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PROFESSIONAL ISSUES

Molecular Testing for Cystic Fibrosis Carrier Status Practice Guidelines: Recommendations of the National Society of Genetic Counselors

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Purpose To provide practice recommendations for genetic counselors whose clients are considering cystic fibrosis (CF) carrier testing or seeking information regarding CF molecular test results. The goals of these recommendations are to: 1) Provide updated information about the natural history, diagnosis, and treatment of CF and related conditions. 2) Supplement genetic counselors' knowledge and understanding of the available carrier screening and diagnostic testing options. 3) Describe the current state of genotype/phenotype correlations for *CFTR* mutations and an approach to interpreting both novel and previously described variants. 4) Provide a framework for genetic counselors to assist clients' decision-making regarding CF carrier testing, prenatal diagnosis, and pregnancy management.

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Keywords Cystic fibrosis carrier screening · Carrier testing, genetic testing · Genetic counseling

Introduction

Since the gene for CF (*CFTR*) and several of its common disease-causing mutations were first identified in 1989, molecular analysis of *CFTR* has been the model for the integration of genetic testing into routine medical care. Data from pilot studies and public policy discourse have led to recommendations by professional organizations that CF screening should be offered to pregnant women and their partners, and to couples planning a pregnancy. Since 2005, when the National Society of Genetic Counselors last approved CF practice recommendations for its membership (Langfelder-Schwind et al. 2005), lessons learned from widespread molecular CF testing have influenced the diagnostic criteria and broadened the scope of CF to include a spectrum of related disorders. To serve as a reliable and educated referral base for health care providers and patients, genetic counselors must have a thorough understanding of the complexities of CF and the implications of CF genetic test results.

The Cystic Fibrosis Disease Spectrum

Cystic Fibrosis (CF) was first defined in 1938 by Dr. Dorothy Anderson in the *American Journal of Diseases of Children.* CF is frequently described as a chronic, life-shortening, autosomal recessive condition that affects approximately 30,000 children and adults in the United States. Mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene lead to decreased chloride transport across the apical membrane of secretory epithelial cells, elevated intracellular sodium, and decreased extracellular water. The result is thickened secretions in affected structures (airways, pancreatic and billiary ducts, intestines, and vas deferens). *CFTR* is expressed throughout the body, but *CFTR* abnormalities predominantly impact the respiratory system, pancreas, sweat glands, and male reproductive system.

Sweat chloride analysis (Di Sant' Agnese et al. 1953), has remained the gold standard for establishing or ruling out the diagnosis (Taylor et al. 2009); however CF diagnostic criteria encompass additional diagnostic methodologies including *CFTR* molecular testing and specialized transepithelial nasal potential difference studies (Rosenstein and Cutting 1998). Widespread implementation of molecular testing for individuals with compatible clinical symptoms and infants with hypertrypsinemia identified through newborn screening (NBS) programs has advanced our understanding of the natural history of cystic fibrosis. As a result, the consensus diagnostic criteria for CF have also evolved, and the CF disease spectrum has been significantly expanded (Farrell et al. 2008; Borowitz et al. 2009) (Table 1).

Cystic Fibrosis

A diagnosis of cystic fibrosis is given to patients who have a positive sweat chloride test (≥60 mEq/L Cl^-) (Table 2) and/or the presence of two disease-causing *CFTR* mutations (Table 1). Individuals with CF have the common constellation of CF symptoms (lung disease, pancreatic insufficiency (PI), and male infertility). Terms found in the literature, such as "non-classic" or "atypical" CF describe a subset of individuals who meet diagnostic criteria but are distinguished from their classically affected counterparts by features such as pancreatic sufficiency (PS), lower sweat chloride concentrations (<60 mEq/L/Cl-), and/or fewer readily apparent CF-related symptoms (Groman et al. 2005).

