Abstract: Introduction: Lipopolysaccharide (LPS) on the Non-typeable Haemophilus influenzae (NTHi) surface plays an important role in interaction of the bacterium with the host and a component of the molecule, phosphocholine (PCho), can affect survival and the ability to cause disease, including otitis media (OM). PCho can bind to Platelet Activating Factor receptor (PAFr) to promote adhesion and evasion of immune responses. C-reactive protein (CRP), an acute phase response molecule whose concentration can rise dramatically in response to inflammatory signals, binds PCho and can initiate bacterial killing. PCho expressed on NTHi can therefore influence the immune response elicited against these bacteria.

Objective: Study the interaction of CRP with NTHi to understand its role in bacterial survival.

Method: NTHi can efficiently infect the Junbo mouse, a characterised model of chronic and acute OM. Serum and middle ear fluid (MEF) were collected from Junbo and wild type (WT) mice inoculated with variant NTHi strains predominantly expressing PCho, not expressing PCho, or unable to express PCho (lic1 mutation). Infection was assessed by bacterial counts and immunoblotting and samples collected from mice at 3 or 7 days post-inoculation. CRP concentrations in MEF and serum samples from mice were examined by ELISA. Binding of CRP to different NTHi strains and their PCho expressing variants and the biological consequences of this interaction were investigated.
Results: CRP was detected at similar levels in MEF samples from Junbo mice infected with each WT NTHi strain and PCho-expressing variant tested; the only notable difference being for the lic1 mutant of strain 375. Higher but comparable levels of CRP were detected in the serum from both Junbo and WT mice infected with each NTHi strain. Control Junbo mice inoculated with PBS showed a baseline level of CRP in MEF that was significantly higher than that observed in mice inoculated with NTHi strains, suggesting that CRP may be being bound to infecting bacteria in vivo. Immunoblotting confirmed the selection of PCho expressing NTHi variants following infection in the Junbo mouse but lic1 mutants of NTHi strains that do not express PCho showed no significant difference in middle ear infection levels compared to WT strains.

Conclusion: PCho expressed on NTHi LPS and its interaction with CRP is one aspect of host-microbial interaction that can contribute to NTHi survival in the normal human host and infection of the middle ear in a surrogate mouse model of OM. Many factors including host cell signalling and the presence of a plethora of other immune molecules can influence this interaction. The interaction of CRP with NTHi is currently under investigation to help advance our understanding of its role in the complex biological processes that influence bacterial killing and the onset and progression of OM caused by NTHi.
Poster # 2

Presenting Author: Michael Binks PhD

Institution(s): Menzies School of Health Research

Title: Otitis Media Hospitalisation in the Northern Territory of Australia: A Decade of Observation (2006-2015)

Abstract: In the Northern Territory (NT) of Australia rates of childhood pneumonia (22% hospitalised in first year), otitis media (OM; 90% at age 18 months) and chronic suppurative lung disease (CSLD; 1 in 68 in Central Australia) are among the world’s highest. Streptococcus pneumoniae and Haemophilus influenzae are leading aetiological pathogens. We describe childhood OM coded hospitalisations in the NT over a 10-year period.

Study design and population: A historical cohort study of NT Indigenous infants born between 1st Jan 2006 – 31st Dec 2015 and followed until age 12 months. Three pneumococcal conjugate vaccines were used in the NT over this period: PCV7 (2006 – 2009); PCV10 (2009 – 2011); and PCV13 (2011 – 2015). Otitis media coded hospitalisations (ICD-10AM H65-H67.8 and H72-H72.9) were identified from the NT Government Hospital Inpatient Activity dataset (ICD-10AM codes: Z38-Z38.8). OM hospitalisation rates (episodes per 100 child-years) were calculated overall, annually, and by PCV era.

Results: The 14,594 Indigenous children born during the study period presented for a total of 13,307 subsequent hospitalisation episodes in their first year of life. Fifty one percent (n=7490) were male, 70% (n=10,175) lived in remote communities and 32% (n=4675) were from the Central desert region. Of the 13,307 episodes, 957 (7%) were coded episodes of OM for 825 infants (6%); 79 (0.6%) had two episodes and 20 (0.15%) had three or more episodes. On 650 occasions (4% overall, 68% of OM episodes) OM was a leading diagnosis (coded 1st, 2nd or 3rd). The frequency of first OM coded hospitalisation peaked at 5 months and remained stable until 12 months. By region, the proportions of children with an OM coded hospitalisation was: remote Central Australia (10%), urban Central Australia (5.5%), remote Top End (5.5%), urban Top End (1.5%). There was no significant trend over calendar time. Over 80% of infants received three or more timely PCV doses before age 12 months and this was consistent over time. Compared to the PCV7 era (7.8 episodes per 100 child-years), OM rates were marginally yet significantly lower in both the PCV10 (6.5 episodes per 100 child-years; IRR 0.84, 95%CI 0.70 to 0.99) and PCV13 eras (6.5 episodes per 100 child-years; IRR 0.83, 95%CI 0.72 to 0.96). These differences were no longer significant when analysis was confined to episodes where OM was a leading diagnosis.

Discussion: One in 20 Indigenous infants in the NT of Australia were hospitalised and coded with a diagnosis of OM annually. These rates remained largely unchanged though the decade 2006-2015. While the majority (70%) of OM coded episodes were leading diagnoses, further investigation of comorbidities is necessary to clarify the clinical significance of the OM. Lower OM coded hospitalisation rates during eras of PCV10 and PCV13 vaccination, compared to the PCV7 era, might suggest a vaccine effect.
Poster # 3

Presenting Author: Oak-Sung Choo

Institution(s): Ajou University School of Medicine

Title: Surgical Outcomes of Ossiculoplasty Following Tympanomastoidectomy and the Comparison with Polycel® and Titanium Prostheses

Abstract: Introduction: Polycel® or titanium materials are widely used as prostheses for ossicular reconstruction. In this study, we reviewed the surgical outcomes of ossiculoplasty following tympano-mastoidectomy, and compared the results according to the materials for prosthesis.

Materials and methods: Only patients with continuous large air-bone gaps after mastoidectomy (± ossiculoplasty) were included in the study. From January 2005 to December 2017, 221 patients received staged ossicular reconstruction after mastoidectomy by a single surgeon. Either Polycel® or titanium was used during the operation. Demographic data of the patients were collected retrospectively, and changes in hearing status before mastoidectomy, after mastoidectomy, and after staged ossiculoplasty were measured by pure tone audiometry and speech audiometry results. Multivariate analysis was also conducted to compare the effects of prosthesis materials.

Results: Titanium prostheses also showed better surgical outcomes in partial ossicular reconstruction (PORP) (35.9% in titanium and 13.0% in Polycel®, p=0.005). Both Polycel® and titanium prostheses represented no difference in total ossicular reconstruction (TORP). In multivariate analysis, the titanium material showed better effect than Polycel® (OR 6.313, 95% confidence interval 2.227 – 17.897) in PORP. Older age and CWD mastoidectomy showed negative effects on the successful rate in PORP. In TORP, staged ossiculoplasty demonstrated better outcomes in multivariate analysis (17.259, 1.921 – 155.045).

Conclusions: If considering PORP as ossiculoplasty after tympano-mastoidectomy, titanium prosthesis can be recommended. If stapes head was missed at the first tympanomastoidectomy, consider staged ossiculoplasty with TORP. Graft material itself may not that important in TORP.
Poster # 4

Presenting Author: Oak-Sung Choo

Institution(s): Ajou University School of Medicine

Title: The Effectiveness of Ventilation Tube Insertion in Cleft Palate Patients

Abstract: Objective: Hearing loss is a common complication in children with cleft palate (CP) due to recurrent otitis media with effusion affecting their hearing abilities. The increased risk for this complication is caused by Eustachian tube dysfunction. Thus, studies have suggested ventilation tube insertion (VTI) for CP patients. The purpose of this study was to identify the proper management of the middle ear in patients with CP to prevent hearing impairment and maintain sufficient middle ear space until the development of adequate E-tube function.

Method: VTI was done simultaneously with CP surgery in 82 patients in Ajou University Medical center between 2005 and 2015. Postoperative follow up loss of 30 patients were excluded. A retrospective review of otology database (otoscopic examination, hearing evaluation, CP type, type of inserted ventilation tube (VT), post-operative complications, and prognosis) was performed.

Results: A total of 52 patients (103 ears) were enrolled in the study. After VTI, complication was noted in 9 cases (8.7%) and recurrence in 29 cases (28.2%). The cases were divided into 3 groups according to the type of CP; submucosal (mild), incomplete (moderate), and complete (severe). Both the submucosal and complete CP group showed no statistical significance in complication or recurrence between the two different types of VT. In the incomplete CP group the recurrence rate was significantly higher in Type I VTI (39.0%) (p=0.017), and the complication rate was significantly higher in Type II VTI (23.3%) (p=0.021).

Conclusion: We concluded that for the submucosal CP with palatoplasty alone and complete CP with a combination of palatoplasty and alveoloplasty, Type I VTI seems to be the treatment of choice. In cases of incomplete CP (moderate type) with palatoplasty alone, Type II VT may be a better option. In conclusion, during VTI for CP patients, consideration of the severity of CP and the type of CP surgery is important in regards to good prognosis and minimal complication.
Poster # 5

Presenting Author: Harvey Coates DM

Institution(s): UNIVERSITY OF WESTERN AUSTRALIA

Title: Ear and Hearing Outcomes from an Ear Bus Program in Western Australia.

Abstract: Introduction. Aboriginal and Torres Strait Islander (ATSI) children have the worst ear health of any community in the world. Chronic Suppurative Otitis Media (CSOM) rates are up to 70% in some communities and on average children experience 32 months of otitis media in their first five years compared to three months in non-ATSI children. These high rates of middle ear disease and associated hearing loss significantly impact the health and education outcomes of these children. The Earbus Foundation of Western Australia is a children's charity that aims to reduce the incidence and impact of otitis media in ATSI and at-risk children in Western Australia. It operates in rural and remote Western Australia bringing together experts from education, health, culture and communities.

Objectives. To document the efficacy of an intensive ear health program delivered remotely by a mobile multidisciplinary team.

Methods. ATSI children are reviewed by a nurse audiometrist, audiologist and community Aboriginal health worker. If necessary, children will have a targeted medical review by a nurse practitioner or general practitioner. Those children with middle ear pathology are seen by an otolaryngologist or have 'store and send' video otoscopic images transmitted to the otolaryngologist together with a brief history and audiological findings. Medical treatment particularly for children with CSOM includes intensive local treatment and referral for surgical treatment when necessary.

Results. Ear and hearing outcomes data of the children seen was collated over a 12 month period. Of 760 patients seen in the Pilbara region there was a reduction of 50% in hearing loss and otitis media referral rate. The prevalence of otitis media with effusion (OME) and CSOM was reduced by 66%. 1200 children seen in the Goldfields region had a 75% reduction in the otitis media referral rate. Hearing loss and the prevalence of OME and CSOM was reduced by 66%.

Conclusion. Intensive ear health management in rural and remote communities by utilisation of an ear bus with a multidisciplinary team resulted in a significant decrease in hearing loss and middle ear disease in ATSI children.
**Poster # 6**

**Presenting Author:** Joshua Earl PhD

**Institution(s):** Drexel University

**Title:** Machine Learning Approaches to Predict Haemophilus influenzae Associated Host Disease State, Host Tissue, and Gene “Dark Matter”

**Abstract:**

Introduction: The characterization of genetic mechanisms of bacterial virulence remain elusive, particularly in species that have diverse phenotype presentation. For example, Non-typeable Haemophilus influenzae (NTHi) is a bacterium implicated in Otitis media and other disease states but can also colonize healthy human hosts. It is also isolated from various biogeographical locations, indicating an ability to successfully adapt to different environments, and present a variety of virulence phenotypes.

Objective: Use the annotated genes from 993 whole genome sequenced (WGS) genomes of NTHi to predict host disease, and body site provenance using two machine learning algorithms. Explore genes both identified as important predictors and annotated as ‘hypothetical’ using deep learning neural networks to apply gene ontology (GO) terms.

Methods: WGS was performed on 993 genomes using multiple different sequencing platforms, though primarily using Nextseq 500 Illumina sequencing. Genomes were assembled using sequencer-appropriate software and automatic gene annotation was performed using Prokka. Pan-genome gene cluster analysis on all strains was performed with Roary. A matrix of 4,207 gene clusters was used as ‘features’ to predict strain isolates from host site, and disease state. Two algorithms used for class prediction were random forest (RF) and neural network (NN). All protein coding gene amino acid sequences were then examined with a deep learning neural network trained on the entire database of Uniprot to apply GO terms to all gene sequences.

Results: Both Random Forests and Neural Networks performed significantly better than the ‘No Information Criteria’ in predicting either body site and disease state, though RF outperformed NN in all cases. Parameter tuning significantly improved RF prediction capability, with a maximum accuracy rate of 85% predicting the disease state of the patient. Initial results on a test group of strains (Moraxella catarrhalis) of the deep learning algorithm provided GO terms with 80% confidence to ~14% of ‘hypothetical’ gene annotations.

Conclusion: We show that gene presence in NTHi strains provides adequate information to predict both ecological niches and associated disease state, up to an accuracy of 85%. Deep learning is shown to be a capable curative step in annotating genes of unknown function, applying GO terms to these annotations with a high degree of confidence. Further work is warranted both to explore additional methods and feature selection.
**Poster # 7**

**Presenting Author:** Mana Espahbodi

**Institution(s):** Medical College of Wisconsin

**Title:** Development and Characterization of Middle Ear Cell Lines for the Study of Otitis Media

**Abstract:** Objective: In vitro cell culture systems are essential for studying the cell and molecular biology of the middle ear (ME) and the pathogenesis of otitis media (OM). Currently, the single human ME epithelial cell line available (HMEEC-1) was established from an adult cochlear implant (CI) patient from whom the phenotypic and genotypic characteristics are unknown. Significant differences exist in innate immunity and ME effusion composition of adults and children. Further, sex and race/ethnicity are risk factors for OM, however studies have not been performed to assess the contribution of intrinsic molecular properties of ME epithelial cells to OM proneness. The objective of this study was to develop ME epithelial cell lines from adult and pediatric CI patients and patients with recurrent OM (ROM) and OM with effusion (OME) of different sex and race to provide tools for comparative analyses of molecular expression and cytokine-responsive signaling relevant to the pathophysiology of OM.

