Pneumococcal and *H. influenzae* protein specific IgG and IgA are present in serum and the middle ear effusion of children with a history of recurrent acute otitis media

Karli Corscadden
The GROMIT study
Investigating the immunology, genetics and microbiology of recurrent acute otitis media in children

**Otitis media cases**
- 201 children < 36 months
- At least 3 episodes of AOM
- Admitted to hospital for grommet insertion

**Healthy controls**
- 89 children < 36 months
- No history of ear disease
- Admitted to hospital for minor surgical procedures

- Blood and saliva samples for immune analysis and nasopharyngeal swabs for culture
- OM children, middle ear effusion (MEE) for bacterial culture and PCR and to investigate the local immune response in the middle ear

- *Streptococcus pneumoniae* and non-typeable *Haemophilus influenzae* (NTHi) were the most common pathogens isolated from nasopharynx – carriage rates in rAOM children were significantly higher compared to healthy children
- NTHi was the predominate pathogen detected in MEE using PCR (culture rates for both *S. pneumoniae* and NTHI were low)
Investigating the immune system of children with a history of rAOM

• To determine whether children with rAOM mount an antibody response to potential vaccine candidate protein antigens from *S. pneumoniae* and NTHi

• Developed a novel bead-based multiplex assay (which requires minimal amount of serum) to measure IgG antibodies to;
  
  − 4 pneumococcal specific protein antigens: pneumococcal surface protein A families 1 (*PspA1*) and 2 (*PspA2*), choline binding protein (*CbpA*) and pneumolysin (*Ply*)
  
  − 3 nontypeable *H. influenzae* outer membrane proteins *P4*, *P6* and *PD*

• Antibody levels for all protein antigens were compared between children with a history of rAOM and healthy children
  
  − Levels correlated with the age of the children and carriage data
Protein specific IgG in serum

- *S. pneumoniae* and NTHi protein specific IgG was detected in the serum of children with a history of rAOM and healthy children
  - Antibodies are produced to these protein antigens
- Children colonised with *S. pneumoniae* or NTHI had higher protein specific IgG antibodies in their serum to the respective bacteria
  - Antibodies are produced during colonisation and these protein antigens are immunogenic in children with rAOM
- Protein specific IgG increased with the age of the child for both groups of children
  - Increased exposure and immune development of the child
Conclusion:
Protein antigens from *S. pneumoniae* and NTHi induce serum IgG in children with a history of rAOM and these levels are comparable to children with no history of rAOM.

Vaccines containing these conserved bacterial proteins antigens could help to reduce the incidents of AOM and decrease the burden of rAOM.

Acute otitis media is a mucosal disease, so what is going on at the site of infection (in the middle ear itself)?

Little is known about the immune response within the middle ear of children with rAOM.

The presence of antibodies in the middle ear may be more important in clearing bacterial infection and preventing recurrent acute otitis media.
Investigate if pneumococcal and NTHi protein specific IgG and IgA are present in the MEE samples from children with recurrent AOM

- MEE samples were collected into saline from children undergoing grommet insertion
- Total IgG and IgA in the MEE samples was determined by nephelometry
  - Used samples with detectable total IgG (n=139) and IgA (n=115)
- Measured *S. pneumoniae* and NTHi protein specific IgG and IgA in the MEE
  - PspA1, PspA2, CbpA (IgG only), PLY, P4, P6 and Protein D
- Protein specific antibodies were reported as a ratio (arbitrary units) of protein specific antibodies within total antibodies to control for variability in sample dilution
- Results were compiled and MEE arbitrary units were correlated with carriage data, age of subject and serum IgG (measuring protein IgA is ongoing)
Pneumococcal and NTHi protein specific IgG is present in the middle ear effusion of children with a history rAOM.
There is a positive correlation between protein specific IgG in serum and MEE for all protein antigens

Suggests that antibodies in the serum transudate to the middle ear
Vaccination with protein vaccines could result in antibodies in the middle ear and the presence of these antibodies could prevent against rAOM
Pneumococcal and NTHi protein specific IgA is present in the middle ear effusion of children with a history rAOM
Pneumococcal protein IgG and IgA in MEE tends to increase with age.

