**Supplementary Information**

# Early nasal microbiota and subsequent respiratory tract infections in infants with cystic fibrosis

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**Supplementary Methods**

Respiratory symptom scores

Symptoms of respiratory tract infections, wheeze and/or cough were recorded in standardized weekly telephone interviews conducted by a study nurse. We calculated a symptom score with high sensitivity for lower respiratory-tract infections (RTIs) ranging from 0 (no symptoms) to 4 (severe respiratory symptoms). The scoring system has been previously used to investigate respiratory symptoms in infants [1,2] (see supplementary table 4). We defined RTIs as cough, wheeze and/or breathing difficulties in combination with upper respiratory tract symptoms or fever for more than two consecutive days and/or symptom score ≥ 3. Day- and night-time symptoms were combined for each interview and the higher score was used. We defined "higher number of RTIs" above the 75th percentile of RTI weeks of controls. We divided infants in two groups: Infants with a "lower number" of RTIs (0-3 weeks of RTI, n=35) and infants with a "higher number" of RTIs (>3 weeks of RTI, n=15).

**Supplementary Tables**

Supplementary Table 1: Included infants per CF Center

| CF Center | Included infants |
|---|---|
| Bern | 16 |
| Zürich | 11 |
| Lausanne | 7 |
| Aarau | 6 |
| Basel | 4 |
| Genf | 2 |
| Lugano | 2 |
| St. Gallen | 1 |
| Luzern | 1 |

Supplementary Table 2: Symptom score calculation

| Symptom score | Day-time symptoms (cough, wheeze, or breathing difficulties) | Night-time symptoms (cough, wheeze, or breathing difficulties) |
|---|---|---|
| 0 | None | None |
| 1 | Slight, no treatment | Slight, sleep not disturbed |
| 2 | Required treatment, but no outside help | Sleep disturbed once, no help required |
| 3 | Severe; required help from GP | Sleep disturbed more than once; child needed help |
| 4 | Very severe; admitted to hospital | Sleep very disturbed or GP called |

Supplementary Table 3: Summary of weeks with RTI and antibiotic treatments

| | CF (n=50) | Controls (n=30) |
|---|---|---|
| Weeks with RTI, all infants (sum (%)) | 145 (15.5) | 64 (11.1) |
| - Of those with AB treatment (%) | 55.2 | 4.7 |
| Weeks with AB, all infants (sum (%)) | 80 (8.6) | 3 (0.5) |
| Weeks with RTI per infant (mean (range)) | 2.9 (0, 12) | 2.1 (0, 8) |
| Weeks with AB per infant (mean (range)) | 1.6 (0, 14) | 0.1 (0, 2) |

*Abbreviations*: RTI = respiratory tract infection; CF = Cystic Fibrosis; AB= antibiotic treatment

Supplementary Table 4: Comparison of within-subject dissimilarity between CF and controls overall, and stratified in groups regarding first RTI and first antibiotic treatment

| Antibiotic treatment | RTI | CF N; consecutive swabs | Controls N; consecutive swabs | Estimate (Controls)† | 95% CI† | *P* value† |
|---|---|---|---|---|---|---|
| All | All | 50; 707 | 30; 442 | -0.053 | -0.084, -0.022 | <0.001 |
| Before | All | 42; 397 | 30; 426 | -0.028 | -0.066, 0.010 | 0.145 |
| All | Before | 43; 305 | 27; 263 | -0.025 | -0.087, 0.038 | 0.441 |
| After * | All | 30; 287 | 30; 442 | -0.090 | -0.049, -0.130 | <0.0001 |
| All | After | 38; 310 | 20; 134 | -0.116 | -0.045, -0.186 | 0.002 |

