of p.Val153Ile, p.Ala171Thr variants in GJB2 gene and p.Ser222= variant in GJB6 gene whose pathogenicity is unclear is 1.38% (1/72). Results showed that GJB2 mutations are an important cause of hearing loss in our region and that similar results have been obtained with studies performed in our country. Further studies with large number cases including more genes associated with hearing loss are required for both the identification of aetiology and for genetic counselling.

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Waardenburg syndrome about 5 cases

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Background: Waardenburg syndrome is an autosomal dominant inherited genetic condition that manifests itself with sensorineural deafness, pigmentation defects of the skin, hair and iris and various defects of neural crest derived tissues. This genetically and clinically heterogeneous disease accounts for 2 % of the congenitally deaf population. We report five newly diagnosed children with Waardenburg syndrome.

Objectives: The aim of our study is to determine epidemiological and clinical characteristics of this group of congenitally deaf pediatric population, in order to improve the management especially for the hearing impairment.

Methods: Five cases were diagnosed during the examination of children with suspected congenital deafness in our department in a period of nine years between December 2008 and January 2017.

Results: The ages of the children ranged between 1,3 years and 5 years, with a median age of 3,6 years. Female cases were predominant. No consanguineous marriage was noticed. Fetal or perinatal history have been reported, there was Family history of premature greying in three cases and a family history of deafness in one case. Two cases were classified as type 1 waardenburg syndrome with a dystopia canthorum, while the other cases were type 2 waardenburg syndrome. There was no case with a Klein or Shah Waardenburg syndrome. the otoscopy was normal in all children. Evoked auditory otoacoustic emissions and tonal audiometry were performed, and showed a profound bilateral sensorineural in all cases. All children had a cochlear implantation with speech reeducation. A genetic consultation was carried out for all patients.

Conclusions: The waardenburg syndrome is a relatively common genetic cause of sensorineural hearing loss. The diagnosis is established in most individuals by physical examination. Molecular genetic testing of relatives at risk allows for early screening of hearing loss in newborns. An early diagnosis and improvement of hearing impairment are the most important for psychological and intellectual development of these children.

Zellweger spectrum disorders in two Moroccan siblings with syndromic hearing loss caused by a new mutation of peroxysomal biogenesis factor 1

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Background: Peroxisomal biogenesis factor 1 (*PEX1*) gene is encoding for a cytosolic AAA ATPase protein Pex1p indispensable in the biogenesis of peroxisomes which are required in many essential metabolic pathways. The *PEX1* gene is usually the gene associated with autosomal recessive inherited diseases known as Peroxisomal Biogenesis Disorders (PBDs) including Zellweger Spectrum Disorders (ZSDs). The ZSDs are characterised in general by development delay, craniofacial dysmorphism, hypotonia, seizures, hearing impairment, vision abnormalities, liver and renal dysfunction.

Objectives: This study aims to establish the appropriate molecular diagnosis in a Moroccan inbred family with two children suffering from syndromic hearing loss.

Methods: The Whole Exome Sequencing (WES) was performed on a HiSeq 2000 (Illumina) sequencer to determine the disease causing gene after proving the absence of the most involved mutations by Sanger sequencing technique in Moroccan families.

Results: In this work, we identified, a new pathogenic homozygous *PEX*1 mutation (p.Leu1026Pro, c.3077T>C) in two Moroccan siblings with syndromic hearing loss. This missense variation is predicted to be deleterious, probably damaging and disease causing respectively by PROVEN, SIFT & POLYPHEN and MUTATION TASTER programs. The p.Leu1026Pro variant is located in P-loop containing nucleoside triphosphate hydrolase witch comprising the most conserved D2 domain of Pex1p protein. This change probably leads to an alteration in the hydrolysis of ATP.

Conclusions: This work report the case of one consanguineous Moroccan family having two affected individuals with many symptoms including profound deafness, impairment vision and development delay. The WES performed reveals a novel deleterious homozygous *PEX1* mutation consistent with the moderate form of ZSDs.

Source of funding: This work was funded by the Pasteur Institute of Morocco and the Pasteur Institute of France.

Pendred syndrome: About 5 cases

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Backgrounds: Pendred syndrome is an autosomal recessive disorder leading to congenital bilateral sensorineural hearing loss and goiter. This disease is linked to a mutation in PDS gene (SLC26A4), encoding Pendrine: a protein expressed in the thyroid gland and the inner ear.

Objectives and Methods: We present a descriptive study of 5 cases of patients with Pendred syndrome that we have diagnosed between January 2010 and December 2017.

Results: The mean age of our patients was 16 years (14-20 years). After the familial survey including genetic trial for each patient, we could not find similar cases. The patients were consulted for goiter with prelingual deafness evolving from the early childhood. Three patients had a history of goiter in the family. The goiter was compressive in two cases. The cervical ultrasound showed a multinodular heterogeneous goiter. Thyroid function was normal in 4 cases. One patient had hypothyroidism. Tonal audiometry showed a severe bilateral neurosensory hearing loss in 4 cases and a moderate perceptive deafness in one case. All patients underwent total thyroidectomy with simple postoperative follow-up. Pathological examination was benign. All patients have benefited from hearing aids with auditory prosthesis and speech reeducation with an improvement of their function.

Discussion and Conclusion: Pendred syndrome accounts for approximately 2 to 10% of syndromic Deafness, a goiter is associated in 50 to 80% of cases. The pathophysiology was elucidated by recent genetic studies. More than 140 mutations of the PDS gene (SLC26A4) have been reported. Once authenticated, a family screening is justified.

Deafness Management In Children With The Charge Association

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Background: CHARGE syndrome consists of a complex cluster of congenital abnormalities, initially described by Hall in 1979 and identified by the acronym CHARGE by Pagon in 1981. CHARGE stands for Coloboma, Heart defects, Atresia of the choanae, Retardation of growth and development, Genital hypoplasia and Ear abnormalities. Blake in 1998 refined the clinical diagnostic criteria by grouping features into major and minor. The major criteria are coloboma, choanal atresia, characteristic ear abnormalities, and cranial nerve abnormalities. Minor criteria are Genital hypoplasia, developmental delay, cardiovascular malformations, orofacial clefts, trachea-oesophagel fistulae and a distinctive face. The presence of these clinical features is variable. Clinical diagnosis requires three or more major criteria alone, or one or more major criteria with at least two minor criteria.Ear abnormalities and hearing loss are common in children with CHARGE and both conductive hearing loss (due to glue ear, ossicular abnormalities or ossicular fixation) and sensorineural hearing loss (due to inner ear abnormalities) may occur. A more number of these children have a moderate or severe hearing loss and are benefist a hearing aids, but a small number of these children have profound hearing loss and are considered for Cochlear implantation.

Objectives: CHARGE syndrome is associated with a variety of temporal boneanomalies and deafness. Charge syndrome is a complex cluster ofcongenital abnormalities, these children may have absent or hypoplasticauditory nerves. The aim of this work is to show the modalitiesof the management of deafness in children with charge syndrome.and objective was to assess outcomes for paediatric hearing aidsrecipients with CHARGE syndrome, to enable better management and family counselling.

Methods: We report the case of a young child of 3 years having as antecedents a surgery for a cardiac malformation and a cryptorchidism. He consults in our service for language development disorders, when examining the child presents a malformation of the external ear, a distinctive face and bilateral deafness 70dB right and 50dB left. The imaging of the ear has objectified a deformity of the internal ear, and after these clinical signs, paraclinical and the antecedents of the children the diagnosis of the charge syndrome was confirmed.

Results: Our patient benefited from a bilateral hearing aid with orthodontic sessions regularly, which allowed the child to develop his language as well as correct schooling. Currently the child is in primary class without scholar difficulty.

Discussion and Conclusions: The audiological management of children with charge syndrome, requiresearly management in order to have a better outcome, for this a collaboration between pediatrician and otorhinolaryngologist and necessary.

Prevention effect of h-IGF1 against progressive hearing loss in DBA/2J mice

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DBA/2J mice are widely used as a model for progressive hearing loss. DBA/2J mice has mutations in cadherin 23 and fascin-2 are responsible for the phenotypes, but the underlying mechanism is unknown. They exhibit high-frequency hearing loss beginning at three or four weeks of age and becoming severe by two to three months of age. IGF1 is crucial for the development and maintenance of the cochlea via two pathways; phosphatidylinositol 3-kinase (PI3K)/Akt pathway and the MEK/ERK pathway. Topical IGF1 application via gelatin hydrogels is reported that it contributes to the recovery of pure-tone audiometry (PTA) levels in patients with sensorineural hearing loss and major recovery of PTA levels occurs within 4 weeks after treatment. To determine whether recombinant human insulin-like growth factor 1 (h-IGF1) can attenuate progressive hearing loss in DBA/2J mice, saline or h-IGF1 was applied with gelatin hydrogels in the round window niche and evaluated by their auditory-evoked brainstem response (ABR) thresholds. Control animals showed progressive hearing loss from 4 weeks old to 8 weeks old. The h-IGF1 group did not showed significant impairment during the period. The saline group showed hearing disturbance at 2 weeks after the operation compared to control animals. The h-IGF1 group ameliorate its hearing level by 15 dB compared to the saline group at 3 weeks after the operation. There are possibilities that h-IGF1 prevents progressive hearing loss in DBA/2J mice and attenuates mechanical hearing disturbance. The mechanism of progressive hearing loss in DBA/2J mice may involve the PI3K/Akt pathway and the MEK/ERK pathway. This work was supported by JSPS KAKENHI 17K11325 and AMED 16ak0101042h0002.

Molecular Diagnosis Of Genetic Deafness

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Introduction: Hearing impairment is the most common sensory disability. Affecting 1 child out of 1,000 live births, deafness can be of genetic or environmental origin. After excluding the main causes of environmental deafness (infections, sound trauma, ototoxic exposure, embryopathy...), a genetic cause should be considered.

Objective: The aim of our work is to provide an update from the literature on recently discovered human deafness genes and to describe the progress made in the complete genetic testing platforms for deafness, all of which have both activated by new massively parallel sequencing technologies.

Discussion: Deafness may be associated with other clinical defects (syndromic deafness) (10%) or isolated (90%).

Each mode of inheritance could be observed: autosomal recessive, autosomal dominant, X-linked, mitochondrial, oligogenic, and even Y-linked.

