1* The effect of the UPR and NMD mechanisms on the response to readthrough treatments

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Readthrough of nonsense mutations generates full length proteins. Variable response to readthrough treatment was found to correlate with CFTR transcript levels. Nonsense Mediated mRNA Decay (NMD) mechanism degrades nonsense carrying transcripts and affects the readthrough response. The CFTR protein is folded in the ER. Accumulation of unfolded proteins in the ER activates the Unfolded Protein Response (UPR), which alters the cellular transcription and translation. Since inefficient NMD leads to translation of truncated proteins that are not correctly folded **we hypothesize** that:

1. Inefficient NMD leads to accumulation of truncated proteins in the ER that activates the UPR.
2. UPR further inhibits the NMD (which is translational dependent).

Results: Downregulation of the NMD by siRNA directed against hUPF1 induced the UPR, indicated by increased levels of UPR markers. Importantly, UPR activation by TM or DTT inhibited the NMD as revealed by increased levels of physiological NMD substrates. Subsequently, we studied 2 sisters homozygous for the W1282X mutations of whom one responded to readthrough treatments and had higher CFTR transcript level. Using SILAC we compared the entire proteome between cells derived from these sisters. Out of 6000 analyzed proteins, 440 showed a significant different level between the cell lines. At least 44 were ER proteins functioning in the UPR. Strikingly, a ~2 fold higher level of each of these proteins was found in the responding sister, indicating a higher UPR activation.

Conclusion: A positive feedback regulation between NMD and UPR pathways regulates the level of transcripts carrying PTCs and the response to readthrough treatments.

2 Expression and DNA methylation of ENaC genes

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In the airways Cl^- ions are secreted by the Cystic Fibrosis Transmembrane conductance Regulator (CFTR) channel that also limits Na^+ absorption by partially repressing the Epithelial Na^+ Channel (ENaC). The mechanisms regulating the transcription of the 3 ENaC genes (SCNN1A, SCNN1B and SCNN1G) are not fully elucidated and only sporadic experimental evidence exists on their possible epigenetic regulation.

We studied the expression of the 3 ENaC genes and the DNA methylation levels of their 5′-flanking regions in H441, MCF10A, 16HBE, CFBE and HACAT *in vitro* cell lines, as well as in nasal brushing, granulocytes, lymphocytes and monocytes of subjects with no Cystic Fibrosis (CF).

We evidenced high expression of all ENaC genes in H441, MCF10A and HACAT cell lines, as well as in nasal brushing. Extremely low, if any, ENaC expression was found in the 16HBE and CFBE cell lines as well as in granulocytes, lymphocytes and monocytes. In these expressing and non-expressing experimental conditions we found an inverse correlation between expression and DNA methylation of each of the 3 analyzed zones of the 5′-flanking the SCNN1A gene. One of the 4 analyzed zones of the 5′-flanking region of the SCNN1B gene was constantly hypomethylated, apart from the gene expression levels; the other 3 zones were instead always methylated. Studies of the methylation pattern of SCNN1G gene are still underway.

These studies point to a role of DNA methylation in the transcriptional control of ENaC genes. They may be of interest for the comprehension of the mechanisms of CFTR–ENaC interaction and possibly for an optimization of therapeutic strategies for CF involving ENaC inhibition.

3* Clinical and molecular characterization of the CF disease modifier syntaxin 1A (STX1A)

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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) has been reported as negative regulator of CFTR and other ion channels. We hypothesized that STX1A acts as CF modifier by influencing the remaining function of mutated CFTR. Applying linear mixed models to data obtained with the Bernese CF cohort revealed STX1A c.204 T>C to influence lung function parameters. Subsequent genotyping of c.204 T>C in the European CF sib and twin study revealed significant differences in allele frequency between mildly and severely affected sib pairs, thus confirming that the polymorphism modifies CF pulmonary disease.

Considering that c.204 T>C does not change the amino acid sequence of STX1A, we investigated its consequences on mRNA level. We show that c.204C reinforces an aberrant partial inclusion of STX1A intron 3, leading to nonsense mediated mRNA decay (NMD). Accordingly, c.204 C should be lower expressed, which is currently investigated by means of allele-specific quantification. Our results further indicate that STX1A mRNA expression is very low in epithelial tissues. Instead, we find STX1C, a splice-isoform of STX1A, to be up to 100 fold higher expressed than STX1A, indicating that not STX1A, but rather STX1C is an important player in CF pathophysiology. This finding additionally suggests that the exact role of STX1A in CFTR regulation should possibly be reconsidered.

4 Whole CFTR gene screening in diagnostics of cystic fibrosis in Serbia

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Since its introduction, molecular diagnostics of CF in Serbia has been performed either by direct detection of the most common 7 mutations (F508del, G542X, G551D, R553X, N1303K, 621+1G>T, 1717–1G>A), use of commercially available kits, or combination of SSCP/DGGE and sequencing of selected exons of CFTR gene. In the last three years, in cases where clinical diagnosis of CF was not confirmed by molecular diagnostics using direct mutation testing, the whole gene sequencing was standardized and introduced as a routine diagnostic method.

Here we present the results of the whole CFTR gene screening by direct DNA sequencing in 14 patients with clinical diagnosis of CF.

Mutation was detected on 24 of 28 chromosomes analyzed. High heterogeneity of detected mutations was observed. Besides F508del, as the most common mutation, 9 other mutations were detected (E585X, 457TAT>G, 1525–1G>A, 4105delA, G126D, R1185X, 2907delTT, 2723delTT, R1070Q). Their distribution was as follows: F508del – 45.8%, E585X – 12.5%, 457TAT>G and 1525–1G>A – 8.3%, each and all others 4.2%, each. With exception of F508del, none of the detected mutations is a part of a core panel of commercially available kits.

These results give support to our strategy for molecular CF diagnostics in Serbian patients which combines direct testing for the presence of F508del as the most frequent mutation in our population, followed by the sequencing of the exons containing the most frequent mutations in our population (1, 3, 4, 5, 6a, 7, 9, 10, 11, 12, 13, 14a, 15, 17b, 19, 20, 21), and whole gene screening if necessary. To further improve the diagnostics of CF in our country we plan to introduce analysis of CFTR gene rearrangements.