Simposio Internacional: Hipoacusias hereditarias: del diagnóstico a la terapia

International Symposium: Hereditary Hearing Impairment: from diagnosis to therapy

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The Clinical Diagnosis of Non-Syndromic Hearing Loss: State of the Art 2015, Richard Smith

Massively parallel sequencing (MPS) has revolutionized human genetics and promises to be the harbinger of personalized medicine. In the treatment of deaf and hard-of-hearing persons, this technology has made comprehensive genetic testing possible and as a result it has changed the clinical evaluation of these persons. The OtoSCOPE® platform, which we developed, uses targeted sequence capture (TSC) paired with MPS to sequence all exons of all genes involved in hearing loss simultaneously. To analyze OtoSCOPE®-generated data, we have developed two complementary tools. The first is a bioinformatics platform, which incorporates allele frequencies of deafness gene variations from multiple ethnically different control populations to facilitate variant calling, and the second is a machine-learning tool called AudioGene, which predicts the genotype from the phenotype and offers clinicians a new way to look at audiograms.
Using OtoSCOPE®, our overall diagnostic rate is ~40% but varies by clinical phenotype from <2% for persons with asymmetric hearing loss to ~60% for persons with bilateral autosomal recessive non-syndromic hearing loss. Each patient's OtoSCOPE® run generates on average 15-million mappable sequence reads. We typically sequence targeted bases to 1,601X depth-of-coverage and cover >99% of targeted bases at our variant calling threshold of 10X. To facilitate variant calling, we have established population-level frequencies of reported deafness-causing variants in over 1,000 controls from six ethnic populations. We have also incorporated the analysis of copy number variations (CNVs) into our pipeline, an important feature since a CNV is implicated in ~20% of genetic diagnoses.
In the coming decade, genetic testing to determine causality of hearing loss will play an integral role in personalized therapies as the foundation upon which gene-and-mutation-specific habilitations options are offered as treatment for hearing loss.

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Hearing solutions: state of the art, Manuel Manrique

Palliative treatment of hearing loss with the use of different types of auditory has suffered a real revolution in the last 30 years. Since then, there are many advances that have undergone this technique for the treatment of conductive, mixed and sensorineural hearing losses. The use of bone conduction implants, middle ear implants, cochlear implants and auditory brainstem implants, has contributed to improve the quality of life of a large group of patients that their auditory disability distanced them of a normal communication life.
The established instructions for each hearing implanted group are defined by the typology and topology of underlying disease and the anatomy-functional and socio-cultural characteristics of each patient. Related to this matter, it must emphasize otorhinolaryngologist role when it comes to making a choice and a follow-up of the treatment.
As general rule, it tries to contribute to the access of the hypoacousic patient to his environmental sound enhancing spoken word comprehension, re-establishing binaurality and at the same time it tries to maintain central auditory pathway plasticity through the provided stimulation for any of these implantable systems.
In this presentation, I will mention the advance especially related these topics: Extension of audiometric indications, bilateral stimulation of the auditory system, the role of the age in the indication of auditory implants, non traumatic surgical techniques.

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Genetic epidemiology of hereditary hearing loss, Ignacio del Castillo

Hearing impairment affects about 1 in every 500-1,000 newborns, and its prevalence reaches 3.5 per 1,000 by adolescence. Over 50% of these cases are due to genetic causes, i.e. mutations in single genes (monogenic disorders). In addition, the prevalence of hearing impairment increases with age, and it amounts to over 50% of people having a hearing disorder by age 80 years. This age-related hearing loss (presbycusis) is multifactorial, due to mutations in multiple genes in combination with environmental factors.
Monogenic forms of hearing impairment are highly heterogeneous. The hearing loss can be part of a genetic syndrome or can be the only clinical sign (non-syndromic hearing impairment, NSHI). This syndromic/non-syndromic border is occasionally diffuse, as clinical signs in other organs can manifest much later than the hearing impairment. Over 300 genes are involved in syndromic forms, while mutations in 83