Table 1 Cystic fibrosis evaluation: possible diagnostic outcomes

Diagnosis	Cystic fibrosis		*CFTR*-related disorder	*CFTR*-related metabolic syndrome
CF symptom(s)	Yes	Yes	Yes	Hypertrypsinemia only
Sweat chloride	Positive	Int. / Normal	Int. / Normal	Int. / Normal
Number of disease-causing CFTR mutations	≤2	2	<2	<2
Recommendations	▪ Follow up in CF Center		▪ If symptomatic, refer to CF Center for comprehensive monitoring ▪ If asymptomatic with two mutations not established as disease-causing, continue to monitor for symptoms	▪ Repeat sweat tests at 2 months and 6 months with follow-up testing and evaluation at a CF Center every 6–12 months until a diagnosis is made or ruled out

(Adapted from Rosenstein and Cutting 1998; Farrell et al. 2008; Borowitz et al. 2009)

Table 2 Guide to sweat chloride result interpretation

Result	Age <6 months (mEq/L Cl^-)	Age >6 months (mEq/L Cl^-)
Positive	≥60	≥60
Intermediate	30–59	40–59
Normal (CF unlikely)	<30	<40

(De Boeck et al. 2006; Farrell et al. 2008; Borowitz et al. 2009)

Chronic pulmonary infections and progressive deterioration of lung function are the major causes of morbidity and mortality. Children and adolescents with CF typically have relatively normal or mildly decreased lung function; however, lung disease tends to progress, and most adults with CF have moderate to severe pulmonary obstruction (Cystic Fibrosis Foundation Patient Registry 2012).

Major gastrointestinal manifestations of CF include meconium ileus (MI) and pancreatic insufficiency. MI affects approximately 15 % of newborns with CF (Massie et al. 2012). Pancreatic insufficiency can cause failure to thrive in infants and contributes to malabsorption, vitamin deficiency, and poor weight gain, which may in turn lead to delays in development. Early detection of CF through newborn screening can mitigate these effects (Koscik et al. 2005).

Although individuals with non-classic presentations have been referred to as having "atypical" or "mild" CF, there are both pancreatic sufficient and insufficient forms of CF with severe pulmonary involvement. Approximately 10–15 % of people with CF demonstrate pancreatic sufficiency (Cystic Fibrosis Foundation 2012) and are at risk for pancreatitis, and/or may develop pancreatic insufficiency (Schibli et al. 2002). In adults, CF-related diabetes also contributes to morbidity (Moran et al. 2010).

CFTR-Related Disorder (CRD)

Individuals with a clinical feature suggestive of CF such as pancreatitis, chronic sinusitis, or absence of the vas deferens may be diagnosed with CFTR-related disorder (CRD) when they do not meet cystic fibrosis diagnostic criteria (see Bombieri et al. 2011). For example, individuals who fall into this category often have intermediate or normal sweat chloride values and fewer than two established disease-causing *CFTR* mutations (Table 1). People with CRD are at an increased risk of developing CF, and should be referred to a CF Center for comprehensive diagnostic testing and ongoing clinical monitoring.

CFTR-Related Metabolic Syndrome (CRMS)

CF screening is a mandated part of all NBS programs in the United States (since late 2009) and preliminary evidence suggests that early diagnosis may favorably change disease course by allowing for improved nutritional support (Farrell et al. 1997; Jones and Helm 2009) and decreasing pulmonary complications (Waters et al. 1999; Howenstine and Montegomery 2009). Asymptomatic infants with an inconclusive diagnostic workup following a positive CF NBS require monitoring and follow up, which leaves the physician with the dilemma of either labeling a symptom-free child with a medical condition or failing to apply an appropriate label in order to justify the additional monitoring and preventive care required. To address this issue, the U.S. Cystic Fibrosis Foundation proposed that individuals in this category be diagnosed with "*CFTR*-Related Metabolic Syndrome" (CRMS) (Borowitz et al. 2009). The diagnosis of CRMS can be made after repeat abnormal sweat chloride values at 2 and 6 months of age or extended genetic analysis that has revealed *CFTR* mutations but fewer than 2 that are known to be disease-causing. Follow-up testing and evaluation should continue at a CF Center every 6–12 months until CF is ruled out or the child is diagnosed with CF or *CFTR*-related disorder (Borowitz et al. 2009).

