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Cystic Fibrosis in the Genomic Era: CFTR Genotyping as a Diagnostic Test

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Abstract

Our insight into cystic fibrosis (CF) disease and diseases associated with CF gene mutations has significantly increased in recent years, particularly after the discovery of the cystic fibrosis transmembrane conductance regulator (CFTR) gene over two decades ago. This has resulted in a widened spectrum of phenotypic manifestations of CF ranging from the classic multisystem disease in infancy and early childhood to adults with single-organ manifestations of CF such as chronic sinopulmonary disease, pancreatitis and obstructive azoospermia. As a consequence, the diagnosis of CF can be difficult to establish or exclude. Extensive CFTR genotyping has been limited by the inability to interpret the functional and clinical significance of a large number of identified CFTR mutations. As identified CFTR mutations can either be "disease-causing", "of varying clinical consequences", "benign" or have unknown consequences, the identification of mutations on both alleles may be insufficient alone to confirm the diagnosis of CF. More recently though, the number of mutations recognized to be disease-causing was greatly expanded by the Clinical and Functional Translation of CFTR (CFTR2) project. This review article discusses the role and yield of CFTR mutation analysis in the diagnosis of CF.

Keyword:

Cystic fibrosis; CFTR; Genotyping; Bronchiectasis; Pancreatitis

Introduction

Cystic fibrosis (CF) is a life-shortening multisystem disease caused by mutations in the CF transmembrane conductance regulator (CFTR) gene [1-3]. It affects 1 in 3000 newborns in white populations [4]. The CFTR gene encodes the CFTR chloride-ion channel that is an essential component of epithelial ion transport systems in many organs, including the lungs, pancreas, intestinal tract, hepatobiliary tract, vas deferens and sweat glands. The epithelia affected by CF exhibit different functions in their native states: volume-absorbing and secreting (e.g. airways and intestine), volume-secreting (e.g. exocrine pancreas and vas deferens) and salt-absorbing (sweat duct). Given this wide range of native activities in diverse organ systems, it is not unexpected that the spectrum of CF disease manifestations is wide.

Significant progress into the knowledge of CF and diseases associated with CFTR mutations has been made since the discovery of the CFTR gene over two decades ago [1-3]. To date, nearly 2000 CFTR mutations have been identified [5]. Furthermore, CF disease is now considered a complex monogenic disease which includes a wide spectrum of phenotypes and clinical presentations ranging from patients affected by the historically described multi-system form of CF disease (previously known as "classic CF") to patients with single organ manifestations of CF (e.g. idiopathic bronchiectasis/chronic sinopulmonary disease and idiopathic recurrent acute/chronic pancreatitis) [6]. The large number of disease-contributing mutations, all of which affects the function of the CFTR protein to varying degrees, is a major contributing factor of the wide phenotypic spectrum in CF. However, the heterogeneity in disease manifestation and severity is not completely explained by mutations in the CFTR gene mutations alone but also from the complex interactions between CFTR and non-CFTR modifier factors (genetic and/or environmental) [7].

The widened phenotypic spectrum of CF

Cystic fibrosis was previously considered as a multisystem disease which presents either at birth with intestinal obstruction (e.g. meconium ileus), or soon after in the first years of life with failure to thrive from maldigestion and malabsorption of nutrients secondary to exocrine pancreatic insufficiency (PI) and recurrent pulmonary infections. Now, individuals with symptoms manifesting in adolescence and adulthood are receiving a diagnosis of CF [8,9]. This has been complicated by the fact that, several diseases that resemble CF at an organ-specific level, namely recurrent acute or chronic pancreatitis, chronic sino-pulmonary disease (e.g. bronchiectasis) and obstructive azoospermia from congenital absence of the vas deferens, have also been found to be strongly associated with mutations in the CFTR gene [10-12].

