Identification of high frequency non-CFTR genetic risk factors
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While many genes have been investigated for their role in pancreatitis, few have been confirmed as risk factors in large cohort studies. Furthermore, pancreatitis associated mutations in these genes are very rare, usually less than 3% in healthy subjects and thus are not relevant for the majority of the patient population in North America. Specifically, rare mutations in the trypsinogen gene (PRSS1) resulting in uncontrolled trypsin activity lead to autosomal dominant hereditary pancreatitis (HP). We hypothesize that common variations in PRSS1 may play a role in other pancreatic etiologies as well.

We genotyped all cases and controls in the NAPS study for variations in PRSS1, with complete data in 952 pancreatitis cases and 646 controls. We compared the frequencies of common SNPs between controls and cases in groups with and without genetic or environmental risk factors. We identified 4 common SNPs that did not change the protein sequence, but formed two frequent haplotypes that account for the majority of alleles (Hap1 60.5%, Hap2 35.4%). The Hap1 allele was identified more frequently in cases than controls (OR 1.31 p=0.001), and the Hap2 allele less frequently (0.71 p=0.002). Hap2 carrier status was a significant protective factor among the entire cohort, especially against chronic disease and those with mutations in CFTR or SPINK1.

Pancreatic tissue samples with at least one Hap2 allele showed lower relative PRSS1 cDNA levels than Hap1 homozygotes, indicating that these haplotypes may result in different trypsin expression levels.

We have identified a common PRSS1 haplotype that is a significant protective factor against genetic pancreatitis.

Comparative quantitative proteomics of wild-type and CF primary nasal and bronchial epithelial cells
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Objectives: In this study we aim to identify novel genes involved in CF pathophysiology by applying a quantitative proteomics approach to compare the proteomes of wild-type and F508del homozygous primary nasal and bronchial epithelial cells.

Methods: Nasal and bronchial epithelial cells, that are obtained by brushing with small cytology brushes, are cultured and harvested at passage 1 or 2 for proteomic analysis. Protein separation is performed by 2-D electrophoresis. Spots, representing differences in relative protein amounts, are compared and identified by MALDI-TOF MS.

Results: Preliminary experiments performed in our group comparing a wild-type (16HBE14o−) and a F508del homozygous (CFBE41o−) cell line led to the identification of 7 proteins which are down-regulated and 4 proteins which are up-regulated at least twofold in the CFBE41o− cell line. By now, we performed several 2-D proteomics gels of primary wild-type and F508del nasal and bronchial cells. We were able to identify 61 protein spots by Maldi-TOF MS. Among them, the protein sperm associated antigen 1 (SPAG1) was found to be present in F508del proteomes but lacking in the wild-type proteomes. Characterization of differentially expressed proteins is ongoing.

Conclusion: To our knowledge, this is the first time that proteome analyses of primary nasal and bronchial epithelial cells have been successfully established. We already found one protein, SPAG1, that might be involved in CF pathogenesis. We are looking for further differentially expressed proteins in CF cells that may help to explain the phenotypic heterogeneity of the disease.