Method: 2mm pinch biopsies were obtained from CI, ROM and OME patients. Blood was obtained for genetic analyses. Effusions and audiometric data were obtained from OM patients. Primary cells established from biopsy were immortalized via lentiviral infection. Cell lines were characterized by karyotype analyses and soft agar assay. Cytokine and mucin expression profile were assessed via qPCR in cells stimulated with pro-inflammatory cytokines and lysate from bacteria involved in OM.

Results: Primary and immortalized cell lines were developed. Soft agar assay demonstrated normal non-tumorigenic phenotype. Preliminary analyses demonstrated differences in basal mucin gene expression between cell lines with further analyses ongoing.

Conclusion: The cell lines developed herein represent the first human ME epithelial cell lines from children with clinical and histopathological confirmation of OME and ROM and the first to permit comparison of intrinsic epithelial cell-based properties across patients of varying disease severity and differing sex and race. These analyses promise to inform our understanding of not only OM, but other conditions impacting the ME and respiratory mucosa in children, and impact interpretation of research findings and their applicability across sex and races with important implications for development of preventative and therapeutic interventions.
Poster # 8

Presenting Author: Marie Gisselsson-Solen MD, PhD

Institution(s): Otorhinolaryngology, Head and Neck Surgery, Lund University Hospital, Lund, Sweden

Title: Quality of life in Swedish children receiving grommets – an analysis of pre- and postoperative results based on a national quality register

Abstract: Background: Otitis media with effusion (OME) and recurrent otitis media (rAOM) are two common diagnoses in childhood, both of which are treated with grommets, or ventilation tubes. It is known that affected children have a worse quality of life (QoL), and various questionnaires have been used to evaluate this. The national Swedish quality register for grommet insertions contains some QoL questions that have hitherto never been analysed.

Methods: Data from 2010-2016 was extracted from the register and analysed with regards to QoL questions, reasons for surgery, hearing levels and number of AOM episodes.

Results: Preoperative QoL data was available for 3835 children. Before surgery, most parents felt that the QoL of their children was negatively affected by the ear disease. Parents of children with OME were more likely to suspect that their child had a hearing loss (ORs 10.1 and 28.2 for suspecting a mild and severe hearing loss, respectively), but less likely to find that the ear disease affected the child’s general wellbeing than did parents of children with rAOM (ORs 0.54 and 0.33 for somewhat and much affected, respectively). Many children underwent surgery despite not fulfilling the criteria for surgery as stipulated in the national guidelines. Those who did fulfil criteria, however, had a more severely affected QoL. A significant improvement was seen in individual QoL scores after surgery (p
Abstract: Background:

In 2018, Otologic Society of Japan, Japan Society for Pediatric Otorhinolaryngology, and Japan Society for Infectious Diseases in Otolaryngology updated "Clinical Guideline for Acute Otitis Media in Children in Japan." The first edition of this guideline was issued in 2006, and this latest edition is the 4th one. The most obvious difference from AAP Guideline might be the treatment options prepared for treatment failures caused by BLNAR (beta-lactamase non-producing ampicillin resistant Haemophilus influenzae). This strategy comes from the high incidence of BLNAR in Japan.

Methods. Treatment outcome of acute otitis media (AOM) in children was assessed when treated with the Guideline. Twelve hospitals in Hokkaido, Japan participated in this study between May and September 2006. Patients were given a diagnosis and treated according to the guideline. Swab samples were obtained from nasopharynx at their first visits to detect pathogens. Patients were requested to visit the clinic 3 days, a week, 2 weeks, and 4 weeks after their first visits.

Results. A hundred forty-six patients with AOM were analyzed on the initial stages, initial scores of TM, isolated bacteria, and treatment information including antibiotics and myringotomy they underwent. Twenty-five patients dropped out of the study, therefore 121 patients were followed until their TM scores reached zero. A hundred ninety-eight bacteria were isolated from 133 patients. Fifty-seven percent of S. pneumoniae were penicillin resistant and 59% of H. influenzae were β-lactamase non-producing ampicillin resistant strains. A hundred twenty-two of 146 patients (84%) underwent antibiotic therapy recommended in the guideline. Sixty-three of 76 recommended cases (83%) underwent myringotomy. Eleven cases (9%) still had inflammatory changes in their TM scores even a week after their first visits. However, 8 of these 11 cases achieved TM score 0 in 2 weeks, and so did the others in 4 weeks after their first visits. Moreover, their symptom scores reached zero in a week for all patients.

Conclusions. Improvement of symptoms and TM findings was satisfactory when patients with AOM in children were treated with the guideline. This guideline would be expected to play an important role to spread the appropriate use of antibiotics in this country.

In 2016, the National Action Plan on Antimicrobial Resistance (AMR) was developed by the Ministerial Meeting in Japan, following endorsement of the Global Action Plan on AMR by the World Health Assembly in 2015. This Action Plan sets a goal of reducing penicillin resistant strains to 15% of all pneumococci by 2020.
A recent increase in multidrug-resistant pathogens in Japan is considered due to inappropriate use of antibiotics, especially overuse of cephalosporins.

The national action plan includes the recommendation on appropriate use of antimicrobial agents. That is summarized as follows: “limit the use to

This new guideline recommends that pediatric patients with acute otitis media are classified into three groups that is mild, moderate, or severe based on the scores for their clinical symptoms and their tympanic membrane (TM) findings. Each group has its own treatment course including not only antibiotic choice but also application of myringotomy.

Objectives. Our goal of this study is to clarify how appropriate this guideline is and whether it has any difficulties in use for daily clinical practice.
Poster # 10

Presenting Author: Takashi Hirano MD,PhD

Institution(s): Oita University, Faculty of Medicine, Department of Otolaryngology

Title: Effect of middle ear inflammation on progression of middle ear cholesteatoma

Abstract: Objective: Middle ear cholesteatoma is characterized by a mass lesion formed by keratinizing squamous epithelium, keratin debris with or without inflammatory reaction. It is known that some cytokines, including interleukin(IL)-17, tumor necrosis factor alpha (TNF-α), induces abnormally excessive growth of the epidermal layer of the skin. In this study, we examined if the inflammatory responses in the middle ear affect progression of cholesteatoma according to classification and staging system in Japan.

Methods: We used 16 granulomas from the middle ear. When we performed tympanoplasty for 16 cholesteatoma cases, we excised samples surgically. Frozen sections were made for HE staining and immunohistochemistry for IL-17 and TNF-α. The number of IL-17 and TNF-α positive cells in immunostaining were classified into three grades, from 1 (low), 2 (moderate) to 3 (high). RNA was also extracted from the granulomas, and IL-6, IL-17A, IL-17F, TNF-α-mRNA expressions in the granulomas were investigated by real-time RT-PCR. We analyzed the relation of the level of these cytokine to classification and staging system in Japan.

Results: The grades of IL-17 staining had positive correlation with the degree of progress of cholesteatoma, and the grade of TNF-α staining had negative correlation with the degree of progress of cholesteatoma. However, there was no statistical difference between the grade of staining and progression of cholesteatoma. In the relationship between the -ΔCT value of each target gene and the degree of progress cholesteatoma, Although there was no statistically significant difference in IL-17A, IL-17F mRNA expression, weak positive correlation was observed. However, significant difference was observed at a significance level of 5% for Spearman’s correlation coefficient in IL-6 mRNA expression.

Conclusion: In this study, IL-17 and TNF-α did not show the evidence to facilitate progression of cholesteatoma in the middle ear. Although IL-6 has been reported to be inflammatory cytokines, it is reported that IL-6 suppress the terminal differentiation of human keratinocytes. IL-6 may be related to inhibition of the expansion of cholesteatoma.
**Poster # 11**

**Presenting Author:** Preben Homøe

**Institution(s):** Zealand University Hospital

**Title:** Parent Satisfaction, Adherence to Guideline and Symptom Relief in Children with Otitis Media undergoing Tympanostomy Tube insertion - a Prospective Multicentre Study

**Abstract:** Objective: This study investigates parent satisfaction, adherence to guideline, and symptom relief in children younger than 12 years undergoing tympanostomy tube (TT) insertion for otitis media (OM) using electronic patient-reported outcome (ePRO) data in private ear-nose & throat (ENT) practice settings.

Methods: A total of 3,572 children aged 0-11 years and registered in the DØNHO database were included. Following parental consent to participate, the Danish National Tympanostomy tube Insertion Questionnaire’s (DANTIQ) consisting of a pre-surgical questionnaire and follow-up questionnaires at 1, 3, 6, 9 and 12 months after surgery were e-mailed to the parents. The pre-operative questionnaire collected information on symptom duration, number of acute OM (AOM) episodes within one year before TT insertion and ear-related symptoms. The post-operative questionnaires collected information on symptom relief, number of AOM episodes and parental satisfaction. Criteria for adherence to guideline were 1) Symptom duration of three months or longer (COME), 2) three or more AOM episodes within six months and 3) four or more AOM episodes within 12 months (RAOM).

Results: Pre- and post-operative questionnaires from 2,462 children were eligible for complete analysis. Before surgery, 89.8% of parents reported a symptom duration of three months or longer and/or recurrent AOM (RAOM) in accordance with the Danish National Guideline. Complete symptom regression was reported in more than half of the children post-operatively. For the rest, significant symptom relief was reported 1-12 months following TT insertion. Parent satisfaction rose from 94.6% to 97.2% throughout the observation period.

Conclusions: We report a high degree of adherence to guidelines, a consistently high rate of symptom relief 1-12 months following TT insertion in Danish children below 12 years of age. Furthermore, parental satisfaction throughout the 12-month observation period was compelling.
Abstract: Introduction: Otitis media (OM) is one of the most common childhood diseases. It is the leading cause of pediatrician visits and antibiotic prescription for children and a significant burden to public health care. Streptococcus pneumoniae (Spn) is one of the primary bacterial pathogens of OM. Pneumococcal conjugated vaccines were introduced into childhood vaccination schedules in the USA in 2000, however immunization attempts have been thwarted by replacement of prevalent pneumococcal serotypes by those not included in the vaccines. Identification of the prevalent pneumococcal serotypes in the OM population will provide information crucial to understanding the epidemiology of OM and development of improved pneumococcal vaccines.

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Methods: A single adenoid swab was collected from each OM patient during tympanostomy tube placement surgery. DNA was extracted from swabs and pneumococcal standard serotype strains. Spn-positive specimens were identified by PCR amplification of pneumococcal 16sRNA and lytA genes, or lytA andcpsA genes. Pneumococcal serotypes in Spn-positive specimens were identified by sequential multiplex PCR using specific primers for genes encoding capsular pneumococcal serogroup/types.

Results: Overall, 41.5% of specimens/cases were pneumococcal-positive. Among the positive cases, at least 14 serotypes were identified. Serotypes included in vaccines, specifically serotypes 4, 5, 6A/B, 19F and 23F, were observed in 58.8% of pneumococcal-positive cases. Serotypes uncovered by vaccines, specifically serotypes 9NL, 10F/33C, 15A/B/C/F, 22A/F, 23B, 24F/A/B, 35A/C/42 and 35B, were observed in 47.1% of pneumococcal-positive cases; none was more predominant than the others. Multiple serotypes were observed in 35.3% of pneumococcal-positive cases.

Conclusions: Although pneumococcal serotypes uncovered by vaccines were detected in OM patients, vaccine-covered serotypes comprised the predominant serotypes in the upper
respiratory tract of OM patients in the Greater Milwaukee Area of Wisconsin. Colonization with multiple pneumococcal serotypes was also common. Further investigation is warranted to assess pneumococcal colonization in a larger population of OM patients relative to healthy control counterparts to aid in evaluating the efficacy of vaccination.
Poster # 13

Presenting Author: Saskia Hullegie MD

Institution(s): Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht

Title: Global Prevalence of Antibiotic Resistance of Bacteria in the Middle Ear from Children with Acute Otitis Media and Ear Discharge: a Systematic Review.

Abstract: Introduction

Around 15%-20% of children with acute otitis media (AOM) present with ear discharge due to a spontaneous perforation of the tympanic membrane (AOMd). A systematic and current overview of the global prevalence of otopathogens and their AMR profiles in children with AOMd is, however, lacking.

Objective: To estimate the global prevalence and the bacterial resistance rates of otopathogens in children with AOMd, and to investigate whether these rates vary over time.

Methods: Systematic searches of PubMed, EMBASE, and the Cochrane Library were performed from inception to June 20, 2018 using a broad search strategy. Two reviewers independently screened titles and abstracts of unique records for eligibility using pre-specified criteria. Next, the same reviewers independently reviewed full texts of potentially relevant articles for inclusion. Non-English studies, animal studies and those from which the full text could not be retrieved were excluded. All studies reporting any prevalence or AMR data of middle ear isolates from children with AOMd were included.

Risk of bias of included studies was assessed by the Joanna Briggs Institute Critical Appraisal quality assessment checklist (for observational studies).

Overall prevalence and AMR rates with 95% confidence intervals of middle ear isolates (S. pneumoniae, nontypeable H. Influenzae, M. Catarrhalis, S. Aureus, P. Aeruginosa, S. Pyogenes) to most commonly prescribed antibiotics will be presented according to the year(s) in which the study was conducted.

Results: The electronic database searches yielded a total of 6,871 records. Removing duplicates left 3,767 unique records. After title/abstract screening, 272 potentially relevant articles remained for full text screening of which 22 were suitable for inclusion. Data extraction is currently ongoing and results will be presented at the conference.

Conclusion: This systematic review will provide a current overview of otopathogens and their AMR profiles in children with AOMd.

