

Genetics of Cystic Fibrosis

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ABSTRACT

Cystic fibrosis (CF) is caused by mutations in the CF transmembrane conductance regulator (CFTR) gene, which encodes a protein expressed in the apical membrane of exocrine epithelial cells. CFTR functions principally as a cyclic adenosine monophosphate (cAMP)-induced chloride channel and appears capable of regulating other ion channels. Mutations affect CFTR through a variety of molecular mechanisms, which can produce little or no functional gene product at the apical membrane. More than 1000 different disease-causing mutations within the CFTR gene have been described. The potential of a mutation to contribute to the phenotype depends on its type, localization in the gene, and the molecular mechanism as well as on interactions with secondary modifying factors. Genetic testing can confirm a clinical diagnosis of CF and can be used for infants with meconium ileus, for carrier detection in individuals with positive family history and partners of proven CF carriers, and for prenatal diagnostic testing if both parents are carriers. Studies of clinical phenotype in correlation with CFTR genotype have revealed a very complex relationship demonstrating that some phenotypic features are closely determined by the underlying mutations, whereas others are modulated by modifier genes, epigenetic mechanisms, and environment.

KEYWORDS: Mutation classification, CFTR mutation screening, genotype-phenotype association, modifier genes, epigenetics

Objectives: Upon completion of this article, the reader should be able to: (1) list the indications for CF genetic testing and the two groups of mutation testing methods; and (2) summarize the important factors contributing to the CF phenotype.

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The past decades have seen a remarkable expansion and development of human genetics, and specifically in molecular genetics, affecting not just the understanding of inherited disorders but the whole of medicine. Methodological and conceptual advances have led to the identification of an impressive number of human disease genes revealing that the traditional understanding of Mendelian inheritance is far more complex. This huge amount of genomic and proteomic information offers

an extraordinary opportunity to gain insight into the biological importance of genetic aberrations and the etiology of diseases permitting early or presymptomatic diagnosis and early initiation of therapeutic interventions. In the coming years, molecular diagnostics will enhance public health worldwide. It will facilitate the detection and characterization of disease, as well as monitoring of the drug response, and will assist in the identification of genetic modifiers and disease susceptibility.

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Cystic fibrosis (CF) has been recognized as a distinct clinical entity for more than 60 years. It is the second most common autosomal-recessive disorder in Caucasians, with an incidence of one in 2500 to one in 1600 and a carrier frequency of 4 to 5%. If two partners are carriers they face a one in four risk of having a child with CF. In African Americans the heterozygote prevalence is estimated at one in 65 and in Hispanic Americans at one in 46, whereas there is insufficient data for the Asian American population.¹ The remarkably high incidence of heterozygotes in the Caucasian population has been proposed to be due to a selective advantage such as an increased resistance to cholera infections² and to typhoid fever.³ Recent data, hypothesizing that CF heterozygosity may prevent melanoma⁴ or breast cancer,⁵ need further population-based and experimental evidence. Although CF is considered a monogenic disorder, studies of clinical phenotype in correlation with the genotype have revealed a very complex relationship. Some phenotypic features are closely determined by the genotype in an essentially monogenic fashion, whereas others are strongly influenced by both modifying genetic factors and the

environment, leading to the realization that a disease phenotype is the sum of variable clinical components that arise from different molecular mechanisms of underlying mutations as well as from influences of secondary disease modifiers (see Dr. Schechter's review of nongenetic influences on clinical outcome in this issue).

CFTR GENE AND PROTEIN

The gene, named the cystic fibrosis transmembrane conductance regulator (CFTR) and identified by positional cloning,^{6,7} is located on chromosome 7q31.2 (Fig. 1A) spanning 250 kb of genomic DNA and containing 27 exons (Fig. 1B). In general, the CFTR gene is transcribed into 6.5 kb messenger ribonucleic acids (mRNAs) (Fig. 1C), encoding a transmembrane protein of 1480 amino acids with a symmetrical, multidomain structure consisting of two membrane-spanning domains, two nucleotide binding folds (NBF1, NBF2), and a central, highly charged regulatory (R) domain with multiple phosphorylation consensus sites (Fig. 1D). These features are characteristic for the large family of adenosine triphosphate