Cystic Fibrosis: Treatment and Care

The U.S. Cystic Fibrosis Foundation (CFF) accredits CF Care Centers of Excellence and establishes the standards of care for the diagnosis and management of CF in the United States. The CFF and its counterparts in Europe and Canada maintain epidemiologic databases for the collection and analysis of clinical data from patients along the disease spectrum.

CFF Guidelines state that, on average, individuals with CF should be seen in an accredited care center at least four times per year for well checkups (Cystic Fibrosis Foundation 1997). Newly diagnosed infants are seen more frequently during the first 12 months of life until care plans for nutritional and respiratory management have been optimized (Borowitz et al. 2009). The need for additional visits, hospitalizations, and/or home intravenous antibiotics varies widely, and increases with disease severity. Daily treatment regimens take between 30 min and several hours per day (Quittner et al. 2008). Improvements in treatment have led to significant increases in median survival age (presently estimated to be 36.8 years (95 % CI 34.7–40.3)) (Cystic Fibrosis Foundation Patient Registry 2012). Lung transplantation is a consideration when pulmonary function falls below 30 % of normal values (Kere et al. 1992), but not all patients choose this option, despite improving outcomes (Morton and Glanville 2009).

Small molecules and other therapeutic approaches to target specific *CFTR* mutations are in various stages of research and development. The compound Ivacaftor has been FDA approved as a treatment for CF patients carrying at least one copy of the G551D *CFTR* mutation and is under investigation as a treatment for patients with other *CFTR* mutations (see www.cff.org; www.clinicaltrials.gov). While the potential for

new therapies provides hope and optimism, a timeframe for the clinical availability of these therapies cannot be reliably predicted.

Quality of Life

Many individuals with CF can maintain a high quality of life in terms of professional and personal achievements, including higher education, hobbies, marriage, and family. CFTR mutations do not cause cognitive deficiencies. Pulmonary exacerbations have been found to be the most significant predictor of decreased perceived quality of life (Sawicki et al. 2010).

Most men with CF (98 %) are infertile due to congenital absence of the vas deferens (Smith 2010), but assisted reproduction techniques have made it possible for men with CF to father biologic children (Schlegel et al. 1995). Women with CF may experience reduced fertility due to poor nutritional status and/or increased viscosity of the cervical mucosa. Pregnant women with CF face an increased incidence of preterm labor and other pregnancy complications, but many are able to achieve pregnancy and deliver without major complications (Whittey 2010). A woman's pre-pregnancy pulmonary and nutritional status correlate with the likelihood of pregnancy having a detrimental impact on her disease course (Fiel 1996).

RECOMMENDATION 1:
COUNSELING ABOUT CF AND THE *CFTR*-RELATED DISEASE SPECTRUM

While most positive CF carrier screening results identify mutations associated with classic CF disease, genetic counselors offering CF carrier screening should ensure that they are providing the most current information to patients regarding the range of symptoms, potential treatment options, and quality of life issues associated with CF and the CFTR-related disease spectrum.

Carrier Screening for Cystic Fibrosis

Cystic fibrosis occurs throughout the world in people of every race and ethnicity (Bobadilla et al. 2002; Mehta et al. 2010), and pre-test carrier risk and test sensitivity vary by ethnicity. Genetic counseling and risk calculations based on ancestry are complicated by the multiethnic nature of the U.S. population and the broad use of terms such as "Hispanic" and "Asian" to describe individuals who may be of varied races or countries of origin (Palomaki et al. 2004).

CF carrier screening has been offered routinely to a subset of pregnant women in the absence of a family history since 2000 (ACOG and ACMG 2001). The American College of Obstetricians and Gynecologists (ACOG) has updated their screening recommendations to move from offering testing primarily to individuals of Caucasian and Ashkenazi Jewish ancestry to offering testing to all women regardless of ancestry (ACOG Committee Opinion No. 486 2011). See Table 3 for indications for CF molecular analyses that fall outside of the scope of routine carrier screening.

RECOMMENDATION 2:
TO WHOM SHOULD CARRIER TESTING FOR CF BE OFFERED?

Carrier testing for CF should be offered to all women of reproductive age, regardless of ancestry; preferably preconceptionally. CF carrier testing should also be offered to any individual with a family history of CF and to partners of mutation carriers and people with CF.