In a study comparing childhood vs. adulthood diagnosis of CF over a 41 year period by the Toronto CF Clinic in Canada [8], the number of patients receiving a diagnosis of CF in adolescence and adulthood has increased over time. Among all patients diagnosed with CF between 1960 and 1989, only 3% were made in adulthood, compared to a proportion of 18% from 1990 to 2001. Patients who were diagnosed with CF in adulthood tended to have more subtle clinical features, present with single-organ manifestations, and are more likely to be pancreatic sufficient (PS) (73%). Among patients who were diagnosed in adulthood between 1990 and 2001, 39%, 26% and 4% of them presented with pulmonary symptoms, male infertility and pancreatitis respectively. From a diagnostic perspective, only 65% of these patients had abnormal sweat test (i.e. sweat chloride levels >60

mmol/L). In effect, as the knowledge of the range of phenotypes associated with CFTR gene mutations has expanded, the demarcation line between individuals with and without CF disease has blurred. Mild CFTR mutations that result in residual CFTR function may be associated with disorders consistent with a CF phenotype but not necessarily supported by evaluation of CFTR function (e.g. borderline sweat test 40-60 mmol/L). Consequently, our ability to establish or exclude CF disease has become increasingly problematic.

Diagnostic criteria for CF

Based on the most recent United States Cystic Fibrosis Foundation (USCFF) Consensus Report [13], a diagnosis of CF can be made according to the following criteria.

One or more characteristic phenotypic features of disease*

PLUS

Sweat chloride ≥ 60 mmol/L

and/or

Identification of CF disease-causing mutations on both alleles

*or in the absence of symptoms, a diagnosis of CF in a sibling is sufficient as a phenotypic criteria

In the large majority of individuals, the diagnosis of CF is relatively straightforward. However, there is a small subset of individuals in whom the diagnosis of CF remains equivocal despite extensive evaluations.

CFTR mutation analysis as a diagnostic tool

There is a clear relationship between the number and functional severity of CFTR gene mutations with the range of CFTR-mediated ion channel abnormalities [10-15]. Generally, the greater the number of mutations identified (i.e. 0 vs. 1 vs. 2) and the more functionally severe the mutation or genotype, the more abnormal the CFTR-related ion channel function changes. Different mutations in the CFTR gene have varying effects on CFTR function. A five class system, devised to predict how mutations influence CFTR-mediated ion channel function [16,17]. Briefly, Class I mutations (e.g. G542X) represent those that fail to produce CFTR or produce a truncated protein, and includes stop codon, frame shift and some splice mutations. In Class II (e.g. F508del), the misfolded mutant protein undergoes intracellular degradation. In Class III (e.g. G551D), the protein reaches the plasma membrane, but there is structural or functional abnormality of the protein. In the case of Class IV mutations (e.g. R117H), the mutated protein reaches the apical membrane but channel conductance is reduced. Class V mutations cause reduced synthesis of normal CFTR by altering splicing efficiency (e.g. 3849+10Kb C>T), or possibly, from the effects of mutations in the promoter region of the CFTR gene.

In general, patients homozygous or compound heterozygous for mutations in Classes I, II, III, which confer absent or loss of CFTR function by different mechanisms, are more susceptible to severe clinical consequences. Most patients with the PI phenotype carry these mutations on both alleles. Class IV or Class V mutations confer some residual, but highly variable CFTR channel function. Patients carrying class IV or V mutations on at least one allele usually have the PS form of CF. While this classification system is useful as a conceptual framework, its limitations are acknowledged. For instance, Class IV and V mutations can have overlapping consequences and inferred properties of many mutations remain to be confirmed by functional studies. In addition and as mentioned, the heterogeneous CF disease spectrum is also influenced by environmental and other genetic modifying factors. More recently, a clinical phenotype based surrogate measure of CFTR mutation severity was developed and validated. This measure, described as the pancreatic insufficiency prevalence (PIP) score [18-21], is based on the premise that the status of the exocrine pancreas (pancreatic sufficient (PS) vs. pancreatic insufficient (PI)) provides the most reliable phenotypic "barometer" of the functional consequences of CFTR mutations. Most CF patients with the pancreatic insufficient phenotype carry functionally severe mutations on both alleles. Patients who carry at least one mild mutation, which confers some residual ion channel function on at least one allele, usually have pancreatic sufficiency [22-24].