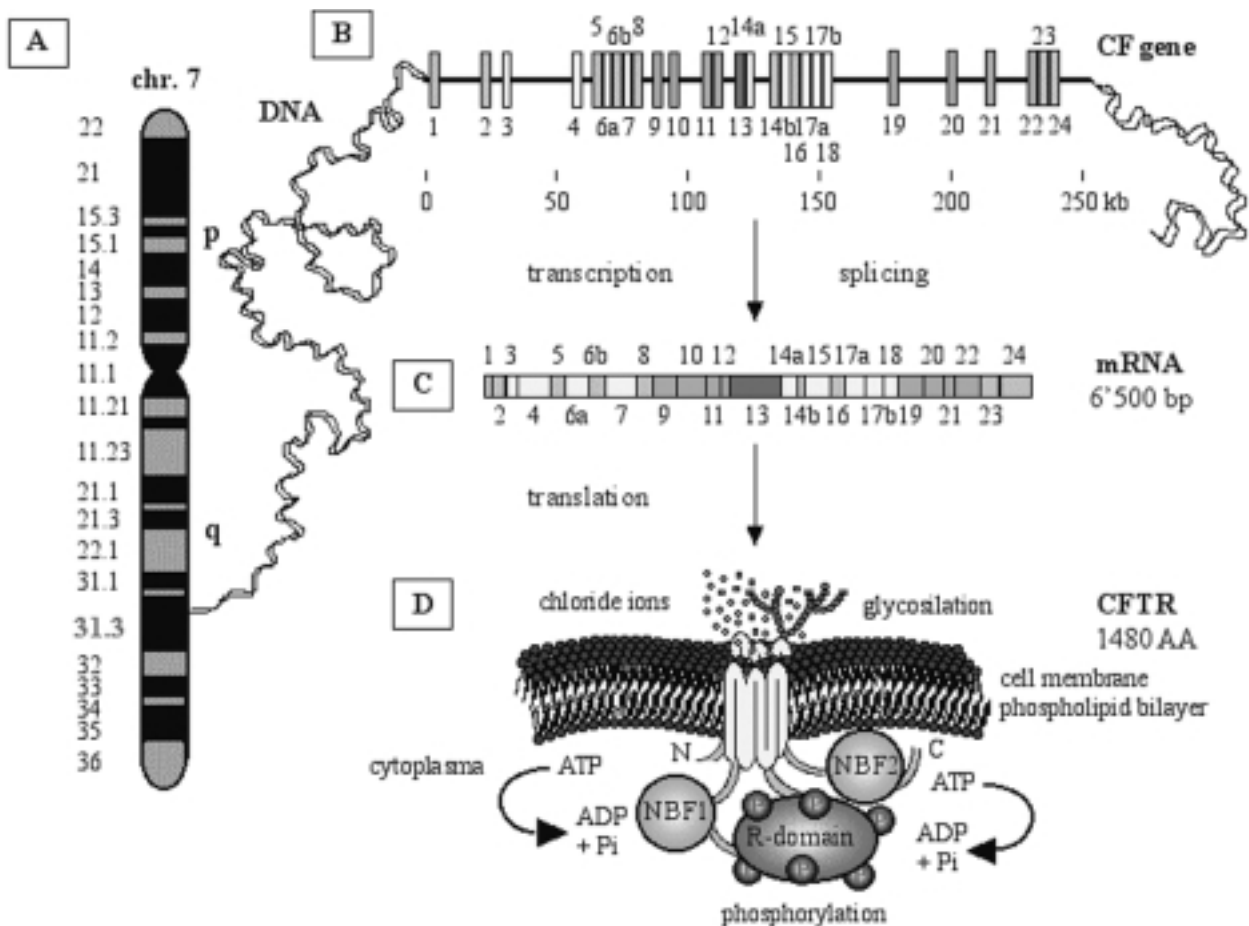


Figure 1 Molecular principle of cystic fibrosis (CF). (A) Chromosomal localization. (B) The CF gene spanning 250 kb of genomic DNA and consisting of 27 exons (coding sequences). (C) The 6.5 kb transcript messenger RNA (mRNA) after processing (capping, splicing, cleavage/polyadenylation). (D) The chloride channel.