Registration PROSPERO: CRD42018100523
Presenting Author: Donggu Hur MD

Institution(s): Gyeongsang National University Changwon Hospital

Title: Otitis Media Without Macrophages

Abstract: Objective: Macrophages are thought to play an important role in otitis media (OM). These phagocytic cells, when classically activated, are important sources of pro-inflammatory mediators and chemotactic factors that attract other leukocyte classes. When alternatively activated into an M2 phenotype, they are anti-inflammatory and can play a major role in the resolution of inflammation. However, given the complex cellular landscape of the middle ear (ME) and OM it is difficult to separate the contributions of a single cell type.

Method: To evaluate the role of macrophages in OM we induced OM in a mouse deficient in CCR2 (CCR2−/−), the major receptor for chemoattraction in macrophages, by intrabullar injection of nontypeable Haemophilus influenzae (NTHi). Some of these mice were also treated intravenously with liposomes containing clodronate (CCR2−/−/clodronate), which induces apoptosis in peripheral blood macrophages, at two day intervals before and after NTHi inoculation. Histological analysis and immunohistochemistry was used to evaluate the MEs of experimental and control (wild-type, inoculated with NTHi, WT; CCR2 KO saline liposome, CCR2−/−/saline) mice for OM.

Results: Mucosal hyperplasia and the number of leukocytes present in the ME were similar across all three groups of mice over the first few days of OM, although CCR2−/−mice showed more initial mucosal growth. However, leukocyte infiltration was significantly greater at 7 days after inoculation in CCR2−/−/clodronate mice. The number of macrophages in the MEs on day 2 after NTHi inoculation was less than 50% of that seen in WT animals in CCR2−/−mice, and only 10% in CCR2−/−/clodronate MEs, with only slight recovery of macrophages by day 3. On day 7, WT mice had only a few macrophages in the ME, while macrophages persisted in CCR2−/− and CCR2−/−/clodronate mice. Numbers of ME neutrophils in the ME, always much higher than the number of macrophages, were higher by more than two-fold in CCR2−/−/clodronate mice compared to WT, and 8-fold higher at day 3. While they were absent at day 7 in WT, they persisted in CCR2−/− and CCR2−/−/clodronate MEs. Finally, while WT mice cleared NTHi by day 7, some CCR2−/− and CCR2−/−/saline, and most CCR2−/−/clodronate MEs were culture-positive at that time.

Conclusion: The results suggest that macrophages play a very significant role in OM, despite the fact that they are typically present in small numbers than neutrophils. They appear to inhibit neutrophil entry into the ME cavity, and to promote the resolution of ME infection and inflammation. Supported by grants DC000129; DC012595 and DC014801 from the NIH/NIDCD and the Veterans Administration.
Poster # 15

Presenting Author: Simon Jespersen

Institution(s): University of Copenhagen

Title: Acute Otitis Media and Pneumococcal Vaccination – An Observational Cross-sectional Study of Otitis Media among Vaccinated and Unvaccinated Children in Greenland

Abstract: Introduction: Streptococcus Pneumoniae (s. pneumoniae) is one of the main pathogens leading to otitis media (OM). In 2010 the 13-valent pneumococcal conjugate vaccine (PCV13) was implemented in the Greenlandic children vaccination programme, but the effect of this change is not yet well documented. The objective of this study is to evaluate the effect of the implementation based on the number of episodes of acute otitis media (AOM).

Methods: Data will be obtained from Greenlandic medical journals. The PCV13 vaccine is given in three doses, when the child is 3, 5 and 12 months old. We will include all children born from January 2015 to December 2016, thus eligible for the three doses of PCV13 including one year of follow-up time. Exclusion criteria will be uncertain vaccination status and predefined comorbidities. The children will be divided into two groups based on vaccination status defined as “Completed vaccination programme”: having received all three doses of PCV13 within the age of 15 months; and “Not vaccinated/uncompleted vaccination programme”: not having received all three doses of PCV13 within the age of 15 months and not fulfilling any exclusion criteria.

Results: In total, we will include 1635 children. From the medical journals we register the following: gender, vaccination dates, the number of AOM-episodes, full pregnancy vs. premature birth, living in a town vs. a settlement and caesarean section vs. vaginal birth. The potential absolute risk reduction and relative risk reduction will be calculated.

Conclusion: Will be presented at the meeting according to the results.
Poster # 16

Presenting Author: Sho Kanzaki

Institution(s): Keio University School of Medicine

Title: Changes of Osteoclasts in ossicles of mouse due to injection of lipopolysaccharide

Abstract: Aim: Repeated otitis media to cause tympanic membrane perforation and ossicular bone erosions, resulting in conductive hearing loss. However, there are few reports on the mechanism of auditory ossicular lesions. We believe that elucidation leads to prevention and treatment.

The aim of this study is that the number of osteoclasts Induced by LPS increase in ossicles and then ossicles are eroded and or fixed.

Method: Lipopolysaccharide (LPS) was injected in the right ear of the mouse and saline was administered to the left ear. We compared the induced amount of osteoclasts in the ossicles on both sides stained by tartaric acid resistant acidic phosphatase, a marker of osteoclasts at one week after injections.

Results: The number of osteoclasts increased significantly in ossicles respectively in LPS-administered ears compared to control ears (p,0.05).

In the middle ear model, osteoclasts involved in ossicular lesions.

Therapeutic drugs such as osteoporosis drugs have been developed and can reduce osteoclasts in ossicular lesions of otitis media and can prevent conductive hearing loss.
Poster #17

Presenting Author: Pawjai Khampang MS

Institution(s): Medical College of Wisconsin

Title: Antibiotic Modulation of Gel-Forming Mucins in Experimental Otitis Media

Abstract: Introduction: Increased mucin expression in Otitis media (OM) is activated by bacteria and proinflammatory cytokines. The prevalence of bacterial biofilm influences inflammation and resolution of OM and may contribute to prolonged mucin production. Specific pathogen influence on mucin expression and development of chronic OM with effusion (OME) remains an area of significant knowledge deficit.

Objective: This study is to assess the relationship between gel-forming mucin (GFM) expression, specific pathogens, middle ear mucosal (MEM) changes, biofilm formation and antibiotic utilization.

Methods: Mixed gender chinchillas were inoculated with nontypeable Haemophilus influenzae (NTHi) strain 86028NP or Streptococcus pneumoniae (SP) strain TIGR4 via transbulla injection. Antibiotics was administered on day 3 to day 5 post inoculation. Mucin expression were measured by quantitative PCR on day 6, day 10 and day 17. Gross biofilm formation was recorded. Inflammatory cell infiltration and middle ear mucosal thickness were histologically measured.

Results: SP infection resulted in higher mortality rate, incidence of biofilm formation and ME effusion compared with NTHi infection. However, NTHi persisted in the middle ear longer than SP with no substantive bacterial clearance detected on day 10 in any NTHi-infected chinchilla compared with 100% clearance on day 10 for half of the SP-infected chinchillas. The infection increased middle ear mucosal (MEM) thickening and inflammatory cell infiltration. NTHi upregulated the mucin genes Muc5AC, Muc5B, and Muc19 on day 10 compared to controls (p = 0.0004, 0.003, and 0.002, respectively). With SP infection, GFMs were upregulated, however, not at statistically significant level. In both NTHi and SP infections, the degree of GFM upregulation has a direct relationship to increased MEM hypertrophy, inflammatory cell infiltration and the presence of biofilm. Antibiotic treatment reduced the incidence of ME effusion and biofilm formation, limited the MEM changes and reversed the GFM upregulation. In NTHi infection, the rate of returning to baseline level of GFMs in treated chinchillas was quicker than those without antibiotic treatment.

Conclusion: Mucin gene expression in an animal OM model is upregulated in conjunction with MEM hypertrophy and biofilm formation. This upregulation is less robust and more quickly ameliorated with appropriate antibiotic therapy in comparison to animals not treated. These findings contribute to the understanding of pathogen specific influences on mucin expression during the development of OM and provide new data which may have implications in how clinicians approach the treatment of OM.
**Poster # 18**

**Presenting Author:** Mathilde Kjeldsen MD

**Institution(s):** Dept. of Otorhinolaryngology and Maxillofacial Surgery, Zealand University Hospital, Lykkebækvej 1, 4600 Køge, Denmark

**Title:** Eradication of Biofilms on Tympanostomy Tubes with Acetic Acid Treatment; An In Vitro Study

**Abstract:**

**Introduction:** Bacterial biofilm is believed to play a part in posttympanostomy tube otorrhea. Biofilm is known for its increased tolerance to antibiotics. Acetic acid has shown promising antibiofilm effect.

**Objective:** To evaluate the eradicative effect of acetic acid on biofilm grown on sterile tympanostomy tubes by in vitro studies.

**Methods:** Biofilms of Pseudomonas aeruginosa and Staphylococcus aureus were grown on sterile tympanostomy tubes for 24 hours. The tubes were treated with acetic acid solutions at various concentrations for 24 hours. Main outcome was viability of bacteria after treatment. Presence of consistently attached biofilm was examined on selected tympanostomy tubes with confocal laser scanning microscopy. Both pH-adjusted and non-pH-adjusted media solutions were applied as control groups.

**Results:** Complete eradication of P. aeruginosa biofilm was obtained with 0.50 % v/v acetic acid. Biofilm of S. aureus was eradicated with 1.25 % v/v acetic acid. Low pH value alone led to decreased growth of already established biofilm but not eradication.

**Conclusion:** Acetic acid showed an eradicating effect on biofilm established on sterile tympanostomy tubes in vitro.
Poster # 19

Presenting Author: Daniel Kohane MD PhD

Institution(s): Boston Children’s Hospital

Title: Trans-Tympanic Delivery of Local Anesthetics for Pain in Acute Otitis Media

Abstract: Objective: Acute otitis media (AOM) commonly causes pain and distress in children. Up to 80% of children with AOM have mild to severe pain during the onset of the infection, of which about 40% have severe pain. Consequently, many AOM guidelines recommend the use of analgesics as an essential part of the treatment. The effectiveness of commercial ototopical products in AOM is also questionable. Nonetheless, local topical pain treatment remains desirable since side effects from systemic drug distribution would be avoided, the pain relief could be faster in onset, be more intense, and last longer than with oral analgesia. Therefore, we aim to develop a local drug delivery system to provide sustained pain relief in patients with AOM.

Method: We hypothesized that the lack of efficient analgesic effects from ototopical drops was due to inability to penetrate the tympanic membrane (TM). The outermost layer in the TM, the stratum corneum, is impermeable to virtually all molecules except the small and moderately hydrophobic ones. Thus, we developed a formulation combining CPEs and known anesthetics to enhance drug flux into and across an intact TM, and achieve effective analgesia for AOM. The CPEs and anesthetics were delivered in a hydrogel-based formulation. It is an easy-to-apply liquid at room temperature, and gels quickly and firmly upon contacting the warm TM, holding the anesthetics and CPEs in place (i.e. on the TM). The sustained release and diffusion of drugs into the middle ear can thus be achieved by a single application of the formulation.

Results: Successful drug delivery across intact tympanic membranes was demonstrated using the amino-amide local anesthetic in current clinical use, bupivacaine, and a highly potent site 1 sodium channel blocker anesthetic, tetrodotoxin, a very hydrophilic compound that blocks the same sodium channel as bupivacaine but at a different site, and has ultrapotent local anesthetic activity. Bupivacaine and TTX are known to strongly increase each other’s anesthetic effects when given in combination. The chemical permeation enhancers incorporated in the delivery system increased the permeability of the to the anesthetics considerably, resulting in high concentration of drug in the middle ear fluid.

Conclusion: A local drug delivery system was developed to provide sustained pain relief from a single application in patients with AOM. A commonly used amino-amide anesthetic, bupivacaine, was successfully delivered across intact TMs, as was a highly potent site 1 sodium channel blocker anesthetic, TTX. The chemical permeation enhancers incorporated in the hydrogel system considerably increased the permeability of BUP and TTX across the TM.
Presenting Author: Kelvin Kong

Institution(s): Hunter Medical Research Institute

Title: Impact of Otitis Media with Effusion (OME) on Quality of Life

Abstract: Waiting to Hear: The Impact of Otitis Media with Effusion (OME) on Quality of Life of Aboriginal and non-Aboriginal Children from Urban, Regional and Rural Areas of New South Wales, Australia
Poster # 21

Presenting Author: Asbjørn Kørvel-Hanquist PHD

Institution(s): Region Sjælland

Title: Determining factors for tympanostomy tube insertion in a country with a high insertion rate

Abstract: Introduction

Tympanostomy tube insertion is the procedure most often performed on children. Although most western countries have a high-level health system, the tympanostomy tube insertion rate differs among these countries.

Objective

To investigate the determining factors for having tympanostomy tubes inserted in children in a western country with a high insertion rate.

Methods

Information was drawn from the Danish National Birth Cohort. This information was interviews from more than 80,000 mothers, recorded prospectively from the first trimester and throughout the pregnancy until the child reached the age of 10 years and included information on OM, prenatal exposures, daycare attendance, breastfeeding, and everyday life. Information were also drawn from the Danish Health registries and the Danish Population Education registry.

Results

In this material regarding 85,565 children, 25,331 of the children had one or more tympanostomy tubes inserted. In the children who were treated with tube insertion, 66% had experienced at least one episode of otitis media compared to 31% of those children not having a tube inserted. In the children who had experienced more than three episodes of otitis media, 75% had a tympanostomy tube inserted.

A total of 24 variables were assessed in a prediction model to detect the risk of receiving treatment with tube insertion.

Conclusion

The variables associated with tube insertion were the same as those variables associated with the child experiencing otitis media. However, it was not possible to predict which children were going to have a tube inserted. The prediction model could not detect the children not going to have a tube inserted, and there was no difference between children with more than 3 episodes of otitis media with had a tube inserted and those not having a tube inserted.
Presenting Author: Oded Kraus

Institution(s): Samson Assuta Ashdod University Hospital

Title: Beyond the Otopscope: Emerging Technologies for the Diagnosis of Otitis Media

Abstract: Objective: To present new experimental technologies for otitis media (OM) diagnosis which have already been tested in clinical settings in humans.