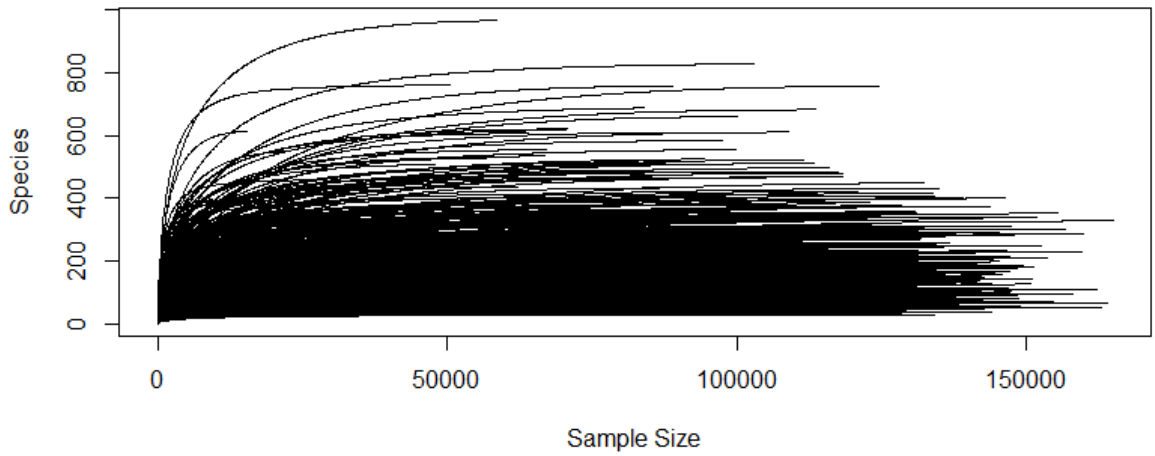
*Abbreviations*: RTI = respiratory tract infection; CF = Cystic Fibrosis; CI = confidence interval; All = all samples were included irrespective of antibiotic treatment or RTI; Before/After = only samples before or after the first antibiotic treatment or RTI were included * CF infants after antibiotic treatment were compared with controls. † gamm model corrected for breastfeeding, siblings, mode of delivery, antibiotic treatment, season (fixed effects), age in weeks (smooth term) and subject (random effect)

Supplementary Table 5: Differential abundance analysis of the most abundant bacterial families between CF and controls in first year of life.

