Online Supplement

FUT2 genotype influences lung function, exacerbation frequency, and airway microbiota in non-CF bronchiectasis

Supplement E1 – non-CF bronchiectasis patient description, inclusion/exclusion criteria

In addition to their description below, details of recruitment and inclusion are provided as part of a separate publication.¹

Non-CF bronchiectasis subject inclusion criteria

1. Able to provide written informed consent.
2. Confirmed diagnosis of bronchiectasis by HRCT within 3 years.
3. Airways obstruction on spirometry (ratio FEV₁/FVC <0.7) and FEV₁ ≥25% predicted.
4. Chronic productive cough with at least 5 mLs sputum production per day.
5. At least two exacerbations of bronchiectasis requiring either oral or intravenous supplemental antibiotic therapy (of at least 7 days on each occasion) in the prior 12 months.
7. Clinically stable for at least four weeks (defined as no symptoms of exacerbation, no requirement for supplemental antibiotic therapy, and FEV₁ within 10% of best recently recorded value where available).

Exclusion criteria

1. Bronchiectasis as a result of CF or focal endobronchial obstruction.
2. Currently active tuberculosis or non-tuberculous mycobacterial (NTM) infection. Subjects with evidence of prior pulmonary NTM infection could be included only if they have completed a course of therapy that is deemed successful on the basis of negative NTM cultures following cessation of therapy. All subjects required a negative NTM culture prior to screening.
3. Any symptoms or signs to suggest recent deterioration in respiratory disease, including exacerbation of pulmonary disease (as previously defined) in the preceding 4 weeks.
4. Any change to medications in the preceding 4 weeks.
5. Prescription of either oral or intravenous antibiotic therapy in the preceding 4 weeks.
6. Cigarette smoking within the preceding 6 months.
7. Any history of malignant arrhythmia (unless in the immediate post-myocardial infarction period and not requiring any regular therapy) or QTc prolongation on baseline ECG.
8. Any of the following within the three (3) months prior to enrolment:
   • Acute MI
   • Acute CVA
   • Major surgery
9. History of any of the following:
• Active malignancy (excepting non-melanoma skin malignancies that have been treated and considered cured)
  • Listed for transplantation
  • Any other significant active illness likely to affect the patient’s survival within 12 months
  • Receiving long-term domiciliary oxygen therapy

10. Allergy to macrolide antibiotics, other than minor, dose-related gastrointestinal intolerance that would not be anticipated to recur with low-dose erythromycin.

11. Any prescription or receipt of long-term macrolide antibiotics, or receipt of a treatment course within 4 weeks.

12. Predominant diagnosis of emphysema (rather than bronchiectasis) on HRCT scan of the chest.

13. Requirement for supplemental oxygen therapy.

14. Inability to complete required study procedures for whatever reason (including 6 minute walk test, hypertonic saline sputum induction).

15. Respiratory symptoms (including cough, sputum production, recurrent exacerbations) not predominantly the result of bronchiectasis in the opinion of the PI; where treatable causes for exacerbations existed, these were treated before considering trial enrolment.

**Excluded medications**

1. Macrolide antibiotics – long-term macrolide use was an absolute exclusion, however subjects who had received a short duration (less than 6 weeks) treatment course were eligible provided they had at least 4 weeks washout.

2. Long term oral antibiotic administration for infection prophylaxis (eg doxycycline).

3. Any other intravenous or oral antibiotic within 4 weeks.

4. While erythromycin in the current study was administered in a low dose, possible drug interactions in all patients entering the study were considered. Subjects using the following medications were not eligible for the study:
   • ergotamine or dihydroergotamine
   • triazolam/ alprazolam
   • sildenafil
   • azole antifungals (ketoconazole, itraconazole, fluconazole)
   • disopyramide
   • quinidine
Concomitant HMG-CoA reductase inhibitor (‘statin’) use was permitted, however subjects on high-dose statins (equivalent to 80 mgs daily of simvastatin) required dose reduction by half at study entry.

Subjects prescribed diltiazem or verapamil were screened to ensure no evidence of clinically relevant increases in levels of these medications.

The following medications were permitted, provided they had been a regular medication for at least 6 months (with the requisite number of exacerbations whilst on this therapy):

1. Inhaled antibiotics in chronic, daily, stable dose.
2. Inhaled mucolytic therapies (hypertonic saline, mannitol, dornase alpha, Nacetylcysteine).
3. Oral mucolytics or expectorants.