nuclear and 2 mitochondrial genes are currently known to be responsible for NSHI, all patterns of inheritance being represented. Many genes still remain unidentified.
Knowledge on the spectra of mutations in the different genes and on their frequency in subjects with NSHI is still scarce in most populations. Studies have focused on the *GJB2* gene (connexin-26) because of its highest contribution (up to 50% of the recessive forms) and easy screening. Other studies, performed in a few populations, indicate smaller but significant contributions (1-5% each) of some other genes (*MYO15A*, *TECTA*, *CDH23*, *OTOF*...), and minimal contributions (less than 1 % each) for the remaining. Mutation spectra include point mutations, small insertions and deletions, and large structural rearrangements. Few mutations are found repeatedly in specific populations and a majority is found each in only one family. This complex genetic epidemiology means that a comprehensive genetic diagnosis of NSHI requires re-sequencing of all the involved genes, and analysis of copy number variation of genes that are prone to large rearrangements. In turn, the progressive introduction of novel genomic technologies into the routine practice of genetic services will provide plenty of data to increase our knowledge on the genetic epidemiology of NSHI of most populations.

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"NGS technologies for the discovery of mutations and diagnosis in genetic diseases", Javier Santoyo-López

Next Generation Sequencing (NGS) technologies have greatly improved our ability to mine variants out of the entire genome. Currently targeted enrichment sequencing and Whole Exome Sequencint (WES) are common approaches to interrogate specific loci or the whole exome in the human genome, that can allow the discovery of variants responsible of disease, specially in monogenic diseases. More recently Whole Genome Sequencing (WGS) is becoming a feasible approach to find genetic variants in the human genome. All of these technologies will produce large lists of candidate variants that will hamper the finding of the causative mutation as there will be a great number of variants that need to be validated.

Finding the disease-causing variant requires to take into account several factors that can affect a successful outcome, therefore different filtering approaches will be needed, depending on the pedigree, disease and population. For example, the analysis of WES from several families with genetic rare diseases, produced a high number of variants no present in any public database, which prevented of having a conclusive candidate gene responsible of the disease. However, the sequencing of healthy individuals, to generate a

database of SNPs from the Spanish population, greatly improved the discovery of mutations responsible of the genetic disease. This showed that the knoledge of the genetic background of the control population can be critical to resolve successfully the cause of genetic diseases when using NGS technologies.

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Sensorineural hearing loss and vestibular disorders: still much to discover, José Antonio López-Escámez

Vestibular disorders can show different clinical phenotypes, including an episodic vestibular syndrome or a persistent vestibular hypofunction with a progressive clinical course. Most patients with vestibular disorders also have sensorineural hearing loss (SNHL), but the cochlear and vestibular symptoms are not temporary associated, being the exception Meniere's disease (MD).
There are several non syndromic deafness which can show vestibular symptoms, such as DFNA9, DFN11, DFNA15 and DFNB102. Furthermore, enlarged vestibular aqueduct syndrome and MD are two endolymph-related disorders that usually manifest with cochlear and vestibular hypofunction. We have identified >90 multicase families with MD and estimated a prevalence of 8.4% of familial MD in a large cohort of spanish patients with MD. Although we have observed genetic heterogeneity, but most families had an autosomal dominant inheritance with anticipation, and no clinical differences were found between sporadic and familial MD, except for an early onset in familial cases.
By whole-exome sequencing, we have identified two heterozygous single-nucleotide variants in *FAM136A* and *DTNA* genes, both in a Spanish family with three affected cases in consecutive generations, highly suggestive of autosomal-dominant inheritance. We have also demonstrated that *FAM136A* and *DTNA* proteins are expressed in the neurosensorial epithelium of the rat *crista ampullaris*. While *FAM136A* gene encodes a mitochondrial protein with unknown function, DTNA encodes a cytoskeleton-interacting membrane protein involved in the formation and stability of synapses with a crucial role in the permeability of the blood – brain barrier.
By reprogramming lymphocytes from patients with these mutations, we have already generated and characterized an induced pluripotent stem cell model to decipher the role of FAM136A and DTNA genes in MD.