Method: We searched the following MeSH terms: ["diagnosis"] AND [all forms of OM] AND ["human"] AND ["ear"] and ["tympanic membrane"] in papers published in various electronic databases between 1/1/2005-4/20/2018. Our search revealed innovative diagnostic technologies which rely on and take advantage of the physical properties of the tympanomastoid cavity components: tympanic membrane (TM) thickness, its translucency and compliance; middle ear fluid characteristics; biofilm presence; increased tissue metabolic activity in OM states and fluid presence in the mastoid cavity. All these parameters are taken into account to establish OM diagnosis in a more objective manner.

Results: Initial search revealed 549 publications, which were then reduced to 47 publications reporting on 12 new technologies for OM diagnosis. We discuss the following technologies: spectral gradient acoustic reflectometry, digital otoscopy, TM image analysis, multi-color reflectance imaging, anti-confocal middle ear assessment, optical coherence tomography, quantitative pneumatic otoscopy, trans-mastoid ultrasound, wideband measurements, TM thickness mapping, shortwave infra-red imaging and wideband acoustic transfer functions.

Conclusion: New experimental technologies are gradually introduced to overcome the difficulties of standard otoscopy. Their main advantage is the objective evaluation of the TM morphology, determination the presence of middle ear fluid and evaluate its content, and thus they can potentially replace standard otoscopy. Because these technologies are still under investigation and are pending widespread clinical use, their implementation in the market depends on their success in clinical trials, as well as on their future cost.
**Poster # 23**

**Presenting Author:** Jaroslaw Krol Dr.

**Institution(s):** Drexel University

**Title:** Species-level bacterial community profiling of the otitis media microbiome using Pacific Biosciences sequencing of full-length 16S rRNA genes

**Abstract:** Introduction: Chronic otitis media with effusion (COME) is one of the major causes of childhood conductive hearing loss. This can result in delayed language acquisition, learning difficulties, and associated socialization deficits. COME pathogenesis is associated with bacteria adopting the biofilm mode of growth which makes them antibiotic resistant. The bacteria that cause acute OM are referred to as otopathogens, and include Streptococcus pneumoniae, non-typeable Haemophilus influenzae and Moraxella catarrhalis. These bacteria originate from the nasopharynx and are capable of migrating to the middle ear via the Eustachian tube. Once in the ME they can cause acute or chronic disease. The role of other bacterial species in COME is, however, an open topic.

Objectives: To identify semi-quantitatively in an unbiased manner all of the bacterial species present in cohort of children ongoing myringotomy and tube placement for COME.

Methods: Seventy children between 5 months and 16 years of age were selected for this study. One hundred thirty four middle-ear effusions (MEE) specimens were collected via Lukens traps with 300 µl of N-acetyl-L-cysteine (NAC) (0.5% solution in 2.93% sodium citrate) buffer. DNA extraction, full length 16S rRNA amplification, and multiplex DNA sequencing were conducted as described (Earl et. al. 2018). The PacBio Sequel demultiplexed FASTQ files were concatenated together for analysis through the MCSMRT pipeline (https://github.com/jpearl01/mcsmrt). Reads with lengths 500>x >2000 bps and those mapped to the host genome were removed as well as reads missing both the forward/reverse primers. The high-quality reads (with an expected error (EE) threshold of 1) were then dereplicated, clustered in OTUs, chimeras were identified and removed, and finally a species level OTU table was created.

Results: Ninety-six samples out of 134 total (70.5%) were positive for the full length 16SrRNA PCR amplification and were sequenced using the Pac-Bio RSII or Sequel instruments. Raw data were filtered and analyzed using the MCSMRT pipeline. The samples with less than 500 total reads or less than 100 OTUs were removed. Finally, 88 samples (65% of original samples) from 55 patients were analyzed. For 35 patients both right and left ears were analyzed, and for 18 patients only a single ear was analyzed. In these samples we identified 126 species (OTUs). The most prevalent species were Alloiococcus otitis (26.5%), Staphylococcus pettenkoferi (18.8%), Haemophilus influenzae (12.8%), Turicella otitidis (12.1%), Moraxella catarrhalis (11.4%), Staphylococcus auricularis (10.4%), Pseudomonas aeruginosa (3.8%), Streptococcus pneumoniae (2.2%) and Staphylococcus simulans (1.9%). Correlations between patients, bacterial strains and sites will be presented and discussed.
Conclusions: It can be concluded by prevalence analyses that in addition to the accepted otopathogens that additional bacterial species including Alloiococcus otitis, Turicellaotitidis, and Staphylococcus pettenkoferiare likely playing a role in the pathogenesis of COME. Further studies should be conducted to identify high probability virulence traits within the genomes of these pathogens to allow more efficient and successful treatment.


*-equal contribution
Title: Acute Otitis Media in Mice with a Human Hematolymphoid System

Abstract: Objective: Otitis media (OM) is one of the largest pediatric health problems, and can have devastating impacts in developing countries. Much of the basic research done on OM pathogenesis, etiology and treatment options owes to animal models. In recent years, “humanized” mice have become a valuable tool with which to study the human immune system in an animal model setting. Here we describe and evaluate their use to model OM.

Method: OM was induced by inoculation of nontypeable Haemophilus influenzae (NTHi) into the middle ears (MEs) of NOD-Scid-IL2Rγnull (NSG) mice that were previously transplanted with human hematopoietic CD34+ cells. Blood and ear tissue were collected at different times post OM induction. Human inflammatory cell mobilization was evaluated by flow cytometry and immunohistochemistry and RNA profile. The ME inflammatory response, leukocyte infiltration, tissue hyperplasia, bacterial clearance and OM recovery were compared to that in wild type (WT) BALB/c mice.

Results: The ME inflammatory response, leukocyte infiltration, tissue hyperplasia, bacterial clearance and OM recovery profile were comparable in humanized NSG (huNSG) to WT. In the huNSGs, we observed the mobilization of human CD45+ immune cells into the ME cavity, suggesting that the human cells are participating in both ME inflammation and NTHi bacterial clearance. At 10 days, all MEs of WT and huNSGs with >30% engraftment were culture-negative, indicating normal recovery from OM. In contrast, animals with
Poster # 25

Presenting Author: Arwa Kurabi PhD

Institution(s): UCSD

Title: Delivery of Antibiotic Through the Intact Tympanic Membrane by Peptide-Mediated Active Transport

Abstract: Objective: While systemic antibiotics are not recommended for the treatment of uncomplicated acute otitis media (OM) in children over two years of age, they are useful in the treatment of younger children and of complicated forms of the disease. Local delivery has also been shown to be efficacious in treating complicated OM, but it requires surgically breaching the tympanic membrane.

Using Phage display, we previously discovered a family of peptides that are actively transported across the intact tympanic membrane (TM), providing a potential mechanism for noninvasive ME delivery. Since these peptides can deliver bacteriophage that are 1 μm in length to the ME, we reasoned that they should also be able to deliver drugs.

Method: As a first attempt at peptide-mediated drug delivery, single amoxicillin molecules were linked to a trans-TM peptide and applied to the rat TM in vivo after ME inoculation of nontypeable Haemophilus influenzae (NTHi). After 8 hours, the ME was opened and the bacteria were titrated. Individual antibiotic molecules linked to peptide only modestly reduced NTHi-load in the ME, suggesting that larger drug packages would need to be delivered in order to be effective. To assess the impact of a substantial drug package, we used EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide/N-hydroxysuccinimide) chemistry to conjugate neomycin to the major coat protein of M-13 phage.

Results: Using this technique, we were able to link several hundred neomycin molecules per phage particle to phage expressing one of two different trans-TM peptides. Application of either neomycin-phage to rat TM in vivo, after ME inoculation with NTHi, resulted in 80% reduction in NTHi in the ME after only 8 hours, when compared to control MEs receiving NTHi but no TM treatment. Additional control MEs, inoculated with NTHi and receiving antibiotic crosslinked to WT phage on the TM, showed no reduction in ME NTHi titer either.

Conclusion: The results indicate that active transport of drug packages by trans-TM peptides can deliver potent concentrations of antibiotics to the ME for the treatment of OM. Supported by grants DC000129; DC012595 and DC014801 from the NIH/NIDCD and the Veterans Administration.
Poster # 26

Presenting Author: Hyun Woo Lim MD

Institution(s): UCSD

Title: Lack of the Hyaluronan Receptor CD44 Affects the Course of Bacterial Otitis Media and Reduces Leukocyte Recruitment to the Middle Ear

Abstract: OBJECTIVE: CD44 is a multifunctional molecule that plays major roles in both leukocyte recruitment and tissue proliferation. Since mucosal hyperplasia and leukocyte infiltration of the middle ear (ME) cavity are major features of otitis media (OM), we evaluated the role of CD44 in the pathophysiology and course of this disease in a mouse model of ME infection.

METHODS: To assess the prevalence and distribution of CD44 in the ME before and during OM, the expression of Cd44 and genes related to its function was evaluated using gene arrays in wild-type mice, and in single-cell transcriptomes from normal and infected wild-type MEs. To evaluate the functional role of CD44 in OM, the MEs of mice genetically deficient in Cd44 were inoculated with non-typeable Haemophilus influenzae (NTHi). Histopathology and bacterial clearance were compared to those seen in wild-type controls.

RESULTS: We observed strong up-regulation of CD44 and of genes related to its role in leukocyte extravasation into the ME, during the course of acute OM. Mice deficient in Cd44 exhibited reduced early mucosal hyperplasia and leukocyte recruitment, followed by delayed resolution of infection and persistent inflammation.

CONCLUSIONS: CD44 plays an important role in both pathogenesis and defense of the ME during OM. Targeted therapies based on CD44 could be useful adjuncts to the treatment of ME infections.

Supported by grants DC000129; DC012595 and DC014801 from the NIH/NIDCD and the Veterans Administration.

Disclosure: Dr. Ryan is a co-founder of, stockholder in and unpaid consultant to Otonomy, Inc., a relationship that has been approved by the UCSD Committee on COI. The company played no part in this research.
Poster # 27

Presenting Author: Audrey Lim BS

Institution(s): University of Pittsburgh School of Medicine

Title: Tubomanometry may describe Passive Properties of Eustachian Tubes in Ears with Intact Tympanic Membranes

Abstract: Introduction: The Forced Response test (FRT) is one of the few Eustachian tube (ET) function (ETF) tests that provides information on the passive and structural properties of the ET. Positive pressure is applied to the middle ear through a non-intact tympanic membranes (TM) until the ET passively opens (opening pressure or OP) and establishes a directional airflow towards the nasopharynx (NP). Tubomanometry (TMM) is a simple in-office test of ETF that can be used in both intact and non-intact TMs. It delivers a controlled bolus of air into the nasal cavity at 30, 40, or 50 mbar and uses the para-tubal muscle assistance during a swallow to open the ET and allow the movement of air from the NP to the middle ear. Based on this knowledge, the authors hypothesize that the OP measured during the FRT test will generally be greater than those in TMM due to the contribution of active function in the latter test and expect that the OP measured on FRT and TMM will not be correlated.

Objective: To determine if the TMM OP can be used as a new parameter of passive ET properties.

Methods: This is a retrospective exploratory cross-sectional study in a group of 14 children tested at a specialized ETD Clinic between 5/6/2013 to 9/11/2018 who underwent FRT and TMM tests. OPs of FRT at 11ml/min and 23ml/min (FRT11, FRT23) were compared to the opening pressures of TMM at 30, 40, and 50mbar (TMM30, TMM40, TMM50). OPs in TMM were defined as the NP pressure at the moment of ET opening during swallows. Data were analyzed using student’s t-tests and Cohen’s d effect size.

Results: The age range of the population was 7.8-18.5 years (mean= 13.1 ± 3.5 years), 9 were males and 13 were white. Data for 15 non-intact ears were analyzed. ET OPs in FRT11 and FRT23 were highly correlated (r=0.824, p
Poster # 28

Presenting Author: Tal Marom

Institution(s):
Samson Assuta Ashdod University Hospital

Title: Acute Otitis Media Treatment Guidelines in the Pediatric Emergency Department: How Adherent Are We?

Abstract: Objective: To quantify the rates of appropriate and inappropriate treatment of uncomplicated AOM visits. Also, we studied the pre-AOM visit and discharge antibiotic prescription rates, the type and duration of antibiotics prescribed for AOM, and the total time spent with antibiotics in each uncomplicated AOM episode.

Method: Records of children 6-36 months of age with AOM visiting a university-affiliated pediatric emergency department (PED) in central Israel between 2014 and 2016 were retrieved and reviewed for the treatment given: watchful waiting (WW) vs. antibiotic treatment. If antibiotics were prescribed, the type and duration were recorded. Overall time spent with antibiotics was also calculated (before, during and after admission). We evaluated appropriate and inappropriate treatment rates of eligible AOM cases, in respect to the local guidelines, published in 2013, which encourage WW in most mild-moderate cases.

Results: Out of 1493 AOM visits, 863 (57.8%) were boys, with a median age of 14.9 months (IQR, 9-19). The overall pre-visit antibiotic rate was 24.1%, but among those children who had been examined by a physician, this rate was 95.2%. Amoxicillin was the most common antibiotic, administered in 66.3% of the cases. Only 21 (5.8%) children had been treated with antibiotics for ≥7 days prior to their visit, and were considered as treatment failure. Antibiotic therapy upon discharge was recorded in 1394/1449 (96.2%) visits, again with amoxicillin as the most common antibiotic therapy, in 80.8% of the cases. Appropriateness of treatment (WW or antibiotics) could be analyzed in 1134 visits; 20.9% were considered as inappropriate. Of them, 98.3% were prescribed with the wrong antibiotic type and duration. The mean duration of the recommended antibiotic therapy upon discharge was 7.84±1.21 days (recommended by the guidelines: 10 days).

Conclusion: Adherence rate to the local guidelines of diagnosis and treatment recommendations for uncomplicated AOM was overall very high, as measured by whether appropriate treatment was given and the type and duration of antibiotics. The total time spent with antibiotics was shorter than recommended but was not associated with a high rate of complications.
Poster # 29

Presenting Author: Tal Marom

Institution(s): Samson Assuta Ashdod University Hospital

Title: Acute Otitis Media Diagnosis and Management Guidelines: How Can We Explain Worldwide Differences?