**Additional information**

While not part of the recruitment inclusion or exclusion criteria, none of the patients in the study had allergic bronchopulmonary aspergillosis, or immunodeficiency (as measured by serum immunoglobulin levels). Other comorbidities, which were not basis for exclusion, are listed in Table 1.
**Supplement E2 – Supplementary methodology**

**Sputum induction procedure**

Subjects were instructed to perform their usual chest physiotherapy regime on the morning of the sputum induction procedure. Prior to commencement of hypertonic saline inhalation, any spontaneous sputum expectorated was collected for standard culture. Sputum induction (SI) was performed after inhalation of 400 ug of albuterol, using 4.5% hypertonic saline nebulised from an ultrasonic nebuliser (output >1 mL/min) for 20 minutes in 4 periods of 5 minutes each, according to the standardised protocol recommended by the European Respiratory Society taskforce. Following mouth-rinsing and expectoration, sputum was collected following each nebulisation period, on each occasion preceded immediately by spirometry. The first sputum sample was refrigerated immediately following collection and frozen at -80°C within an hour. A cold chain was maintained up until the point of DNA extraction.

Ten, 15 and 20 minute samples were pooled and an aliquot from this placed on ice immediately and transferred for inflammatory cell count processing within 60 minutes. Sputum was processed according to the methods of the US Cystic Fibrosis Therapeutics Development Network Standard Operating Procedure. Briefly, an equal volume of sterile 10% dithiothreitol (DTT) (Sputolysin; Calbiochem-Novabiochem Corp., San Diego, CA), was added to the sputum, then incubated in a shaking water bath at 37°C for 5-10 min, and mixed using a transfer pipette at 5-min intervals. A further three times the volume of both DTT and phosphate-buffered saline (Dulbecco’s; Gibco BRL, Grand Island, NY) was added and the mixture incubated again in the 37°C shaking water bath for another 5-10 min. 10 μl of homogenized sputum samples, mixed with Trypan Blue, was used to calculate total cell counts using a standard hemacytometer. A further 0.25-0.50 ml of both samples was used to prepare cyto spin slides for differential cell counts. After staining the slides with Wright’s stain, 300 non-squamous cells were counted and cell differentials calculated.

**Endobronchial Biopsy Collection and Processing**

Subjects were fully informed about the potential risks of the procedure and provided written consent. Bronchoscopy was performed as an outpatient procedure in the endoscopy unit of the operating theatres of the Mater Adult Hospital, using an Olympus flexible fibre-optic bronchoscope according to the safety standards of the Thoracic Society of Australia and New Zealand, with details of the research bronchoscopy procedure adapted from prior methods. Subjects fasted for 6 hours before the procedure. The procedure was performed transorally, under light sedation using intravenous midazolam and fentanyl to ensure patient comfort. Topical lignocaine was applied to the vocal cords and bronchi by instillation through the bronchoscope. Endbronchial biopsies were then taken from subsegmental carinae of the lower lobes using Boston scientific Radial Jaw 3 single-use biopsy forceps (diameter 1.8 mm), starting at 5th order airways and working proximally as far as the 3rd order bronchi if necessary (bifurcation of segmental and subsegmental bronchi). Subjects were observed for 2 hours after the bronchoscopy before being allowed home.
Clinical measures of lung disease severity

Leicester Cough Questionnaire

The Leicester Cough Questionnaire (LCQ) is a valid, repeatable 19 item self-completed quality of life measure of chronic cough which is responsive to change. LCQ was employed as described previously.\textsuperscript{8}

St George’s Respiratory Questionnaire

The St. George's Respiratory Questionnaire (SGRQ) is a disease-specific measure used to assess patients with mild to severe airway disease. This measure is a disease-specific instrument designed to measure impact on overall health, daily life, and perceived well-being. It was developed for use by patients with fixed and reversible airway obstruction. The measure consists of 50 (76 responses) items that produce three domain scores and one overall score measuring: Symptom (frequency and severity); Activity (activities that cause or are limited by breathlessness); and Impacts (social functioning, psychological disturbances resulting from airways disease). The SGRQ has been validated and approved as a self-administered instrument, and was applied here as described previously.\textsuperscript{9}

Physician Defined Pulmonary Exacerbations

Using a modification of the Anthonisen criteria,\textsuperscript{10} a subject was considered to have a pulmonary exacerbation when they had persistent (> 24 hour) increase in sputum volume or purulence in addition to deterioration in at least 2 other, different of the following symptoms: sputum volume, sputum purulence, cough, dyspnoea, chest pain, new haemoptysis (to meet criteria required 3 separate symptoms overall; ie if a subject described increase in sputum volume, then this symptom could not also be counted as one of the ‘additional’ symptoms, although increased sputum purulence could be counted as a second, separate symptom if present).

