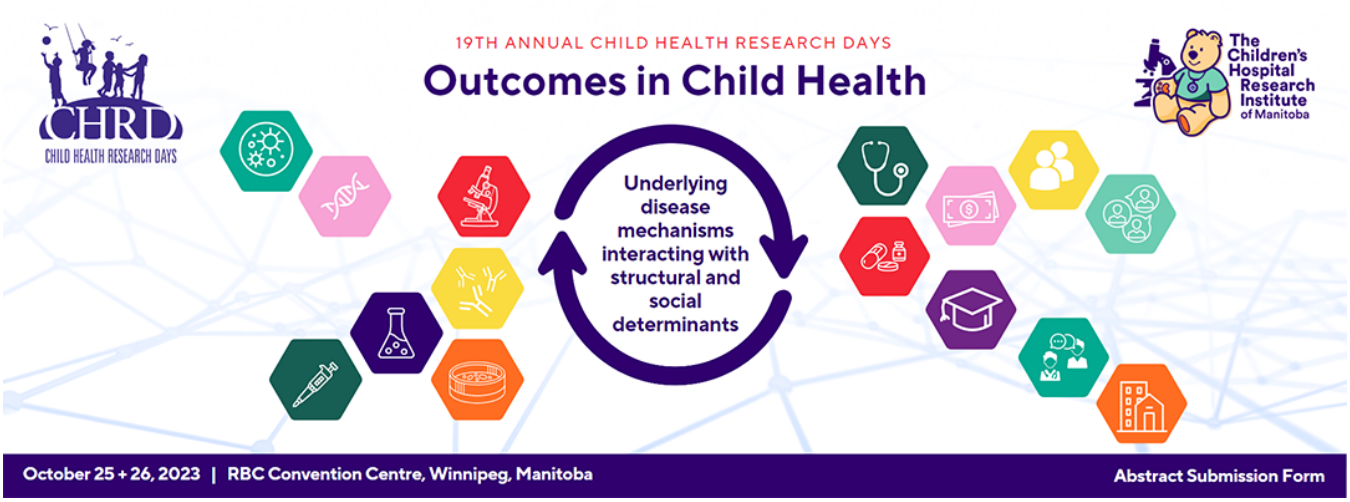

CHRD 2023: Abstract Submission Form

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Presenter Status
Post-Doctoral Fellows

Research Category
Basic Science

Role in the project
Design
Perform Experiments
Analyze Data
Write Abstract

Title
Modification of antimicrobial responses by CFTR interactions with bitter taste receptor T2R14.

Background
Cystic Fibrosis (CF) is caused by mutations in CFTR (Cystic Fibrosis Transmembrane Conductance Regulator) gene, and CF patients suffer from opportunistic microbial lung infections. T2R14 (one of >25 chemosensory bitter taste receptors-T2Rs) is highly expressed in bronchi, where it recognizes and responds to microbial quorum sensing molecules (QSMs) from CF-associated pathogens. T2Rs and CFTR are functionally coupled in epithelia. However, the mechanism by which CFTR influences antimicrobial signaling from T2R14 is not known.

Objective
We hypothesize that specific domain(s) of CFTR interact with T2R14, which modulates downstream signaling and influences antimicrobial responses.

Methods
We used HEK293-T/CHO-K1 cells overexpressing CFTR, T2R14 and Gα constructs, singly or in combination with each other. Agonist-mediated changes in secondary messengers (cAMP, Ca2+) were measured following treatment of cells with T2R14-specific agonist (diphenhydramine) or microbial QSMs (acyl homoserine lactones, autoinducer peptide 1, tyrosol & farnesol). Direct protein interactions were queried by BRET (Bioluminescence Resonance Energy Transfer). The ability of individual CFTR domains to interact with T2R14 was examined by engineering SPASM (Systematic Protein Affinity Strength

Modulation) sensors.

Results
First, we observed that T2R14 interacts directly with CFTR, as evaluated by BRET. Second, using SPASM sensors suggest that the N-terminus lasso loop of CFTR governs interaction with T2R14. Next, we find that T2R14 can interact with gustducin, Gαi and Gαq, but not with Gαs, upon stimulation with DPH. Finally, CFTR is capable of direct interaction with Gαi and Gαq.

Conclusion
Our results demonstrate that T2R14 interacts directly with CFTR, through the N-terminus lasso loop. We predict that in CF patients (Class I &II), the lack of plasma-membrane localized CFTR precludes T2R14 interaction, which affects T2R14 function. T2R14 exhibits multiplicity of signaling pathways, and the effects of CFTR interaction on G protein bias and downstream signaling are being investigated. G-protein – CFTR interactions introduce novel avenues for inquiry.

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