cholesteatoma. One patient (4%) had displacement of the ossicular prosthesis.

Conclusion: Cholesteatomas restricted to the attic and/or mesotympanum can be removed in a one-stage technique with no residual visible at 1 year and closure of ABG by 50%.

doi:10.1017/S0022215116004102

Inflammation in the middle ear: initiation, regulation and pathophysiology (K823)

ID: 823.1

Inflammation in the middle ear: Initiation, regulation and pathophysiology

Presenting Author: **Allen F. Ryan**

Allen F. Ryan
University of California, San Diego

Learning Objectives: Inflammatory reactions in the middle ear (ME) are significant contributors to otologic disease, including cholesteatoma and otitis media. A major source of ME inflammation is the activation of pattern recognition receptors (PRRs), either by bacteria, viruses or damage-associated molecules patterns (DAMPs) released from dying cells. Ligand binding to PRRs, including Toll-like (TLR), NOD-like (NLR) and C-type lectin receptors, in turn activates pro-inflammatory signaling pathways including the NFkB and JNK cascades. This leads to the production of pro-inflammatory cytokines, chemokine leukocyte chemoattractants, and growth factors that enhance tissue hyperplasia. Studies in mice with deletion of genes encoding PRRs, downstream signaling molecules and their major transcriptional targets clarify the relative roles of PRRs in mediating ME inflammation. These studies implicate TLR signaling via MyD88 and NOD receptor signaling via RIPK2 as major mediators of ME inflammation. They further indicate that the cytokines TNF alpha and IL-1 beta, and the chemokine CCL3, are critical effector molecules downstream from PRRs. Transcriptome analysis of the ME following activation of PPRs further clarifies the nature and timing of ME inflammatory events, with a large number of PRRs and pro-inflammatory mediators rapidly up-regulated. Expression profiles also highlight the role of anti-inflammatory genes, which are activated in response to PRR activation with similar kinetics to that observed for pro-inflammatory mediators. These serve to blunt inflammation and prevent bystander injury to ME tissues. Inflammation also down-regulates tissue growth suppression genes in the ME, including the transmembrane oncogene ecrg4. ECRG4 protein is also enzymatically cleaved in response to inflammation, further eliminating growth suppression and releasing an extracellular fragment with growth-promoting activity. In addition, the fragment complexes with the TLR4/CD14/MD2 endotoxin receptor, forming another link between tissue growth and inflammation. The inflammatory pathways activated in cholesteatoma include up-regulation of TLRs, NLRs and their downstream signaling molecules. This includes TLR4, which has been linked to cholesteatoma pathogenesis. TLR4 functions not only as a receptor for bacteria that may disperse from cholesteatoma biofilms but also for DAMPs released from necrotic cells, such as S100A and HMGB1 both of which are up-regulated in cholesteatoma. Understanding the complex intracellular web that regulates ME inflammation provides potential targets for manipulation as pharmacological interventions. Supported by grants DC000129 and DC012595 from the US NIH/NIDCD.

Inflammation in the middle ear (ME) contributes to disease including cholesteatoma and otitis media. Activation of pattern recognition receptors (PRRs) by bacteria, viruses or damage-associated molecules patterns (DAMPs) activate PRRs, including Toll-like (TLR), NOD-like (NLR) and C-type lectin receptors These in turn activate pro-inflammatory signaling including the NFkB and JNK cascades, inducing pro-inflammatory cytokines, chemokines, and growth factors that contribute to pathogenesis.

Studies in gene deletion mice clarify the roles of various PRR signaling molecules in ME inflammation, while transcriptome analysis following PPR activation further reveals the nature and timing of ME inflammatory events, with a large number of PRRs and pro-inflammatory mediators rapidly up-regulated. Anti-inflammatory genes are activated with similar kinetics, to blunt inflammation and prevent bystander injury to ME tissues. Inflammation also down-regulates tissue growth suppression genes in the ME, including the transmembrane oncogene *ecrg4*. The ECRG4 protein is also enzymatically cleaved in response to inflammation, further eliminating growth suppression and releasing an extracellular fragment with growth-promoting activity. In addition, the fragment complexes with the TLR4/CD14/MD2 endotoxin receptor, forming another link between tissue growth and inflammation.

Inflammatory pathways in cholesteatoma include TLRs, including TLR4 which has been linked to cholesteatoma pathogenesis, NLRs and their downstream signaling molecules. TLR4 functions not only as a receptor for bacteria but also for DAMPs released from necrotic cells, such as S100A and HMGB1 both of which are up-regulated in cholesteatoma.

Understanding the complex intracellular web that regulates ME inflammation provides potential targets for manipulation as pharmacological interventions.

doi:10.1017/S0022215116004114

Genetics in Otology (R831)

ID: 831.1

Genetics of Cholesteatoma Project

Presenting Author: **Peter Prinsley**

Peter Prinsley[1], Barbara Jennings[2], Carl Philpott[2], Mahmoud Bhutta[3]
[1]James Paget University Hospital, [2]Norwich Medical School, [3]University College London

Learning Objectives: The support of BSO to identify affected families is sought.

