Review

‘CFTR-opathies’: disease phenotypes associated with cystic fibrosis transmembrane regulator gene mutations

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Abstract

Cystic fibrosis is a genetic disease that is associated with abnormal sweat electrolytes, sino-pulmonary disease, exocrine pancreatic insufficiency, and male infertility. Insights into genotype/phenotype relations have recently been gained in this disorder. The strongest relationship exists between ‘severe’ mutations in the gene that encodes the cystic fibrosis transmembrane regulator (CFTR) and pancreatic insufficiency. The relationship between ‘mild’ mutations, associated with residual CFTR function, and expression of disease is less precise. Atypical ‘mild’ mutations in the CFTR gene have been linked to late-onset pulmonary disease, congenital bilateral absence of the vas deferens, and idiopathic pancreatitis. Less commonly, sinusitis, allergic bronchopulmonary aspergillosis, and possibly even asthma may also be associated with mutations in the CFTR gene, but those syndromes predominantly reflect non-CFTR gene modifiers and environmental influences.

Keywords: asthma, cystic fibrosis (CF), cystic fibrosis transmembrane regulator (CFTR), mutations, pancreatitis, phenotype

Introduction

Cystic fibrosis (CF) is a recessive genetic disease that is caused by mutations on both CFTR alleles, resulting in abnormal sweat electrolytes, sino-pulmonary disease, male infertility, and pancreatic exocrine insufficiency in 95% of patients [1,2]. In its classic form, the disease is easily diagnosed early in life, through a combination of clinical evaluation and laboratory testing (including sweat testing, and CFTR mutation analysis) [3]. Depending on the ethnic background of the populations tested, common genetic mutations are identified in the majority of cases of CF. In the USA, two-thirds of patients carry at least one copy of the ΔF508 mutation, with approximately 50% of CF patients being homozygous for this mutation [4].

A wide spectrum of molecular abnormalities may occur in the CFTR gene, and uncommon mutations that result in partial (residual) CFTR function may be associated with nonclassic presentations of disease. Overall, 7% of CF patients are not diagnosed until age 10 years, with a proportion not diagnosed until after age 15 years; some of these patients present a considerable challenge in establishing a diagnosis of CF. Moreover, the phenotype in these patients may vary widely [5,6]. The focus of the present review is on nonclassic phenotypes associated with mutations in the CFTR gene, which may manifest as male infertility (congenital bilateral absence of the vas deferens [CBAVD]), mild pulmonary disease and idiopathic chronic pancreatitis (ICP). These phenotypes are included within the definition of ‘atypical CF’.

ABPA = allergic bronchopulmonary aspergillosis; CBAVD = congenital bilateral absence of the vas deferens; CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane regulator; IB = idiopathic bronchiectasis; ICP = idiopathic chronic pancreatitis.
Cystic fibrosis transmembrane regulator: the relationship between gene mutations and function

CFTR is a transmembrane spanning protein with multiple activities that are related to normal epithelial cell function [2]. Mutations in CFTR result in abnormalities in epithelial ion and water transport, which are associated with derangements in airway mucociliary clearance and other cellular functions related to normal cell biology [7]. Depending on the molecular abnormality, the defect in CFTR may be the equivalent of that associated with a ‘null’ mutation, or may be ‘mild’, with partial/residual function [4]. At one end of the spectrum of severity, ‘null’ or ‘severe’ mutations reflect nonsense, frame-shift or splice mutations; these result in absence of production of functional CFTR, which correlates strongly with pancreatic exocrine insufficiency, but less strongly with severity of lung disease. At the other end of the spectrum, ‘mild’ mutations may result in some production of functional CFTR protein at the apical membrane, with partial CFTR channel function, and are generally associated with pancreatic sufficiency and milder pulmonary disease.

The molecular basis for the severity of mutations may derive from the extent to which normal mRNA transcription or protein synthesis takes place; for example, splice mutations may influence the efficiency of normal/abnormal CFTR mRNA transcription to varying degrees. In turn, the severity of the abnormality in CFTR may relate directly to the phenotypic expression of disease, with absent function causing more severe disease, whereas some residual function may modulate the severity of disease in different organ systems. Clinically, this may be reflected in normal or borderline sweat chloride values in patients with atypical CF. Other factors, including non-CFTR gene modifiers and environmental influences, are probably also associated with the severity of disease. Given this background, it is not surprising that disease expression is complex and that nonclassic CF phenotypes exist.

Phenotypes associated with atypical cystic fibrosis

Table 1 provides a schema of how mutations on one or both alleles of the CFTR gene might relate to nonclassic phenotypic expression of disease. ‘Atypical CF’ includes those clinical phenotypes that have the strongest associations with mutations in the CFTR gene: CBAVD in males, mild pulmonary disease and ICP.

