

Atypical Cystic Fibrosis and CFTR-Related Diseases

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Abstract Cystic fibrosis (CF), which is among the most common life-shortening recessive illnesses, is caused by mutations of the CF transmembrane conductance regulator (CFTR) and typically involves chronic infection and progressive obstruction of the respiratory tract as well as pancreatic exocrine insufficiency. Disease severity, to some extent, correlates with organ sensitivity to CFTR dysfunction and to the amount of functional protein, which is influenced by the type of mutation. Atypical CF represents approximately 2% of affected individuals, and includes cases presenting in adolescence or adulthood with pancreatic exocrine sufficiency, normal or borderline sweat chloride concentrations, or with a single predominant clinical feature. This review briefly describes diagnostic methods and phenotypic characteristics of classic and atypical CF, as well as CFTR-related diseases, conditions in which mutated CFTR may contribute to the pathogenesis but do not strictly fit established diagnostic criteria.

Keywords Cystic fibrosis · CF transmembrane conductance regulator · Mutations

Introduction

Cystic fibrosis (CF) is among the most common life-shortening recessive genetic diseases, with approximately

30,000 affected individuals in the US. The incidence varies by ethnic background, and in Caucasians, is estimated to be 1:3,200; in African Americans 1:15,000; and in Asian Americans 1:31,000. It results from mutations of the CF transmembrane conductance regulator (CFTR), which functions as a cyclic adenosine monophosphate (cAMP) chloride channel at the apical epithelial cell surface [1]. Over 1,500 mutations have been identified to date and categorized into distinct classes reflecting abnormalities of protein structure or function [2]. The degrees of organ involvement, and to some extent, disease severity correlate with the organ's sensitivity to CFTR dysfunction and to the amount of functional CFTR, which is influenced by the type of mutation [3]. The primary organ systems involved are the lungs, in which mucus plugging and inflammation lead to progressive small airways obstruction and bronchiectasis; the gastrointestinal tract, liver, and exocrine pancreas, in which inspissation of viscid secretions leads to intestinal obstruction, cholestasis, and malabsorption of fat and protein; and the male reproductive tract, in which obstructive azoospermia leads to infertility.

A 1998 Cystic Fibrosis Foundation Consensus Panel established diagnostic criteria [4] based on: (1) the presence of one or more characteristic phenotypic features of chronic sinopulmonary disease, gastrointestinal and nutritional abnormalities, salt depletion syndromes, and male urogenital abnormalities; (2) a history of CF in a sibling; or (3) a positive newborn screening test with laboratory evidence of a CFTR abnormality such as an elevated sweat chloride concentration, identification of mutations in each CFTR gene known to cause CF, or in vivo demonstration of characteristic ion transport abnormalities across the nasal epithelium. Atypical cases are those in which the genotype-CFTR functionality-phenotype correlation is less clear-cut and results in wide variability in disease presentation,

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which in turn is exaggerated further because of the large number of identified CFTR mutations.

The increased recognition of atypical or nonclassic CF, which represents approximately 2% of affected patients [3], has made clinical diagnosis much more challenging. Non-classic cases include those presenting in adolescence or adulthood, those with pancreatic exocrine sufficiency and/or normal or borderline sweat chloride concentrations, or those with a single predominant clinical feature. Of particular interest are those individuals with incompletely penetrant mutations, as these variants can also influence CFTR function and result in variable phenotypes.

This review will briefly describe classic and nonclassic CF as well as some CFTR-related diseases, which do not strictly fit the established diagnostic criteria, although mutated CFTR may have a role in the pathogenesis. Methods of diagnosis, including genotype analysis, and the contributions of poly-T variants and modifier genes, will be discussed, in addition to objective tests that define *in vivo* ion transport abnormalities. The phenotypic characteristics of nonclassic CF will also be reviewed.