Introduction: This research project seeks to identify genetic pathways predisposing to cholesteatoma. Familial clustering of cholesteatoma has been observed in East Anglia (Prinsley 2009). DNA sequencing has advanced so that whole exome sequencing of affected and unaffected individuals is now feasible.

Methods: A database of East Anglian families with cholesteatoma forms the core recruitment group for this study. However, the British Society of Otology (BSO) network could help identify other families. Pedigree charts and blood/saliva samples will be obtained from affected families for DNA extraction.

In the second stage, exome sequencing will be coupled to a linkage analysis in the families in which cholesteatoma is segregating. In conjunction with the pedigree mapping, we will have an opportunity to identify genetic polymorphisms predisposing to formation of cholesteatoma, and by using multiple affected families, to identify recurrent pathways or genes identified through this methodology.

Results: A research team of clinicians and scientists has been assembled and a systematic literature review has been carried out. Data extracted from the literature review will be used to identify pathways to focus on during the filtering steps to identify variants of interest that co- segregate with the disease phenotype. Funding has been secured from the Royal College of Surgeons of England and from the Rosetrees Foundation. The project will be adopted on to the NIHR Portfolio subject to Research Ethics Approval. The whole exome sequencing and analysis will be performed at The Genome Analysis Centre in Norwich.

Conclusions: A project has been created to identify genetic causes of cholesteatoma.

By selecting the right families, the project has potential to yield information that may widen our understanding of the disease pathophysiology.

doi:10.1017/S0022215116004126

Genetics in Otology (R831)

ID: 831.2

Gene expression profiling reveals expression of tumor-relevant

Presenting Author: **Johannes Greiner**

Johannes Greiner, Janine Müller, Jörg Ebmeyer, Holger Sudhoff

Klinikum Bielefeld Mitte

Learning Objectives: Cholesteatoma is a destructive, potentially life-threatening lesion of the middle ear Cholestatoma tissue expresses tumor markers SERPINB3 and SERPINB4 Oncogenes like Lipocalin 2 are upregulated in Cholestatoma tissue, while tumor suppressor gene are downregulated.

Introduction: Cholesteatoma is a gradually expanding destructive epithelial lesion within the middle ear, which leads to extensive tissue destruction in the temporal bone followed by conductive and sensorineural hearing loss and facial nerve palsy. To develop new treatment strategies, gaining further insights into the complex gene regulation and signaling underlying the formation and progression of cholesteatoma are mandatory.

Methods: Gene expression profiling of cholesteatomas and regular external auditory skin from 17 patients via full genome micro-arrays containing 19,596 human genes followed by validation using real time PCR analysis.

Results: Full genome micro-arrays showed significantly increased expression of 811 genes in cholesteatoma tissue compared to regular external auditory skin, while 334 were found to be downregulated. Next to matrix metalloproteases MMP9, MMP10 and MMP12, the anti-apoptotic genes BCL2L1 and A20 were upregulated in cholesteatoma tissue. Providing a further linkage to tumorigenic tissue, expression of the tumor markers SERPINB3 and SERPINB4 as well as the oncogene Lipocalin 2 was increased in cholesteatoma tissue in comparison to external auditory skin. Accordingly, downregulation of the cell adhesion molecule cadherin 18 as well as the tumor suppressor gene inhibitor ID4 was observed in cholesteatoma tissue. Linking the characteristic expression of tumor-relevant genes in cholesteatoma to inflammation, the inflammation-related calcium binding protein S100A7A was found to be highly upregulated.

Conclusions: The Expression profile of cholesteatoma was found to be similar to a tumorigenic and chronically inflamed tissue, giving new insights in the complex biology of cholesteatoma.

doi:10.1017/S0022215116004138

Genetics in Otology (R831)

ID: 831.3

Molecular pathology of cochlear gap junction in GJB2 associated hearing loss

Presenting Author: **Kazusaku Kamiya**

Kazusaku Kamiya, Ichiro Fukunaga, Katsuhisa Ikeda

Juntendo University Faculty of Medicine

Learning Objectives:

Introduction: Hereditary deafness affects about 1 in 2000 children and GJB2 gene mutation is most frequent cause for this disease. GJB2 encodes connexin (Cx) 26, a component in cochlear gap junction. We recently demonstrated that the drastic disruption of gap junction plaque (GJP) macromolecular complex composed of Cx26 and Cx30 are critical pathogenesis starting before hearing onset (Kamiya *et al.,* 2014, *J Clin Invest* 124, 1598–1607). To develop the effective therapy for GJB2 associated hearing loss, restoration of gap junction plaque (GJP) macromolecular complex using virus vectors or multipotent stem cells such as induced prulipotent stem (iPS) cells and mescenchimal stem cell (MSC) are expected to rescue the hearing function of GJB2 related hearing loss.