Congenital bilateral absence of the vas deferens

Although not all males with CBAVD have mutations in the CFTR gene, approximately 50% have abnormal CFTR alleles [8]. Generally, one ‘severe’ allele is combined with one ‘mild’ allele, such that the ‘mild’ allele appears to dominate and cause the milder phenotype (e.g. ΔF508 in combination with R117H). Routine screening for common mutations that does not take into account milder or rarer mutations may miss many of the mild mutations associated with this particular clinical expression of disease [8]. This combination of mutations may occur in other forms of atypical CF (see below).

One particular abnormality deserves a special mention – the various alleles of the polythymidine tract in the intron 8 (IVS8) of the CFTR gene [9]. Of the three alleles that have been identified in IVS8 (5T, 7T and 9T), the 9T allele is associated with the most efficient usage of the intron 8 splice acceptor site. This efficiency decreases with shorter
polythymidine tracts (5T and 7T), which results in a lower than normal level of full-length CFTR mRNA and presumably in a decrease in mature, functional CFTR protein. For example, the mild CFTR mutation R117H is influenced by the polythymidine tract sequence, such that an R117H-bearing allele in cis with a 7T allele may result in CBAVD, whereas when R117H is associated with the 5T allele the phenotypic expression may be associated with atypical CF. R117H with a 9T allele may exhibit a normal phenotype. The 5T allele under the influence of other sequence variants in the CFTR gene may also be associated with atypical CF [10].

Although males with CBAVD may present to urology clinics, with no discernable lung or other organ presentation of disease, a careful work-up should be carried out to determine whether subtle lung disease is present. Evidence of CFTR dysfunction may be found on laboratory testing, with abnormal or borderline sweat chloride levels and/or abnormal CFTR-mediated chloride conductance in nasal epithelia [11,12]. Whether lung disease may develop later in life in these generally young males remains to be determined, but they should at least be counseled regarding lung health and cigarette smoking.

Mild pulmonary disease
Older patients with mild pulmonary disease, including bronchiectasis, may not present with symptoms until later in life, but are found to have atypical CF when appropriate investigations are carried out, including normal or borderline sweat chlorides and pancreatic sufficiency [10]. Thus, as with CBAVD, a careful work-up is mandatory. This should include not only a standard diagnostic work-up, including a sweat chloride and radiologic screening for subtle lung disease, but also nasal potential difference measures in order to evaluate CFTR at a physiologic level, and screening for mild and rare CFTR mutations [10]. A ‘severe’ mutation may be found on one allele, with a ‘mild’ mutation, such as the 5T abnormality (with or without other abnormalities in the CFTR gene), on the other allele. The level of expression of full-length mature CFTR may be less than that in CBAVD, with adverse consequences for the lung, albeit with a later presentation [10]. Although the pulmonary disease is milder than that with classic CF, these patients generally exhibit phenotypic similarities to CF; for example, the distribution of radiographic changes often involve the upper lobe, and mucoid Pseudomonas aeruginosa may be present in the lower airway.

Idiopathic bronchiectasis (IB) could loosely be defined as bronchiectasis in which no clear cause has been found, and in which the clinical pattern differs from CF and other known causes of bronchiectasis. Two studies [13,14] suggested that IB may be linked to mutated CFTR. In one study [13], five out of 16 patients with IB harbored the 5T allele in the CFTR gene. Of those, two were 5T/5T homozygotes. Insufficient data were supplied regarding the clinical phenotype in the five patients harboring the 5T allele to draw any firm conclusions as to whether they would otherwise fulfill rigorous diagnostic criteria for CF [3]. In the second study [14], from France, 13 mutations were found in 16 CFTR alleles in 32 patients with idiopathic bronchiectasis. Only six of the 13 mutations were confirmed to be CF-causing mutations, with the remainder hypothesized as being ‘potentially’ CF causing. Four patients were compound heterozygotes, and all 11 of the patients who harbored mutations had abnormal sweat chloride levels (>60 mmol/l), with apparently no clear-cut evidence of CF otherwise (‘isolated bronchiectasis’). Girodon et al. [14] speculated that IB might be related, at least in part, to mutated CFTR, with possible other factors at play. In any such population, atypical or variant CF is likely to be present in a proportion of patients studied in detail.

