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REVIEW

Cystic Fibrosis

A Review of Associated Phenotypes, Use of Molecular Diagnostic Approaches, Genetic Characteristics, Progress, and Dilemmas

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Cystic fibrosis (CF) is an autosomal recessive disease with significant associated morbidity and mortality. It is now appreciated that the broad phenotypic CF spectrum is not explained by obvious genotype-phenotype correlations, suggesting that CF transmembrane conductance regulator (*CFTR*)—related disease may occur because of multiple additive effects. These contributing effects include complex *CFTR* alleles, modifier genes, mutations in alternative genes that produce CF-like phenotypes, epigenetic factors, and environmental influences. Most patients in the United States are now diagnosed through newborn screening and use of molecular testing methods. We review the molecular testing approaches and laboratory guidelines for carrier screening, prenatal testing, newborn screening, and clinical diagnostic testing, as well as recent developments in CF treatment, and reasons for the lack of a molecular diagnosis in some patients. *(J Mol Diagn 2016, 18: 3—14; http://dx.doi.org/10.1016/j.jmoldx.2015.06.010)*

Although some genetic conditions already highlight the potential of precision medicine, much is yet to be learned. In this review, we discuss the current understanding and complexity of cystic fibrosis (CF) genetics. CF is a relatively common, autosomal recessive, and frequently lethal condition caused by mutations in the CF transmembrane conductance regulator gene (*CFTR*). *CFTR* consists of 27 exons, spanning approximately 250 kb on 7q31.2.[1] *CFTR* is a member of the ATP-binding cassette transporter family and encodes an anion transporter protein in the epithelium with five domains. Two membrane-spanning domains form a chloride channel pore that plays a role in chlorine and bicarbonate transport and have secondary effects on sodium transport. CFTR protein dysfunction leads to increased salt concentration in sweat and thickened secretions in various organ systems. Numerous genetic mutations have been identified; their characterization and contribution to disease pathogenesis are discussed below. The clinical presentation ranges from multiorgan symptoms, such as chronic respiratory tract infections, failure to thrive, and pancreatic insufficiency starting in infancy, to single-organ manifestations, such as male infertility or chronic sinusitis in adulthood.[1] The broad phenotypic spectrum is not fully explained by genotype-phenotype

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correlations. *CFTR*-related disease may arise because of multiple combining effects, such as complex alleles, modifier genes, mutations in genes that can mimic CF phenotypes, and additional effects, such as those influenced by epigenetic and environmental factors. Birth prevalence of CF approximates 1:2300 for non-Hispanic whites, 1:13,500 for Hispanic whites, 1:2270 for Ashkenazi Jews, 1:15,100 for African Americans, and 1:35,100 for Asian Americans.[2]

CF Phenotype

The consequences of CFTR dysfunction often commence before birth. Effects of CFTR dysfunction include incomplete embryologic formation of the Wolffian structures, causing congenital bilateral absence of the vas deferens (CBAVD), which causes infertility in virtually all males with CF. Females do not have structural abnormalities, but may face fertility issues as a result of thickened cervical secretions. Fetal ultrasonographic findings of hyperechogenic bowel with or without meconium peritonitis, bowel dilation, or an undetectable gallbladder are concerning for CF. Meconium ileus occurs in up to 20% of CF-affected newborns and is strongly correlated with CF (90% of such cases occur in CF patients).[1] The analogous condition in children and adults with thickened intestinal secretions is distal intestinal obstructive syndrome (10% to 47% of patients). Pancreatic insufficiency is a manifestation in 85% of patients, and fat malabsorption can be measured in 90% of affected infants by 1 year of age. Pancreatic dysfunction contributes to generalized malnutrition, failure to thrive, and suboptimal bone mineral content. CF-related diabetes (25% by the age of 20 years; 50% in adulthood) and pancreatitis are other manifestations.[3]

As the infant grows, additional symptoms present. Some, such as the CF hallmark of failure to thrive, are non-specific. High temperatures risk electrolyte abnormalities because of excess losses in sweat. Respiratory tract symptoms are highly variable and can look non-specific but are the most recognized complication. Most patients develop sinus opacification, and up to 30% will have nasal polyps. Impaired pulmonary function is an early finding in some.[4] Bronchiectasis, mucus plugging, and air trapping have been documented by 6 to 12 months. The CF respiratory phenotype progresses because of static mucus and chronic bacterial colonization, infection, and inflammation, with progressively deteriorating lung function. Over the years, significant improvements have been made in diagnosis, delivery of care, and treatment modalities, such that the median life expectancy is now 36.8 years.[5] With the increasing life span, however, hepatobiliary dysfunction is becoming increasingly prevalent.[3]

Diagnostic Criteria

The diagnosis of CF is on the basis of characteristic symptoms in addition to evidence of CFTR dysfunction (Table 1).[6]

Table 1 Diagnostic Criteria for CF

Diagnostic Criteria for CF
Criteria are met in the presence of (at least one):
Organ system symptoms consistent with CF, such as the following
Chronic sinopulmonary disease
Characteristic gastrointestinal and nutritional abnormalities
Salt loss syndromes
Obstructive azoospermia
Sibling with CF
Positive newborn screening result
Criteria are met in combination with (at least one)
CFTR dysfunction indicated by elevated sweat chloride levels (≥60 mmol/L, performed in accord with practice guidelines and adjusted for age) on two tests
Nasal potential difference consistent with CF
Presence of two pathogenic *CFTR* mutations on different alleles

CF, cystic fibrosis; CFTR, CF transmembrane conductance regulator.

Historically, it was on the basis of presenting clinical symptoms with sweat test verification. Over time, however, increasingly the diagnosis is solidified by molecular testing that identifies both symptomatic and presymptomatic patients. In this transition toward more frequent identification through screening and molecular analysis, several observations have emerged.

First, within the CF spectrum, a variety of symptoms and sweat chloride levels can be seen. Symptoms range from single-system (eg, CBAVD) to multiple-system involvement. As evidenced in approximately 2% of patients who meet diagnostic criteria, even in individuals with clinical CF, sweat chloride values can be normal (≤29 mmol/L) or indeterminate (30 to 59 mmol/L).[7] Such values only become a diagnostic conundrum when patients who are clinically suspected to have CF do not meet diagnostic criteria. These cases have long puzzled clinicians and have been variably designated as atypical, non-classic, non-traditional, or mild variant CF.[8]

Second, CFTR dysfunction encompasses the spectrum of CF, *CFTR*-related diseases, and CFTR-related metabolic syndrome. Individuals with *CFTR*-related disease (including chronic rhinosinusitis, idiopathic bronchiectasis, allergic bronchopulmonary aspergillosis, and chronic idiopathic pancreatitis) and CFTR-related metabolic syndrome have come to medical attention for clinical signs or screening results but have indeterminate sweat chloride or nasal potential difference values and do not meet diagnostic criteria. CFTR-related metabolic syndrome is a designation given with an initial positive CF newborn screen (CFNBS) but no symptoms on follow-up and either normal sweat chloride results and two *CFTR* mutations, with at least one being a variant of uncertain clinical relevance,[7] or an intermediate sweat result and one or zero *CFTR* mutations.[7,8] On longitudinal assessment, most of these children will not develop symptoms. In the past, children with CFTR-related metabolic syndrome who developed CF symptoms might have fallen into the atypical or mild variant CF

Table 2 CFTR Mutation Frequency in CF Patients by Ethnic Group and in a Panethnic US Population