CF Carrier Screening Test Selection

Factors influencing the selection of a CF carrier test include: pregnancy status, fetal gestational age, family history, ethnicity, race, institutional policies, and practical concerns, such as insurance coverage and out-of-pocket expense. As with most carrier screening, an individual's risk to be a carrier of CF or to have a child affected with CF is not completely eliminated following a negative test result. Therefore, communicating effectively about pre-and post-test reproductive risks is a crucial component of CF carrier screening.

RECOMMENDATION 3:
PRE-TEST RISK ASSESSMENT

Pre-test risk assessment should include an estimate of CF carrier frequency based on the individual's family history, ethnic background, and the predicted residual risk to have a child with CF if the test is negative.

Approaches to CF Molecular Analysis

There is significant variability in the size, composition, and methodology of CF mutation panels (Eshaque and Dixon 2006). This is likely to evolve as an increasing number of *CFTR* mutations are characterized as disease-causing (Sosnay et al. 2011). Genetic counselors and other clinicians should have a full understanding of test limitations prior to determining which method is best suited for any given patient (Castellani et al. 2008; Grody et al. 2007). If the family history is positive for CF, ensuring that the familial mutation is included in the chosen panel is critical to effective post-test counseling.

Table 3 Other indications for CF mutation analysis

- Diagnostic testing of symptomatic individuals
- Fetal echogenic or dilated bowel or peritoneal calcifications identified on prenatal ultrasound
- Positive CF newborn screening result
- Family History of CF

1) Standard Carrier Screening: *The American College of Medical Genetics–CF Mutation Panel*

In 2001, the American College of Medical Genetics (ACMG) and ACOG jointly recommended and later reaffirmed a limited mutation panel for CF carrier screening currently comprised of 23 known disease-associated mutations (Grody et al. 2001; Watson et al. 2004; ACOG Committee Opinion No. 325, 2005). By employing a threshold for mutation inclusion of >0.1 % frequency in the general U.S. population, this panel was designed to identify the majority of CF carriers in the United States. Generally, in the setting of a negative family (Lebo and Grody 2007), a basic mutation panel is appropriate.

2) Expanded Mutation Panels

CF mutation analysis using a mutation panel expanded beyond the ACMG/ACOG panel may afford a higher mutation detection rate for individuals of minority ethnic backgrounds (Rohlfs et al. 2011), particularly those associated with population-specific mutations that have a frequency above 1 % (Dequeker et al. 2009). Some mutations included in expanded panels, however, may not be as well-characterized in terms of disease liability as those in the standard panel.

3) *CFTR* Sequencing

Full sequencing of *CFTR* is reported to detect ~98.7 % of both disease-causing mutations and variants of unclear clinical significance (Strom et al. 2003). While sequencing may identify rare disease-causing mutations, it may also identify novel or rare *CFTR* variants for which clinical significance has not been assessed, or variants associated with a broad phenotypic spectrum. Mérelle et al. (2006) estimated that a novel variant is identified in approximately 1 in 500 samples tested for carrier status using mutation identification assays, such as sequencing.

Approximately 1–2 % of *CFTR* mutations are the result of large intragenic deletions, duplications or rearrangements (Svensson et al. 2010), which are not detectable through sequence analysis. Deletion/duplication testing is generally not performed as part of routine sequence analysis, but is offered as supplemental testing and may be requested.

RECOMMENDATION 4:

CF CARRIER TEST SELECTION

Carrier testing panels should include the mutations recommended by ACOG and ACMG. For individuals of non-Northern European descent, pan-ethnic panels that include additional mutations more commonly identified in minority populations are appropriate to consider. Focus general population CF screening practices on identifying carriers of established disease-causing CFTR mutations.