Put in a different way, PDPE required deteriorations in at least 3 of the following, with at least 1 of the major criteria:

Major criteria: increase in sputum volume or sputum purulence.

Minor criteria: cough, dyspnoea, chest pain or haemoptysis.

(ie PDPE= 1 major and 2 minor or 2 major and 1 minor)

In order to be counted as separate PDPE’s, sequential episodes required unequivocal resolution of symptoms from the first event AND >14 days from the end of one event to the commencement of the subsequent event. If both criteria were not met, the exacerbation was counted as a single, continuing event.

Subjects were provided with 24 hour contact details and directed to contact study staff (Megan L Martin or DJS) at any time (including after hours) should they develop either symptoms of exacerbation or feel that they required antibiotic therapy. Subjects were directed to ensure that antibiotic prescription was provided by and directed through study staff rather than alternative sources. Criteria for PDPE were adjudicated by DJS and MLM.

Criteria for exacerbation were adjudicated by telephone if subjects contacted study staff outside trial visits, and in those meeting criteria for PDPE a prescription was faxed to the subject’s local pharmacy for collection. Subjects were not routinely reviewed at the study
centre in such circumstances, unless symptoms were inconsistent with an exacerbation, the subjects was too unwell to be managed as an outpatient or if symptoms failed to subsequently respond appropriately to therapy. Antibiotic prescriptions were directed by DJS, provided by study staff and standardized according to microbiology and antibiotic tolerance.

Antibiotic selection for PDPE’s:

All antibiotic prescriptions were directed by DJS, were provided by study staff and were standardised according to the results of the most recent respiratory microbiology sample and subject antibiotic tolerance. Oral antibiotic selection was based upon the most recent microbiology result, according to the following guide (subjects were instructed to cease antibiotics once symptoms of exacerbation resolved, even if antibiotic supply remained; microorganisms not discussed below had antibiotic selection determined according to the results of antimicrobial susceptibility testing):

‘Normal flora’/ no respiratory pathogens isolated – oral amoxycillin 500 mg/ clavulanic acid 125 mg, one tablet twice daily for 10 days

- If penicillin allergic (simple), cephalxin 500 mg, one capsule four times daily for 10 days

- If penicillin anaphylaxis, antibiotic selection individualised by DJS according to results of prior airway microbiology, prior antibiotic history and tolerability, etc

*P. aeruginosa* – oral ciprofloxacin 250-750 mg (generally 500 mg) twice daily for 14 days

*H. influenzae* – either oral amoxicillin 500 mg thrice daily for 7-14 days or oral amoxicillin 500 mg/ clavulanic acid 125 mg, one tablet twice daily for 10 days according to results of antimicrobial susceptibility testing

- if penicillin allergic, oral cotrimoxazole (sulphamethoxazole 800 mg/ trimethoprim 160 mg) one tablet twice daily for 10 days

If subjects had persistent symptoms of exacerbation at the completion of the course of antibiotics, repeat respiratory microbiology was obtained and the following antibiotics continued until microbiology results were available:

‘Normal flora’ or *H. influenzae* – same antibiotics as original course, for 5 to 10 days; in the event that symptoms persisted following this further course, oral ciprofloxacin 250-750 mg (generally 500 mg) was commenced twice daily for 14 days for suspected occult gram negative airway infection *P. aeruginosa* – oral ciprofloxacin was continued and oral cotrimoxazole (sulphamethoxazole 800 mg/ trimethoprim 160 mg) twice daily added, for a further 10 to 14 days.

**Serum CRP measurements**

Subjects were asked to provide a blood sample to be analysed at Mater Pathology laboratory. CRP measurement was performed using an Architect ci16200 latex-enhanced immunoturbidimetric assay (Abbott Diagnostics, Abbott Park, IL).