The objectives of molecular biology in cases of deafness are as follows:

- Establishment or exclusion of a genetic defect
- Search for related anomalies
- Establish a prognosis for evolution
- Assessment of the risk of recidivism in the family or in the next generation
- Discover other affected members in the family

Genetic diagnosis has traditionally been difficult due to extreme genetic heterogeneity.

For these reasons, complete genetic screening platforms have been developed with the use of massively parallel sequencing.These technologies are also accelerating the pace of gene discovery for deafness. Because genetic diagnosis is the basis of molecular therapies, these advances provide the foundation for the clinical care of deaf and hard of hearing people in the future.

Genetic deafness involving connexin 26

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Introduction: Genetic deafness accounts for more than half of the child's perceptual deafness, manifested in multiple clinical aspects depending on the presence of other organs, the mode of genetic transmission, and the degree and age of onset of hearing loss. Diagnosis is based on family history, the systematic search for a syndrome associated with deafness, and verification of the audiogram of parents and siblings.

Objective: We report the case of a child with a genetic deafness due to a mutation of the connexin 26 gene; we show the interest of this molecular test in screening, genetic counseling and the management of this type of deafness.

Observation: This is a 14-year-old child from a non-consanguineous marriage, but the parents lived in the same area.

The child has had hypoacousia since birth, with no associated signs.

Detailed parental examination, clinical examination of the patients, and ophthalmological examination with a fundus of eye allowed us to eliminate an infectious or traumatic origin and to rule out syndromic deafness, in particular Usher's syndrome. None of the patients had any notion of delayed walking.

The tonal audiogram reports a deafness of bilateral perception; cerebral MRI and rocks are without anomaly. Parental consent was obtained to carry out the molecular study

Result: The genetic analysis by DNA sequencing method according to Sanger on ABI 3130 is in favor of a c.35delG mutation of the GJB2 gene in the homozygous state.

Which confirms the diagnosis of autosomal recessive deafness related to the connexin gene 26.

Discussion: In Morocco, deafnesses of environmental origin remain frequent (deafness secondary to congenital infections, perinatal anoxia, jaundice, meningitis and trauma). However, with the improvement of the health system and because of the frequent inbreeding (nearly 25% of marriages), the relative proportion of genetic deafness, although poorly estimated, would be increasing.

Conclusion: This work underlines the interest of the systematic research of this mutation, in deaf children in the absence of certain environmental causes. The identification of this genetic anomaly signals the diagnosis of the cause of deafness, which allows for proper genetic counseling, and better patient management

Auditory Ageing In *Drosophila:* A New Model For Age-Related Hearing Loss

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Hearing loss affects ~10% of the general population and ~70% of the over 70s. Here we introduce the hearing organs of the fruit fly *Drosophila* as a model to explore ageing and sensory homeostasis in an adult ear. The fly's 'inner ear' is formed by the Johnston's Organ (JO). JO harbours ~500 ciliated neurons, which mediate the sensations of sound, gravity and wind.

Using RNA-Seq of dissected JOs, we profiled the auditory transcriptome across different ages. The regulatory landscape of the 1,000 most age-variable genes was explored using the i-cis-Target/i*Regulon* software package, which predicts (upstream) transcription factors and (downstream) regulons based on binding domain analyses. Four master regulators emerged from our analyses, which show expression in JO and are conserved in humans (orthologues in brackets): *onecut* (ONECUT1-3), *optix* (SIX1-6), *wor* (SNAI2), *amos* (ATOH7). *Optix* and *onecut* are predicted to be direct transcriptional regulators of the mechanosensory ion channels NompC (=TRPN1), Inactive (Iav) and Nanchung (Nan), which are all essential components of *Drosophila* hearing.

Auditory function was quantified *in vivo* by means of Laser Doppler Vibrometry (LDV). Our results show a marked age-related hearing loss in wildtype flies, consisting of (i) reduced energy injection, (ii) loss of frequency tuning, and (iii) reduced frequency selectivity in the ears of 70 days old flies. Up to the age of 50 days, however, fly hearing fluctuates around normal (healthy) baseline values.

RNAi-mediated knockdown of *onecut* and *amos* caused dramatic declines in auditory function resembling those seen during age-related hearing loss; knockdown of *optix* and *wor*, in contrast, improved hearing.

The consequences of adult-specific RNAi knockdowns were further investigated by means of qPCR. The results show that *iav* and *nan* are downregulated after knockdown of *onecut*, whereas knockdown of *Optix* leads to *nan* and *iav* upregulation. *NompC* expression, in turn, was upregulated in both *Optix* and *onecut* RNAi knockdowns.

Main conclusions of this work are: (i) fly ears display age-related hearing loss; (ii) a homeostatic transcriptional program maintains hearing during large parts of the flies' life course, its eventual breakdown results in auditory decline; (iii) four transcription factors could be identified as core components of the underlying homeostatic network, corresponding knockdowns either accelerated auditory ageing or protected hearing in older flies. (iv) *onecut* and *Optix* emerged as positive and negative regulators of *nan* and *iav*, respectively, and both act as negative regulators of *NompC*.

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Usher Syndrome: About 4 Cases

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Introduction: Usher syndrome is a heterogeneous group of affections associating congenital sensorineural hearing loss and progressive visual loss secondary to retinitis pigmentosa. Several clinical types are distinguished by the severity of hearing loss, the presence or absence of balance disorders, and the age at which signs and symptoms appear.

Materials and methods: We report 4 cases of children with congenital hearing loss associated with retinitis pigmentosa.

Results: The average age is 9 years. The sex ratio is 3. Two siblings were born of a non-consanguineous marriage. They had a low vision since a young age leading to blindness in the boy and a progressive perceptive deafness. Both have hearing aids. Two boys were born from a consanguineous marriage. They had a profound sensorineural congenital hearing loss and a progressive decrease of the visual acuity. One child also had a delay in walking acquisition. Both children underwent cochlear implantation with a good hearing improvement. The ophtalmological examination in 4 cases showed spicule intraretinal pigmentation, and optic nerve head pallor. A genetic counselling was carried out in three families:

In the first family, the affected child has 3 siblings, born of a consanguineous marriage. Both parents are heterozygous for the faulty allele. They are a healthy heterozygote carrier. No other affected individual has been observed among ascendants, which is explained by the autosomal recessive inheritance.

In the second family, the affected child has 4 siblings, born of a consanguineous marriage. His paternal grandfather had albinism, and his maternal grandmother had ocular involvement, which could not be connected to retinitis pigmentosa.

In the third family, the affected children had 6 siblings, born of a non-consanguineous marriage. No other affected individual has been observed among ascendants.

Discussion: The association of congenital sensorineural hearing loss and retinitis pigmentosa in a context of consanguinity suggests the diagnosis of Usher syndrome in our patients. The presence of a profound congenital deafness preventing the delay in language acquisition and vestibular disorders is favorable to type 1 of this syndrome.

Conclusion: Usher syndome is an unrecognized and incurable pathology whose only therapeutic hope remains gene therapy. Rehabilitation of low vision and cochlear implantation are a palliative treatment to improve the living conditions of these patients who are deafblind.

It seems important to offer families the opportunity to use genetic counseling and molecular diagnosis.

Comorbidity of vestibular and anxiety disorders is observed only after moderate vestibular hair cell ablation in mice

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Background: Vestibular deficiency is associated with comorbid balance and anxiety disorders. We used mice with moderate or severe ablation of vestibular hair cells (HCs) to test the relationship between the extent of HCs ablation and the emergence of elevated anxiety.

Methods: Vestibular HCs were ablated by injection of diphtheria toxin (DT) to adult mice engineered to express DT receptors in HCs ($Pou4f3^{DTR/+}$). The vestibulo-autonomic reflex was monitored as a response of core body temperature to rotation. Mice were rotated on a horizontal plate, resulting in max centrifugal acceleration of 0.5 or 1.0G. Stress was monitored as the response of core body temperature to confinement in a tight cylinder.

Results: Two weeks after DT, HC numbers in the utricle and lateral crista were 31-40% of uninjected mice after moderate-dose DT and 4-8% after high-dose DT. Ten weeks after injection, HC numbers were 26% for moderate-dose DT and 21% for high-dose DT, the increase due to HC regeneration. $Pou4f3^{DTR/+}$ mice showed a dose-related decrease in temperature response to rotation over all time points after DT injection. $Pou4f3^{DTR/+}$ mice injected with a moderate dose of DT showed an increase in temperature response to confinement stress over successive sessions with maximal response at 20 wks after injection.

Conclusions: Short vestibular and stress challenges trigger a reliable autonomic response. Moderate and severe losses of vestibular HCs decrease the autonomic response to a vestibular challenge in a dose-related manner. Shortly after HC damage, both lesions enhance the autonomic response to stress, implying elevated anxiety. Several months after HC damage, elevated anxiety prevailed only in mice with surviving original HCs. We are searching for clinical relevance of these findings in patients with full damage to the vestibular peripheral organs.

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DNA Methylation During Development and Maturation of the Mammalian Inner Ear

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Background: Epigenetic modifications, such as DNA methylation, are essential for tissue development and maturation and have a regulatory role during inner ear formation. High-throughput sequencing, coupled with technologies such as MethylC-seq, RNA-seq and ChIP-seq, enable us to study the epigenome of the auditory system, using small amount of inner ear sensory epithelium as input.

Objectives: We studied DNA methylation at three key developmental time points that represent key turning points during development and maturation of inner ear sensory epithelium, embryonic day 16.5 (E16.5), postnatal day 0 (P0) and P22.

Methods and Results: We found that the sensory epithelium exhibits an accumulation of methylation in a non-CpG context at P22, while none is shown at earlier time points; non-CpG methylation is not characteristic of differentiated mature tissue, with the exception of brain neuronal cells. Our methylome analysis showed time specific Un-Methylated Regions (UMRs), associated with gene promoters, and Low-Methylated Regions (LMRs), located in intronic or distal intergenic region and associated with putative enhancers. GO analysis of the putative regulatory element associated genes exhibits a connection between UMR and LMR regulatory regions and known and novel biological pathways and functions taking part in sensory epithelium development and maturation. We analyzed our data for Differentially Methylated Regions (DMRs) that are either hypo-methylated (loss of methylation) or hyper-methylated (gain of methylation). Overlaying UMR and LMR location with DMR gave us a new perspective of the regulatory processes occur during development and maturation of the inner ear sensory epithelium.

Conclusions: Understanding epigenome dynamics, specifically methylation, will help us discover gene specific and systematic modes of gene expression regulation, leading us to derive regulatory networks that form a fully functional organ of Corti.

