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Consensus on the use and interpretation of cystic fibrosis mutation analysis in clinical practice

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Abstract

It is often challenging for the clinician interested in cystic fibrosis (CF) to interpret molecular genetic results, and to integrate them in the diagnostic process. The limitations of genotyping technology, the choice of mutations to be tested, and the clinical context in which the test is administered can all influence how genetic information is interpreted. This paper describes the conclusions of a consensus conference to address the use and interpretation of CF mutation analysis in clinical settings.

Although the diagnosis of CF is usually straightforward, care needs to be exercised in the use and interpretation of genetic tests: genotype information is not the final arbiter of a clinical diagnosis of CF or CF transmembrane conductance regulator (*CFTR*) protein related disorders. The diagnosis of these conditions is primarily based on the clinical presentation, and is supported by evaluation of *CFTR* function (sweat testing, nasal potential difference) and genetic analysis. None of these features are sufficient on their own to make a diagnosis of CF or *CFTR*-related disorders.

Broad genotype/phenotype associations are useful in epidemiological studies, but *CFTR* genotype does not accurately predict individual outcome. The use of *CFTR* genotype for prediction of prognosis in people with CF at the time of their diagnosis is not recommended.

The importance of communication between clinicians and medical genetic laboratories is emphasized. The results of testing and their implications should be reported in a manner understandable to the clinicians caring for CF patients.

Keywords

Cystic fibrosis; *CFTR*; Genetic analysis; Diagnosis; Genotype/phenotype correlation

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A huge amount of information has been published on the cystic fibrosis (CF) transmembrane conductance regulator (*CFTR*) gene since it was identified in 1989 [1–3]. Remarkable developments in molecular analysis techniques have resulted in the identification of more than 1500 *CFTR* mutations [4], and high sensitivity mutation panels are now available for many populations or ethnic groups. The great strides in molecular genetics need to be matched by equal progress in clinical interpretation and communication. The meaning of rare mutations in the diagnostic field, genotype/phenotype correlation in the single individual, and the clinical relevance of complex alleles and modifier genes are all issues which often raise more questions than answers. It is often challenging for the clinician interested in CF to interpret the molecular results, and to integrate them in the diagnostic process.

A Consensus Conference organized by the European Cystic Fibrosis Society (www.ecfsoc.org), with the partnership of the European Society of Human Genetics

(www.eshg.org), and the EuroGentest Network of Excellence (www.eurogentest.org), took place in Garda, Italy, on March 23–24, 2007. Its main purpose was to provide the CF clinician with information relevant for the best use of CF genetic testing. Thirty experts in cystic fibrosis and molecular genetics from Europe and North America were involved in pre-conference consultations and in the drafting of preliminary documents, and twenty-three attended the meeting. The conference addressed a wide range of issues, including *CFTR* analysis technical standards, distribution of mutations, complex alleles, the use of genetic testing for the diagnosis of cystic fibrosis, genotype/phenotype correlation including potential impact of modifier genes, and proper communication of the genetic test result. This document is the result of the Consensus Conference.

1. Mutations in the *CFTR* gene and their analysis

1.1. Distribution of mutations

Cystic fibrosis is caused by mutations in the *CFTR* (ABCC7) gene [1–3]. The most common mutation is ΔF508del, previously termed ΔF508, which accounts for approximately two thirds of all *CFTR* alleles in patients with CF, with a decreasing prevalence from Northwest to Southeast Europe [5–10]. The remaining third of alleles are substantially heterogeneous, with fewer than 20 mutations occurring at a worldwide frequency of more than 0.1% [4,11]. Some mutations can reach a higher frequency in certain populations, due to a founder effect in religious, ethnic or geographical isolates [12,13] (Table 1 and Table 2).

The majority of *CFTR* mutations have been associated with European-derived populations [6]. There are also *CFTR* mutations in non-European populations, such as African and East Asian populations [14], but no alleles have reached the high frequency of F508del.

Overviews of the distribution of *CFTR* mutations causing CF have been produced by WHO [11] and Bobadilla et al. [15]. In approximately two thirds of all instances the entire *CFTR* coding region has been analysed, and therefore in these reports some mutations which were easier to screen for are over-represented, compared to those which are more difficult to determine from the technical point of view. The reviews do not contain data on intra-*CFTR* rearrangements, and have limited information on mutations occurring in non-European-derived populations. A project for updating the previous WHO review is currently in progress.

To date more than 1500 sequence alterations have been identified in the *CFTR* gene and their listing is continuously updated within the Cystic Fibrosis Genetic Analysis Consortium (CFGAC) database [4]. In the database missense mutations account for 42%, frameshift for 15%, splicing for 12%, nonsense for around 10%, inframe insertions/deletions for 2%, large insertions/deletions for 3%, promoter mutations for 0.5%, and sequence variations which are not predicted to be disease-causing for 15% of all alleles. *De novo* mutations and uniparental disomy of chromosome 7 bearing a mutated *CFTR* gene are exceptional events.

Conclusion

CFTR mutations vary in their frequency and distribution in different populations. Very few mutations have a worldwide frequency above 0.1%, but some can reach high prevalence in selected populations.