Abstract: Objective: In order to reduce acute otitis media (AOM) burden and limit unnecessary antibiotic use, guidelines and consensus statements have been published in many countries over the past decades. We review the differences between AOM guidelines worldwide and attempt to track the origins of these dissimilarities.

Method: The key-words: ‘acute otitis media’ AND ‘children’ AND [‘treatment’ or ‘management’] AND [‘guideline’ or ‘consensus’] were used in various electronic databases between 1/1/1989 through 9/30/2018. Overall, 99 sources from 64 countries were retrieved. According to the United Nations Annex definition, 60 were from 26 developed countries, and 39 were from 38 developing countries. We performed a qualitative review for the following key points: diagnosis, treatment decision algorithm, antibiotic treatment options, role of complementary treatment, treatment failure, salvage and preventive strategies.

Results: Tympanic membrane bulging, opacity and presence of middle ear fluid are essential for diagnosis, as marginal/uncertain cases are not accepted. Guidelines from developed countries offer the use of pneumatic otoscopy and tympanometry to aid diagnosis. Withholding antibiotic therapy and a ‘watchful waiting’ (WW) approach in mild-moderate cases are preferred in settings where follow-up visits are both possible and attainable, mostly in developed countries. While amoxicillin is mostly accepted as the 1st-line antibiotic therapy, options for 2nd- and 3rd-line antibiotics vary, according to local bacteriology and antimicrobial susceptibility data and costs. Other treatments, such as complementary and alternative medicine, steroids or anti-histamines, are either rejected or ignored. Reduction of known risk factors and call for vaccinations (influenza, pneumococcal conjugate vaccines) are encouraged mostly in developed countries, where such immunizations have been implemented in National Immunization Programs.

Conclusion: Despite regional differences, AOM guidelines worldwide share common grounds on various matters concerning diagnosis and management: diagnosis based on TM findings using otoscopy and/or pneumatic otoscopy in conjunction with tympanometry, WW approach in appropriate cases, oral analgesic treatment using ibuprofen/paracetamol, reduction of risk factors and preventive measures to reduce AOM burden.
**Presenting Author:** Robyn Marsh PhD

**Institution(s):** Menzies School of Health Research

**Title:** Candidatus Ornithobacterium hominis: an Important but Under-Recognised Nasopharyngeal Bacterium?

**Abstract:**

*Introduction:* DNA-based studies have revealed the presence of a new nasopharyngeal bacterium recently named Candidatus Ornithobacterium hominis (OH). This uncultured bacterium has been detected at high relative abundance in NP microbiota datasets from around the world and was shown by a PCR-based study to be highly prevalent and persistent in a Thai paediatric population at high-risk of respiratory infection. Furthermore, the closest known OH relative (Ornithobacterium rhinotracheale) is a respiratory pathogen of birds. Although OH genomes can be derived from metagenomic data, isolates are needed to deepen understanding of the bacterium’s role in human infections. The aim of this study was to isolate OH and determine its optimal culture conditions.

*Methods:* Culture was performed using biobanked NP swabs from four children (age 1-2 years) with chronic lung disease. All were OH-positive by 16S rRNA gene sequencing at 5-55% relative abundance. Colonies were screened using OH-specific PCR targeting the 16S rRNA and toxA genes. Presumptive OH colonies were confirmed using genome sequencing.

*Results:* OH was successfully cultured from all four NP swabs. The isolate genomes had average nucleotide identity of 97.86-98.23% with draft genomes OH-22767 and OH-22803 (derived from metagenomic data), indicating they are members of the same species. Optimal primary culture was achieved using Tryptic Soy Agar with 5% Sheep Blood incubated in a microaerophilic atmosphere at 35°C for up to 5 days. Under these conditions, OH colonies ranged in size from 1-3 mm and were pleomorphic, glistening, grey and concave. All isolates were pleomorphic Gram-negative bacilli and were oxidase-positive and catalase-negative. All isolate genomes contained distinct lipopolysaccharide (LPS) biosynthesis clusters. All produced β-lactamase and contained genes encoding efflux pumps associated with multi-drug resistance.

*Conclusion:* OH isolates were successfully recovered from all of the NP swabs. The optimal culture conditions included a microaerophilic atmosphere and prolonged incubation time; both conditions which are not part of standard culture conditions used to recover respiratory pathogens. Heterogeneity among the LPS cluster is suggestive of multiple capsular types, consistent with observations from earlier DNA-based studies. The presence of antibiotic resistance genes in all OH isolates suggests a potential capacity to indirectly affect treatments targeting pathogenic species. We propose a global study to more deeply assess OH diversity, distribution and prevalence and to determine whether this species may directly or indirectly contribute to OM pathogenesis.
Poster # 31

Presenting Author: Robyn Marsh PhD

Institution(s): Menzies School of Health Research

Title: Respiratory Pathogens are among the Dominant Taxa in Nasopharyngeal Microbiota of One-month Old Indigenous Australian Infants.

Abstract: Introduction: Nasopharyngeal (NP) pathogen carriage is a known otitis media (OM) risk factor, whereas dominance of the NP microbiota by Corynebacterium sp. and Dolosigranulum pigrum is potentially protective against OM. No studies have examined NP microbiota in Indigenous Australian children; a population at high-risk of early-onset, persistent and severe OM.

Objective: The aim of this study was to compare the NP microbiota (including analysis of potentially protective commensal taxa) in infants with or without early-onset OM.

Methods: NP swabs were collected from Indigenous Australian infants aged 28-38 days. OM (any acute otitis media or otitis media with effusion) was diagnosed by research nurses using tympanometry. Swabs were cultured using standard methods. Total and species-specific bacterial loads were determined by qPCR. Sequencing of the 16S rRNA gene V4 region was done with kitome exclusion included in the analytic pipeline.

Results: 111 infant NP swabs were included in the analysis. OM was diagnosed in 33% infants. Total bacterial load was similar in infants with or without OM (Wilcoxon p=0.2). After kitome exclusion, swabs from 87 infants had sufficient reads for microbiota analysis; 31% of these infants had OM. Swabs from infants with and without OM had similar alpha (Wilcoxon p>0.9) and beta diversity (PERMANOVA p=0.16). Thus, bacterial density, community structure and overall NP microbiota composition were not different in children with or without early-onset OM.

The microbiota of 86/87 swabs clustered into six profiles which all included combinations of pathogenic and commensal taxa. Streptococcus pneumoniae (Spn), Haemophilus influenzae (Hi), Moraxella catarrhalis (Mc), and Staphylococcus aureus (Sa) were detected by PCR and/or culture in 50%, 48%, 48% and 49% of swabs, respectively, with 89% of infants colonised by at least one of these species. Co-colonisation by multiple pathogens was detected in 31% of infants with 5% positive for Spn and Hi; 11% positive for Spn, Hi and Mc; 6% positive for Spn, Hi and Sa; and 9% positive for Spn, Hi, Mc and Sa. Pathogen carriage rates (either alone or in combination) were similar in infants with and without OM (all chi-square adj-p>0.2).

Analysis of potentially protective taxa showed lower D. pigrum relative abundance in infants with OM (median 0.05%, IQR 0-3%) compared to those with no OM (median 4%, IQR 0.2-9%; Wilcoxon p=0.02), whereas Corynebacterium relative abundance was similar in both groups (median 46% and 44%, respectively; Wilcoxon p=0.8).
Conclusion: Pathogens were among the dominant taxa in most NP swabs from this cohort of one-month old infants with or without OM. Commensal taxa were also present in all swabs, but at lower relative abundance than studies from other populations. Development of microbiota modifying agents (e.g. probiotics) for preventing pathogen colonisation and subsequent OM in high-risk populations will require formulations suitable for use during the neonatal period.
Poster # 32

Presenting Author: Takayuki Matsunaga MD

Institution(s): Oita University, Faculty of Medicine, Otolaryngology Head and Neck Surgery

Title: Phase Variation of Nontypeable Haemophilus influenza Affects Mucosal Immune Responses in The Nasopharynx

Abstract: Objective: Nontypeable Haemophilus influenzae (NTHi) are considered the chief pathogens in both acute otitis media and otitis media with effusion (OME), and most strains cultured from middle ear effusions are identical with those found in the nasopharynx of patients with OME. NTHi has phosphorylcholine (ChoP) on the surface of lipooligosaccharides (LOS), and our previous study revealed that the sickness-period of otitis media with effusion caused by ChoP (+) NTHi was longer than that of ChoP (-) NTHi in clinical cases. In this study, we investigated the host immune responses to phase variation by using mouse models of nasopharyngeal clearance with clinically isolated NTHi strains.

Methods: Animals were inoculated with ChoP (+) and ChoP (-) NTHi strains into the nose, and then euthanized at 12 hours, day 1, 3, and 7 after inoculation. Nasopharyngeal washes (NW) were collected, and then used for viable bacterial counts and an estimation of various inflammatory cytokines by Bio-Plex® assay.

Results: The numbers of ChoP (+) NTHi in NWs from 12 hours to day 7 were higher than of ChoP (-) NTHi, and the levels of interleukin-1β (IL-1β), IL-6, KC and tumor necrosis factor-α (TNF-α) increased in NWs in both ChoP (+) and ChoP (-) NTHi strains, and the levels of these cytokines were significantly higher in ChoP (+) NTHi than those in ChoP (-) NTHi at 12 hours and day 1 after inoculation, however, the level of these cytokines was not different between ChoP (+) and ChoP (-) NTHi at day 3 and 7.

Conclusion: Chop expression of NTHi affected mucosal immune responses in nasopharynx, as well as adhesion on nasopharynx. Chop expression of NTHi may be related to prolongation of the duration of OME by not only nasopharyngeal adherence but also mucosal inflammation.
Abstract: Introduction: Otitis media (OM) is the most common middle ear disease especially affecting children. Although the pathogenesis of OM caused by most common pathogens namely, Streptococcus pneumoniae, Non-typeable Haemophilus influenzae (NTHi) and Moraxella catarrhalis is well characterized, the molecular mechanisms underlying middle ear infection induced by microbes such as Pseudomonas aeruginosa are not known. This lack of understanding about pathogenetic events can be attributed to the lack of a good animal model.

Objective: The aim of this study was to establish a mouse model of P. aeruginosa induced OM and characterize the middle ear inflammatory responses as well their impact on inner ear.

Methods: Otopathogenic P. aeruginosa was inoculated into the middle ears of mice through transtympanic administration. Animals that received phosphate buffered saline (PBS) as well as naïve animals served as the control groups. Mouse middle ears were harvested at different post-infection time periods to determine bacterial load, recruitment of inflammatory cells, cytokine production and fluid accumulation. Infected animals were also subjected to auditory brainstem recordings (ABRs) to determine hearing thresholds. Inner ears harvested from infected mice were subjected to confocal microscopy and scanning electron microscopy to evaluate the effects of middle ear infection on inner ear.

Results: We observed that otopathogenic P. aeruginosa can successfully colonize the middle ear of normal mice and induce characteristic features of OM without any surgical manipulation. We also observed that P. aeruginosa middle ear infection damages sensory cells in the inner ear leading to hearing loss in our mouse model.

Conclusions: The results of our study suggests that otopathogenic P. aeruginosa can induce OM in a normal mouse ear. The availability of P. aeruginosa induced OM mouse model established in this study will open up avenues to understand the molecular mechanisms involved in the pathogenesis of disease as well as to develop novel treatment modalities.
Poster # 34

Presenting Author: Elaine Mokrzan PhD

Institution(s): The Research Institute at Nationwide Children’s Hospital

Title: Contact with Host Respiratory Tract Epithelial Cells Upregulates NTHI Expression of the Vaccine Candidate Antigen PilA

Abstract: Background: The Type IV pilus (Tfp) of nontypeable Haemophilus influenzae (NTHI) plays a major role in bacterial adherence/colonization, motility and biofilm formation, processes that contribute significantly to the pathogenesis of NTHI-induced otitis media (OM). The major protein subunit of Tfp, PilA, is a promising vaccine immunogen for use against OM due to NTHI. As such, it is important to understand how microenvironmental cues encountered in the nasopharynx (34°C) and middle ear (37°C), the sites of NTHI asymptomatic colonization and infection during OM, respectively, affect the expression of this vaccine candidate. Here, we examined how epithelial cells influenced NTHI Tfp expression. Methods: We used an NTHI reporter construct in which GFP expression is driven by the pilA promoter, and monitored relative fluorescence intensity over time to estimate pilA promoter activity and by inference, Tfp expression. NTHI were incubated in epithelial cell culture medium (CCM) alone or together with human airway epithelial cells. Results: CFU of NTHI incubated in CCM only remained at or above initial values for 12 h, during which time pilA promoter activity did not increase. In contrast, NTHI incubated in CCM with epithelial cells increased in both CFU/well and pilA promoter activity over time at both 34°C and 37°C (p
Poster # 35

Presenting Author: Ryan Nolan M.Eng., CCRP

Institution(s): PhotoniCare

Title: Development of a Handheld Optical Coherence Tomography Device with an Otoscopy Probe for In-office Non-invasive Imaging and Characterization of Middle Ear Effusions

Abstract: Accurate determination and characterization of middle ear effusions (MEEs) associated with otitis media (OM) is a major diagnostic challenge. While standard and pneumatic otoscopy have been historical methods for making this assessment, they rely on the subjective interpretation of the surface view of the eardrum. Consequently, OM is the leading cause for antibiotic prescription and over-prescription, as well as surgery in children, in the U.S.

Our objective was to develop and clinically test the ability of a new in-office diagnostic imaging device with an otoscopy probe. This compact, portable device utilizes an advanced optical imaging technique called optical coherence tomography (OCT) to collect human middle ear images for interpretation via blinded reader quiz, and compare to that of a cart-based traditional OCT device with an otoscopy probe.