Evolution of Test Panels and Their Interpretation: The Example of I148T/3199del6

Large-scale population carrier screening is providing new information regarding the frequency of alleles in healthy and patient populations. A key example is the I148T/3199del6 mutation. Initially, I148T was included on the ACOG/ACMG standard mutation panel (ACOG and ACMG 2001). However, it was later learned that I148T only behaved as a deleterious mutation when in *cis* with 3199del6. Individuals with a known deleterious mutation in *trans* to an I148T mutation in the absence of a *cis* 3199del6 were asymptomatic (Monaghan et al. 2004). As a result, I148T was removed from the ACMG standard mutation panel (Rohlfs et al. 2002; Buller et al. 2004).

RECOMMENDATION 5:

CHANGES IN TESTING PANELS AND INTERPRETATION

The inclusion and exclusion of mutations on available CFTR mutation screening panels remains a dynamic process as new information is learned about the pathogenicity of CFTR mutations. When individuals present for genetic counseling with prior carrier screening results, those results should be reviewed and re-interpreted, if necessary, in light of current knowledge.

Residual Risk

Most CF carrier screening results will be negative for the first partner screened, and no further screening of the couple is performed. If one partner has had a negative screening result and the second is untested, Bayesian analysis should be used to calculate the couple's residual risk to have a child with CF. Such analysis should consider the racial and ethnic background of both individuals and the detection rate of the mutation panel.

In cases where one member of a couple has been identified as a CF carrier and the second has had negative carrier test results, the counseling issues may become more challenging. Framing the residual risk in alternative formats may be helpful (Uhlmann et al. 2009). Some patients may have negative emotional, cognitive and/or behavioral responses to information they find to be ambiguous (O'Neill et al. 2006), and it can be helpful to explore feelings and perceptions about what it would be like to have a child with CF. It is also important to explore whether there are moral, religious, or cultural factors playing a role in the response (Weil 2000).

Informing patients about the role CF newborn screening in identifying CF patients with rare mutations and preparing patients for the potential to have a (likely false) positive CF newborn screen if their newborn carries one

mutation may help to alleviate post-partum concerns. (See Tluczek et al. 2011 for discussion of a tailored model of genetic counseling specific to counseling for parents of infants with a positive CF newborn screening result).

RECOMMENDATION 6:

COMMUNICATING NEGATIVE and +/− CARRIER SCREENING RESULTS

Clients who have had a negative CF carrier screening test result should be informed of their reduced or residual risk to have a child with cystic fibrosis, and the possibility of their child having an abnormal CF newborn screen if one partner is a CF carrier.

Prenatal Diagnosis

Couples in which both partners are carriers of disease-causing CF mutations have a 1 in 4 risk of having a child with CF. Prenatal diagnosis by amniocentesis or chorionic villus sampling (CVS) can be performed to evaluate the mutation status of a fetus. Typically, laboratories performing prenatal diagnosis for CF will require a sample of both parents' blood as a positive control. Alternatively, pre-implantation genetic diagnosis and in vitro fertilization may be preferable to some couples in order to minimize the risk of having a child with CF while circumventing decisions about pregnancy termination. Some couples opt to forego pre-implantation genetic diagnosis or invasive prenatal diagnostic testing.

Couples who are at increased risk for or are expecting a child with CF (3.6 % of newly diagnosed infants in 2010, Cystic Fibrosis Foundation Patient Registry 2011) may benefit from a referral to a local accredited CF caregiver team, including a genetic counselor specializing in CF, in order to gain a balanced and realistic perspective of CF as they work through decisions regarding the management of a current or future pregnancy. Some couples may also wish to speak with a parent of a child with CF. It is important to note that finding a genotypic "match" is unnecessary, and may even be misleading due to disease variability. There are a number of reliable online resources to which patients and healthcare providers can be directed (Table 4).

Genetic counselors should further support couples at risk to have a child with CF through anticipatory guidance regarding a pre- and perinatal plan. The prenatal plan would include serial ultrasounds and preparing for the possibility of neonatal surgery for meconium ileus (see Ogino et al. 2004; Carlyle et al. 2012 for additional information). Planning for neonatal sweat testing, newborn screening, and/or CF mutation analysis will help to ensure that the newborn's CF status is clarified as quickly as possible and appropriate treatment is initiated for affected infants.