1.2. Methods in genetic testing

CFTR genetic analysis *panels* should have a mutation detection rate superior to 95%, but the heterogeneity of *CFTR* mutations in various populations makes this goal extremely challenging using the current molecular genetic techniques. Every effort should be made to determine the

frequency of specific CF mutations within the target population and to provide testing with reasonably high sensitivity levels. Laboratories that service a particular ethnic population should consider including ethnic-specific mutations into their panel [16]. Different testing panels might be employed for identification of *CFTR* mutations in patients diagnosed with CF, as opposed to relatives of CF patients, where the family specific mutation should be tested first. An extended mutation panel may be appropriate for certain diagnostic testing purposes, but it is not currently recommended for routine carrier screening [17].

Evidence has accumulated that a group of *intragenic rearrangements* (i.e. large deletions and to lesser a extent insertions) [18] account for about 1–3% of all *CFTR* mutations. The large deletions and/or insertions so far detected are mainly localised around *CFTR* exons 2–3 and 17b. These deletions are rare, with the exception of CFTRdele2,3 (21kb), which accounts for 6% of all CF alleles in Slavic populations [19]. These alleles are optimally examined either by Multiplex Ligation-dependent Probe Amplification (MLPA) or Quantitative Fluorescent Multiplex PCR [20,21].

Mutation *scanning* approaches (DGGE, DHPLC, HRMCA) have been designed to explore the gene exon by exon for abnormalities, most often as a second-step analysis after screening for frequent mutations. Sequencing is then used to characterize the abnormal pattern. Scanning techniques are still widely used although it has become feasible to sequence the entire *CFTR* gene in the diagnostic setting [22]. However, mutation scanning techniques result in a considerable risk of missing mutations, in particular homozygous alleles, and even with complete gene sequencing a small risk remains. Sequencing is also used when the mutation under investigation cannot be detected by alternative direct techniques, e.g. after the population specific panel has been exhausted and/or mutations that occur at a frequency of at least 0.5% have been excluded. Once a rare missense mutation has been identified, caution has to be exercised in order not to over interpret its significance to disease causation.

The combined use of all these techniques cannot guarantee to detect both mutated alleles in all patients with CF [18,23–25], 1–5% of CF alleles remaining undetermined. Currently used *CFTR* mutation analysis protocols are not capable of identifying *CFTR* regulatory mutations located in sites distant from the gene or embedded in the genes' non-coding regions [26]. At present, research is ongoing to identify eventual mutations in potential regulatory elements, including the gene promoter [27], highly conserved DNA segments, DNAse hypersensitive sites, and/or distant regulatory elements [28]. Mutations of this type are expected to abolish or reduce production of functional *CFTR* [29], and could be associated with a clinical spectrum from classic CF to *CFTR*-related disorders.

In a small number of CF families, in which only one or no *CFTR* mutations have been identified, *segregation analysis* of *CFTR* polymorphic non pathogenic markers may provide further helpful information for identifying disease genes. Multiple informative markers are available both in the *CFTR* gene and flanking the gene. It is recommended that more than one marker be included in the analysis, and the diagnosis of CF in the index case should be clearly defined. Ideally, one should use intragenic and extragenic markers, since the risk for unidentified recombination between marker and mutation is lower if flanking markers are concordant. Analysis of variable markers at loci on different chromosomes should also be performed when offering prenatal diagnosis as an added safeguard for maternal contamination.

Conclusion

Current analytic methods to detect mutations in the *CFTR* gene range from panels containing the most common disease-causing alleles to extensive scanning/sequencing protocols that enable the detection of novel mutations and to methods to identify large rearrangements.

However, even the most extensive *CFTR* mutation screening tests fail to detect all CF alleles in most CF populations.

1.3. Quality control

DNA-based analyses are carried out in a variety of settings, including academic research laboratories, clinical molecular genetics laboratories, and private laboratories. As commercial kits are only available for limited number of mutations and are expensive, many laboratories, especially in less developed countries, use their own in-house test methodologies, which are usually not amenable to automation and rigorous standardization. These approaches have a higher risk of producing invalid results.

In Southern European populations, which show high molecular heterogeneity, commercial mutation panels cover only 50–75% of alleles and scanning/sequencing techniques may be used in genetic analysis performed for CF diagnostic purposes [8,30].

The diagnostic services should be organized at two levels: Level 1 (i.e. “local testing”) where only frequent mutations are tested, ideally reaching 75–85% detection rate of CF-causing alleles in patients with a clearly defined phenotype, and Level 2, represented by a limited number of national and/or regional expert laboratories that test for less frequent mutations using more sophisticated technologies in order to achieve the highest possible mutation detection rate [31–33]. Each laboratory must show that its tests can consistently deliver the correct genotype under normal working conditions. In order to evaluate the quality of genetic testing for cystic fibrosis, a Cystic Fibrosis External Quality Assessment (EQA) scheme has been set up [34]. EQA schemes seek to educate participants, so that they can achieve high quality standards, and regular participation in EQA is a vital part of the quality assurance process and a requirement for laboratory accreditation. Different sources of errors include administration errors, technical errors or misinterpretation of technically correct results. The use of a commercial kit alone does not ensure high accuracy of mutation analysis, which stresses the importance of validating all genetic tests in the laboratory, and the need for laboratory networks at regional or national levels.