Diagnostic criteria

The 1998 Consensus Panel established the diagnostic criteria with the intention of refining and updating them as new knowledge is obtained regarding the CF phenotype, identification of additional CF-causing genetic mutations, and reference ranges for diagnostic studies such as sweat tests and nasal potential difference measurements [4]. The correlation between genotype and phenotype has been extensively reviewed given the discovery of over 1,500 CFTR mutations [2, 5–7], in addition to other work suggesting that factors other than dysfunctional CFTR could produce “variant” CF [8, 9]. The present understanding of the definition of CF involves identification of two disease-causing CFTR mutations, laboratory evidence of CFTR dysfunction either by sweat chloride or nasal potential difference measurements, and demonstration of the CF phenotype in at least one organ system [4].

Genotypic criteria are primarily based on identification of two alterations in the CFTR gene. CF-causing mutations should fulfill at least one of the following: (1) alteration of the CFTR sequence to affect protein synthesis and function; (2) introduction of a premature termination signal, such as an insertion, deletion, or nonsense mutation; (3) alteration of the intron splice sites; and 4) creation of a novel amino acid sequence that does not occur in the normal CFTR genes of carriers from the patient's ethnic group [4]. To date, more than 1,500 CFTR mutations have been identified [2] that are categorized into six distinct classes (I–VI) reflecting errors in protein synthesis or trafficking from the

endoplasmic reticulum to the cell membrane [6]. This classification roughly organizes CFTR mutations by their molecular mechanisms and consequences for CFTR biogenesis and functionality. Overlap of mutation class, defect in biogenesis, and the cumulative effect of two mutations on the eventual CF phenotype will ultimately define the severity of involvement. Any discordance between the CFTR mutation classification and clinical phenotype may be related to the specific amino acid change, the adjacent sequence of amino acids, or functional contributions from other mutations or modifiers present on the same or alternate allele [10]. Class I, II, III, and VI mutations are generally associated with severe CF phenotypes. Class IV and V mutations are typically associated with pancreatic exocrine sufficiency and milder disease.

Class I mutations are associated with either absent or defective protein biosynthesis resulting in abnormal protein variants. Class II mutations arise from variants that are not properly processed or transported to the apical cell membrane. Class III mutations affect the regulation of CFTR function by preventing ATP hydrolysis at the nucleotide binding domains 1 and 2, thereby hindering chloride channel activation. Class IV mutations result in a normal amount of CFTR with reduced function at the apical membrane because of decreased chloride conductance. Class V mutations are associated with reduced synthesis of fully active CFTR resulting in reduced expression at the apical membrane because of ineffective trafficking, or aberrant splicing or promoter mutations. Class VI mutations are defined by decreased stability of a fully processed and functional CFTR variant and likely result from nonsense or frameshift mutations leading to truncation of the carboxyl terminus of CFTR [6].

Variations in the alleles of the polythymidine (poly-T) tract sequences in intron 8 of the CFTR gene can result in a reduced amount of full-length CFTR mRNA, and by extension, abnormalities, or decreased amounts of the mature, functional CFTR protein. Three alleles, 5T, 7T, and 9T, have been identified with the intron 8 splice site. The 9T allele is associated with the most preserved function of the intron 8 splice site, and this degree of preservation is reduced with the two shorter 5T and 7T poly-T alleles [11].

The presence of these alleles in conjunction with a CFTR mutation can influence CFTR expression and result in either a mild or nonclassic CF phenotype. For example, the mild mutation R117H/7T can result in CBAVD and R117H/5T in nonclassic CF, whereas R117H/9T exhibits a normal phenotype. Differential splicing in tissues may in part be responsible for the lack of symptoms in other organs in men affected with CBAVD [6]. Representative mutations causing atypical CF lung disease and pancreatic sufficiency or late-onset pancreatic insufficiency are listed (Table 1).

