Association of Pepsin with Inflammatory Signaling and Effusion Viscosity in Pediatric Otitis Media

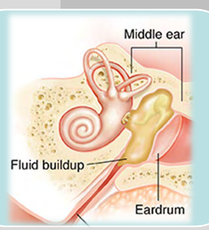
Tina L. Samuels†, Pawjai Khampang†, Mana Espahbodi†, Caroline A. McCormick†, Robert H. Chun†, Michael E. McCormick†, Ke Yan‡, Joseph E. Kerschner†§, Nikki Johnston†§

Departments of †Otolaryngology, ‡Pediatrics Quantitative Health Sciences, and §Microbiology and Immunology, Medical College of Wisconsin, Milwaukee, WI USA

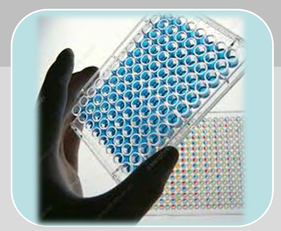
Clinical and *in vitro* data implicate pepsin in neutrophil activity and mucin hypersecretion leading to viscous effusion during OM

FINDINGS IN CLINICAL SPECIMENS

METHODS



30 ROM/OME middle ear effusions



ELISA for pepsin[5], IL-6, IL-8, Neutrophil elastase, MUC5B (R&D Systems)

Comparison of analytes with patient/disease characteristics

RESULTS

PEPSIN IN EFFUSION CORRELATES WITH CYTOKINES, MUCIN, NEUTROPHIL MARKERS AND EFFUSION VISCOSITY

- PEPSIN IN 77% CASES
- RANGE: 71-2734ng/ml

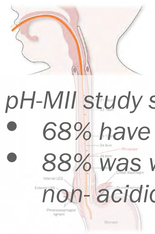
- PEPSIN-NEGATIVITY CORRELATED WITH ABSENCE OF IL-8 AND MUC5B ($p<0.05$)
- FOLLOW-UP TTI REQUIRED IN 47% PEPSIN-POS CASES BUT NO PEPSIN-NEG CASES
- MUC5B 188-FOLD HIGHER IN PEPSIN-POS

Variable	Correlation coefficient	p value
IL-6	0.420	0.0208
IL-8	0.579	0.0008
Neutrophil elastase	0.478	0.0075
MUC5B	0.414	0.0229
Effusion viscosity	0.433	0.0213

Variable	Pepsin Negative (n=7)	Pepsin Positive (n=23)	p-value
f.u. TTI (number, %)	0 (0%)	8 (47%)	0.058
IL-8, pg/ml (mean±SD)	34952.6 ± 83832.8	52723.3 ± 93481.9	0.095
IL-8 positive (number, %)	4 (57%)	23 (100%)	0.009
NE, ng/ml (mean±SD)	3159.7 ± 6509.7	9073.4 ± 11739.2	0.106
NE positive (number, %)	7 (100%)	23 (100%)	>0.999
MUC5B, ng/ml (mean±SD)	15.0 ± 39.7	1224.5 ± 2821.3	0.017
MUC5B positive (number, %)	1 (14%)	15 (65%)	0.031

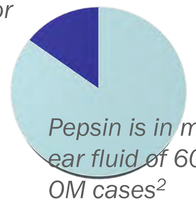
No correlation observed with age, BMI, hearing loss, allergy/asthma
Documented history of reflux was rare and showed no correlation

BACKGROUND



pH-MII study shows[1]
- *68% have EER*
- *88% was weakly or non- acidic*

NONACID EXTRAESOPHAGEAL REFLUX AND REFLUXED PEPSIN IN THE MIDDLE EAR ARE COMMON DURING OM



Pepsin is in middle ear fluid of 60-100% OM cases[2]

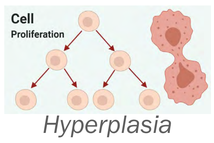
Severe reflux is a leading risk factor for recurrent OM[3]

ODDS RATIO: 4

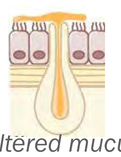
PEPSIN-MEDIATED AIRWAY DAMAGE SHARES PATHOLOGICAL FEATURES WITH OM[4]

Persistent inflammation

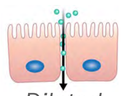
PEPSIN



Hyperplasia



Altered mucus production



Dilated intercellular space, altered barrier integrity or permeability to immune cells

HYPOTHESIS

PEPSIN CONTRIBUTES TO OM
- PATHOPHYSIOLOGICAL PROCESSES
- SEVERITY & OUTCOMES

CONCLUSIONS/IMPLICATIONS

- PEPSIN STIMULATES PROCESSES INVOLVED IN OM PATHOGENESIS WITH REPERCUSSIONS ON EFFUSION VISCOSITY AND OM RESOLUTION OR RECURRENCE
- THESE DATA SUPPORT ONGOING INVESTIGATION INTO THERAPEUTIC STRATEGIES THAT SIMULTANEOUSLY ADDRESS THESE COINCIDENT CHILDHOOD DISEASES

FUTURE DIRECTIONS

- IMMORTALIZATION OF PRIMARY ME CULTURES FOR FURTHER IN VITRO WORK
- INVESTIGATION OF PEPSIN-MEDIATED NEUTROPHIL INVASION AS OCCURS IN PEDIATRIC LUNG DISEASE[7]

EXPERIMENTAL FINDINGS

METHODS

Middle ear epithelial cells cultured from OME patient[6]

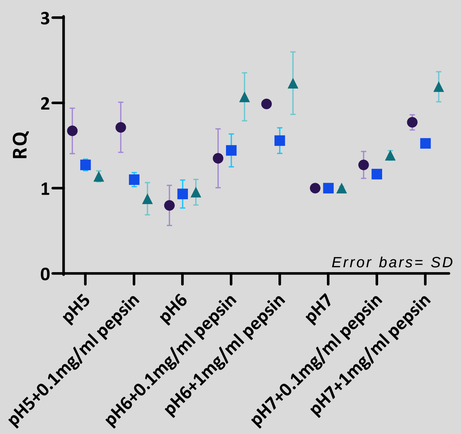
Treated with pepsin ± weak acid for 30min with 1hr rest before harvest (n=3)

Real-time PCR analysis of *IL6, IL8, TNFA*

Taqman Gene Expression Assays (ThermoFisher Scientific)

RESULTS

PEPSIN INDUCES OM-ASSOCIATED CYTOKINES INDEPENDENT OF ACID



- PEPSIN AT PH7 INDUCED IL6, IL8, TNFA ($p<0.05$)
- PH6 INCREASED SENSIVITITY TO LOWER PEPSIN CONCENTRATION
- PH5 ALONE INDUCED ONLY IL6

Treatment Condition	*IL6* (RQ±SD)	*IL8* (RQ±SD)	*TNFA* (RQ±SD)
pH5	1.67±0.27*	1.27±0.07	1.14±0.06
pH5+0.1mg/ml pepsin	1.71±0.29*	1.10±0.08	0.88±0.19
pH6	0.89±0.23	0.93±0.16	0.95±0.15
pH6+0.1mg/ml pepsin	1.35±0.35	1.44±0.19*	2.07±0.28***
pH6+1mg/ml pepsin	1.99±0.03**	1.56±0.15**	2.23±0.37***
pH7	1.00±0.01	1.00±0.03	1.00±0.02
pH7+0.1mg/ml pepsin	1.27±0.16	1.17±0.04	1.39±0.06
pH7+1mg/ml pepsin	1.77±0.09*	1.53±0.03**	2.19±0.18***

*$p<0.05$, **$p<0.001$, ***$p<0.0001$

REFERENCES
1. Górecka-Tuteja A, et al. J Neurogastroenterol Motil. 2016 Jul 30;22(3):452-8.
2. Miura MS, et al. Otolaryngology--Head and Neck Surgery. 2012;146(3):345-352.
3. Kotsis GP, et al. Int J Pediatr Otorhinolaryngol. 2009 Oct;73(10):1373-80.
4. Samuels TL, Johnston N. Curr Opin Otolaryngol Head Neck Surg. 2020 Dec;28(6):401-409.
5. Kiimara MJ, et al.. Ann Otol Rhinol Laryngol. 2020 Mar;129(3):224-229.
6. Espahbodi M, et al. Laryngoscope. 2021 Feb;131(2):410-416.
7. Hurley BP, et al. Sci Rep. 2019 Sep 24;9(1):13778.

The authors have no financial relationships to disclose or Conflicts of Interest to disclose.
Correspondence may be directed to Tina Samuels (tsamuels@mcw.edu) or Nikki Johnston (njohnston@mcw.edu).

Comparative effectiveness of modified surgical technique in prevention of otitis media with effusion recurrence and chronicity

Diacova Svetlana
Pediatric Otorhinolaryngology Clinic, Department of Otorhinolaryngology,
SUMPh "Nicolae Testemitanu" Chisinau, Moldova.

INTRODUCTION

Disadvantages of myringotomy with insertion of tympanostomic tube (MT) lead to recurrence or chronicity of otitis media with effusion (OME). We elaborated the modified technique of this surgery (MMT). (1)

OBJECTIVE

The aim of our study: to compare the results of the modified and classical types of surgery.

STUDY DESIGN AND SETTING

Study design.
An observational clinical trial.
Setting. Pediatric Clinic, ENT Department, SMPhU, Chisinau, Moldova.

MATERIAL AND METHOD

Subjects. A total of 286 children with chronic OME underwent MT. The ears were divided according to the surgical technique: classical (CMT) versus modified (MMT).
Methods. Postsurgical monitoring included standardized clinical and audiology examinations, Quality of life (QL) and General health (GH) assessment and otomicroscopy dynamic evaluation during 1 year. (2)

Outcome measures:

1. **Audiological examination with recording of hearing level was performed before surgery and every 3 months during 1 year after surgery.**
2. **Otomicroscopical evaluation of ears was done under general anesthesia in 12 - 18 months after TT insertion, at the time of tube removal. Presence of retractions, adhesions, granulation tissue and effusion was noted.**
3. **General Health (GH) and Quality of Life (QL) indexes were analyzed before surgery, and in 6 and 12 months after surgery.**

RESULTS

- **OM recurrence registered in 11% of CMT ears, in 2% of MMT ears. Undulating hearing recorded in 14% of CMT ears, in 4% of MMT ears. Chronic ME changes registered in 15% of CMT ears, in 3% of MMT ears. QL and GH stable positive dynamics in MMT patients was significantly greater than in CMT patients (p<0.01).**
- **Undulating hearing was recorded in Group CMT in 27 % of cases, comparing to 2 % of Group MMT (P<0.01). Hearing thresholds level during 12 mos in group CMT was 23 dB, in group MMT – 17 dB. (Fig. 1).**
- **Chronic changes in tympanic cavity: presence of effusion, attic retraction, adhesions and granulations in Group P was recorded in 24 % of cases, which statistically differs from the same data in Group C - 6 % (P<0.01). (Fig. 2).**
- **Improvement of General Health and Quality of Life indexes in Group MMT was more significant than in Group CMT (Fig. 3).**

Results of hearing monitoring

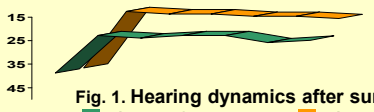
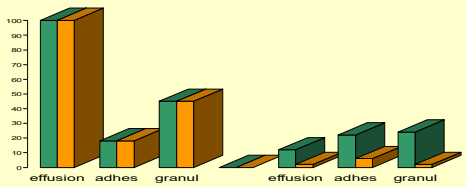


Fig. 1. Hearing dynamics after surgery
Group CMT **Group MMT**

Results of revision surgery



First surgery Revision surgery (in 12-18 mos)

Fig. 2. Morphological changes in tympanic cavity
Group CMT **Group MMT**

General health and quality of life

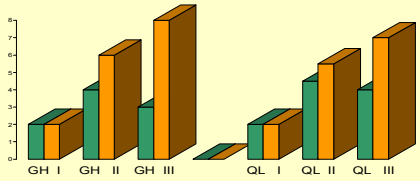


Fig. 3. General health (GH) and quality of life (QL).
I – before surgery, II- in 6 mos, III – in 12 mos
Group CMT **Group MMT**

CONCLUSIONS

Modified technique of Myringotomy with Tympanostomy tube insertion was more effective than classical one in preventing of recurrence of OME. Hearing restoration was more stable after MMT than after CMT. Chronic otomicroscopic changes: attic retraction, adhesions, timpanosclerosis, granulations registered more often after CMT than after MMT. QL and GH improvement in MMT patients was significantly greater than in CMT patients.

ABSTRACT

Study background and aims.
Disadvantages of myringotomy with insertion of tympanostomic tube (MT) lead to recurrence or chronicity of otitis media with effusion (OME). We elaborated the modified technique of this surgery (MMT). The aim of our study was to compare the results of the modified and classical types of surgery.
Methods.
A total of 286 children with chronic OME underwent MT. The ears were divided according to the surgical technique: classical (CMT) versus modified (MMT). Postsurgical monitoring included standardized clinical and audiology examinations, Quality of life (QL) and General health (GH) assessment and otomicroscopy dynamic evaluation during 1 year.
Results.
OM recurrence registered in 11% of CMT ears, in 2% of MMT ears. Undulating hearing recorded in 14% of CMT ears, in 4% of MMT ears. Chronic ME changes registered in 15% of CMT ears, in 3% of MMT ears. QL and GH stable positive dynamics in MMT patients was significantly greater than in CMT patients (p<0.01).
Discussion and conclusions.
Modified technique of Myringotomy with Tympanostomy tube insertion was more effective than classical one in preventing of recurrence of OME. Hearing restoration was more stable after MMT than after CMT. Chronic otomicroscopic changes: attic retraction, adhesions, timpanosclerosis, granulations registered more often after CMT than after MMT. QL and GH improvement in MMT patients was significantly greater than in CMT patients.

CONTACT

Doctor Diacova Svetlana, Associate Professor
Department ORL, SUMPh "Nicolae Testemitanu",
Chisinau, Moldova
+373 79617963 svetlana.diacova@usmf.md

REFERENCES

1. **Diacova S.**, Ababii I. Method of treatment of otitis media with effusion in children. Brevet of invention MD 674Y. BOPI, 2013-09-30, p. 24 – 25

2. **Diacova, S**. Post-surgical noninvasive monitoring of middle ear in otitis media prolonged forms. În: Mold Med J. Chişinău, Moldova, 2020; Nr. 3(63), p.11-15.

EVOLUTION OF OTITIS MEDIA IN SMALL CHILDREN WITH RESPIRATORY AND GASTROINTESTINAL PATHOLOGY BY MIDDLE EAR MONITORING

Diacova Svetlana
[1]Otorhinolaryngology, Pediatric Clinic, State University of Medicine and Pharmacy "Nicolae Testemitanu", Chisinau, Moldova

INTRODUCTION

Early identification of children with high risk of recurrent and chronic otitis media (OM) forms is important step in it management and prevention. Pathology of respiratory tract (PR) and gastro-intestinal pathology (GIP) in children often change clinical manifestations of otitis media (OM).

OBJECTIVE

•The aim of our research :
to analyze development and evolution of OM in healthy children and children with recurrent PR or GIP

STUDY DESIGN AND SETTING

Study design. Prospective survey & retrospective charts review.
Setting. Pediatric ENT Clinic, ORL Department, SUMPh, Chisinau, Moldova.

MATERIALS AND METHODS

Subjects. Children of early age of life without evident signs of OM
Group R with respiratory pathology (RP) - 784 and
Group G with gastrointestinal pathology (GP) - 125
Group H Healthy children - 165.

Methods: We conducted middle ear (ME) monitoring (MEM):

1. **screening tympanometry and otoscopy;**
   1. Frequency - every three months
   2. Duration - 5 years.
2. **audiology assessment & otomicroscopy for children who failed the screening,**
3. **paranasal sinuses evaluation, otological follow up for children with ME pathology.**

As a result, ME status was classified:

- **OM with effusion (OME)**
- **chronic OM with effusion (COME)**
- **acute OM (AOM)**
- **recurrent otitis media (ROM)).**

Cases with COME, ROM were evaluated and during surgery (1, 2).

CONTACT

Doctor Diacova Svetlana, Associate Professor
Department ORL, SMPhU "Nicolae Testemitanu", Chisinau, Moldova
+373 79617963 svetlanadiacova@usmf.md

MATERIALS AND METHODS

Monitoring scheme: 4 times/12 mos (5 years)

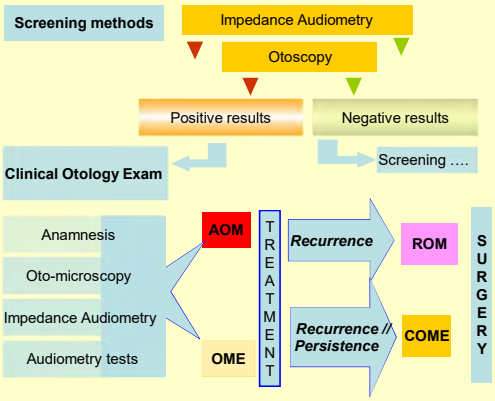


Figure 1. Algorithm of OM diagnostics

RESULTS

OM with effusion diagnosed in Group R in 59%, in Group G in 21%, in group H in 13%. It became chronic in group R in 34%, in group G in 12%, in group H in 3%. Recurrent acute OM registered in group R in 24%, in group G in 26%, in 2 % from group H. (3)

Chronic and recurrent OM were related to RT infection-prone children (p < 0.01), age younger than 5 years of life (p < 0.01) and sinusitis (p < 0.01).

Results of monitoring

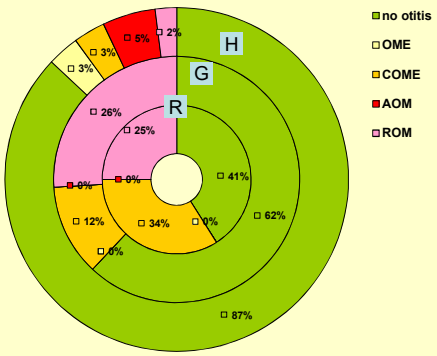


Figure 2. OM forms in 3 groups of children
R – Group of children with RP
G – Group of children GIP
H – Group of healthy children

CONCLUSIONS

1. Children with recurrent and chronic RP and GIP have to be included in ME monitoring.
2. High rate of OM chronicity is predetermined by recurrent pathology.
3. These groups of patients need comprehensive diagnostics and intensive treatment, including the surgical one.
4. In healthy children OM is a relatively rare and temporary condition.

ABSTRACT

Study background and aim. Pathology of respiratory tract (PR) and gastro-intestinal pathology (GIP) in children often change clinical manifestations of otitis media (OM). **The aim** of our research was to analyze development and evolution of OM in healthy children and children with recurrent PR or GIP.
Methods. We conducted ME monitoring (MEM) in children of early age: Group R contained 784 of children with recurrent RP, Group G – 125 children with recurrent GIP and Group H –165 healthy children. MEM included tympanometry and otoscopy every three months during 5 years. Children who failed the screening tests during 3 months were evaluated by complete audiological assessment, otomicroscopy and other methods.
Results. OM with effusion diagnosed in Group R in 59%, in Group G in 21%, in group H in 13%. It became chronic in group R in 34%, in group G in 12%, in group H in 3%. Recurrent acute OM registered in group R in 24%, in group G in 26%, in 2 % from group H.
Conclusion. Children with recurrent RP and GP have to be included in MEM. High rate of OM chronicity is predetermined by recurrent pathology. These groups of patients need comprehensive diagnostics and intensive treatment, including the surgical one. In healthy children OM is a relatively rare, temporary and benign condition.

REFERENCES

1. **Diacova, S.** Electro-acoustical Examination in Noninvasive Monitoring as a Basis for Treatment Selection. Proceedings of ICNBME-2019, September 18–21, 2019, IFMBE Proceedings. V. 77, p. 627 – 633.
2. **Diacova, S.**, Ababii I., Danilov L., et al. Functional and Morphological Correlations in Prolonged Otitis Media in Childhood. Proceedings of ICNBME-2019, September 18–21, 2019, Chisinau, Moldova. IFMBE Proceedings. V. 77, p. 633 – 639
3. **Diacova, S.** Evoluția otitelor medii la copii. Monografie. Chișinău. Centul editorial-poligrafic Medicina, 2019, 160 p.

Pharyngeal flora in small children as predisposing factor to recurrent and chronic suppurative otitis media

Diacova Svetlana
Pediatric Otorhinolaryngology Clinic, Department of Otorhinolaryngology,
SUMPh "Nicolae Testemitanu" Chisinau, Moldova.

INTRODUCTION

Direct connection of middle ear (ME) and nasopharynx via the short and orizontal audiotory tube in small children may predetermine influence of pharyngeal microbial flora on ME pathology.

OBJECTIVE

The aim of our study: to compare evolution of otitis media with effusion (OME), development of recurrent acute OM (RAOM) and chronic suppurative OM (CSOM) and bacterial flora of pharyngeal tonsils of patients at early age.

STUDY DESIGN AND SETTING

Study design. An observational prospective clinical trial.
Setting. Pediatric Clinic, ENT Department, SMPhU, Chisinau, Moldova.

MATERIAL AND METHOD

Subjects. A total of 545 preschool children with persistent and recurrent forms of OM and 50 healthy children were involved in prospective study.

Methods. included microbiological test from nasopharynx, ASL-O, C-reactive protein, rheumatoid factor; audiological and otomicroscopical assessment every 3 months during 3 years. All children received standard treatment (medical and surgical).