Clinicians should be aware that the quality of the test is associated with diagnostic validation procedures and continuous improvement of laboratory quality using the latest ISO norms or National Molecular Genetic Agreements. The minimal standard for countries without a national agreement system is “certification” (e.g. ISO 9001), while optimally the laboratory should be accredited according to ISO 17025 or 15189, which assures sustained and continuous improvement of the standards of laboratory testing. The EuroGentest FP6 Network of Excellence (www.eurogentest.org), together with Orphanet (www.orpha.net), is launching a Quality Assurance database where the referring clinicians will be able to select a laboratory that is accredited or certified.

Recommendation

Referring clinicians should use molecular genetic diagnostic services from certified and/or accredited laboratories.

2. The role of genetic analysis for establishing the diagnosis of CF

The diagnosis of CF is based on a consistent phenotype plus evidence of *CFTR* channel dysfunction (abnormal sweat chloride concentration or nasal potential difference), or identification of two CF-disease causing mutation in *trans* [35]. The sweat test with chloride determination is still the gold standard for confirming a CF diagnosis [35,36]. In the majority

of cases the diagnosis of CF is clear and easily made: the clinical features are typical, and the abnormal sweat chloride values support the clinical diagnosis. In such situations, genetic analysis is not strictly necessary, although it may be useful to confirm the diagnosis, and to enable carrier testing and prenatal diagnosis within the family. In a smaller fraction of patients, particularly those that carry one or more mutations that confer exocrine pancreatic sufficiency, sweat chloride concentrations may be within the reference range for unaffected controls. In some of these patients, provided they have consistent clinical features, genetic analysis may support a diagnosis of CF [35–37].

From a formal genetic perspective, the term “mutation” is defined as a molecular alteration in the DNA sequence of a gene. Accordingly, this definition makes no assumptions concerning the potential effect of a mutation on the expression or function of the protein product. Thus, in terms of clinical consequences mutations can be neutral, deleterious or even beneficial. Another much used term, polymorphism, is defined as a DNA sequence alteration which has a frequency of at least 1% in the general population. Given their high frequency, polymorphisms were previously thought to have no clinical consequences. This assumption is clearly incorrect. Several polymorphisms in *CFTR* are known to influence disease severity in subjects with CF and CFTR-related disorders [38,39]. Furthermore, polymorphisms in other genes, termed modifier genes, clearly contribute to the severity of CF disease.

CFTR mutations may be clustered into four groups according to their predicted clinical consequences:

- **A.** Mutations that cause CF disease
- **B.** Mutations that result in a CFTR-related disorder
- **C.** Mutations with no known clinical consequence
- **D.** Mutations of unproven or uncertain clinical relevance.

There is some overlapping of groups A and B, as some mutations may sometimes be detected in association with pancreatic sufficient CF, some other times with CFTR-related, mono-symptomatic disorders. Individuals carrying mutations like D1152H together with a CF-causing mutation like F508del may show a clinical spectrum ranging from CBAVD to CF with sufficient pancreatic function but fully expressed lung disease. Factors such as the age related progression of the disease, the environment, and modifier genes, all play a role in the clinical heterogeneity of patients carrying these “borderline” mutations.Table 3 lists some *CFTR* mutations with regard to their clinical consequences.

Conclusion

Mutation analysis may be used for making a diagnosis of CF in individuals with a consistent clinical picture. *CFTR* mutations may: A. cause CF; B. be associated with CFTR-related disorders; C. have no clinical consequences; D. have unknown or uncertain clinical relevance.

2.1. Mutations that cause CF

Ex vivo functional evaluation of the *CFTR* protein can help to predict the disease-causing implications of a sequence variation. *In vivo* assessment of *CFTR*-mediated chloride transport can be indirectly assessed by sweat chloride concentrations, nasal potential difference or rectal biopsy measurement of chloride transport.

Empiric evidence for the most common mutations and/or a clear pathogenic molecular mechanism for *insertions*, *deletions*, and *nonsense mutations*, strongly indicate that such mutations belong to the CF-causing group.

Splicing mutations that completely abolish exon recognition (such as 621 + 1G > T, 711 + 1 G > T and 1525-1G > A) give complete absence of correctly spliced transcripts, and thus belong to the CF-causing group [40,41]. Splicing mutations that still result in a fraction of correctly spliced transcripts, together with aberrantly spliced transcripts, may belong to the CF-causing or *CFTR*-related disorders associated group (e.g. 3849+10kbC>T, 2789+5G>A, 3272-26A>G, IVS8-T5) [42–44]. Patients carrying these mutations often have a relatively mild phenotype, yet with variable disease expression, from minimal lung disease, pancreatic sufficiency and male fertility to a relative severe disease in all the involved organs [45,46]. This variable disease expression is inversely correlated with the quantity of correctly spliced transcripts, i.e. lower levels are associated with a severe disease, while higher levels are associated with a milder phenotype (Table 4).

