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.

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Genetics in Otology (R831)

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Gene expression profiling reveals expression of tumor-relevant

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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.

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Genetics in Otology (R831)

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Molecular pathology of cochlear gap junction in GJB2 associated hearing loss

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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.