Increased exposure to pneumocococcus over time and also immune development.

Different ratios of IgG and IgA within proteins:
- PspA1 → Greater IgA
- PspA2 → Greater IgG
- PLY → Greater IgG but both are present
NTHi protein specific IgG and IgA tended to increase with age

Increased exposure to NTHi over time and also immune development

**Different ratios of IgG and IgA within proteins**
- **P4** → Greater ratio of IgA
- **P6** → Greater ratio of IgG
- **PD** → Equal levels of IgG and IgA
Children colonised in the nasopharynx by the respective bacteria have higher IgG and IgA antibodies to bacteria specific proteins. 

Trend the same as seen for serum IgG → rAOM children produce antibodies in response to colonisation → these proteins are immunogenic in rAOM children.
Children with NTHi detected in their MEE sample have higher IgG and IgA antibodies to NTHi specific proteins.

Trend the same as seen for serum IgG and MEE IgG → children colonised at the time of sample collection have higher IgG and IgA antibodies to bacteria proteins.

rAOM children produce antibodies to these bacteria during colonisation → These proteins are immunogenic in rAOM children.
Conclusions

- Children with a history of rAOM are able to produce IgG antibodies against pneumococcal and NTHi proteins in serum at least as well as healthy children.
- In rAOM children, IgG and IgA antibodies are present in the middle ear and IgG correlates well with serum IgG.
  - Suggests antibodies in the serum, which could be induced by vaccination, can enter the middle ear space and could potentially help in clearing these bacteria at the site of infection.
- Protein specific IgG and IgA levels increase in the middle ear with age.
  - May be due to increased exposure to these bacteria over time.
- rAOM children colonised during sample collection tended to have higher protein antibodies in the middle ear.
  - Suggesting rAOM do produce antibodies to these proteins.
- Pneumococcal and NTHi protein antigens are immunogenic in rAOM children and vaccines including these proteins may provide species wide protection and may help to reduce the incidence and burden of rAOM.
Future work

• Measure protein specific IgA in the serum sample collected from rAOM and healthy children
  – correlate IgA in the serum with IgA in the middle ear
• Measure protein specific IgG and IgA in the saliva samples collected from rAOM and healthy children
  – Investigate the local mucosal response in rAOM children
• Investigate the functionality of these protein antibodies in the serum and saliva samples (Ply functionality in serum already done)
  – If antibodies are present are they functioning correctly and able to protect against infection with these bacteria
• Evaluate these protein antibodies responses in Aboriginal children
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All the children and parents who participated in the GROMIT Study
# Demographics (don’t think need to include)

<table>
<thead>
<tr>
<th></th>
<th>rAOM</th>
<th>Healthy</th>
<th>p-value</th>
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<tbody>
<tr>
<td>Number</td>
<td>169</td>
<td>77</td>
<td>n/a</td>
</tr>
<tr>
<td><strong>Mean age (months)</strong></td>
<td>21.1</td>
<td>16.9</td>
<td><strong>0.01</strong></td>
</tr>
<tr>
<td><strong>Male (%)</strong></td>
<td>61.5%</td>
<td>75.3%</td>
<td><strong>0.034</strong></td>
</tr>
<tr>
<td>AOM episodes total (n=157)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 – 2</td>
<td>-</td>
<td>77 (100%)</td>
<td></td>
</tr>
<tr>
<td>3 – 4</td>
<td>50 (31.8%)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>5 – 7</td>
<td>27 (17.2%)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>8 – 9</td>
<td>57 (36.4%)</td>
<td>-</td>
<td></td>
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<tr>
<td>≥ 10</td>
<td>23 (14.6%)</td>
<td>-</td>
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<tr>
<td><strong>Median # AOM episodes</strong></td>
<td>7</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>Day care attendance (&gt;4hrs)</td>
<td>67.7%</td>
<td>29.3%</td>
<td><strong>&lt;0.001</strong></td>
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<tr>
<td>Currently on antibiotics</td>
<td>24.4%</td>
<td>16.0%</td>
<td>0.15</td>
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<tr>
<td>Have siblings</td>
<td>70.3%</td>
<td>67.6%</td>
<td>0.68</td>
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