The quantity of correctly spliced transcripts may also differ among various organs of the same patient, contributing to differential organ disease severity [28,47–52]. Several groups studied the IVS8-T5 allele in the respiratory epithelium and epididymis [49,50], showing that in CBAVD males the level of correctly spliced transcripts is lower in the epididymis than in the respiratory epithelium (10–24% versus 26–37%), whereas in an infertile male CF patient with a severe lung disease the level of correctly spliced transcripts was low in both tissues. A similar correlation was also shown for the 3849 + 10kbC > T mutation [51].

The extent of abnormal splicing in a patient is not determined in routine diagnostics tests, because cells expressing *CFTR* are difficult to obtain in sufficient quantities, and their analysis is very laborious. Besides, the percentage of correctly spliced transcripts in patients with mild disease varies within a large range of 4% to 50%, whereas severe disease is caused by lower levels of correct transcripts.

The clinical role of *missense mutations* is extremely difficult to assess. The identification of a missense mutation in a single family/individual makes it impossible to determine with certainty whether it has any consequential effect. Several missense mutations are commonly seen in the general population but also, more frequently, in patients with CFTR-associated disorders. For a small fraction of mutations, the functional consequences have been determined in research projects, and these findings can be used to predict the most likely consequences of such a mutation.

These difficulties account for the lack of a clearly established or substantiated knowledge of the pathogenic potential of the majority of the known *CFTR* mutations. However, a general acknowledgement [36] has been agreed upon that a sequence variation may be predicted to cause CF if:

- it causes a change in the amino acid sequence that severely affects *CFTR* synthesis and/or function
- it introduces a premature termination signal (insertion, deletion or nonsense mutation)
- it alters the invariant nucleotide of intron splice sites.

Other criteria can be used to asses the pathogenic potential, but they provide a lower degree of certainty. Some of them include:

- the sequence variation causes a novel amino acid sequence that does not occur in the normal *CFTR* gene from at least 100 carriers of CF mutations from the patients ethnic group

- the sequence variation is detected in a set number of unrelated individuals with CF
- the sequence variant changes a highly evolutionarily conserved amino acid residue
- the sequence variation creates a novel/cryptic splice site
- similar sequence variations are found in other *ABC* genes.

Indirect evidence that a *CFTR* sequence variations does not cause CF is:

- the other allele is carrying a well know CF-causing mutation in a clearly asymptomatic individual
- a silent exonic sequence variation, without *a priori* splicing modification
- an intronic sequence variation outside the known consensus sites and which does not create a splicing site
- frequency in the general population equal to or above 0.4% [53].

Conclusion

Mutation analysis is not the answer to every diagnostic dilemma: its limitations and role must be understood by the clinician, who has to interpret and use it in the context of the clinical setting. The majority of the *CFTR* mutations have not been functionally characterized, and for most the pathogenic potential is not clear.

2.2. Complex alleles

Genetic analysis may result in the identification of a common CF-causing mutation and a rare *CFTR* mutation or of two *CFTR* mutations. Under these circumstances, segregation analysis should be performed in the family of the patient. If the two mutations are on the same parental *CFTR* gene, it is said they are in *cis*; if each mutation is on a different parental *CFTR* gene, it is said they are in *trans*. When the two mutations are in *cis*, CF may not be confirmed, and the search of another allelic mutation, located in *trans* of the other two, should be continued. *CFTR* genes that carry at least two functional DNA alterations in *cis*, are called “complex alleles”. The best understood complex alleles of the *CFTR* gene are the associations of intron 8 (IVS-8) variants TG13-T5, TG12-T5 and TG11-T5, of R117H-T5 and R117H-T7, and of mutations I148T and 3199del6.

A. TG13-T5, TG12-T5 and TG11-T5 variants—Two polymorphic tracts are found in front of exon 9, a T(n) tract and TG(n) tract. At the Tn locus, three common alleles may be detected: T5, T7 and T9, and a much rarer T3 [54] (respectively a stretch of 5, 7, 9 and 3 T-residues). A T5 *CFTR* allele can be associated with TG11, TG12, TG13, and, exceptionally, TG15 (respectively 11, 12, 13, and 15 TG repeats) [55]. The number of T and/or TG repeats within these polymorphic tracts affect the extent of correct splicing of exon 9: low numbers of T-residues and high numbers of TG repeats give rise to less efficient splicing [42,56,57]. Transcripts that lack exon 9 sequences fail to mature [58,59].

About 5% of the *CFTR* genes in the general Caucasian population carry the T5 allele [60]. In most T5 *CFTR* genes, the number of TG repeats found in *cis* determines whether the amount of functional *CFTR* proteins that will be translated does fall above or below the critical level for normal *CFTR* function [56,38]. A TG12-T5 or TG13-T5 *CFTR* gene found in compound heterozygosity with a CF-causing mutation, or possibly even in homozygosity, will in general result in a *CFTR*-related disorder, such as Congenital Bilateral Absence of the Vas Deferens (CBAVD) or chronic idiopathic pancreatitis. Some CBAVD patients may develop mild lung symptoms. In exceptional cases, TG12-T5 and TG13-T5, may cause a mild form of CF [61].

