

Orphan CFTR mutations – from disease mechanisms to novel therapeutic opportunities.

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Objectives: This work aims to better characterize orphan CF-causing mutations, having as a basis the great heterogeneity found in CF patients. It will also contribute to the understanding of the molecular and cellular mechanisms underlying this disease and to repurpose approved mutation-specific therapies. It comprises the following specific goals:

- 1) To characterize orphan *CFTR* mutations in novel cellular models at the molecular, cellular and functional levels, including repurposing existing therapeutic corrective strategies for *CFTR* mutations, by testing *CFTR* responses in adequate cellular models.
- 2) To clarify disease mechanisms for CF, correlating rare mutations with common ones, in terms of global patterns of gene expression, protein interactions and trafficking networks.

Methodology:

The proposed work will involve two main tasks

Task 1 – Characterization of rare CFTR mutations

For this, novel cellular models will be generated based on BHK, HEK or CFBE41o- cell lines with heterologous expression of *CFTR* cDNA or *CFTR* mini-genes (cDNA with the relevant introns) containing the mutation of interest (generated by in vitro mutagenesis). CFBE-based models will be preferred as they are a model closer to the patient situation.

To assess the effects of *CFTR* mutations in these cellular models, different assays will be carried out, namely assessing *CFTR* mRNA, cell surface localization of *CFTR* mutants, *CFTR* function/ activation as a Cl⁻ channel (e.g. iodide efflux assay as described for the semi-quantitative assessment of Cl⁻ transport and/or ion transport measurements in Ussing chamber), stability and efficiency of maturation of *CFTR* mutants.

The existing therapeutic strategies will be tested to assess if they can be repurposed and used also in patients bearing the rare variants. The compounds to be tested were previously shown to correct *CFTR* dysfunction for specific mutations – but not for uncommon variants.

Task 2 – CFTR proteins: one size fits all?

A second task will include the characterization of the *CFTR* protein variants with rare mutations in the context of the cell. Our lab has been involved in several high-scale approaches that identify *CFTR* trafficking regulators, *CFTR* interactors, modulators of other ion channels (ENaC and anoctamins), differentially expressed genes in CF and other respiratory diseases.

The rare variants will be analysed in order to place them within these complex networks – detection of interacting proteins, identification of trafficking modulators, quantification of gene expression. Selected targets identified as critical in the previous and ongoing screens will be tested for the rare mutations and results will be used to establish a correlation between the molecular phenotype and drug response (see above Task 1) and the position in gene/protein/traffic networks.



Characterization of the disease mechanisms will also include testing the response to rescuing strategies (see above compounds, but also genetic rescue – with second site mutations/revertants, testing their validity in rare mutations).

Additionally, specificity of the observed mechanisms will be assessed by comparison with an unrelated membrane protein with defective traffic (e.g. MC4R, a GPCR involved in hereditary juvenile obesity, and its trafficking-incompetent variants).

Type of fellowship: National only