25* Molecular and functional characterization of CBAVD-causing mutations located in both NBDs of the CFTR protein

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Introduction: In a previous screening of the whole *CFTR* gene in order to establish the spectrum of mutations in a representative sample of Portuguese patients with CBAVD, we identified 3 novel disease-causing mutations in both nucleotide binding domains (NBDs). P439S lies within NBD1, P1290S and E1401K in NBD2. The present study analysed the effects of the 3 mutations on CFTR processing and intracellular transport. In addition, cAMP-regulated Cl– conductances produced by the 3 mutants were assessed.
Methods: Mutations generated in an expression plasmid were transiently over-expressed in HEK 293 cells. Biosynthetic pathways were analyzed by Western blotting and confocal microscopy. Measurement of the cAMP-regulated Cl– conductance was assessed by iodide influx assay and whole-cell patch-clamp.
Results: Mutant CFTR exhibited a maturation pattern similar to the wild-type CFTR, but the total amount of mature P439S-CFTR and P1290S-CFTR was reduced. E1401K-CFTR was localized in cell membrane, whereas P439S-CFTR and P1290S-CFTR were mainly detected in cytoplasm. All mutants showed reduced cAMP-activated Cl– conductances.
Conclusions: Decreased Cl– conductances detected for P439S-CFTR and P1290S-CFTR are explained by reduced amounts of mature protein in cell membrane, whereas for E1401K-CFTR a defective channel was confirmed. The reduced Cl– conductance of all mutants is sufficient to affect the normal development of a susceptible tissue such as the vas deferens, although not to cause cystic fibrosis.

26* Does male fertility impairment due to idiopathic semen hyperviscosity depend on CFTR gene mutations?

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Idiopathic seminal fluid hyperviscosity (ISHV), a major cause of impaired fertility in human males, has a prevalence of approximately 4%. Its pathogenesis is usually unknown, but some previous results (Rossi et al., Fertil. Steril. 2004;82:1316–22) have suggested that an altered CFTR genotype be involved, at least in some cases. The variability of the CFTR gene was investigated in 40 ISHV patients and in 125 random subjects by performing an extensive sequencing of the coding and of the flanking intronic regions of this gene, searching for: (a) pathogenic mutations; (b) Splicing-Error Prone (SEP) haplotypes, namely the $(TG)_{12}T_5$ and $(TG)_{12}T_7$ repeats in intron 8, known to cause a substantial increase in the frequency of incorrectly spliced RNA transcripts; (c) genomic variants with a still undefined functional role. We have sofar sequenced, in all our samples, 11 CFTR exons (i.e. 1855 coding bp out of 4443) and about 880 non-coding bp of the adjacent intronic regions. The ISHV group, as compared to controls, was found to have a significantly higher frequency of subjects with at least one CFTR pathogenic mutation or a SEP haplotype (0.525 ± 0.079 vs 0.176 ± 0.034) or with at least one CFTR variant (0.825 ± 0.060 vs 0.456 ± 0.045).
Although the functional relevance of genomic variants is still to be assessed, the higher frequency, in the ISHV target population, of classic mutations, SEP haplotypes and/or variants in the CFTR gene supports the hypothesis that this overall genomic specificity be significantly associated to the ISHV phenotype.

27* Transcription factors: new therapeutic targets in cystic fibrosis lung disease?

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The lung disease of cystic fibrosis (CF) is characterized by a self-sustaining cycle of airway obstruction, infection, and inflammation. Such processes are in part regulated by transcription factors. Among them, NRF2 (NF-E2-related factor) plays a pivotal role in protection against oxidative stress-induced pulmonary inflammation and IRFs (Interferon regulatory factors) are involved in bacterial infection response. Our laboratory evidenced that both ubiquitous (such as SRF and YY1) and tissue-specific (such as FoxA2) transcription factors are implicated in the transcriptional regulation of the CFTR gene. Recently and interestingly, in the CFTR minimal promoter we identified new cis-regulatory elements including an overlapping binding site for NRF2 and IRFs. By using EMSAs, we demonstrated the following DNA/protein bindings: DNA/NRF2 and DNA/IRFs. A functional analysis, by co-transfections assays, showed while NRF2 and IRF1 are repressors, IRF2 is an activator. Although additional studies are required to further elucidate the regulatory mechanisms contributing to the CFTR expression control, we present the first evidence that transcription factors implicated in inflammation and infection processes, regulate the CFTR gene transcriptional activity. Better understanding of the role of these factors could identify new therapeutic targets for a more exquisite regulation of both inflammatory and infectious pathways that ultimately contribute to lung damage in CF. It will be interesting to test whether ceramids, already involved in different therapeutic aspects of the CF disease (host-defense against *P. aeruginosa*, inhibition of IL-8 disease and reduction of airway anion secretion), could be more beneficial therapeutic agents, for instance through NRF2 cytoplasmic relocalization.
Supported by: VLM.

28* Study of the unfolded protein response (UPR) in cystic fibrosis (CF)

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Cystic fibrosis transmembrane conductance regulator (CFTR) functions as a chloride (Cl–) channel in the apical membrane of epithelial cells. The most common mutation in CF is a deletion of a phenylalanine residue at position 508 (ΔF508). The ΔF508-CFTR protein is incorrectly folded and accumulates in the endoplasmic reticulum (ER). This accumulation could trigger the UPR pathway which leads to a global decrease of the protein synthesis in cells and to apoptosis. Our aim was to determine whether UPR is triggered in ΔF508-CFTR expressing cells. Because infection and inflammation observed in CF can trigger UPR we studied the cell susceptibility to exogenous UPR inducers tunicamycin (Tu) and thapsigargin (Tg). UPR was studied by the expression of two specific markers: the glucose-regulated protein 78 (GRP78) and the transcription factor 6 (ATF6) which were compared in wild type (Wt) and in ΔF508-CFTR transfected cells. The model was validated by immunofluorescence and by Cl– channel function studies (SPQ) and we showed by western blotting that both GRP78 and ATF6 are over-expressed in ΔF508-CFTR cells. Furthermore ATF6 was activated in ΔF508-CFTR cells since it was cleaved and relocated to the nuclei. The SPQ results indicated that the inhibition of GRP78 expression by a specific siRNA had no significant effect upon the CFTR activity whereas the inhibition of ATF6 expression induced an increased Cl– channel function of CFTR and ΔF508-CFTR. The response of Wt and ΔF508-CFTR cells to Tu and Tg were not different. In conclusion we show that UPR is triggered in ΔF508-CFTR cells and that a decreased ATF6 expression leads to an increased CFTR and ΔF508-CFTR function. We propose that the decreased ATF6 expression is a therapeutic target.
Supported by: VLM.