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Next generation sequencing approaches for the identification of novel hearing loss genes, Margit Schraders

Identification of the causative mutation in families with hearing loss can be difficult due to a high degree of genetic heterogeneity. Therefore diseases like hereditary hearing impairment, in which the causative mutations or even the causative gene may be private, have already greatly benefited from the recent technological advances in target-enrichment and next generation sequencing (NGS). Over fifteen novel nonsyndromic deafness genes, identified using these techniques, have been published in the last four years. Although NGS studies have thus been successful in the field of hearing loss, estimated success rates for NGS in diagnostics are as low as 25% for uncovering the pathological variant underlying the disease. This is often due to the large number of candidate variants remaining after common filtering strategies making the identification of the causative mutation difficult. The use of NGS approaches for the identification of novel deafness genes will be discussed as well as the difficulties in data analysis and the sometimes unexpected findings. Finally, some examples will be given of newly identified candidate genes for deafness.

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Myosins and regulation of mechanotransduction: role in hearing and deafness, Bechara Kachar

Stereocilia are specialized actin-protrusions of inner ear sensory epithelial cells that are unique in that they have extraordinarily tightly and differentially regulated lengths. The result is that each stereocilia bundle has a distinctive staircase organization, vital for its role in mechanotransduction: the conversion of mechanical stimuli such as sound and head-movement, to neural signals. The number of known auditory and vestibular deficits caused by abnormal stereocilia morphology highlights the importance of stereocilia structure and organization to hair cell mechanotransduction. With lengths that can range from less than 1 µm to sometimes over 100 µm in a single cell, stereocilia depend on a range of coordinated molecular-level interactions to build and regulate their structure and function. These include: i) active molecular transport from the cell soma to distal tips of stereocilia; ii) local self-regulatory mechanisms within distinct confined compartments of stereocilia, particularly at the distal tips where mechanotransduction takes place; and iii) fine tuning steady-state lengths of adjacent stereocilia to generate a precise graded pattern. In this presentation we will highlight the roles of several unconventional myosins and cargos associated with inherited deafness in regulating stereocilia functional architecture. We will also discuss how these myosin motor systems transport integral components of the mechanotransduction channel complex to the distal tips of stereocilia.

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Tectorial Membrane Alterations Associated with Hearing Loss and Audiogenic Seizures, Guy Richardson

The tectorial membrane (TM) is a prominent extracellular matrix that lies over the endolymphatic surface of the organ of Corti. It is composed of collagens that are typical of cartilage (Types 2, 9 and 11) and a number of non-collagenous glycoproteins that are only expressed at high levels in the inner ear (Tecta, Tectb, Ceacam16, otogelin and otogelin like). Mutations in Type 11 collagen, Tecta, Ceacam16, otogelin and otogelin are known to cause human hereditary deafness, with missense mutations in TECTA being one of the more common causes of autosomal dominant non-syndromic hearing loss. Tecta is large modular protein composed of various predicted protein domains and is required, along with Tectb and Ceacam16, for the formation of the striated-sheet matrix within which the collagen fibrils in the core of the TM are imbedded. A small allelic series of transgenic mice lines is now available that reveals how recessive null mutations and some of the ~40 dominant missense mutations thus far reported in TECTA affect the structure of the TM. The residual TMs of mice with effective null mutations in Tecta are only comprised of collagen fibrils, are completely detached from the lumenal surface of the organ of Corti and are associated, instead, with Reissner's membrane. The compound action potential threshold audiograms reveal hearing thresholds for 20 kHz tones are elevated by ~70 dB. A genotype-phenotype correlation is observed in mice with dominant missense mutations in Tecta. Mutations in the zona pellucida domain cause a distinctive change in shape, an enlargement of the subtectorial space, a delamination of Kimura's membrane, a displacement or detachment of the marginal band, and a loss of striated-sheet matrix in the sulcal zone. Mutations in the zonadhesin domain primarily cause a fragmentation of the marginal band and a reduction of the covernet fibrils. In response to a click stimulus, auditory brainstem response thresholds of are, on average across all ages and strains tested, elevated by 30 to 50 dB in mice carrying these dominant missense mutations. Despite elevated auditory thresholds, mice carrying both null and missense mutations in Tecta all exhibit audiogenic seizures in response to 8-16 kHz broad band noise at sound pressure levels ≤84dB SPL. Recent findings reveal mice carrying null mutations in either Tectb or in otoancorin, a product of the DFNB22 locus required for the attachment of the TM to the spiral limbus of the cochlea, are also susceptible to audiogenic seizures. Possible reasons for the common behavioural phenotype of mice with a wide variety of structural alterations affecting the TM will be discussed.