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Goldenhar syndrome: About 3 cases

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Introduction: Goldenhar syndrome, a term that is often used synonymously with “Oculo-Auriculo-Vertebral (OAV) spectrum” is a craniofacial developmental disorder affecting the development of the structures derived from the 1st and the 2nd branchial arches during embryogenesis, with consequential maxillary, mandibular, and ear abnormalities. The disorder is characterized by a wide spectrum of symptoms and physical features that may vary greatly in range and severity from case to case Through these 3 observations, we discuss the pathogenic, clinical and genetic aspects of this rare clinical entity.

Material and Methods: We reviewed the records of a retrospective study, from September 2012 to June 2016, including 3 patients with Goldenhar syndrome. Clinical data regarding age, sex, ethnicity, background, genealogical trees, symptoms, treatment and outcome were collected.

Results: 2 patients were male, the ages ranged from 2 to 13 years. Microtia was the most frequent finding (n=2), anotia (1 case), agenesis of the external auditory canal (1cas), enchondromes and pretragian fistulas (2 cases) were the main otologic manifestations. Dermoid of the limbus (two cases) and coloboma of the upper eyelid (two cases) were the ophtalmological symptoms. 2 patients had mandibular hypoplasia with dental articular abnormal. The vertebral anomalies were kyphosis (1 cas) and scoliosis (1cas). Audiological explorations have objectified a conductive deafness in all patients. The scan of the petrous bones showing external agenesis of the auditory canal (2 cases) and an ossicular malformation in all our patients. The scan of the petrous bones was carried out in all patients showing external agenesis of the auditory canal (1 cas), hypoplasia of the middle ear (2 cas), and an ossicular malformation in all our patients.

Discussion and Conclusion: Goldenhar syndrome is a rare entity. Its management is above all multidisciplinary and must be early on to allow the best psychomotor, speech and visual rehabilitation. More over, only a specific genetic diagnosis can be used to evaluate the risk of recurrence of the condition for the relatives.

Prevalence of genetic origin in childhood congenital deafness

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Background: Deafness is the most common sensory disability in children. Establishing an etiological diagnosis is an important step that has major prognostic and therapeutic implications. The etiologies are varied and require a rigorous and complete assessment.

Objective: Determine the etiological profile of childhood deafness in Marrakech.

Methods: Retrospective study of the various causes of deafness in children under the age of 15 years taken care of in our hospital between January 2012 and December 2016

Results: A total of 535 cases of deafness were recorded (average of 107 cases per year). The average age of diagnosis was 4.2 years and 54.4% of the patients were female, the deafness was prelingual in 79% of the cases and bilateral in 87.5%. Genetic causes accounted for 18%, alleged acquired causes in 35%, and 47 % are unknown causes. Of the genetic causes, syndromic deafnesshas been encountered in 32% of cases, remainder being non-syndromic. The main causes of acquired hearing loss are otitis (29%), meningitis (22%) and neonatal diseases (19%). Consanguineous families has been encountered in 12% of the total sample.

Conclusion: The study of deafness in children is very important in terms of public health. The impact of deafness on the possibilities of communication and language development depends on the importance of deafness and the speed of management, hence the need for a positive and etiological diagnosis as early as possible.

Whole exome sequencing identified three causative mutations in EDNRB, SLITRK6 and ATP6V1B1 associated with hearing loss and additional phenotypic features in three Moroccan syndromic families

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Hearing loss (HL) is one of the most common sensorineural disorders. In this study, we present the utility of whole exome sequencing (WES) to identify candidate genes in three Moroccan families with hearing loss. After the exclusion of mutations previously reported in Moroccan deaf patients, we performed whole exome sequencing in patients affected with severe to profound deafness. Sanger sequencing was used to validate mutations in the candidate genes. Our results disclosed a heterozygous missense variant NM_001201397 (p.Arg319Trp) in *EDNRB* gene in SF181 family, one base deletion; frame shifting mutation leading to a premature stop codon NM_032229 (c.696delG) in *SLITRK6* gene in SF120 family and a novel homozygous mutation NM_001692 (c.1155dupC, p.Ile386Hisfs*56) in the *ATP6V1B1* gene in SF193 family. A precise molecular diagnosis obtained from these results will allow us to guide family planning for better management of heritable deafness.

Keywords: EDNRB; SLITRK6; ATP6V1B1; Hearing Loss; Whole Exome Sequencing; Morocco.

Sudden hearing loss : a retrospective study about 36 cases

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The aim of this study is to describe our experience on the management of sudden hearing loss by highlighting the notion of urgency and by showing the factors affecting the probability of recovery. We report a retrospective study including 36 patients treated in the Department of Otolaryngology – Head and Neck Surgery at the Avicenne Military Hospital in Marrakech, Morocco, between January 2010 and December 2015. Only unilateral sudden hearing loss was included in our study (21 right ears and 15 left ears). The clinical data were collected by the interview and the full clinical examination. Hearing impairment was evaluated at admission, every 48 hours and after treatment with pure-tone audiometry. All our patients underwent auditory brainstem response (ABR), 09 of them a computed tomography. MRI was performed in a single case. The therapeutic protocol included corticosteroids and vasodilators. Only 16.6% of patients recovered the entire initial hearing loss. The auditory brainstem response (ABR) detected a case of acoustic neuroma confirmed by imaging.

Cochleo- vestibular dysplasia

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Background: Cochleovestibular dysplasia or Mondini malformation is exceptional. Cochlear dysplasia is due to early cessation of the development of the inner ear during embryonic stage.

Objective: We report two cases with inner ear malformations of cochlear dysplasia type

Patient and Methods: One patient was a 1-year-old-girl who had bilateral cochleovestibular dysplasia associated with Chiari II malformation. The second patient was a 4-year-old-boy who had bilateral cochleo-vestibular dysplasia.

Results: The child without neurologic malformation was successfully implanted without any complication whatsoever.

Conclusion: Although inner ear malformation was once regarded as a contraindication for cochlear implantation, several studies have now proved that this treatment is not only feasible surgically, but also beneficial for the children from an audiological point of view.

Syndromic hearing loss (Hurler Syndrome: A case report)

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Introduction: The mucopolysaccharidose type 1(MPS1), recently known as Hurler syndrom, is a genetic pathology due to a deficit in L-iduronidase enzyme. The hearing impairment appears in the first years of life. Our aim in this work, is to describe types of deafness and their mechanisms in this syndrome through a case followed in the ENT department of the hospital of specialities in Rabat.

Materiels and Methods: It's about a 9 years old child who was diagnosed, based on morphological and biological signs. Which his family reports a language delay and a hearing loss.

Discussion: The hairing impairement appears in the first years of life. Next to the respiratory infections which complicate the seromucous otitis, the osteoarticular involvement which affects also the ossicular chain, in addition to the sensorineural affection, all that explains the variation of hearing loss types in this disease: conductive deafness, perceptive deafness or a mixed hearing loss.

Conclusion: The MPS1, as the majority of genetic pathologies needs a multidisciplinary care. However, the role of the ENT physician is really important for a better life quality.

Psychological aspects of chronic tinnitus

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Background: Tinnitus is defined as a subjective acoustic perception in the absence of any external source, its prevalence is estimated to 10-16% for chronic tinnitus in the adult population; and increases with age.

Objectives: The objectives of our study is to assess the different psychological aspects of chronic tinnitus, including psychological impact on patients and the types of psychological interventions.

Methods: A bibliographic review based on Medline and Scopus database research

Results: While the majority of the population is unaffected by tinnitus, 0.5-3% of the adult population experience distress and impairment in everyday life. Moreover, distressing tinnitus is often associated with psychological problems such as anxiety and depressive symptoms.

Cognitive behavioural therapy is the most common intervention conducted by the researchers. The length of therapy ranges from six weeks to three months. Psychological interventions are more effective in reducing psychological impacts of tinnitus than non-psychological interventions such as the use of tinnitus maskers. Nevertheless, the combination of the treatments gives superior outcomes.

Conclusions: Tinnitus acceptance plays an important role for patients with chronic tinnitus. Increased levels of acceptance are related to better quality of life and less psychological distress. Simplified version of psychological intervention that can be implemented by other clinical professionals should be developed to treat tinnitus.

Source of funding: All authors are state employees.

Therapeutic aspects of chronic tinnitus

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Background: Tinnitus is defined as a subjective acoustic perception in the absence of any external source, its prevalence is estimated to 10-16% for chronic tinnitus in the adult population; and increases with age.

Objectives: The objectives of our study is to assess the different therapeutic aspects of chronic tinnitus, including pharmacological therapy and the types of psychological interventions.

Methods: A bibliographic review based on Medline and Scopus database research

Results: While the majority of the population is unaffected by tinnitus, 0.5-3% of the adult population experience distress and impairment in everyday life. Most widely used treatments for tinnitus involve counselling, and best evidence is available for cognitive behavioural therapy. New pathophysiological insights have prompted the development of innovative brain-based treatment approaches to directly target the neuronal correlates of tinnitus. The use of pharmacotherapy is not well supported by prospective, randomized, placebo-controlled clinical trials. Various drugs have been shown to be effective in some studies, but the clinical evidence is limited.

Conclusions: A large variety of therapeutic interventions is already available, which can efficiently reduce tinnitus severity. Several innovative treatment approaches are currently under development.

Source of funding: All authors are state employees.

Alport Syndrome: Three Cases Report

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Background: Alport syndrome is a rare hereditary disorder characterized by the association of progressive hematural glomerulopathy, deafness of perception of progressive evolution and ocular abnormalities are frequently associated.

Objective: to describe the clinical and paraclinical profile of Alport syndrome about 3 cases.

Methods: We report three cases of Alport syndrome patients with renal, auditory and ocular manifestations.

Results: The average age of our patients has been 21 years (15, 19, 30 years). They are all male with a history of hematuria progressing to chronic end-stage renal disease requiring hemodialysis. One case of our patients had a family history of end-stage renal disease in her mother. Renal biopsy showed irregular thickening of the glomerular basement membrane in all three cases. Two patients had moderate bilateral perception deafness corrected by hearing aids and one patient had mild bilateral deafness. A decrease in visual acuity was noted in all three patients. Lens damage in the form of anterior lenticone was found in two patients and retinal involvement in the form of a dark red macula in one patient. Genetic study has been done for all patients.