Legacy/cDNA name/HGVS protein	Non-Hispanic white	Hispanic white	African American	Asian American	Ashkenazi Jewish	Panethnic
DelF508/c.1521_1523delCTT/p.Phe508del	72.42	54.38	44.07	38.95	31.41	66.31
G542X/c.1624G>T/p.Gly542*	2.28	5.10	1.45	0.00	7.55	2.64
G551D/c.1652G>A/p.Gly551Asp	2.25	0.56	1.21	3.15	0.22	1.93
621+1G>T/c.489+1G>T/-	1.57	0.26	1.11	0.00	0.00	1.30
W1282X/c.3846G>A/p.Trp1282*	1.50	0.63	0.24	0.00	45.92	2.20
N1303K/c.3909C>G/p.Asn1303Lys	1.27	1.66	0.35	0.76	2.78	1.27
DelI507/c.1519_1521delATC/p.Ile507del	0.88	0.68	1.87	0.00	0.22	0.90
R553X/c.1657C>T/p.Arg553*	0.87	2.81	2.32	0.76	0.00	1.21
R117H/c.350G>A/p.Arg117His	0.70	0.11	0.06	0.00	0.00	0.54
3849+10kbC>T/c.3718-2477C>T/-	0.58	1.57	0.17	5.31	4.77	0.85
1717-1G>A/c.1585-1G>A/-	0.48	0.27	0.37	0.00	0.67	0.44
2789+5G>A/c.2657+5G>A/-	0.48	0.16	0.00	0.00	0.10	0.38
R347P/c.1040G>C/p.Arg347Pro	0.45	0.16	0.06	0.00	0.00	0.36
711+1G>T/c.579+1G>T/-	0.43	0.23	0.00	0.00	0.10	0.35
R560T/c.1679G>C/p.Arg560Thr	0.38	0.00	0.17	0.00	0.00	0.30
3659delC/c.3528delC/p.Lys1177Serfs	0.34	0.13	0.06	0.00	0.00	0.28
A455E/c.1364C>A/p.Ala455Glu	0.34	0.05	0.00	0.00	0.00	0.26
G85E/c.254G>A/p.Gly85Glu	0.29	0.23	0.12	0.00	0.00	0.26
R1162X/c.3484C>T/p.Arg1162*	0.23	0.58	0.66	0.00	0.00	0.30
2184delA/c.2052delA/p.Lys684Asnfs	0.17	0.16	0.05	0.00	0.10	0.15
1898+1G>A/c.1766+1G>A/-	0.16	0.05	0.06	0.00	0.10	0.13
R334W/c.1000C>T/p.Arg334Trp	0.14	1.78	0.49	0.00	0.00	0.37
3120+1G>A/c.2988+1G>A/-	0.08	0.16	9.57	0.00	0.10	0.86
Total	88.40	71.90	64.51	48.93	94.14	84.00

Adapted and modified from Watson et al,[11] with permission from Nature Publishing Group (*Genetics in Medicine*, copyright 2004).
CF, cystic fibrosis; CFTR, CF transmembrane conductance regulator; HGVS, Human Gene Variation Society; -, not applicable.

diagnostic classes. Such categories remain relatively vague because they lack strict criteria and, therefore, these terms are used inconsistently or interchangeably. Nevertheless, regular evaluation remains important.[9]

Last, although *CFTR* mutations are identified in 95% of CF patients, genotype-phenotype correlations are as yet limited. CF and *CFTR*-related disorders exhibit varied clinical manifestations for the same *CFTR* genotypes, even within families.

CF Carrier Screening

CF carrier frequency is highest in non-Hispanic whites and Ashkenazi Jews (1:29 for each), followed by Hispanic Americans (1:46), African Americans (1:65), and Asian Americans (1:90).[10] As expected, most parents who have a child with CF have no family history. The American College of Medical Genetics and Genomics (ACMG),[11] American College of Obstetricians and Gynecologists,[12] and Human Genetics Society of Australia[13] recommend molecular carrier screening for CF be offered to inform prospective parents of their risk of having a child with CF (*http://www.hgsa.org.au/about/hgsa-committees/genetic-services-committee*, last accessed June 22, 2015).[14] A European consensus statement has also been published.[15]

Carrier screening[16] can be pursued by screening the female, or by sequential or direct couples-based testing. The approach to pursue is influenced by personal preference, time frame, privacy, and economic considerations. If testing is not obtained before conception, parental carrier screening can be performed during pregnancy, with reflex testing to prenatal diagnostic testing of fetal cells by means of chorionic villus sampling or amniocentesis, if indicated.

The mutation panel recommended by the ACMG in 2001 included 25 *CFTR* mutations on the basis of an allele frequency of $\geq$0.1% in CF patients in the United States. The updated 2004 guidelines recommended that 23 mutations continue to be included[11] (Table 2). The 1078delT (c.948delT; p.Phe316fs) mutation was removed because it was found to occur in only 0.03% of CF patients, and thus it did not meet the allele frequency threshold. I148T (c.443T>C; p.Ile148Thr) was removed because it was present at >100-fold frequency in the general population compared with CF patients, and is itself not associated with CF.[11,17] I148T is in linkage disequilibrium with 3199del6 (c.3067_3072delATAGTG), which is a rare mutation seen in <1% of I148T carriers. It does not meet criteria for panel inclusion. The 5T variant upstream of legacy exon 9 is a modifier of the R117H (c.350G>A; p.Arg117His) mutation and should only be offered as a reflex test, given that CF carrier screening aims to identify the risk of classic CF.[11]

A normal carrier screening result in someone with a family history of CF renders a higher residual risk than

Table 3 Risk of Having a Child with CF in an Asymptomatic Mother with a Negative ACMG Carrier Screening Panel Result, Listed by Ethnic Group

	Non-Hispanic white*	Hispanic white	African American	Asian American	Ashkenazi Jewish
Birth prevalence	1:2300	1:13,500	1:15,100	1:35,100	1:2270
Carrier risk (pre test)	1:29	1:46	1:65	1:90	1:29
Carrier detection rate (%)	72—90	57	69	25	97
Carrier risk (post test/neg test)	1:100—1:280	1:105	1:207	1:120	1:934
Fetal risk (neg mother, untested partner)	1:11,600—1:32,480	1:19,320	1:53,820	1:43,200	1:108,344
Fetal risk (neg test, both partners)	1:40,000—1:313,600	1:44,100	1:171,396	1:57,600	1:3,489,424

Adapted and modified from Lebo and Grody,[10] with permission from the publisher, Mary Ann Liebert, Inc.
*Approximate values available for US whites (origin unspecified) and European whites.
ACMG, American College of Medical Genetics and Genomics; CF, cystic fibrosis; neg, negative.

would be the case for an individual without it, unless the mutations in the relative were included in the screen.[10,18] Bayesian risk calculations can be applied to convey the most accurate information.[10,19] The risk of having an affected child after testing negative with the 23-mutation panel with a negative family history and with a negative test result in the partner is approximately 1 in 44,100 for Hispanic whites, 1 in 57,600 for Asian Americans (estimated, with further studies required), 1 in 171,396 for African Americans, 1 in 3,489,424 for Ashkenazi Jews, and 1 in 78,400 in US whites(when origin is not further specified)[10] (Table 3).

The limited mutation detection rate (clinical sensitivity) in certain ethnic groups[2] and the high US frequency of mixed ethnicities has led to development of expanded mutation panels and other mutation detection approaches. The issues with such panels include potentially conveying a false sense of security with normal results, and a false sense of danger with identified variants of uncertain clinical significance. This is compounded by uncertain allele frequencies, admixture of one or more ethnic backgrounds, and arbitrary selection of rare variants that often do not have genotype-phenotype correlation data. With an increasing number of mutations thus selected, minimal improvement in sensitivity is achieved.[20] In addition, the mutation spectrum is still not well characterized for several ethnic groups. Until the mutation spectrum is better characterized, clinicians can consider CF in these ethnic groups as a genetic disorder that is not sufficiently screened by existing common frequency mutation panels. Examination of sensitivity and specificity combined with family history and ethnicity are key to assessing which test(s) to pursue and to providing targeted, informative, and cost-effective carrier screening care on a population scale. For the individual with a family history of CF, general carrier screening is not the appropriate workup because sequencing and/or deletion or duplication analysis may be appropriate next steps. When the affected individual in a family does not have a known molecular diagnosis and when the individual with a family history of CF has a partner who is a carrier, sequencing and/or deletion or duplication analysis may be

Table 4 Examples of Common Mutation Molecular Testing Methods for CF

Method	Advantages and limitations
Allele-specific PCR	Testing for known single-nucleotide variants. Initial setup/design must be performed carefully because mutation detection is on the basis of absence of PCR products.
Array-based testing	Rapid, scalable testing for known single-nucleotide variants.
Invader chemistry	Invader and primary nucleotide probe hybridize to target region, and fluorescent signal is generated in second reaction. Suitable for known single-nucleotide variants and small insertions and deletions.
Oligonucleotide ligation assay	Testing for known single-nucleotide variants. On the basis of differentially modified oligonucleotides for wild-type and variant alleles.
Mass spectrometry	Testing for unknown variants on the basis of mass size differences. Relatively expensive setup.
Sequencing	Gold standard for detection of unknown variants. Sequence variants are verified by sequencing of the opposite strand.
Multiplex ligation-dependent probe amplification	Forward and reverse probes must ligate for subsequent amplification. Used for identification of deletions and duplications of one or multiple exons, missed by most other approaches, including sequencing.
Next-generation sequencing	High output and economical on a per-base basis. Shorter reads and complex interpretation. Relatively expensive setup.