Use of the compact OCT device for in-office non-invasive imaging and characterization of MEE shows promise for improving physician assessments in diagnosing presence of MEE, but with a smaller, more in-office appropriate form factor than cart-based traditional OCT systems.
Poster # 36

Presenting Author: Laura Novotny MS

Institution(s): The Research Institute at Nationwide Children’s Hospital

Title: Antibody Against a Novel Chimeric Peptide Immunogen that Targets a Bacterial DNA-Binding Protein Integral to Biofilm Structural Integrity Resolves Experimental Otitis Media

Abstract: Objective: The chronic nature of many diseases, including otitis media (OM), is largely due to the formation of bacterial biofilms. An abundant component of the biofilm is bacterial extracellular DNA, arranged in a lattice and maintained by the DNABII family of bacterial DNA-binding proteins. One member, integration host factor (IHF) binds to biofilm DNA via its DNA-binding ‘tip’ regions, and thus the amino terminal (‘tail’) region is exposed. As a consequence, the natural immune response is directed against the exposed tail region of IHF, however we’ve shown that antibody directed to this region does not disrupt biofilms. In contrast, antibody that targets the occluded tip regions of IHF induces significant biofilm disruption in vitro and collapses biofilms in the middle ear during experimental OM. Herein, we designed a novel chimeric peptide immunogen that specifically re-routes the immune response to target the protective tip regions of IHF and compared the therapeutic potential for anti-tip chimer peptide antibodies versus anti-native IHF protein to disrupt bacterial biofilms in vitro and in vivo.

Methods: NTHI biofilms were formed in the middle ears of chinchillas prior to delivery of IgG-enriched rabbit anti-IHF tip chimer peptide, anti-native IHF, or as negative controls, IgG from naive serum or against a peptide that targets the non-protective tail region of IHF, directly into this anatomical site. The relative bacterial load, amount of NTHI biofilm and mucosal inflammation within each middle ear was assessed 1 day after receipt of the second dose of antibody, and again a week later. In vitro, NTHI biofilms were established and incubated with the aforementioned antibodies for 5 to 120 min and biofilm disruption examined.

Results: Animals administered anti-tip chimer peptide or native IHF had 4-log fewer NTHI in their middle ears 1 d after receipt of the 2nd dose of antibody, compared to controls (P ≤ 0.05). Also, significantly less biofilm was observed (P ≤ 0.001) and 50% less biofilm remained in ears treated with anti-tip chimer peptide compared to anti-native IHF. In vitro, biofilms incubated with anti-tip chimer peptide had 50% less biomass compared to anti-native IHF after 5 min (P ≤ 0.05). As proof, whereas immunization of chinchillas with native IHF induced the production of antibodies directed primarily toward the non-protective tail region (as occurs during natural disease), delivery of tip chimer peptide induced a complete shift in antibody response toward the protective tip region with rapid resolution of disease, as by design.

Conclusions: These data revealed that a therapeutic approach toward biofilm disruption is achieved by targeting an essential structural component, the DNABII proteins. The continued refinement of candidate immunogens and delivery strategies has tremendous potential as highly effective and broadly applicable means to reduce the burden of chronic diseases, including those under the spectrum of OM. NIH R01 DC011818
Poster # 37

Presenting Author: Sharon Ovnat Tamir

Institution(s): Assuta Ashdod University Hospital

Title: Biofilm Distribution on Ventilating Tubes: An in vivo Study

Abstract: In this study we wanted to determine the topographic distribution of biofilms on the surface of VT’s, while comparing both the medial and lateral surfaces of VT’s. We secondarily, sought out to detect VT "prone zones" for biofilm adherence.

Our secondary objectives was to establish a clear association between biofilms and recurrent/chronic infections and post-tympanostomy tube otorrhea.

Our results showed that bacterial biofilms were more likely to be formed on the perpendicular junction and in the internal lumen of the VT.

When VTs were sliced into medial and lateral parts, there were more biofilm colonies on the medial part facing the middle ear mucosa, and when VTs were extracted from children due to persistent otorrhea we noted to have considerably more biofilm adhesions on the entire VT surface.

In conclusion VT “weak” zones are the perpendicular junctions and the internal lumen. These “weakness zones” can be the future target areas for changes in VT geometry or can be specifically coated with anti-biofilm materials to decrease biofilm adherence.
Poster # 38

Presenting Author: Kwang Pak PhD

Institution(s): UCSD

Title: In Vitro Analysis Demonstrates that Peptides are Actively Transported Across the Human Tympanic Membrane

Abstract: Objective: Using phage display, we identified a family of peptides that can actively transport bacteriophage across the intact tympanic membrane (TM) of rats and other mammalian species. However, it was not clear whether the peptides require the phage for transport, or whether they can cross the TM independently. It was also not clear whether the peptides would mediate transport across the human TM.

Methods: Using an in vitro assay consisting of a modified Ussing chamber into which a TM could be mounted between an upper and lower fluid chambers, we applied a trans-TM peptide linked to a DNA oligo template on the TM surface. After 2 hours, fluid was recovered from the lower chamber and qPCR was used to quantify the amount of peptide present. Controls were peptide lined to phage at the same molar concentration. In a second study, TM fragments from human patients undergoing TM reconstruction procedures were place in the device. Phage expressing trans-TM peptides were applied to the human TM and 2 hours later, fluid was collected from the lower chamber and phage was tittered to evaluate concentration present. Control human TMs were exposed to untargeted WT phage.

Results: In the first experiment, the amount of DNA-tagged peptide recovered from the lower chamber was comparable to the amount of phage recovered. In the human TM experiments, recovery of trans-TM phage from the lower chamber was similar to that seen with the TMs of rat or other mammalian species used.

Conclusions: The results indicate that transport of trans-TM peptides across the TM is not dependent on phage cargo. Moreover, transport is also independent of size since recovery amounts were similar between the peptides linked to DNA-oligo and peptide linked to a 1um phage particle. This would be consistent with a transcytotic transport mechanism as opposed to a transmembrane transporter or paracellular movement. Finally, active peptide transport across the TM occurs in the human TM ex vivo which is critical for any use of peptides in drug delivery. Supported by grants: DC000129; DC012595 and DC14801 from the NIH/NIDCD and the Veterans Administration.
Poster # 39

Presenting Author: Kwang Pak BS

Institution(s): UCSD

Title: Delivery of Antibiotic Through the Intact Tympanic Membrane by Peptide-Mediated Active Transport

Abstract: While systemic antibiotics are not recommended for the treatment of uncomplicated acute otitis media (OM) in children over two years of age, they are useful in the treatment of younger children and of complicated forms of the disease. Local delivery has also been shown to be efficacious in treating complicated OM, but it requires surgically breaching the tympanic membrane. Using Phage display, we previously discovered a family of peptides that are actively transported across the intact tympanic membrane (TM), providing a potential mechanism for noninvasive ME delivery. Since these peptides can deliver bacteriophage that are 1 µm in length to the ME, we reasoned that they should also be able to deliver drugs.

As a first attempt at peptide-mediated drug delivery, single amoxicillin molecules were linked to a trans-TM peptide and applied to the rat TM in vivo after ME inoculation of nontypeable Haemophilus influenzae (NTHi). After 8 hours, the ME was opened and the bacteria were titered. Individual antibiotic molecules linked to peptide only modestly reduced NTHi-load in the ME, suggesting that larger drug packages would need to be delivered in order to be effective. To assess the impact of a substantial drug package, we used EDC chemistry was used to conjugate neomycin to the major coat protein of M-13 phage. Using this technique, we were able to link several hundred neomycin molecules per phage particle to phage expressing one of two different trans-TM peptides. Application of either neomycin-phage to rat TM in vivo, after ME inoculation with NTHi, resulted in 80% reduction in NTHi in the ME after only 8 hours, when compared to control MEs receiving NTHi but no TM treatment. Additional control MEs, inoculated with NTHi and receiving antibiotic linked to WT phage on the TM, showed no reduction in ME NTHi titer.

The results indicate that active transport of drug packages by trans-TM peptides can deliver effective concentrations of antibiotics to the ME for the treatment of OM.

Supported by grants DC000129; DC012595 and DC014801 from the NIH/NIDCD and the Veterans Administration.

Disclosure: Dr. Ryan is a co-founder of, stockholder in and unpaid consultant to Otonomy, Inc., a relationship that has been approved by the UCSD Committee on COI. The company played no part in this research.
**Poster # 40**

**Presenting Author:** Janak Patel

**Institution(s):** Novus Therapeutics Inc.

**Title:** A Phase 1 Safety Study of Repeated Doses of Intranasal OP0201Metered Dose Inhaler Compared to Placebo in Healthy Adults: A Potential Treatment for Otitis Media

**Abstract:**

Introduction: OP0201 nasal aerosol is a novel surfactant being developed to treat and prevent otitis media. OP0201 is a 20:1 fixed combination of dipalmitoylphosphatidylcholine (a phospholipid surfactant) and cholesteryl palmitate (a neutral phospholipid spreading agent) suspended in propellant. Intranasal OP0201 is intended to absorb to mucosal air-liquid interface and reduce Eustachian tube (ET) interfacial surface tension and in turn reduces ET passive opening pressure resulting in ‘de-sticking’ and restoring ET physiologic activity. ET dysfunction is an important underlying cause of otitis media.

Objective: To evaluate safety and tolerability of 14 days of intranasal OP0201 (30 mg per day) compared to placebo in healthy adult volunteers.

Method: Phase 1, randomized, double-blind, placebo-controlled, parallel-group, dose-escalation study in adults at 1 study center in the United States. Two dose cohorts are planned (N=15 per cohort). Within each cohort, subjects are randomized 4:1 to receive OP0201 or placebo (stratified by gender; male versus female). Eligible subjects are admitted to the study center and randomized. Subjects remain in resident at the study center until all Day 14 assessments are completed. A Day 21 follow-up visit is planned. Study treatment will be administered to the subjects by the study center staff three times a day for 14 days. Safety endpoints include adverse events (AEs), otoscopy, tympanometry, nasal and epipharynx endoscopy, olfactory test, pure tone hearing test, electrocardiogram, physical examination and clinical laboratory tests.

Results: Fifteen subjects were randomized to Cohort A (30 mg per day); all completed the study as planned. Subjects were Caucasian (60%) or Black (40%), female (53.4%), with a mean age of 34 years (range 20-49). No severe or serious AEs occurred during the 14 days of treatment or during follow up. All AEs resolved without sequelae. Twelve mild or moderate AEs occurred; 3 reported as not related to blinded study treatment (pain upper right arm, somnolence, toothache) and 9 reported to be related to blinded study treatment (headache [5 reports in 4 subjects], nasopharynx dryness/irritation [3 reports in 3 subjects, all mild] and common cold [1 report]. All safety results were within normal limits or changes were rated as not clinically significant. After all subjects in Cohort A completed the Day 14 visit, a Safety Review Committee reviewed all the blinded safety data and determined the doses administered were safe and well tolerated, and recommended escalation to the next cohort (Cohort B 60 mg/day).

Conclusion: In this Phase 1 study, OP0201 was found to be safe and well tolerated in adult volunteers. Future development studies are planned to evaluate OP0201 to treat acute and chronic otitis media in infants and children.
Poster # 41

Presenting Author: Yoon Chan Rah MD

Institution(s): Korea University

Title: Surgical management of large-sized labyrinthine fistula caused by cholesteatoma: a long term outcome and hearing changes

Abstract: Objective:

To evaluate long-term hearing changes and recurrence after surgical management of cholesteatoma matrix overlying large-sized labyrinthine fistula (≥ 2 mm)

Method: A retrospective review of medical records was carried out for 26 patients who underwent surgical resection. Among them, 25 patients underwent complete resection of the cholesteatoma matrix. A long-term recurrence and hearing changes were assessed with an investigation of associated complications.

Results: For complete resection group, average bone conduction threshold was changed from 38.6 to 45.1 dB immediate postoperatively (p=0.073) with 5 cases (20%) with more than 10 dB loss although 3 of them already had more than 70 dB preoperative hearing level. Minimal hearing changes (46.5 dB, p=0.353) were observed thereafter over 46.8 months (15-87 months) without recurrent case. The risk of postoperative hearing loss was significantly increased as the size of fistulae increases (p=0.011), however, a definite cut-off size cannot be determined. Two cases (8%) with large fistulae (3.1 mm, 3.8 mm) experienced intraoperative perilymph leakage with resultant significant additional hearing loss in one case. In a case, cholesteatoma matrix was partially removed because of extension to oval window.

Conclusion: Complete removal of cholesteatoma matrix could also be applied even for large-sized fistula (≥ 2mm) with successful long-term preservation and disease control. Intraoperative hearing loss could be minimized by considering preoperative hearing level and great cautions for cases with larger than 3 mm fistula.
Poster # 42

Presenting Author: Allen Ryan PhD

Institution(s): UCSD

Title: Active Transport Across the Intact Tympanic Membrane is Mediated by Transcytosis

Abstract: Local delivery of antibiotics has been found to be effective in treating OM, but requires surgically breaching the tympanic membrane (TM). We previously discovered a family of peptides expressed on bacteriophage that are actively transported across the intact tympanic membrane (TM), providing a potential mechanism for noninvasive drug delivery. However, the mechanism by which this transport occurs is not known. We therefore analyzed these peptides to identify features associated with transport. Common peptide features included a central lysine residue, isoelectric point of 0.0 at physiologic pH and a hydrophobic C-terminus. Two conserved amino acid motifs were present in the peptides: SxDSTK and PxxP. 3-D structural analysis showed that peptides exhibiting the highest transport rates presented these motifs at the free end of the M-13 PIII N-terminus. Those in which the motifs were predicted to be closer to the phage pIII protein exhibited lower rates. These structural analyses strongly implicate the presentation of the conserved motifs, and especially SxDSTK, in determining peptide transport characteristics.

Reasoning that the conserved motifs might point to the mechanism by which peptides transit the TM, we used the Motif Alignment and Search Tool (MAST) to search for proteins with related motifs. The highest predicted alignments were for exocyst complex component 1, which mediates docking of exocytic vesicles with fusion sites on the plasma membrane, and importin subunit alpha 6. The importin complex is involved in transport of protein across intracellular compartments and is capable of bidirectional transport. These identifications implicate transcytosis, by which cargoes are transported across polarized cells, as the transport mechanism.