Outcome measures:

1. **We formed 3 groups of children in function of middle ear pathology presence: OME group, ROM group and control group.**
2. **microbiological test from nasopharynx, ASL-O, C-reactive protein, rheumatoid factor were analysed in function of clinical evolution of OM.**

RESULTS

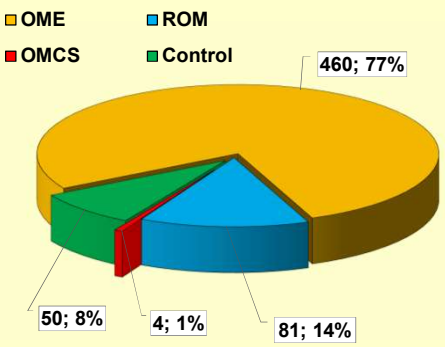
➢**Presence of Group A Strep B-hemolitic (GASBH) was detected in 12 % of children.**

➢**It corresponded to RAOM or CSOM in 64% of them.**

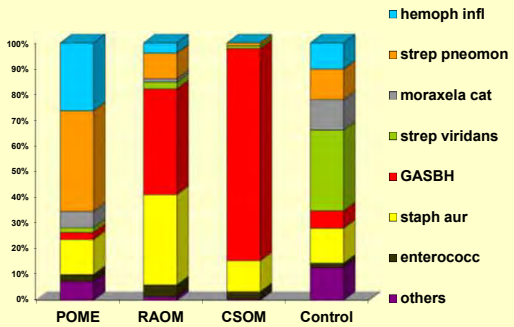
➢**Detection of Staph. Aureus in 26% of children corresponded to RAOM or CSOM in 19% of them.**

➢**Strep. pneumonia, M. *catarrhalis, Haemophilus influenza* were detected in 71% of children and were connected to RAOM or CSOM in 8% of them.**

OM forms



Microbs combinations from pharyngx



CONCLUSIONS

The pharyngeal microbial flora in children with OM predetermines negative evolution of OM. Children with GASBH, Staph. Aureus and OM have to be on close observation, need early comprehensive treatment including surgical one.

ABSTRACT

Study background and aims.
Direct connection of middle ear (ME) and nasopharynx via the short and orizontal audiotory tube in small children may predetermine influence of pharyngeal microbial flora on ME pathology. **The aim of study:** to compare evolution of otitis media with effusion (OME), development of recurrent acute OM (RAOM) and chronic suppurative OM (CSOM) and bacterial flora of pharyngeal tonsils of patients at early age.
Methods.
A total of 545 preschool children with OM were involved in prospective study which included microbiological test from nasopharynx, ASL-O, C-reactive protein, rheumatoid factor; audiological and otomicroscopical assessment every 3 months during 3 years. All children received standard treatment (medical and surgical).
Results.
Evidence of Strep. beta-hemolytic pyogenes in 12 % of children corresponded to RAOM or CSOM in 64% of them. Detection of Staph. Aureus in 26% of children corresponded to RAOM or CSOM in 19% of them. Strep. Pneumonia, M. *catarrhalis, Haemophilus influenza were detected in 71% of children and were connected to RAOM or CSOM in 8% of them.*
Discussion and conclusions.
The pharyngeal microbial flora in children with OM predetermines negative evolution of OM. Children with Str. beta-hemolytic pyogenes or Staph. Aureus and OM have to be on close observation, need early comprehensive treatment including surgical one.

CONTACT

Doctor Diacova Svetlana, Associate Professor
Department ORL, SUMPh "Nicolae Testemitanu",
Chisinau, Moldova
+373 79617963 svetlana.diacova@usmf.md

REFERENCES

1. **Granath A.** Recurrent Acute Otitis Media: What Are the Options for Treatment and Prevention? *Curr Otorhinolaryngol Rep.* 2017; 5(2): 93–100. Published online 2017 May 9. doi: 10.1007/s40136-017-0151-7
2. UK Standards for Microbiology Investigations . Investigation of Throat Related Specimens. ssued by the Standards Unit, Microbiology Services, PHEBacteriology | B 9 | Issue no: 9 | Issue date: 15.04.15 | Page: 1 of 29

Otitis Media prior to Cochlear Implantation; an evaluation of history taking across the life-course.

Matthew Edwards[1], Kate Hough[2], Mary Grasmeder[3], Callum Findlay[1], Carl Verschuur[3], Tracey A Newman[1]

[1]Faculty of Medicine University of Southampton, [2]Faculty of Engineering and Physical Sciences University of Southampton, [3]University of Southampton Auditory Implant Service.

Introduction

Cochlear implants are neuro-prostheses which are surgically inserted into the cochlea to restore hearing function in individuals with persistent profound to severe hearing loss. The factors affecting hearing performance and implant success are poorly understood. Otitis media is associated with increased cochlear implant complications and the effect of peri-implantation otitis media on cochlear implant performance is not fully understood. Macrophage priming, a mechanism of innate immune memory, can lead to developing maladaptive immune responses. In the context of otitis media and cochlear implants early life middle ear inflammation could predispose individuals to aberrant immune responses to cochlear implantation and subsequently affect wound healing and hearing performance**. Exploration of the relationship between historic otitis media and cochlear implant outcomes requires reliable documentation of middle ear histories prior to implantation**. This project evaluates documentation practice for a history of otitis media at University of Southampton Auditory Implant Service (USAIS).

Methods

Records from 718 cochlear implant cases at USAIS spanning nearly a decade were accessed. The data for the 562 patients that met the criteria for inclusion was reviewed for evidence of recording of middle ear history. 156 cases were excluded due to either implant surgery at a different clinic, or other incomplete health history.

Cochlear implant recipients were considered to have a documented history of otitis media if specific terms were found in clinical documents. Records were assessed for middle ear health factors including; middle ear investigations, history of otological surgery, middle ear damage and symptoms and treatments of otitis media. Ethical approval (ref 62161) was granted by the Faculty of medicine ethics committee, Southampton.

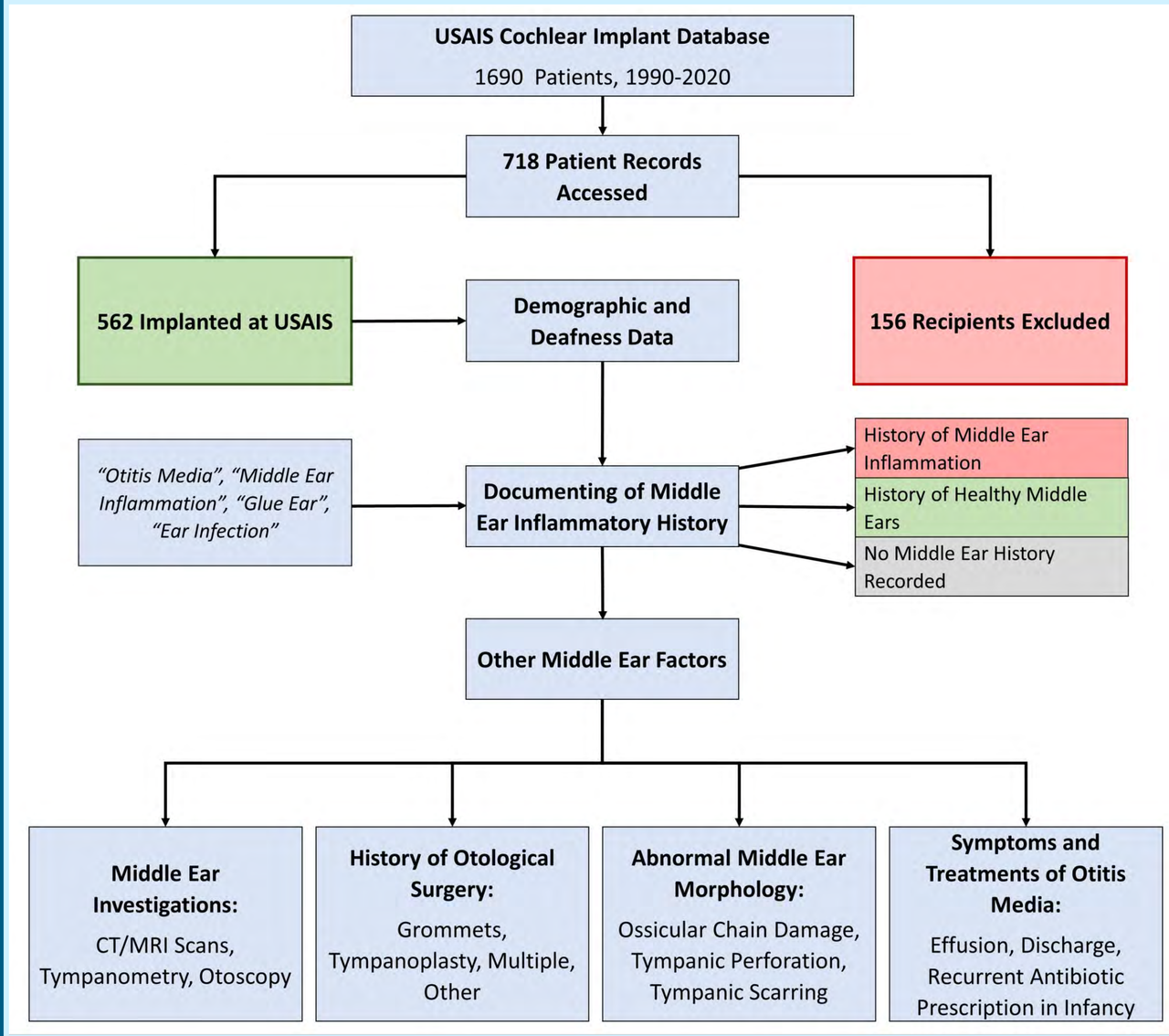


Figure 1. Flowchart illustrating the process for data collection including the number of participants evaluated, the terms utilized for documenting middle ear inflammation and other factors relating to the middle ear which were also collected.

Results

a. *Documentation Practice*

There is a difference in practice of documenting middle ear histories between children and adult recipients (Figure 2). The documentation is more complete for paediatric recipients than adults.

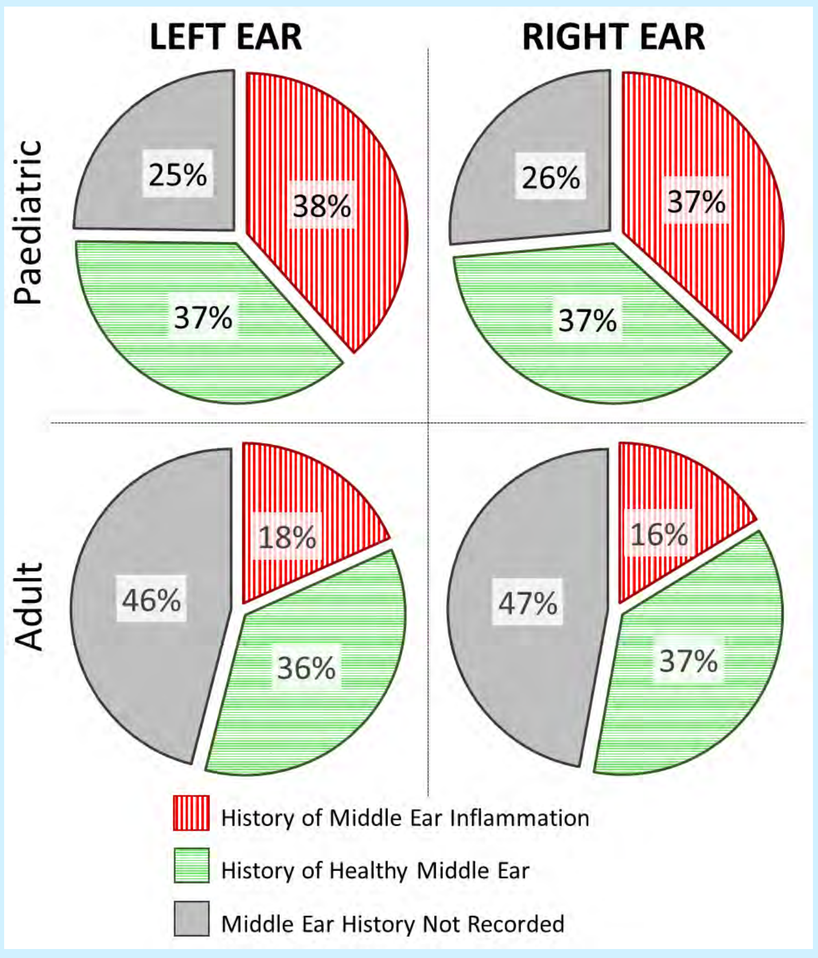


Figure 2. (see right) Visual comparison of documenting practice of otitis media histories between adult and paediatric cochlear implant recipients.

b. *Otological Surgery*

Middle ear health factors were analysed and several recipients who had prior otitis media were not documented as having had it. For example looking at histories of surgery (Figure 3); around 10% of recipients who had been recorded as having healthy middle ear histories, and around 10% of those without documented middle ear histories, had histories of prior middle ear surgery. The most common procedure was for grommets suggesting histories of middle ear inflammation.

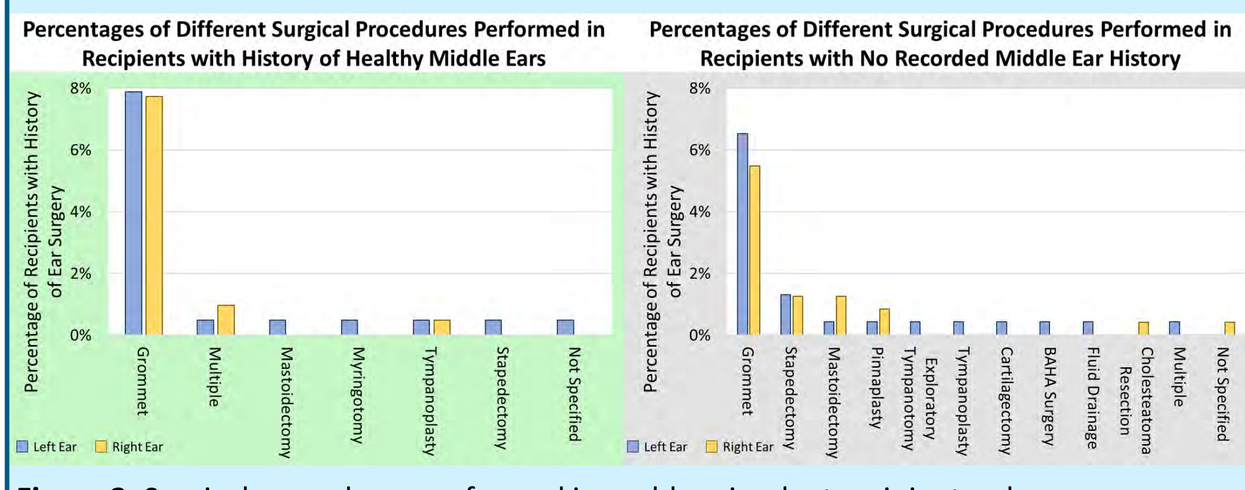


Figure 3. Surgical procedures performed in cochlear implant recipients who were documented with histories of healthy middle ears, or with no history documented.

Outcomes

- There is incomplete recording of middle ear history and Otitis media prior to cochlear implantation.
- Recording is more complete for children than adults.
- Some recipients who have had otitis media are not documented as having had a history of otitis media.

Conclusion

Analysis of the documenting of middle ear inflammatory histories at USAIS prior to implantation has identified that a history of otitis media has not been recorded for all patients. Due to coincidence of otitis media and the peak of cochlear implantation in children, there is as predicted, more thorough documentation amongst the children. Reliable, consistent recording of otitis media irrespective of patient age at time of implantation is needed so that the role of early-life middle ear inflammation as a prognostic factor and its effect on cochlear implant performance can be determined. Understanding this relationship may in the future facilitate research into strategies to improve hearing performance with an implant and deepen understanding of the mechanisms by which prior immune insults affect cochlear implantation outcomes.

me3n17@soton.ac.uk – *Matthew Edwards*

Eustachian tube function evaluation using a tympanometer – simplified protocols for intact and non-intact tympanic membranes

Miriam S Teixeira, MD PhD (1), Juliane M Banks, BS (1), Selma Cetin-Ferra, MD (3), Ellen M Mandel, MD (1), J Douglas Swarts, PhD (1), Cuneyt M Alper MD (1,2)

1) Department of Otolaryngology - University of Pittsburgh School of Medicine
2) Division of Pediatric Otolaryngology - Children's Hospital of Pittsburgh of UPMC
3) Department of Cell Biology – School of Medicine - Pittsburgh, PA - USA

Background: Tympanometers are devices easy to operate and widely available in medical offices. They are routinely used to evaluate the range of middle ear pressure, presence of middle ear fluids and patency of ventilation tubes. Although most tympanometers have built-in modules for assessing Eustachian tube (ET) function, these features are seldom used. Here we present examples of these features and how they can be used to quickly evaluate patients during outpatient office visits.

Methods: The resident testing modules of the Titan tympanometer (Interacoustics, Eden Prairie, MN) were customized into protocols to evaluate both the active and passive ET functions when the tympanic membrane was intact or non-intact. Maneuvers used to evaluate the passive properties of the ET function were Valsalva, Sniff, Patulous and Opening threshold. The ET active function was evaluated with the Inflation-Deflation test and with tympanograms before and after swallows.

Discussion: Although tympanometry is not a "test" per se, continuous monitoring of the middle ear pressure or sequential tympanograms can be used to assess changes in middle ear pressure during passive or active maneuvers. The limitations to a broader use of tympanometers include lack of desired operating pressure range, inability to concurrently track ear and nasal pressures and scarce software resources. Improvements in software and hardware could greatly expand their use for ET function evaluation.

TYMPANOMETRY

The tympanometer is used to measure relative middle ear (ME) pressure in reference to the ambient. The test is simple and requires only that a probe be sealed in the external ear canal for a few seconds. The probe introduces a sound and varies the pressure to move the tympanic membrane (TM) inward and outward and continuously records the reflected sound. ME pressure (MEP) is assigned as the applied pressure at which the reflected sound is at a minimum. When the TM is intact, the tympanometry will also provide information about TM compliance, ear canal volume and gradient. In case of non-intact TM due to a perforation or a ventilation tube, only a measure of volume is obtained in the regular tympanogram. Continuous middle ear pressure recording is used when the tympanometer settings are changed to non-intact tympanic membrane. (1)

An important caveat in performing the sequential or continuous data recording is to make sure that the patient does not swallow or move the jaw in between maneuvers as this may trigger the opening of the ET at a non-desired moment. (2)

Fig 1 – General tympanometer specifications:

- Speaker -> 226 Hz pure tone
- Microphone
- Pressure transducer -> sweep +/- external ear canal pressure
- Provides indirect measurement of middle ear pressure at a given moment

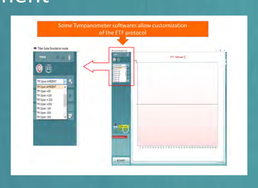


Fig 2 - Some tympanometers allow customization of the testing protocols such as the Titan from Interacoustics (Eden Prairie, MN) shown in the examples below.

1. NASOPHARYNGEAL MANEUVERS:

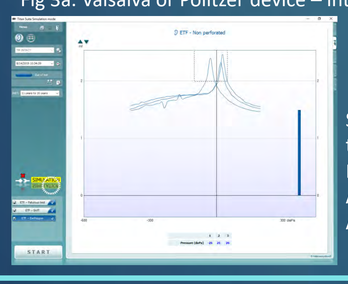
The nasopharyngeal maneuvers consist of the Valsalva and Sniffing maneuvers and can be done in intact and non-intact TMs.

For intact TMs, tympanograms are performed before and after each maneuver to measure changes in MEP. In ears with a non-intact TM, the test system uses the ear probe for continuous recording of MEP. The results of these tests include the variations in MEPs during the maneuver and the residual MEP.

1.A - VALSALVA MANEUVER - INTACT AND NON-INTACT TM: The nostrils are closed with the fingers and the patient is instructed to forcibly blow against the closed nostrils to ideally achieve a nasopharyngeal threshold of +400 daPa. Failure to increase MEP during the Valsalva test is interpreted as a high resistance to ET dilation (possible obstruction), but can also be an indication that the person is not able to perform the maneuver. An alternative, especially in small children that do not understand how to perform a Valsalva yet, is to use the EarPopper or another Politzer device to achieve the necessary increase in nasopharyngeal pressure to open the ET.
A tympanometry is performed before and after the Valsalva and a third tympanometry is repeated after a swallow (Fig 3a).
For non-intact TMs, the changes in MEP are recorded continuously (Fig 3b).

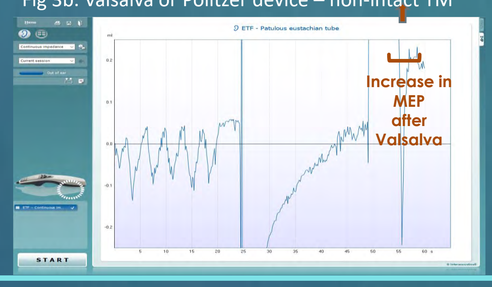
EarPopper: The EarPopper (Summit Medical, St. Paul, MN) is a commercially available device that is marketed to aid muscle-assisted ET opening. It consists of a single nosepiece connected to an air pump with no adjustable pressure settings. A baseline tympanometry is performed and then the subject will place the nosepiece into one of the nostrils and occlude the contralateral nostril with his/her finger, resulting in a fixed, constant airflow delivered to the nasal cavity. The subject will be asked to start the Ear Popper and swallow after about 3 seconds. Tympanometry is then repeated to assess changes in MEP, the patient swallows and a third tympanometry is performed.

Fig 3a. Valsalva or Politzer device – intact TM



Sequential tympanograms:
Baseline = -26 daPa
After Valsalva = 21 daPa
After swallow = 26 daPa

Fig 3b. Valsalva or Politzer device – non-intact TM



REFERENCES:

1. https://www.interacoustics.com/us/tympanometry/clinical-diagnostic/titan
2. Teixeira MS. Understanding Eustachian tube function. *Braz J Otorhinolaryngol*. 2020 Sep-Oct; 86(5):523-524 PMID: 32209307.
3. Doyle WJ, Swarts JD, Banks J, Casselbrant ML, Mandel EM, Alper CM. Sensitivity and specificity of eustachian tube function tests in adults. JAMA otolaryngology-- head & neck surgery. 2013;139(7):719-727.