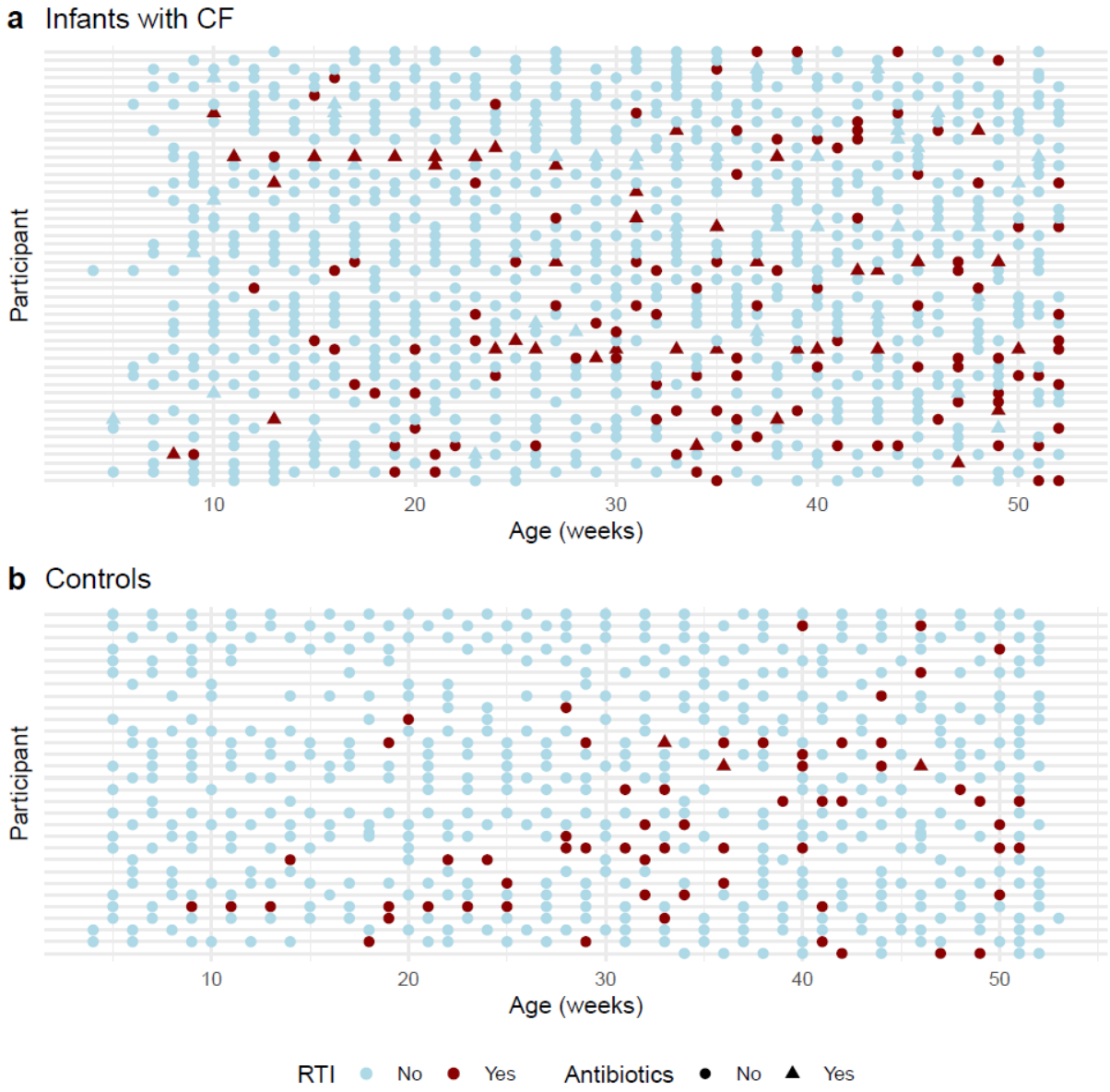
| **Feature** | **Value** | **Coef** | **Stderr** | **pval** | **qval*** |
|---|---|---|---|---|---|
| Micrococcaceae | Controls | -1.70 | 0.32 | <0.0001 | <0.0001 |
| Staphylococcaceae | Controls | -2.07 | 0.47 | <0.0001 | <0.0005 |
| Propionibacteriaceae | Controls | -1.32 | 0.39 | <0.005 | <0.01 |
| Gemellaceae | Controls | -1.20 | 0.39 | <0.005 | <0.05 |
| Carnobacteriaceae | Controls | 1.34 | 0.38 | <0.001 | <0.005 |
| Moraxellaceae | Controls | 1.05 | 0.34 | <0.005 | <0.01 |
| *Before first RTI* | | | | | |
| **Feature** | **Value** | **Coef** | **Stderr** | **pval** | **qval** |
| Carnobacteriaceae | Controls | 1.71 | 0.54 | <0.005 | <0.05 |
| Staphylococcaceae | Controls | -2.34 | 0.72 | <0.005 | <0.05 |
| *Before first antibiotics* | | | | | |
| **Feature** | **Value** | **Coef** | **Stderr** | **pval** | **qval** |
| Micrococcaceae | Controls | -1.54 | 0.36 | <0.0001 | <0.005 |
| Staphylococcaceae | Controls | -1.78 | 0.53 | <0.005 | <0.05 |
| Carnobacteriaceae | Controls | 1.23 | 0.42 | <0.005 | <0.05 |
| *Before first antibiotics, after RTI* | | | | | |
| **Feature** | **Value** | **Coef** | **Stderr** | **pval** | **qval** |
| Staphylococcaceae | Controls | -2.63 | 0.69 | <0.001 | <0.01 |
| *Before RTI, after antibiotics* | | | | | |
| **Feature** | **Value** | **Coef** | **Stderr** | **pval** | **qval** |

*Differential abundance analysis was performed with MaAsLin2, default settings. The reported significant q-values are corrected for multiple testing (Benjamini-Hochberg) and considered as significant if q<0.05.