As transcytosis is initiated by endocytosis, we applied wortmannin, a potent inhibitor of receptor-mediated endocytosis, to the TM for 1 hour prior to applying a TM-transiting phage peptide. The inhibitor decreased phage recovery from the ME by >90%. Wortmannin is also know to inhibit other aspects of the endocytotic pathway, including vesicle fusion and perinuclear transport, and has been shown to inhibit both apical-to-basolateral and basolateral-apical transcytosis in polarized epithelial cells. The data strongly support transcytosis as the mechanism by which trans-TM peptides more actively across the membrane, initiated by binding to a cell surface receptor with transition into the endocytotic pathway.

Supported by grants DC000129; DC012595 and DC014801 from the NIH/NIDCD and the Veterans Administration.

Disclosure: Dr. Ryan is a co-founder of, stockholder in and unpaid consultant to Otonomy, Inc., a relationship that has been approved by the UCSD Committee on COI. The company played no part in this research.
Presenting Author: Regie Santos-Cortez MD PhD

Institution(s): University of Colorado School of Medicine

Title: A2ML1 and otitis media: novel variants, differential expression and relevant pathways

Abstract: A genetic basis for otitis media is established, however the role of rare variants in disease etiology is largely unknown. Previously a duplication variant within A2ML1 was identified as a significant risk factor for otitis media in an indigenous Filipino population and in US children. In this report exome and Sanger sequencing was performed using DNA samples from the indigenous Filipino population, Filipino cochlear implantees, US probands, and Finnish and Pakistani families with otitis media. Sixteen novel, damaging A2ML1 variants identified in otitis media patients were rare or low-frequency in population-matched controls. In the indigenous population, both gingivitis and A2ML1 variants including the known duplication variant and the novel splice variant c.4061+1G>C were independently associated with otitis media. Sequencing of salivary RNA samples from indigenous Filipinos demonstrated lower A2ML1 expression according to carriage of A2ML1 variants. Sequencing of additional salivary RNA samples from US patients with otitis media revealed differentially expressed genes that are highly correlated with A2ML1 expression levels. In particular RND3 is upregulated in both A2ML1 variant carriers and high-A2ML1-expressors. These findings support a role for A2ML1 in keratinocyte differentiation within the middle ear as part of otitis media pathology and the potential application of ROCK inhibition in otitis media.
Presenting Author: Gayatri Sharma PhD

Institution(s):

Title: Cationic Nanoparticle Enhanced trans-Tympanic Membrane Drug Delivery for Noninvasive Treatment of Otitis Media

Abstract:
RGD (https://rgd.mcw.edu) is a multispecies resource for translational research and comparative genomics. Most notably for researchers working with chinchilla as a model for otitis media (OM), RGD now contains all of the data and functionality of the previous Chinchilla Research and Resource Database, and more.

Results: The full incorporation of chinchilla data into RGD provides chinchilla researchers with the advantage of being able to leverage a wealth of both human data and data associated with commonly used model organisms such as rat and mouse. RGD curators manually review the literature for information on the associations between genes and diseases, pathways and phenotypes, as well as information on gene functions, processes and subcellular localization. The information is standardized and assigned to the appropriate genes for the species studied, then propagated to the orthologous genes in other species. This data is augmented by the output of a number of automated pipelines which regularly import a substantial volume of data from sources such as Mouse Genome Informatics for phenotype annotations based on mouse knockouts, and OMIM, ClinVar and the Comparative Toxicogenomics Database for human disease annotations. The consolidation of data from multiple sources, including the sharing of data across species, gives researchers easy access to a substantial amount of data even when their gene or genes of interest have not been studied in their organism of choice.

For easy access to data and tools of interest to chinchilla researchers, RGD has added a new species-specific portal for chinchilla at https://rgd.mcw.edu/wg/chinchilla/. The Chinchilla Portal gives links directly to tools such as the OLGA Object List Generator and Analyzer for finding chinchilla genes that match specified criteria, and the PhenoMiner tool to query for quantitative phenotype data in chinchilla. For researchers interested in viewing a gene of interest in its genomic context, the portal provides a link to the chinchilla JBrowse genome browser. The portal also supplies direct links to data of interest to chinchilla researchers such as the ontology report page for otitis media which lists all of the genes associated with OM in chinchilla and other species, including human.

Conclusion: The RGD website provides seamless access for a wide variety of data types across multiple species, including chinchilla, making RGD an invaluable resource for researchers interested in chinchilla specifically, and in otitis media and related diseases in general.
Abstract: Introduction: Down syndrome (DS) is a common chromosomal abnormality involving full or partial trisomy of human chromosome 21 (Hsa21). DS has a variety of phenotypes, including craniofacial defects and learning difficulties. 96% of DS children also suffer from hearing loss due to fluid accumulation in the middle ear cavity (Otitis Media with Effusion (OME)), which further compounds their learning difficulties. The standard treatment for chronic OME, grommet insertion, is not possible for children with DS due to their increased susceptibility to infection. Therefore, we need to understand how they are genetically predisposed to OME to allow the development of effective therapies for hearing loss in DS.

The mouse orthologues to the genes from Hsa21 are present on mouse chromosomes 16 (Mmu16), 10 (Mmu10) and 17 (Mmu17). Our collaborators have developed mouse models of DS with full or partial duplication of the homologous regions to Hsa21. Upon determining the OM phenotype of these mice, we found that half of the Dp9Tyb mice and all of the Dp5Tyb mice have OM. The Dp5Tyb mice are of particular interest as they only have 12 genes present in 3 copies.

Objective: To investigate which of these 12 genes plays a role in the OM phenotype of Dp5Tyb mice.

Methods: We designed primers for the genes in the Dp5Tyb region and tested their fidelity on wild-type (WT) mouse tissues known to express these genes. PCRs were then carried out on RNA extracted from WT middle ear epithelial cells (ECs) to study the expression of the genes in the middle ear.

Based on the known role of these genes we selected two proteins encoded by Hsa21 genes, ERG and ETS2, to look for protein localisation by immunohistochemistry (IHC) on control tissue and head sections from Dp5Tyb and WT mice.

A western blot was carried out to identify the presence of ERG and ETS2 in lung lysate then WT middle ear EC lysate.

Results: The PCR results showed that Erg and Ets2 are expressed in WT middle ear ECs.

IHC indicated that ERG is localised in very few middle ear ECs in WT mice, but more positive cells were seen in Dp5Tyb mice. ETS2 was expressed much more strongly than ERG in the middle ear EC lining of both WT and Dp5Tyb mice. In addition, ETS2 positive cells were seen in the middle ear cavity fluid of Dp5Tyb mice with OM. The preliminary data from the western blot analysis showed high levels of both proteins in WT lung lysate and low levels, especially for ERG2, in the middle ear EC lysates.
Conclusion: The expression data for the 12 Dp5Tyb genes will help us understand their role in the development of OME. The next step will be to analyse the genes in the Dp9Tyb region using the same methods. Identifying the gene(s) which in three copies result in the development of OM could allow therapies to be developed to treat OME in DS children, improving their hearing and therefore their ability to learn and interact with their surroundings.
**Poster # 47**

**Presenting Author:** Sean Stacey Ph.D.

**Institution(s):** Nationwide Children’s Hospital

**Title:** Rapid Gene Regulation by nontypeable Haemophilus influenzae Resulted in Altered Adherence to Respiratory Tract Epithelial Cells and Mucus

**Abstract:** **OBJECTIVE:** Nontypeable Haemophilus influenzae (NTHi) is a pathobiont of the human respiratory tract. NTHi attaches to and colonizes the human nasopharynx via a wide array of adhesive proteins, or adhesins, and resides therein as a commensal organism. However, NTHi is also one of the three major pathogens of middle ear infection (otitis media; OM), and is the major causative agent of chronic OM, recurrent OM, and OM in which treatment has failed. The environments of the nasopharynx and middle ear are somewhat unique and NTHi has developed a powerful mechanism to successfully adapt to and persist in these unique microenvironments termed the ‘phasevarion,’ for phase variable regulon. Phasevarions allow NTHi to rapidly and reversibly regulate the expression of multiple gene products. Phasevarion switching results in two genetically identical but phenotypically unique subpopulations (e.g., ‘ON’ and ‘OFF’). The capacity for diversification markedly increases the adaptability and survivability of NTHi in its’ human host. While phasevarions have been identified in the genomes of multiple human mucosal pathogens, the role they play in adherence has not yet been fully characterized. We showed that NTHi regulates expression of several adhesins via its phasevarion, thereby we hypothesized that this outcome would also influence adherence to respiratory tract epithelial cells and mucus. We investigate whether phasevarion regulation affects the ability of multiple NTHi clinical isolates to adhere to cells and mucus of the respiratory tract.

**METHODS:** To study the effects of the phasevarion, genetically modified variants, termed ‘locked ON’ or ‘locked OFF,’ of four NTHi clinical isolates, which possess four of the five phasevarions most commonly associated with OM, were assayed. These locked variants are unable to phase- vary which allows us to separate the two phenotypic subpopulations. We can also assess how NTHi uses the phasevarion to respond to biological cues or conditions without the complication of ongoing phase-variation. Variants were assayed for relative ability to adhere to mucus harvested from human airway epithelial (HAE) cells or to the apical surface of both HAE and chinchilla middle ear epithelial (CMEE) cells.

**RESULTS:** We observed that the ability of NTHi to adhere to mucus, HAEs, or CMEEs varied significantly among the tested isolates, and that differential regulation of adhesins by these four phasevarions affected how well each NTHi strain adhered to the tested targets.

**CONCLUSIONS:** To date, we know that the phasevarions of NTHi regulate expression of gene products that affect pathobiology; for example, response to oxidative stress, resistance to opsonization and therefore phagocytosis by host immune cells, and biofilm formation. However, ours is the first study to investigate the possible effects the phasevarion has on the ability of NTHi to adhere to human airway epithelial cells and the mucus they produce. The
phasevarion is a potent tool that NTHi has evolved to adapt to variable microenvironments and persist in its' human host. A complete understanding of the phasevarion of NTHi will reveal how NTHi causes disease, and aid in the development of more efficient therapies and cures.
Support: NIH/NIDCD R01DC015688
Poster # 48

Presenting Author: Hilda Tateossian Dr

Institution(s): MRC Harwell Institute

Title: Otitis Media in Down Syndrome: Towards Identification of the Gene for OM Using Mouse Models

Abstract: Down syndrome (DS) is caused by an extra copy of some or all of the genes of human chromosome 21 (Hsa21). This condition results in a number of phenotypes one of which is hearing loss as a result of the development of otitis media with effusion (OME).

To understand better the genetic basis for OME in DS we are currently studying mouse models of DS at the MRC Harwell Institute. The orthologs to the genes from Hsa21 are spread in three regions on the mouse genome located on chromosomes 10 (Mmu10), 16 (Mmu16) and 17 (Mmu17) and we have undertaken detailed studies of a large number of mice carrying genome duplications across larger and smaller segments of regions syntenic to Hsas1. Mutants with duplication of the genes from Mmu10 (Dp2Yey) and Mmu17 (Dp3Yey) revealed no middle ear inflammation. However, mice with duplication of the genes from Mmu16 (Dp1Tyb) all had bilateral OME, suggesting that this region contains the gene(s) that contribute to the OME phenotype. We tested the mice for hearing loss by performing click-evoked auditory brainstem response (ABR). At two months of age, the Dp1Tyb carriers had elevated mean thresholds (+27.5 dB SPL) compared with wild-type mice. In addition assessment of the inner ear of the mice by scanning electron microscopy and histology revealed no indication of a sensorineural element to the hearing loss. These results suggested that the Dp1Tyb mutants have conductive hearing loss. Further examination revealed that they also had thickened middle ear mucosa, exudate rich in neutrophils and macrophages, intact tympanic membranes and craniofacial defects. To narrow down genes which in three copies result in OME, we first examined the phenotypes of duplication mice, Dp2Tyb, Dp3Tyb and Dp9Tyb, each of which comprises a separate segment of Dp1Tyb. While we detected low levels of OME in Dp9Tyb mice compared to Dp1Tyb mice and no OME in Dp2Tyb mice, we observed substantial OME in Dp3Tyb mice. We have proceeded to examine mice with duplication of smaller segments of the Dp3Tyb region for OME - Dp4Tyb, Dp5Tyb and Dp6Tyb – and have found that only Dp5Tyb mice had OME. The Dp5Tyb region contains only 12 genes. We are currently analysing the expression data of these 12 genes in the middle ear and are crossing the Dp5Tyb carriers to single gene knockouts to see if the OME phenotype is rescued in the double mutants.

The identification of the causative gene(s) from the Dp5Tyb region will lead to a better understanding of the mechanisms leading to middle ear inflammation in DS and potentially aid the development of new therapeutic approaches for this condition.
Poster # 49

Presenting Author: Roderick Venekamp MD PhD

Institution(s): Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht

Title: Pain Management in Acute Otitis Media: a Qualitative Study of Parents’ Views and Expectations

Abstract: Introduction. Earache is a key symptom of acute otitis media (AOM). For unclarified reasons, parents tend to be cautious about administering analgesics to their children, potentially leading to suboptimal management of AOM symptoms. We aim to understand parents’ views and expectations of pain management in childhood AOM.

Methods. Qualitative study alongside a cluster-randomized controlled trial (PIM-POM study) aimed at optimizing pain management in childhood AOM. We purposefully sampled 14 parents of children diagnosed with AOM by their general practitioner (GP), who were recruited to the trial between November 2017 and May 2018. Semi-structured interviews were held at home in the first two weeks after trial enrollment. Interviews were audio-recorded, transcribed and analyzed thematically.