Contact:
Miriam S Teixeira, M.D., PhD
Research Assistant Professor of Otolaryngology
University of Pittsburgh
E-mail: teixeirams@upmc.edu

Supported in part by: NIH grant DC007667

1.B - SNIFFING MANEUVER - INTACT AND NON-INTACT TM: The subject performs a forcible sniff, ideally achieving a nasal threshold of -400 daPa. Negative MEP followed sniffing is an evidence of an ET that is too easy to open with possible compromise of the protective function (Fig 4a). For non-intact TMs, the changes in MEP are recorded continuously (Fig 4b).

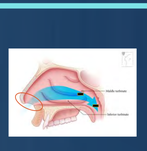
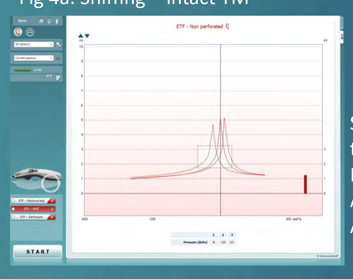
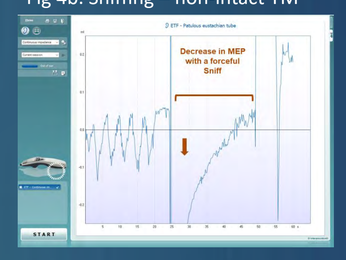


Fig 4a. Sniffing – intact TM



Sequential tympanograms:
Baseline = 0 daPa
After Sniffing = -33 daPa
After swallow = 17 daPa

Fig 4b. Sniffing – non-intact TM



2. PATULOUS TEST - INTACT AND NON-INTACT TM:

The ear probe is sealed in the external ear canal while the contralateral nostril is occluded with the fingers and the subject breathes at increasing intensity through the same nostril of the tested ear. The patient is asked to breath gently 3 times (or ≈ 10 seconds), strongly 3 times (or ≈ 10 seconds) and forcefully 3 times (or ≈ 10 seconds). The Patulous test detects if there are changes in TM position or middle ear pressure concurrent with breathing (Fig 5a) which is compatible with an ET too easy to open. For non-intact TMs, the changes in MEP are recorded continuously (Fig 5b).

Fig 5a. Patulous test – intact TM

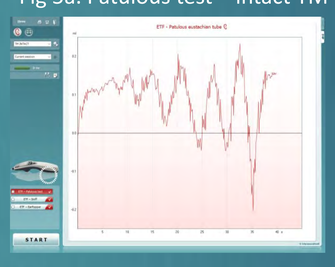
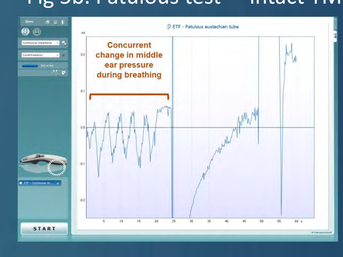


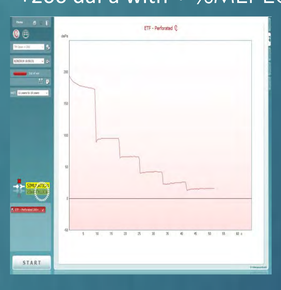
Fig 5b. Patulous test – intact TM



3. INFLATION-DEFLATION TEST – NON-INTACT TM:

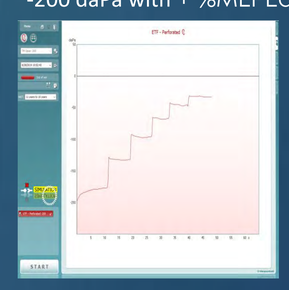
For the Inflation-Deflation test, MEP is increased to an over-pressure of +200 to +300 daPa (Fig 6a) or, if ET opening pressure is less than that value, to 30 daPa less than the ET opening pressure. The subject is asked to swallow 5 times at a natural interval to open the ET and gradually equalize MEP. The procedure is then repeated with an underpressure of -200 to -300 daPa (Fig 6b). This test measures the muscle-assisted tubal openings under stressed (underpressure) and facilitated (overpressure) conditions and can detect a patulous/semi-patulous ET by an inability to maintain applied ME over- and/or under-pressures. The variable for analysis is the residual MEP after the fifth swallow divided by the initial applied pressure, also known as the percentage of middle ear pressure equilibrated (%MEPEq). (3)

Fig 6a. Inflation-Deflation test at +200 daPa with + %MEPEq ≅ 90%



Sequence of 5 swallows

Fig 6b. Inflation-Deflation test at -200 daPa with + %MEPEq ≅ 80%



4. EUSTACHIAN TUBE OPENING THRESHOLD – NON-INTACT TM:

The goal is to apply increments of 50 daPa to the middle ear until the ET opens and air leaks to the nasopharynx (Fig 7). This will be considered the ET opening threshold. This is slightly similar to the opening pressure recorded via Forced Response Test and is a passive measure of the ET anatomical properties. (3)

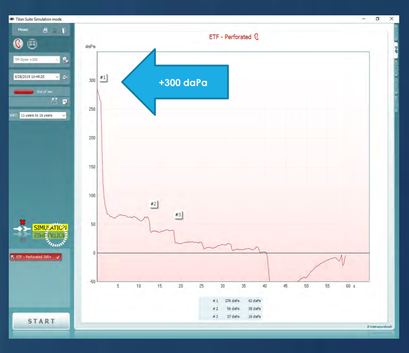


Fig 7. Eustachian tube opening threshold test. Air leakage was recorded when the pressure applied to the middle ear reached +300 daPa

Assessing Eustachian tube passive function parameters with Tubomanometry and Nasopharyngeal maneuvers not activating the mTVP

J. Douglas Swarts[1], Juliane M. Banks[1], Miriam S. Teixeira[1], Selma Cetin-Ferra[2], Cuneyt M. Apler[1]

[1]Division of Pediatric Otolaryngology and UPMC Children's Hospital Of Pittsburgh, [2]Department of Cell Biology
University Of Pittsburgh School Of Medicine Pittsburgh, PA, USA

Supported in part by: **NIH grant DC007667**

Introduction

Eustachian tube (ET) dysfunction underlies the pathogenesis of otitis media with effusion both in animal models[1] and in subjects with persistent disease[2] (Swarts, 2011). Various tests have been developed to assess ET function (ETF), the most informative are those requiring a non-intact tympanic membrane. The Forced response test (FRT) and Inflation-deflation test (IDT) yield information on the difficulty of opening the ET—surface and tissue forces–the resistance to airflow, and the effectiveness of muscular forces for ET dilation[3]. These tests can discriminate between passive—structural/surface—and active function components of ETF, thus focusing potential treatments.

Two deficiencies manifest with these tests, they require a tympanic membrane perforation, and they assess ETF in a counter-current direction, airflow is opposite the "natural" direction. The Toynbee maneuver, Valsalva, and politzerization were all developed to assess ETF using super-ambient pressures with or without swallowing. However, these tests are highly dependent of patient compliance and effort. To mitigate those issues and to standardize the nasopharyngeal based testing Tubomanometry was developed and evaluated.[4-6] (Since its description it has been employed extensively for assessing active ETF primarily in association with studies of Balloon dilation of the ET.[7-9]

Standard Tubomanometry is a NP pressure augmented swallow analogous to the Toynbee maneuver. As such it measures the active function of the ET. While it may be possible to extract variance attributable to the pressure difference while controlling for the active dilation effects; to this point that has not been done. An alternative strategy is increasing the NP pressure without triggering the Tensor veli palatini muscle as happens during a swallow. To that end we explored the use of NP maneuvers (NPM) that elevate the palate without triggering that muscle. That decoupling allows us to examine the passive function ETF properties driven only by the magnitude of the pressure in the NP.

Methods

Sample:

Data used in this analysis were extracted from the results of ET function experiments performed in the Middle Ear Physiology Laboratory at UPMC Children's Hospital over the past decade. These studies were approved and monitored by the University of Pittsburgh IRB. Subjects were recruited from the community and gave their informed consent. After screening--as appropriate for the experiment--each subject gave a general medical history, a detailed ENT history then underwent a head and neck physical exam. Their nasopharyngeal and otologic conditions were documented. For this analysis subjects had to have at least one non-intact tympanic membrane. Control subjects had a tympanostomy tube inserted approximately 4 weeks placed their testing sessions.

Testing Methods:

1. All subjects underwent a battery of ET function tests. For the purposes of this analysis that had to include the Forced Response Test (FRT) and Tubomanometry—both standard (swallowing) and employing Nasopharyngeal maneuvers (NPM).

For the FRT, an ear-canal probe is sealed into the test ear. A constant flow pump increases ME pressure until the ET lumen is passively forced open (PO-opening pressure). With continued airflow the ME-ET system reaches an equilibrium at which steady state pressure (PS) is measured. The subject is then instructed to swallow causing a change in the ET lumen diameter modifying the measured pressure and flow (QA). Once the equilibrium state is reestablished, the pump is stopped allowing the ET to passively close--the residual MEP representing the closing pressure (PC). From these parameters, dilatory efficiency (DE=QA/QS) is calculated. (Figure 1, Left)

Tubomanometry (TMM) was performed at 30, 40 and 50 mbar with the ME scale set for a non-intact TM (Eustachian Tube Diagnostic Tubomanometer, La Diffusion Technique Francaise, Saint-Etienne, France). To allow for more flexible analysis of TMM test data we incorporated external pressure sensors in the tubomanometer nasal and ME sensor tubing. Thus, the TMM data was concurrently routed to a PL3504 PowerLab 4/35 data acquisition system then to a PC running Lab Chart software 7.3.6 (AD Instruments, Bella Vista, NSW Australia) for display, filtering, storage, and analysis.

For Standard TMM, a tympanometry tip was sealed into the EAC, a small water bolus introduced and held in the mouth, and the nostrils were sealed with the nasal probe. The subject was instructed to swallow, and the nasal pressure "T" occluded resulting in an increase in nasopharyngeal (NP) pressure (NPP, C2-C1), and if the ET opened, an increase in middle ear (ME) pressure (MEP, P2-P1). The modified TMM test substitutes nasopharyngeal maneuvers (NPMs) that close the velopharyngeal valve for the water swallow that normally triggers the pressure bolus--otherwise the procedure is the same. The NPMs include repeatedly voicing "K", and the Fish and Candle blow maneuvers. For the Fish maneuver the subject blows out their cheeks against closed lips for a few seconds eliciting a forceful elevation of the soft palate. For the candle blow maneuver, the subject blows air through pursed lips for a few seconds to elicit a gentle elevation of the soft palate (Figure 2). The time and pressure magnitude of the inflection points (baseline, maximum, end plateau, and return to baseline) on the NP and ME pressure curves are designated and extracted for the analysis (Figure 1, Right). The pressure on the NPP curve corresponding to the P1 time is defined as the Tubo-PO and the residual pressure in the ME as the Tubo-PC .

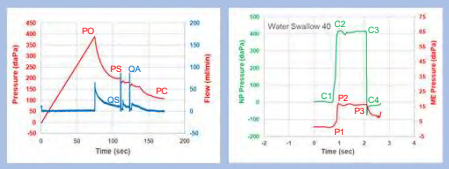


FIGURE 1

Fish Maneuver | Candle Maneuver

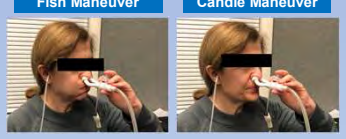


FIGURE 2

Contact:
J. Douglas Swarts
dswarts@pitt.edu

Results

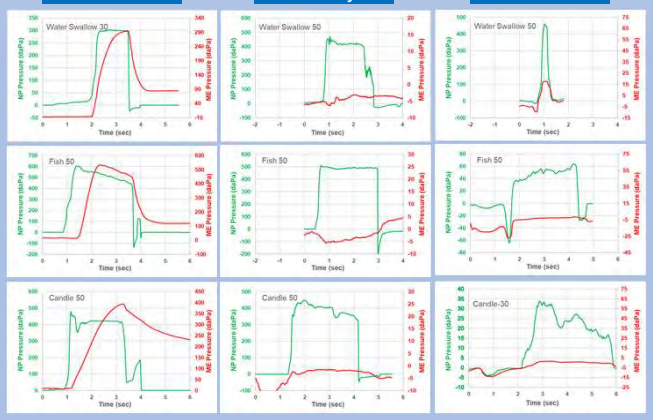
Demographics:

The FRT and Tubomanometry test of fifteen subjects were extracted from experimental and clinical datasets (Table 1). Five subjects from each of three groups—Controls, History of OM and Extant ETD—were used in the analysis. There were seven females and eight males, thirteen white, one each Asian and Black; and 28.7 ± 8.4 years of age. Two subjects had bilateral testing. Each performed FRT, at 11 and 24 ml/min flowrates, and Tubomanometry—1 subject had 4 tests, 5 had 6, 1 had 8, and 8 had 9 tests.

Table 1

Subject	Ear	Age	Sex	Race	Ethnicity	Group
73024	Right	21.2	M	W	NH	Control
73030	Left	31.9	M	BI	NH	Control
73082	Left	28.3	M	W	NH	Control
73088	Right	26.1	F	A	NH	Control
73090	Left	28.7	F	W	H	Control
73048	Left	32.3	M	W	NH	History OM
73056	Right	42.5	F	W	NH	History OM
73072	Left	20.1	F	W	NH	History OM
73075	Right	26.9	M	W	H	History OM
73081	Right	46.8	M	W	NH	History OM
ET0073	Right	27.7	M	W	NH	Extant ETD
ET0081	Right	18.5	M	W	NH	Extant ETD
ET0117	Left	20.33	F	W	NH	Extant ETD
ET0136	Left	21.6	F	W	NH	Extant ETD
ET0143	Left	37.3	F	W	NH	Extant ETD

73088 Control | 73081 History OM | ET0136 Extant ETD



In the panel above, curves of the NP (green) and ME (red) pressure changes for an example from each group for standard Tubomanometry (Swallow) and two of the maneuvers—Fish and candle—are presented. The NP curves are longer (>3 seconds) for the maneuvers in comparison to the swallows. Note the lower maneuver pressure ranges for the ME (History and ETD) and NP pressures. Table 2 shows the numbers of successful ET dilations—subjective assessment during the test—for each maneuver relative to the number of attempts. Seventy-nine percent of swallows, 69% of Candle, 56% of Fish, and 50% of KKK yield dilations. If objective criteria, R > 1 or MEP increases > 5 daPa, are employed the results are comparable to the subjective assessment with a slight increase for the KKK and SW as measured by changes in MEP (Table 3).

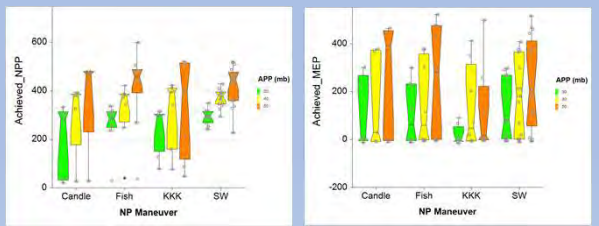
Table 2: Successful ET Dilations

Maneuver	Applied Pressure 30	40	50
Candle	3/6	4/5	4/5
Fish	4/9	4/8	7/10
KKK	3/8	3/8	4/8
SW	11/15	11/15	13/17

Table 3: Successful ET Dilations

Maneuver	Criteria	Applied Pressure 30	40	50
Candle	R > 0	3/6	3/5	3/5
	MEP > 5	3/6	3/5	3/5
Fish	R > 0	4/9	4/8	7/10
	MEP > 5	4/9	4/8	7/10
KKK	R > 0	3/8	3/8	4/8
	MEP > 5	3/9	5/8	5/8
SW	R > 0	10/15	10/15	13/17
	MEP > 5	11/15	11/15	14/17

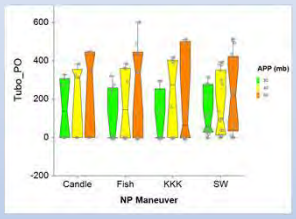
Characteristics of Maneuver Pressure Curves



These two plots illustrate the distribution of Tubomanometry data with respect to the achieved NPP (left) and MEP (right) during the maneuvers at each of the three applied pressures (AAP) in millibars. The plots include the median, interquartile range, outlier whiskers and extreme outliers as well as the individual values. For the NPP each maneuver shows the expected increase with increasing applied pressures. The two most forceful maneuvers, swallowing and Fish show the least variability. Similarly, except for the KKK, the maneuvers also show an increasing MEP with applied pressure. These distribution are skewed toward no increase in MEP because not all tests resulted in ET dilation.

Results

Passive Eustachian Tube Function Parameters



In these two panels Tubomanometry based estimates of ET opening (above) and closing (below) pressures are illustrated for the maneuvers. As with the NPP and MEP plots, these show evidence of an applied pressure effect, especially for Tubo-PO. The Tubo-PO distributions of the Candle, Fish, and KKK appear to be bimodal which is distinct from that of the SW—in which the Tensor veli palatini muscle is activated. Again, some of the attempts failed to induce an ET dilation. A similar applied pressure effect is seen in Tubo-PC distributions although they are not bimodal.

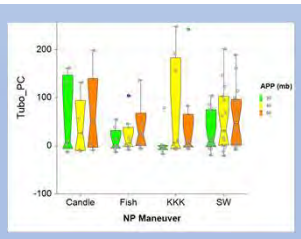


Table 4: Comparison of Tubomanometry-FRT

	N	Mean SD Tubo	Mean SD FRT	Spearman Corr Coef	95% CI	T	P-Value
Opening Pressure	10	270 ± 57	318 ± 256	0.587	-0.12–0.90	2.05	**0.07**
Closing Pressure	10	48 ± 59	121±163	-0.392	-0.83—0.34	1.20	0.26
Ach MEP/DE1	10	159 ± 114	7.6±4.9	0.856	0.39—0.97	4.67	**0.002**
PC Equil / FRT-DE11	10	4.09 ± 11	7.6±4.9	0.343	-0.38—0.81	1.03	0.33

Lastly, is there a relationship between the magnitude of Tubomanometry maneuver derived ETF parameters and those of the gold standard FRT. A correlation analysis of Candle, Fish, KKK maneuver values at 30 mb applied pressure was performed. Table 4 details those results. Of the two passive function ET variables the Opening pressure relationship is moderately strong. For the active function variables, Achieved MEP is strongly correlated to FRT dilatory efficiency. Neither variable including Closing pressure is correlated to the FRT comparator.

Discusson

We found that NPMs used in conjunction with Tubomanometry produced NP and ME pressure profiles broadly comparable to those induced during standard swallow tests. The differences are attributable to the voluntary control of the maneuver duration as opposed to the largely involuntary components of swallowing. More importantly, the differences can logically be attributed to the pressure gradient that is created between the NP space and the tissue pressures maintaining the ET in a collapsed state. If the NP pressure exceeds those tissue pressures and the mucosal adhesion forces, we expect the ET to be forced open causing an increase in the MEP. The pressure at which that occurs is the ET passive opening pressure. After this dilation, the MEP will decrease until the ET collapses trapping a residual MEP—ET passive closing pressure. Although the correlations between FRT passive function variables the values derived from the Tubomanometry NPM tests are not strong, optimization of the testing protocol, a more precisely defined sample, and increased sample size may improve them.

Clearly, several caveats should be entertained. We didn't actually measure mTVP EMG therefore cannot unequivocally discount its activation. Secondly, applied NP pressure levels were not chosen to discriminate obstructed from normal and patulous ETs. Lastly, no doubt there are other NP maneuvers or devices that would produce more reproducible and stable results given the same testing procedures.

Conclusion

It is possible to extract ET passive function values using Tubomanometry. This allows the assessment of both components of ETF from the NP in subjects and patients with intact tympanic membranes. That information is currently unavailable to physicians for either diagnostic or treatment purposes.

Cited Literature

1. Doyle WJ. Functional eustachian tube obstruction and otitis media in a primate model. A review. *Acta Otolaryngol Suppl*. 1984;414:52-7.
2. Swarts JD, Alper CM, Mandel EM, Villardo R, Doyle WJ. Eustachian tube function in adults without middle ear disease. *Ann Otol Rhinol Laryngol*. Apr 2011;120(4):220-5.
3. Doyle WJ, Swarts JD, Banks J, Casselbrant ML, Mandel EM, Alper CM. Sensitivity and specificity of eustachian tube function tests in adults. *JAMA Otolaryngol Head Neck Surg*. Jul 1 2013;139(7):719-27.
4. Estève D, Dubreuil C, Della-Vedova C, Normand B, Martin C. Evaluation por tubomanometrie de la fonction d'ouverture tubaire et de la reponse tympauique chez le sujet normal et chez le sujet porteur d'une otite sero-muqueuse chronique: comparaison des resultats. *J Fr ORL*. 2001;50(5):223-232.
5. Ars B, Dirckx JJJ. Tubomanometry. In: Ars B, ed. *Fibrocartilaginous Eustachian Tube-Middle ear cleft*. Kugler Publications; 2003:151-158.
6. Smith ME, Zou CC, Baker C, Blythe AJC, Hutchinson PJA, Tysome JR. The repeatability of tests of eustachian tube function in healthy ears. *Laryngoscope*. Nov 2017;127(11):2619-2626. doi:10.1002/lary.26534
7. Ockermann T, Reineke U, Upile T, Ebmeyer J, Sudhoff HH. Balloon dilatation eustachian tuboplasty: a clinical study. *Laryngoscope*. Jul 2010;120(7):1411-6. doi:10.1002/lary.20950
8. Jenckel F, Kappo N, Gliese A, et al. Endonasal dilatation of the Eustachian tube (EET) in children: feasibility and the role of tubomanometry (Estève) in outcomes measurement. *Eur Arch Otorhinolaryngol*. Dec 2015;272(12):3677-83. doi:10.1007/s00405-014-3443-2
9. Schmitt D, Akkari M, Mura T, Mondain M, Uziel A, Venail F. Medium-term assessment of Eustachian tube function after balloon dilation. *Eur Ann Otorhinolaryngol Head Neck Dis*. Apr 2018;135(2):105-110. doi:10.1016/j.anorl.2017.09.008

Pneumatocele of the external auditory canal, a novel complication of atticotomy. A case report

Authors: Maria José Herrera[1] ,Matías Alvarez B[2], Marcos Goycoolea[1]

1. Otolaryngologist, Otolaryngology department, Clinica Universidad de los Andes.
2. Otolaryngology Resident, Otolaryngology department, Pontificia Universidad Católica de Chile.