(ATP)-binding cassette (ABC) transporters found in bacteria, archea, and eukaryotes.⁸ The R domain, however, is unique for CFTR. The principal function of CFTR is that of cyclic adenosine monophosphate (cAMP)-dependent protein kinase A (PKA)-regulated chloride conductance at the apical membranes of epithelial cells⁹ but has also been implicated in many other processes such as regulation of other ion channels,¹⁰ membrane trafficking, and pH regulation.¹¹ Alterations in the synthesis or sequence of the CFTR gene product lead to a loss of these functions and to the aberrant electrolyte and fluid transport that are hallmarks of the pathology.

CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR MUTATIONS

CF-causing mutations have existed for more than 50,000 years¹² and many are strongly associated with specific European populations. The most common CFTR defect is the ΔF508 mutation,¹³ a 3-bp deletion in exon 10 causing the loss of phenylalanine at the amino acid position 508 of the protein. Worldwide, this allele accounts

for ~66% of all CF chromosomes,¹⁴ with considerably variable frequencies depending on populations and geographical locations. There is a clear northwest to southeast gradient in ΔF508 frequency across Europe, with a maximum of 87.5% in Denmark and a minimum of 24.5% in Turkey. Caucasians in Canada and North America present with a ΔF508 frequency of 68 to 70%, whereas Hispanic populations and American blacks show significantly lower frequencies (47% and 36%, respectively). Worldwide, there are 24 relatively common mutations (identified in more than 50 CF chromosomes) (Table 1) and a few mutations have been found with an unusually high frequency in specific populations (Table 2) indicating founder effect genetic drift. However, there is a broad spectrum of all types of mutations represented by a large number of rare alleles and distributed throughout the entire gene. To date, more than 1000 different alleles have been reported as proven or putative disease-causing mutations to the Cystic Fibrosis Genetic Analysis Consortium (CFGAC; www.genet.sickkids.on.ca/cftr/).

Mutations (missense, nonsense, frameshift, splice, small and large in-frame deletions or insertions) con-

Table 1 Distribution of the Worldwide 24 Most Common Cystic Fibrosis Mutations^a

Mutation	Exon/ Intron	Northern Europe	Southern Europe	North America	South America	Austral- asia	Africa	Asia	Relative Frequency
G85E	E 03	30	14	16	n.a.	n.a.	0	7	0.15
R117H	E 04	62	3	61	n.a.	7	0	0	0.30
621+1G→T	I 04	97	37	154	n.a.	27	0	0	0.72
711+1G→T	I 05	15	13	21	n.a.	n.a.	n.a.	0	0.11
1078delT	E 07	53	2	1	n.a.	1	n.a.	0	0.13
R334W	E 07	18	21	12	n.a.	2	0	0	0.12
R347P	E 07	55	24	26	n.a.	1	0	0	0.24
A455E	E 09	35	0	27	n.a.	n.a.	n.a.	0	0.14
ΔI507	E 10	57	5	20	2	9	0	0	0.21
ΔF508	E 10	14,866	4007	6901	342	2309	351	173	66.02
1717-1G→A	I 10	160	65	44	n.a.	12	0	3	0.65
G542X	E 11	439	259	234	38	56	9	27	2.42
S549N	E 11	18	2	5	1	3	1	0	0.07
G551D	E 11	356	37	206	1	117	0	0	1.64
R553X	E 11	165	44	96	5	11	1	0	0.73
R560T	E 11	40	0	24	0	3	0	0	0.15
1898+1G→A	I 12	41	10	2	n.a.	n.a.	n.a.	0	0.12
2184delA	E 13	14	7	8	n.a.	n.a.	n.a.	0	0.07
2789+5G→A	I 14b	27	10	17	n.a.	n.a.	n.a.	0	0.12
R1162X	E 19	36	68	19	0	2	0	0	0.28
3659delC	E 19	39	1	14	n.a.	n.a.	n.a.	0	0.12
3849+10kbC→T	I 19	23	8	57	n.a.	n.a.	n.a.	16	0.24
W1282X	E 20	120	43	245	n.a.	6	2	120	1.22
N1303K	E 21	209	179	130	11	23	8	29	1.34
Chromosomes screened		21,154	7281	10438	758	3095	515	608	
Detection rate		80.2	66.7	79.9	52.8	83.7	72.2	61.7	

^aAccording to the Cystic Fibrosis Genetic Analysis Consortium, <http://www.genet.sickkids.on.ca/cftr/>. In total 43,849 CF chromosomes have been screened with a mean detection rate of 71%. n.a., not analyzed.