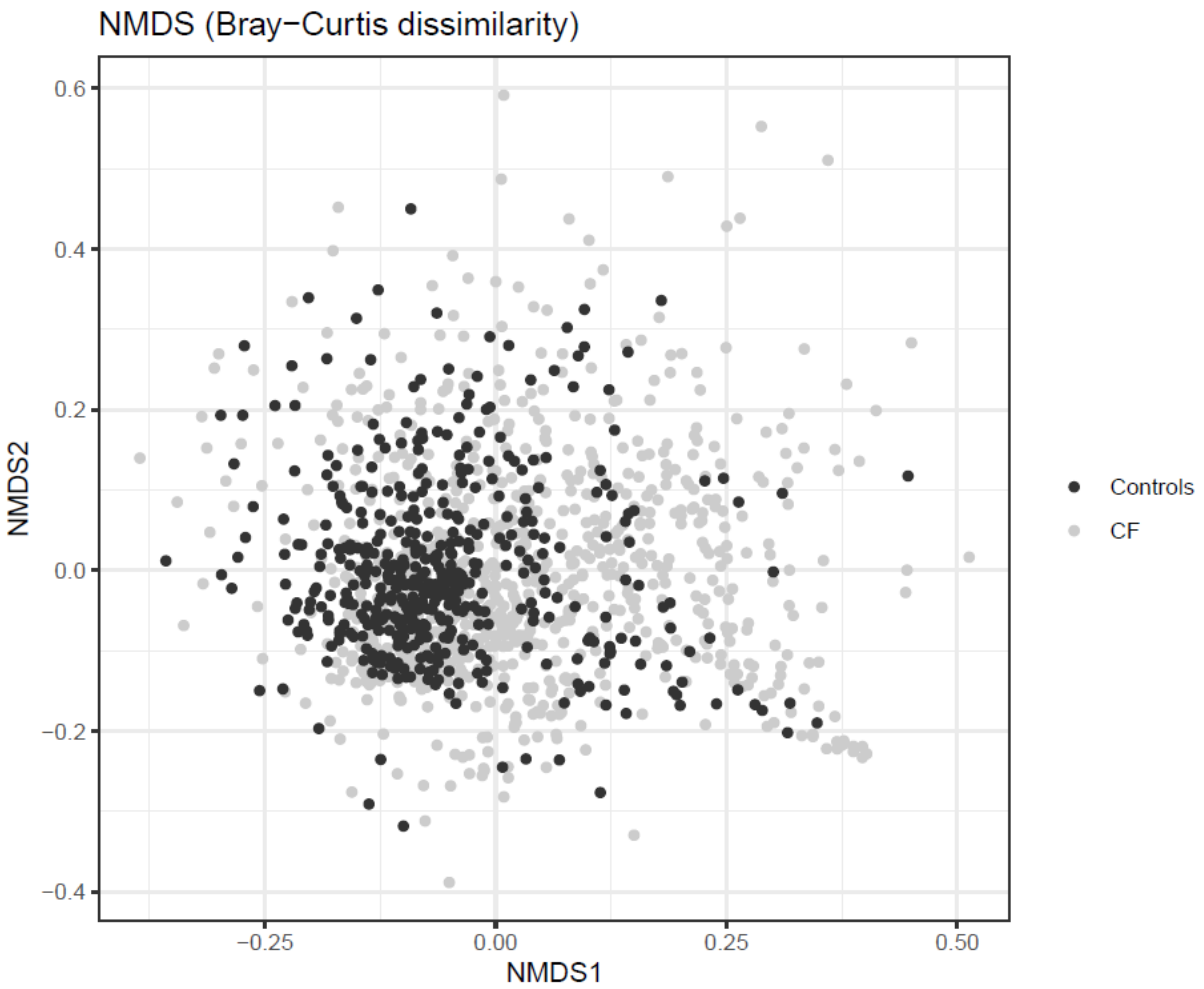
**Supplementary Figures**



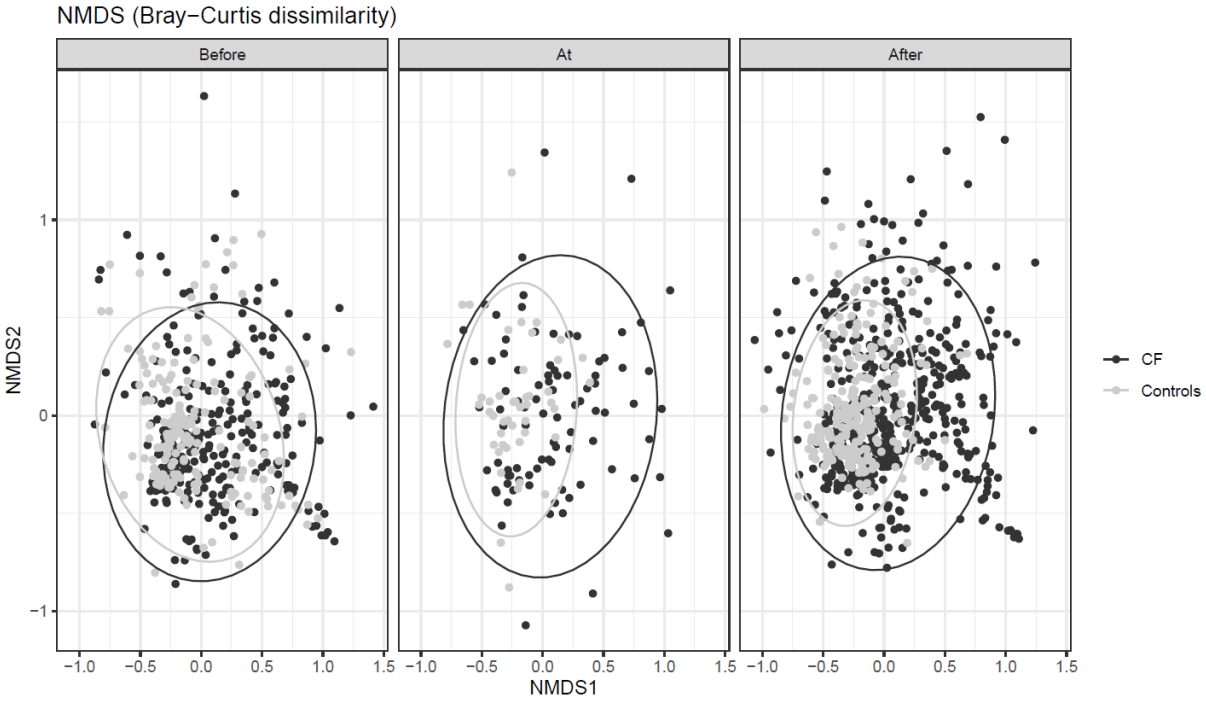

Supplementary Figure 1: Rarefaction curves. Sample size is displayed on the x-axis and Species on the y-axis. We included samples with at least 3000 reads.