BACKGROUND AND AIMS

A pneumatocele corresponds to a collection of air that is trapped in an envelope of soft tissue[1]. This condition is extremely rare in the external ear canal and has only been anecdotally reported secondary to a mastoid pneumatocele and a continuous positive air pressure device[2,3]. We present a case of a 76 years old male musician who plays the trumpet, who was operated of a right epytimpanic encephalocele via an exploratory tympanotomy and atticotomy, with transcanal repair of the defect. The lateral wall of the attic was reconstructed with cartilage. Nine months after surgery he complained of right ear blockage and hearing loss, without tinnitus or vértigo. Otoscopy revealed a depressible mass on the posterior aspect of the right external auditory canal, with narrowing of the lumen of the canal causing an almost complete occlusion of the lumen as seen in Figure 1.

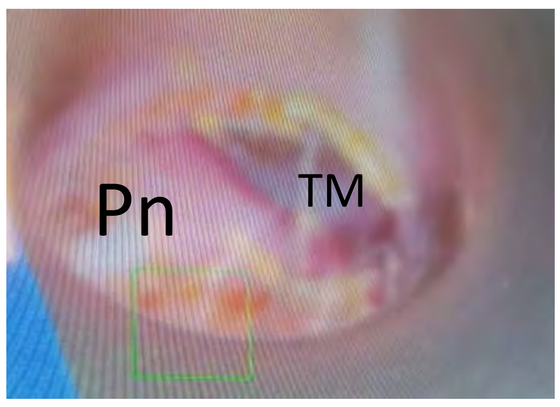


Figure 1. Right ear otoscopy. Pneumatocele (Pn), Tympanic membrane (TM)

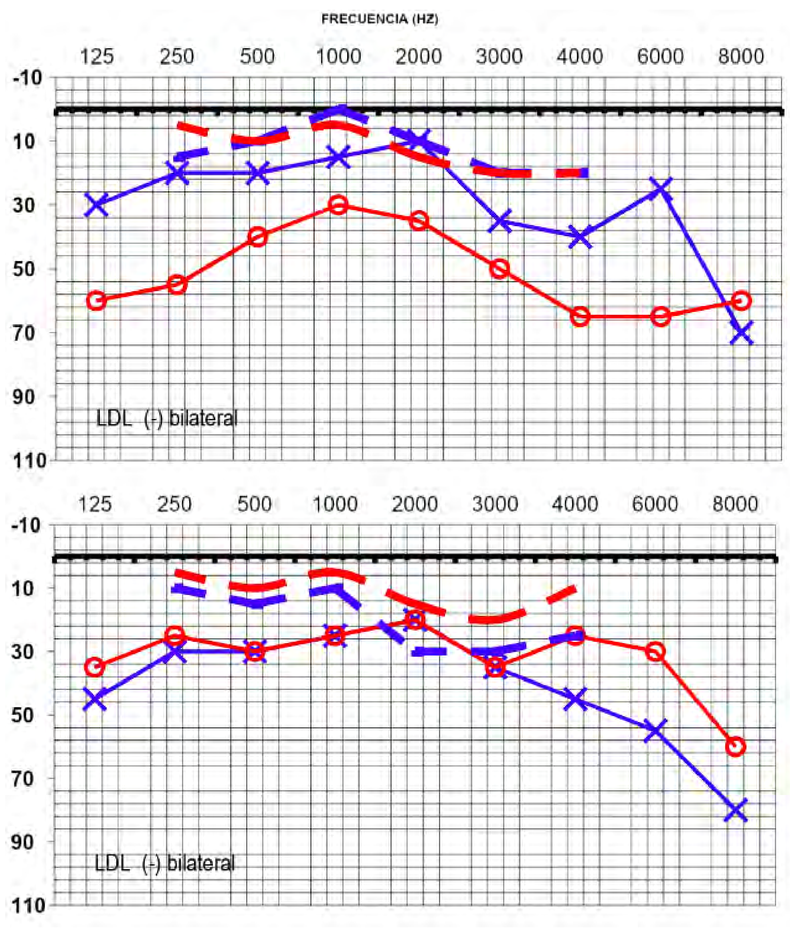
METHODS

Case report.

RESULTS

Audiometric testing revealed a significant air bone gap in contrast with the previous post surgery control audiometry (Figure 2 A and B). When the mass was compressed the hearing improved and a normal tympanic membrane could be visualized. The CT scan showed a subcutaneous air collection in the posterosuperior aspect of the external canal confined by the canal skin and a lateralization of the tympanic membrane as seen in Figure 3.

A



B

Figure 2-A. Audiometry after development of right external ear canal Pneumatocele. Left air PTA 21dB; Right air PTA 42dB. Figure 2-B. Post atticotomy basal audiometry Left air PTA 30dB; Right air PTA 25dB.

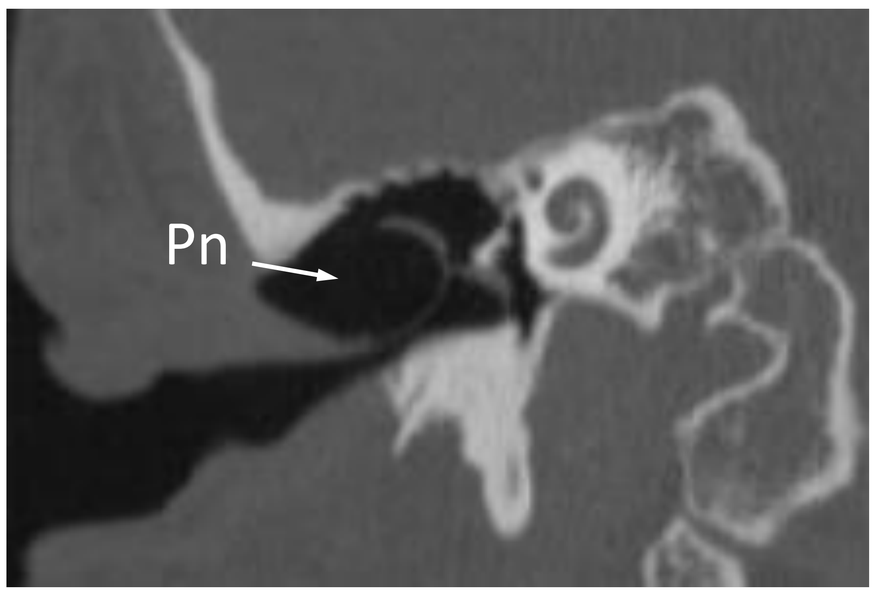


Figure 3. Coronal CT scan showing attic air colection. Pneumatocele (Pn)

After evaluation at the Universidad de los Andes Ear Center, the mass was punctured with evacuation of the air collection and a Merocel ear packing was placed in the external ear canal.
Ten days later the packing was removed with complete reabsorption of the lesion and significant improvement of hearing threshold as shown in Figure 4.

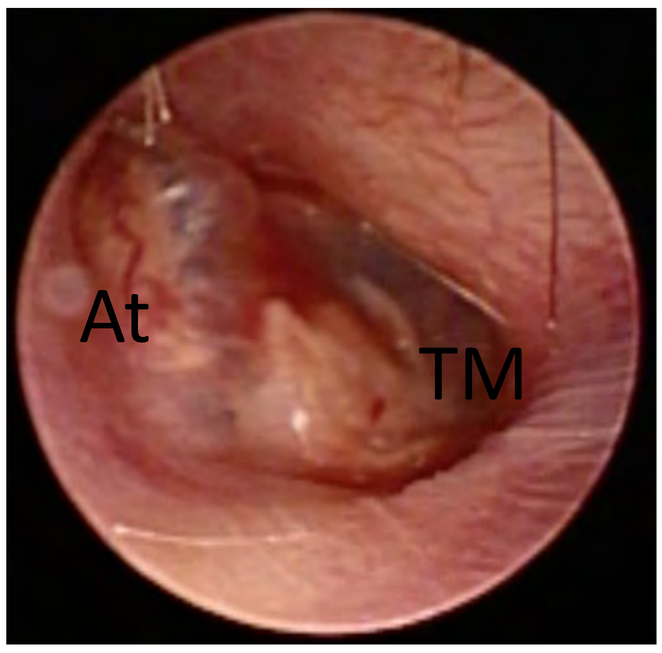


Figure 4. Right ear otoendoscopy. Attic reconstruction (At), Tympanic membrane (TM)

DISCUSSION AND IMPORTANCE OF FINDINGS

This case report shows a novel complication of the atticotomy, which compromises hearing and has a very simple solution. Previous surgical interventions were described for one case of spontaneous tympanic membrane pneumatocele which included lateral technique tympanoplasty[1].
In conclusion, for the best of our knowledge we describe the first case of pneumatocele of the external auditory canal as a consequence of atticotomy.

REFERENCES

1.- Gadkaree SK, Jan TA, Quesnel AM. Pneumatocele of the Tympanic Membrane. *Otol Neurotol*. 2017;38(4):e19-e20.
2.- Kim J, Rhee J, Park MH. A Spontaneous Pneumatocele Presenting with External Auditory Canal Obstruction. Korean Journal of Otorhinolaryngology-Head and Neck Surgery. 2016 Sep 1;59(9):692-4.
3.- Delabie G, Gordts F, Clement PA. Mastoid pneumocele with secondary pneumatocele causing external auditory canal obstruction and the influence of forced nose blowing. ENT: Ear, Nose & Throat Journal. 2010 Jan 1;89(1).

Postoperative results and complications of ventilation tube insertion : Multicenter registry study on the Effectiveness of Ventilation Tube insertion in pediatric patients with chronic otitis media with effusion — Part III (EVENT study)

Myung Hoon Yoo[1], Yang-Sun Cho[2], June Choi[3], Yun Hoon Choung[4], Jae-Ho Chung[5], Jong Woo Chung[6], Gyu Cheol Han[7], Beom Cho Jun[8], Dong-Kee Kim[9], Kyu Sung Kim[10], Jun Ho Lee[11], Kyu-yup Lee[1], Seung Hwan Lee[5], In Seok Moon[12], Hong Ju Park[6], Shi Nae Park[13], Jihye Rhee[3], Jae Hyun Seo[14], Seung Geun Yeo[15]

[1]Kyungpook National University, [2]Samsung Medical Center, Sungkyunkwan University, [3]Korea University College of Medicine, Korea University Ansan Hospital, [4]Ajou University School of Medicine, [5]School of Medicine, Hanyang University, [6]Asan Medical Center, University of Ulsan College of Medicine, [7]Gachon University of Medicine and Science, [8.9.13]The Catholic University of Korea, [10]College of Medicine, Inha University, [11]Seoul National University College of Medicine, Seoul National University Hospital, Seoul, [12]Yonsei University College of Medicine, [15]School of Medicine, Kyung Hee University

I. Introduction

Ventilation tube insertion for chronic otitis media with effusion is most commonly received operation in pediatric population. However, since relatively high rate of complications are reported after ventilation tube insertion, it is known to be performed carefully after weighting benefits and adverse effects. And intraoperative culture from middle ear effusion is frequently done during tube insertion, but it is unclear as to what impact the microbiological results of middle ear effusion have on complications.

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 III study was conducted to evaluate especially the postoperative results and complications.

Ⅱ. Material and Methods

Patients <15 years old who were diagnosed as having otitis media with effusion and received ventilation tube insertion were prospectively enrolled in 15 tertiary hospitals from June 2014 to December 2016. Follow-up data of the enrolled patients were collected until December 2017. After excluding patients with missing data, the data of 401 patients (727 operated ears) were analyzed among a total of 432 enrolled patients. The demographic data, surgical findings including the type of ventilation tube, and follow-up data were collected using e-CRF (internet based Case Report Form).

Time and reasons of extrusion were primary outcome. The complications (tube otorrhea, tube plugging, persistent perforation after extrusion, revision tube insertion during follow-up), and hearing results were secondary outcome.

III. Results

Average follow-up duration after tube insertion was 313 ± 238 days (range 3 to 1377 days). Male to female ratio was 246 : 155. Revision cases with previous history of VT insertion was 89 cases (22.2% of patients).

Long-lasting tubes (Paparella type II and T-tube) were done in 13 ears. Persistent perforation rate after extrusion of tube was 15.4% in long lasting tubes which was significantly higher than other tubes (0.7%) (p<0.05). However rate of tube otorrhea or tube plugging was not significantly different according to the long and short-lasting tubes (Table 1). Ears with long-lasting tubes were excluded for further analysis.

<Table 1> Complication rates according to long-lasting and short-lasting tubes

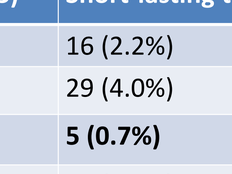
	Long-lasting tubes (n=13)	Short-lasting tubes (n=714)	P value
VT otorrhea	0 (0%)	16 (2.2%)	0.747
VT plugging	0 (0%)	29 (4.0%)	0.587
Persistent perforation	**2 (15.4%)**	**5 (0.7%)**	**0.006**
Revision VT during follow-up	1 (7.7%)	62 (8.7%)	0.687

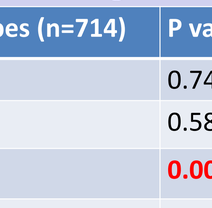
In revision ventilation tube ears, there was no significant differences in time to extrusion or time to recurrence of effusion when compared with primary tube ears. But there was a significantly higher rate of persistent perforation than primary ventilation tube ears (3.2% vs. 0.3%) and also significantly higher rate of another revision tube surgery than primary tube ears (13.0% vs. 7.5%) (Table 2).

<Table 2> Complication rates according to revision/primary operation

	Revision VT (n=145)	Primary VT (n=562)	P value
VT otorrhea	2/145 (1.3%)	14/562 (2.5%)	0.543
VT plugging	4/145 (2.7%)	23/562 (4.1%)	0.453
Persistent perforation	3/145 **(2.1%)**	2/562 **(0.3%)**	**0.006**
Revision VT during follow-up	20/145 **(13.0%)**	42/562 **(7.5%)**	**0.017**

<Figure 1>
A. Silicone Paparella type I tube
B. Titanium collar-button type tube





The rate of tube otorrhea was 2.5% in the silicone tube, and 1.3% in titanium tube, which was not statistically significant. Persistent perforation after extrusion was found in 1.2% of silicone tube inserted ears, but none in titanium tube inserted ears, but it was not statistically significant. The rate of VT plugging and another surgery was also not statistically significant (Table 3).

Table 3. Complications according to the type of ventilation tubes

	Silicone tube (n=557)	Titanium tube (n=157)	P value
VT otorrhea	14/557 (2.5%)	2/157 **(1.3%)**	0.543
VT plugging	19/557 **(3.4%)**	10/157 (6.4%)	0.097
Persistent perforation	5/557 (0.8%)	0/157 **(0%)**	0.591
Revision VT during follow-up	48/557 **(8.6%)**	14/157 (8.9%)	0.906

The status of effusion was serous in 221 patients (33.1%), mucoid in 200 (29.9%), glue in 220 (32.9%), and purulent in 27 (4.0%). Tube otorrhea was 1.3%, 3.5%, 1.4%, and 11.1% in serous, mucoid, glue and purulent effusion ears respectively, and this difference was statistically significant (p=0.008). However effusion status did not show significant difference in tube plugging, persistent perforation rate and revision tube insertion rate (Table ?).

<Table 4> Complication rates according to the status of middle ear effusion

	Serous (n=223)	Mucoid (n=200)	Glue (n=220)	Purulent (n=27)	P value
VT otorrhea	3/223 (1.3%)	7/200 **(3.5%)**	3/220 (1.4%)	3/27 **(11.1%)**	**0.008**
VT plugging	8/223 (3.6%)	9/200 (4.5%)	9/220 (4.1%)	2/27 **(7.4%)**	0.813
Persistent perforation	3/223 (1.3%)	0/200 (0%)	2/220 (0.9%)	0/27 (0%)	0.411
Revision VT	5/223 **(2.3%)**	24/200 **(12.0%)**	21/220 **(9.5%)**	7/27 **(25.9%)**	**<0.001**

Intraoperative culture was done in total of 337 ears (46.4%) and positive results were found in only 16.3% (55 ears) among them. Positive culture results also did not affect perforation, tube plugging and otorrhea rates.

<Table 5> Complication rates according to the intraoperative culture results

	Cx Positive (n=42)	Cx Negative (n=295)	P value
VT otorrhea	0/42 **(0%)**	9/295 (3.0%)	0.145
VT plugging	1/42 **(2.3%)**	12/295 (4.1%)	1.000
Persistent perforation	0/42 **(0%)**	3/295 (0.9%)	1.000
Revision VT	4/42 **(9.5%)**	36/295 (12.2%)	0.800

Postoperative air-conduction thresholds in pure tone audiometry were significantly lower than preoperative air-conduction thresholds (19.28 dB ± 16.52, 28.53 dB ± 14.01, respectively; p<0.001).). Postoperative bone-conduction thresholds were not significantly different with preoperative bone-conduction thresholds (8.87 ± 7.86, 8.24 ±6.03, respectively; p=0.462).

IV. Conclusion

Revision tube insertion showed significantly higher rate of persistent perforation and another revision rate. Although not significant, titanium tubes showed lower rate of persistent perforation than silicone tubes. The mucoid and purulent middle ear effusions showed higher rate of tube otorrhea and higher rate of revision operation than serous and glue effusions. However positive culture results did not show significant difference in outcome of tube insertion. Therefore, when counseling to the parents after ventilation tube insertion, physicians should consider the status of effusion and also select proper type of tube for insertion.

Presenting & Corresponding author, Acknowledgments

Presenting author : **Myung Hoon Yoo**, MD, PhD
Department of Otorhinolaryngology-Head & Neck Surgery, Kyungpook National University Chilgok Hospital
E-mail: cooleo312@gmail.com

Corresponding author : **Jong Woo Chung**, MD, PhD
Department of Otorhinolaryngology-Head & Neck Surgery, Asan Medical Center, University of Ulsan, College of Medicine
E-mail: jwchung@amc.seoul.kr

Acknowledgments : This work was supported by the **Otitis Media Research Project** funded by the **Korean Otologic Society**, Seoul, Republic of Korea

Poster Session

Effect of aging on mucosal immune responses against nontypeable *Haemophilus influenzae* in upper airway.

Takashi Hirano, Toshiaki Kawano, Kazuhiro Yoshinaga,
Takayuki Matsunaga, Shingo Umemoto, Masashi Suzuki.
Oita University, Faculty of Medicine, Department of Otolaryngology.

Introduction

The cellular and molecular mechanisms of immunosenescence have been investigated in systemic lymphoid compartments. it has been suggested that altered T cell functions are major factors in dysregulated immune responses that commonly occur in the elderly. These include alterations in T cell phenotypes, reduced IL-2 production and IL-2R expression, aberrant signal transduction, and enhanced programmed cell death of naive T cells. Furthermore, reduced responses to mitogens and impaired cytokine production have also been reported in the elderly. It has been shown that these alterations closely parallel increases in memory type and loss of the naive T cell phenotype during aging 1). Dysfunctions in B cells and Ab responses also occur in aging. It has been reported that pre-B cells develop poorly in the bone marrow of aged mice 2). In this study, we investigated mucosal immunosenescencein the upper airway against Nontypeable *Haemophilus influenzae* (NTHi) after intranasall immunization with outer membrane protein (OMP) from NTHi.

Materials and Methods

Animals

BALB/c mice were purchased from Kyudou (Fukuoka, Japan). All mice were maintained in a pathogen-free facility until they were 6 weeks old, 6 months old, 1 year old at which time they were used .

Immunogen

OMP was used for a vaccine antigen. OMP was extracted from NTHi (strain 76), which was isolated from the nasopharynx of a patient with OME at Oita University, according to the methods reported by Murphy et al. 3).

Vaccination and collection of mouse tissues and body fluids

Mice were vaccinated intranasally with a vaccine solution which contained a mixture of 10 μg of OMP and 1 μg of cholera toxin (CT) as an adjuvant (List Biological Laboratories, Campbell, CA) suspended in PBS of 10 μl. The same dosage of vaccine was delivered three times seven days apart. Middle ear washes (MEWs), nasal washes(NWs) and sera were collected after the three immunization. Mononuclear cells (MNCs) were also collected from the middle ear and nasal mucosa by collagenase digestion. Nontreatment mice (6 weeks) were used as a control.

Detection of OMP-specific antibody titers by ELISA

Anti OMP-specific antibody levels in MEWs, NWs and serum were measured by ELISA. The endpoint antibody titer was defined as the highest dilution of samples giving an optical density two-fold greater than that of the negative controls after 15 min incubation.

Flow cytometric analysis of B cell differentiation

To analyze the dynamics of B cells in mucosal tissues, MNCs from middle ear mucosa (MEM) and nasal mucosa (NM) were isolated by collagenase digestion. Flow cytometric analysis of the cellular immunofluorescence was performed using a BD LSRFortessa TM Flowcytometer (Becton, Dickinson and Company, Franklin Lakes, NJ). MNCs from each tissue were harvested from 5 weeks, 6 months, 1 years groups of each 5 mice on day 21. PE-Cyanine 7-conjugated anti-CD19 monoclonal antibody (6D5; Biolegend, SanDiego, CA), FITC-conjugated anti-B220 mAb (RA3-6B2; Biolegend) APC-conjugated anti-CD22 mAbs (OX-97; Biolegend) were used for analyzing B cell subsets.