CF, cystic fibrosis.

wanted and referral to a genetics professional for assessment and genetic counseling is warranted.

Prenatal Genetic Diagnosis and Screening

When familial mutations are known, preimplantation genetic diagnosis or screening is an option. Preimplantation genetic diagnosis for CF allows couples to avoid decisions regarding continuation of an affected pregnancy. However, insurance coverage for *in vitro* fertilization and preimplantation genetic diagnosis varies, and there are often significant associated personal costs. After conception, prenatal CF testing can be performed on chorionic villus sampling specimens at 10 to 12 weeks and on amniocentesis samples at 16 to 18 weeks. Cell-free fetal DNA testing using a maternal peripheral blood sample instead of an invasive procedure is not yet clinically available for CF, but will likely be an option in the future. Coordination with the molecular diagnostic laboratory before sending specimens is important given the need for timely results regarding an ongoing pregnancy.

CFNBS

Newborn screening[21] was initially used for detection of rare inborn errors of metabolism, but has expanded to commonly include endocrine, hematologic, and hearing disorders. Discussions surrounding the addition of CF to newborn screening date back to the 1970s, but it was not until the new millennium that the consensus was reached that CFNBS was warranted on the basis of moderate benefit and low risk of harm.[22] CFNBS leads to early diagnosis, which improves clinical outcomes. By 2010, all US states had approved CFNBS.[23] Most patients (59% in 2011, as opposed to 9.4% in 2001) are now identified by CFNBS for immunoreactive trypsinogen (IRT), with clinical and molecular follow-up when elevated.[5]

CFNBS samples are collected via heel stick puncture, typically within 48 hours of birth. Laboratory tests for CFNBS include IRT enzyme testing, pancreatitis-associated protein (PAP) testing, DNA mutation analysis, and sweat chloride testing.[24] Infants with CF have elevated IRT levels, hypothesized to be because of pancreatic duct dysfunction in both pancreatic-sufficient and pancreatic-insufficient infants. False negatives are occasionally seen with meconium ileus, and therefore IRT is not used as a diagnostic test. False positives are more commonly seen, particularly in cases of prematurity, perinatal stress, low Apgar scores, and African ethnic origin.[25] PAP is a non-specific stress protein elevated in the blood of newborns with CF, and the combination of IRT with PAP is being examined in several populations in Europe.[26]

A key issue in CFNBS design is to select appropriate mutations for the molecular follow-up, to reflect the ethnicities of the region. Approximately 20 *CFTR* mutations have a worldwide frequency of 0.1%.[1,21] The remainder are less common overall and may exhibit regional effects. For example, splice site mutation 3120+1G>A (c.2988+1G>A) is relatively uncommon in the US population overall, but is the second most common mutation in African American patients, with a prevalence of >12%.[27] The sweat chloride test has been the most widely used test to confirm the diagnosis of CF. Sweat chloride values decrease in the first few weeks of life, which requires that age-specific interpretation ranges be used.[6]

CFNBS algorithms differ by state and country. In the United States, all CFNBS programs use IRT as the initial test. Programs individually define an elevated IRT by using either an established cutoff value or a percentile threshold. A positive IRT screen is triaged to second-tier testing, which is repeat enzyme testing, DNA mutation testing, or both. Each test and algorithm has its own sensitivity and specificity. Repeat IRT testing becomes more specific for CF because IRT decreases with age in a healthy infant.[22] The single IRT and IRT + IRT repeat testing algorithms are reported to have a sensitivity of 85% to 90%, with many false positives (less specificity).[28,29] An alternative screening algorithm involves IRT testing with DNA mutation analysis, either IRT + DNA, IRT + IRT + DNA, or IRT + DNA + IRT. A newborn is considered positive if one or more *CFTR* mutations are observed, or one or more *CFTR* mutations with repeat elevated IRT occur after mutation testing. The combination of IRT and DNA mutation testing improves sensitivity compared with other testing algorithms.[30,31] The increased sensitivity is potentially associated with more referrals of individuals who have an initial elevated IRT and happen to carry a single *CFTR* mutation but do not have CF. Last, another CFNBS algorithm is on the basis of IRT + PAP screening, which has been evaluated in conjunction with IRT + DNA testing in several European countries. This approach provides easy testing of both samples simultaneously, may save cost, and is less likely to identify unaffected carriers, but conversely also misses milder cases.[26,32]

Whichever algorithm is followed, infants with a positive screening result are referred for sweat chloride testing, further clinical evaluation, and additional molecular testing if clinically indicated.

Molecular Diagnostic Testing

The purpose of molecular diagnostic testing (in contrast to molecular testing as part of population screening) is to provide genetic characterization of individuals with clinical or suspected CF. Reasons for pursuing such diagnostic testing include prenatal diagnosis in a carrier couple, newborn screening follow-up, clinical symptoms consistent with CF phenotypes, and a family history of a relative with CF or with a CF-like condition. The benefits of testing include earlier and definitive diagnosis, improved CF-specific care, clarification of atypical cases, and attainment of the information required for providing counseling regarding recurrence risk and fertility options.

CFTR molecular testing is technically straightforward, most commonly using a blood sample and test modalities that include established mutation panels, sequencing, and methods that can detect deletions and duplications usually missed by sequencing. Examples of current commonly used methods for CF testing are listed in Table 4. Next-generation sequencing will provide numerous variants and has the potential to combine sequencing with the detection of deletions and duplication. Variants of unknown significance will inevitably present; however, this is seen with Sanger sequencing, as well. Clinicians need to be aware of the limitations of the test being ordered and pay particular attention to test ordering (eg, provide ethnic background, family history of CF, and familial mutations, if available). Often times, however, this information is not available or is incompletely provided. Clinicians should also be aware that interpretation can be challenging because of ethnic background, variants of unknown clinical significance, and residual risk resulting from assay specifications. In evaluating nuances presented by individual cases, genetic counseling is an appropriate resource.

The US and European diagnostic guidelines for CF are not identical but are in concordance.[28,33,34] US guidelines classify individuals as having CF, having *CFTR*-related disorder, and being unlikely to have CF, whereas the European guidelines classify their diagnoses as classic CF, CFTR dysfunction (which includes atypical/non-classic cases), inconclusive, or unlikely CF. For patients with symptoms of CF or a family history, the US guidelines recommend sweat chloride testing with age-appropriate screening thresholds (20 mmol/L, <6 months of age; 40 mmol/L, >6 months of age). If the sweat chloride result is lower than the threshold and two *CFTR* mutations were not identified with appropriate testing, then the individual is unlikely to have CF.[6] If the sweat chloride result is lower than the threshold in the presence of two CF-causing mutations, then the diagnosis of CF can be made. If the sweat chloride test result is higher than the threshold, the diagnosis can be made by confirmatory sweat chloride testing (≥60 mmol/L) or by identification of two *CFTR* mutations, which is often initially pursued using the ACMG panel, originally designed for carrier screening and, in many ethnic groups, suboptimal for diagnostic testing. With an indeterminate sweat chloride result (higher than the threshold but <60 mmol/L), more extensive *CFTR* mutation testing is recommended and if two *CFTR* mutations are then identified, the diagnosis of CF is made. If, however, zero or one *CFTR* mutation is identified, then *CFTR*-related disorder or CF both remain possibilities and additional nasal potential difference testing may help distinguish between the two.