Table 1 Mutations, sites, and molecular consequences associated with either an atypical presentation of CF respiratory disease or pancreatic sufficiency or late-onset pancreatic insufficiency (<http://www.genet.sickkids.on.ca>)

Mutation	Site	Consequence
Atypical presentation		
M1210I	Exon 19	Met to Ile at 1210
S1455X	Exon 24	Ser to Stop at 1455
1811+18G→A	Intron 11	mRNA splicing defect
L346P	Exon 7	Leu to Pro at 346
Y161D	Exon 4	Tyr to Asp at 161
R31C	Exon 2	Arg to Cys at 31
I752S	Exon 13	Ile to Ser at 752
2811G/T	Exon 15	Sequence variation
Pancreatic sufficiency or late-onset pancreatic insufficiency		
R600G	Exon 13	Arg to Gly at 600
D1152H	Exon 18	Asp to His at 1152
Y89C	Exon 3	Tyr to Cys at 89
R117H	Exon 4	Arg to His at 117
D110E	Exon 4	Asp to Glu at 110
296 + 3insT	Intron 2	mRNA splicing defect
E217G	Exon 6a	Glu to Gly at 217
V392G	Exon 8	Val to Gly at 392
N1088D	Exon 17b	Asn to Asp at 1088
S737F	Exon 13	Missense
1716+1G→A	Intron 10	mRNA splicing defect
R334W	Exon 7	Arg to Trp at 334
R347P	Exon 7	Arg to Pro at 347
A455E	Exon 9	Ala to Glu at 455
P574H	Exon 12	Pro to His at 574
3850-3T→G	Intron 19	mRNA splicing defect

Environmental exposures to infectious pathogens and inhaled pollutants and genetic factors can modulate pulmonary disease and influence the clinical outcome in CF. For example, exposure to passive exhaled tobacco smoke may significantly affect the extent of lung disease in subjects who have the same genotype. Bacterial infection poses a complex problem both in terms of the risks of exposure between patients and of their genetic adaptability to evade airway host defenses and survive in the airway microenvironment. Genetic modifiers have long been implicated in affecting the severity of disease; they may be tissue or organ specific and involve multiple genes and pathways [12]. Thus far, the study of CF genetic modifiers has included linkage studies and testing of candidate genes [13]. A recent study [5] estimated the genetic contribution to variation in CF lung disease by comparing the similarity of lung function in monozygous twins, dizygous twins, and sibling pairs, and found that a significant portion of the variability in CF lung disease is attributable to modifier genes independent of the CF genotype.

The sweat test is considered to be the gold standard for confirming the diagnosis of CF [4]. Testing must be

performed by experienced personnel in accredited laboratories using standardized methods to maintain quality control. The method and reference values must be known to accurately interpret the sweat chloride measurements. The quantitative pilocarpine iontophoresis sweat test is the only acceptable procedure, where a minimum volume (15 μ L for the Macroduct coil system) or weight (75 mg for the Gibson–Cooke method) of sweat must be collected over 30 min to ensure an average sweat rate of 1 g m^{-2} min^{-1} . Alternative procedures such as a paper-patch indicator system or direct-reading conductivity measurements should never be used to definitively diagnose CF because these methods are associated with high false-positive and false-negative results.

Elevation of sweat chloride over 60 mEq/L is consistent with a CF diagnosis, whereas levels greater than 160 mEq/L are physiologically impossible and suggest improper sweat collection or analysis. Because elevated sodium levels can be demonstrated in individuals with diseases other than CF, measurement of sodium concentration alone is not advised. Both sodium and chloride concentrations are proportionately elevated in CF within 15 mEq/L and the ratio of chloride to sodium concentrations is almost always greater than 1.0 [4].

Sweat chloride results less than 40 mEq/L are considered negative, although studies are currently underway to determine if there is an age-dependent physiologic variability in sweat electrolyte levels, particularly in newborns. A systematic review [14] identified 17 studies of sweat chloride analysis over a 50-year period and revealed that there is no definitive study that quantifies sweat electrolyte concentrations in a normal, unaffected population. This study concluded that further work is required to establish reference ranges for sweat electrolytes using a standardized testing method with accurate statistical analysis to determine age-dependent or physiologic variability in normal subjects and CFTR mutation analysis to exclude carriers.