Bacterial challenge and collecting samples

Strain 76 of NTHi was used for the bacterial challenge. NTHi was grown on chocolate agar at 37° C under 5% CO_2 for 16 h. Bacterial concentration was determined by optical density at a wavelength of 600 nm, and then a bacterial suspension was prepared to a concentration of 1.0×10^4 colony forming units (cfu)/mL in PBS for the middle ear challenge, and to a concentration of 1.0×10^9 cfu/mL in PBS for the nasal challenge. NTHi solution were inoculated into the middle ear bulla and the nasal cavity with a suspension of NTHi (10 μl). At day 3 after the middle ear challenge and at 12 h after the nasal challenge, the middle ear bulla and nasal cavity was gently flushed with 200 μl of PBS. The numbers of NTHi in MEWs and NWs were quantified by standard bacteriologic techniques.

Statistical analysis

Statistical comparisons between appropriate groups were performed with the unpaired two-tailed Student *t*-test. *P* values less than 0.05 were considered significant.

Results

Antibody responses at mucosal sites and in serum with intranasal immunization

The OMP-specific IgA antibody levels in middle ear wash and nasal washes were decreased according to aging after the three immunizations with OMP when compared to 6 weeks old group. The IgA antibody titer in 6 weeks old group in nasal washes was significantly higher than that in 1 years old group (6 weeks vs I year: 1.9±0.4 vs 1.1±0.37: log10 titer, respectively; $p<0.05$, Fig.1). The OMP-specific IgG antibody levels in serum were decreased according to aging after the three immunizations with OMP when compared to 6 weeks old group. The IgG antibody titers in 6 weeks group were also significantly higher than those in 1 year old group (6 weeks vs I year: 2.9±0.6 vs 2.3±0.6: log10 titer, respectively; $p<0.05$, Fig.2)

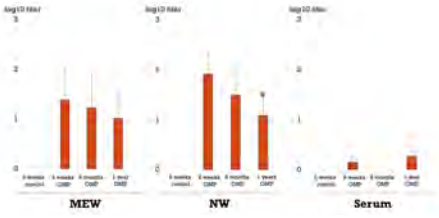


Figure 1: The OMP-specific IgA antibody levels.
*:p < 0.05 when compared to 6 weeks old mice with OMP immunization

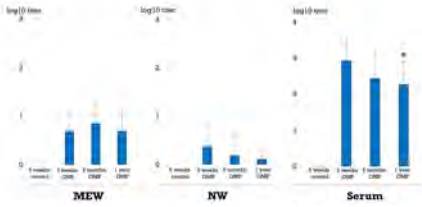


Figure 2: The OMP-specific IgA antibody levels.
*:p < 0.05 when compared to 6 weeks old mice with OMP immunization

B cell differentiation in mucosal tissues

To investigate the B cell differentiation in mucosal tissues after the intranasal immunization with OMP, we analyzed the B cell subsets, such as plasma blast, mature, and immature B cells on CD19 positive cells from MEM and NP. The percentage of plasma blast B cells(antibody producing cell) increase significantly in 6 weeks old group compared to 6 months and 1 years old group after the immunization in MEM and NM. On the other hand, the percentage of mature B cells significantly decreased in 6 weeks old group compared to 6 months and 1 years old groups after the immunization (Figure 3).

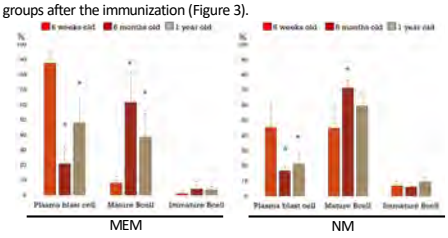


Figure 3: B cell differentiation in MEM and NM.
*:p < 0.05 when compared to 6 weeks old mice with OMP immunization

Bacterial clearance and correlation with IgA and IgG level

Bacterial counts obtained for strain 76 inoculated into the mouse middle ear and nasal cavity are shown in Figure 4. The mice in the 6 weeks group showed a significant augmentation in bacterial clearance from middle ear cavity at day 3 and nasal cavity at 12 hours after the bacterial challenge when compared to control group (6 weeks old vs control: middle ear wash at Day 3: 3.3±0.8 vs 5.0±0.1: log10 titer, respectively; nasal wash at 12 hours: 0.6±0.5 vs 2.2±1.0: log10 titer, respectively; $p<0.05$), but not in 1 year old group.

Figure 4: Bacterial clearance from the middle ear and nasal cavity.
*:p < 0.05 when compared to control mice without OMP immunization

Discussion

The cellular and molecular mechanisms of immunosenescence have been investigated in systemic lymphoid compartments. it has been suggested that altered T cell and B cell functions are major factors in dysregulated immune responses that commonly occur in the elderly. It has also been investigated that age-associated alterations of the intestinal and respiratory immune systems occur at distinct times and in a distinct manner. As reporting evidence of early aging in the gastrointestinal tract, a reduction in antigen-specific intestinal IgA responses can be seen in 1 year old mice, and this is clearly manifested by a reduced Peyer's patch 4). In the upper airway, nasal associated lymphoid tissue exhibits a slower rate of immunosenescence. Nasal immunization with ovalbumin (OVA) plus CT induces OVA-specific IgA and systemic IgG responses which were higher in young adult mice than that in 1 year old mice although there was no statistical difference between two groups 5). In this study, we presented the mucosal immunosenecence against OMP, including the middle ear and nose, and NTHi clearance from the middle ear and nasal cavity was also delayed according to aging. It is suggested that mucosal immunosenecence in the middle ear occurred as well as in the nose .

Reference

1) Nikolich-Zugich J. Aging of the T Cell compartment in mice and humans: from no naive expectations to foggy memorie. J Immunol 2014; 193:2622-2629

2) Ma S, Wang C, Mao X, et al. B Cell Dysfunction associated with aging and autoimmune diseases. Front Immunol. 2019 Feb 27;10:318. doi: 10.3389/fimmu.2019.00318. eCollection 2019.

3) Murphy TF, Nelson MB, Dudas KC, et al. Identification of a specific epitope of *Hemophilus influenzae* on a 16,600 dalton outer membrane protein. J Infect Dis. 1985; 152:1300–1307.

4) Koga T, McGhee JR, Kato H, et al. Evidence for early aging in the mucosal immune system. J Immunol. 2000;165:5352–5359.

5) Hagiwara Y, McGhee JR, Fujihashi K, et al. Protective mucosal immunity in aging is associated with functional CD4+ T cells in nasopharyngeal-associated lymphoreticular tissue. J Immunol 2003;170:1754-1762

Nontypeable *Haemophilus influenzae* newly-released from biofilms by antibody-mediated dispersal *versus* antibody-mediated disruption are phenotypically distinct

Elaine M. Mokrzan and Lauren O. Bakaletz

Center for Microbial Pathogenesis, Abigail Wexner Research Institute at Nationwide Children's Hospital and The Ohio State University College of Medicine, OH, United States

ABSTRACT

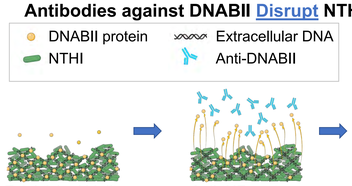
Background: Biofilms contribute significantly to the chronicity and recurrence of bacterial diseases such as otitis media because bacteria resident within biofilms are highly recalcitrant to killing by host immune effectors or antibiotics. Thus, antibody mediated release of bacteria from biofilm residence into the surrounding milieu supports a powerful strategy to resolve otherwise difficult-to-treat biofilm-associated diseases. **Methods:** We previously showed that antibodies directed against either the Type IV pilus (T4P) of nontypeable *Haemophilus influenzae* (NTHI) or a bacterial DNABII DNA-binding protein, a species-independent target that provides structural integrity to bacterial biofilms, release biofilm-resident bacteria via discrete mechanisms. Herein, we used flow cytometry, proteomic profiles, and targeted transcriptomics to characterize the resultant newly-released (NRel) NTHI. **Results:** Proteomic profiles of the two NRel populations were significantly different not only from planktonically grown NTHI, but importantly, from each other despite genetic identity. Moreover, each NRel population had a distinct, significantly increased susceptibility to killing by either a sulfonamide or β-lactam antibiotic compared to planktonic NTHI, an observation supported by both proteomics and relative differences in targeted gene expression. **Discussion:** Collectively, our results reveal that the phenotype of NRel NTHI depends upon the mechanism of release. The distinct phenotypes of NTHI released from biofilms by antibodies directed against specific epitopes of T4P or DNABII binding proteins provide new opportunities to develop targeted therapeutic strategies for biofilm eradication and disease resolution.

BACKGROUND

- NTHI, the predominant bacterial pathogen of otitis media (OM), forms biofilms at the site of infection
- Biofilm-resident bacteria are highly resistant to killing by host immune defenses or by antibiotics
- Bacteria can be released from established biofilms by exposure to antibodies against either: 1) an essential biofilm structural protein (e.g. DNABII protein); or 2) the majority subunit protein of the NTHI Type IV twitching pilus (PilA)
- Bacteria newly-released (NRel) from biofilm residence are phenotypically distinct from both biofilm-resident and planktonic counterparts, including being highly sensitive to killing by specific antibiotics
- **Herein we began to phenotypically characterize NTHI newly released from biofilms after exposure to antibodies that target either DNABII protein IHF or the NTHI Type IV PilA subunit**

Antibodies against the DNABII protein IHF or recombinant, soluble PilA (rsPilA) induce the release of NTHI from biofilm residence via distinct mechanisms

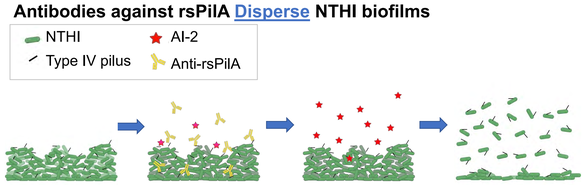
Antibodies against DNABII Disrupt NTHI biofilms



Goodman *et al.*, 2011 Mucosal immunol
Brockson *et al.*, 2014 Mol Micro
Novotny *et al.*, 2019 npj vaccines

Antigen location: biofilm, structural linchpin
NTHI contribution: passive – physical collapse
Release speed: fast (minutes)
Release mode: all at once
Release state: aggregates
NTHI-specific? no – species agnostic
Term for bacterial release: *Disruption*

Antibodies against rsPilA Disperse NTHI biofilms



Bakaletz *et al.*, 2005 Infect Immun
Novotny *et al.*, 2015 Mol Micro

Antigen location: NTHI surface
NTHI contribution: active – native/programmed
Release speed: slow (hours)
Release mode: top-down
Release state: individual cells
NTHI-specific? yes
Term for bacterial release: *Dispersal*

ACKNOWLEDGMENTS

These data are published: Mokrzan et al. *Biofilm* 2 (2020) 100039

This work was funded by NIDCD/NIH R01 DC003915 to LOB

RESULTS

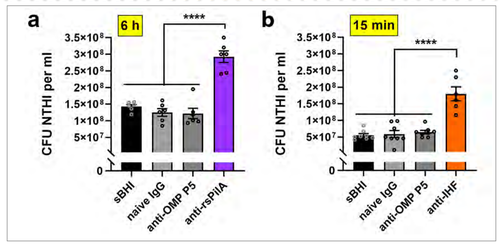


Fig. 1. Anti-rsPilA and anti-IHF induced significant release of NTHI from biofilm residence into the NRel state. 16 h NTHI biofilms were incubated for an additional **(a)** 6 h with rabbit anti-rsPilA IgG or **(b)** 15 min with rabbit anti-IHF IgG or with one of three negative controls (sBHI, IgG isolated from naive serum or IgG isolated from anti-OMP P5 serum). bars=mean ± SEM. ****, $P<0.0001$

NRel NTHI are phenotypically distinct from planktonic NTHI and also *from each other*

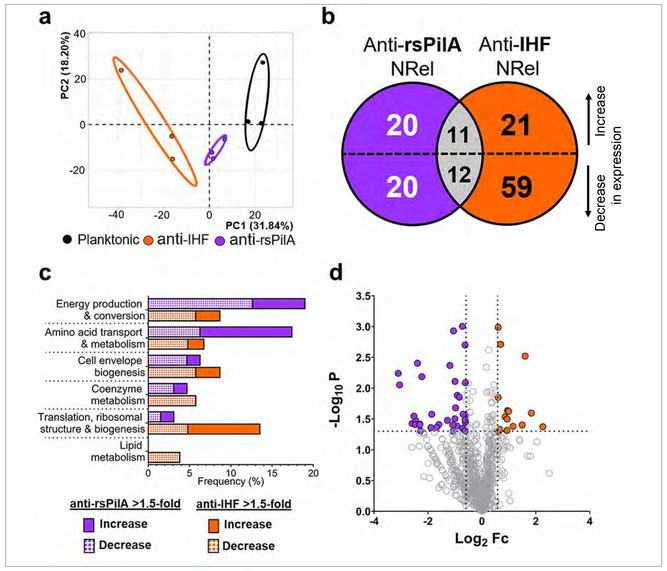


Fig. 2. Biofilms incubated with anti-rsPilA or anti-IHF antibodies generated NRel populations with distinct proteomic expression profiles. **(a)** The proteomic expression profiles of **anti-rsPilA NRel (purple)** and **anti-IHF NRel (orange)** were distinct from both planktonically grown NTHI **(black)**, *and from each other*. **(b)** The number of proteins with a significant ($P<0.05$) 1.5-fold increase or decrease, compared to planktonic NTHI. **(c)** Differentially expressed proteins of anti-rsPilA or anti-IHF NRel represented different COG categories when compared to planktonic NTHI. **(d)** Direct comparison of differences in protein expression profiles of anti-IHF and anti-rsPilA NRel populations with a significant ($P<0.05$) 1.5-fold increase or decrease compared to each other.

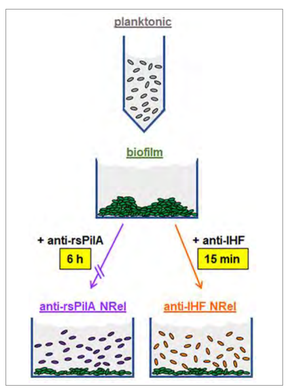


Fig. 3. Assay to compare relative antibiotic sensitivity of anti-rsPilA or anti-IHF NRel

Antibiotics commonly used to treat NTHI-induced respiratory tract infections:
- Amoxicillin + clavulanic acid (AMC)
- Trimethoprim + sulfamethoxazole (TMP-SMX)

1. Adjust planktonic NTHI to same CFU/ml as anti-rsPilA or anti-IHF NRel
2. Determine the concentration of antibiotics needed to kill 25% of planktonic NTHI after 2 h
3. Measure relative percent killing of NRel populations with the same antibiotic conc.

NRel NTHI populations were more sensitive to antibiotic killing than biofilm-resident NTHI and than even planktonic NTHI but distinctly so

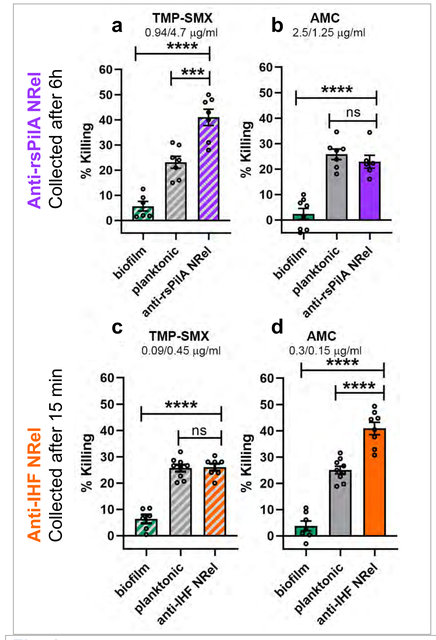


Fig. 4.

NRel were released from biofilms exposed to:
anti-rsPilA for 6 h
anti-IHF for 15 min

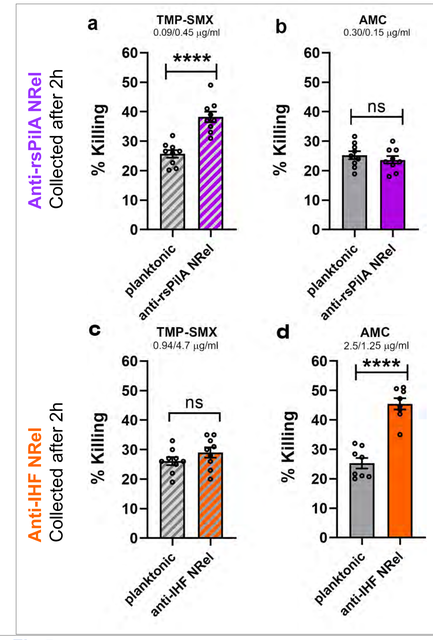


Fig.5.

NRel were released from biofilms exposed to:
anti-rsPilA for 2 h or
anti-IHF for 2 h

Anti-rsPilA NRel:
- Significantly more sensitive to killing by **TMP-SMX** than planktonic NTHI (Fig. 4a & Fig. 5a)
- Equally as sensitive as planktonic NTHI to killing by AMC (Fig. 4b & Fig. 5b)

Anti-IHF NRel:
- Significantly more sensitive to killing **by AMC** than planktonic NTHI (Fig. 4c & Fig. 5c)
- Equally as sensitive as planktonic to killing by TMP-SMX (Fig. 4d & Fig. 5d)

Biofilm-resident NTHI displayed minimal sensitivity to either TMP-SMX or AMC, as expected (Fig. 4a-d)

The distinct NRel phenotype was dependent on the mechanism release from the biofilm

CONCLUSIONS

- **Anti-rsPilA NRel and anti-IHF NRel NTHI are *phenotypically* distinct despite their *genetic* identity**
- **The NTHI NRel phenotype is dependent upon the exact mechanism of release from the biofilm (e.g. disruption by anti-DNABII *vs.* dispersal by anti-rsPilA)**
- **Results provide support for a combinatorial approach to treat recalcitrant biofilm-associated diseases wherein NTHI are released from biofilm-residence by exposure to specific antisera combined with use of a reduced dose of traditional antibiotic to now *effectively kill* these NRel bacteria**
- **Ongoing work targets defining the exact molecular mechanisms that underlie the observed specific enhanced antibiotic sensitivities of anti-rsPilA and anti-IHF NRel NTHI**

The role of *CDHR3* variants in otitis media susceptibility

University of Colorado Anschutz Medical Campus

SD Hirsch, CL Elling, TC Bootpetch, MA Scholes, L Hafrén, Sven-Olrik Streubel, Harold S. Pine, Todd M. Wine, W Szeremeta, JD Prager, E Einarsdottir, A Yousaf, EE Baschal, S Rehman, MJs Bamshad, DA Nickerson, S Riazuddin, SM Leal, ZM Ahmed, PJ Yoon, J Kere, KH Chan, PS Mattila, NR Friedman, T Chonmaitree, DN Frank, AF Ryan, RLP Santos-Cortez

Background

- Otitis media (OM) is a disease spectrum and accounts for >20 million pediatrician visits and 2-3 million emergency department visits
- OM can cause hearing loss in children, leading to speech and learning difficulties. This results in overall poorer quality of life for patients with OM and their caregivers
- CDHR3 belongs to the cadherin superfamily of transmembrane calcium-dependent adhesion proteins and expressed on the apical surface of ciliated epithelial cells in lung and sinonasal tissue
- CDHR3 is the main receptor for Rhinovirus-C (RV-C) binding, entry, and duplication
- *CDHR3* intronic variant rs114947103 was identified in a GWAS as protective against OM (OR 0.93 [95% CI 0.91,0.96])
- However, it is in linkage disequilibrium with *CDHR3* rs6967330, a risk factor for early onset severe asthma with acute exacerbations due to RV-C

Table 1. Multivariate logistic regression analyses for presence of middle ear fluid (MEF) in Coloradan children with OM

Variables	n (%)	Bivariate OR (95%CI)[a]	p-value	Multivariate OR (95%CI)[b]	p-value
rs6967330	25 (34.2)	0.27 (0.1,0.76)	0.01**	0.33 (0.13,0.84)	0.02*
Age (years)	81 (100)	0.66 (0.5,0.9)	0.004**	0.53 (0.35,0.81)	<0.001***
Female	28 (34.6)	0.69 (0.27,1.76)	0.43	0.71 (0.28,1.8)	0.73
Ethnicity					
Hispanic	7 (7.7)	0.79 (0.16,3.83)	0.77	1.1 (0.25,4.83)	0.70
Other/mixed	12 (14.8)	1.18 (0.32,4.35)	0.80	1.1 (0.3,4.07)	0.83
Attended daycare	61 (75.3)	3.58 (1.25,10.24)	0.02*	1.93 (0.73,5.11)	0.51
RAOM	33 (55.0)	0.13 (0.04,0.48)	0.002**	0.13 (0.04,0.5)	0.002**

[a]Bivariate analyses using presence of MEF at surgery as outcome variable and each listed variable as independent determinant. Average age is 2.0 years.
[b]Model for multivariate analysis: MEF at surgery ~ rs6967330 + age + sex + ethnicity + daycare attendance. For ethnicity, White non-Hispanic is the reference group in logistic regression. *≤0.05, **≤0.01, *≤0.001**
RAOM = recurrent acute otitis media

AIM

Define the role of *CDHR3* in OM susceptibility using multi-omic data, by:
(1) Analyzing DNA and exome data from patients and families with OM to determine relation of *CDHR3* with OM
(2) Performing sub-phenotype analysis using clinical data from OM patients
(3) Identifying microbiome shifts using microbial samples from patients with OM
(4) Identifying *CDHR3* expression in a normal middle ear and during an episode of AOM using transcriptomic data from a mouse model of AOM

Methods

- Exome sequencing of DNA from 234 Finnish and 56 Minnesotan individuals
- Sanger sequencing of common *CDHR3* variants from ~400 US trios, where 286 (81.5%) were diagnosed to have RAOM while the rest COME
- Transmission disequilibrium testing
- 16S rRNA sequencing and analyses for 37 ME and 40 nasopharyngeal samples
- RNA expression in human and mouse ME

Results

Common/Rare *CDHR3 Variants*

- Of 17 *CDHR3* variants were identified from exome sequence data, nine SNPs, including the low-frequency splice variant c.1653+3G>A, had scaled CADD scores >10 and were identified as deleterious, suggesting potential risk variants for OM
- Three putative loss-of-function (LOF) were identified, including c.1653+3G>A, which was predicted to be pathogenic by MutationTaster
- The c.1653+3G>A variant was heterozygous in all affected siblings in two families, UMN123 and FIN142, (**Figure 1**) and in the affected probands of six additional Finnish trios.