Table 4 Online information for patients and providers

Genetics Home Reference	ghr.nlm.nih.gov/condition/cystic-fibrosis
Cystic-L – Cystic Fibrosis Information and Support	www.cystic-l.org
CysticFibrosis.com	www.cysticfibrosis.com
CFvoice (by Novartis)	www.cfvoice.com
Cystic Fibrosis Foundation	www.cff.org
Canadian Cystic Fibrosis Foundation	www.cysticfibrosis.ca
European Cystic Fibrosis Society	www.ecfs.eu
Cystic Fibrosis Trust	www.cftrust.org.uk
CFTR2	www.cftr2.org
For providers only	
GeneTests/GeneReviews	www.genetests.org
Cystic Fibrosis Mutation Database	www.genet.sickkids.on.ca

RECOMMENDATION 7:

COUNSELING COUPLES AT RISK TO HAVE A CHILD WITH CF

When both parents are known carriers for CF, available prenatal and pre-implantation diagnostic testing should be offered. Prenatal facilitation of a monitoring plan should begin for couples at risk or who continue a pregnancy known to have CF, and postnatal evaluation through sweat testing and state NBS programs, should be discussed.

CFTR Mutations: Naming Conventions

Guidelines have been established to standardize the way in which sequence variants at the DNA and protein level are named. (To learn more, see http://www.HGVS.org/mutnomen/recs.html.) To date, the CF literature has primarily referred to mutations and polymorphisms by their original ("legacy") names, which were established prior to standardization. As standard nomenclature is increasingly utilized, genetic counselors will need to familiarize themselves with both sets of nomenclature in order to review literature and patient reports and access mutation information. (See www.genet.sickkids.on.ca/app and www.cff.org/cftr2 for more information.)

CFTR: Complex Alleles

A complex allele is one in which two or more variants exist on the same allele (in *cis*), with each variant contributing differentially to the clinical presentation. Many complex alleles have been reported in *CFTR*. (For more about common complex alleles, see Claustres et al. 2004; Groman et al. 2005; Massie et al. 2001.)

R117H/PolyT

The disease-causing potential of the R117H mutation is influenced by the *cis* status of a haplotype that includes a polymorphic tract of thymidines (5T, 7T and 9T) and a variable length TG repeat tract (see below) in intron 8 of *CFTR* near the splice site for exon 9 (Kiesewetter et al. 1993). Errors in intron 8/exon 9 splicing lead to skipping of exon 9, resulting in a CFTR protein that lacks chloride channel function. The 5T variant is associated with increased intron 8 splicing errors and a significant reduction in the amount of functional CFTR protein, while the 7T variant is associated with more modest reductions in functional CFTR protein as compared to 5T. (Kiesewetter et al. 1993). Individuals with a disease-causing *CFTR* mutation in *trans* to R117H/5T typically have PS cystic fibrosis, whereas individuals with a *CFTR* mutation in *trans* to R117H/7T are more likely to be asymptomatic (possibly CRMS if an elevated IRT was detected on the CF NBS) or have a phenotype consistent with a *CFTR*-related disorder (Massie et al. 2001; Kiesewetter et al. 1993). R117H/9T is highly unlikely to act as a disease-causing mutation (CFTR2, 2013).

Infants with a positive CF NBS diagnosed with CRMS are commonly found to have a genotype consisting of one disease causing mutation and either R117H-7T or 5T *in trans* to that mutation (Borowitz et al. 2009). Current laboratory guidelines state that intron 8 polyT status should be assessed as a reflex test when R117H is detected, since clarifying the polyT status in individuals with R117H can help delineate the range of potential phenotype (Grody et al. 2001; Watson et al. 2004).

Intron 8 5T/TG Tract

In Caucasians, the 5T variant has a carrier frequency of ≥10 % (Kiesewetter et al. 1993). Because the 5T variant is associated with a significant reduction in functional CFTR protein, it has the potential to be clinically significant not only in *cis* with another variant (like R117H) but also as a stand-alone mutation. However, most information about symptomatic individuals with the 5T allele is based on case reports rather than population-based studies, and must therefore be interpreted with caution.