Table 2 Unusually Common Cystic Fibrosis Mutations in Specific Populations^a

Mutation	Exon/ Intron	Ethnic Origin	Number Observed	Total Number Screened	Frequency (%)
296+12T→C	intron 02	Pakistani	02	24	8.33
E60X	exon 03	Belgian	06	394	1.52
G91R	exon 03	French	04	266	1.50
394delTT	exon 03	Scandinavian	78	1588	4.91
457TAT→G	exon 04	Austrian	04	334	1.20
Y122X	exon 04	Réunion Island	14	29	48.27
I148T	exon 04	French Canadian	06	66	9.09
711+5G→A	intron 05	Italian (North East)	06	225	2.67
1078delT	exon 07	Celtic	27	475	5.68
1161delC	exon 07	Pakistani	02	24	8.33
T338I	exon 07	Italian, Sardinian	04	86	4.65
Q359K/T360K	exon 07	Georgian Jews	07	8	87.50
R347H	exon 07	Turkish	04	134	2.98
1609delCA	exon 10	Spanish	03	96	3.12
1677delTA	exon 10	Bulgarian	05	222	2.25
S549I	exon 11	Arabs	02	40	5.00
Q552X	exon 11	Italian (North East)	03	225	1.33
A559T	exon 11	African-American	02	79	2.53
1811+1.2kbA→G	intron 11	Spanish	22	1068	2.06
1898+5G→T	intron 12	Chinese	03	10	30.00
1949delB4	exon 13	Spanish	02	136	1.47
2143delT	exon 13	Russian	04	118	3.39
2183AA→G	exon 13	Italian (North East)	21	225	9.33
2184insA	exon 13	Russian	03	118	2.54
3120+1G→A	intron 16	African-American	14	112	12.50
3272-26A→G	intron 17a	Portugese, French	06	386	1.55
R1066C	exon 17b	Portugese	05	105	4.76
R1070Q	exon 17b	Bulgarian	04	166	2.41
Y1092X	exon 17b	French Canadian, French	11	725	1.52
M1101K	exon 17b	Hutterite	22	32	68.75
3821delT	exon 19	Russian	03	118	2.54
S1235R	exon 19	French (South)	04	340	1.18
S1251N	exon 20	Dutch, Belgian	11	792	1.39
S1255X	exon 20	African-American	02	79	2.53
3905insT	exon 20	Swiss	45	982	4.58
		Amish, Arcadian	13	86	15.12
W1282X	Exon 20	Jewish-Ashkenazi	50	95	52.63
R1283M	exon 20	Welsh	03	183	1.64

^aAccording to the Cystic Fibrosis Genetic Analysis Consortium, <http://www.genet.sickkids.on.ca/cftr/>.

tribute to the phenotype by their type and position in the gene. Therefore, they can be grouped into different classes based on their known or predicted molecular mechanisms of functional consequences for the protein. CFTR mutations have first been subdivided into four groups,^{15,16} and subsequently been expanded and refined to the following six classes.^{17,18}

Class I: Defective Protein Synthesis

This category includes mainly nonsense, frameshift, and splice site mutations resulting in premature termination signals or defective splicing and, as a consequence, producing truncated, deleted, or elongated protein variants. Such proteins tend to be unstable and rapidly degraded and cleared from the cell. In effect, virtually no func-

tional CFTR is reaching the apical membrane of epithelial cells, and therefore, class I mutations are expected to cause severe phenotypes.

Class II: Abnormal Processing and Trafficking

The second class of mutations contains many missense mutations as well as in-frame deletions or insertions, including the $\Delta F508$ deletion. The corresponding proteins fail to be properly processed to a mature glycosylated form and will not, or only exceptionally, appear at the apical membrane. Interestingly, some of the class II mutations (e.g., $\Delta F508$), if correctly processed, possess residual chloride (Cl) channel activity and may lead to a milder phenotype. For this reason, mutations in this group are targets of potential therapies, aimed at correcting the processing and delivery of a mutated CFTR protein to the apical membrane.