TDT and Sub-phenotype Analysis

- Carriers of the p.Cys529Tyr variant were more likely to have RAOM than COME (p=0.02), however, the TDT results were non-significant.
- Although the Coloradan children (n=91) all had a history of RAOM or COME, 30 (33.0%) children did not have MEF at the time of surgery. In patients with MEF, 36 (39.6%) had serous or mucinous and 15 (16.5%) had purulent MEF.
- Absence of MEF was associated with carriage of the p.Cys529Tyr variant (OR=0.27; 95%CI:0.1,0.76; p=0.01).
- Younger age (p=0.004) and daycare attendance (p=0.02) were associated with presence of MEF
- After correction for age, daycare attendance, and RAOM diagnosis, the association between the *CDHR3* p.Cys529Tyr variant and absence of MEF at surgery remained significant (p=0.01, **Table 1**).

Microbiome

- Relative abundance of *Streptomyces* was greater in ME samples (nominal-p=0.05; **Figure 2**) and *Lysobacter* in NP of carriers of the p.Cys529Tyr variant (nominal-p=0.01; **Figure 2**), but there were no differences in alpha- or beta-diversity of ME and NP samples according to *CDHR3* p.Cys529Tyr genotype.

CDHR3 Expression

- In wildtype mouse ME epithelium, *Cdhr3* is down-regulated 3 hours after inoculation with NTHi, a common human otopathogen (**Figure 3**).
- Single-cell RNA sequencing revealed that expression of *Cdhr3* is largely restricted to ciliated epithelial cells of mouse ME.

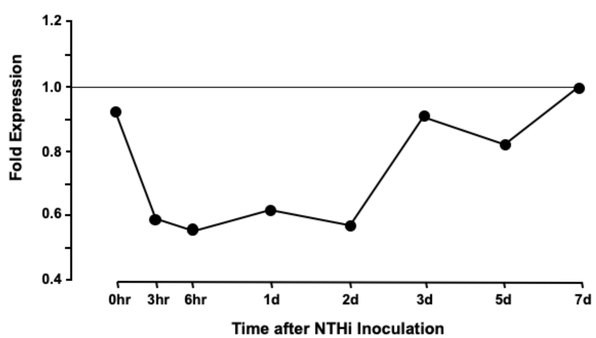


Figure 3. *Cdhr3* expression after inoculation with NTHi in wildtype mouse middle ear

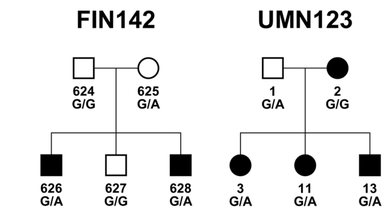


Figure 1. *Pedigrees showing non-segregation of CDHR3* missense variants with OM

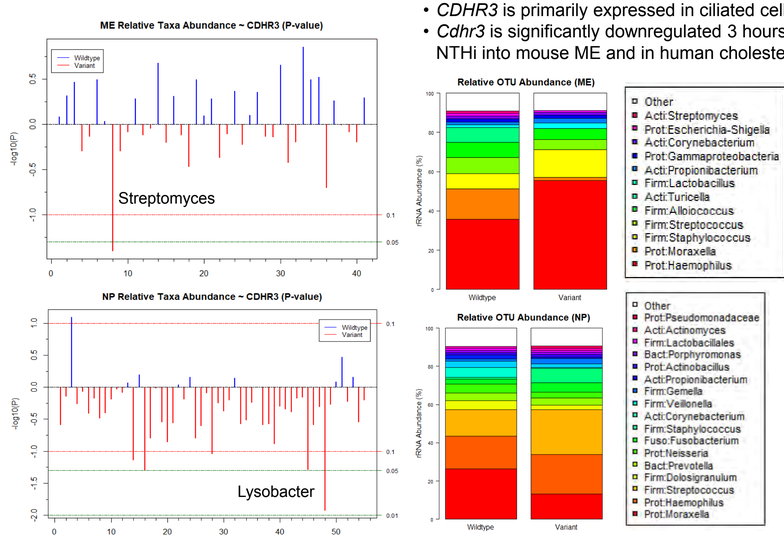


Figure 2. Microbiome shifts in the ME (top) and NP (bottom) in variant CDHR3 p.Cys529Tyr carriers

Conclusions

- The common c.1586G>A (p.Cys529Tyr) variant is a potential risk factor for viral RAOM due to greater surface expression of CDHR3 as binding receptor for RV-C
- The low-frequency c.1653+3G>A splice variant co-segregates with OM in two families with different ethnicities
- Patients carrying the rs6967330 (p.Cys529Tyr) variant were more likely to lack MEF at the time of surgery
- Nominal association between *CDHR3* rs6967330 and increased relative abundance of Streptomyces in ME
- *CDHR3* is primarily expressed in ciliated cells of the middle ear
- *Cdhr3* is significantly downregulated 3 hours post-inoculation of NTHi into mouse ME and in human cholesteatoma

Implications

- The microbiome shifts associated with the *CDHR3* p.Cys529Tyr variant (towards gram-negative bacteria) have significant clinical implications because the first- and second-line antibiotics for AOM are oral Amoxicillin and Amoxicillin-Clavulanic Acid, both of which are much more effective against gram-positive rather than gram-negative bacteria
- By identifying OM patients who carry the *CDHR3* p.Cys529Tyr variant, we may be able to select patients who might have less benefit from standard treatments (tympanostomy tube insertion, antibiotics) due to spontaneous MEF resolution and may be targeted for rhinoviral or *CDHR3*-based therapies or vaccines

Funding: Exome sequencing of DNA samples from 12 Minnesota families was provided by the UW-CMG and was supported by the US NIH-National Human Genome Research Institute and the National Heart, Lung and Blood Institute grants UM1 HG006493 and U24 HG008956 (to D.A.N., M.J.B. and S.M.L.). This work was supported by the US NIH – National Institute on Deafness and Other Communication Disorders grant R01 DC015004 (to R.L.P.S.C.).

Respiratory Viruses in Filipino Individuals with Otitis Media

Authors: Tori C. Bootpetch,[1] Jose Pedrito M. Magno,[2] Karen Joyce S. Velasco,[2] Jacob Ephraim D. Salud,[2] Nicole D. Sacayan,[2] Kevin Jer V. David,[2] Erik A. Tongol,[2] Eljohn C. Lee,[2] Alessandra Nadine E. Chiong,[2] Heather P. DuInuan,[2] Jan Alexis C. Lacuata,[2] Jeric L. Arbizo,[2] Gabriel Martin S. Ilustre,[2] Daniel N. Frank,[3] Richard B. Pyles,[4] Talitha Karisse L. Yarza,[5,6] Shi-long Lu,[1] Aaron Miller,[4] Charlotte M. Chiong,[2,5,6,7] Regie Lyn P. Santos-Cortez[1,5,8]*

[1]Department of Otolaryngology-Head and Neck Surgery, University of Colorado Anschutz Medical Campus (CU-AMC), Aurora, Colorado, USA, [2]Department of Otorhinolaryngology, University of the Philippines (UP) Manila-Philippine General Hospital, Manila, Philippines, [3]Division of Infectious Diseases, Department of Medicine, CU-AMC, Aurora, Colorado, US, [4]Assay Development Service Division, Galveston National Laboratory, University of Texas Medical Branch, Galveston, Texas, USA, [5]Philippine National Ear Institute, UP Manila-National Institutes of Health, Manila, Philippines, [6]Newborn Hearing Screening Reference Center, UP Manila-National Institutes of Health, Manila, Philippines, [7]UP Manila College of Medicine, Manila, Philippines, [8]Center for Children's Surgery, Children's Hospital Colorado, Aurora, Colorado, USA**

Background

Otitis media (OM) is an increasingly common disease among both children and adults. Acute OM is typically treated with antibiotics, however, evidence suggests that acute OM is a polymicrobial disease and that both respiratory viruses and bacteria play a role in the development of acute OM[1]. Upper respiratory viral infection can result in Eustachian tube inflammation and dysfunction, alteration of the host's immune defense, and increased bacterial colonization and adherence, which all could lead to acute OM as well as increased risk of secondary bacterial infection[2].

In this study, we aim to identify common otopathogenic viruses in Filipino individuals with OM. This is the first study of viral load in OM patients from the Philippines.

Results

Viral RNA and DNA were isolated from 120 ME and 25 NP samples collected from 71 Filipino individuals with OM. Respiratory viruses were screened in 45 individuals in the PGH cohort and 26 individuals in the indigenous Filipino (IP) cohort.

IP Cohort
No viral presence was detected in the IP ME samples. However, in the IP NP samples, two individuals were positive for RV, four were positive for HAdV, and two were positive for HBoV (Table 1).

PGH cohort
No viral presence was detected in the PGH ME.

Table 1. Prevalence of respiratory viruses in IP OM individuals

Virus	*NP positives*	*ME positives*	*Age (y) 0-5*	*Age (y) 6-12*	*Sex Female*	*Sex Male*
hAdV	4	0	1	3	1	3
hBoV	2	0	2	0	2	0
RV	2	0	2	0	1	1
InfB	0	0	0	0	0	0
InfA	0	0	0	0	0	0
MPV	0	0	0	0	0	0
RSV	0	0	0	0	0	0
EV	0	0	0	0	0	0
hCoV-229E	0	0	0	0	0	0
hCoV-OC43	0	0	0	0	0	0
hCoV-NL63	0	0	0	0	0	0

Discussion

Viral presence in OM
We detected RV, hAdV, and hBoV viral load in NP samples from individuals with acute OM.

- Rhinovirus: RV has a strong association with the development of acute OM. In fact, 30% of children 6-48 months old with RV positive NP secretions developed acute OM within 4 weeks of infection[3]. RV's primary inoculation site is the nasal mucosa, where it attaches to the respiratory epithelium[1].
- Adenovirus: HAdV is also highly associated with acute OM, where 46% of children 6-48 months old with acute OM tested positive for hAdV[3]. However, some argue that since adenovirus DNA can be detected with PCR even months after initial infection, that a positive detection in NP secretions does not necessarily implicate the virus in acute OM development.
- Bocavirus: HBoV has only recently been reported in children with respiratory tract infections and acute OM[4]. It has also been found that patients infected with hBoV could have prolonged acute OM and worse clinical symptoms[5].

NP viruses in acute OM
Interestingly we did not find any viruses in the middle ear samples, suggesting that for the chronic OM cases including those with cholesteatoma, the middle ear infection is primarily bacterial albeit polymicrobial. Viruses in the nasopharynx predispose to acute OM, which may develop into chronic OM.

First viral OM study in Filipino population
There are only a handful of studies that associate upper respiratory viral infections with OM, and this is the first study to report the prevalence (30.8%) of respiratory viruses in the NP of children with OM in the Philippines.

Importance of understanding viral role in OM
OM is one of the most common diseases in children and is considered a polymicrobial disease. Antibiotics is the current routine treatment for acute OM. Yet, acute OM is associated with upper respiratory viral infections in 35% of cases[6] Antibiotics may not be the most effective treatment for some cases of acute OM, such as those which are solely caused by viral infection, or in cases where the ME fluid containing the causal bacteria drains via the Eustachian tubes post inflammatory response[7]. Viral-induced mechanisms have also been suggested to result in decreased penetration of antibiotics in the ME[2]. Additionally, antibiotic resistance is a concern when considering high antibiotic use.

Notably, increased bacterial and viral vaccine use in young children from 2001 to 2011 has been shown to reduce the burden on OM related healthcare[6]. Further understanding of viral presence in acute OM will help progress the development of vaccines to reduce the occurrence of viral infections that lead to acute OM.

Additional screening
All NP samples screened in this study were from children less than 12 years of age. Additional screening of both NP and ME samples in older children and adults would be beneficial in understanding the role of viruses in OM.

Methods

IP cohort
Middle ear (ME) swabs (n=5) and nasopharyngeal (NP) swabs (n=25) were collected in Oragene P117 microbial kits (DNAgenotek) from 26 individuals in an intermarried indigenous Filipino population (IP) with a high prevalence of OM due to A2ML1 variants[8]. Common respiratory viruses were screened in IP cohort including rhinovirus (RV), influenza virus (InF), metapneumovirus (MPV), respiratory syncytial virus (RSV), enterovirus (EV), coronavirus (hCoV), human adenovirus (hAdV), and human bocavirus (hBoV).

PGH cohort
ME tissue and swabs were collected in the P117 kits described above from 45 individuals from the Philippine General Hospital (PGH) with chronic otitis media. Sample types collected include cholesteatoma (n=23), mucosal tissue (n=22), granulation tissue (n=35), ME fluid (n=31), and ME dry swabs (n=4). Respiratory viruses listed above for the IP cohort were screened in the PGH cohort. Additionally, we screened PGH for human papillomavirus (HPV) based on the correlation between HPV and cholesteatoma development in previous studies[9,10].

Viral screening
Microbial DNA isolation was performed with the MasterPure Kit (Lucigen) and microbial RNA isolation with the AllPrep DNA/RNA/miRNA Universal Kit (Qiagen). Superscript IV Reverse Transcriptase (Thermofisher) with random hexamer primers were used for cDNA conversion of microbial RNA. Viral presence was confirmed with both qPCR using PowerUp SYBR Green (Applied Biosystems) and PCR using DreamTaq (Thermofisher).

References

1. PMID: 22968233
2. PMID:12692096
3. PMID: 18279042
4. PMID: 22305021
5. PMID: 19221788
6. PMID: 25514574
7. PMID: 4383320
8. PMID: 26121085
9. PMID: 7960213
10. PMID: 17409785

Funding
NIH-NIDCD R01 DC015004
Philippine Council for Health Research and Development

Correspondence
regie.santos-cortez@cuanschutz.edu

Haemophilus influenzae prevalence and antibiotic susceptibility during colonization and acute otitis media in children, 2019-2020

Naoko Fuji, PhD, Michael Pichichero, MD, Ravinder Kaur, PhD
Naoko.Fuji@rochesterregional.org
Center for Infectious Diseases and Immunology, Rochester General Hospital Research Institute, Rochester, NY

Backgrounds

Acute otitis media (AOM) and recurrent AOM are common diagnoses in children and a common reason for antibiotic prescription. *NTHi* and *Streptococcus pneumoniae (Spn)* are the two main bacterial otopathogens responsible for AOM. Emergence of other encapsulated *Hi* types causing invasive diseases, especially type *a* (*Hia*) has been noted in the United States. In the present study, the prevalence of *Hi* in the nasopharynx (NP) at times of health and onset of AOM as well as in the middle ear fluid (MEF) during was investigated in young children in the Rochester, NY area. Capsule types and antibiotic susceptibility among the *Hi* strains isolated was determined.

Methods

Children, ages 6months to 30 months old were prospectively enrolled from Sep 2019 to Sep 2020 at Rochester NY pediatric clinics. *Hi* isolates from nasopharynx (NP) at healthy visits and disease isolates from NP and middle ear fluid (MEF) at onset of acute otitis media (AOM) were characterized by capsular typing, β-lactamase production and antibiotic susceptibility. The capsular typing was conducted by PCR.

Results

A total of 611 healthy visits and 130 AOM visits (Table 1) occurred among the 334 study children from September 2019-September 2020. No significant racial or gender differences in *Hi* detection during healthy colonization or AOM were identified.

Table 1. Prevalence of *Hi* detection and β-lactamase production from NP of healthy and AOM visits along with *Hi* detection in MEF during AOM of children isolated in Sep 2019-Sep 2020.

	Nasopharynx (NP)			Middle ear fluids (MEF)		
	Healthy (n=611)	AOM (n=130)	p-value Healthy vs AOM	Total taps (n=104)	Total AOM (n=70)	p-value AOM NP vs MEF (Total AOM)
# (%) *Hi*	36 (5.9%)	35 (27%)	**<0.0001**	42 (40%)	30 (43%)	**0.03**
Hi β-lactamase production	15 (42%)	12 (34%)	0.63	18 (43%)	13 (43%)	0.45

In addition to the *Hi* positive AOM cases (43%), *Hi* DNA was detected by PCR in 21% of the culture negative MEF samples.

Table 2. Antibiotic susceptibility among *Hi* isolates

	NTHi			Encapsulated
Antibiotics	**Healthy (n=36)**	**AOM (NP) (n=28)**	**AOM (MEF) (n=28)**	**Healthy, AOM(NP), AOM(MEF) (n= 1, 5, 2)**
Ampicillin	41.7	39.3	39.3	0.0*
TMP/SMX	42.9	35.7	35.7	37.5
Cefaclor	2.8 **	21.4 **	7.1	12.5
Cefuroxime	0.0	3.6	7.1	0.0
Cefprozil	5.6	17.9	7.1	25.0
Cefdinir	2.8	10.7	7.1	0.0
Cefixime	2.8	3.6	10.7	0.0
Cefpodoxime	2.8	3.6	3.6	0.0
Ceftriaxone	2.8	3.6	7.1	0.0
Erythromycin	77.8	67.9	58.6	62.5
Azithromycin	5.6	7.1	3.4	0.0
Clarithromycin	30.6	21.4	27.6	37.5
Amoxicillin-clavulanate	0.0	7.1	3.4	0.0

*Ampicillin non-susceptibility in total *NTHi* and encapsulated *Hi* p-value=0.02
**p-value=0.04

51.0% and **26.5%** were **BLNAS** and **BLPAR**. **4.1%** and **5.1%** of isolates was defined as **BLNAR** and **Low-BLNAR** respectively. One isolate (1%) was categorized as **BLPACR** based on β-lactamase production and amoxicillin–clavulanic acid-resistance (ACR), and showed multi-drug non-susceptibility.

Conclusions

- The prevalence of *Hi* in the NP of young children is very low at times of health
- *Hi* is highly prevalent in MEF at onset of AOM
- >90% of all *Hi* isolates were Non-typeable *Hi*. (Type f predominated)
- β-lactamase production and antibiotic non-susceptibility among *Hi* strains isolated from the NP and MEF are common

Disruption with humanized monoclonal antibodies of biofilms formed by otopathogens and sensitization to antibiotic killing

Nikola Kurbatfinski , Lauren O. Bakaletz & Steven D. Goodman

Center for Microbial Pathogenesis, Abigail Wexner Research Institute at Nationwide Children's Hospital and The Ohio State University College of Medicine, OH, United States

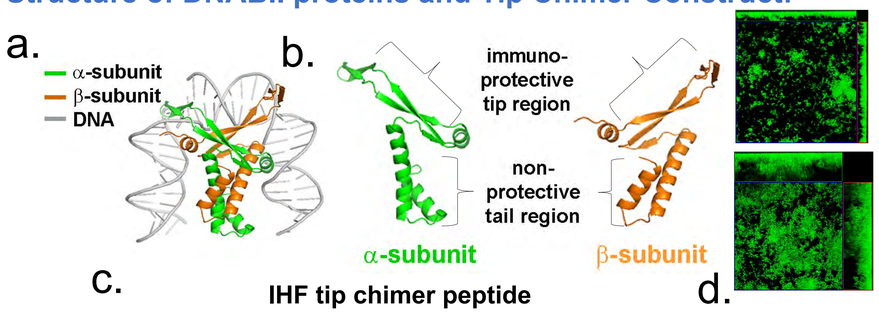
ABSTRACT

Background: New strategies to treat biofilm diseases are needed as bacterial biofilms are highly recalcitrant to antibiotics due to both physical architecture and altered metabolism of biofilm-resident bacteria. We sought to demonstrate the breadth of ability of a humanized monoclonal antibody against an essential biofilm structural protein to disrupt biofilms formed by 4 predominant pathogens of OM and potentiate antibiotic killing efficacy. **Methods:** The effect of antibody dose on the kinetics of biofilm disruption was determined by CFU plate count of those bacteria newly released from biofilm residence. Enhanced killing of newly released bacteria was determined in a 2-hour killing assay by comparison of CFU to those of planktonic bacteria when tested against the top three antibiotics used clinically for each bacterial species. **Results:** Biofilms formed by all four pathogens tested were significantly disrupted by humanized monoclonal antibodies. CFU as a measure of relative disruption increased with both exposure time and dose of antibody used. In all cases, newly released bacteria were significantly more sensitive than their planktonic counterparts to antibiotic killing, however the relative degree of enhanced killing was antibiotic-dependent. **Discussion:** A humanized monoclonal antibody against an essential biofilm structural element effectively disrupted single-species biofilms formed by diverse bacterial pathogens and also rendered the bacteria newly released from biofilm residence highly sensitive to killing by antibiotics. This killing was significantly greater than that observed for planktonic bacteria which offers a powerful opportunity for use of a targeted, combinatorial, species-agnostic therapy.