Approximately 10% of CF patients do not receive a molecular diagnosis. Some of this may result from limited molecular testing (ie, a mutation panel as opposed to sequencing of promoter, exons, and exon/intron boundaries and deletion or duplication analysis). However, even with extensive testing, some patients do not have two mutations identified. Genetic explanations include deep intronic and other mutations that can affect splicing, alternative genes (eg, the epithelial sodium channel genes), epistatic/synergistic heterozygosity relationships, mutation(s) in an unrecognized or untested regulatory element, unidentified insertion or deletion mutations, and dominant negative or epigenetic effects. For example, recent evidence suggests a role for epigenetic mediators, such as miRNAs, histone modification, and DNA methylation in CF.[35] Technical

Table 5 Standards and Guidelines for CF Molecular Testing in the United States

Carrier Screening and Prenatal Testing
Laboratory Standards and Guidelines for Population-Based Cystic Fibrosis Carrier Screening[13] describes the original 25-mutation ACMG *CFTR* carrier screening panel (*http://www.acmg.net/StaticContent/SGs/Population-based%20Cystic%20Fibrosis.pdf*)
Cystic Fibrosis Population Carrier Screening: 2004 Revision of ACMG Mutation Panel[11] revises the original ACMG *CFTR* carrier screening panel and is the current recommended panel (*http://www.acmg.net/StaticContent/StaticPages/CF_Mutation.pdf*)
ACOG Committee Opinion Number 325, December 2005: Update on Carrier Screening for Cystic Fibrosis and the updated opinion *ACOG Committee Opinion No. 486: Update on Carrier Screening for Cystic Fibrosis*[12] outline guidelines for obstetricians-gynecologists and include screening scenarios and clinical guidelines (*http://www.acog.org/Resources-And-Publications/Committee-Opinions/Committee-on-Genetics/Update-on-Carrier-Screening-for-Cystic-Fibrosis*)
Technical Standards and Guidelines for CFTR Mutation Testing is a disease-specific guideline that supplements carrier screening guidelines by inclusion of diagnostic testing, prenatal screening, and prenatal diagnostic testing, and technique-specific guidelines (*http://www.acmg.net/StaticContent/SGs/CFTR%20Mutation%20Testing.pdf*)
Molecular Diagnostic Approach for Screened Newborns, Children, and Adults
Guidelines for Diagnosis of Cystic Fibrosis in Newborns through Older Adults: Cystic Fibrosis Foundation Consensus Report[5] discusses clinical, laboratory, and genetic techniques for CF diagnosis
Technical Standards and Guidelines for CFTR Mutation Testing is a disease-specific guideline to accompany ACMG standards and guidelines for clinical laboratories; it also supplements carrier guidelines by inclusion of diagnostic testing, prenatal screening and prenatal diagnostic testing, and technique-specific guidelines (*http://www.acmg.net/StaticContent/SGs/CFTR%20Mutation%20Testing.pdf*)
Genetic Counseling Regarding CF Molecular Testing
Molecular Testing for Cystic Fibrosis Carrier Status Practice Guidelines: Recommendations of the National Society of Genetic Counselors[18] provides practice recommendations for carrier screening and molecular testing

ACMG, American College of Medical Genetics and Genomics; CF, cystic fibrosis; CFTR, CF transmembrane conductance regulator.

Table 6 Resources for Laboratory Testing and Proficiency Testing in the United States

Laboratory Standards

ACMG Standards and Guidelines for Clinical Genetics Laboratories provides an overview regarding use of molecular techniques to determine hereditary basis of disease (*https://www.acmg.net/ACMG/Publications/Laboratory_Standards___Guidelines/ACMG/Publications/Laboratory_Standards___Guidelines.aspx?hkey=8d2a38c5-97f9-4c3e-9f41-38ee683bcc84* and *https://www.acmg.net/StaticContent/SGs/Section_G_2010.pdf*)

The CAP molecular pathology checklist is available free to members of CAP or for purchase directly from CAP

Development of Genomic Reference Materials for Cystic Fibrosis Genetic Testing[40] describes reference material available (eg, to serve as CF mutation controls)

Proficiency Testing

US Centers for Disease Control and Prevention Newborn Screening Quality Assurance Program (NSQAP) CF Mutation Detection PT Program provides CFTR mutation proficiency testing materials for dried-blood spot testing (*http://www.cdc.gov/labstandards/nsqap.html*)

CFTR mutation proficiency testing (MGL2 and MGL5) is available via the CAP (2015 Surveys and Anatomic Pathology Education Programs, *www.cap.org/web/home/lab/catalogs-ordering-shipping*)

Methods-based proficiency testing for sequencing and for next-generation sequencing is provided by the CAP (2015 Surveys and Anatomic Pathology Education Programs, *www.cap.org/web/home/lab/catalogs-ordering-shipping*)

ACMG Clinical Laboratory Standards for Next-Generation Sequencing[38] and *College of American Pathologists' Laboratory Standards for Next Generation Sequencing Clinical Tests*[41] have been published

Variant Interpretation for Clinical Reporting

ACMG Recommendation for Standards for Interpretation of Sequence Variations and ACMG Recommendations for Standards for Interpretation and Reporting of Sequence Variations: Revisions 2007[37] provide recommendations for interpretation and reporting of sequence variants

CFTR Variant Databases

CF mutation database (*http://www.genet.sickkids.on.ca/app*) is a repository of variants submitted by laboratories worldwide

CFTR2 database (*http://www.cftr2.org*) summarizes variants, clinical phenotypes, and functional data in an effort to determine pathogenicity of variants

The North American Gene Modifier Consortium and European Cystic Fibrosis Society Gene Modifier groups examine genetic modifiers of CF

Tools for Variant Pathogenicity Prediction Analysis

PolyPhen-2 (Polymorphism Phenotyping version 2; *http://genetics.bwh.harvard.edu/pph2*), SIFT (Sorting Intolerant from Tolerant; *http://sift.jcvi.org*), and Provean (Protein Variation Effect Analyzer; *http://provean.jcvi.org/genome_submit.php*) are examples of tools used to predict functional consequences of predicted amino acid changes in the protein sequence

Splice site variant prediction programs include Spliceman (*http://fairbrother.biomed.brown.edu/spliceman*), ASSEDA (*http://splice.uwo.ca*), and the Berkeley Drosophila Genome Project (*http://www.fruitfly.org/seq_tools/splice.html*)

Nomenclature

Naming conventions for genetic variants (*http://www.hgvs.org/mutnomen/recs.html*)

CAP, College of American Pathologists; CF, cystic fibrosis; CFTR, CF transmembrane conductance regulator.

issues that can prevent mutation detection include allele dropout in testing methods using PCR. Allele dropout is typically the result of unequal allelic PCR amplification because of preferential annealing of a primer to the matched allelic sequence over a mismatched allelic sequence.

CF molecular testing guidelines do not encompass all scenarios. This can be because of reasons including lack of consensus, limited applicability, and unanticipated findings. Genetic professionals can provide assessment, patient counseling, and critical insight in these situations for the patients, their families, and the clinicians. For example, the workup for the patient with a family or a personal history of CF and a negative ACMG 23 mutation panel can differ. We suspect that most would argue that sequencing and deletion or duplication of the *CFTR* gene would be appropriate in these two cases of personal and family history of CF. However, in all patient interactions, clinical judgment, knowledge of strengths and limitations of different test modalities, and the best interest of the patient are essential.

Laboratory Guidelines

Laboratory standards and guidelines for CF carrier screening, prenatal screening, newborn screening, and diagnostic testing are summarized in Table 5. Recommendations were first formulated for carrier screening and have since expanded to diagnostic testing. In 2001, the ACMG published *Laboratory Standards and Guidelines for Population-Based CF Carrier Screening.*[13] Recommendations included offering universal screening with appropriate counseling for non-whites. Patient ethnicity, indication for testing, mutations tested, method of testing, residual risk, and a summary of CF test results to convey to the patient with referral to counseling, if needed, should be included in the laboratory report. In 2004, these guidelines were updated to incorporate new information and to modify the panel from 25 to 23 mutations.[11]

In 2002, the ACMG published *Standards and Guidelines for CFTR Mutation Testing*,[36] a report that is CF specific and addresses technical laboratory issues related to CF testing, including diagnostic and prenatal testing. This text