In addition, there is another variable length tract within intron 8, a thymidine/guanine (TG) tract, which includes three common variants: TG11, TG12 and TG13. The number of TG repeats further modifies splicing efficiency and thus pathogenicity of the 5T variant (Cuppens et al. 1998). The longer the TG tract, the less efficiency there is in exon 9 splicing. In a cohort of men with a 5T variant in *trans* to a ΔF508 or other common disease-causing *CFTR* mutation, the 5T/TG12 and the 5T/TG13 alleles were more commonly seen in men with isolated CBAVD or PS CF, whereas the 5T/11TG allele was most often commonly seen in fertile fathers of patients *with CF* (Groman et al. 2004). In newborns with a positive CF NBS result and a ΔF508 mutation, the presence of 5T/TG12 or 5T/TG13 variant *in trans* is associated with higher sweat chloride levels than 5T/TG11 *in trans* (Keiles et al. 2011). However, due to the phenotypic variability associated with the poly T/TG tracts, evaluation of the poly T and the TG tract are not recommended as part of population-based carrier testing protocols (Strom et al. 2003; Watson et al. 2004; Groman et al. 2004).

RECOMMENDATION 8:

THE R117H/POLY T and 5T/TG TRACT ALLELES

If a client is found to carry an R117H mutation, it is important to ensure the testing laboratory performs reflex testing for poly T status along with studies to determine the cis/trans orientation of the poly T alleles. In the absence of an R117H mutation, assessment of the intron 8 poly T or TG tracts is not recommended for routine CF carrier testing.

CFTR Genotype and Phenotype

Establishing Phase

Over 1,800 mutations have been identified in *CTFR* (www.sickkids.on.ca/cftr), but only a small number of mutations, including ΔF508 (p.Phe508del*), are responsible for the majority of known CF diagnoses. There is substantial variability in phenotype even among patients with the same genotype (McKone et al. 2003; Drumm et al. 2005). The fact that two *CFTR* alterations have been identified in apparently healthy individuals following carrier testing (Rohlfs et al. 2001) highlights the importance of determining whether two variants are in *cis* with one another (within the same allele) or in *trans* to one another (on opposite alleles).

RECOMMENDATION 9:

INDIVIDUALS WITH ≥2 MUTATIONS IDENTIFIED BY CARRIER SCREENING

Identification of two or more mutations in a patient referred for routine carrier screening should lead to a referral for clinical diagnostic evaluation. If the mutations identified are uncommon CFTR sequence variants, the likelihood of pathogenicity may be refined through determination of phase (cis/trans orientation).

Genotype/Phenotype

When considering genotype/phenotype correlations, the potential clinical impact of homozygosity for established CF disease-causing mutations, such as ΔF508, or nonsense mutations, can be distinguished from genotypes that include

Table 5 Classes of CFTR mutations

Class	Effect	Example(s)
I	Nonsense or frameshift mutations that prevent the transcription and translation of the full *CFTR* protein.	G542X, W1282X
II	Cause structural alterations to the *CFTR* protein and prevent it from moving to the cell surface.	ΔF508
III	CFTR reaches the cell surface but results in little or no functional *CFTR* channel activity.	G551D
IV	CFTR reaches the cell surface but chloride secretion is reduced.	R334W, R347P
V	Lead to a quantitative defect in the amount of *CFTR* protein that reaches the cell surface due to decreased stability of mRNA.	3849+10 kb C-T
VI	Cause rapid turnover of the *CFTR* proteins that reach the cell surface which decreases the overall amount of functional CFTR.	4279insA

CFTR mutations with unknown or unclear clinical significance. Therefore, when interpreting a result, it is useful to determine if functional analysis and confirmation of disease association for each mutation has occurred (Claustres et al. 2004). As this information is not available for most reported mutations (www.CFTR2.org), knowing the class of the mutation(s) an individual has may provide information about the nature of the molecular defect, the impact on chloride channel function, and presence of the CFTR protein at the cell surface (Table 5). Counselors should keep in mind that these classifications have been designed primarily for research purposes and were not meant to be used as clinical predictive tools (Castellani et al. 2008).