A TG11-T5 *CFTR* gene is highly unlikely to cause disease. Approximately 90% of the T5 *CFTR* genes found in CBAVD patients associate with TG12 or TG13, while about 10% associate with TG11 [56,38,62].

B. R117H-T5 and R117H-T7 complex alleles—R117H is a relatively frequent mutation in CF patients worldwide [11]. R117H can be in *cis* with either T5 or T7 [63]. R117H-T5 will result in less functional *CFTR* than R117H-T7 [42]. When found in compound heterozygosity with a CF-causing mutation, or possibly even in homozygosity, R117H-T5 generally results in pancreatic sufficient CF, while R117H-T7 may result in a mild form of CF, obstructive azoospermia, or no disease at all.

In newborn screening programs, up to 7% of the newborns who have an elevated immunoreactive trypsinogen test and two mutations are compound heterozygous for R117H-T7 and a CF-causing *CFTR* mutation [64]. In their first years of life, these children have shown no major signs of CF, although it cannot be excluded that they may develop manifestations of CF disease in adulthood [65].

C. I148T-3199del6 complex alleles—Soon after the identification of the *CFTR* gene, the detection of mutations was time-consuming and expensive. If a missense mutation which involved a highly evolutionarily conserved amino acid, and which was not found in a set of control individuals, was identified in a CF patient, it was defined as a CF-causing mutation. In most instances, such a *CFTR* allele was then not further investigated, since the underlying mutation was assumed to have been identified. Besides, there was a general consensus that only a limited number of mutations would be found in CF patients.

On these grounds, the I148T sequence variation [66] was initially classified as a frequent CF-causing *CFTR* mutation. The American College of Medical Genetics (ACMG) therefore included the I148T mutation in its mutation panel of 25 mutations that were recommended to be screened in CF carrier testing [67]. Since then, however, it has been found that I148T is a neutral polymorphism [68–70]. A second mutation, 3199del6 is the underlying mutation in *cis* in most I148T *CFTR* genes of CF patients. Since only 1% of all I148T *CFTR* genes in the general Caucasian population carry the 3199del6 mutation, I148T should not be routinely screened for. In 2004, ACMG recommended to remove I148T from the mutation panel [17].

Conclusion

A TG13-T5 or TG12-T5 CFTR gene found in compound heterozygosity with a CF-causing mutation may result in CFTR-related disease, or even in a mild form of CF. ATG11-T5 CFTR gene is highly unlikely to cause disease.

When R117H is detected, the T5 or T7 status should also be established. In compound heterozygosity with a CF-causing mutation, or in homozygosity, R117H-T5 generally results in pancreatic sufficient CF, while R117H-T7 may result in a mild form of CF, CBAVD, or no disease at all.

I148T alone is a neutral polymorphism and does not cause CF disease.

2.3. Non-CFTR genes causing CF-like clinical syndrome

Not in every patient with CF can a mutation be identified on both *CFTR* genes. This happens in 1–1.5% of the *CFTR* alleles of patients with fully expressed disease from Northern Europe and in an even higher proportion of *CFTR* alleles from Southern Europe [11]. As mentioned before, the inability to detect these mutations may be partially explained by the fact that the currently most advanced *CFTR* genetic tests study only the coding region and the adjacent

exon/intron junctions. Mutations located in the intronic and promoter regions as well as distant regulatory sequences are not routinely screened for, and may therefore be missed.

However, there is emerging evidence that genes other than *CFTR* may cause a disease clinically indistinguishable from CF. In a German family, no mutation could be identified in both *CFTR* genes of a CF patient, and his sister, who had inherited the same *CFTR* genes from their parents, was not affected [71]. In two American families, each of them having two affected sibs, no mutations could be found on both *CFTR* genes, while both sibs did not inherit the same parental *CFTR* genes [25].

CF is characterized not only by defective chloride secretion, but also by increased sodium absorption in the airways. Sodium transport is mediated through the amiloride sensitive epithelial sodium channel, ENaC, which is made of the 3 subunits SCNN1A, SCNN1B and SCNN1G. Over expression of SCNN1B results in increased airway epithelial sodium absorption and CF-like lung disease in mice [72]. There is evidence that mutations in SCNN1B may cause CF-like disease in a small fraction (about 10%) of CF patients in whom a *CFTR* mutation cannot be found on both *CFTR* genes [73]. The genetic factor(s) causing CF-like disease in the other patients remains unknown.

Conclusion

There is some evidence that other genes may cause CF-like disease in a small fraction of patients. These findings are too preliminary to be used in a clinical context.

3. Genetic analysis for outcome prediction

CF is characterized by a wide range of clinical expression. If not detected by neonatal screening, patients are diagnosed with various modes of presentation, involving a variety of CF-affected organs, at different ages, from birth to adulthood. In addition, there is considerable variability in the severity and rate of disease progression of the involved organs. Although the majority of CF patients are diagnosed in the first year of life with signs and symptoms of malnutrition, pancreatic insufficiency (PI) or typical lung disease, an increasing number of patients have been diagnosed in adolescence or adulthood.

