## 1* ENaC mutations in patients with CF-like disease

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We investigated if mutations in SCNN1A, SCNN1B, SCNN1G, which code for the different subunits of the amiloride sensitive epithelial sodium channel (ENaC), explain disease in patients in whom a mutation cannot be found on both CFTR genes. While ENaC mutations were not the underlying basic defect in the majority of these patients (more than 90–95%), evidence was found for an involvement in disease in some patients, such as p.V114I in SCNN1A. The p.W493R variant in SCNN1A was found at an increased incidence in the tested patients. We found that p.W493R-SCNN1A led to a 4-fold increase in ENaC current. Given the finding of an incidence of 2% of p.W493R-SCNN1A in the general population, and given the incidence of CF carriers of 1/30, about 1 in 3000 individuals is expected to be heterozygous for p.W493R-SCNN1A and a CF-causing CFTR mutation. This, possible partial penetrant, genotype may be sufficient to cause, or to predispose to, CF-like disease in some individuals.

## 2* The β-defensin region affects CF lung disease severity

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β-defensins 2–6 are antimicrobial peptides which form part of a repeat region that is polymorphic between individuals and therefore the dosage of these defensin genes/proteins varies.

We determined the number of repeats in 146 F508del homozygous CF patients from Belgian, Czech and Italian origin. For each patient group, a higher number of repeats was found in the group of patients with milder disease (FEV1 >70%) compared to those with more severe disease (FEV1 <70%) (Student T test, P-values of 0.0006, 0.03 and 0.019).

Furthermore survivors (older than 30 years of age) had a higher number of repeats than younger CF patients (10–29 years of age) in different CF populations (Belgian, Hannover, Verona, Copenhagen and Manchester; Chi square P-values where 0.05, 0.03, 0.11, 0.12 and 0.05).

To evaluate this, we cultured nasal epithelial cells individuals with a low or high number of repeats. The cells were stimulated with 10 ng TNF-α. DEFB4 expression, as measured by the extent of transcription, was only upregulated by TNF-α in cells with a high number of repeats (P-value = 0.015).

We also tested the antimicrobial activity of the epithelial cells. We challenged the epithelial cells with a laboratory strain (PA01) of *Pseudomonas aeruginosa* and a clinical isolate, with or without TNF-α. After 3 h, surviving bacteria were counted. Cells that were stimulated with TNF-α 12 h prior to the bacterial infection were more bactericidal when a high number of repeats was present.

## 3 Disease severity in cystic fibrosis is modified by variants of the syntaxin 1A gene

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Disease severity in cystic fibrosis (CF) varies greatly among patients, even when they carry the same CFTR mutation. This might partially be due to environmental factors, yet sibling studies strongly indicate that genes other than CFTR modify CF disease outcome.

Syntaxin 1A (STX1A) is a negative regulator of CFTR and other ion channels. Considering that even severe CFTR mutations do not lead to a complete loss of CFTR function, we hypothesized that CF disease outcome might be influenced by variants of the STX1A gene that either increase or hamper STX1A functionality, leading to a further reduction or enhancement of the remaining CFTR function by the regulatory activity of STX1A.

We thus screened for SNPs in the complete coding sequence and the adjacent intronic regions of the STX1A gene in 62 phenotypically well-characterized patients being homozygous for the most common CFTR mutation, F508del. Two SNPs which were in strong linkage disequilibrium were found to be significantly associated with the lung parameters lung clearance index (LCI) and forced expiratory fraction ($FEF_{50}$). In an expanded population (n = 93, all F508del homozygous), p-values reached 0.002 (LCI) and 0.047 ($FEF_{50}$) for SNP 1 and 0.004 and 0.006 for SNP 2, respectively. No association was found in non-F508del CF patients (n = 71), presumably because the contribution of the CFTR genotype on disease expression outweighs that of the STX1A genotype.

Based on the assumption that STX1A modifies CFTR function, the two SNPs are currently genotyped in a population with defined nasal potential difference values. In addition, the two SNPs are functionally characterized.

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## 4 COX2 as a protective modifier of CF pulmonary disease severity

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The clinical outcome of CF pulmonary disease varies remarkably even in patients with the same *CFTR* genotype. That is why searching for genetic modifiers located outside the *CFTR* gene is provided.

94 CF patients, F508del mutation homozygotes were included into the project. In order to consider and compare patients' condition several clinical parameters were recorded: chest radiography according to the Schwachmann-Kulczycki score, forced expiratory volume in one second ($FEV_1$) and microbiological testing. For the purpose of this study a unique clinical score has been created by clinicians: disease severity status (DSS), which combines many clinical parameters of each patient and shows the degree of lung function impairment in different age groups.

To consider the effect of non-*CFTR* genetic polymorphisms on the clinical course of lung disease in CF patients we have studied molecular variants of 7 genes: *PI*, *MBL2*, *IFNG*, *GSTT1*, *GSTM1*, *PTGS1* and *PTGS2*.

Analysis of *PTGS1* and *PTGS2* genes as potential *CFTR* modifiers has been provided for the first time in this study. Results of *PTGS2* gene analysis were most informative and confirmed hypothesis that COX2 protein is a protective modifier of CF severity.

| Gene / Molecular defect | Variant effect | Clinical associations |
|---|---|---|
| *PI* / 1237G>A | ↓ gene expression, compromised AAT acute-phase response | GA: higher $FEV_1$, lower DSS score |
| *MBL2* / −550C>G | ↑ gene expression, ↑ protein production | LH: lower $FEV_1$ |
| *MBL2* / any mutation in the coding region | ↓ levels of functional MBP protein | A0+00: less *Paeruginosa* infections, higher SC score, lower DSS score |
| *IFNG* / +874A>T | ↑ protein production | TT: higher DSS score |
| *IFNG* / +1480 ins. T | unknown | ins−/ins+: lower DSS score |
| *GSTT1* / null deletion | lack of protein production | del+/del+: higher SC score, lower DSS score |
| *PTGS1* / 639C>A | unknown | CA: higher $FEV_1$ |
| *PTGS1* / any mutation in the coding region | destabilization of protein structure | AM: more *P. aeruginosa* infections |
| *PTGS2* / −765G>C | ↓ gene expression | GC: higher $FEV_1$ |
| *PTGS2* / 8473T>C | degradation of PTGS2 mRNA, ↓ protein concentration | TC: higher $FEV_1$, less *P. aeruginosa* infections, higher SC score, lower DSS score |