Class III: Defective Regulation

Mutations in this class affect the regulation of CFTR function by preventing ATP binding and hydrolysis at NBF1 and NBF2 required for channel activation. Alterations within NBF1, such as the missense mutation G551D, may additionally prevent the regulation of other channels associated with CFTR.¹⁹

Class IV: Decreased Conductance

The fourth class of mutations involves amino acids located within the membrane-spanning domain, which is implicated in forming the pore of the channel and results in a CFTR channel with defective conductive properties. The missense mutations R117H, R334W, and R347P were shown to form a chloride channel with a normal phosphorylation and ATP-dependent regulation, but with reduced single-channel currents.²⁰ Alleles in this class are typically associated with a milder clinical phenotype.

Class V: Reduced Synthesis and/or Trafficking

Various mutations are associated with reduced biosynthesis of fully active CFTR due to partially aberrant splicing (3849+10kbC→T, T5),^{21,22} promoter mutations or inefficient trafficking (A455E).²³ These mutations result in reduced amounts of functional gene products and thus in milder CF phenotypes.

Class VI: Decreased Stability

Nonsense and frameshift mutations (e.g., Q1412X, 4326delTC, 4279insA) causing a 70- to 100-bp truncation of the C-terminus of the CFTR lead to a marked

instability of an otherwise fully processed and functional variant,²⁴ and as a consequence to a severe CF presentation.

The preceding classification categorizes CFTR mutations according to their molecular mechanisms and consequences and can allow the association of a mutation with more than one class. However, the potential of a mutation to contribute to the phenotype depends not only on its type, localization in the gene, and molecular mechanism, including the net molecular effect, but also on its interaction with the second mutation and with intragenic modulators as well as on modifications by epigenetic and environmental factors.

GENETIC TESTING FOR CYSTIC FIBROSIS

Genetic testing should be done in the context of appropriate genetic counseling, and it is the laboratory's responsibility to explain CF testing to the health care provider such that meaningful informed consent from the patients may be obtained. To perform a molecular genetic diagnostic test is a complex process requiring internal quality control systems such as good laboratory practice (GLP) procedures or some form of accreditation. A wide range of mutation testing methods are currently used in diagnostic laboratories²⁵ and can be divided into two groups: specific mutation detection based on the well known spectrum of CF mutations in a defined ethnic group or population and mutation screening methods with a high detection rate and the ability to identify novel mutations independent from allele frequencies and ethnic origin. The most commonly used specific mutation detection methods for CF are heteroduplex (HD) analysis; restriction enzyme analysis; reverse dot-blot; the commercial kits INNO-LiPA CF2, CFTR12, CFTR17+Tn (Innogenetics nv, Gent, Belgium); Elucigene CF4, CF12, CF20 (AstraZeneca Diagnostics, Abingdon, Oxfordshire, UK); and OLA Cystic Fibrosis Assay (PE Applied Biosystems, New Jersey, USA).²⁶ Table 3 indicates the mutations detected by commercial kits. Denaturing gradient gel electrophoresis (DGGE), denaturing high performance liquid chromatography (DHPLC), single-strand conformation polymorphism (SSCP), two-dimensional DNA electrophoresis, and sequencing are mostly used as CF mutation screening methods.^{27,28} There exists no standardization or general preference as to which method should be used, and all available methods have advantages as well as disadvantages requiring considerable skills and experiences. Whatever the method used in a diagnostic laboratory, it has to be thoroughly validated and must be rapid, efficient, and inexpensive while also avoiding the use of radioactivity and toxic reagents.