The heterogeneous range of phenotypes can be attributed to various factors, such as *CFTR* genotype, modifier genes and environmental factors, including the beneficial and harmful effects of treatment [74]. The beneficial contribution of therapy is exemplified by the progressive improvement in survival of CF patients over the last 5 decades, which can be attributed entirely to changes in approach to therapy of patients, including provision of pancreatic enzymes, intensive nutritional support, and antibiotics for pulmonary infections, aggressive physiotherapy and multidisciplinary care [75]. Although these environmental modifiers have had a profound effect upon the survival of CF patients as a whole, considerable variation in outcome continues to exist among individual patients, even among those with the same *CFTR* genotype receiving similar levels of care.

There is substantial interest in the influence of the *CFTR* genotype on phenotype. The relative impact of *CFTR* genotype on clinical phenotype is organ specific, with the vas deferens being most sensitive to a small reduction in *CFTR* function and the lung less influenced [76]. The initial studies of genotype and phenotype relationships focused on patients carrying F508del. Patients homozygous for F508del usually have more pronounced clinical manifestations compared to compound heterozygotes and genotypes without F508del [77–80], although these differences are highly variable [77,81,82]. Patients homozygous for the F508del mutation have

an earlier diagnosis of disease, higher sweat Cl^- levels, younger current age and are more likely to be pancreatic insufficient.

In order to collect adequate numbers of patients for comparative analyses, genotype–phenotype correlations have been undertaken using the *CFTR* mutations classification of five classes, which is shown in Table 5 [83–85]. In general, patients homozygous for class I–III mutations exhibit a phenotype associated with pancreatic insufficiency, higher frequency of meconium ileus, premature mortality, earlier and more severe deterioration of lung function, higher incidence of malnutrition and severe liver disease. Class IV–V mutations are usually associated with milder lung disease, older age at death, pancreatic sufficiency [77,86,44]. Class IV–V mutations are phenotypically dominant when occurring in combination with class I–III mutations. As discussed in more detail below, these differences are not fully explained by clinical measures of lung function, nutrition, and pancreatic insufficiency, suggesting that the *CFTR* genotype is an independent predictor of survival.

These broad genotype/phenotype associations are useful in epidemiological studies which relate the severity of CF to the class of mutations. While this functional classification of *CFTR* mutations is widely used in the scientific literature to establish statistical associations, it was not developed as a clinical tool for individual prognostic predictions. Since there is considerable variability in phenotype, even with patients carrying the same genotype, the use of genotype to make statements of prognosis is not recommended. First, the number of mutations so far identified in the *CFTR* gene is exceedingly high, and most of them are too rare to draw statistical conclusions regarding phenotype. Second, only a fraction of them have been characterized according to their functional consequences. Third, a number of *CFTR* mutations have several functional consequences, and cannot be assigned to one particular class. Finally, patients homozygous for mutations, like F508del, classically associated with a severe and complete form of CF, exhibit an extremely wide range of severity of pulmonary disease mild disease. Such clinical variability is likely to be even more pronounced by clustering together different mutations belonging to the same class.

Conclusion

The CF phenotype is affected by the *CFTR* genotype as well as by other genetic and environmental factors. When patients who are homozygous, or compound heterozygous for a class I–III *CFTR* mutation, are compared to patients who carry at least one class IV–V CFTR mutation, the latter group tends to have less severe disease. The categorization of *CFTR* mutations in five classes is a research tool, and is not predictive of clinical outcome in individual patients. Thus, *CFTR* genotype/phenotype correlations may be useful at a population level to determine associations, but should not be used to indicate prognosis in individual patients.

3.1. CF genotype and pancreatic status

Pancreatic insufficiency (PI) and sufficiency (PS) are clinical terms describing the degree of pancreatic function and are determined by measurement of fat absorption. Pancreatic insufficiency occurs when less than 2% of the exocrine pancreas is functioning, which gives rise to fat malabsorption, thereby requiring exogenous pancreatic enzyme replacement therapy. Pancreatic sufficiency means that the residual exocrine pancreatic function is sufficiently preserved to allow normal fat absorption and therefore enzyme replacement is not required. Pancreatic status is measured by fecal fat, fecal elastase, serum trypsinogen, or quantitative studies of the pancreas. The presence of clinical symptoms suggestive of malabsorption is not an accurate way to determine pancreatic status [87].

There is a strong relationship between CF genotype and pancreatic status: Class I–III mutations (also called “severe” mutations) are associated with pancreatic insufficiency, and Class IV and V mutations (also called “mild” mutations) with pancreatic sufficiency. A class I–III allele, if paired with another class I–III allele, is associated with PI but the presence of one class IV or V allele is usually enough to confer PS [88].

