New putative cis-acting regulatory variations in the CFTR gene

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Understanding the range of putative effects of nucleotide variants on cis-regulatory elements is of increasing interest for several reasons. It should help to distinguish disease-causing mutations from linked neutral variations. It might explain some of the reported allelic imbalance, thus leading to interindividual phenotype variability such as protein levels. It should greatly advance our understanding of CFTR transcriptional control.

The aim of this study was to evaluate the functional relevance of a few nucleotide variants identified within the untranslated CFTR regions. Some of these nucleotide variants have been shown to be associated with CF and CBAVD diseases (the 48 C>G variant and the allelic variation at an intronic tetranucleotide microsatellite, respectively), others have only been identified in controls (the 99C>T variant).

We report an in-depth in silico characterization of these variants, including RNA secondary structure analysis. By using basic molecular techniques such as EMSA, ChIP and luciferase reporter constructs assays, we provide experimental evidence that the studied nucleotide variants have a substantial impact on the basal rate of transcription of the CFTR gene. We discuss the importance of mapping and characterizing genetic variations controlling CFTR gene expression for both clinical diagnosis and research fields.

This study should provide a wealth of data for understanding cis-acting elements variability affecting the CFTR regulation and ultimately pinpointing therapeutic targets.

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Exonic sequence variations affecting splicing within exons 3, 4 and 5

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Within the CFTR gene, some sequence variations have been shown to affect pre-mRNA splicing. We are interested in sequence variations located within exons affecting exon recognition and inclusion. Such events have been described for exons 12 and 13, where nucleotide substitutions can disrupt exon splicing enhancer or silencer elements, preventing or favoring the recruitment of splicing factors. These nucleotide variations affect the amount of full length mRNA, thus reducing total CFTR protein expression and modulating the severity of the disease.

We aim to identify exonic sequence variations within exons 3, 4 and 5 affecting exon splicing enhancers and their associated SR proteins. We cloned into a splicing reporter minigene (pET01 vector) exons 3, 4 and 5 from DNA of patients bearing either WT or mutated sequences and transfected them in various cell lines. By bioinformatic analysis, Ser/Arg rich proteins (SFRS) able to bind to CFTR exonic sequences were identified. By this approach, we have unmasked two nucleotide substitutions (R74W and R75Q) as creating an alternative splicing of exon 3 and points to altered binding of SFRS1 and SFRS6. These results were confirmed by mRNA analysis from nasal cells of healthy volunteers carrying R75Q after separation of the PCR product by DHPLC.

The identification of nucleotide substitutions affecting exon recognition will give a better understanding of CFTR RNA processing and will lead to reconsider some neutral variants as disease-causing, thus improving diagnosis accuracy.

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ETR-3 is a major regulator of CFTR pre-mRNA splicing

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CF is the most common autosomal recessive disorder in Caucasians, caused by mutations in the Cystic Fibrosis Transmembrane conductance Regulator (CF) gene. Some milder forms of CF are due to the skipping of CFTR exon 9, which results in an aberrant protein. This skipping is under the control of a polymorphism in intron 8, which can be bound by the splicing protein TDP-43.

Our aims were to elucidate the antagonist effect previously reported between TDP-43 and ETR-3 splicing proteins and to look for other regulator(s) of CF exon 9 skipping, which could explain the differences in the observed phenotypes.

We show that ETR-3, which was reported to decrease CFTR exon 9 exclusion, dramatically increases its exclusion in our minigenes, more strongly than TDP-43. In addition, CUGBP-1, a structurally close member of the ETR-3 protein, has the opposite effect on CFTR exon 9 splicing. Hence CUGBP-1 reduces exon 9 exclusion, yet both proteins bind onto the intron 8 polymorphic sequence. We identified the protein domain responsible for the opposite effect between ETR-3 and CUGBP-1 as a domain included between the RNA Recognition Motif 2 (RRM2) and the RRM3 in the so called Divergent Domain, which shows low sequence conservation.

As a whole, we describe 2 new splicing regulators of CFTR exon 9, ETR-3 and CUGBP-1. These factors, like TDP-43, which are implicated in another genetic disease (Myotonic Dystrophy), can bind intron 8 UG-repeats. The interplay between each of these factors in different tissues could explain the variability in CFTR exon 9 skipping phenotypes.

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Over-representation of NFκB binding sites in CF human airway epithelial spheroid genes vs. nonCF

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It is well-known that the expression of several genes is altered in CF. However, it is not known how much of the altered gene expression is a consequence of the CFTR mutation and how much is due to inflammation caused by bacterial over-growth in CF-airways. The aim of the project was to characterize gene expression differences between CF and nonCF airway epithelia, derived from human explants. Nasal polyps from 7 patients (4 CF and 3 nonCF) were used to form airway epithelial spheroids which were preserved under defined bacterial-free conditions for 14 days. Genes that are differentially expressed between CF and nonCF samples were identified by DNA microarray analysis. The promoters of the affected genes were analyzed for over-represented transcription factor binding sites in order to identify the transcription factors regulating the differential expression.

Results: The expression profiles of CF vs. nonCF airway epithelial spheroids showed over 2000 genes up- or down-regulated, by more than 2-fold. Among these we found several genes involved in the inflammatory response: e.g. TNFSF9, TNFRSF10, GLS, CYP1A1 were up-regulated, and IL8, IL6R, SOCS3 were down-regulated. Furthermore, we found a significant (p < 0.05) over-representation of NFκB binding sites in the promoter regions of up-regulated genes in CF-spheroids.

Discussion: The spheroid-model is unique for maintaining and analyzing CF airway epithelium ex vivo in a bacterial-free environment. Our data gives a further insight into molecular processes involved in CF, and the possibility of an intrinsic inflammation preceding infection, involving activity of NFκB.

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