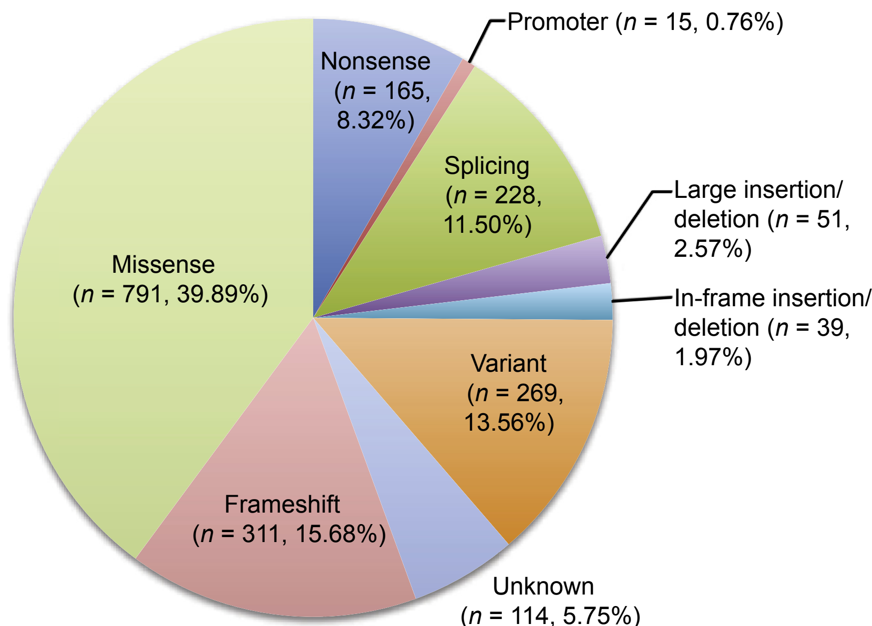

Figure 1 Types of cystic fibrosis transmembrane conductance regulator (*CFTR*) variants. Summary of 1983 *CFTR* variants by category in the CF mutation database (*http://www.genet.sickkids.on.ca/app*), as reported on October 31, 2014. Further studies by Sosnay et al[48] have classified 1044 variants from 39,696 individuals with CF. Allele frequency, functional testing, and parental transmission were used to characterize variants as consistent with causing disease, non-disease causing, or indeterminate in effect. Number of variants in CF mutation database: 1983; number of variants in CFTR2 database: 1044. Allele frequency >0.01%: 159; +Met clinical and functional criteria: 127; +Neutral effect: 12; +Indeterminate effect: 20.

was updated in 2008, with an online revision in March 2011. ACMG guidelines on variant interpretation[37] comprise sample preparation, sequencing platforms, and data analysis, including variant reporting. With the advent of next-generation sequencing, the ACMG and Association for Molecular Pathology published documents that are applicable to use of this technology for *CFTR* testing.[38,39]

PT Data

Clinical laboratories performing molecular testing in the United States must follow the regulations of the Clinical Laboratory Improvement Act and comply with the standards of the College of American Pathologists (CAP) or another deemed entity. Clinical Laboratory Improvement Act regulations, which are overseen by the Centers for Medicare and Medicaid Services, encompass appropriate sample documentation, assay validation, general proficiencies, and quality control measures and lead to Clinical Laboratory Improvement Act certification. CAP accreditation requires a thorough method validation (for Laboratory Developed Procedures) or verification (for Food and Drug Administration−cleared assays). CAP also requires a range of additional quality measures, including proficiency testing (PT) (Table 6).

The CAP offers biannual external PT for *CFTR*: MGL-5, which is a stand-alone PT for CF, and MGL-2, which is offered in a survey with several other genetic conditions. For each of these surveys, DNA samples with the associated clinical scenario of a child with failure to thrive are sent to the participating laboratories twice a year, and a laboratory uses their validated method for analysis, evaluating only the ACMG panel. Laboratories must provide the correct sample genotypes and clinical interpretations. The PT data from the most recent 11 years of this program have been published.[42] CAP PT also includes methods-based external PT for Sanger sequencing, which is suitable for *CFTR* PT when applied in combination with the analyte-specific PT.[43] Methods-based next-generation sequencing PT will be offered as of 2015. The US Centers for Disease Control and Prevention Newborn Screening Quality Assurance Program offers quality control and PT surveys in a dried-blood spot matrix, specifically for CFNBS. It provides coded specimens with *CFTR* mutations, including, but not limited to, the ACMG panel.

Current Therapies

Although the median life expectancy for patients in the United States is 36.8 years of age,[5] an individual born with CF in 2000 has an anticipated life expectancy of >50 years.[44] Reasons for the improved projected longevity include pancreatic replacement enzymes, antimicrobials, including azithromycin and inhaled tobramycin, recombinant human DNase, hypertonic saline, improved clearance techniques or devices, the development of drugs that enhance CFTR function,[45,46] and availability of specialized clinical care at CF-focused centers. If lung disease becomes severe, lung transplantation is an option.[5]

The heterogeneity of *in vivo* transepithelial potentials and symptoms has led to the observation that even partial CFTR function improves a patient's likelihood of manifesting less severe symptoms and spurred the development of targeted molecular CFTR medication.[46,47] New therapies include at least three classes of drugs: i) Potentiators, targeted at improving chloride channel activity (ivacaftor; Vertex Pharmaceuticals, Boston, MA). Ivacaftor increases chloride transport of the G551D (c.1652G>A; p.Gly551Asp) mutation seen in 4% of CF patients and is the first US Food and Drug Administration−approved molecular therapy.[47] ii) Correctors, to chaperone or regulate the protein and minimize

proteostasis resulting from mis-processing mutations (lumacaftor and VX-661; Vertex Pharmaceuticals). iii) Suppressors, aimed at promoting ribosomal read through at premature termination codons (ataluren; PTC Therapeutics, South Plainfield, NJ). The long-term impact of these medications and potential use prenatally and in early childhood remain to be determined. In addition, mutations can have multiple effects, such as delF508 (c.1521_1523delCTT; p.Phe508del), which affects trafficking, open channel time, and membrane half-life.[47] The combinations of these types of medications may enhance therapeutic effects.

Gene therapy has long been a hope in the CF community. Cationic liposomes and plasmid DNA vectors are among the approaches being explored to improve CFTR function.[48] Non-CFTR targets are also being pursued as new pulmonary therapies. This includes efforts focused on reduction of sodium and fluid absorption via epithelial sodium channel inhibition, activation of alternative chloride channels, and osmotic agents to increase airway surface liquid hydration.[46,49]

Categorization of Genetic Variants

There have been almost 20 years of international cooperation, cataloging, and investigating *CFTR* sequence variants. The CF Mutation Database (CFTR1; *http://www.genet.sickkids.on.ca/app*; last accessed October 31, 2014) was initiated in 1990 as a repository for *CFTR* sequence variants (Figure 1). This wealth of information, and its application to genetic testing, is complicated, however, because patient studies and experimental data that would meet stringent standards for classification have only been determined for a portion of these variants to date.[11,50] In addition, as with many other genetic disorders, *CFTR* mutation nomenclature and exon numbering have changed over the years, and adherence to consensus nomenclature (Table 6) as well as clear communication to clinicians of the correlation with familiar mutation and exon names are essential.

The CFTR2 project compiled clinical data and *CFTR* variants from CF patients enrolled in national registries and large clinical centers from 24 countries to address the need for clinical interpretation. Variants were evaluated for allele frequency (with a threshold of >0.01%), clinical phenotype, including sweat chloride measurements, lung function, and pancreatic status, and functional consequences. In instances that did not meet either clinical or functional criteria, disease penetrance was determined via parental alleles to assist in interpretation. One-hundred fifty-nine *CFTR* variants are estimated to represent approximately 96% of CF disease-causing alleles in a predominantly (95%) white cohort.[50] For variants at lower allele frequencies or not present in the database at time of analysis, the interpretation still defaults to laboratory assessment of predicted effects on the basis of information in various databases, computational analysis, and literature review (Table 6). The CFTR2 database is updated, however, on an ongoing basis. The CFTR2 database is also an excellent example of functional characterization of variants. It is in alignment with the ACMG variant classification recommendations that variants be characterized on the basis of all available clinical and experimental information to designate their status as pathogenic, likely pathogenic, variant of unknown significance, likely benign, and benign.

Genotype-Phenotype Correlations

Historically, *CFTR* mutations have been categorized on the basis of predicted effects on CFTR biosynthesis, trafficking, channel gating, channel conductance, and decreased transcript levels.[51] Class I mutations cause protein termination codons, resulting in little or no CFTR protein. Class II mutations cause trafficking issues with misfolding, or mislocalization, resulting in low levels of apical plasma membrane CFTR. delF508 (c.1521_1523delCTT; p.Phe508del) epitomizes this class. Class III mutations affect channel gating and result in decreased ion transport. Class IV mutations affect channel activity. Class V mutations affect splicing, resulting in decreased protein levels. Class VI mutations cause short residence time at the plasma membrane in the presence of truncation mutations. This classification system has the advantage of characterizing mutations by (often predicted) functional defect, and helps categorize the targets of the new classes of molecular therapies. It is not used in clinical predictions, however, because this classification system has significant limitations because of poor correlation of mutation class with clinical characteristics, despite some useful generalizations that have been observed. In general, class I to III mutations tend to have minimal function and more severe disease consequences. Severe mutations are associated with pancreatic insufficiency,[52] meconium ileus,[53] CF-related diabetes,[54] and CF liver disease.[55] Classes IV and V represent mutations with a milder phenotype. The presence of at least one allele with residual function confers improved nutritional status and less severe lung disease.[55]