These correlations are not absolute, and some mutations may be associated with either pancreatic sufficiency or insufficiency. Patients identified by neonatal screening and who carry class I–III mutations on both alleles may be pancreatic sufficient at diagnosis, but develop PI within the first 1–2years of life [89]. Later in life, although most patients appear to maintain PS for many decades, a small percentage of patients with pancreatic sufficiency appear to develop insufficient digestive function as they grow older. Recurrent acute pancreatitis or chronic pancreatitis is seen in 20% of PS patients, and PI can result from the repeated inflammatory parenchymal damage [90].

Table 6 lists the pancreatic status associated with the most frequent *CFTR* mutations.

Conclusion

Patients who carry class I–III mutations on both alleles are likely to be pancreatic insufficient, whereas those carrying at least one class IV–V CFTR mutation has a high probability of being pancreas sufficient. Pancreatic sufficient patients tend to have better nutritional status, but are also at significant risk for developing pancreatitis.

3.2. CF genotype and pulmonary involvement

The lung has the greatest variability of disease severity of all organs involved in CF. Modifier genes, environmental and treatment factors all play important roles [78,80,82,88,79,81,91–94]. CF twin and siblings studies show that the proportion of CF variance that can be attributed to genetic factors, i.e. the heritability, ranges from 0.6 to 0.8 [95,96]. This indicates that modifier genes are primarily responsible for variation in pulmonary disease severity in patients living in the same household.

Pulmonary phenotype of CF can be determined by pulmonary function tests (e.g. FEV_1), bacteriological markers (e.g. age at first acquisition of *Pseudomonas aeruginosa*, pulmonary exacerbations requiring IV antibiotics), measurement of structural changes (e.g. High Resolution Computed Tomography scores), and by survival. Since CF lung disease is progressive change over time must be taken into account. Large studies have shown that patients with Class I, II and III mutations have a steeper rate of decline in FEV_1 than patients who carry one Class IV or V mutation [97]. However, the variance in FEV_1 in patients within these two groups is considerable and thus prediction of progression of lung disease in individual patients is not possible [82]. A patient who is homozygous or compound heterozygous for class I–III *CFTR* mutations may present with mild lung disease. On the other hand, patients who carry at least one class IV–V mutations may present with severe disease later in life. Thus, for individual patients, it is impossible to predict reliably the severity of pulmonary disease based only on *CFTR* genotype.

Conclusion

Since the *CFTR* genotype is not a useful predictor of the severity of lung disease in the individual patient, it should not be used as an indicator of prognosis.

3.3. CF mutations and other disease manifestations

Other manifestations of CF include gastrointestinal complications, CF related diabetes, and male infertility.

a. Gastrointestinal complications of CF—These are mainly meconium ileus (MI), distal intestinal obstruction syndrome (DIOS), and CF liver disease. Genotype–phenotype studies of these CF complications are hampered by small sample sizes, as these complications are seen in 5–20% of CF patients, and by difficulty in determining a common definition. This is especially true for CF liver disease, which is insidious in onset, and with no commonly agreed diagnostic criteria. Nevertheless, liver disease, MI and DIOS, occur almost exclusively in patients carrying severe class I–III mutations on both alleles. However, there is no evidence of a phenotype relationship with specific gene mutations [98,99]. Modifier genes seem to play a prominent role in development of MI [100] and CF-associated liver disease [101].

b. Diabetes—The prevalence of CF-related diabetes (CFRD) increases with patient age with an overall prevalence of ~ 5%. Around 15–20% of CF adults have CFRD, almost exclusively PI patients, and thus diabetes is strongly associated with Class I, II and III mutations [98, 102].

c. Male infertility—Obstructive azoospermia is present in the greatest majority of male patients with CF [103,104], mainly because of congenital bilateral absence of the vas deferens (CBAVD), but also following other obstructive anatomic abnormalities [105]. *CFTR* is very important in the normal development of the vas deferens, as relatively minor disruption of *CFTR* function appears to cause CBAVD. Fertile CF men often carry the 3849 + 10kbC > T mutation. This correlation is very strong and does allow prediction of infertility in men with CF who carry mutations other than 3849+10kbC>T.

Conclusion

There is no correlation between any specific *CFTR* mutation and meconium ileus, DIOS, liver disease, and CF related diabetes. These manifestations almost never occur in the presence of a PS mutation.

3.4. CFTR mutations in the context of CFTR-related disorders

The best studied CFTR-related disorders are CBAVD and other forms of obstructive azoospermia, recurrent acute or chronic pancreatitis, and diffuse bronchiectasis [106–108]. A prevalence of *CFTR* mutations higher than expected in the general population has been reported also in rhinosinusitis, allergic bronchopulmonary aspergillosis, and sclerosing cholangitis [44,109–112].