SSCP analysis is one of the most popular methods for the detection of sequence variants in polymerase chain reaction (PCR) amplified DNA fragments.²⁹ The princi-

Table 3 Cystic Fibrosis Mutations Detected by Commercial Kits

INNO-LiPA	Mutations
CF2	Δ F508, Δ I507, G542X, 1717-1G→A, G551D, R553X, W1282X, N1303K
CFTR12	Δ F508, Δ I507, G542X, 1717-1G→A, G551D, R553X, W1282X, N1303K, S1251N, R560T, 3905insT, Q552X
CFTR17+Tn	394delTT, G85E, 621+1G→T, R117H, 1078delT, R347P, R334W, E60X, 2183AA→G, 2184delA, 711+5G→A, 2789+5G→A, R1162X, 3659delC, 3849+10kbC→T, 2143delT, A455E, (5T/7T/9T)
Elucigene	
CF4	Δ F508, G542X, G551D, 621+1G→T
CF12	Δ F508, G542X, G551D, N1303K, W1282X, 1717-1G→A, R553X, 621+1G→T, R117H, R1162X, 3849+10kbC→T, R334W
CF20	1717-1G→A, G542X, W1282X, N1303K, Δ F508, 3849+10kbC→T, 621+1G→T, R553X, G551D, R117H, R1162X, R334W, A455E, 2183AA→G, 3659delC, 1078delT, Δ I507, R345P, S1251N, E60X
CF Poly-T	5T/7T/9T
OLA	
CF OLA assay	Δ F508, F508C, Δ I507, Q493X, V520F, 1717-1G→A, G542X, G551D, R553X, R560T, S549R, S549N, 3849+10kbC→T, 3849+4A→G, R1162X, 3659delC, W1282X, 3905insT, N1303K, G85E, 621+1G→T, R117H, Y122X, 711+1G→T, 1078delT, R347P, R347H, R334W, A455E, 1898+1G→A, 2183AA→G, 2789+5G→A b

ple is based on the assumption that the electrophoretic mobility of single-stranded DNA in non-denaturing polyacrylamide gels (PAG) depends not only on its size but also on its sequence-dependent folded structure. A single base change can alter this secondary structure and, as a consequence, the electrophoretic mobility of the single strands resulting in band shifts on the gel (Fig. 2). In our laboratory we have developed a two buffer PAGE (polyacrylamide gel electrophoresis) system-based protocol combining SSCP and HD analysis and allowing the detection of at least 97% of all mutations within the entire coding sequence of the CFTR gene (including intron/exon boundaries) independent of their nature, frequency, and geographic or ethnic origin.^{30,31} This protocol represents a general model for rapid and reliable mutation analysis in any genetic disorder and has already been successfully established for 20 different nuclear gene loci^{32,33} as well as for the entire mitochondrial genome.^{34,35}

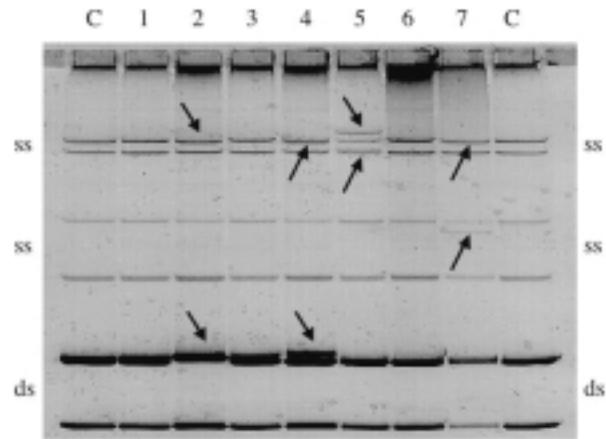


Figure 2 Mutation screening of exon 19 of the cystic fibrosis transmembrane conductance regulator (CFTR) gene using polymerase chain reaction (PCR) followed by single-strand conformation polymorphism/heteroduplex (SSCP/HD) analysis on a silver-stained polyacrylamide gel. ss, single strands; ds, double strands; C, control samples; lanes 1, 3, 6: CF patients without any sequence variants within exon 19; lane 2: CF patient homozygous for the intronic polymorphism nt3600-65 A/A; lane 4: CF patient heterozygous for the nonsense mutation R1162X; lane 5: CF patient heterozygous for the polymorphism nt3690 A/G; lane 7: CF patient heterozygous for the missense mutation S1235R.

For patients with pathological or borderline sweat test and/or typical CF symptoms, where no or only one CF mutation is identifiable on the gene (DNA) level, we offer quantitative and qualitative transcript (RNA) analysis allowing the detection of large deletions, alternatively spliced transcription products, as well as reduced transcriptional efficiency.^{36,37}

Indications for Genetic Testing in Cystic Fibrosis and Related Disorders

Whereas the quality of the laboratory method is a prerequisite for accurate testing, determining the correct indication is an integral part of a successful test result and reliable diagnosis.