Results. Parents experienced difficulties in recognizing earache and other symptoms of an ear infection. They consulted the GP for a diagnosis, for reassurance and for management advice. Parents shared that, prior to consultation, they had insufficient knowledge of the benefits of correctly dosed pain medication at regularly scheduled intervals. Parents valued GP’s advice on pain management, and were happy to accept pain medication as standalone therapy, provided that the GP explained why antibiotics would not be needed. Parents’ views and expectations of pain management in AOM were shaped by previous experiences of AOM within their family; those with a positive experience of pain medication are more likely to use it in subsequent AOM episodes.

Conclusions. Parents of children with AOM consult the GP to help cope with uncertainties in recognizing symptoms of AOM, and receive management advice. It is important that GPs are aware of parents’ lack of understanding of the role of pain medication in managing AOM, and that they address this during the consultation.
Poster # 50

Presenting Author: Pratik Vikhe PhD

Institution(s): Medical Research Council - Harwell

Title: NTHi Phosphocholine Induces Immune Tolerance in the Bulla Fluid of the Junbo Mouse Model of Acute Otitis Media.

Abstract: Introduction: Phosphocholine is a minor component of the lipopolysaccharide (LPS) expressed on the surface of NTHi but bacterial variants expressing it are more efficient in causing infection. The phosphocholine on the LPS is known to play an important role in host interactions and helps bacteria evade the immune response against them through evasion of antibody binding and modulation of cytokine expression. NTHi is a major OM pathogen but the mechanisms(s) behind the immune tolerance required for sustained and chronic infection are not clear.

Objective: The objective of the current study was to elucidate the T-reg mediated immune tolerance induced by phosphocholine expressed on the surface of NTHi.

Methods: Phosphocholine expressing variants of NTHi strains were assayed for their effect on T-reg cell induction in-vivo using the Junbo mouse, a well-characterised chronic and acute otitis media infection model, and in-vitro using mouse bone marrow derived dendritic cell (DC) culture followed by a T-cell polarisation assay. Multicolour flow-cytometry and intracellular staining were used to characterise the immune cell population.

Results: The cellular immune response in the bulla fluid of the Junbo mouse infected with NTHi expressing phosphocholine showed a rise in the T-reg (CD4+CD25+FoxP3+) cell population; this rise was not observed upon infection with NTHi variants (NTHilic1 – knockout and NTHiPCho-ve – natural non-expressing variant) deficient in phosphocholine on their LPS. In vitro stimulation of mouse bone marrow derived dendritic cells (DC) by NTHi strains showed variable levels of CD86 and CD80 expression that was dependent on NTHi phosphocholine levels. The DC's stimulated with phosphocholine deficient NTHi variants significantly reduced T-reg cell polarization compared to phosphocholine expressing NTHi.

Conclusion: These observations indicate a novel mechanism used by NTHi to induce immune tolerance through T-reg mediated immune suppression, which could play an important role in long term and recurrent acute otitis media. Further characterisation of cytokine/chemokine signalling is on-going to provide a more detailed insight into the signalling cross talk induced by NTHi phosphocholine; this will help our understanding of the molecular mechanism(s) involved.
**Poster # 51**

**Presenting Author:** Brett Wiesen

**Institution(s):** University of Colorado School of Medicine

**Title:** The Relationship between ABO Variants and Otitis Media in Finnish Families

**Abstract:**

Introduction: Previously an intronic variant rs781822643 within the ABO locus was identified as a genome-wide significant protective factor against otitis media. Earlier studies demonstrated an association between blood type and otitis media, such that type O is protective against otitis media with effusion, while type A increased risk for acute or secretory otitis media.

Objective: To determine if there was any association between ABO variants or blood types and otitis media.

Methods: DNA samples from 214 probands from Finnish families with recurrent acute (RAOM) and/or chronic otitis media with effusion (COME) were submitted for exome sequencing. Of these Finnish probands, 43 had blood types available from clinical records. Using the exome data, ABO blood type was predicted for the rest of the Finnish cohort: (1) Heterozygous genotypes for a haplotype consisting of three ABO variants [c.703G>A(p.Gly235Ser);c.796C>A(p.Leu266Met);c.803G>C(p.Gly268Ala)] conferred a B phenotype. (2) For ABO c.260insG (p.Val87Thr88fs*), the wildtype genotype resulted in type O while homozygous genotype conferred the A phenotype. (3) Type AB was assigned if heterozygosity for the 3-variant haplotype and homozygosity for c.260insG both occurred. (4) All other genotype combinations were assigned as type A. For the general Finnish population, the minor allele frequency for the c.260insG and c.703G>A were derived from the Finnish genotypes in the genome Aggregation Database (gnomAD). Fisher exact tests were performed when (a) comparing frequencies of ABO genotypes in the Finnish probands with otitis media vs. counts in gnomAD Finnish, and (b) within the Finnish family cohort, comparing occurrence of RAOM vs. COME according to ABO genotype/haplotype and predicted blood type. Age and sex were also included in the study as potential covariates in logistic regression analysis.

Results: Fifteen coding variants within the ABO gene were identified, of which the [c.703G>A(p.Gly235Ser);c.796C>A(p.Leu266Met);c.803G>C(p.Gly268Ala)] and c.260insG variants were the most significant eQTLs for airway or mucosal tissue in the GTEx database. The proportions of predicted blood types within the Finnish family cohort was comparable to the general Finnish population according to national data. Female sex is protective against having both RAOM and COME (OR=0.51; p=0.02). No association between otitis media and genotypes for the ABO c.260insG and c.703G>A genotypes were identified. Type O was protective against RAOM (OR=0.33; p=0.04). On the other hand, type A was associated with increased risk for COME (OR=2.20; p=0.03). These findings remained significant even when adjusted for age and sex.
Conclusion: Within the Finnish family cohort, type O is protective against RAOM while type A increases risk for COME. This suggests that the association between the ABO locus and otitis media is specific to blood type, otitis media type and cohort.
Abstract: Introduction: Prevalence of chronic suppurative otitis media (CSOM) among children in remote Australian Aboriginal communities remains high. Clinical failure is over 70% using recommended treatment. This study evaluated combination therapy to improve polymicrobial pathogens that cause CSOM. Objective: An RCT took place in 28 remote and urban communities of the NT to determine among Aboriginal children with CSOM if either of the following strategies given twice daily (in addition to standard treatment—twice daily ear cleaning and ciprofloxacin drops) reduced the proportion of children with discharging perforations after 16 weeks of treatment; A) antiseptic ear wash (>= 20mls povidone-iodine 0.5% solution syringed into the ear canal) or no ear wash, or B) oral antibiotics (cotrimoxazole 4mg/kg per dose of trimethoprim component) or placebo. Methods: The Indigenous Healthy EARS using BEtadine, Tissues and Antibiotics (I HEAR BETA) study was a parallel group, factorial design, randomised (allocation concealed), assessor blinded, controlled clinical trial. With a total of 280 children and control failure rate of 70%, there was ~88% power to detect an effect size of 20% for each intervention, assuming 10% loss to follow up. Participant eligibility was age 6 months to 17 years with CSOM (discharge > 2 weeks and perforation size > 2%). Results: Of the 1732 children screened in 28 communities, 355 (20%) had discharging perforations and 280 (16%) were randomised. Mean age at randomisation was 7 years. Primary outcome was clinical failure (presence of any ear discharge) after 16 weeks of treatment; 66 (49.3%) in the betadine group versus 69 (50.7%) in the no betadine group (RD= -0.5% (-12,11), p= 0.88) and 56 (41.8%) in the cotrimoxazole group versus 79 (58.1%) in the placebo group (RD=16% (-28,-4), p=0.015), after adjusting for the other intervention and stratification variables. Across all groups, 50% of children had dry ears in the study after 16 weeks. Conclusion: Cotrimoxazole may play an important role in reducing the burden of CSOM and these results may contribute to much needed evidence-based management of CSOM in this population. Compliance and microbial data will be further examined to determine the role of both cotrimoxazole and betadine in reducing discharge and microbial carriage at 16 weeks and at 12 months.
**Poster # 53**

**Presenting Author:** Jianghong Xu MD

**Institution(s):** ENT Institute and Otorhinolaryngology Department, Affiliated Eye and ENT Hospital, Fudan University, Shanghai, China

**Title:** The Molecular Microbiological profile of Otitis Media with effusion in Chinese Children

**Abstract:** Introduction: Otitis media with effusion (OME) is one of the most common pediatric diseases worldwide. Some studies have found the diversity of microbiome in middle ear effusions from developed countries. But no microbiological studies of middle ear effusions from Chinese children with OME have been reported. The study aimed to characterize the middle ear and nasopharyngeal microbiological profile in children with OME and compare the microbial flora of nasopharynx from patients with OME and children without otitis media.

Methods: Middle ear effusions and adenoid swabs were acquired from 15 children undergoing ventilation tube insertion and adenoidectomy. Nasopharyngeal swabs from 15 patients without ear diseases were controls. Samples were analyzed using 16S rRNA sequencing on an IlluminaHiSeq2500 platform.

Results: The total numbers of operational taxonomic units (OTUs) were similar among the middle ears (TM) and nasopharynx (TN) of OME subjects and nasopharynx of controls (CN). But the microbiota of TM was found to be dissimilar to that of the TN (P = 0.001), whereas the TN and CN microbiota were similar (P = 0.06) (Analysis of similarities). The middle ear effusion was dominated by Haemophilus (14.75%), Staphylococcus (9.37%), Streptococcus (5.76%), Halomonas (7.85%), Bacteroides (6.27%). The bacterial composition of nasopharynx in OME groups was dominated by Haemophilus (21.87%), Streptococcus (19.65%), Neisseria (5.8%), and Moraxella (5.16%). The bacterial composition of nasopharynx in control groups was dominated by Haemophilus (15.96%), Streptococcus (13.33%), Moraxella (12.28%), Neisseria (4.79%). The significant differential taxa between the nasopharynx and the middle ear in OME patients were Streptococcus, Moraxella and Neisseria.

Conclusions: Our results confirmed the microbiota diversity of middle ear effusions in Chinese children. But the dissimilar microbiome composition between the adenoid and the middle ear question the theory that the nasopharynx serves as a reservoir microbiota of middle ear in children with otitis media with effusion.
Presenting Author: Rong Yang PhD

Institution(s): Boston Children's Hospital

Title: Synergy between Chemical Permeation Enhancers and Drug Permeation across the Tympanic Membrane

Abstract: Chemical permeation enhancers (CPEs) can enable antibiotic flux across the tympanic membrane for the treatment of otitis media. Here we study whether combinations of CPEs (sodium dodecyl sulfate, limonene, and bupivacaine hydrochloride) are synergistic and whether they could increase the peak drug flux. Synergy is studied by isobolographic analysis and combination indices. CPE concentration-response (i.e. trans-tympanic flux of ciprofloxacin) curves are constructed for each CPE, isobolograms constructed for pairs of CPEs, and synergy demonstrated for all three pairs. Synergy is much greater at earlier (6 hours) than later (48 hours) time points, although the effect sizes are greater later. Synergy is also demonstrated with the three-drug combination. Combinations of CPEs also greatly enhance the maximum drug flux achievable over that achieved by individual CPEs.
Poster # 55

Presenting Author: Myung Hoon Yoo

Institution(s): Department of Otorhinolaryngology-Head and Neck Surgery, School of Medicine, Kyungpook National University

Title: Extrusion rate and complications according to the type of ventilation tube: Multicenter registry study on the effectiveness of ventilation tube insertion in pediatric patients with chronic otitis media with effusion—Part II

Abstract: Introduction: Ventilation tube insertion for chronic otitis media with effusion is most commonly received operation in pediatric population. Various types of tube are used, but it is unclear as to what impact the tube shape and material have on extrusion and complication rates.

Objective: This multicenter registry study aimed to investigate the effectiveness of ventilation tube insertion and the microbiology of otitis media with effusion in children. This part II study was conducted to evaluate the postoperative results especially according to the type of ventilation tube.

Methods: Patients
**Poster # 56**

**Presenting Author:** Kazuhiro Yoshinaga MD

**Institution(s):** Oita University, Faculty of Medicine, Department of Otolaryngology

**Title:** Effect of cholera toxin on Group 2 innate lymphoid cell in mucosal and lymphoid tissues.

**Abstract:**

Objective: Acute otitis media (AOM) is one of the most common infectious diseases in children, and Nontypeable Haemophilus influenzae (NTHi) is Gram-negative bacteria that are considered major pathogens of AOM and respiratory tract infections. Previously, we reported that intranasal immunization with outer membrane protein or P6 from NTHi and mucosal adjuvants can induce antigen-specific immune responses in systemic and upper airway, and it enhances NTHi clearance from the nose and middle ear. Mucosal adjuvants, such as cholera toxin, are important to elicit mucosal and systemic immune responses to bacterial antigens. In recent years, innate lymphoid cell (ILC), is known to participate in various mucosal immune responses. In this study, we investigate the effect of mucosal adjuvant via nasal route on Group 2 innate lymphoid cell (ILC2) in mucosal tissues, cervical lymph nodes and spleen.

Methods: BALB/c (male: 5-6 weeks old) mice were intranasally administered 1 μg cholera toxin (CT) once a week for 1 or 2 times. Control mice were administered phosphate-buffered saline alone. At 1 week after CT administration, these mice were sacrificed to collect nasal mucosa, lung, cervical lymph nodes and spleen. Mononuclear cells (MNCs) were isolated from these tissues by enzymatic digestion or physical disassociation. ILC2 were concentrated by using the Mouse ILC2 Enrichment Kit. The collected ILC2 rich cells were stained with Pacific blue-labeled anti-CD 45 antibody, FITC-labeled anti Linear (+) antibody, PE-labeled CD 278 antibody, and flow cytometric analysis was performed to examine the proportion of ICL2 in ILC2 rich cells.

Results: The percentage of ICL2 in the nasal mucosae increased according to the number of doses compared to the control, but no change was observed in the cervical lymph nodes and the spleen. In the lung tissues, the proportion of ICL2 was slightly increased in a single dose, but not in 2nd dose compared to the control.

Conclusion: CT has been used as a potent adjuvant for inducing mucosal immune responses. Our study indicated that nasal administration of CT adjuvant may affect ICL2 population in the nasal mucosa, and ICL2 may be related to inducing mucosal immune responses to antigen.