Discussion / Conclusion: Alport syndrome is clinically and genetically heterogeneous, with the most frequent mode of transmission being the dominant X-linked mode. It is secondary to a structural abnormality of collagen IV. Mutations of the COL4A5 gene have been characterized in more than 300 patients with X-linked Alport syndrome. Mutations in the COL4A3 and COL4A4 genes are involved in the autosomal forms of the disease. Deafness is observed in about 80% of families, it is a bilateral, often progressive, deafness, which occurs in half of the cases before the age of ten years, which can be the source of a school discomfort. It is therefore necessary to know how to recognize this syndrome given its familial nature, its severity and consequently the importance of genetic counseling.

Two novel homozygous missense mutations identified in the BSND gene in Moroccan patients with Bartter's syndrome

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Hearing loss (HL) is one of the most common sensorineural disorders. In the present study using whole-exome sequencing, we identified two novel missense mutations in *BSND* gene causing Bartter syndrome type IV which is a genetic disease with an autosomal recessive transmission, characterized by hypokalaemia, metabolic alkalosis, an elevation in plasma renin activity and hyperaldosteronism as well as sensorineural deafness. The two novel homozygous mutations p.Arg8Gly (c.22 C>G), p.Thr36Asn (c.107C>A) in exon 1 of *BSND* gene which encodes barttin were identified in 7 patients belonging to two unrelated families originated from central region of Morocco.

Waardenburg Syndrome: Case Report

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Introduction: Waardenburg Syndrome is a polymalfortive genetic syndrome characterized by an oculo-dermato-hearing impairment; it has an autosomal dominant transmission. It's the most frequent cause of syndromic deafness with dominant transmission. It was originally described in 1951 by the Dutch ophthalmologist Petrus Johannes Waardenburg.

Presentation of the case: A 3-year-old boy born from a non-consanguineous marriage, he's the only son. The pregnancy was completed, the delivery was uneventful. It's reported the absence of a similar case in the family. He's admitted to the otorhinolaryngology department of Rabat for a bilateral deafness. At the inspection we find a white forelock and sky blue eyes, the otoscopic examination is without particularity. The diagnosis of the Waardenburg syndrome was discussed. A paraclinic assessment was performed, auditory evoked potentials demonstrate a bilateral cophosis, CT scans of the temporal bone revealed a normal external, middle and internal ear, a genetic study has found a mutation of the SOX 10 gene. The OCT performed objectivized a dystopia canthorum. A pre-implantation MRI was normal. The patient benefited from a right cochlear implantation which was carried out without any incident with simple surgical procedures.

Discussion: Sensoryneural deafness, impaired iris pigmentation, hair hypopigmentation and dystopia canthorum with a Waardenburg index> 1.95 are the major criteria of the syndrome. The minor criteria are congenital leukoderma, enlargement of the base of the nose, hypoplasia of alae nasi, Premature graying of the hair-predominance of white scalp hairs before age 30 years and synophritis. The diagnosis is selected according to 2 major criteria or 1 major criterion and 2 minor criteria. Our patient presents three major criteria of the syndrome, that are profound and bilateral sensoryneural deafness, white forelock and the last criterion is the dystopia canthorum that allows to classify our case as Waardenburg syndrome type 1. Waardenburg syndrome is classified into 4 types according to the clinical criteria and the genes involved. The management of the syndrome is mainly symptomatic in order to offer these patients a better quality of life. Detection of deafness at an early age should be a priority especially with the development of hearing aids and cochlear implants. Our patient benefited from a cochlear implant that allowed a clear improvement in the hearing and a better interactivity with the entourage.

Conclusion: Waardenburg syndrome is a congenital anomaly, hence the importance of genetic counseling. Cochlear implantation is a good rehabilitation method for children with Waardenburg syndrome.

A case of sensorineural hearing loss in children with neurofibromatose type 1

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Introduction: Neurofibromatosis type 1 (NF1) is an autosomal-dominant neurocutaneous disorder with an estimated prevalence of two to three cases per 10,000 population. Sensorineural hearing loss with this rare disease is rarely reported.

Aim of this report: To present a pediatric case of neurofibromatose type 1 with sensorineural hearing loss and review literature data concerning this topic.

Case report: A 11 years old child who consult for progressive hearing loss from 2 years ago. In his medical history we found that he was diagnosed with neurofibromatosis type 1 (NF1) at the age of 3 years, on the basis of café-au-lait spots, cutaneous neurofibromas and axillary freckling, without a family history.

Otoscopy examination was normal. Initial audiogram documented bilateral sensorineural deafness. Magnetic resonance imaging showed multiples heterogeneous abnormalities of signal of brain correlated with Neurofibromatosis, there was no image of vestibular schwanomma or inner ear malformation. The patient benefited from hearing aids and after 3 month of following we have noted a moderate improvement of hearing.

Discussion: Sensorineural hearing loss is more common with Neurofibromatosis type 2 because the frequent association with the vestibular shwanommas; but, this deafness is uncommon with Neurofibromatosis type 1 and its pathogenesis is not well understood. Cochlear and auditory brainstem implantation are proposed for management of the hearing deafness in this disease.

Management of sensorineural deafness in temporal bone trauma: A report of 23 cases

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Introduction: Temporal bone fractures often lead to loss of audio-vestibular function. Otic capsule violating fractures are associated with higher incidence of Sensorineural hearing loss than otic capsule sparing fractures. A rapid assessment, early diagnosis and treatment improve the prognosis.

Materials and methods: Prospective study conducted at the Mohammed VI University Hospital center of Marrakech, from January 2013 to June 2017, covering 23 cases of sensorineural hearing loss in the context of Temporal bone trauma. The objective of this study is the evaluation of the clinical, paraclinical, therapeutic and evolutionary data of this population.

Results: The mean age was 31 years (14-56 years), with a clear male predominance (sex ratio 21/2). Clinical symptomatology was characterized by hearing loss in all patients, otorrhagia in 20 cases, vertigo in 14 cases, facial paralysis in 12 cases, and otoliquorrhea in 5 cases. CT showed labyrinthine fractures in all patients. Two cases of pneumolabyrinthe and one case of bilateral fracture was noted. The audiometry showed sensorineural hearing loss in 9 cases and mixed in 14 cases. In one case the hearing loss was bilateral with severe sensorineural hearing loss on one side and moderate on the other side. Treatment was most often conservative by corticosteroids, prophylactic antibiotics, and vasodilators. Hearing aids are prescribed based on the type and the importance of the hearing loss. For the patient with bilateral fracture, a bilateral hearing aid has been recommended with strict supervision, a cochlear implant will be the alternative in case of deterioration of the hearing. The evolution after one month of the trauma was marked by the spontaneous improvement of the mixed deafness in 6 cases. The persistence of sensorineural deafness in the other cases with evolution towards cophosis in 4 cases. Regression of vertigo was noted in 11 cases, however 3 cases have kept moderate instability. No patient presented with meningitis during the surveillance.

Discussion and Conclusion: Temporal bone fracture is one of the most common traumatic injuries that cause loss of auditory and vestibular function. The consequence is the cochleo-vestibular destruction with a loud symptomatology. The diagnosis must be made within a few hours after the trauma, as the auditory prognosis depends on the take-over time. Auditory sequelae can go as far as cophosis.

Galvanic Vestibular Stimulation: a therapeutic tool for patients with postural instability

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Background: Recent studies have shown galvanic vestibular stimulation (GVS) as a useful tool for postural instability rehabilitation. The GVS can stimulate the central nervous system and create new neuronal links which enable partial or total recovery of that function which has been lost due to neurological disorder. The disease under this study was Human T-cell lymphotropic virus type 1 (HTLV-1)-associated myelopathy (HAM). The geographic distribution of the virus is characterized by clusters of seropositivity, with highest prevalence in Japan, Africa, the Caribbean islands, and Brazil. This slowly progressive myelopathy is characterized by medullary inflammatory alterations and it causes postural instability in 30% of infected subjects.

Objective: As there is no treatment for HTLV-1 infection until now, this study aimed at evaluating if GVS could bring any benefit to balance disorders of patients with HAM.

Methods: Three patients were submitted to five series of GVS, once a week, during a four-week period. All of them were evaluated before and after the GVS treatment by using cervical, ocular and solear vestibular evoked myogenic potentials, Romberg test, Berg Balance Scale, Visual Analog Scale, and Time up and go test.

Results: After GVS sessions, all patients presented instability improvement: higher stability at Romberg test, 26% of improvement in Berg Balance Scale values, a decrease of 39% in instability impact on daily activities (Visual Analog Scale) and better performance during the walking test with increase of 30% on the length of the walking at Time up and go test.

Conclusion: This pilot study indicates GVS as a possible new therapeutic tool, particularly simple and with few side effects for HAM patients, but it still requires further investigation.

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Miller Syndrome: About A Case

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Introduction: Oto-mandibular dysplasias are malformations involving hypoplasia or agenesis of the ear and mandibular hypoplasia. On the genetic level, it is a group of very diverse, sometimes hereditary, affections with different possible or sometimes isolated modes of transmission within a family.

Materials and Methods: We report a case of Miller syndrome (Genée-Wiedemann syndrome) in a 12-year-old girl associated with perceptual deafness.

Clinical observation: the patient presented facial dysmorphism with bird head facies. There is also a hyperthelorism with effacement of the nasogeneic fold and an important micrognatie and mandibular retrognatia. At the otological examination, microtitis is present with low implanted and poorly hemmed ears without pretragian tubercles or preauricular fistulas. The acoustic external meatus is stenosed, which does not allow the eardrums to be visualized. The auditory balance shows a right endocochlear involvement with abolished PEA on the left. On the CT of temporal bone we notice a bilateral malformed aspect of the ossicular chains with hypoplasia of the long processes of anvils and stirrups more marked in the left side. The genetic opinion is in favor of an oto-mandibular syndrome: Miller given the presence of malformations of the upper right extremity.

Discussion: Associated extrafacial malformations that are often present but unrecognized as well as the nature of the facial involvement and the symmetrical or asymmetrical character will allow to recognize certain specific genetic syndromes. Only the constitution of a family tree, a detailed clinical examination and sometimes some complementary examinations make it possible to identify these syndromic forms. The presence of abnormalities of the extremities associated with the facial involvement makes it possible to distinguish acrofacial dysostoses (Miller and Nager syndrome) which are genetically distinct from other oto mandibular dysplasias.

Key words: acrofacial dysostoses; Miller syndrome; Genie-Wiedemann syndrome.

Sudden hearing loss, How to manage it : a retrospective study about 22 cases

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Background: Sudden hearing loss is a medical emergency requiring rapid and appropriate management of patients with this disorder. It poses a problem of etiopathogenesis and therapeutic protocol.