1. Diagnostic testing in (a) patients with a definite or possible clinical diagnosis of CF, (b) infants with meconium ileus, (c) males with proven congenital bilateral absence of vas deferens (CBAVD) or suffering from primary infertility
2. Carrier testing in (a) individuals with positive family history, (b) partners of proven CF carriers, (c) gamete donors
3. Prenatal and preimplantation diagnostic testing (on request and after genetic counseling) (a) if both parents are proven carriers and both mutations have been identified, (b) in fetuses presenting with echogenic bowel during the second trimester

Carrier and Neonatal CF Screening

It is now technically feasible to screen large populations for CF carrier status. The central question, however, is whether screening can be implemented in a way that is consistent with widely supported ethical principles. There are guidelines and recommendations created by the American College of Medical Genetics (ACMG), the American College of Obstetricians and Gynecologists (ACOG), and the National Institutes of Health (NIH) agreeing that mutations with a carrier frequency of at least 0.1% in the general population should be screened for, resulting in a panel of 25 mutations for carrier testing.³⁸ CF carrier screening should be made available to any ethnic and racial group following correct information of mutation detectability, informed consent process, and other efficient methods as well as taking into account that genetic screening is always a voluntary personal decision and not a routine medical test.

CF neonatal screening as a method of early diagnosis has been implemented in several states of North America and in different European countries.^{26,39–41} The evolution of tests has featured a progression from immunoreactive trypsinogen (IRT) testing to a two-tier combination of trypsinogen and DNA analysis for either the $\Delta F508$ allele or multiple CFTR mutations.⁴²

In conclusion, although the use of genetic information for population and neonatal screening has great potential, the ultimate goals for any CF screening program must first be identification of the population-specific CF mutational arrays, second, the expansion of counseling and support networks, and third, the implementation of a population-tailored screening protocol. For CF screening programs, ensuring more good than harm is the ultimate objective and duty.

GENOTYPE-PHENOTYPE ASSOCIATIONS AND DISSOCIATIONS

The large number of CFTR mutations, the variable impact of these mutations on the protein, and the heterogeneity of phenotypic expression prompted the search for a correlation between the molecular defect (genotype) of the CFTR gene and the clinical course of the disease.

Gastrointestinal Expression

Of the various gastrointestinal manifestations in CF, exocrine pancreatic insufficiency (PI) is the most common symptom, being present in 85 to 90% of CF patients. There is a close relationship between the CFTR genotype and the pancreatic phenotype, revealing two categories of alleles: (1) “severe” mutations, such as $\Delta F508$ as well as nonsense, frameshift, and splice site mutations, being associated with PI,^{43,44} and (2) a series of missense mutations associated with pancreatic sufficiency (PS) and

defined as “mild” with reference to pancreatic status.^{45,46} There is growing evidence that early identification of patients (e.g., through newborn screening and providing early nutritional support and pancreatic enzyme supplementation) is beneficial for patients with PI.⁴⁷

Expression of other less common gastrointestinal complications such as meconium ileus, liver disease, and diabetes is not as genotype-dependent as the pancreatic function; however, these manifestations are almost exclusively observed in patients with PI and severe genotypes.

Pulmonary Expression

Lung disease is the main cause of morbidity and mortality in CF and most difficult in terms of treatment. Moreover, because of its complexity and patient exposure to a multitude of endogenous and exogenous factors, pulmonary outcome is clinically the most variable as well as the most unpredictable component of the CF phenotype. Studies focusing on the pulmonary status as a function of the $\Delta F508$ allele reported a wide range of effects from detectable impact of CFTR genotype^{43,48} to none or statistically insignificant.^{49–51} Other studies using more refined assessment of CFTR mutations have shown statistically significant correlations between CFTR genotypes and pulmonary status,^{52,53} whereas still others have failed to detect significant association.^{54–56} From our own studies we can conclude that the frameshift 3905insT is associated with a severe pulmonary disease, whereas patients carrying the nonsense mutation R553X present with milder symptoms. However, the group of $\Delta F508$ homozygotes shows the most considerable variation in severity of pulmonary disease.^{57–60} All published data strongly suggest that the pulmonary phenotype may be modulated by other factors such as environment, genetic background (modifier genes, polymorphisms), and epigenetic mechanisms.