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La senescencia celular programada durante el desarrollo embrionario del oído interno y en su envejecimiento, Isabel Varela-Nieto

The sensory organs responsible of hearing and balance have a common embryonic origin in the otic placode. Lineages of neural, sensory and support cells are generated from common otic neuroepithelial progenitors. The sequential generation of the cell types that will structure the adult inner ear requires the dynamic coordination of cell proliferation with cell differentiation programs; as well as the strict regulation of cell survival, apoptosis, autophagy and senescence to refine cell numbers and sculpt the organs. A network of intracellular signals orchestrates the transcriptional response to the extracellular input. Understanding the molecular clues that direct otic development is fundamental for the design of novel

strategies for the protection and repair of hearing loss (HL) and balance disorders. Some forms of genetic HL are rare diseases, in striking contrast, presbyacusis, affects approximately half of the population over 60 years old. HL occurs when the sensory cells and neurons of the cochlea degenerate and die. Genetic and environmental factors contribute to the progression of HL, being noise the main environmental noxious agent for human hearing. There is no restorative treatment for deafness but functional replacement by means of prosthesis. Therefore, prevention and treatment of HL is an unmet medical need. The described pathophysiological mechanisms involved in HL include oxidative stress, excitotoxicity and inflammation, resulting in synaptic loss, axonal degeneration, and apoptosis of spiral ganglion neurons. These mechanisms are shared with other neurodegenerative disorders. Neuroinflammation is an essential element in the progression of injury and cell loss, and a target for cell protection strategies. Autophagy and senescence are cellular processes involved in development and ageing, whose contribution to inner ear structures and functions will be discussed in the context of their regulation by IGF-1.

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What mouse mutants tell us about deafness, Karen Steel

Progressive hearing loss is very common in the human population and can start at any age from the first decade of life onwards. Single gene mutations have been implicated in progressive hearing loss in a handful of extended families where linkage analysis can be used to pinpoint the causative mutations, but for most cases there are no clues to the causes. It is likely that a combination of environmental factors and genetic predisposition underlies hearing loss in many cases, making it difficult to study directly. Mouse mutants offer an alternative approach to identifying genes that are essential for maintenance of normal hearing.

We have generated a large number of new mouse mutants with known genes inactivated and screened them for hearing deficits by Auditory Brainstem Response recording (ABR) at 14 weeks old. Out of the first 900 new mutant lines screened, 24 have shown raised thresholds. Several of these have been followed up with ABR at different ages and show progressive increases in thresholds with age. Examples of primary defects in the hair cells, in synapses below inner hair cells, and in maintenance of endocochlear potential have been discovered, emphasising the heterogeneous nature of progressive hearing loss. These genes represent good candidates for involvement in human progressive hearing loss.

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Investigating future forms of hearing loss treatments, Pascal Senn

In this presentation, two ongoing EU-FP7-funded, collaborative projects will be reviewed. The first project NANOCI (www.nanoci.org) uses a combination of regenerative medicine, biomedical engineering and nanotechnology to improve current cochlear implant technology. The core of the concept is to attract peripheral processes of the auditory neurons to the cochlear implant electrode array surface. We hope to increase the number of independent channels of cochlear implant systems for better auditory performance and to substantially reduce energy consumption. In the second project, OTOSTEM (www.otostem.org), a collaborative approach by a consortium of stem cell researchers aims at finding new therapy forms of hearing loss. The concepts of these two initiatives will be reviewed and some examples of ongoing experiments of both projects in the laboratory of the presenter will be reviewed.

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