Objectives: The aim of this study is to report our experience in the management of sudden hearing loss by highlighting the concept of this emergency and focusing on our therapeutic management.

Results: The average age of our patients was 40 years (22 to 80 years). The sex ratio was 1.75. The average time for consultation was seven days. Influenza-like illness was found in six cases. Among our patients, five were diabetic, four hypertensive and two had hypercholesterolemia as antecedent. Deafness was associated with tinnitus in 07 cases and vertigo in 2 cases. Otoscopy was normal in all our patients. A deficient peripheral vestibular syndrome was found in one case. The audiogram carried out in all patients showed a deafness of less than 70 dB in 13 cases, a loss between 70 and 90 dB in 7 cases and a total deafness in two cases. The videonystagmogram made in 9 cases showed, on the side of the deafness, vestibular areflexia in two cases. MRI was performed in 17 cases, it was normal in 15 cases; showed a homolateral vasculo-nervous conflict in one case and homolateral acoustic neurinoma in one case. After control of cardiovascular risk factors, all our patients received corticosteroid therapy according to the Stennert protocol. Antiviral drugs were prescribed in all cases for an average of 10 days.

The audiometric control showed almost complete recovery in 03 patients (14.28%), partial recovery in 12 patients (57.14%), and aggravation with total deafness in 01 case (4.76%). For the other cases the hearing remained stable.

Conclusion: The etiology of sudden hearing loss is still unknown and therefore its treatment not yet codified. Corticosteroids are the only treatment with proven clinical efficacy at present.

Characteristics Of Sensori – Neural Hearing Loss After Irradiation

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Sensori – neural hearing loss (S.N.H.L.) after irradiation of malignant tumors located in nasopharynx, parotid gland or facial sinus are a special entity with specific characteristics and difficulties in their management

This kind of deficiencies are neglected and confused with main lesions like external or middle ear lesions at first. The sensorineural deafness is well documented now regarding to the generalization of audiometric tests in the clinical check – up in the following up of all patients who received external irradiation

About this clinical study conducted by ENT and oncologists specialists. Sixty (60) cases of SNHL are documented between 2006 and 2014 where the study was done. There is no difference between males and females and the age of this population is over 45 years. The majority of patients were treated for malignant nasopharyngeal tumors where the U.C.N.T. type is predominant in our country. Less than 7 patients were treated for parotid cancers and sinuses.

All patients have at least 3 audiometric tests in their following up (at 2, 4 and 6 years after the end of radiotherapy). These tests showed a progressive hearing impairment since the first examination to the final one where high frequencies were regularly affected. In the third of cases middle ear was involved by existence of effusion after chronical Eustachian tube dysfunction.

The management of cases offer difficulties regarding to 1, possible cerebral ischemia located near inner ear and 2, osteitis of temporal bone. Medical treatment and prosthetic rehabilitation are the first line. Two thirds of patients received a prolonged corticotherapy since the deafness signs or persistent tinnitus appears. In 5 cases f sudden hearing loss and hyperbaric oxygenotherapy was added. Cochlear implantation has often difficulties in relation with the mastoid bone quality.

Besides the direct responsibility of external irradiation of inner ear and the extreme vulnerability of the organ Corti to high doses of Cobalt, the new protocols of chemotherapy have another impact where the first signs are noted few weeks or months in comparison with radiotherapy where the delay of deafness is longer.

Management of neurosensorial hearing loss by cochlear implantation: Our experience About 106 cases

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Background: A cochlear implant is a surgically implanted electronic device for the management of severe-to-profound sensorineural hearing loss whether acquired or congenital.

Objectives: The purpose of the study is to report our experience in the management of sensorineural hearing loss, by focusing on the factors influencing the results in our practice.

Material and Methods: This is a retrospective study between September 2007 to September 2017,during this period, 106 patients were implanted.

Results: Our series consists of 50 girls and 56 boys suffering from severe-to-profound sensorineural hearing loss whose 80 cases of congenital pre-lingually deaf. The average age of implantation was 5.15 years. All the patients received a unilateral cochlear implantation. The intervention was followed by regular adjustments and aural rehabilitation. The evaluation was carried out by the same team each month during the first 6 months, and then every 6 months. All children has a significant benefit from their implants with a mean of 30.86 months. The good results were correlated with early implantation, an important parental investment and a good follow-up of aural rehabilitation.

Discussion and conclusions: The cochlear implantation has revolutionized the management of severe-to-profound sensorineural hearing loss, is a safe, effective when aimed at properly selected populations and require the organization of a multidisciplinary team. So efforts must be done to improve the management of deaf children.

Albinism-deafness syndrome: about 2 cases

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Background: albinism-deafness syndrome associates deafness with pigmentation disorder. It's a rare autosomal syndrome due to a mutation of the basic region of the MITF gene resulting in a defect in the synthesis of melanocytes.

Objectives: the aim of these observations is to discuss with the literature data two cases of albinism-deafness syndrome.

Methods: We report two observations of two female cousins aged 5 years and 4 months for the first and 20 months for the second, having a history of first degree inbreeding, followed in our service for a bilateral profound congenital deafness with an oculo-cutaneous albinism.

Results: The diagnosis was supported by the results of the auditory evoked potentials for the two girls, associated with the data of the dermatological examination which found diffuse hypopigmentation, blond hair with blue eyes. Ophthalmologic examination demonstrated chorio retinal bilateral atrophy associated with photophobia and strabismus in both girls. The remainder of the morphological assessment showed that in the 20-months-old infant many cardiac malformations: the association of mitral insufficiency and inter-atrial communication for which she is in the course of the assessment, she also presents malformations of the locomotor system .No malformation of the inner ear is detected in the CT scan. A cochlear implantation in programmed in both cases.

Discussion and Conclusions: albinism-deafness syndrome is due to a mutation of the basic region of the MITF gene. This mutation results is the absence of melanocytes in the skin, iris and vascular striae of the cochlea that's why, in this syndrome, the patients are born white and develop a certain degree of pigmentation. They present in the adult a clear skin, from blond to white hair with eyelashes and white eyebrows, they all have blue eyes. Deafness is neurosensory, always bilateral, congenital and profound.

Ear malformations in syndromic deafness: Ct scan Data

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Introduction: In terms of congenital deafness, temporal bone CT scan is the first exam to realize. It may show abnormalities in 6.8% to 12% of cases according to studies; these abnormalities may be isolated or associated with syndromic and / or genetic disorders. The purpose of our study is to evaluate diagnostic value of temporal bone CT in term of genetic deafness.

Materials and Methods: We conducted a retrospective study involving 116 patients who presented severe presumed genetic deafness, from January 2010 to January 2017. All patients performed a temporal bone CT scan. We collected and analyzed medical history, physical examination and CT scan outcomes.

Results: The average age of our patients was 10 years, with a slight female predominance 62 F/ 54M. Medical history data revealed 36 cases of consanguineous marriage (31%), family history of severe deafness was found in 32 cases (28%), no history of meningitis, traumatism, ototoxicity, otitis or otological surgery were noted. 36 children with syndromic deafness (31%) (31 cases of oculo-auditory syndrome and 5 cases of Pendred syndrome). Of the 116 patients, 11 (9%) had abnormalities of the ear in CT scan. within 36 patients with syndromic deafness, 16% (6 cases) had ear abnormalities and 6% (5 cases) had malformations among 80 cases without syndromic deafness.
The malformations found in 116 cases were as follows: Inlarged vestibular acqueduct is the most frequent malformation (6 cases), three cases of type 1 Mondini syndrome including a waardenburg syndrome, 2 cases of internal auditory canal stenosis, 2 cases of middle ear hypoplasia (otomandibular syndrome), 3 cases of external auditory canal atresia including 2 cases of Goldenhar syndrome and a case of Charge syndrome showing an expansion of the external semi-circular duct.

Comments / Conclusion: In the light of these results, ear malformations are often isolated and not associated with syndromic involvement, but the presence of these abnormalities, in an evocative context, should lead a physician to look for a genetic syndrome, hence the interest of the realization of temporal bone CT before for every congenital deafness in children.

Dehiscence of upper semicircular canal revealed by vertigo

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Background: The dehiscence of the upper semicircular canal or the minor syndrome is a rare malformation. It is better identified today by the development of novel neuro-ontological tests. It manifests itself clinically by: vertigo, deafness, and / or tinnitus.

Objectives: Development on this rare disease that can be treated by surgery or medical treatment.

Methods, Results: Study of the case of a 56-year-old patient without significant pathological histrory admitted for fluctuating hypoacousia for 6 years with homolateral affections and a dizziness especially with the exertion. The clinical examination finds a normal eardrum. The audiometry reveals mixed left deafness with loss of 50 db. The ipsilateral and counter -lateral stapedial reflex are present. The video nystagmography is favor of a periferic origin with a right deficit of 20% to the caloric test. The diagnosis is confirmed by a CT of the rocks which showed a focal discontinuity of the bone wall of the superior left semicircular canal.

The patient was provided with an external auditory prosthesis for deafness and vestibular rehabilitation for balance disorders.

Conclusions: The dehiscence of the upper semi-circular duct is the most frequent in humans. The symptomatology is variable according to the seat and the size. It associates a mixed deafness with a normal stapedian reflex, a hyperacusis or pulsatile tinnitus. The vertigo is triggered by the effort that changes the intracranial or the middle ear pressure. The scanner confirms the diagnosis and eliminates an associated meningocele.

Enlarged vestibular aqueduct syndrome

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Introduction: Enlarged vestibular aqueduct (EVA) is the most common CT scan abnormality of the inner ear in children with sensorineural hearing loss (SNHL). It predisposes patients to early onset of SNHL and vestibular disorders. Hearing loss usually presents at birth and initially at high frequencies. Often it progresses during early life and could be sudden, fluctuating or a stepwise fashion.

Case presentation: A 10-year- old female presented sudden and persistent hearing loss after minimal head trauma. Physical examination was significant for normal auricles and external ear canal, normal mobile tympanic membranes with no syndromic facial features. No goiter was found at neck examination. Audiogram demonstrated a bilateral severe SNHL. A CT scan of the temporal bones was ordered which revealed bilateral enlarged vestibular aqueducts measuring 2.4 mm on the right and 2.3 mm on the left without other inner ear abnormalities. MRI confirmed bilateral enlargement of vestibular aqueducts and endolymphatic sac. Her family history was significant for her older sister who also had bilateral moderate SNHL at age 12 and had been fitted with hearing aids since then. The EVA was confirmed in the older sibling with the right and left vestibular aqueducts. The hearing aids were fitted for the patient. Additionally, they were sent for genetic mutation testing including pendrin, and connexin 26.