Measurements between 40 and 60 mEq/L are associated with atypical or nonclassic CF and warrant further diagnostic evaluation, which may include transepithelial nasal potential difference measurement, gene mutation analysis, and clinical phenotypic correlation. Analysis of sodium concentration may also be of benefit when borderline sweat chloride measurements are obtained. A retrospective study [15] demonstrated that misdiagnosis of CF, in terms of false-positive and false-negative sweat test results, was lessened when sodium levels were concurrently analyzed. In this study, normal subjects with borderline sweat tests were found to have sodium concentrations higher than chloride levels, whereas CF subjects showed higher chloride concentrations compared to sodium.

Determination of the transepithelial nasal potential difference has been beneficial in establishing a CF

diagnosis in many atypical cases. Three features of nasal potential difference measurements are characteristic of CF: (1) a raised basal potential difference reflecting enhanced sodium transport across a chloride-impermeable barrier; (2) a decrease in potential difference after perfusion with amiloride, a sodium channel inhibitor; and (3) little or no change in potential difference after perfusion with a chloride-free solution in conjunction with isoproterenol, which proves the absence of CFTR-mediated chloride secretion. Nasal potential difference measurements are carried out only in established laboratories and centers and are not considered to be a standard diagnostic testing method. Several technical considerations must be addressed to ensure valid and reproducible results, as the sites of potential difference measurements are critical for accuracy. False-negative results may be obtained in the presence of nasal polyposis or mucosal inflammation. Furthermore, interpretation of the measurements requires a clear understanding of the ion transport properties of the nasal and respiratory epithelium to definitively confirm or exclude a diagnosis of CF [16–18].

Both the value of early diagnosis and the limitations of sweat testing have been recognized since the 1970s, when Dr. Harry Shwachman advocated for screening newborns and Dr. Jeanette Crossley demonstrated that dried newborn blood specimens could be tested for immunoreactive trypsinogen (IRT), which remains persistently elevated in CF [19]. The IRT is the first test for all CF neonatal screens, and in some regions, is the sole screening test. The single IRT, or IRT/IRT combination, is 85–90% sensitive, but also has a high false-positive rate. DNA analysis is also used in place of a second IRT measurement, although a high IRT with a negative DNA analysis is still considered highly suspicious for CF. The current two-tier testing method utilizing IRT measurements and DNA analysis has helped to identify affected infants earlier, including heterozygotes that may present with atypical symptoms; increase sensitivity, specificity, and positive predictive value compared to IRT alone; and identify carriers whose families may potentially benefit from genetic counseling [20].

Classic CF

The majority of children with CF will present with the classic clinical triad, which includes a history of: (1) chronic and recurrent sinopulmonary infections, (2) steatorrhea, and (3) failure to thrive. Pancreatic insufficiency is present in approximately 85% of affected individuals. The diagnosis is typically confirmed by an elevated (>60 mEq/L) sweat chloride as well as the identification of two CFTR mutations [21].