Genotype-phenotype correlations suggest that additional genetic factors (eg, complex alleles and modifier genes) and/or nongenetic factors significantly influence CF and *CFTR*-related disorders. Complex alleles have two variants on one chromosome, and one variant modulates the effect of the other. For example, the 5T allele in intron 8 is a variant with variable penetrance for inefficient splicing of exon 9. In that location, common alleles are 7T (85%), 9T (10%), and 5T (5%). The TG tract that immediately precedes the poly-Ts further modulates when 5T is present. The combination of p.Phe508del on one chromosome and 5T preceded by 11TG on the other chromosome is unlikely to develop classic CF. The combination of p.Phe508del and 5T preceded by >11TG, however, is more likely to do so.[56] 5T also modulates the effect of the p.Arg117His mutation, in that these two variants in *cis* add up to one pathogenic mutation, whereas in *trans* they are both considered mild and likely to result in CBAVD. Thus, reflex testing for the 5T allele is

recommended when p.Arg117His is identified on a carrier screening panel.[11]

Modifier and Candidate Genes

CF is a monogenic disorder. However, manifestations range from single to multisystem involvement and from mild to severe disease, largely without the strong genotype-phenotype correlations that would explain the observed variability. This makes conveying clinical prognosis difficult and illustrates the benefits to patients and their families of genetic counseling to learn about these complex issues. This suggests that multiple factors influence the phenotype, acting in the manner of a complex disorder. The observed phenotypic heterogeneity may then easily be because of multiple additive effects, including those contributed by complex alleles with multiple mutations, modifier genes, mutations in alternative genes that produce CF-like phenotypes, epigenetic factors, and influences from the environment.

Heritability estimates are a means of conveying the proportion of variance in a trait in a particular population that is because of genetic factors, as opposed to environmental or stochastic variation. CF manifestations have different heritability estimates, reflecting that some features, such as CBAVD, are highly determined by genetic factors, whereas others, such as respiratory tract problems, have additional influencers. Genetic factors are estimated to account for 54% to 100% of phenotypic variability in respiratory tract symptoms.[55,57,58] Heritability estimates for chronic *Pseudomonas aeruginosa* infection are also high (76% to 85%).[59] The age of onset, disease extent, and degree of multisystem involvement vary even within homozygous p.Phe508del patients.[60] Genetic variation has also been shown within twin pairs, suggesting that environmental influences do play a substantial role in the heterogeneity of observed manifestations.[61]

Genome-wide association studies have provided evidence for modifying loci at 11p13 and 20q13.2.[62] The list of candidate genes that may influence the phenotypic aspects of CF includes genes associated with inflammatory response, infectious predisposition, signaling, and colocalizing proteins.[63,64] Candidate gene modifiers of clinical phenotypes for lung function that have been replicated include immunology and/or inflammation factors mannose-binding lectin 2, transforming growth factor-β1, IL-8, interferon-related developmental regulator 1, and endothelin receptor.[55]

The heritability estimates of 88% to 100% for meconium ileus indicate a strong genetic influence.[53,54] Candidate modifier genes include solute carrier proteins *SLC26A9*, *SLC9A3*, and *SLC6A14*, as well as multiple apical membrane constituents.[65] Distal intestinal obstruction has an association with meconium ileus, but nongenetic factors appear to be dominant there.[53] When the modifier genes for meconium ileus were examined as modifiers for other CF manifestations, *SLC26A9*, *SLC9A3*, and *SLC6A14* were pleiotropic (ie, each having multiple different phenotypic effects) for meconium ileus and pancreatic damage, lung disease, and lung disease and age of *P. aeruginosa* infection, respectively.[66]

CF-related diabetes has heritability estimates at virtually 100%.[67] In this case, however, some of the heritability appears to come from other type 2 diabetes genes as opposed to solely from *CFTR*. Nutritional status affects lung function and has a heritability estimate of approximately 60%.[55,68] Finally, CF liver disease with portal hypertension occurs in a small proportion of patients (5%), and at a median age of 10 years. Heritability has not yet been formally estimated, and non-CFTR factors are thought to contribute.[69]

Conclusions

CF has long been at the forefront of genetics research and has served as a model for both research investigation and diagnostics. Databases to catalog and characterize *CFTR* variants were established and served as an example for other conditions, and the first universal population carrier screen in the United States was for CF. Novel molecular targeted therapies for CF have resulted from a basic science understanding of the gene and will serve as a template to other conditions. Despite these advances, obtaining sufficient unequivocal experimental and clinical evidence to fully interpret the approximately 2000 *CFTR* variants is challenging and often not feasible, leaving us with variants of unknown significance. This, of course, is a reality in common with many other genetic conditions as well. Lessons learned in the context of CF emphasize both the difficulties and the enormous potential of continued genetic studies. We have come a long way, and can forge ahead with a sense of excitement and with confidence in the tremendous progress yet to be made.

References

1. Welsh MJ, Ramsey BW, Accurso F, Cutting GR: Cystic fibrosis. Metabolic and Molecular Bases of Inherited Disease, 8th Edition, Volume 3. Edited by Scriver CR, Beaudet AL, Sly WS, Valle D. New York, McGraw-Hill, 2013, pp 5121−5188
2. Palomaki GE, FitzSimmons SC, Haddow JE: Clinical sensitivity of prenatal screening for cystic fibrosis via CFTR carrier testing in a United States panethnic population. Genet Med 2004, 6:405−414
3. Katkin JP, Schultz K. Cystic fibrosis: overview of gastrointestinal disease in UpToDate, Post TW (Ed), In UpToDate [Internet]. Copyright Wolters Kluwer, Waltham, MA. Available at http://www.uptodate.com/contents/cystic-fibrosis-overview-of-gastrointestinal-disease, last revised July 15, 2015
4. Pillarisetti N, Williamson E, Linnane B, Skoric B, Robertson CF, Robinson P, Massie J, Hall GL, Sly P, Stick S, Ranganathan S; Australian Respiratory Early Surveillance Team for Cystic Fibrosis: Infection, inflammation, and lung function decline in infants with cystic fibrosis. Am J Respir Crit Care Med 2011, 184:75−81
5. Cystic Fibrosis Foundation Patient Registry: Annual Data Report to the Center Directors. Bethesda, MD, 2011
6. Farrell PM, Rosenstein BJ, White TB, Accurso FJ, Castellani C, Cutting GR, Durie PR, Legrys VA, Massie J, Parad RB, Rock MJ, Campbell PW: Guidelines for diagnosis of cystic fibrosis in newborns

through older adults: Cystic Fibrosis Foundation consensus report. J Pediatr 2008, 153:S4−S14

