Cystic Fibrosis Foundation

View Abstract

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Abstract Details

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Pathogens Exposure - Abstract Submission: I acknowledge that I have read and understand the Foundation's attendance policy for events and will do my part to minimize the spread of germs at NACFC.

Abstract

TITLE: ANTISENSE OLIGONUCLEOTIDES TO IMPROVE CFTR FUNCTION FOR PEOPLE WITH THE INTRON 9 5T POLYMORPHISM

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ABSTRACT BODY:

Abstract Body: Introduction: Over 2000 mutations in the Cystic fibrosis transmembrane conductance regulator (*CFTR*) gene causes cystic fibrosis (CF) with variable clinical phenotypes. The length of the poly T tract in intron 9 influences exon 10 selection and can manifest as mild or severe disease depending on other *CFTR* mutations. Manipulation of *CFTR* pre-RNA splicing using antisense oligonucleotides (AOs) is a potential therapy for selected CF-causing mutations. We aim to develop splice modulating AOs to rescue CFTR function in CF patients that carry the shorter 5T polymorphism in intron 9. AOs could strengthen exon 10 selection or weaken the selection of flanking exons. As seen with specific cases of Duchenne muscular dystrophy, removing a block of exons can restore more functional dystrophin protein over the removal of a single exon.
Methods: Multiple AOs targeting *CFTR* intron 9 and the flanking exons; 9 and 11 were designed and initially optimised using 2'-O-Methyl modified bases on a phosphorothioate backbone (2OMe) and transfected into primary airway epithelial cells from a child with p.508del/Arg117His;5T CF. After 48 hours RNA was collected, and PCR was used to determine the ratio of altered transcript compared to full-length product. CFTR protein size was determined by western blot analysis. CFTR functional outcomes were measured using Ussing chamber studies utilising Air-Liquid Interface primary airway cell cultures.
Results: Of the 32 2OMe AOs tested for exon 10 inclusion, none reduced the intron 9 5T induced exon 10 skipping. Of the 8 AOs designed to skip exon 9, the highest efficiency was 24% from both the p.Phe508del allele and intron 9 5T allele. Of the 6 AOs designed to skip Exon 11, the highest efficiency was 22% from the intron 9 5T allele. CFTR protein size was determined on western blot and CFTR function was determined by response to Forskolin (Change in Isc).
Conclusion: We propose that skipping the exons flanking exon 10 (9 and/or 11) on the *CFTR* 5T allele could improve CFTR function in CF patients carrying selected mutations, either alone or in combination with current therapeutics.
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