Certain manifestations occur in infancy and early childhood and are strongly suggestive of the diagnosis of CF. In the prenatal period, ultrasonographic findings consistent with hyperechoic bowel suggest intestinal obstruction; approximately 10% of fetuses with this finding will have CF. In neonates, the presence of abnormal meconium with a high protein concentration can result in the delayed passage of meconium, meconium plug syndrome, or meconium ileus, which occurs in 15–20% of affected patients [21]. Resulting from the inspissation of meconium in the small intestine, meconium ileus is generally associated with abdominal distension and the presence of dilated bowel loops on abdominal imaging studies. Approximately 50% of cases are complicated by conditions such as peritonitis or intestinal perforation. Treatment generally requires surgical intervention. Rectal prolapse occurs in approximately 20% of affected children, typically between the ages of 6 months and 3 years, and likely results from malnutrition, malabsorption, poor muscle tone, and the elimination of bulky, voluminous stools. Other less common clinical features presenting in infancy include: (1) edema, hypoproteinemia, and acrodermatitis enteropathica, which is associated with either breast milk or soy protein feedings and likely results from malabsorption; (2) salt depletion syndrome, which manifests as a hypokalemic, hypochloremic metabolic alkalosis, particularly in warm seasons or climates, (3) prolonged neonatal jaundice secondary to intrahepatic bile stasis and extrahepatic bile duct obstruction; and (4) hemorrhagic disease of the newborn secondary to vitamin K deficiency [21].

Clinical findings seen in older children and adolescents that are suggestive of CF can include both respiratory and gastrointestinal conditions. Common examples typical of respiratory involvement are recurrent infections of the sinuses and respiratory tract, refractory asthma, nasal polyposis, bronchiectasis, and the presence of digital clubbing. Gastrointestinal features at diagnosis in this age group may include a history of poor growth and weight gain, steatorrhea, rectal prolapse, chronic intestinal obstruction, or even intussusception.

The diagnosis is typically confirmed with an elevated sweat chloride measurement along with identification of two CFTR mutations. Approximately 70% of affected individuals have one copy of $\Delta F508$, the most common mutation, with about 25% of these being homozygous for $\Delta F508$. Affected individuals with a compound heterozygous genotype will demonstrate typical phenotypic characteristics with less than 5% of functioning CFTR [1]. Treatment is based on the control of chronic airways infection, airway clearance to remove the thick, tenacious secretions, and aggressive nutritional support to treat the malabsorption resulting from pancreatic exocrine insufficiency and other gastrointestinal manifestations.

Nonclassic CF

Individuals demonstrating a CF phenotype in at least one organ system with normal (<40 mEq/L) or borderline (40–60 mEq/L) sweat chloride values are described as having nonclassic disease. Generally, these patients are pancreatic sufficient with milder lung disease compared to those with classic CF and carry two CFTR mutations, at least one of which results in partial protein expression or function. Whereas the symptoms of nonclassic CF may have been present to a lesser degree in infancy or early childhood, they tend to become clinically significant after the first decade of life or during adulthood [22]. Affected patients may, alternatively, present with single organ involvement, such as focal biliary cirrhosis with portal hypertension, recurrent pancreatitis, chronic sinusitis, nasal polyposis, or infertility secondary to congenital bilateral absence of the vas deferens (CBAVD).

Previous studies of the relationship between CFTR mutations and recurrent idiopathic pancreatitis have demonstrated that 10–20% of individuals carry two abnormal CFTR genes, at least one of which is classified as a mild mutation [23, 24]. Although pancreatitis is unusual in individuals with typical CF, its incidence is increased in those who are pancreatic sufficient. These individuals generally have little or no evidence of CF lung disease, along with normal or borderline sweat chloride measurements and abnormal ion transport measured across the nasal epithelium. Indeed, idiopathic pancreatitis may represent a condition of single organ involvement since genetic studies have demonstrated not only a greater frequency of CFTR mutations, but also associated candidate genes [6, 11].

Another example of single organ involvement is CBAVD, which occurs with a frequency of approximately 1–2% in infertile males and is a closely investigated cause of obstructive azoospermia [6]. CFTR mutations are present in about 50% of affected men. The genotype typically includes a mild mutation and results in 10% of residual CFTR function, which explains the spectrum of mild phenotypic features. Mutations are missense or splice variants of Class IV or Class V. A significant proportion of these affected men have chronic sinusitis as well as abnormal nasal epithelial potential difference measurements, although the exact prevalence of these coexisting conditions is not known. Although these affected individuals meet the established consensus criteria for diagnosis of CF, the medical, psychosocial, and financial impact of such a diagnosis can be burdensome [3].

