WS20.1 Role of transcription factors and microRNAs in *CFTR* gene expression

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Objectives: *CFTR* gene, described as a housekeeping gene, displays a tightly temporal and developmental regulation. In lung, *CFTR* transcripts are abundant during fetal compared to adult stage, where only two copies per cell are detected. The aim of this work is the characterization of regulatory elements acting on the amount of *CFTR* transcripts *(transcription and stability)* to explain the regulation of *CFTR* gene expression in lung.

Methods: 3′UTR and 5′UTR regions of *CFTR* gene were analysed thanks to bioinformatics tools *(TS Search, Consite, AREsite, miRBase)*. To evaluate the importance of each predicted motif for transcription factors *(FTs)* and microRNAs *(miRNAs),* functional studies were realized *(Luciferase assays, siRNA, RTq-PCR, constructions containing or not degenerated motifs for trans-regulators...).* Based on the identification of miRNAs binding sites on *CFTR* mRNA, we also propose to test new potential therapeutic tools for CF patients by designing oligonucleotides TSB *(Target Site Blockers).*

Results: We characterized new motifs for the binding of FTs on *CFTR* mRNA, key regulators in the pulmonary development (FOX, C/EBP, Nkx2), and miRNAs (miR101, miR145) explaining the modulation of *CFTR* transcrits rate in lung. In addition, the first findings on the use of oligonucleotides TSB led to increased of *CFTR* mRNA and protein level in CF patients.

Conclusion: We propose a molecular network involving FTs and miRNAs to explain difference in the *CFTR* transcrit level, in fetal and adult lungs. Identification of *cis*-regulatory motifs led to envision new tools for CF therapy.

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WS20.2 Over-expression of miR-494 and miR-145 correlates with CFTR and SMAD3 down-regulation in cystic fibrosis patients

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Objectives: microRNAs (miRNAs) have recently been emerged as important regulatory molecules in Cystic Fibrosis (CF) disease in which airways tissues/organs are often characterized by severe inflammation and infections. In order to comprehend the role of miRNAs in the CF pathogenesis, we analyzed the expression of specific miRNAs in nasal epithelia of CF patients and healthy controls.

Methods: Quantitative real time PCR (Q-PCR) showed that both miR-145 and miR-494 were significantly up-regulated in nasal epithelial tissues from CF patients compared with healthy subjects ($p < 0.001$ and $p < 0.01$, respectively). Only miR-494 levels correlated with a reduced CFTR mRNA expression ($r = -0.42$, $p < 0.05$, Pearson's correlation), supporting the negative regulatory role of this miRNA on CFTR synthesis. Using customized bioinformatic pipelines, we identified SMAD3, a key element of the TGF-beta1 inflammatory pathway, as a putative target of miR-145. Indeed, luciferase reporter assays showed that miR-145 synthetic mimics suppressed by approximately 40% the expression of a reporter construct containing the SMAD3 3′-untranslated region. In addition, we observed an inverse correlation between SMAD3 mRNA expression and miR-145 in CF nasal tissues ($r = -0.68$, $p < 0.001$, Pearson's correlation).

Conclusion: Our data confirm the importance of miRNAs in the CF pathogenesis and suggest that miRNA deregulation plays a role in the airway disease severity by modulating CFTR levels as well as the expression of molecules involved in the inflammatory response. Therefore, miR-494 and miR-145 may be not only novel biomarkers but also potential tools in order to target specific CF clinical manifestations.

WS20.3 Transcriptomics of native nasal epithelium expressing F508del-CFTR and intersecting data from comparable studies

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Microarray studies of cystic fibrosis (CF) airway gene expression have made little progress in defining a robust molecular signature associated with F508del-CFTR expression. Differences in platform and experimental design have led to widely differing conclusions [1].

We carried out a whole genome microarray study of gene expression in human native nasal epithelial cells from F508del-CFTR homozygotes and non-CF controls. Superficial comparison of our data with five other published microarray datasets showed that the number of differentially expressed genes shared between independent studies was often no greater than that expected by chance. To reduce underlying methodological variability we therefore performed a more in-depth meta-analysis of our dataset alongside two other CF studies using only native airway tissue samples [2,3], which allowed us to compare data from bronchial and nasal tissues, and from patients with mild and severe CF. For this analysis we developed a novel statistical methodology based on a permutation test.

We conclude that independent studies of *in vivo* gene expression provide interesting clues to CF pathogenesis based on the significant differential expression of a few key genes. However, detection of a consistent molecular signature by comparison of datasets obtained in different platforms remains elusive.

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WS20.4 The rs1078761 polymorphism is associated with reduced SPLUNC1 expression and increased pulmonary disease severity in cystic fibrosis patients

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Objectives: A genome-wide association study of CF modifier genes identified the rs1078761 polymorphism (MAF = 0.310) in the 20q11 region as a marker associated with lung disease severity ($p = 1.3 \times 10^{-4}$). The identified region contains two genes, LPLUNC1 and SPLUNC1, which are proposed to play a role in innate immunity in the lungs. We hypothesized that the G allele of rs1078761, which is associated with more severe disease, reduces expression of the SPLUNC1 and/or LPLUNC1 genes, resulting in an impaired immune response in CF patients.

Methods: mRNA expression was measured in peripheral lung sections from genotyped CF and non-CF patients using quantitative PCR (100 patients) and microarray analysis (1111 patients). Saliva samples were collected and genotyped from 55 healthy volunteers. Western blotting and ELISA were used to measure protein levels in lung and saliva samples.

Conclusions: LPLUNC1 and SPLUNC1 had 8 and 30 fold higher levels of mRNA expression, respectively, in CF lung samples compared to non-CF samples ($p < 0.0001$). The G allele of rs1078761, which was associated with increased CF severity, was associated with lower mRNA levels of SPLUNC1 but not LPLUNC1 in CF ($p = 0.0181$) and non CF lungs ($p = 0.0086$). Although rs1078761 was not significantly associated with SPLUNC1 protein levels in saliva or lung tissue ($p = 0.3767$ and 0.2382, respectively) there was a trend towards reduced SPLUNC1 protein with the G allele. Preliminary data show that recombinant SPLUNC1 reduced growth of *P. aeruginosa* in a dose dependent manner. Therefore, low levels of SPLUNC1 may be detrimental to the immune response in CF and SPLUNC1 may be an important target for augmentation therapy.