Significant phenotypic differences have been reported among cohorts of CF patients when they were grouped according to functional class (McKone et al. 2003). Typically, Classes I, II and III mutations are associated with a classic CF phenotype, including pulmonary disease, elevated sweat chloride, pancreatic insufficiency, and male infertility. In contrast, classes IV and V mutations tend to be associated with a pancreatic sufficient phenotype and a later onset of symptoms (Mickle and Cutting 1998; Zielenski et al. 2000) or *CFTR*-related disorder.

Research to characterize *CFTR* mutations based on the nature of the molecular defect and the impact on chloride channel function is ongoing (Sosnay et al. 2011). While functional analysis provides general information about the chloride conductance and presence of the protein at the cell surface, most *CFTR* mutations are too rare to safely extrapolate from published functional or phenotypic data, or have not yet been investigated (Castellani et al. 2008). With that said, some broad correlations are possible, and are summarized in Table 6.

Additional Influences on CF Phenotype: Environmental and Genetic Modifiers

In addition to the nature of the *CFTR* mutation, modifying features within *CFTR* or other genes and environmental factors also influence the clinical manifestation of CF. In

Table 6 Generalized genotype/phenotype correlations in cystic fibrosis

System	Genotype/phenotype correlation	Comments
Reproductive	Infertility is present in ≥98 % of males with 2 *CFTR* mutations in *trans.*	Male fertility has been reported in association with compound heterozygosity for 3849+ 10KbC-T or IV8-5T-TG11 and a second mutation in *trans.*
Gastrointestinal	There is a strong correlation between pancreatic insufficiency and having two mutations (in *trans*) that both lead to a failure of the *CFTR* protein to reach the cell surface and/or block chloride conductance.	If one of the two mutations leads to a CFTR protein with residual chloride conductance, the patient is usually pancreatic sufficient.
	Meconium ileus is predominantly associated with pancreatic insufficient mutations.	Non-genotypic factors also contribute to development of MI.
Endocrine	Pancreatic insufficient mutations confer the highest risk for CF-related diabetes.	CF-related diabetes occurs in both pancreatic insufficient and pancreatic sufficient patients.
Respiratory	Chronic, progressive lung disease is the predominant clinical feature of CF. CF-associated lung disease is highly variable, even in patients with the same genotype, and is influenced by both environmental factors and modifier genes.	Individuals with pancreatic insufficient mutations may have shorter survival and more rapid progression of lung disease than patients with pancreatic sufficient mutations.

(Based on Castellani et al. 2008; Moskowitz et al. 2008; Vanscoy et al. 2007; McKone et al. 2006; Cleveland et al. 2009; Mussaffi et al. 2006; Thauvin-Robinet et al. 2009; Groman et al. 2004; Sebro et al. 2012)

fact, environmental exposures and epigenetic modifiers may have more influence on an individual's phenotype than the specific combination of *CFTR* mutations (Moskowitz et al. 2008), and unlike the patient's genotype, some environmental exposures (early treatment, medical compliance, and the avoidance of smoking and second-hand smoke) can be controlled. Counseling families about the positive effects of an optimal environment for individuals with CF can prove empowering to parents and other caregivers (For a review, see Collaco and Cutting 2008). Prophylaxis against early RSV infection may also impact the progression of pulmonary disease.

RECOMMENDATION 10:
CF GENOTYPE/PHENOTYPE CORRELATIONS

While some broad correlations can be made between genotype and anticipated phenotype, genetic counselors should not counsel regarding severity of disease course based on published case reports or individual patient experience.

Conclusion

Cystic fibrosis was once a textbook example of simple Mendelian inheritance in human genetics. However, the relationship between *CFTR* genotype and CF phenotype has since proven to be complex and variable. CF, with its accompanying spectrum of *CFTR*-related disorders, is now a paradigm for the intricacies of molecular analysis interpretation, multifactorial influences on disease, and in some cases, diagnostic dilemmas. Genetic counselors are trained to employ client-centered strategies and are experienced in communicating with clients about uncertainty. Genetic counseling provides the opportunity for clients to receive accurate and current information as well as support for informed decision-making. As the number and types of disorders available for carrier screening continues to increase, lessons learned from over a decade of CF population carrier screening will continue to inform future policies.

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