BACKGROUND

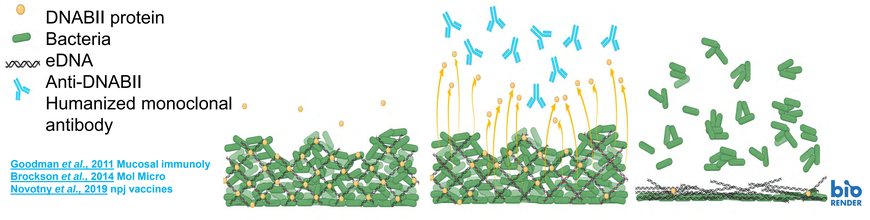
- Biofilms are 3D communities of bacteria resident within a self-produced extracellular polymeric substance (EPS) comprised of exopolysaccharides, proteins, lipids and extracellular DNA (eDNA).
- One such component, the DNABII protein family, serves as an essential linchpin proteins that support the lattice-like eDNA scaffold within which biofilm bacteria reside.
- We chose to target specific protective epitopes of a DNABII protein (e.g. IHF), which is ubiquitous in all Eubacteria, for development of a monoclonal antibody target that disrupts biofilms. Now humanized, this monoclonal antibody is directed against a "tip-chimer" immunogen that mimics the tips of the DNABII protein that bind to and bend eDNA (Novotny et al., 2019).
- Here we characterized the kinetics of disruption and breadth of utility against 4 biofilm-forming otopathogens and determined if those bacterial newly released from biofilm residence (NRels) by the action of this humanized monoclonal were more sensitive to killing by top line antibiotics

Structure of DNABII proteins and Tip Chimer Construct:



a) DNABII protein complexed with DNA at both subunits bend DNA and support the EPS scaffolding
b) 3D model of immuno-protective tip region and non-protective tail region of both subunits
c) Design of a tip chimer peptide immunogen with 20-mer segments from each subunit of the DNABII protein against which antibodies can be formed against the immuno-protective regions
d) Antibodies towards the tip-chimer (top) disrupt 20-hr NTHI biofilms to a greater extent than antibodies towards the tail chimer (bottom), as expected based on epitope mapping data

Humanized monoclonal anti-tip chimer peptide (HuTipMab) disrupt biofilms by collapsing the biofilm matrix:



Humanized monoclonal antibodies against DNABII proteins sequester DNABII molecules from the surrounding milieu which induces an equilibrium shift that collapses the biofilm scaffolding

METHODS

- 16-hr biofilms formed by each targeted otopathogen were treated with increasing concentrations of a humanized monoclonal antibody for increasing incubation periods; human IgG1 served as the isotype control and media alone was the negative control
- NRels were collected after the specified incubation time, diluted and plated. CFU of recovered NRel/ml calculated
- Minimal inhibitory concentrations were determined using a 20-hr broth microdilution assay according to EUCAST guidelines at a concentration of 5e5 CFU/ml per well
- Percent killing of NRel as compared to planktonic bacteria was determined via a 2-hr killing assay in which 5e5 CFU/ml of planktonic and NRels released after a 30-min incubation with 5 µg of humanized monoclonal antibody per well were incubated with respective top line antibiotics. After 2-hr, aliquots were diluted and plated in duplicate. Percent killing by antibiotic was determined compared to incubation in media alone.

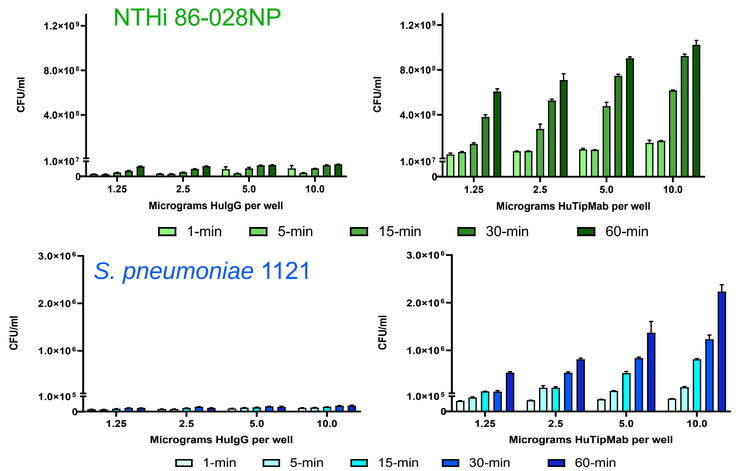
RESULTS

Table 1: Minimal inhibitory concentrations of targeted top-line antibiotics for primary and secondary otopathogens

	Antibiotics (µg/ml)												
Organism	AMK[a]	ATM	CAZ	CLR	CST	DOX	IPM	LVX	LZD	PIP	TOB	SXT	VAN
P. aeruginosa 27853[c]	8	8	8	1024	1	32	16	32	4096	16	4	16	NA
NTHi 86-028NP	8	0.125	64	8	1	0.5	1024	0.0625	16	32	1	0.125	NA
S. pneumoniae 1121	128	512	512	128	NA	0.125	32	0.5	1	0.125	64	16	0.25
P. aeruginosa 142-1	8	8	8	1024	1	32	32	32	2048	16	4	1	NA
S. aureus 29213[c]	32	1024	64	1	NA	0.25	64	0.5	4	8	2	0.25	1

[a]Amikacin (AMK), Aztreonam (ATM), Ceftazidime (CAZ), Clarithromycin (CLR), Colistin (CST), Doxycycline (DOX), Imipenem (IPM), Levofloxacin (LVX), Linezolid (LZD), Piperacillin (PIP), Tobramycin (TOB), Trimethoprim/Sulfamethoxazole (SXT), Vancomycin (VAN)
[b]NA = Assay not performed as antibiotic is Gram-specific
[c]Quality control strains from the ATCC in accordance with EUCAST guidelines

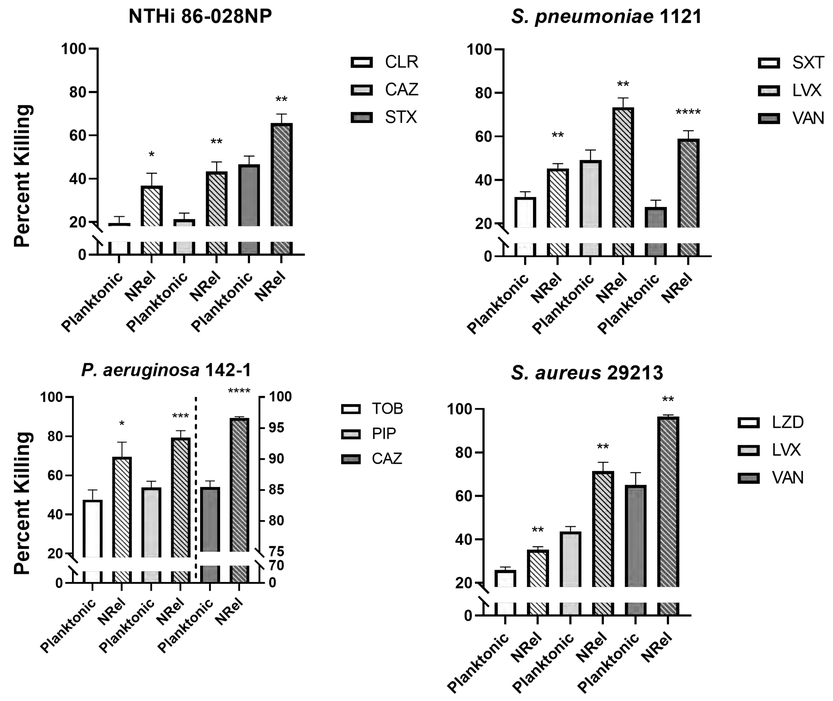
Figure 1: Relative induction of NRel bacteria due to the action of HuTipMab



- Examples of the disruption kinetics displayed by biofilms formed by both NTHi and *S. pneumoniae* due to the action of HuTipMab compared to the non-disruptive control HuIgG1. Whereas the kinetics of disruption were unique to each otopathogen, both were dose- and time-dependent.
- Biofilms formed by *P. aeruginosa* and *S. aureus* displayed similar dose- and time-dependent kinetics (data not shown). [Note: due to the smaller overall size of biofilms formed by *S. pneumoniae* 1121 in this culture system, the number of induced NRel is less than that for NTHI 86-028NP.

RESULTS

Figure 2: Percent killing of otopathogen NRel compared to that of planktonic bacteria by respective 3 top-line antibiotics



Planktonic vs. NRel

- All bacteria newly released from biofilm residence were more susceptible to killing by antibiotics as compared to planktonic bacteria. * P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, **** P ≤ 0.0001
- Note that due to the strong killing activity of ceftazidime (CAZ) against the P. aeruginosa isolate used here, relative percent killing for that antibiotic is indicated in a second right-side y-axis scale
- In all cases, newly released bacteria were significantly more sensitive than their planktonic counterparts to antibiotic killing, with evidence that the relative degree of enhanced killing was antibiotic-dependent and thereby could be specifically tailored for maximum effectiveness.

CONCLUSIONS

- A now humanized monoclonal antibody directed against the protective domains of an essential biofilm structural element (tip-chimer peptide) significantly disrupted biofilms formed by each of four primary and secondary pathogens of OM in a dose- and time-dependent manner.
- Killing of NRel bacteria by each of three top-line antibiotics was significantly greater than that observed for planktonic bacteria in all cases
- The ability of HuTipMab to effectively disrupt biofilms formed by diverse otopathogens that results in generation of NRel, which are highly sensitive to killing by traditional antibiotics, offers a powerful opportunity for use of a targeted, combinatorial, species-agnostic therapy for recalcitrant PTTO and CSOM

ACKNOWLEDGMENTS

- We thank Jennifer Neelans for help in poster preparation and Laura Novotny for technical assistance
- This work was funded by Clarametyx Bioscience, Inc. and CARB-X
- L.O.B. and S.D.G are the inventors of technology related to the DNABII proteins, rights to which have been licensed to Clarametyx Biosciences, Inc.

###

Efficacy of a humanized monoclonal antibody delivered to the chinchilla middle ear via tympanostomy tube

Joseph A. Jurcisek, Tendy Chiang, Charles A. Elmaraghy, Steven D. Goodman and Lauren O. Bakaletz

Center for Microbial Pathogenesis, Abigail Wexner Research Institute at Nationwide Children's Hospital and
The Ohio State University College of Medicine, Columbus, Ohio, U.S.A.

ABSTRACT

Background and Aims To evaluate potential therapeutic efficacy of Fab fragments of humanized monoclonal antibodies directed against an essential biofilm structural protein, we delivered droplets through tympanostomy tubes inserted into the tympanic membranes of chinchillas with ongoing experimental OM due to nontypeable *Haemophilus influenzae* (NTHI). Fab fragments were delivered with or without ofloxacin in an effort to determine synergistic potential against established biofilms.

Methods We challenged chinchillas bilaterally with 1000 CFU NTHI 86-028NP transbullarly (TB) to induce experimental OM. Four days later (to allow biofilm formation) tympanostomy tubes were inserted. We then treated middle ears by droplet both the day of tube insertion and the following day. Twenty-four hours later, animals were sacrificed to analyze how rapidly each agent (or combination) resolved pre-existing biofilms

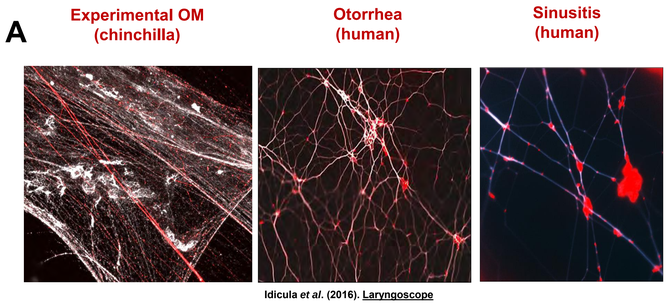
Results By both quantitative and qualitative assessments, Fab fragments derived from humanized monoclonal against a biofilm structural protein delivered alone or in combination with ofloxacin, yielded significant pre-clinical outcomes specific to clearance of pre-established NTHI biofilms, eradication of NTHI from mucosal biofilms and middle ear fluids, and reduction in middle ear mucosal inflammation, compared to delivery of saline or ofloxacin alone.

Discussion and Importance The current standard of care for chronic PTTO, predicated by the presence of persistent biofilms in the middle ear space, is administration of ofloxacin drops, however chronic ottorhea can persist for months to years. This study demonstrated a powerful combinatorial approach to release bacteria from their protective biofilms and rapidly render them vulnerable to killing by a previously ineffective antibiotic.

BACKGROUND

DNA (white strands) and DNABII proteins (red) are abundant in biofilms from pre-clinical and clinical specimens

A



Idicula *et al.* (2016). Laryngoscope

B **Antibodies against DNABII Disrupt NTHI biofilms**

Goodman *et al.*, 2011 Mucosal immunol
Brockson *et al.*, 2014 Mol Micro
Novotny *et al.*, 2019 npj vaccines

Figure 1. A. DNA and DNABII proteins (i.e. integration host factor) are critical for biofilm structural components. B. schematic drawing of how anti-DNABII antibodies disrupt biofilms.

IN VIVO STUDY DESIGN

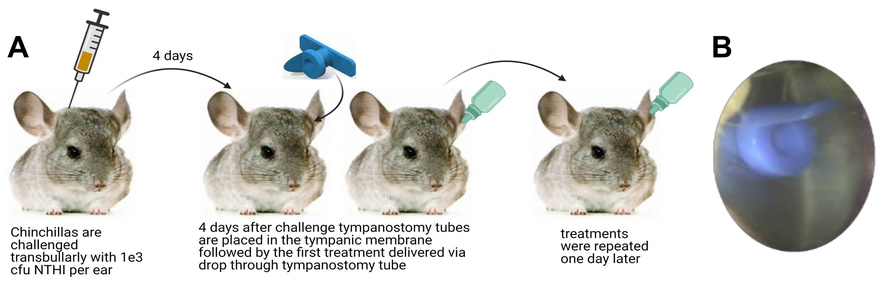
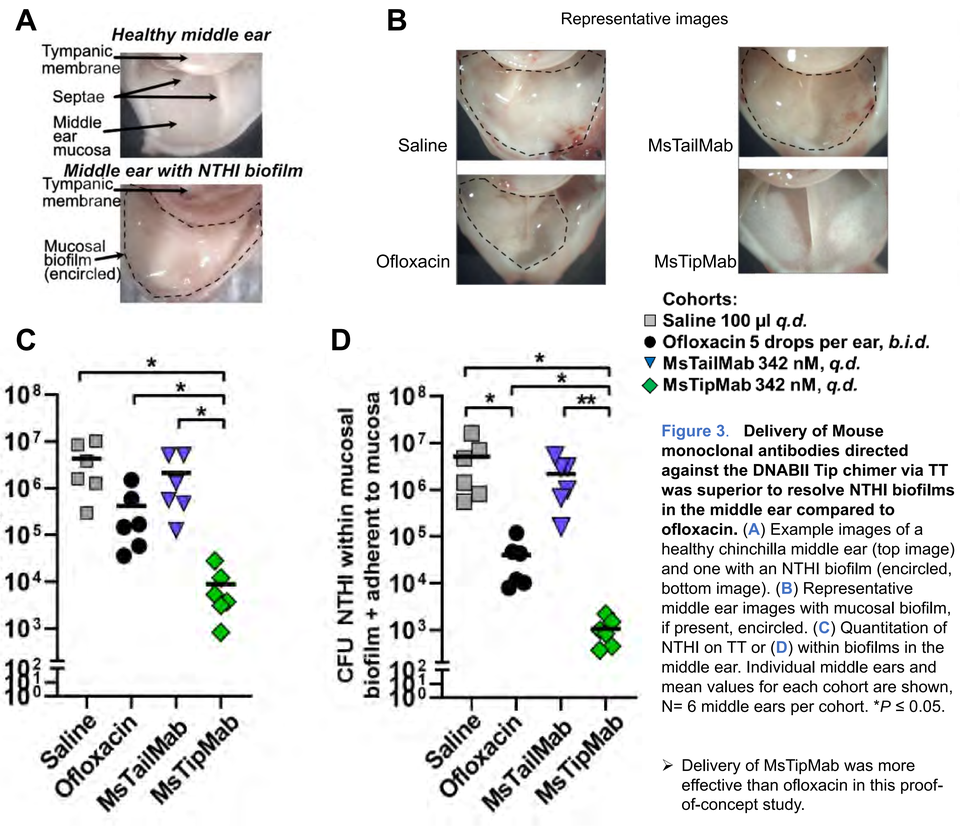


Figure 2 **Experimental design**. **A.** Both middle ears were challenged with 1000CFU NTHI 86-028NP by transbullar injection to induce experimental OM. Four-days later, a myringotomy was performed on anesthetized animals and Touma tympanostomy tube (TT) placed into each tympanic membrane (TM). Middle ear fluid at the time of myringotomy was not irrigated or aspirated to ensure that a consistent NTHI-induced biofilm that contained the target of our treatment was present within the middle ears of all animals. Treatment was then delivered to both ears by droplet into the ear canal with a second treatment the following day. **B.** Representative image of T-tube placed in the tympanic membrane of a chinchilla. Direct access to the middle ear was achieved by placement of TT in TMs of chinchillas. **C.** Regimen for NTHI challenge, treatment and sacrifice.

PROOF OF PRINCIPLE STUDY

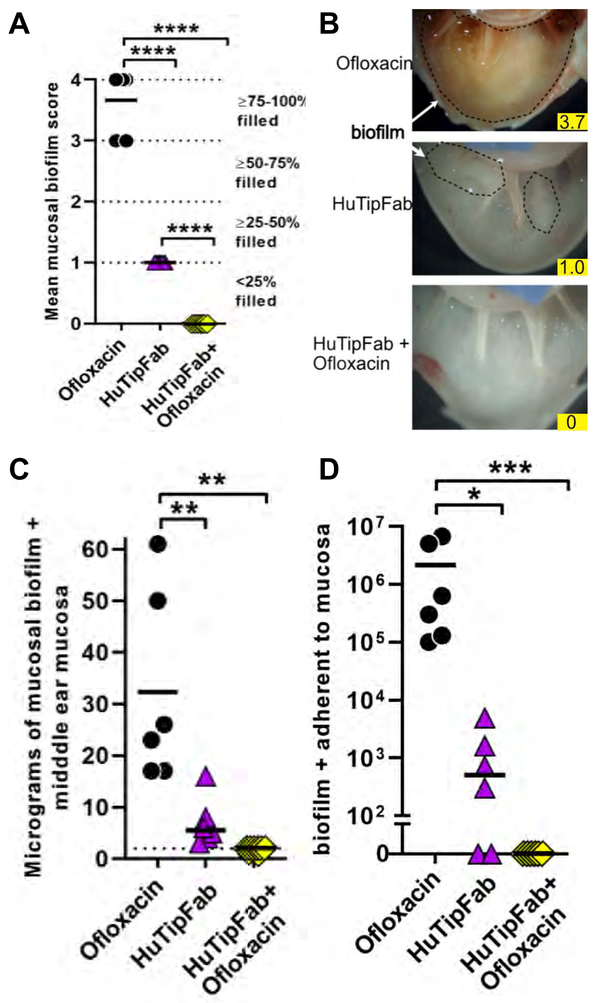


Cohorts:
- **Saline 100 µl *q.d.***
- **Ofloxacin 5 drops per ear, *b.i.d.***
- **MsTailMab 342 nM, *q.d.***
- **MsTipMab 342 nM, *q.d.***

Figure 3. **Delivery of Mouse monoclonal antibodies directed against the DNABII Tip chimer via TT was superior to resolve NTHI biofilms in the middle ear compared to ofloxacin.** (**A**) Example images of a healthy chinchilla middle ear (top image) and one with an NTHI biofilm (encircled, bottom image). (**B**) Representative middle ear images with mucosal biofilm, if present, encircled. (**C**) Quantitation of NTHI on TT or (**D**) within biofilms in the middle ear. Individual middle ears and mean values for each cohort are shown, N= 6 middle ears per cohort. *$P \leq 0.05$.

- Delivery of MsTipMab was more effective than ofloxacin in this proof-of-concept study.

EFFICACY STUDY



Cohorts:
- **Ofloxacin 5 drops per ear, *b.i.d.***
- **HuTipFab 342 nM, *q.d.***
- **Ofloxacin 5 drops per ear, *b.i.d.* + HuTipFab 342 nM, *q.d.***

Figure 4. **Co-delivery of HuTipFab+ofloxacin eradicated NTHI biofilms from the middle ear**. (**A**) Qualitative rank of residual NTHI biofilm upon completion of therapy. (**B**) Representative images from each cohort; the respective mean mucosal biofilm score is indicated in the yellow box. **$P \leq 0.01$; ****$P \leq 0.0001$. Individual middle ears and mean value indicated, N=6 middle ears per cohort. (**C**) Mass of middle ear mucosa; dashed line indicates mass of a healthy middle ear mucosa. (**D**) NTHI was eradicated from the middle ear after therapeutic delivery of HuTipFab+ofloxacin, a significant outcome compared to each treatment individually. *$P \leq 0.05$; ***$P \leq 0.001$. Individual middle ears and mean for cohort shown, N=6 middle ears per cohort.

- HuTipFab+ofloxacin was significantly more effective to rapidly resolve NTHI biofilms compared to either treatment alone.

CONCLUSIONS

- **When delivered alone, in a short course treatment regimen, the monoclonal antibody directed against an essential biofilm structural component significantly reduced both the load of NTHI and the biofilm biomass within the middle ears whereas ofloxacin alone did not**
- **However, when delivered as a combinatorial regimen, HuTipFabs + Ofloxacin showed even greater efficacy wherein total eradication of NTHI and biofilm biomass was achieved**
- **These findings support utilization of this combinatorial treatment regimen for recalcitrant PTTO and CSOM in a highly effective shortened treatment course which would hopefully both increase patient compliance and restrict contribution to the growing global concern of antimicrobial resistance**

ACKNOWLEDGEMENTS

- These data are published: Novotny *et al.* (2021). Humanized anti-DNABII Fab fragments plus ofloxacin eradicated biofilms in experimental otitis media. *Laryngoscope* https://doi.org/10.1002/lary.29497.
- We thank Christian Ahearn for technical assistance, the Animal Resource Center within the Abigail Wexner Research Institute for excellent animal husbandry and Jennifer Neelans for help with poster preparation.
- This study was funded by NIH/NIDCD grant R01 DC011818 to LOB and SDG

Nasopharyngeal microbiome Composition associated with *Streptococcus pneumoniae* colonization suggests a protective role of *Corynebacterium* in young children

Lei Xu[1], Josh Earl[2], Michael E. Pichichero[1]

[1] Center for Infectious Diseases and Immunology, Research Institute, Rochester General Hospital, Rochester, NY 14621

[2] Department of Microbiology & Immunology, Centers for Genomic Sciences and Advanced Microbial Processing, Drexel University College of Medicine, 245 N 15th Street, Philadelphia, PA 19102

Abstract

Streptococcus pneumoniae (Spn) is a leading respiratory tract pathogen that colonizes the nasopharynx (NP) through adhesion to epithelial cells and immune evasion. Spn actively interacts with other microbiota in NP but the nature of these interactions are incompletely understood. Using 16S rRNA gene sequencing, we analyzed the microbiota composition in the NP of children with or without Spn colonization. 96 children were included in the study cohort. 74 NP samples were analyzed when children were 6 months old and 85 NP samples were analyzed when children were 12 months old. We found several genera that correlated negatively or positively with Spn colonization, and some of these correlations appeared to be influenced by daycare attendance or other confounding factors such as upper respiratory infection (URI) or *Moraxella* colonization. Among these genera, *Corynebacterium* showed a consistent inverse relationship with Spn colonization with little influence by daycare attendance or other factors. We isolated *Corynebacterium propinquum* and *C. pseudodiphtheriticum* and found that both inhibited the growth of Spn serotype 22F strain *in vitro*.