Mild pulmonary disease as well as idiopathic bronchiectasis in older patients warrants a thorough evaluation for nonclassic CF, including measurement of sweat chloride, assessment of CFTR function through nasal epithelial potential difference, and genotype analysis particularly for

mild CFTR mutations. The pulmonary disease may be less severe compared to classic CF but still exhibits phenotypic similarities, such as chronic *Pseudomonas aeruginosa* infection of the lower airways and predominant upper lobe involvement. Previous studies [25–27] have linked idiopathic bronchiectasis to CFTR, in which mutations were identified in patients with mild lung disease, but did not definitively conclude whether the study population would meet CF diagnostic criteria because two mutations were rarely identified. These studies speculated that the etiology of idiopathic bronchiectasis is likely multifactorial with some contribution of mutated CFTR.

The occurrence of less common phenotypes with less multisystem involvement and unusual CFTR mutations, particularly in an older adolescent or adult patient, may be indicative of nonclassic CF, but also makes confirmation of the diagnosis less straightforward [3, 22]. As is the case with patients with classic CF, identifying these affected individuals early in the course of disease will have a profound impact on their overall survival and quality of life and provide a more accurate estimate of the prevalence of nonclassic CF.

CFTR-related diseases

Another category of diseases includes those respiratory conditions, namely chronic sinusitis, allergic bronchopulmonary aspergillosis, and asthma, which neither fit CF criteria nor follow a Mendelian inheritance pattern. Mutated CFTR may play a role in their pathogenesis, but more likely these conditions result from multifactorial non-CFTR genetic and nongenetic environmental influences.

Whereas chronic sinus involvement is present in nearly all CF patients, previous studies have demonstrated that the presence of CFTR mutations in unaffected subjects may increase the risk of chronic sinusitis. In one study of 147 non-CF subjects with chronic sinusitis, 7% carried CFTR mutations compared with 2% of healthy controls [28]. Another study [29] showed CFTR mutations in 12% of pediatric subjects with chronic rhinosinusitis. With a low frequency of CFTR mutations in both of these studies, it is likely that the pathogenetic mechanism of chronic sinusitis is multifactorial.

Allergic bronchopulmonary aspergillosis (ABPA), a hypersensitivity response to the ubiquitous fungus *Aspergillus fumigatus*, is present in approximately 2% of CF and asthma patients. It should be suspected in patients who present with wheezing, expectoration of brown mucus plugs, and in those with poor response to conventional therapies or unexpected deteriorations in lung function. Diagnostic criteria generally include elevation of serum IgE, a positive skin test response to *Aspergillus* antigen or

elevated *Aspergillus*-specific IgE, or isolation of *Aspergillus* on sputum culture [21]. CFTR mutations have been implicated in isolated ABPA [27]; in a screening study of ABPA patients who had normal sweat chloride concentrations, there was an increased frequency of CFTR mutations compared to the general population and also to patients with chronic bronchitis [30]. Abnormalities of CFTR, either alone or in combination with other genetic or environmental influences, may play a role in the pathogenesis of ABPA, but further studies are needed to establish this correlation.

In a similar manner, there is conflicting evidence supporting a link between CFTR mutations and asthma. One Danish study found a higher frequency of the $\Delta F508$ mutation among subjects with obstructive pulmonary diseases, of whom 9% reported having symptoms of asthma [31]; however, other smaller studies showed either a negative or no association with the $\Delta F508$ allele. Other studies carrying out a more detailed mutation analysis found an increased frequency of CFTR mutations but no correlation to asthma severity with the presence or absence of mutations [11].

Management of nonclassic CF and CFTR-related diseases

In general, the management of CF has centered on the control of chronic infection and inflammation of the airways, therapies to facilitate mucus clearance, and aggressive nutritional support to treat malabsorption and malnutrition. Given the wide spectrum of disease presentation of nonclassic CF and CFTR-related conditions, it follows that accepted treatments of the pulmonary and gastrointestinal manifestations will be beneficial, although these conclusions are based on limited data.