7. Borowitz D, Parad RB, Sharp JK, Sabadosa KA, Robinson KA, Rock MJ, Farrell PM, Sontag MK, Rosenfeld M, Davis SD, Marshall BC, Accurso FJ: Cystic Fibrosis Foundation practice guidelines for the management of infants with cystic fibrosis transmembrane conductance regulator-related metabolic syndrome during the first two years of life and beyond. J Pediatr 2009, 155:S106−S116
8. Groman JD, Meyer ME, Wilmott RW, Zeitlin PL, Cutting GR: Variant cystic fibrosis phenotypes in the absence of CFTR mutations. N Engl J Med 2002, 347:401−407
9. Mayell SJ, Munck A, Craig JV, Sermet I, Brownlee KG, Schwarz MJ, Castellani C, Southern KW; European Cystic Fibrosis Society Neonatal Screening Working Group: A European consensus for the evaluation and management of infants with an equivocal diagnosis following newborn screening for cystic fibrosis. J Cyst Fibros 2009, 8:71−78
10. Lebo RV, Grody WW: Testing and reporting ACMG cystic fibrosis mutation panel results. Genet Test 2007, 11:11−31
11. Watson MS, Cutting GR, Desnick RJ, Driscoll DA, Klinger K, Mennuti M, Palomaki GE, Popovich BW, Pratt VM, Rohlfs EM, Strom CM, Richards CS, Witt DR, Grody WW: Cystic fibrosis population carrier screening: 2004 revision of American College of Medical Genetics mutation panel. Genet Med 2004, 6:387−391
12. American College of Obstetrics, Gynecologists Committee on Genetics: ACOG Committee Opinion No. 486: update on carrier screening for cystic fibrosis. Obstet Gynecol 2011, 117:1028−1031
13. Grody WW, Cutting GR, Klinger KW, Richards CS, Watson MS, Desnick RJ; Subcommittee on Cystic Fibrosis Screening Accreditation of Genetic Services Committee, ACMG, American College of Medical Genetics: Laboratory standards and guidelines for population-based cystic fibrosis carrier screening. Genet Med 2001, 3:149−154
14. Delatycki M, Burke J, Cristie L, Collines F, Gabbet M, George P, Haan E, Ioannou L, Massie J, McKenzie F, O'Leary P, Scoble-Williams N, Turner G, Human Genetics Society of Australia Genetic Services Committee. [Internet] Position statement. Population based carrier screening for cystic fibrosis. Copyright Human Genetics Society of Australasia, Sydney, Australia. Available at documents/item/1282, last revised October 2013
15. Castellani C, Macek M Jr, Cassiman JJ, Duff A, Massie J, ten Kate LP, Barton D, Cutting G, Dallapiccola B, Dequeker E, Girodon E, Grody W, Highsmith EW, Kaariainen H, Kruip S, Morris M, Pignatti PF, Pypops U, Schwarz M, Soller M, Stuhrman M, Cuppens H: Benchmarks for cystic fibrosis carrier screening: a European consensus document. J Cyst Fibros 2010, 9:165−178
16. Grody WW, Thompson BH, Gregg AR, Bean LH, Monaghan KG, Schneider A, Lebo RV: ACMG position statement on prenatal/preconception expanded carrier screening. Genet Med 2013, 15:482−483
17. Monaghan KG, Highsmith WE, Amos J, Pratt VM, Roa B, Friez M, Pike-Buchanan LL, Buyse IM, Redman JB, Strom CM, Young AL, Sun W: Genotype-phenotype correlation and frequency of the 3199del6 cystic fibrosis mutation among I148T carriers: results from a collaborative study. Genet Med 2004, 6:421−425
18. Langfelder-Schwind E, Karczeski B, Strecker MN, Redman J, Sugarman EA, Zaleski C, Brown T, Keiles S, Powers A, Ghate S, Darrah R: Molecular testing for cystic fibrosis carrier status practice guidelines: recommendations of the National Society of Genetic Counselors. J Genet Couns 2014, 23:5−15
19. Ogino S, Wilson RB, Gold B, Hawley P, Grody WW: Bayesian analysis for cystic fibrosis risks in prenatal and carrier screening. Genet Med 2004, 6:439−449
20. Grody WW, Cutting GR, Watson MS: The Cystic Fibrosis mutation "arms race": when less is more. Genet Med 2007, 9:739−744
21. Zinn AB: Inborn errors of metabolism. Edited by Fanaroff AA, Martin RJ. Neonatal-Perinatal Medicine. ed 7. St Louis, Mosby, 2002, pp 1468
22. Grosse SD, Boyle CA, Botkin JR, Comeau AM, Kharrazi M, Rosenfeld M, Wilfond BS; CDC: Newborn screening for cystic fibrosis: evaluation of benefits and risks and recommendations for state newborn screening programs. MMWR Recomm Rep 2004, 53:1−36
23. Wagener JS, Zemanick ET, Sontag MK: Newborn screening for cystic fibrosis. Curr Opin Pediatr 2012, 24:329−335
24. Deucher A, Schrijver I: Newborn screening for cystic fibrosis. Cystic Fibrosis: Etiology, Diagnosis and Treatments. Hauppauge, NY, Nova Science Publishers Inc., 2009, pp 1−28
25. Rock MJ, Mischler EH, Farrell PM, Bruns WT, Hassemer DJ, Laessig RH: Immunoreactive trypsinogen screening for cystic fibrosis: characterization of infants with a false-positive screening test. Pediatr Pulmonol 1989, 6:42−48
26. Sommerburg O, Krulisova V, Hammermann J, Lindner M, Stahl M, Muckenthaler M, Kohlmueller D, Happich M, Kulozik AE, Votava F, Balascakova M, Skalicka V, Stopsack M, Gahr M, Macek M Jr, Mall MA, Hoffmann GF: Comparison of different IRT-PAP protocols to screen newborns for cystic fibrosis in three central European populations. J Cyst Fibros 2014, 13:15−23
27. Bobadilla JL, Macek M Jr, Fine JP, Farrell PM: Cystic fibrosis: a worldwide analysis of CFTR mutations−correlation with incidence data and application to screening. Hum Mutat 2002, 19:575−606
28. Farrell MH, Farrell PM: Newborn screening for cystic fibrosis: ensuring more good than harm. J Pediatr 2003, 143:707−712
29. Padoan R, Genoni S, Moretti E, Seia M, Giunta A, Corbetta C: Genetic and clinical features of false-negative infants in a neonatal screening programme for cystic fibrosis. Acta Paediatr 2002, 91:82−87
30. Narzi L, Lucarelli M, Lelli A, Grandoni F, Lo Cicero S, Ferraro A, Matarazzo P, Delaroche I, Quattrucci S, Strom R, Antonelli M: Comparison of two different protocols of neonatal screening for cystic fibrosis. Clin Genet 2002, 62:245−249
31. Comeau AM, Parad RB, Dorkin HL, Dovey M, Gerstle R, Haver K, Lapey A, O'Sullivan BP, Waltz DA, Zwerdling RG, Eaton RB: Population-based newborn screening for genetic disorders when multiple mutation DNA testing is incorporated: a cystic fibrosis newborn screening model demonstrating increased sensitivity but more carrier detections. Pediatrics 2004, 113:1573−1581
32. Krulisova V, Balascakova M, Skalicka V, Piskackova T, Holubova A, Paderova J, Krenkova P, Dvorakova L, Zemkova D, Kracmar P, Chovancova B, Vavrova V, Stambergova A, Votava F, Macek M Jr: Prospective and parallel assessments of cystic fibrosis newborn screening protocols in the Czech Republic: IRT/DNA/IRT versus IRT/PAP and IRT/PAP/DNA. Eur J Pediatr 2012, 171:1223−1229
33. Ooi CY, Dupuis A, Ellis L, Jarvi K, Martin S, Gonska T, Dorfman R, Kortan P, Solomon M, Tullis E, Durie PR: Comparing the American and European diagnostic guidelines for cystic fibrosis: same disease, different language? Thorax 2012, 67:618−624
34. De Boeck K, Wilschanski M, Castellani C, Taylor C, Cuppens H, Dodge J, Sinaasappel M; Diagnostic Working Group: Cystic fibrosis: terminology and diagnostic algorithms. Thorax 2006, 61:627−635
35. Chan HC, Jiang X, Ruan YC: Emerging role of cystic fibrosis transmembrane conductance regulator as an epigenetic regulator: linking environmental cues to microRNAs. Clin Exp Pharmacol Physiol 2014, 41:615−622
36. Richards CS, Bradley LA, Amos J, Allitto B, Grody WW, Maddalena A, McGinnis MJ, Prior TW, Popovich BW, Watson MS, Palomaki GE: Standards and guidelines for CFTR mutation testing. Genet Med 2002, 4:379−391
37. Richards CS, Bale S, Bellissimo DB, Das S, Grody WW, Hegde MR, Lyon E, Ward BE; Molecular Subcommittee of the ALQAC: ACMG recommendations for standards for interpretation and reporting of sequence variations: revisions 2007. Genet Med 2008, 10:294−300
38. Rehm HL, Bale SJ, Bayrak-Toydemir P, Berg JS, Brown KK, Deignan JL, Friez MJ, Funke BH, Hegde MR, Lyon E; Working Group of the American College of Medical Genetics, Genomics