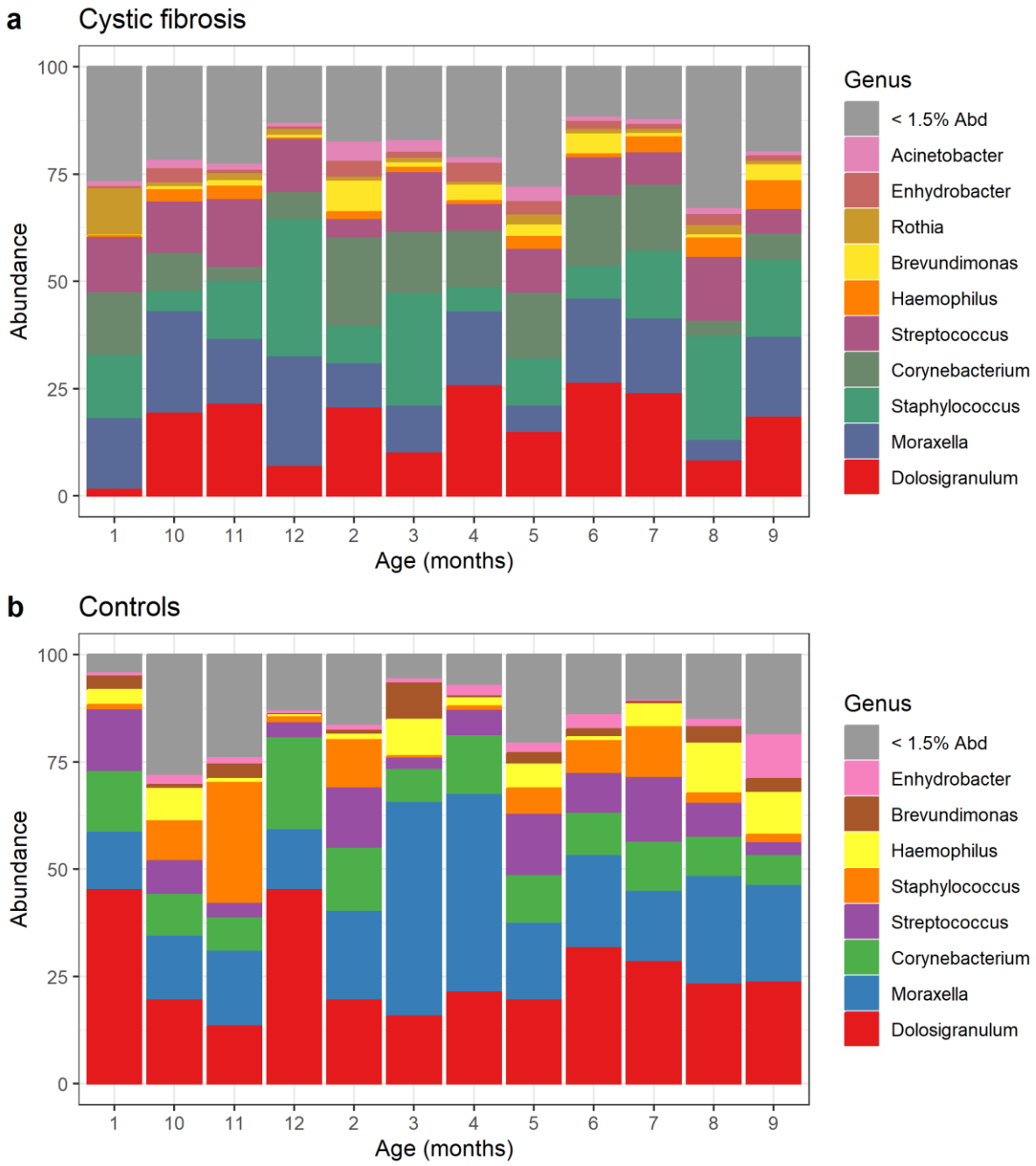

Supplementary Figure 2: Longitudinal display of study cohort. Each row of the y-axes displays a study participant. The x-axes show the age in weeks. Each symbol shows a data point (nasal swab and interview). Red colours show that RTIs were reported and triangles show antibiotic treatment.