Discussion and conclusion: EVA is a relatively frequent entity and must be suspected in patients with unilateral or bilateral progressive or sudden SNHL. Some authors have suggested that the vestibular aqueduct could be more easily evaluated on CT scans or MR imaging. We recommend that a complete family history should be obtained and suspected siblings undergo evaluation for EVA when there is a diagnosis within the family. Early detection and diagnosis can prevent deterioration of hearing.

Key words: Inner ear. Sensorineural hearing loss. Computed tomography. Magnetic resonance imaging. Hearing aids.

Neurobrucellosis: a treatable cause of sensorineural hearing loss not to ignore

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Introduction: Brucellosis is a rare disease with neurological manifestations that are polymorphous and multifocal. It can make the diagnosis difficult. We present a clinical case of deep bilateral hearing loss associated with headaches leading to the diagnosis of neurobrucellosis.

Note: A 45-year-old man, a farmer, has consulted with a bilateral hearing loss for 2 months associated with moderate temporo-frontal headaches. Audiometry had confirmed a perceptional hearing loss. An MRI made initially did not show any cerebral abnormalities. Considering the symptomatology of the patient and his profession, a brucellosis serology was positive in the serum and cerebrospinal fluid. The patient was put on quinolones and cyclins for 3 months. The clinical course was favorable with complete regression of the neurological signs and satisfactory improvement of his hearing loss.

Discussion and Conclusion: Brucellosis is a frequent infection in the Mediterranean area. Its neurological symptomatology is highly polymorphic and non-specific, and the hearing loss is a sign frequently encountered during this disease. In unusual neurologic disorders brucellosis should be kept in mind especially in endemic areas.

The inner ear malformations

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Background: The inner ear malformations constitute a vast and complex pathology requiring a perfect knowledge of anatomy and embryology. Generally no obvious cause is found, however genetic abnormalities (chromosomal aberration, parental consanguinity), as well as some teratogenic or viral agents may be incriminated. These malformations are most often isolated or may be part of a polymalformative syndrome.

Objectives: The aim of our study is to report our experience with cases presenting malformations of the inner ear.

Materials and methods: This was a retrospective study between January 2009 and December 2016 that included all patients with a diagnosis of congenital malformation of the inner ear, followed in the Otorhinolaryngology Department of hospital center Hassan II of Fez. The clinical and audiological data, the results of the computed tomography scan of the temporal bone as well as those of magnetic resonance imaging were analyzed for each patient.

Results: 52 malformations of the inner ear were diagnosed in 28 patients. They are dominated by dilated vestibule (15 cases), large vestibular aqueduct (9 cases) and an incomplete Cochlear Partition Type II (8 cases). The discovery circumstances are mainly represented by hearing loss, language delay, and behavioral disorder in respectively 36%, 32% and 18% of cases. The degree of hearing loss is deep in 54%, severe in 25%, and moderate in 21%. The genetic abnormalities were: a SLC26A4 mutation (4 cases of Pendred syndrome), a CHD7 mutation (2 cases of CHARGE syndrome), and a mutation of the EYA1 gene (1 case of Branchio-oto-renal syndrome).Cochlear implantation was performed in 3 patients: "2 cases of hypoplasia of the cochlear nerve and 1 case of Mondini dysplasia".

Conclusion: The inner ear malformations are often revealed by a congenital hearing loss which will directly affect the development of the language and whose early detection is necessary with adequate care.

Sensorineural Hearing Loss in chronic otitis media with cholesteatoma

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Background: The middle ear cholesteatoma is a particularly aggressive form of Chronic otitis media. The audiometry is an integral part of its appraisal. It usually shows a conductive hearing loss due to a tympanic and an ossicular damage; more rarely it reveals a mixed hearing loss or even a cophosis.

Objectives: The aim of our study is to report our experience with cholesteatoma cases presenting a sensorineural hearing loss and to identify the factors that may explain this association.

Materials and Methods: We performed a retrospective analysis on 72 patients who underwent surgery for middle ear cholesteatoma at our otolaryngology department of university hospital center Hassan II of Fez over a 4 years from January 2013 to December 2016. The clinical and audiological data, the results of the computed tomography scan of the temporal bone as well as those of the surgical exploration were analyzed for each patient.

Results and Discussion: A total of 72 patients with middle ear cholesteatoma were included in our study. 29 of them had sensorineural hearing loss (9 of them had bilateral cholesteatoma while 1 had congenital cholesteatoma), their age ranged from 5 years to 65 years and majority (33%) of them belonged to the age group between 30 to 39 years. Among the patients with sensorineural hearing loss, 8 had intracranial or extracranial cholesteatoma complications. Labyrinthine fistula was found in 4 patients with mixed hearing loss at the surgical exploration. Our study have not demonstrate any factors that could explain the association between cholesteatoma and sensorineural hearing loss. However, there are multiple advanced physiopathological mechanisms to clarify this association in the literature.

Conclusion: The sensorineural hearing loss due to the cholesteatoma is a highly debated topic whose causes are still poorly understood. Other clinical and experimental observations are necessary to better determine why and how this hearing loss occurs. This will help develop new bone conduction hearing aids specifically designed for patients with chronic otitis media.

The labyrinthine concussion (about 1 case)

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Introduction: The labyrinthine concussion is a non-specific term that implies an injury of the membranous labyrinth resulting from the forces of acceleration and deceleration on the bony labyrinth during a trauma of the petrous bone. Hearing loss, dizziness and tinnitus are the characteristic symptoms and often progress towards improvement. Through this study we recall the different clinical and paraclinic characteristics as well as the means of management of the labyrinthine concussion.

Clinical Case: We report the case of a 38-year-old patient who suffered a road accident with head trauma. The patient presented a brief initial loss of consciousness and a left temporal wound. When he was admitted to the emergency department, he reported rotatory vertigo with nausea and vomiting. The clinical examination has demonstrated a destructive peripheral vestibular syndrome with a strictly normal otological examination. In the presence of this symptomatology, craniofacial CT and subsequently cerebral and labyrinthine MRI were performed and did not show brain lesions or fractures in the temporal bone, notably signs of intralabyrinthic bleeding or fistula labyrinthine or lesions of the middle ear. The patient was put on antivertiginous. A tonal audiometry performed 2 days after the trauma was normal whereas videonystagmography revealed a left ductal arterflexia. A control VNG was performed 3 months after the trauma that returned normal.

Conclusion: Despite the normality of imaging in the case of petrous bone trauma, the diagnosis of labyrinthine concussion must be evoked before any un explained cochleo-vestibular symptomology. Although the prognosis of this condition is good, some patients require a long recovery period.

Sensorineural Hearing Loss (SNHL) in Children and Perspectives of its Treatment

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Introduction: Despite carrying out variety of scientific research on the treatment of SNHL, most of them remains at the level of laboratory studies, the results have not found their clinical applying and require further elaboration.

Purpose: Based on our own clinical observations and analyzes of the results of experimental research that published in the world literature over the past 10 years, to clarify the current state of the problem.

The material and methods: include the analysis of the supervision of children with SNHL who have been observed during 50 years and the results of clinical and experimental studies that have been published in the world literature in the last 10 years. The cause of SNHL was the influence of ototoxic antibiotics, drugs, viral and bacterial infections, congenital factors. Studies included the use of audiometry, otoacoustic emission, the registration of AEP (auditory evoked potentials), CT of labyrinth.

Results:

1. The main cause of SNHL is the lesion of cochlear hair cells (CHC), comparatively less – deafferentation of spiral ganglion neurons (SGN), disorders of stria vasculari and spiral ligamentum, absence of ability to endogenous spontaneous regeneration of CHC.
2. The effectiveness of treatment of patients with SNHL depends on the etiology, the time that past after hearing loss, the morpho-functional state of cochlea when patients visit a doctor.
3. If the cochlea saves its certain function and the CHC do not completely disappear, it is necessary to stimulate their survival, which requires rational pharmacological molecules and the way to deliver them to target cells.
4. With the death of hair cells and the safety of their progenitors, is possible to choose the pathway of induction to transdifferentiation of supporting cells to ciliary cells, which also requires experimental and clinical elaboration for choosing of the best inhibitors of the Notch signaling pathway and for promoting the expression and generation of the hair cell gene Atoh1, as well as for delivering of pharmacological molecules, peptides, proteins directly into cochlear cells. It is possible choosing of induction to proliferation of supporting cells by inhibition of the cell cycle inhibitors, and then its transdifferentiation. Under these conditions, the activation of endogenous stem cells (SC) by expression, inhibition, drugs and genes is considered as most perceptiveness. However, the quality of the regenerated cells remains unsatisfactorily and the elaboration of new therapeutic exposure is required.
5. With degeneration and loss of large amount of support cells and their insufficiency for transdifferentiation, transplantation of exogenous SC into the cochlea is considered promising, the clinical realization of which requires the teamwork of specialists of various field. This method takes on a great perspective if implanted hearing aids remain ineffective.
6. The well-known cellular mechanisms underlying SNHL, the target for exposure of pharmacological molecules opens the way to new developments to intensify their clinical applying and testing for safety. Obtaining of best results is expected by the using of cochlear implantation together with pharmacological and gene therapy.

Conclusion: It is necessary to improve the clinical diagnosis of the lesion of separate structures of cochlea by electrophysiological, audiological, morphological, beam methods, to create a new screening and treatment program for patients with SNHL. The main effort should be directed to preventing loss of CHC, elaboration of effective methods for inhibiting their death, improving cell survival, restoring steroociliary bundles by using of SC, genes, drugs, neurotrophic factors to achieve the regeneration of CHC and SGN and their functional connection with brain structures.

Endogenous repair of vestibular synapses: an opportunity to restore gait and balance following acute deafferentation?

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Vertigo is a booming health problem and a unmet medical need. In France as in the United States it is the third motive for consultation to the general practitioner doctor and 5 % of hospital emergencies. In over 80 % of cases these conditions result from direct impairment of the vestibule, the organ of balance located in the inner ear. Synaptic contacts between mechanoreceptors and vestibular primary neurons that form the vestibular nerve are the most sensitive area. Acute unilateral vestibular deafferentation is believed to be involved in several vestibular syndromes such as vestibular neuritis, labyrinthitis, vertigo of ischemic origin, as well as in Meniere disease. There is currently no targeted and efficient therapy to effectively protect or repair vestibular synapses under pathological conditions.