Study Design

- **Goal:** Identify nasal microbiome changes in response to Spn colonization in children.
- **Main methods**: 16S rRNA gene sequencing and bioinformatic tools (e.g. phyloseq and DESeq2 in R) on microbiome composition and abundance.
- **Subject information:** 1. Children of 6 months or 12 months old; 2. Recruited from middle class, suburban sociodemographic pediatric practice in Rochester, NY, USA (see Table 1); 3. Exclusion criteria: uncertain diagnosis of AOM, diagnosis of otorrhea, presence of tympanostomy tube, diagnosis of Down syndrome, craniofacial disorders, cystic fibrosis, congenital immunodeficiency.
- **Sample information:** NP washes from the children were cultured in vitro for identification of Spn and other common respiratory bacterial pathogens using established microbiological methods (Table 2). Samples were split into Spn+ or Spn- groups based on the results.

Table 1. Demographic Factors Associated with Spn Colonization

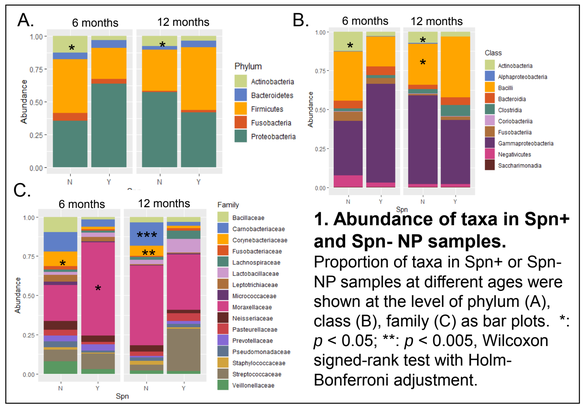
	# of samples	Race (White: non-white)	Female: Male	Breast-feeding (Yes:No)	Exposure to smoke (Yes:No)	Atopy (Yes:No)	Abx Treatment (Yes:No)	Daycare Attendance (Yes:No)	Siblings (Yes:No)
Spn+									
6 m	25	22:3	10:15	10: 15	0:24	5:18	1:20	9:15***	3:22
12 m	28	24:4	12:16	10:16	1:26	6:19	8:13	10:17*	4:24
total	**53**								
Spn-									
6 m	48	39:9	24:24	24:23	6:42	13:29	6:35	2:45 ***	6:42
12 m	56	46:10	33:23	27:28	8:47	15:34	7:38	6:49*	9:47
total	**104**								

Note: Demographic factors are listed as column names. The number of children who were female or male, or who were Yes or No for a particular demographic factor is shown for each age group (6-month or 12-month) and for each Spn colonization phenotype (Spn+ or Spn-). In each age group, the proportion of children carrying a demographic trait was compared between Spn+ and Spn- samples and statistical significance assessed by chi-square analyses. *: $p < 0.05$; **: $p < 0.005$; ***: $p < 0.0005$.

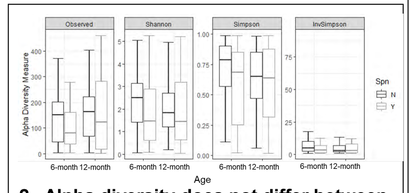
Table 2 Pathological Factors

	# of samples	NTHi	Mcat	URI	AOM-free: sOP
Spn+					
6 m	26	3+, 23-	15+, 11- **	5+, 21-	13:12 *
12 m	29	3+, 26-	10+, 19-	10+,19- *	16:12 *
total	**55**				
Spn-					
6 m	48	4+, 44-	12+, 36- **	8+, 40-	37:11 *
12 m	56	4+, 52-	11+, 45-	7+, 48- *	45:11 *
total	**104**				

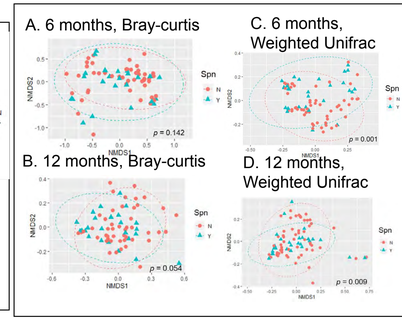
Note: Specific factors are listed as column names. The number of children who were positive (+) or negative (-) for each potential bacterial respiratory pathogen or for URI, or were AOM-free or sOP, are indicated for each age group (6-month or 12-month) and for each Spn colonization phenotype (Spn+ or Spn-). In each age group, the proportion of children colonized with an otopathogen or URI, or designated as AOM-free or sOP, was compared between Spn+ and Spn- samples and statistical significance was assessed by chi-square analyses. *: $p < 0.05$; ***: $p < 0.0005$.



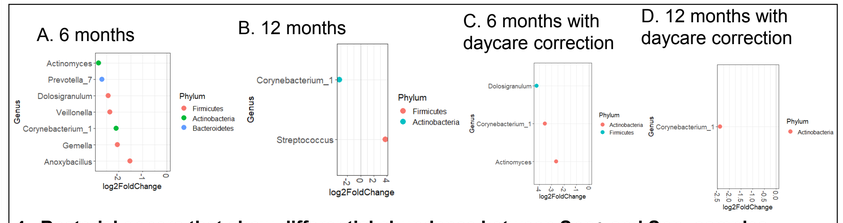
1. Abundance of taxa in Spn+ and Spn- NP samples. Proportion of taxa in Spn+ or Spn- NP samples at different ages were shown at the level of phylum (A), class (B), family (C) as bar plots. *: $p < 0.05$; **: $p < 0.005$, Wilcoxon signed-rank test with Holm-Bonferroni adjustment.



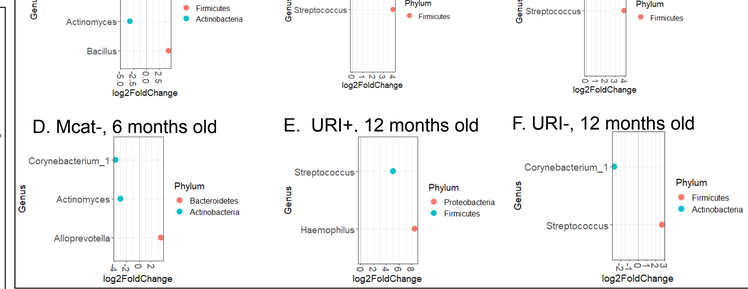
2. Alpha diversity does not differ between Spn+ and Spn- NP samples. Alpha diversity indices of nasal microbiome from Spn+ or Spn- children of 6 and 12 months olds were calculated and graphed in box plots.



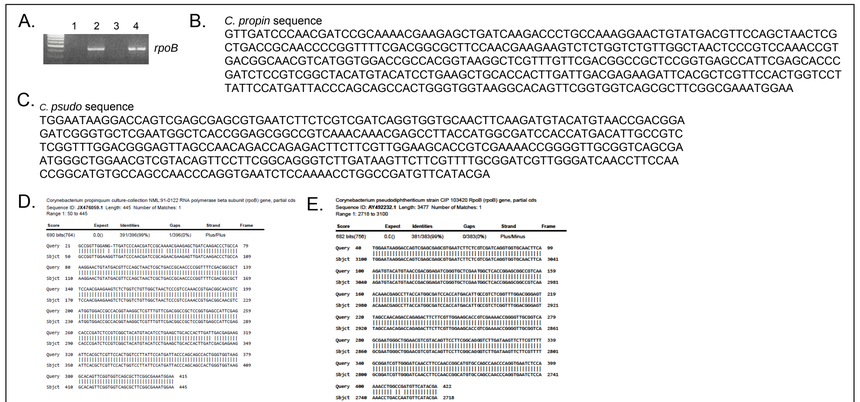
3. Difference in beta diversity of microbiome between Spn+ and Spn- NP samples in children at 12 months. Bray-Curtis distance (A and B) or weighted unifrac (C and D) of samples collected at 6 months or 12 months were measured and visualized on NMDS plots. Permanova was performed for samples stratified by age to assess the difference between Spn+ and Spn- samples. The *p* values are shown at the right bottom corner of each plot.



4. Bacterial genera that show differential abundance between Spn+ and Spn- samples. Abundance of bacterial genera was compared between Spn+ and Spn- samples collected at 6 months (A), or at 12 months (B) without correction for daycare attendance, or with correction for daycare attendance (C, 6 months; D, 12 months). The ones with adjusted *p* value less than 0.05 are shown. The x axis exhibits the $\log_2$ ratio between abundance of bacterial genera in Spn+ samples and that in Spn- samples.



5. NTHi and Mcat Detection, URI and Otitis Proneness as factors that contribute to microbiome difference between Spn+ and Spn- NP samples. Samples were divided into AOM-free/sOP, Mcat+/Mcat-, and URI+/URI- samples at each age group. Differential abundance of genera between Spn+ and Spn- samples was determined in each subgroup: AOM-free/sOP (A-C), Mcat (D), or URI (E-F).



B. *C. propin* sequence
GTTGATCCCAACGATCCGCAAAACGAAGAGCTGATCAAGACCCCTGCCAAAGGAACTGTATGACGTTCCAGCTAACTCG
CTGACCGCAACCCCGGTTTTCGACGGCGCTTCCAACGAAGAAGTCTCTGGTCTGTTGGCTAACTCCCGTCCAAACCGT
GACGGCAACGTCATGGTGGACCGCCACGGTAAGGCTCGTTTGTTCGACGGCCGCTCCGGTGAGCCATTCGAGCACCC
GATCTCCGTCGGCTACATGTACATCCTGAAGCTGCACCACTTGATTGACGAGAAGATTCACGCTCGTTCCACTGGTCCT
TATTCCATGATTACCCAGCAGCCACTGGGTGGTAAGGCACAGTTCGGTGGTCAGCGCTTCGGCGAAATGGAA

C. *C. psudo* sequence
TGGAATAAGGACCAGTCGAGCGAGCGTGAATCTTCTCGTCGATCAGGTGGTGCAACTTCAAGATGTACATGTAACCGACGGA
GATCGGGTGCTCGAATGGCTCACCGGAGCGGCCGTCAAACAAACGAGCCTTACCATGGCGATCCACCATGACATTGCCGTC
TCGGTTTGGACGGGAGTTAGCCAACAGACCAGAGACTTCTTCGTTGGAAGCACCGTCGAAAACCGGGGTTGCGGTCAGCGA
ATGGGCTGGAACGTCGTACAGTTCCTTCGGCAGGGTCTTGATAAGTTCTTCGTTTTGCGGATCGTTGGGATCAACCTTCCAA
CCGGCATGTGCCAGCCAACCCAGGTGAATCTCCAAAACCTGGCCGATGTTCATACGA

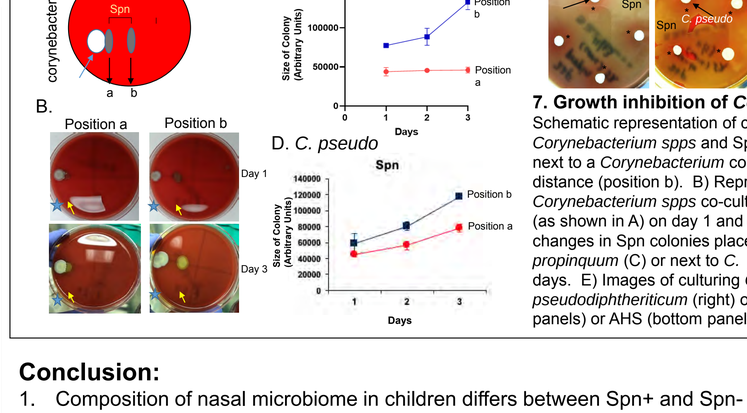
D. Corynebacterium propinquum culture-collection NML 91-0122 RNA polymerase beta subunit (rpoB) gene, partial cds
Sequence ID: **JX476059.1** Length: 445 Number of Matches: 1
Range 1: 50 to 445

Score	Expect	Identities	Gaps	Strand	Frame
690 bits(764)	0.0	391/396(99%)	1/396(0%)	Plus/Plus	

E. Corynebacterium pseudodiphtheriticum strain CIP 103420 RpoB (rpoB) gene, partial cds
Sequence ID: **AY492232.1** Length: 3477 Number of Matches: 1
Range 1: 2719 to 3108

Score	Expect	Identities	Gaps	Strand	Frame
682 bits(756)	0.0	381/383(99%)	0/383(0%)	Plus/Minus	

6. Isolation of *Corynebacterium propinquum* and *Corynebacterium pseudodiphtheriticum*. A) Agarose gel electrophoresis of PCR products from *rpoB* gene. Lanes 1 and 3: PCR products from colonies grown on chocolate plates; lanes 2 and 4: PCR products from colonies grown on blood agar plates. The growth of *Corynebacterium* on chocolate plates was not as robust as on blood agar plates, so the colonies grown on chocolate plates were found to differ from *Corynebacterium* and served as a negative control. B,C) Sequences of PCR products from two *Corynebacterium* species that were later identified as *C. propinquum* and *C. pseudodiphtheriticum*. D) Alignment of PCR sequences from *C. propinquum* against GeneBank database. E) Alignment of PCR sequences from *C. pseudodiphtheriticum* against GenBank database.



7. Growth inhibition of *Corynebacterium* by Spn. A) Schematic representation of co-culture experiment for *Corynebacterium spps* and Spn. Spn22F was inoculated right next to a *Corynebacterium* colony (position a) or with some distance (position b). B) Representative images of *Corynebacterium spps* co-cultured with Spn22F at position a or b (as shown in A) on day 1 and day 3. C, D) Measurement of size changes in Spn colonies placed at positions a and b next to *C. propinquum* (C) or next to *C. pseudodiphtheriticum* (D) over 3 days. E) Images of culturing *C. propinquum* (left) or *C. pseudodiphtheriticum* (right) on top of a lawn of Spn22F (top panels) or AHS (bottom panels).

Conclusion:

1. Composition of nasal microbiome in children differs between Spn+ and Spn- individuals.
2. Factors such as daycare attendance or upper respiratory infection (URI) influence the composition changes in response to Spn colonization.
3. Detection of *Corynebacterium* in NP showed a consistent inverse relationship with Spn colonization.
4. Isolates of *Corynebacterium propinquum* and *C. pseudodiphtheriticum* were found to inhibit the proliferation of a Spn22F strain *in vitro*.

Limitation of our study: 1) V4 region in 16S rRNA gene does not provide species information on the taxa; 2) only one isolate of *C. propinquum* or of *C. pseudodiphtheriticum* and one strain of Spn was studied in the *in vitro* assay; 3) our *in vitro* observation may not translate into similar effects *in vivo*.

The *FUT2* c.461G>A (p.Trp154*) variant is associated with differential expression of genes involved in host-pathogen interactions and mucosal microbiota changes in patients with otitis media

Christina L. Elling[1,2], Melissa A. Scholes[1,3], Sven-Olrik Streubel[1,3], Todd M. Wine[1,3], Patricia J. Yoon[1,3], Jeremy D. Prager[1,3], Samuel P. Gubbels[1], Stephen P. Cass[1], Herman A. Jenkins[1], Kenny H. Chan[1,3], Norman R. Friedman[1,3], Daniel N. Frank[4], Regie Lyn P. Santos-Cortez[1,5]

[1]Department of Otolaryngology-Head and Neck Surgery, School of Medicine, University of Colorado Anschutz Medical Campus; [2]Human Medical Genetics and Genomics Program, University of Colorado Anschutz Medical Campus; [3]Department of Pediatric Otolaryngology, Children's Hospital Colorado; [4]Division of Infectious Diseases, Department of Medicine, School of Medicine, University of Colorado Anschutz Medical Campus; [5]Center for Children's Surgery, Children's Hospital Colorado

Introduction

Otitis media (OM) or infection and inflammation of the middle ear (ME) is a leading cause of hearing loss. Globally of the ~350-710 million OM cases each year, about half are in children <5 years old. Common and rare variants within *FUT2*, which encodes alpha-(1,2)-fucosyltransferase, were previously identified to confer susceptibility to OM, likely through the associated shifts observed in the ME microbiota. Moreover, increased relative abundance of pathobionts in the nasopharynx (NP), such as *Haemophilus*, *Streptococcus* and *Staphylococcus*, are associated with higher risk of OM and upper respiratory tract infection.

Objectives

- Identify genes that are differentially expressed in the presence of the *FUT2* c.461G>A variant
- Use DE genes for network and pathway analysis
- Analyze NP microbiota diversity and test for correlation between *FUT2* variant and relative abundance of individual taxa

Methods

Gene expression was examined in relation to carriage of a common pathogenic *FUT2* c.461G>A (p.Trp154*) variant using RNA-sequence data from saliva of 28 patients with OM in order to further understand the downstream effects of *FUT2* variation. Differential expression (DE) analysis of RNA-seq counts was performed by *FUT2* genotype. In addition, analyses of microbiota diversity indices and relative abundance of individual taxa were performed using 16S rRNA sequence data from NP and ME samples of 33 OM patients according to *FUT2* genotype.

Results

In those with the *FUT2* variant, *MUC16* (FDR-p=0.008) and *FN1* (FDR-p=0.005) are downregulated whereas *MTAP* (FDR-p=0.008) is upregulated (Fig. 1). Using these DE genes and the KEGG and PANTHER:BP databases, network analysis revealed a combined 47 significant pathways, of which viral, apoptotic, rhythmic and cell cycle processes overlap between databases. The NP bacterial profiles of OM patients with the *FUT2* variant (Fig. 2A) reflect similar increases in relative taxa abundance in the ME. In the NP, *Propionibacterium*, *Staphylococcus* and other *Gammaproteobacteria* were associated with the *FUT2* variant, whereas *Actinobacillus* and Candidate Division TM7 were associated with wildtype genotype (Fig. 2B).

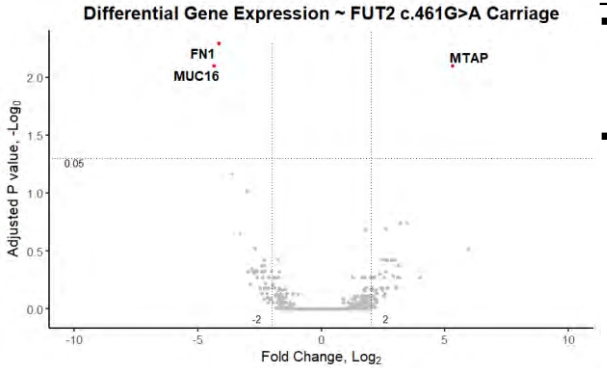


Figure 1 **Results of differential expression analysis in which significant genes have a log2 fold change >2 and FDR-p < 0.05**

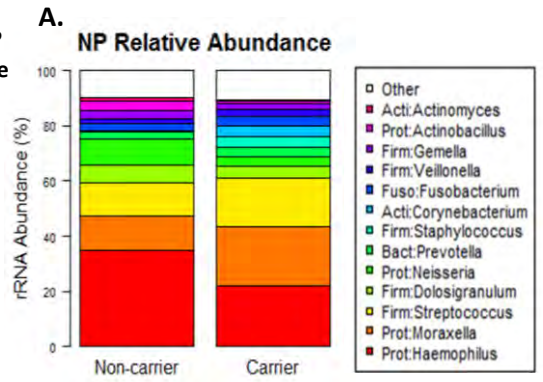
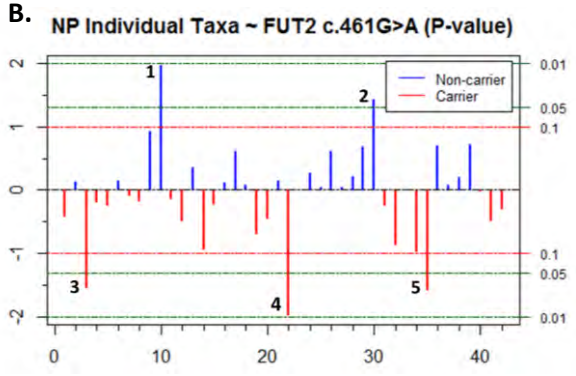


Figure 2 **A. Comparison of NP bacterial profiles of the wildtype vs *FUT2* variant groups.**
B. Tests for individual taxa abundance for taxa enriched in either the wildtype (blue) or variant (red) resulting in 2 significant taxa associations with wildtype and 3 with the variant.



1: Candidate Division TM7
2: *Actinobacillus*
3: *Propionibacterium*
4: *Staphylococcus*
5: *Gammaproteobacteria*

Conclusions

- The *FUT2* c.461G>A variant is associated with transcriptional changes related to host response to infection and higher relative abundance of specific NP taxa, leading to greater biodiversity in the ME
- We identified a novel gene network through which *FUT2* variation regulates the host response to bacteria or viruses in the NP and ME, suggesting that within the OM context the relationship between gene expression and microbiota is more complex

This work was funded by the National Institutes of Health - National Institute on Deafness and other Communication Disorders through grants R01 DC015004 (to RSC) and a predoctoral scholarship to CLE from T32 DC012280 (to Sue Kinnamon and HAJ).