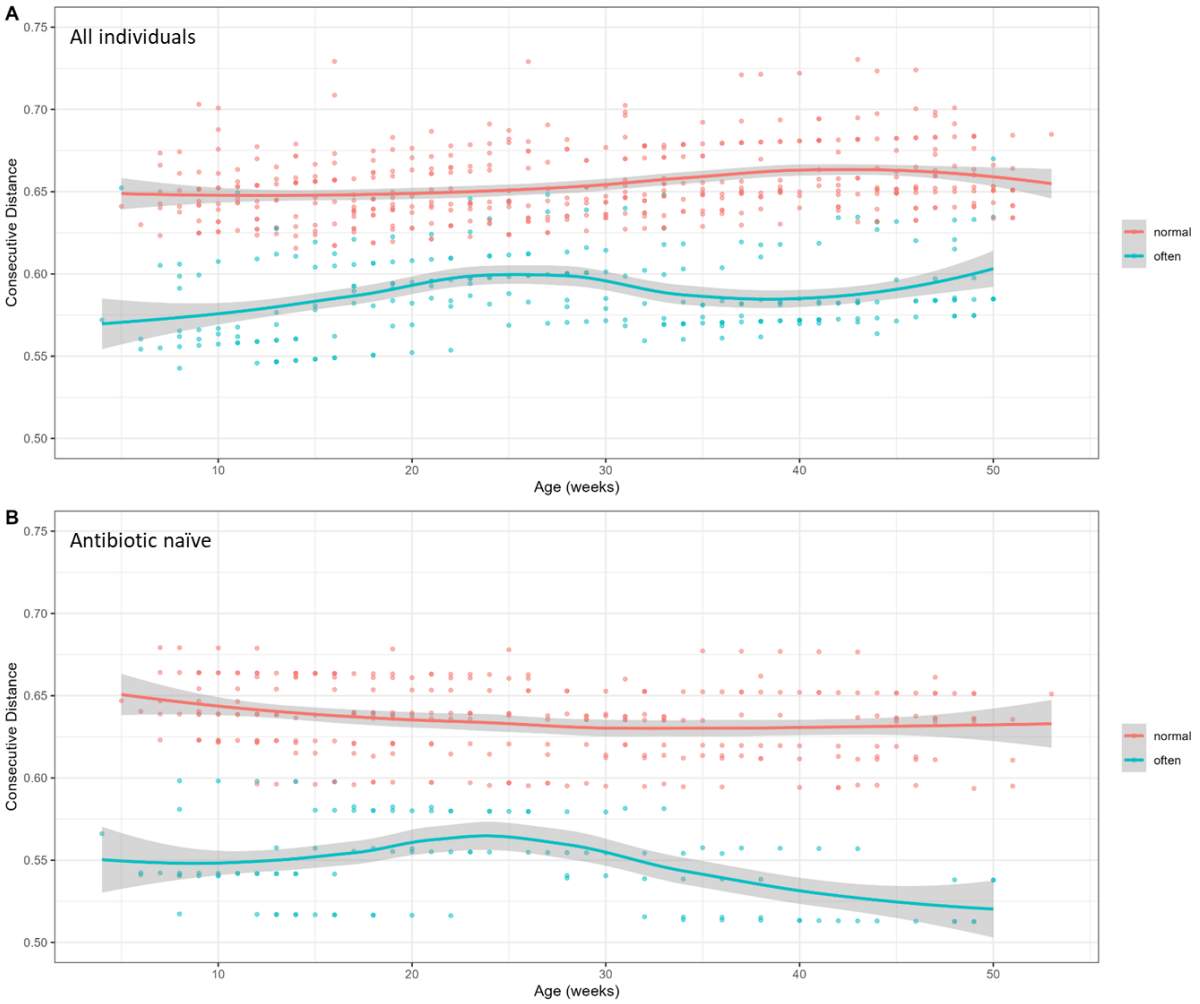

Supplementary Figure 3: NMDS showing compositional difference between infants with CF and controls measured as Bray-Curtis dissimilarity (PERMANOVA $R^2$=0.016, p=0.001).




Supplementary Figure 4: NMDS showing compositional difference between infants with CF and controls measured as Bray-Curtis dissimilarity before, at and after first RTI. Compositional difference increases in infants with CF after first RTI compared to before (PERMANOVA $R^2$=0.009, p<0.001).




Supplementary Figure 5: Most prevalent and abundant bacterial genus in our dataset. Mean relative abundance was plotted on the x-axis and prevalence on the y-axis. Most abundant and prevalent genera were *Dolosigranulum, Moraxella, Streptococcus, Staphylococcus, Corynebacterium and Haemophilus*.




Supplementary Figure 6: Within subject dissimilarities in infants with CF with higher and lower number of RTIs in the first year of life. x-axes display the age of infants in weeks and y-axes display model fitted values of the median Bray-Curtis dissimilarity between consecutive time-points within the same subject. β-diversity differed between the two groups including (A) all swabs and (B) swabs taken before the first antibiotic treatment.

**References**


1. Korten, I. *et al.* Respiratory symptoms do not reflect functional impairment in early CF lung disease. *J Cyst Fibros* **20**, 957-964 (2021).
2. Latzin, P. *et al.* Prospectively assessed incidence, severity, and determinants of respiratory symptoms in the first year of life. *Pediatr Pulmonol* **42**, 41-50 (2007).

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