Idiopathic chronic pancreatitis
Recent reports [5,6,15,16] suggest that patients with an ICP phenotype have an increased incidence of mutations in CFTR. Such patients generally present with symptoms of pancreatitis at an older age than those patients with classic CF. Because CF carriers represent 3–4% of the general population, it is important to know whether one or two mutations predispose to ICP. Although the data initially appeared to suggest that patients with one mutation in CFTR were at risk, subsequent studies have borne out the observation of a link between mutated CFTR on both alleles and ICP.

A rigorous search was conducted for other mutations in patients with one CFTR mutation, and CFTR function in nasal epithelia was assessed in vivo in patients with ICP [17]. Sequencing of the CFTR gene indicated that nine out of 41 patients with ICP had two abnormal CFTR alleles; again the combination of ‘severe’ and ‘mild’, and having two mutations increased the risk for ICP 40-fold. ICP patients with two abnormal CFTR alleles had reduced CFTR-mediated chloride conductance in nasal epithelia as compared with ICP control individuals. The number of CFTR heterozygotes with ICP was no higher than is expected in the general population. These data strongly suggest that abnormalities on both alleles are required for expression of ‘CF-related ICP’, perhaps with some added influence from mutations in pancreatic inhibitor genes (PRSS1, PST1) [18].

Other phenotypes associated with mutations in the cystic fibrosis transmembrane regulator gene
Other sino-pulmonary syndromes have been studied to test for a link to mutated CFTR; sinusitis, allergic bronchopulmonary aspergillosis, and asthma. However, the likelihood is that they predominantly reflect non-CFTR gene modifiers and environmental influences.
Sinusitis
In a recent study [19], DNA from 147 patients with chronic rhino-sinusitis was screened for 16 CFTR mutations, including the 5T sequence, and patients with a mutation had their DNA screened over the entire coding region. Eleven patients had a mutation in CFTR (all severe mutations, and one patient eventually developed CF), as compared with two out of 123 control individuals, whereas there was no difference in the incidence of the 5T allele between controls and study subjects. There was also a higher frequency of the M470V polymorphism on the opposite allele to that containing a severe mutation as compared with control individuals. Physiologic testing in the sinusitis patients showed normal indices of nasal epithelial sodium transport, with a slight reduction in CFTR-mediated chloride conductance. The authors of that report concluded that the combination of a severe mutation on one allele with a sequence variant that is not normally associated with CF on the opposite allele may be responsible. An analogy is again drawn with the other non-classic phenotypes, with enough residual CFTR function to protect against early, classic sino-pulmonary disease and a pancreatic phenotype, but clearly other non-CFTR factors may also be at play (Table 1).

Allergic bronchopulmonary aspergillosis
Although Aspergillus fumigatus is ubiquitous in nature, allergic bronchopulmonary aspergillosis (ABPA) occurs in only a small number of patients with asthma and CF; thus, genetic factors may play a role in the pathogenesis of ABPA in some patients. A study from several years ago [20] showed that, in a small number of patients who met criteria for ABPA, there was a higher frequency of abnormal CFTR alleles than expected. The authors of that report speculated that mutations in CFTR may play a role in the pathogenesis of ABPA, either as a result of heterozygosity alone (and 50% CFTR function), or heterozygosity plus other genetic factors that were not detected by the methods used in the study. The situation is probably similar to that in asthma, with genetic factors outside of CFTR, together with environmental influences, playing major roles.

Asthma
There are conflicting data as to whether mutations in the CFTR gene are over-represented in patients with asthma [21–23]. In Denmark, a questionnaire study was carried out in a cohort of carriers of the ΔF508 mutation in CFTR [24]. Of 250 adults studied, it appeared that 9% reported symptoms of asthma, as compared with 6% of control non-carriers, with airways obstruction being present in those carriers with symptoms of asthma. However, there are clear limitations in a study of this kind, relying solely on a questionnaire for diagnosis. A second study investigated 144 patients with documented asthma [22], and identified 15 missense mutations in the CFTR gene of 15 patients, compared with none in a small control group. When tests were carried out in a larger control group, however, the differences lost significance. In contrast, several other studies failed to show a link between mutations in CFTR and asthma, and if anything show a protective effect [23]. Thus, there is little evidence to support a link between asthma and abnormalities in CFTR, such that, if there is a link, then it plays a small role in the overall pathogenesis of disease, with a much larger role played both by genetic factors outside of CFTR and by environmental influences (Table 1).

Conclusion
Mutated CFTR may be associated with an atypical CF phenotype in the sino-pulmonary tract, pancreas, and male genital tract, with reduced CFTR epithelial function. Although abnormalities in the CFTR gene may play a minor role in the pathogenesis of asthma, sinusitis, and ABPA in subsets of patients, these diseases predominantly result from genetic (non-CFTR) and nongenetic environmental influences.

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