**Neutrophils in sputum as a percent of total non-squamous cells**

Collected sputum was placed on ice immediately and transferred for processing within 60 minutes. Sputum was processed according to the methods of the US Cystic Fibrosis Therapeutics Development Network Standard Operating Procedure. Briefly, an equal volume
of sterile 10% dithiothreitol (DTT) (Sputolysin; Calbiochem-Novabiochem Corp., San Diego, CA), was added to the sputum, then incubated in a shaking water bath at 37°C for 5-10 min, and gently mixed using a transfer pipette at 5-min intervals. A further three times the volume of both DTT and phosphate-buffered saline (Dulbecco's; Gibco BRL, Grand Island, NY) was added and the mixture incubated again in the 37°C shaking water bath for another 5-10 min. Ten microliters of the homogenized sputum samples, mixed with Trypan Blue, was used to calculate total cell counts, using a standard hemacytometer. A further 0.25-0.50 ml of both samples was used to prepare cytopsin slides for differential cell counts. After staining the slides with Wright’s stain, 300 cells were counted and cell differentials calculated.

Diagnostic microbiology data

Pyrosequencing

Bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) was performed as described previously using Gray28F 5’-TTTGATCNTGGCTCAG-3’ and Gray519r 5’-GTNTTACNGCGGCCGTCG-3’). Initial generation of the sequencing library involved a one step PCR of 30 cycles, using a mixture of Hot Start and HotStar high fidelity Taq DNA polymerase, as described previously. Tag-encoded FLX amplicon pyrosequencing analyses utilized Roche 454 FLX instrument with Titanium reagents, titanium procedures performed at the Research and Testing Laboratory (Lubbock, TX, USA) using RTL protocols (www.researchandtesting.com).

Sequence processing pipeline

The following information is as described in protocol documentation provided by Molecular Research DNA, Texas, USA (www.mrdnalab.com). Custom software written in C# within a Microsoft.NET (Microsoft Corp, Seattle, WA, USA) development environment was used for all post sequencing processing. Quality trimmed sequences obtained from the FLX sequencing run were derived directly from FLX sequencing run output files. Tags were extracted from the multi-FASTA file into individual sample-specific files based upon the tag sequence. Tags which did not have 100% homology to the sample designation were not considered. Sequences which were less than 150 bp after quality trimming were not considered. All failed sequence reads, low quality sequence ends and tags and primers were removed. Sequences with ambiguous base calls, sequences with homopolymers > 6bp were removed. Further, any non-bacterial ribosomal sequences and chimeras using B2C2 as described previously.

Individual samples were assembled using CAP3 after parsing the tags into individual FASTA files. The ace files generated by CAP3 were then processed to generate a secondary FASTA file containing the tentative consensus (TC) sequences of the assembly along with the number of reads integrated into each consensus. TC were required to have at least 2-fold coverage.

To determine the identity of bacterial species in the remaining sequences, sequences were denoised, assembled into OUT clusters at 97% identity, and queried using a distributed .Net algorithm that utilizes Blastn+ (KrakenBLAST www.krakenblast.com) against a database of high quality 16S rRNA gene bacterial sequences. Using a .NET and C# analysis pipeline the resulting BLASTn+ outputs were compiled, data reduction analysis performed, and sequence identity classification carried out, as described previously.
Based on the described protocols, identification at the species level should only be considered putative.

**Determining Secretor Status**

**FUT2 Polymorphism Genotyping**

Genomic DNA was extracted from patients’ serum using Mammalian Genomic DNA Miniprep Kit (Sigma-Aldrich), according to manufacturer’s instructions. An 1162bp region of the FUT2 gene was amplified using primers F: 5’-CGTGTCGTTGTTCTCCTCC, R: 5’-AGAGAGATGGGTCTGCTCAT. Each reaction contained 5 µL of KAPA Taq HotStart Buffer (KAPA Biosystems, MA, USA), 2.5 µL of MgCl₂, 0.5 µL of dNTP, 0.5 µL of each primer (10 µM), 0.2 µL KAPA Taq HotStart polymerase, 14.8 µL dH₂O and 1 µL of genomic DNA. Cycling conditions were 95°C for 3 min followed by 40 cycles of 95°C for 30s, 60°C for 15s, 72°C for 30s. A final elongation was allowed at 72°C for 5 min. Successful amplification was confirmed by gel electrophoresis, and the PCR products were purified using a DNA acid phosphatase-exonuclease kit (New England BioLabs) according to the manufacturer’s instructions.