Over the last decade, we developed several experimental models with the aim to better understand how deafferentation occurs and how its characteristics (severity, stage) govern the heterogeneity of the symptoms that compose the acute vestibular syndrome (AVS). We developed rodent models of excitotoxic-type vestibular insult, through transtympanic administration of glutamate receptors agonists (TTK model). TTK administration induces in the rat as in the mouse a strong alteration of the vestibulo ocular reflex (VOR) accompanied by the expression of an acute vestibular syndrome (AVS) whose the characteristics mimics those encountered in human. Both the alteration of the VOR and the AVS are transient and reverse within hours and days depending on the parameter considered. This process is correlated with a massive repair of the synaptic contacts between vestibular hair cells and the terminal processes of the vestibular primary (Scarpa's ganglion) neurons. The strong propensity of synapse repair observed *in vivo*, confirms the observation previously reported *in vitro* on co-culture models of vestibular sensory epithelia and Scarpa's ganglion. In the presentation that will be given at the 1rst ISIET2017, we will present data from the VERTIDIAG project currently running in Marseille. We will also discuss the perspective and aims of this project in term of potential clinical transfer.

The Antioxidant N-Acetyl-L-Cysteine (Nac) As A Pharmacological Candidate For Age-Related Hearing Loss

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Introduction: Age-related hearing loss (ARHL) is the most common sensory disorder in the elderly population. The senescence-accelerated prone strain 8 (SAMP8) mouse model present accelerated senescence and have been identified as a model of gerontological research. SAMP8 displays sequential degeneration of cochlear hair cells, spiral ganglion neuron and stria vascularis which mimic human ARHL. The molecular mechanisms associated with SAMP8 senescence involve oxidative stress and altered levels of antioxidant enzymes leading to chronic inflammation and apoptosis. Here, we studied the effect of NAC, an antioxidant, on SAMP8 hearing loss to determine the potential interest of this model in the study of new therapies.

Material & Methods: To characterize hearing loss in SAMP8, we added NAC in the drinking water at 61 mM and we measured the auditory brainstem response (ABR) and the distortion product otoacoustic emissions (DPOAEs), two auditory parameters, every two weeks during two months.

Results: we observed a strong decrease of ABR thresholds at all frequencies and a significant increase of DPOAE amplitude in NAC treated group compared to vehicle.

Conclusion: NAC reduces the accelerated senescence process by decreasing ABR thresholds and protecting cochlear hair cells, strongly suggesting that antioxidants could be a pharmacological target for ARHL.

The improvement of the human hearing capacity by irradiation of the inner ear with Low Level Laser Light

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During the last years the amount of people who suffer from hearing loss is continuously increasing in all levels of age.

Until now is the widespread opinion that no non - invasive medical method is able to improve the human hearing capacity. In opposite to this point of view I experience over more than 30 Years that it is possible to improve the human hearing capacity by irradiation of the inner ear with high quality of Low Level Laser Light.

The irradiation is done in a lying position and reaches the inner ear via ear canal, middle ear and mastoid. Every ear is treated 30 min. The parameters of the laser light are a light power of 3000 mW with a wave length of 830 Nano meters

(= near infrared) and a light power of 100 mW with a wave length of 650 Nano meters (= visible red light) at the same time. The treatment is applied every day over a period of 10 days. Audiometry is done before the first session and before the last session.

The amount of patients is more than 11000 over a period of 30 years. The patients are taught to use during and after the therapy ear protection against daily civilization volume. Side effects where not observed.

The average improvement is 15 until 20 dB over all audio frequencies. The audiometric improvements correspond to the individual hearing experiences of the patients.

It is possible to improve hearing loss with a non-invasive medical method. Further investigations should follow.

No fundings.

Place of hyperbaric oxygen therapy in the treatment of sudden deafness

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Background: Sudden deafness (SD) or sudden hearing loss (SHL) is defined as a suddenly appearing deafness of perceptual type, that is usually unilateral and of unknown etiology, associated or not with tinnitus and/or vertigo. It constitutes a medical emergency requiring a rapid, comprehensive and adapted management of the patients affected. In fact, all current therapeutic modalities tend to increase the oxygen concentration in endolymphatic fluids in order to interrupt the anoxic process and stop the release of ototoxic anions and glutamate at the level of the synapse and thus stop the extension of the lesions. The balance sheet must systematically include the realization of the PEA-TC of which the alteration is synonymous of the request for an MRI (which should be currently systematic).

Objectives: The goal of this work is to evaluate the efficiency of hyperbaric oxygen therapy (HBOT) in the treatment of sudden deafness through a series of 28 cases.

Methods: This is a retrospective study carried out in the ENT Department, in collaboration with the Hyperbaric Chamber Service, at Mohammed V Military Academic Hospital in Rabat, between February 1999 and December 2009. Were included in our study all the patients presenting sudden deafness and who were treated with hyperbaric oxygen therapy (10 sessions) in combination with corticosteroids and vasodilators perfusion. HBOT sessions were performed in a hyperbaric multi-place chamber for 90 minutes at a pressure of 2.5 ATA. After the HBOT sessions the patients had been subject to an oral vasodilator therapy for 30 days. Efficiency control was based on the comparison of the audiograms performed every 2 days. After the 10 HBOT sessions, it was possible to calculate for each patient the relative gain R which is the ratio between the mean total gain (MTG) and the mean initial loss (MIL) on the frequencies of 500, 1000, 2000 and 4000HZ.

Results: A series of 28 patients: 17 women, 11 men, with an average age of 43 years (range: 15-75 years). All our patients have well tolerated the sessions of the hyperbaric oxygen therapy (HBOT). For the 28 patients subjected to the study, we have achieved 72% of good results, 21% of average results and 7% of negligible results. These results confirm the literature data advocating the efficiency of HBOT in the management of sudden deafness provided it is applied in a very early stage.

Conclusion: Hyperbaric oxygen therapy has never been performed alone. It is necessary to conduct prospective studies which only accept as an imperative criterion of inclusion the subjects of other treatments' failure.

Compromised blood flow induces hidden cochlear vulnerability

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The cochlea is metabolically dependent on oxygen supply to maintain its normal function, particularly its endocochlear potential that drives sound-induced ion currents through mechanotransduction channels. This oxygen supply is only provided by the labyrinthine artery, a terminal branch of the ipsilateral antero-inferior cerebellar artery. A large number of conditions may compromise its blood flow, from compressive cerebellopontine-angle tumors to microangiopathies, either genetic (Fabry disease), possibly inflammatory (Susac syndrome) or related to high-prevalence general diseases (diabetes mellitus, excessive blood pressure). The simplest model is a mechanical one that simulates what happens during cerebellopontine surgery, in which postoperative deafness is often attributed to intraoperative damage to the labyrinthine artery. In this study, ferromagnetic microspheres were injected to Mongolian gerbils through a carotid line. A powerful magnet placed at varying distances from the exposed porus acusticus produced reversible sequences of reduced cochlear blood flow (CBF), on a time scale of tens of minutes. The CBF was monitored with a laser Doppler flowmeter and the cochlear function (using auditory evoked potentials -AEP) by a round-window electrode.

We found that for long-lasting CBF reductions of up to 60% and even more, AEPs remained unchanged. However, when the animals with compromised CBF were transiently exposed to loud sounds at normally totally harmless levels such as those used for collecting suprathreshold AEPs, cochlear function was severely impaired within minutes. These observations suggest that in patients with conditions that may decrease CBF, the finding that their audiological tests detect no abnormality does not preclude an inordinately fragile cochlear function, with a permanent threat of developing sudden sensorineural hearing loss. This situation extends the concept of hidden hearing loss already brought forward for noise-induced synaptopathies, to a broader concept of hidden susceptibility to hearing loss. Yet its diagnosis would open the way for possibly simple vasoactive therapies.

Why Human Spiral Ganglion Neurons Persist?

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Human spiral ganglion (SG) neurons possess unique survival properties and maintain electric excitability for a long time after even a complete disconnection with organ of Corti, such as in individuals suffering from profound deafness after loss of the sensory epithelia. This is essential for hearing rehabilitation using cochlear implantation. In recent years, we analyzed and compared immunohistochemically gap junction (GJ) channels (e.g., connexin 43, Cx43), presence or absence of myelin sheath (myelin basic protein, MBP), expression of neurotrophic factors (BDNF and GDNF) and their receptors in SG neurons as well as in their satellite glial cells in man and animals. Recently we used super-resolution microscopy in analyzing cells and molecules that might be associated with the neuron protection and preservation. In man, the non-myelinated SG neurons can survive as monopolar cells with unbroken central projections following dendrite degeneration and consolidation of the dendritic poles against the neuron cell bodies. The Cx43-mediated GJ signaling between cells in human SG may play a key role in this “healing” process in addition to neurotrophic pathway represented by BDNF and GDNF.

The study of human materials was approved by the local ethics committee (no. 99398, 22/9 1999, cont, 2003, Dnr. 2013/190), and patient consent was obtained. The study adhered to the rules of the Declaration of Helsinki. This study was performed in collaboration with MedEl, Innsbruck, Austria; Our research forms part of the European Community Research: Human stem cell applications for the treatment of hearing loss, Grant Agreement No. 603029, Project acronym: OTOSTEM. We are grateful to SciLife Laboratories and the BioVis Platform at the Uppsala University for providing SR-SIM equipment and technical support during the entire study. We also acknowledge the generous donations of private funds by Börje Runögård and David Giertz and kind support from Tysta Skolan, Sweden

A model of P1 chick organotypic culture for single-cell RNA-Seq analysis

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Introduction: The regenerative capacity of mammalian auditory hair cells (HCs) is limited after birth, resulting in the loss of ability to recover from sensorineural hearing loss (SNHL) in mammals. In contrast, birds are able to regenerate damaged HCs through the proliferation or trans-differentiation of supporting cells (SCs), providing us a hint to achieve the recovery from SNHL in mammals. To reveal the HC regeneration mechanism, single-cell RNA-Seq has emerged as a powerful tool. Single-cell analysis is more useful than whole-tissue analysis because HCs and SCs are aligned within sensory epithelia next to each other. Comprehensive analysis of gene expression will provide the information about the network of molecules involved in the HC regeneration. To achieve this purpose, we established the HC regeneration model using the organotypic culture of chick basilar papilla (BP) for single-cell RNA-Seq analysis.