There was great optimism that genotyping would supersede the traditional sweat test after the CFTR gene was identified. Instead, whilst the ability to identify mutations is becoming increasingly straightforward, the ability to accurately interpret genotype results is now recognized to be complex. The term "mutation" simply refers to a molecular alteration in the DNA sequence of a gene, without inference made regarding the effect of this alteration on gene expression or function of the protein product. Mutations can be neutral (non-disease-causing), deleterious (disease-causing or -modifying) or potentially even beneficial. Therefore, the diagnosis of CF cannot be made based upon identification of CFTR mutations on both alleles alone, especially when one or both are not designated as disease-causing mutations.

The clinical interpretation of CFTR mutations has been controversial. The United States CF Foundation (USCFF) consensus reports in 1999 and 2008 recommended the interpretation of mutations in clinical practice according to whether the identified mutation(s) were pathogenic or not [13,25]. For a mutation to be considered disease-causing, the mutation must cause either a change in amino acid sequence that severely affects CFTR synthesis and/or function, introduce a premature termination signal, or alter invariant nucleotides of intron splice sites. Until recently, the USCFF recommended a list of 23 mutations as proven CF-causing mutations [13]. A major issue was this small number of known CF-causing mutations when patients presenting with diagnostic dilemmas (e.g. borderline sweat chloride concentrations) typically carry mutations that are not included in the list of disease-causing mutations. For this primary reason, the Clinical and Functional Translation of CFTR (CFTR2) project was established [26]. By combining phenotypic evidence with functional analysis from a database of more than 39,000 individuals with CF from 24 countries in North America and Europe, the CFTR2 study expanded the list of disease-causing mutations from 23 to 140 mutations [26]. CFTR2 also described mutations of "varying clinical consequence", because they had been identified in subjects with and without CF.

In a large prospective study of 202 patients with single organ manifestations of CF, the diagnostic yield of CFTR genotyping was compared against other diagnostic tests in CF, namely sweat test and nasal potential difference (NPD) test [11]. The subjects from this study comprised of 68 individuals with idiopathic chronic sinopulmonary disease, 42 patients with idiopathic pancreatitis and 92 subjects with obstructive azoospermia from congenital absence of vas deferens. Ninety-one individuals were identified to carry 2 CFTR mutations on extensive genotyping. Genotyping was the least sensitive diagnostic test, compared to sweat test and NPD, even when extensive

genotyping was performed. Based upon the original 23 CF-causing mutations, only 7 of 202 (3.5%) were diagnosable as CF, all of whom were diagnosable by the traditional sweat test. Using CFTR2's expanded list of CF-causing mutations (a list of 122 mutations was defined as CF-causing mutations at the time of the study), 10 (4.9%) more subjects became diagnosable by genotyping, giving a total of 17 out of 202 (8.4%) patients. In contrast, sweat test and NPD diagnosed CF in 35 of 202 (17.3%) and 67 of 202 (33.2%) of patients. In addition, genotyping could not establish or exclude the diagnosis of CF in 74 of 91 (81.3%) with 2 CFTR mutations. Forty-seven of 91(51.7%) subjects with 2 CFTR mutations carried at least one mutation of varying clinical consequence: 44/91 (48.4%) carried a CF-causing mutation together with a mutation of varying clinical consequence, while 3 (3.3%) carried mutations of varying clinical consequence on both alleles. Of the 10 additional subjects with 2 CF-causing mutations designated by CFTR2, the diagnosis of CF could also be established by least another diagnostic test (sweat test and/or NPD). Sweat testing alone missed 3/10 patients while NPD testing alone missed 2 patients. In short, the diagnostic role of extensive genotyping remains limited in exactly the situations it was envisaged to resolve for the time being. In the same study, extensive genotyping was also performed in established PI and PS CF patients [11]. Compared to the list of 23 CF-causing mutations, the expanded number of CF-causing mutations based on CFTR2 increased the number of PI and PS patients fulfilling the diagnostic criteria for CF by genotype alone from 86% to 90.7% and 28.1% to 45.3% respectively.