Amplicons were then sequenced by Sanger Sequencing using two internal primers (5’-TGCTGTCGTTGTTCTCCTCC and 5’-CCATCTTCAAACTCACCCTG) by Flinders Sequencing Facility (SA pathology, Bedford Park, SA). Readouts were aligned to a reference sequence using Clustal W multiple alignment using UGENE, where polymorphisms were detected by mismatched alignment. Polymorphisms were then cross-referenced with the literature to determine loss-of-function polymorphisms.

**Secretor Status Bronchial Phenotyping by Immunohistochemistry**

*Ulex Europaeus* lectin 1 (HRP-UEA1) staining was performed on 32 endobronchial biopsies. After removal of paraffin and rehydration of biopsies, endogenous peroxidase was inhibited by 20 min incubation in 3.0% H₂O₂. Sections were rinsed twice for 5 min in PBS/0.3% Tween-20. Tissue was blocked with 200 µl of 1% BSA/PBS for 30 min at room temperature. Sections were then incubated with 200 µl of 5 µg/mL HRP-UEA1 for 1 hour (EY Laboratories, San Mateo, CA, USA). Sections were then rinsed in PBS/Tween-20, 3 times for 5 min, and AEC substrate (Vector Laboratories, Burlingame, CA, USA) added for 30 min before addition of haematoxylin counterstain (all steps at room temperature).
**Table E3.** Summary of relative abundance of the dominant infective microbe in sputum of bronchiectasis patients

<table>
<thead>
<tr>
<th>Genus</th>
<th>Number of patients</th>
<th>% Average relative abundance (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Haemophilus</em></td>
<td>33</td>
<td>85.6 (18)</td>
</tr>
<tr>
<td><em>Pseudomonas</em></td>
<td>25</td>
<td>87.0 (14)</td>
</tr>
<tr>
<td><em>Veillonella</em></td>
<td>9</td>
<td>33.6 (8)</td>
</tr>
<tr>
<td><em>Prevotella</em></td>
<td>9</td>
<td>40.3 (13)</td>
</tr>
<tr>
<td><em>Streptococcus</em></td>
<td>4</td>
<td>44.2 (21)</td>
</tr>
<tr>
<td><em>Pasteurella</em></td>
<td>2</td>
<td>93.0 (9)</td>
</tr>
<tr>
<td><em>Stenotrophomonas</em></td>
<td>1</td>
<td>80.8</td>
</tr>
<tr>
<td><em>Staphylococcus</em></td>
<td>1</td>
<td>25.6</td>
</tr>
<tr>
<td><em>Porphyromonas</em></td>
<td>1</td>
<td>72.6</td>
</tr>
<tr>
<td><em>Neisseria</em></td>
<td>1</td>
<td>18.0</td>
</tr>
<tr>
<td><em>Moraxella</em></td>
<td>1</td>
<td>79.1</td>
</tr>
<tr>
<td><em>Leptotrichia</em></td>
<td>1</td>
<td>34.6</td>
</tr>
<tr>
<td><em>Flavobacterium</em></td>
<td>1</td>
<td>16.7</td>
</tr>
<tr>
<td><em>Burkholderia</em></td>
<td>1</td>
<td>58.1</td>
</tr>
<tr>
<td><em>Bordetella</em></td>
<td>1</td>
<td>54.2</td>
</tr>
<tr>
<td><em>Achromobacter</em></td>
<td>1</td>
<td>52.7</td>
</tr>
<tr>
<td><em>Abiotrophia</em></td>
<td>1</td>
<td>92.7</td>
</tr>
</tbody>
</table>
Supplement E4 - Fungal Supplementary data

Samples were each assigned as either positive or negative for *C. albicans* or *A. fumigatus* based on qPCR CT score, referenced to culture positive standard curves. Details of each standard curve and detection and cut-off thresholds are detailed below. Approximate copy numbers for standards were calculated based on DNA concentration of positive control (measured by Qubit fluorometer), fungal genome size, and number of gene copies per genome. Samples were run in triplicate and averaged.