Although treatment of chronic bacterial infection is beneficial regardless of the severity of disease, the advantages of other antiinflammatory and airway clearance therapies warrant further study. For the treatment of CF or CF-related lung disease and bronchiectasis, the agents best studied for their antiinflammatory properties are the inhaled corticosteroids and the macrolide antibiotics. Four small clinical trials reported the reduction of sputum volume as a parameter for decreasing airway inflammation [32]. Based on their efficacy in the treatment of diffuse panbronchiolitis (DPB), the macrolide antibiotics have been shown to improve lung function, sputum volume, and symptom control in diseases such as asthma and CF and reduce nasal polyps and secretions in chronic sinusitis. The effects of macrolides include decreasing mucus production, altering the release of inflammatory mediators, and inhibiting virulence factors and *Pseudomonas* biofilm formation [32,

33]. Airway clearance therapies and the use of inhaled hyperosmolar agents have been proven to be of benefit in individuals with CF regardless of lung disease severity, but require further study in the form of randomized, controlled clinical trials to determine efficacy and maximize patient adherence [34–36].

Non-CFTR-related diseases

Whereas classic and nonclassic CF are associated with CFTR mutations, variant CF phenotypes can also result from factors other than dysfunctional CFTR. In an extensive genetic analysis of 74 patients with nonclassic CF [9], 29 had two mutations, 15 had one mutation, and 30 had none. This suggests that factors other than CFTR mutations could produce phenotypes that are clinically indistinguishable from nonclassic CF. Another study [8] demonstrated that in subjects with nonclassic CF, the presence of a common CF-causing mutation, absence of the vas deferens, or *Pseudomonas aeruginosa* infection were predictive of deleterious CFTR mutations, whereas the presence of steatorrhea alone was likely to have an etiology other than CFTR dysfunction.

Diffuse panbronchiolitis (DPB) is an idiopathic, suppurative, inflammatory lung disease that is well-recognized in Japan and is associated with chronic inflammation of the respiratory bronchioles and infection with such pathogens as *Haemophilus influenzae* and *Pseudomonas aeruginosa*. Although DPB has been frequently compared to CF, and has served as a model for the implementation of antiinflammatory therapies in CF lung disease [33], it is known to be a separate clinicopathologic entity that is not characterized by pancreatic exocrine insufficiency or obstructive azoospermia and male infertility. DPB is not associated with the $\Delta F508$ mutation [37], although the contribution of minor CFTR mutations has not been excluded [38].

Comment

The variation in the clinical presentation of CF underscores the importance of the concept of genotype–phenotype correlation in establishing and confirming a diagnosis, assessing severity, and ultimately in managing disease. Classic CF is a monogenic disorder resulting from two distinct CFTR mutations. Nonclassic CF may be caused by disease-causing mutations of variable severity and result in either single or multiorgan involvement based on each organ's sensitivity to dysfunctional CFTR. Alternatively, nonclassic CF phenotypes may result from a single CFTR mutation in combination with alleles of other genes or the

presence of other nongenetic and environmental factors. This possibility suggests that individuals with single CFTR mutations are at higher risk than the general population for having clinical features of CF, and raises the question of whether certain conditions, such as asthma and chronic rhinosinusitis, are more prevalent in carriers. Although the consideration of non-CFTR variant CF further implicates the contribution of other genetic factors in manifesting a disease phenotype, it is difficult to characterize CF without some evidence of a genetic mutation or alteration in ion transport properties [39]. Taken together, understanding the genetic basis of CF, the contribution of other candidate and modifier genes and disease-modulating factors, advancing the current knowledge of ion transport properties and molecular dysfunction, and defining the variability of clinical phenotypes may lead to reestablishing diagnostic criteria but will all ultimately positively influence disease management and outcomes.

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