It is worth noting that the majority of newly identified disease-causing mutations by CFTR2 have abnormal sweat chloride concentrations and the PI phenotype. Hence, most patients carrying these mutations could be identified by ion channel measurements alone (e.g. sweat test). It is also not surprising that a large subset of patients with single-organ manifestations of CF were found to carry mutations designated by CFTR2 as "varying clinical consequences". Identification of at least one mutation of varying clinical consequences in a symptomatic patient with a CF-like phenotype should prompt at least a sweat test (if not already done). Furthermore, most of the mutations reported in the CF Mutation Database [5] are missense mutations, and CFTR2 remains unable, to date, to assign either a disease-causing or a benign designation. Future studies are needed to determine functional and clinical consequences of rare CFTR mutations/variants, but these studies need to be designed to minimize ascertainment bias.

Despite the aforementioned limitations of genetic analysis as a diagnostic tool, genotyping is not indispensable. There are well recognized mutations (e.g. 3849+10kb C>T) that lead to severe lung disease but are associated with normal or borderline ion channel measurements. Identification of 2 mutations without necessarily being able to confirm the diagnosis of CF should encourage clinical follow-up of patients over time by CF physicians. Furthermore, the diagnostic consequences (benign vs. disease-causing) of any CFTR mutations of "unknown" consequences identified at present may not be apparent until later on, when new genetic information and updated reclassification of CFTR mutations becomes available.

In addition, there are patients reported in the literature with clinical CF but without identifiable CFTR mutations, despite gene sequencing [27,28]. Up to 5% of mutations may be missed despite gene sequencing, especially for CFTR regulatory mutations located in sites distant from the gene or embedded in the non-coding regions of the gene [29]. Furthermore, loss of function in the epithelial sodium channel (ENaC) has been linked with a CF-like phenotype [30] and also shown to correlate with the severity of CFTR dysfunction. In a study of men with congenital bilateral absence of the vas deferens (CBAVD), the NPD composite parameter of the total change in potential difference in response to consecutive perfusions with amiloride, chloride-free solution and isoproterenol (ΔAmil+Cl-free +Iso), demonstrated the sequential continuum of CFTR dysfunction according to the number of CFTR mutations better than other NPD parameters; ΔAmil+Cl-free+Iso values were significantly different between CBAVD men with 0 vs. 1 vs. 2 mutations [15]. It is also noteworthy that the various composite ion channel measurements, which are mainly based on nasal potential difference measurements and associated with "improved" diagnostic performance for CF, incorporate interactions between sodium and chloride channel function.

Lastly, there are individuals whose diagnosis of CF will remain equivocal despite comprehensive testing (including the use of various ion channel measurements and composite scores). Clinical monitoring and repeat ion channel measurements should be considered. Adjunctive tests for CF such as exocrine pancreatic function testing, lung function testing, search for CF-related pathogens and testing for obstructive azoospermia should be considered. Treatment for lung disease should be considered irrespective of the diagnostic label.

Conclusion

The diagnosis of CF may remain challenging. There is a role for CFTR mutation analysis in the diagnosis of CF but the diagnostic yield of genotyping currently remains somewhat limited compared to "functional studies" of ion channel measurements including the traditional sweat test. It is anticipated that the role and yield of genotyping, as a diagnostic test, will improve in the future as our understanding the functional consequences of greater number of mutations increase.

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