### *C. albicans* isolate standard

<table>
<thead>
<tr>
<th>Sample</th>
<th>Copy #</th>
<th>Av CT</th>
<th>std</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dil1:10^0</td>
<td>621600</td>
<td>22.901</td>
<td>0.118</td>
</tr>
<tr>
<td>Dil1:10^1</td>
<td>6216</td>
<td>27.790</td>
<td>0.288</td>
</tr>
<tr>
<td>Dil1:10^2</td>
<td>621.6</td>
<td>31.247</td>
<td>0.165</td>
</tr>
<tr>
<td>Dil1:10^3</td>
<td>62.16</td>
<td>34.211</td>
<td>0.737</td>
</tr>
<tr>
<td>Dil1:10^4</td>
<td>6.216</td>
<td>37.231</td>
<td></td>
</tr>
</tbody>
</table>

% amplification efficiency: 92.77%

R^2: 0.9879

*Detection limit of 35 CT (or 36.4 copies) assigned to *C. albicans* qPCR based on standard curve

^a 1:10^0 dilution failed to produce sufficient CT, likely due to interference with PCR inhibitors or buffer concentration imbalance. No samples produced a CT <27 therefore this dilution was ignored.

### *A. fumigatus* isolate standard

<table>
<thead>
<tr>
<th>Sample</th>
<th>Copy #</th>
<th>Av CT</th>
<th>std</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dil1:10^0</td>
<td>3763200</td>
<td>13.022</td>
<td>0.009</td>
</tr>
<tr>
<td>Dil1:10^1</td>
<td>376320</td>
<td>16.392</td>
<td>0.003</td>
</tr>
<tr>
<td>Dil1:10^2</td>
<td>37632</td>
<td>19.916</td>
<td>0.024</td>
</tr>
<tr>
<td>Dil1:10^3</td>
<td>3763.2</td>
<td>23.392</td>
<td>0.107</td>
</tr>
<tr>
<td>Dil1:10^4</td>
<td>376.32</td>
<td>27.237</td>
<td>0.153</td>
</tr>
<tr>
<td>Dil1:10^5</td>
<td>37.632</td>
<td>30.927</td>
<td>0.500</td>
</tr>
</tbody>
</table>

% amplification efficiency: 90.02%

R^2: 0.9995

*Detection limit of 34 CT (or 4.76 copies) assigned to *A. fumigatus* qPCR based on standard curve
Figure E5: Endobronchial biopsy stain by *FUT2* genotype. UEA-1 lectin staining, which is specific for α(1,2) fucosylated Lewis glycans, of bronchial biopsies from non-cystic fibrosis bronchiectasis patients with *sese* (A), *Sese* (B), and *SeSe* (C) genotypes. Staining is located on apical cell surfaces and within secretory vesicles of *Sese* and *SeSe*, but absent in *sese* biopsies. There was no clear difference in staining intensity between biopsies from *Sese* and *SeSe* patients.

Figure E6: Effect of *FUT2* genotype on FEV$_1$ %. Box and whisker plots show median, IQR, and 5th and 95th percentiles (dots show outliers) of pre-bronchodilator FEV$_1$ as a percentage of the predicted value. *p*=0.023 by Tukey’s post-hoc test.
**Figure E7:** Effect of *FUT2* genotype on physician defined pulmonary exacerbation count. Proportion of patients who had a physician defined pulmonary exacerbations (PDPE) over the 48 weeks of the trial. Colours indicate number of PDPEs. Left – All patients, Right – Patients in the placebo group only. P values calculated by Wald test.

**Figure E8:** Effect of *FUT2* genotype on time on antibiotics. Number of days on antibiotics due to pulmonary exacerbation were recorded over the course of trial. Showing total patients (receiving either placebo or erythromycin). Time on antibiotics in 10 day groups, for purpose of graphing. P values calculated by Wilcoxon rank-sum test based on ungrouped data.
Figure E9: Effect of FUT2 genotype on Alpha diversity measures of non-dominant bacterial species. Shannon Weiner Index and Simpson’s Complement Index of rescaled relative abundance (excluding Pseudomonas aeruginosa and Haemophilus influenzae, when dominant). P values calculated by ANOVA (left) and Kruskal-Wallis (right). This indicates no significant difference of within patient microbiome diversity of evenness between secretor genotypes.

Figure E10: Effect of FUT2 genotype on Principal Coordinate (PCO) analysis of Bray-Curtis distances. PCO1 and PCO2 plot of Bray-Curtis similarity of rescaled relative abundance (excluding Pseudomonas aeruginosa and Haemophilus influenzae, when dominant) between sese (dark blue), Sese (green), and SeSe (light blue) patients. This indicates that the microbiota of patients, excluding P. aeruginosa and H. influenzae, when dominant, is not different. PERMANOVA p=0.777.
Figure E11: Effect of *FUT2* genotype on Airway fungal predominance. Detection of *Candida albicans* (top) and *Aspergillus fumigatus* (bottom). P values calculated by Fishers exact test.
References