Expression in Other Organs

The impact of CFTR genotypes has also been investigated in other organs such as the sweat glands and the reproductive tract in men, indicating that expression of CFTR in different organ systems of the same individual may be highly variable.^{21,61,62} Therefore, although elevated sweat chloride concentration is diagnostic for CF, there are cases that present with normal or borderline sweat electrolyte concentrations, despite the manifestation of other typical symptoms of CF, and, because more than 95% of males with CF suffer from CBAVD or other forms of obstructive azoospermia, it seems that the male reproductive tract is the most sensitive system of all CFTR-affected tissues. Moreover, recent advances in mutation screening technology have led to the detection of an increased incidence of CF mutations in clinical

monosymptomatic diseases resembling the CF phenotype such as obstructive azoospermia, idiopathic pancreatitis, disseminated bronchiectasis, allergic bronchopulmonary aspergillosis, atypical sinopulmonary disease, diffuse bronchiectasis, and sarcoidosis. Taken together, all these findings point to the understanding that a CFTR genotype constitutes only a potential (predisposition) for CF disease, which will to various degrees be expressed and translated into CF pathophysiology. Thus, classic genotype-phenotype studies are important but not sufficient for answering the question of how specific genotypes cause a disease; extended search for modifying factors is needed.

MODIFIER GENES AND EPIGENETIC FACTORS

A multitude of genetic loci and genes have been investigated as modifiers of CF expression at the pulmonary, gastrointestinal, and liver levels, suggesting that several genes could interact in different ways to produce the highly variegated CF phenotype. Some of the genes currently under study as potential modifiers of CF include human leukocyte antigen (HLA) class II antigens, mannose-binding lectin, $\alpha(1)$ -antitrypsin and $\alpha(1)$ -antichymotrypsin, glutathione-S-transferase, nitric oxide synthase type I, as well as inflammatory cytokines and angiotensin I-converting enzyme.^{63,64} A study on highly concordant or discordant $\Delta F508$ homozygous sib pairs showed evidence for the existence of CF modulating genes in the partially imprinted region 3' of CFTR determining stature, food intake, and energy homeostasis.⁶⁵ However, further investigations on large numbers of cases with homologous genotypes and well-defined phenotypes are required to give more reproducible and final data.

Epigenetic regulation involves the maintenance of a particular state of gene expression. Molecular mechanisms for many of the epigenetic phenomena have been elucidated and include DNA methylation, chromatin remodeling, histone modification, and RNA interference. In complex diseases such as CF contribution of epigenetic factors might be substantial. Therefore, DNA sequence variation within genes should be investigated in parallel with epigenetic regulation. In CF, it is well established that polymorphisms in a poly-T tract and a TG repeat in intron 8 of the CFTR gene cause alternative splicing resulting in variable exon 9 skipping.^{22,66} Moreover, several polymorphisms have been reported to lead to alterations in transcription factor binding and therefore to be involved in the modulation of CFTR transcription.^{67,68} A very recent study presents a model of CFTR-promotor-dependent alternative splicing regulation suggesting a kinetic link between transcription and splicing.⁶⁹ In conclusion, it is very likely that a significant number of polymorphisms, transcription factors,

and splicing factors interact to effect the complex, tissue-specific regulation of the CFTR gene.

CONCLUDING REMARKS

Studies of clinical phenotype in CF in correlation with CFTR genotype have revealed a very complex relationship. The extent by which CF phenotype is determined varies considerably from organ to organ. Some phenotypic features are closely determined by the CFTR genotype in a rather monogenic fashion, whereas others are strongly influenced by secondary genetic factors and the environment. Thus, more specific predictions can be made when all available clinical and molecular data are evaluated, intensifying the significance of mutation screening and qualitative and quantitative transcript analyses in indicated cases. In summary, studies in CF make us aware that phenotypic variability in a Mendelian disease is best explained not only by different molecular mechanisms of underlying mutations but also by the involvement of secondary disease modifiers.

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