

substantial morbidity and decreased quality of life. We previously showed that the novel and unique biopolymer poly (acetyl, arginyl) glucosamine (PAAG15A), a polycationic polysaccharide that inter-digitates with human mucus and interacts with purified mucin to normalize their structure, decreased the viscosity, elasticity, and adhesivity of CF airway mucus, substantially improving mucociliary transport. We have also shown that a related polymer, PAAG11D, prevented the incidence of DIOS in CFTR<sup>-/-</sup> mice, significantly improving survival and growth. We have turned to the CFTR<sup>-/-</sup> rat to investigate whether PAAG11D can prevent the development of DIOS and also treat documented obstruction. The rat will allow us to evaluate endpoints including diagnostic imaging. A DIOS treatment study is in progress.

**Methods:** In a natural history study, intestine from CFTR<sup>-/-</sup> rats euthanized at 3 or 4 weeks was collected for gross pathology and histopathologic analysis. Stool and serum were collected for ongoing evaluation of inflammation. For the prevention study, due to the early onset of DIOS, CFTR<sup>-/-</sup> rats at 2 weeks of age were randomized and administered PAAG (40 mg/kg/d) by oral gavage once daily (or Golytely or vehicle control) for 21 days while on a regular diet. Body weights and general health were monitored daily.

**Results:** Evaluation of CFTR<sup>-/-</sup> rats at 3 and 4 weeks determined DIOS occurs as early as 2 weeks of age, indicating that intervention is needed at an earlier age than observed in our CFTR<sup>-/-</sup> mice studies. With early PAAG11D treatment, survival was improved and gross examination of intestine displayed obvious obstruction due to DIOS in untreated control rats, compared to healthy-appearing intestines in PAAG11D- and Golytely-treated CFTR<sup>-/-</sup> rats. Histological evaluation of intestine of control rats indicated epithelial cell sloughing and crypts dilated with adherent mucus. There also appeared to be increased bacterial load and prominent mucosal inflammation.

**Conclusions:** Currently, no treatments are intended to address the underlying adhesive properties of CF mucus that cause impacted mucus, stool, and associated inflammation, representing a significant unmet medical need. CFTR<sup>-/-</sup> rats develop DIOS as early as 2 weeks of age. Intervention with the novel biopolymer PAAG11D prevented the development of DIOS in CFTR<sup>-/-</sup> rats, improving survival and growth. These data provide a strong basis to evaluate the effect of PAAG11D and other anti-adhesive mucolytic therapies on CF intestinal disease in humans.

## 259

### STRATIFYING YOUNG CHILDREN WITH CF FOR DISEASE SEVERITY USING INTESTINAL ORGANOID SWELLING, INTESTINAL CURRENT MEASUREMENT OR SWEAT CHLORIDE CONCENTRATION AS CFTR-DEPENDENT BIOMARKER

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**Introduction:** Forskolin-induced swelling (FIS) can be used to measure individual CFTR residual function. The aim of this study is 1) to compare CFTR residual function in intestinal organoids with current diagnostic tools sweat chloride concentration (SCC) and intestinal current measurement (ICM), and 2) to validate the predictive capacity of this model for clinical outcome parameters in infants with CF. This study is part of the HIT-CF program.

**Methods:** In the CF clinics of the University Medical Center Utrecht and Erasmus MC Rotterdam, infants with CF who are identified by newborn screening, are enrolled in a monitoring protocol, which is pioneered

by the Australian CF research group (AREST-CF). This protocol includes biannual bronchoscopy with collection of BALF and chest CT, starting at the age of 1. At the age of 1 year patients also undergo a rectal biopsy for ICM. After performing ICM, organoids are cultured from the residual materials and FIS is measured. Severity of CF lung disease is scored using a PRAGMA score method which reflects bronchiectasis, bronchial thickening, air trapping and mucus plugging.

**Results:** CFTR measurements and clinical parameters of 17 infants with 12 different genotypes are analyzed. FIS correlates with ICM and SCC,  $r = 0.78$  and  $-0.64$ , respectively, both  $p < 0.01$ . Children with high FIS values have lower values of IRT (immunoreactive trypsinogen), are more often pancreatic sufficient and have lower CT PRAGMA scores at age of 1 year when compared to children with lower FIS values: 165 (134-213) versus 123 (79-141)  $\mu\text{g/mL}$ ,  $p = 0.05$ ; 100 versus 22%,  $p = 0.02$  and 0.89 (0.62-2.85) versus 3.81 (1.76-7.62)% disease,  $p = 0.03$ , respectively. High versus low SCC is only significant related to fecal elastase values ( $p < 0.01$ ), while ICM has no relation with any clinical parameter.

**Conclusions:** FIS of intestinal organoids correlates well with currently established CFTR-dependent biomarkers SCC and ICM. Stratification for FIS appears to better identify subgroups that differ in pulmonary and gastrointestinal clinical outcome parameters when compared to SCC or ICM.

## 260

### STRATIFICATION FOR CF DISEASE SEVERITY IN ADULTS WITH CF WITH HOMOZYGOUS F508DEL MUTATIONS BY INTESTINAL ORGANOID

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**Introduction:** Forskolin-induced swelling (FIS) of intestinal organoids can be used to measure individual CFTR residual function. The aim of this study is to analyse relations between FIS and clinical outcome parameters in adults with CF with homozygous F508del mutations. This study is part of the HIT-CF program.

**Methods:** Multicentre observational study. During a study visit subjects underwent a rectal biopsy as well as chest CT and pulmonary function tests. Relevant diagnostic and clinical parameters were collected from all outpatient visits during the five years before the study visit and at the age of 12. Forskolin-induced swelling (FIS) is measured in cultured intestinal organoids as parameter for CFTR activity.

**Results:** Data of 38 patients with CF were collected and analysed. Subjects whose organoids demonstrate higher FIS values ( $n=19$ ) versus lower FIS values have higher BMI (19.6 (17.9-22.5) versus 18.2 (16.4-19.2)  $\text{kg/m}^2$ ,  $p = 0.008$ ), and tend to have lower Crispin-Norman scores at chest X-ray (18.0 (11.5-20.5) versus 21.5 (17.3-23.8),  $p = 0.059$ ) and higher mean FEV1 during the last year (2.6 (2.1-3.2) versus 1.9 (1.3-3.0) ltr,  $p = 0.08$ ).

**Conclusion:** Preliminary data suggest that FIS of intestinal organoids can define clinically distinct subgroups in adult homozygous F508del subjects, implicating CFTR residual function as modifier of disease in subjects with identical CF-causing mutations.