CFTR protein analysis of splice site mutation 2789+5 G-A

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Abstract

Ex vivo biochemical analysis of rectal biopsies of a carrier of the mild 2789+5 G-A CFTR frameshift splice site mutation revealed mutant truncated CFTR of expected size and an imbalance of more core-glycosylated and less mature full-length CFTR. This first immunoblot analysis of a non-F508del CFTR mutant protein derived from human tissue demonstrates that splice site mutations should not only be investigated at the mRNA, but also at the protein level to properly interpret the associations between genotype, molecular pathology and disease.

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1. Introduction

More than 1500 mutations and sequence variations have been identified in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene (http://genet.sickkids.on.ca/cftr/), the majority of which causes cystic fibrosis (CF) [1]. The mutant phenotype has been studied for numerous mutations in patients’ specimens at the mRNA transcript level [1]. Mutant CFTR protein, however, was mainly investigated in heterologous model systems. The few immunocytochemical or biochemical studies on human specimens focussed on the major mutation F508del [2,3], mainly because of the lack of sufficiently sensitive and specific tools and protocols. According to our knowledge, this is the first report of the biochemical phenotype of a non-F508del mutant CFTR protein in native human epithelial tissue.

We developed a protocol that analyses both CFTR function and protein in the same sample ex vivo. Freshly obtained rectal suction biopsies mounted in mini-Ussing chambers are characterized in their chloride secretory responses towards secretagogues and inhibitors (Fig. 1A) [4]. Immediately thereafter the mucosal biopsies are lysed, immunoprecipitated with a cocktail of polyclonal anti-hCFTR antibodies, and CFTR immunoreactive bands are visualized on immunoblot with monoclonal antibodies against hCFTR (Fig. 1B) [5].

The splice mutation 2789+5 G-A in the human CFTR gene is known to be associated with mild CF [6–8]. The initial analysis of affected members of a large inbred pedigree showed that the G-to-A substitution at position +5 of the splice donor site in intron 14b leads to skipping of the 38-bp exon 14b and a frameshift mutant CFTR mRNA. Homozygosity for 2789+5 G-A produced approximately 4% of normally spliced CFTR mRNA transcript [6].

2. Case report

We had the opportunity to study the consequences of the 2789+5 G-A mutation on CFTR protein. The 6-year old
male index case had experienced recurrent episodes of bronchitis during the last three years. Since CF was suspected as a probable cause of his respiratory disease, the boy was referred to the CF center to make a diagnosis. No clinical symptoms of CF were observed in the exocrine pancreatic sufficient boy. The screening for common mutations and the complete sequencing of all CFTR exons and exon/intron boundaries uncovered heterozygosity for 2789+5 G-A. Pilocarpine iontophoresis sweat tests on several occasions yielded chloride concentrations in the borderline range of 30 to 60 mM chloride. Intestinal current measurements [4] of rectal suction biopsies demonstrated normal chloride secretory responses (Fig. 1A) consistent with the genetic diagnosis of a heterozygous carrier of a CFTR mutation. The immunoblot [2,5] of the proband’s mucosal biopsies revealed three interpretable CFTR immunoreactive bands (Fig. 1B). Interestingly, CFTR band B that under standard conditions was barely visible in samples taken from 18 healthy individuals, was a prominent band in the index case as strong as the band C of mature CFTR [9] indicating that the sample contained substantial amounts of the immature core-glycosylated CFTR isoform. The third immunoreactive band was observed in the 100–115 kDa range. Signal intensity was stronger in the index case than in healthy non-CF controls (Fig. 1B). The band was interpreted to represent a superposition of degradation forms of CFTR designated P in Fig. 1B [10,11] (all tissue samples) and mutant CFTR protein designated M in Fig. 1B (index case only). Skipping of exon 14B leads to a CFTR mRNA transcript that encodes a truncated 892 amino acids large 102 kDa CFTR protein that lacks the consensus N-glycosylation sites at N894 and N900 [9]. CFTR forms of slightly lower molecular weight result from limited proteolysis at predisposed sites in the R domain [10,11].

The authors have examined nine individuals with CF at our clinic who are compound heterozygous for 2789+5 G-A and another PI CF allele (seven F508del, one 2043delG, one E92X [7]). All individuals were exocrine pancreatic sufficient, had normal weight throughout their lifetime and showed elevated chloride concentrations of 80–130 mM chloride in sweat tests. The number of patients with normal lung function declined from four by age 20 to two by age 30 and one by age 40, respectively. Three patients died from pulmonary disease at the age of 27, 38, and 41 years, and two patients received lung transplants at the age of 29 and 35 years. The alive non-transplanted patients were 2007 25, 34, 45, and 48 years old. Two F508del/2789+5 G-A compound heterozygotes with mild pulmonary manifestation treated at another CF clinic are the oldest living CF siblings in Germany [12]. Nasal potential difference measurements...
demonstrated substantial chloride conductance indicative for residual CFTR function in the upper airways. In summary, consistent with clinical data from the French CF registry [8], the 2789 +5 G-A splice mutation is associated with normal anthropometry, salt loss by sweat glands and progressive pulmonary disease.

3. Discussion

The rather uniform genotype–phenotype associations in 2789 +5 G-A compound heterozygous patients indicate that truncated CFTR and/or small amounts of normal CFTR may provide some tissue-specific rescue from manifestation of clinical symptoms. The mutant protein contains a complete N-terminal half and the R domain. The recombinant N-terminal portion of CFTR (D836X) forms proper chloride channels in vitro [13], albeit the cooperation between the two non-equivalent NBDs in the binding and hydrolysis of nucleotides cannot take place [9]. If this in vitro data reflects the situation in vivo, the small portion of full-length protein and the major portion of truncated TM1-NBD1-R CFTR derived from the 2789 +5 G-A allele may be sufficient for proper function of some epithelial tissues such as the intestine but not for that of others such as the sweat gland where CFTR is involved in both secretion and re-absorption of salt.

The ex vivo CFTR protein analysis showed an unexpected influence of the 2789 +5 G-A splice site mutation on the relative amounts of full length CFTR isoforms demonstrating that in contrast to common practise splice site mutations should be studied at the mRNA and protein levels to properly interpret the associations between genotype, pathophysiology and clinical manifestations of CF.

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