Laboratory Quality Assurance Committee: ACMG clinical laboratory standards for next-generation sequencing. Genet Med 2013, 15:733−747
39. Schrijver I, Aziz N, Farkas DH, Furtado M, Ferreira-Gonzalez A, Greiner TC, Grody WW, Hambuch T, Kalman L, Kant JA, Klein RD, Leonard DGB, Lubin IM, Mao R, Nagan N, Pratt VM, Sobel ME, Voelkerding KV, Gibson JS: Opportunities and challenges associated with clinical diagnostic genome sequencing: a report of the Association for Molecular Pathology. J Mol Diagn 2012, 14:525−540
40. Pratt VM, Caggana M, Bridges C, Buller AM, DiAntonio L, Highsmith WE, Holtegaard LM, Muralidharan K, Rohlfs EM, Tarleton J, Toji L, Barker SD, Kalman LV: Development of genomic reference materials for cystic fibrosis genetic testing. J Mol Diagn 2009, 11:186−193
41. Aziz N, Zhao Q, Bry L, Driscoll DK, Funke B, Gibson JS, Grody WW, Hegde MR, Hoeltge GA, Leonard DG, Merker JD, Nagarajan R, Palicki LA, Robetorye RS, Schrijver I, Weck KE, Voelkerding KV: College of American Pathologists' Laboratory Standards for Next-Generation Sequencing Clinical Tests. Arch Pathol Lab Med 2015, 11:186−193
42. Lyon E, Schrijver I, Weck KE, Ferreira-Gonzalez A, Richards CS, Palomaki GE: Molecular genetic testing for cystic fibrosis: laboratory performance on the College of American Pathologists external proficiency surveys. Genet Med 2015, 17:219−225
43. Richards CS, Palomaki GE, Lacbawan FL, Lyon E, Feldman GL; CAP/ACMG Biochemical and Molecular Genetics Resource Committee: Three-year experience of a CAP/ACMG methods-based external proficiency testing program for laboratories offering DNA sequencing for rare inherited disorders. Genet Med 2014, 16:25−32
44. Hurley MN, McKeever TM, Prayle AP, Fogarty AW, Smyth AR: Rate of improvement of CF life expectancy exceeds that of general population: observational death registration study. J Cyst Fibros 2014, 13:410−415
45. Rowe SM, Verkman AS: Cystic fibrosis transmembrane regulator correctors and potentiators. Cold Spring Harb Perspect Med 2013, 3. doi:10.1101/cshperspect.a009761. pii: a009761
46. Hanrahan JW, Sampson HM, Thomas DY: Novel pharmacological strategies to treat cystic fibrosis. Trends Pharmacol Sci 2013, 34:119−125
47. Clancy JP, Jain M: Personalized medicine in cystic fibrosis: dawning of a new era. Am J Respir Crit Care Med 2012, 186:593−597
48. Gill DR, Hyde SC: Delivery of genes into the CF airway. Thorax 2014, 69:962−964
49. Donaldson SH, Galietta L: New pulmonary therapies directed at targets other than CFTR. Cold Spring Harb Perspect Med 2013, 3. doi:10.1101/cshperspect.a009787. pii: a009787
50. Sosnay PR, Siklosi KR, Van Goor F, Kaniecki K, Yu H, Sharma N, Ramalho AS, Amaral MD, Dorfman R, Zielenski J, Masica DL, Karchin R, Millen L, Thomas PJ, Patrinos GP, Corey M, Lewis MH, Rommens JM, Castellani C, Penland CM, Cutting GR: Defining the disease liability of variants in the cystic fibrosis transmembrane conductance regulator gene. Nat Genet 2013, 45:1160−1167
51. Rowe SM, Miller S, Sorscher EJ: Cystic fibrosis. N Engl J Med 2005, 352:1992−2001
52. Ooi CY, Dorfman R, Cipolli M, Gonska T, Castellani C, Keenan K, Freedman SD, Zielenski J, Berthiaume Y, Corey M, Schibli S, Tullis E, Durie PR: Type of CFTR mutation determines risk of pancreatitis in patients with cystic fibrosis. Gastroenterology 2011, 140:153−161
53. Blackman SM, Deering-Brose R, McWilliams R, Naughton K, Coleman B, Lai T, Algire M, Beck S, Hoover-Fong J, Hamosh A, Fallin MD, West K, Arking DE, Chakravarti A, Cutler DJ, Cutting GR: Relative contribution of genetic and nongenetic modifiers to intestinal obstruction in cystic fibrosis. Gastroenterology 2006, 131:1030−1039
54. Adler AI, Shine BS, Chamnan P, Haworth CS, Bilton D: Genetic determinants and epidemiology of cystic fibrosis-related diabetes: results from a British cohort of children and adults. Diabetes Care 2008, 31:1789−1794
55. Knowles MR, Drumm M: The influence of genetics on cystic fibrosis phenotypes. Cold Spring Harb Perspect Med 2012, 2:a009548. doi:10.1101/cshperspect.a009548
56. Lebo RV, Grody WW: Variable penetrance and expressivity of the splice altering 5T sequence in the cystic fibrosis gene. Genet Test 2007, 11:32−44
57. Stanke F, Becker T, Kumar V, Hedtfeld S, Becker C, Cuppens H, Tamm S, Yarden J, Laabs U, Siebert B, Fernandez L, Macek M Jr, Radojkovic D, Ballmann M, Greipel J, Cassiman JJ, Wienker TF, Tummler B: Genes that determine immunology and inflammation modify the basic defect of impaired ion conductance in cystic fibrosis epithelia. J Med Genet 2011, 48:24−31
58. Vanscoy LL, Blackman SM, Collaco JM, Bowers A, Lai T, Naughton K, Algire M, McWilliams R, Beck S, Hoover-Fong J, Hamosh A, Cutler D, Cutting GR: Heritability of lung disease severity in cystic fibrosis. Am J Respir Crit Care Med 2007, 175:1036−1043
59. Emond MJ, Louie T, Emerson J, Zhao W, Mathias RA, Knowles MR, Wright FA, Rieder MJ, Tabor HK, Nickerson DA, Barnes KC; National Heart Lung, Blood Institute GO Exome Sequencing Project, Lung GO, Gibson RL, Bamshad MJ: Exome sequencing of extreme phenotypes identifies DCTN4 as a modifier of chronic Pseudomonas aeruginosa infection in cystic fibrosis. Nat Genet 2012, 44:886−889
60. Cutting GR: Modifier genes in Mendelian disorders: the example of cystic fibrosis. Ann N Y Acad Sci 2010, 1214:57−69
61. Collaco JM, Blackman SM, McGready J, Naughton KM, Cutting GR: Quantification of the relative contribution of environmental and genetic factors to variation in cystic fibrosis lung function. J Pediatr 2010, 157:802−807
62. Wright FA, Strug LJ, Doshi VK, Commander CW, Blackman SM, Sun L, et al: Genome-wide association and linkage identify modifier loci of lung disease severity in cystic fibrosis at 11p13 and 20q13.2. Nat Genet 2011, 43:539−546
63. Davies J, Alton E, Griesenbach U: Cystic fibrosis modifier genes. J R Soc Med 2005, 98 Suppl 45:47−54
64. Collaco JM, Cutting GR: Update on gene modifiers in cystic fibrosis. Curr Opin Pulm Med 2008, 14:559−566
65. Sun L, Rommens JM, Corvol H, Li W, Li X, Chiang TA, Lin F, Dorfman R, Busson PF, Parekh RV, Zelenika D, Blackman SM, Corey M, Doshi VK, Henderson L, Naughton KM, O'Neal WK, Pace RG, Stonebraker JR, Wood SD, Wright FA, Zielenski J, Clement A, Drumm ML, Boelle PY, Cutting GR, Knowles MR, Durie PR, Strug LJ: Multiple apical plasma membrane constituents are associated with susceptibility to meconium ileus in individuals with cystic fibrosis. Nat Genet 2012, 44:562−569
66. Li W, Soave D, Miller MR, Keenan K, Lin F, Gong J, Chiang T, Stephenson AL, Durie P, Rommens J, Sun L, Strug LJ: Unraveling the complex genetic model for cystic fibrosis: pleiotropic effects of modifier genes on early cystic fibrosis-related morbidities. Hum Genet 2014, 133:151−161
67. Blackman SM, Commander CW, Watson C, Arcara KM, Strug LJ, Stonebraker JR, Wright FA, Rommens JM, Sun L, Pace RG, Norris SA, Durie PR, Drumm ML, Knowles MR, Cutting GR: Genetic modifiers of cystic fibrosis-related diabetes. Diabetes 2013, 62:3627−3635
68. Bradley GM, Blackman SM, Watson CP, Doshi VK, Cutting GR: Genetic modifiers of nutritional status in cystic fibrosis. Am J Clin Nutr 2012, 96:1299−1308
69. Bartlett JR, Friedman KJ, Ling SC, Pace RG, Bell SC, Bourke B, Castaldo G, Castellani C, Cipolli M, Colombo C, Colombo JL, Debray D, Fernandez A, Lacaille F, Macek M Jr, Rowland M, Salvatore F, Taylor CJ, Wainwright C, Wilschanski M, Zemkova D, Hannah WB, Phillips MJ, Corey M, Zielenski J, Dorfman R, Wang Y, Zou F, Silverman LM, Drumm ML, Wright FA, Lange EM, Durie PR, Knowles MR; Gene Modifier Study Group: Genetic modifiers of liver disease in cystic fibrosis. JAMA 2009, 302:1076−1083