

Transient correction of the basic defect in sweat glands in an individual with cystic fibrosis carrying the complex *CFTR* allele F508del-R553Q

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ABSTRACT

The molecular pathology of mutant F508del *CFTR* is partially corrected in vitro by the secondary amino acid substitution R553Q in the ABC signature motif. An individual with the *CFTR* genotype R553X/F508del-R553Q showed the typical symptoms and electrophysiological anomalies of cystic fibrosis in the airways and intestine. Sweat chloride concentrations were normal early in life, but were later raised into the range that is diagnostic for cystic fibrosis, suggesting that R553Q could temporarily correct the basic defect in sweat glands. R553Q caused a delay in diagnosis because of false negative sweat tests but was not a disease reverting suppressor mutation as had been inferred from cellular models.

Complex alleles in monogenic disease pose a challenge for clinicians because they are often associated with uncommon diagnostic and clinical features. Here we report on the 30 year course of a basic defect and disease in an individual with cystic fibrosis (CF) who was compound heterozygous for the cystic fibrosis transmembrane conductance regulator (*CFTR*) mutations R553X¹ and the complex allele F508del-R553Q.²

The amino acid substitution R553Q resides within the ABC signature motif of *CFTR*.³ R553Q has been shown in heterologous model systems to partially correct the defective processing and anomalous ion channel gating of mutant F508del *CFTR*.⁴ Hence R553Q has been classified as a disease reverting suppressor mutation. Investigation of the affected subject, however, revealed a more complex manifestation of the basic defect of anomalous epithelial chloride conductance.

The sweat test is still the gold standard to diagnose CF⁵ whereby elevated sweat chloride concentrations of more than 60 mmol/l are considered to be diagnostic of CF. The R553X/F508del-R553Q index case showed a strong increase in sweat electrolytes from infancy to adulthood. Quantitative pilocarpine iontophoresis sweat tests yielded chloride concentrations below 30 mmol/l during infancy, 30–50 mmol/l by the age of 4–5 years, 60–65 mmol/l by the age of 7 years and 100–120 mmol/l by the age of 17–29 years. Nasal potential difference measurements⁶ revealed a typical CF phenotype of a high basal potential difference (–46 mV) and a large response of +30 mV on superfusion with the sodium channel blocker amiloride. No chloride diffusion potential (+3 mV) was induced by chloride free solution and isotroterenol, indicating that *CFTR* mediated

chloride conductance was absent in the patient's upper respiratory epithelium. Intestinal current measurements in rectal biopsies⁷ uncovered a small transient chloride secretory response to carbachol, suggesting that some residual *CFTR* mediated chloride secretion within the typical CF range was operating in the patient's intestinal epithelium.

Clinically, the female patient presented with typical manifestations of CF. Since birth she had been suffering from growth retardation, steatorrhoea, and chronic cough and wheezing. The parents' persistent suspicion that their child was affected by CF was discarded by medical professionals because of repeated false negative sweat tests in early childhood. At the age of diagnosis, 7 years, the exocrine pancreatic insufficient girl was below the third percentile for height and weight. Symptomatic treatment achieved normal anthropometry and lung function during school age. Concurrent with the emergence of type III diabetes mellitus during adolescence, lung function was gradually deteriorating so that the patient needed to receive a lung transplant by the age of 27 years. She is now a full time working clerk.

DISCUSSION

Two major conclusions can be drawn from this singular case. Firstly, CF is a clinical diagnosis and should not be denied by apparently normal sweat electrolytes. Several disease causing *CFTR* mutations that are associated with sweat test values below 60 mmol/l are known,^{8,9} and correspondingly contemporary algorithms explicitly require thorough investigations for making a diagnosis in individuals with sweat test values within the borderline range of 30–60 mmol/l chloride.¹⁰ Secondly, the partial correction of the basic defect observed in transfected cells⁴ was only seen in the patient's sweat glands, but not in her respiratory and intestinal epithelia. The clinical characteristics of the R553X/F508del-R553Q subject were similar to those of R553X/F508del compound heterozygotes.² Moreover, even in the sweat gland, the basic defect was corrected only early in life, but faded over the years, indicating that aging mechanisms abolished the rescue by R553Q in the morphologically inconspicuous tissue.

In summary, R553Q rescued the molecular pathology of F508del *CFTR* in a cellular model, but it was not a disease reverting suppressor mutation in the affected individual.

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Lung alert

Pathogenetic mechanisms involved in viral-induced exacerbations of COPD

Viruses have been shown to be important causes of exacerbations in patients with chronic obstructive pulmonary disease (COPD). Evidence suggests that innate immune responses play a key role in the pathogenesis of airway inflammation in cigarette smoke (CS)-exposed individuals. However, the precise immune pathways and viral pathogen-associated molecular patterns that trigger these responses have not been previously elucidated.

This study used dsRNA (polyinosine-polycytidylic acid (poly(I:C))) as a surrogate to define responses elicited by viral infections in a murine model. Significantly increased airway inflammatory responses and increased alveolar remodelling were observed in CS-exposed mice receiving poly(I:C) compared with mice breathing room air (RA). Poly(I:C) was a potent stimulator of bronchoalveolar lavage fluid levels of type I and II interferon (IFN), interleukin (IL)-18 and IL-2/IL-23 p40 (all known to have roles in viral responses in patients with COPD). These responses were significantly greater in CS-exposed mice than in RA-exposed mice.

Furthermore, CS + poly(I:C)-induced inflammation and alveolar remodelling was significantly diminished in the airways of mice with separate null mutations of IL-18R α , IFN γ , PKR (double-stranded RNA-dependent protein kinase), TLR-3 (toll-like receptor 3) or MAVS (mitochondrial antiviral signalling protein) compared with wild-type mice.

This intriguing study provides a novel insight into the mechanisms involved in viral-induced exacerbations of COPD and suggests potential therapeutic targets to control virus-induced responses in smokers with COPD. However, the applicability of this murine model to human patients who have been exposed to many years of CS and a variety of respiratory viruses remains to be seen.

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