Methods: BPs were dissected from post-hatch day 1 chicks and cultured in DMEM with 1%FBS for 8 days. The explant was treated with/without 78 mM streptomycin (SM) for the first two days. BP samples were collected before culture and days 2 and 8 after culture, fixed with 4%PFA and stained with the marker for HCs and SCs (myosinVIIa, sox2, phalloidin, and DAPI). HC numbers were counted in 3 regions at the distance of 20%, 30% and 40% from the distal end of BP. For the single-cell analysis, the sensory epithelium was isolated from the cartilaginous plate using thermolysin, and single-cell suspensions were obtained by incubating with Accutase and TrypLE Select.

Results: Complete HC loss was observed with two-day SM treatment for BP. In the control samples, HCs labeled with anti-myosinVIIa antibody, phalloidin and DAPI, looked normal in three distal regions, except the proximal end of BP. There are statistically significant differences in HC numbers on day2 between control and SM- treated samples, or non-culture samples and SM-treated samples in the distal region. As for BP samples of cultured for 8 days after SM treatment, regenerated HCs, double-labeled with anti-sox2 and anti-myosinVIIa antibodies were observed. After the cell dissociation, 8,000 to 10,000 single-cells were collected from four BPs of non-culture or SM-treated samples on day 8. The quality of RNA prepared from these samples was good enough to perform single-cell RNA-Seq analysis of BP.

Conclusion: We have established the HC regeneration model of P1 chick BP cultures, which provides sufficient cell number and quality of RNA for single-cell RNA-Seq analysis.

Resveratrol and N-acetylcysteine combined treatment modulates the expression of oxidative stress response genes and ameliorate cochlear damage in a ototoxicity rat model

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Background: Aminoglycoside antibiotics are widely used in medicine but they show ototoxic side-effects. The generation of reactive oxygen species is a central element in ototoxicity, leading to oxidative stress, inflammation and ultimately activation of caspase-dependent apoptosis. Several antioxidants have been used independently in clinical trials against ototoxicity, with positive but limited effects. The combination of antioxidant drugs with complementary mechanisms of action is a novel approach that could provide stronger ROS scavenging potentiate the otoprotection and prevent the oxidation of the drugs themselves.

Objective: To evaluate the protective effect of a treatment with resveratrol plus N-acetylcysteine (NAC) on the ototoxic actions of kanamycin and furosemide in the rat.

Methods: Resveratrol (10 mg/kg) and NAC (400 mg/kg) were administered together intraperitoneally to male 2 month-old Wistar rats on 5 consecutive days. The second day, a concentrated solution of kanamycin and furosemide was placed unilaterally on the round window by bullostomy to induce ototoxicity. Auditory brainstem responses were registered before and 5, 16 and 23 days after the beginning of the treatment. Cochlear samples were taken at day 5 and 23 to analyze oxidative balance and inflammation related genes by targeted PCR arrays or RT-qPCR, respectively. The cytoarchitecture and the presence of apoptosis, oxidative stress and inflammation markers were evaluated in cochlear sections.

Results and Conclusions: Co-administration of resveratrol plus NAC reduced the threshold shifts induced by ototoxic drugs, although this protective effect fades after the cessation of the treatment. The treatment modulated the expression of genes involved in the cellular oxidative (*Gpx1, Sod1, Ccs* and *Noxa1*) and inflammatory (*Il1b, Il4, Mpo* and *Ncf*) responses to injury.

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Immunohictochemical mapping of BK and SK2 channels at the OHC efferent synapse of the mouse cochlea

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Outer hair cells (OHCs) of the mammalian cochlea receive cholinergic inhibitory synapses from medial olivocochlear neurons originating in the brainstem. Recent electrophysiological and immunohistochemical observations show that calcium entry through the OHC nAChR activates both small conductance calcium-dependent potassium channels (SK2) and large conductance, calcium-sensitive, voltage-gated (BK) channels (Rohmann *et al* 2015). This activity occurs in the postsynaptic membrane underlying the large efferent terminals, further specialized by a co-extensive near-membrane cistern that delimits a constant 14 nm cytoplasmic gap throughout the postsynaptic extent. The gap contains regularly-spaced (16 nm center-to-center) unidentified electron densities (Fuchs *et al* 2014). The presynaptic efferent terminal contains thousands of vesicles that focus onto a few dozen discrete active zones scattered throughout the microns square terminal. How do these ultrastructural elements relate to known molecular components of the synapse? In particular, are the postsynaptic SK and BK channels intermingled or segregated? If clustered, does their spacing correspond with that of presynaptic active zones or that of other elements? To address these and other questions we are developing molecular mapping of postsynaptic BK and SK channels using TIRF-STORM microscopy of fluorophore-tagged antibodies that specifically label each channel type.

Supported by NIDCD R01 DC015309 to R. Winslow and P. Fuchs.

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STRIATIN: A novel tight junction protein with a role in auditory function

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Background: Tight junctions (TJs) provide important adhesive contacts between epithelial cells and are crucial for epithelial adhesion, integrity and barrier functions in many tissues. TJs are crucial for maintaining the endocochlear potential in the inner ear and thus disruption of these cell-cell junctions has a deleterious effect on hearing. We have previously shown that members of the striatin interaction phosphatases and kinases (STRIPAK) complex, which are multi-domain scaffolding proteins, are expressed in TJs. Recently, we have found that striatin has a role in junctional integrity and is expressed in the cochlea of neonatal mice.

Objectives: To study the role and understand the molecular and functional connection between striatin and auditory function.

Methods and Results: By western blot and immunofluorescence analysis we show that in the inner ear, striatin is localized to cell-cell junctions of Hensen supporting cells, co-localizes with actin in Claudius supporting cells and is highly expressed in spiral ganglion cells. Endogenous pulldown of striatin by immunoprecipitation recovered RIPOR2 (FAM65B, DFNB104). Our preliminary results demonstrate that striatin interacts with occludin, a TJ protein. Moreover, striatin depletion by siRNA leads to reduced levels of occludin in the junctions. Auditory brainstem response (ABR) revealed that Striatin$^{-/-}$ mutant mice are hearing impaired in the lower frequencies, further supporting striatin's role in auditory function. Currently we are using different biochemical, cell culture and mice models to elucidate the molecular and functional connection between the STRIPAK complex and inner ear function.

Conclusions: Our data reveals that Striatin is a tight junction protein expressed and localized in the mouse inner ear. Striatin also interacts with proteins associated with deafness and localized to the same inner ear cells. This result is further corroborated by defective hearing in Striatin-null mice.

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The CIB2/USH1J protein, defective in isolated deafness without vestibular and retinal deficits, is key for auditory hair cell mechanotransduction and survival

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Defects in CIB2, the calcium- and integrin-binding protein 2, cause isolated deafness, DFNB48, and the Usher syndrome type-IJ, characterized by congenital profound deafness, balance defects and blindness. We show here that a ubiquitous, early gene inactivation (*CIB2*$^{-/-}$ mice) of CIB2 in mouse causes profound deafness without any sign of balance and retinal dysfunctions. In these mice, the mechanoelectrical transduction currents are totally abolished in the auditory hair cells, whilst they remain unchanged in the vestibular hair cells. The hair bundle morphological abnormalities of *CIB2*$^{-/-}$ mice, unlike those of mice defective for the other five known USH1 proteins, begin only after birth, and leads to regression of the stereocilia and rapid hair cell death. The mislocalisation of two key CIB2-partners in the hair bundle, whirlin and integrin α8, in *CIB2*$^{-/-}$ mice point to a key scaffolding role of CIB2 in the hair bundle. Unlike other USH1 mutant mice, the structural abnormalities in *CIB2*$^{-/-}$ mice began after birth, leading to stereocilia regression and, rapidly afterwards, to hair cell death. This essential role of CIB2 in mechanotransduction and cell survival that, we show, is restricted to the cochlea, probably accounts for the presence in *CIB2*$^{-/-}$ mice and *CIB2* patients, unlike in Usher syndrome, of isolated hearing loss without balance and vision deficits.

Clearing the cochlea in research and diagnosis

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In recent years, several chemically clearing techniques have become available to make rodent or human tissues optically transparent (1, 2). These new clearing technologies are of high interest to analyze normal or pathological organs both in fundamental biology and medical sciences. Clearing organs can be imaged in 3D with a high resolution without time consuming tissue sectioning. Native or immunolabelled organs can be used and the techniques is well-adapted for transgenic animals (YGF, GFP, Tomato).

Here we report on clearing cochleas from mouse and rat by the CLARITY method (3) to visualize these organs' cellular and architectural structures. After decalcifying the otic capsule bone, the clarity process renders the entire bone transparent without affecting the endogenous fluorescence (4), making this approach compatible with reporter adult mice or rats, xenografted (DiL cellular tracer)(5) or immunolabelled (Myosin 7A, parvalbumine). Reconstruction is performed by multiphoton or light sheet ultramicroscopy.

The Clarity technique is able to provide fast and comprehensive visualization of biological processes such as ischemia, ototoxicity, effects of drugs, etc. We show here the high potential of tissue clearing supporting its usefulness in research and diagnosis (6).

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Long non-coding RNAs in the auditory and vestibular systems

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Background: Hearing loss, a common sensory disability, is often caused by genetic mutations that interrupt the normal development of the human ear. Recent advances in next generation sequencing (NGS) techniques have sparked interest in the role of regulatory elements of the genome in the development of the auditory and vestibular systems.

Objectives: We sought to identify and illuminate the role of long non-coding RNAs (lncRNAs) in the mouse inner ear on a comprehensive level for the first time, which have been linked to development, epigenetic processes and disease.

Methods: We performed RNA-seq at two developmental stages, embryonic day 16.5 and at birth. RNA was isolated from the sensory epithelium of 20 inner ears of 10 C57BL/6 mice. cDNA libraries were generated and pair-end RNA-seq was performed.

Results: Each of the four conditions had a distinct transcriptome profile, and samples from similar tissue and stage had similar transcriptomes. Of 3,239 lncRNA genes identified, three were examined further, Malat1, linc_Gata3, and linc_miR96. The expression of these candidates was validated using qRT-PCR and the spatial pattern of their expression was evaluated by *in situ* hybridization.

Conclusions: Our findings indicate that lncRNAs are expressed in a spatial and temporal manner in the inner ear and are likely to be involved in the regulation of key pathways in the development of the auditory and the vestibular systems.

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