To assess the association between *CFTR* mutations and these disorders is often difficult. The prevalence of *CFTR* mutations depends on the number of mutations tested (e.g. a limited panel or full gene sequencing) and on patients' selection criteria. Besides, these clinical entities may have other non *CFTR* etiologies.

a. Congenital bilateral absence of the vas deferens—Obstructive azoospermia, in which CBAVD is the most common phenotype, is an important cause of infertility and accounts for 1–5% of male infertility. The molecular basis of this condition has been investigated by several groups worldwide with common findings [39,106,107,113–115,116,117]. Shortly after the discovery of the *CFTR* gene, Dumur et al. showed that a large proportion of sterile men with CBAVD carried F508del [106]. Later studies determined that 80–90% of men with

CBAVD carry at least one and 50–60% have two *CFTR* mutations (including the T5 variant). For those men with 2 *CFTR* mutations, usually one is CF-causing, and the other is a CFTR-related disorder associated mutation. The F508del mutation is found in 40% of CBAVD patients, followed by the T5 variant in about 35% [39,62,107,115,116,118,119]. R117H was also frequently reported (about 30%) in these patients [107], usually in association with the T7 variant [63].

b. Pancreatitis—The evidence of an association of *CFTR* mutations with CBAVD led Sharer and Cohn to evaluate the possible role of CFTR in chronic pancreatitis and to show that the frequency of a single mutation was 11 fold greater than the expected frequency in a control population [113,114]. These data were confirmed later by different groups with a frequency of about 30% of chronic pancreatitis patients carrying one *CFTR* mutation and 10–15% being compound heterozygotes [120–124]. F508del was the most common severe mutation with the T5 variant, R117H-T7, L206W, D1152H, R1070Q, R347H, R334W, and 2789+5G>A also seen.

c. Bronchiectasis—*CFTR* mutations were found in 10–50% of series of patients with idiopathic bronchiectasis [108,125–130] This variability may be due to the more heterogeneous nature of bronchiectasis and may depend upon how exhaustive was the search for other etiologies. Although the specific *CFTR* mutations were similar to those seen in men with CBAVD, fewer patients were found to be compound heterozygotes. If the patient is a compound heterozygote, only one mutation is a CF-causing mutation.

Conclusion

A frequency of CFTR mutations higher than in the general population is found in individuals with clinical presentations that do not fulfill the diagnostic criteria of CF, thus called CFTR-related disorders. CF-causing mutations can be detected, but not in both CFTR genes.

3.5. Modifier genes

Identification of genetic modifiers and determination of their clinical effect on CF is expected to become an integral part of assessing disease prognosis, and in some cases may lead to alternative approaches to therapy. Over 30 studies on CF modifier genes have been published since 1998. Most of these studies ascertained very small populations and have not been replicated. Consequently, there have been a number of diverse and contradictory conclusions. The current strategy to clarify this area is to conduct studies on modifiers in large CF patient or family cohorts, in order to increase statistical power of association analyses for various CF clinical traits and specific covariates.

In spite of some discordance in the most representative studies results [131–135], a few genes are well-proven to be CF modifiers (TGFbeta1, MBL2). More research has to be done on these and other putative CF modifiers, and therefore caution has to be exercised in interpreting these results. We do not know yet whether the heritability of CF lung disease variation is due to a few genes having major effects, or many genes with minor effects, or some combination of the two. The present level of knowledge does not allow us to use the results so far achieved in a clinical context. However it is anticipated that advances in this area of CF research will provide a basis for prognostic and therapeutic applications for CF management in the not distant future.

Conclusion

Modifier candidate gene studies have so far identified alleles of real but apparently minor effect. At present time, it does not seem appropriate to test routinely for modulating mutations/genes, as there is no clear clinical relevance, nor therapeutic implications.

4. Communication of the analysis results

CFTR mutation analysis is among the most common tests in molecular genetics laboratories. In spite of the frequency of the test, considerable differences exist in the extent of mutation testing. The way in which the clinician interprets the report will have a major impact on the further clinical management of the tested individual and his family. Therefore it is crucial that the results are presented in a clear and unambiguous way. Different entities, including the International Organization for Standardization [136], the Organization for Economic Co-operation and Development (OECD) [137], and some scientific societies [138,139] have drafted guidelines describing the information that should be included in the molecular genetics laboratory report to ensure that clinicians and patients receive adequate and understandable information.

It is important that the information flows from the laboratory to the clinician, and also from the clinician to the molecular genetics laboratory. In fact, depending on the indications for testing, the analysis and the report will have to be adapted as to include information pertaining specifically to the reasons why the test was requested, and what its potential clinical utility is.

4.1. From the clinician to the molecular analysis laboratory

The Best Practice Guidelines for Reporting Molecular Testing Results included in the OECD guidelines for Molecular Testing [137] states that “as the utility of a genetic test report is often dependent on the accuracy and adequacy of information provided to the laboratory, a request for genetic testing should include all information necessary for the laboratory to perform appropriate testing and interpret results”.

These general guidelines also apply to CF genetic testing. It is very important for the molecular genetic laboratory to obtain information on the ethnicity of the individual to be tested, and clear indications for testing, before proceeding to the analysis. This information will help the molecular genetic laboratory to employ the best possible “targeted” diagnostic protocol. Mutations which are found in CFTR-related disorders, like CBAVD or chronic idiopathic pancreatitis, may be different from those in PI CF. Similarly, some mutations may be rare in the local population, but relatively frequent in other ethnic groups.

The indications for testing can be reduced to confirming a clinically proven CF, investigating a suspicion of CF or of a CFTR-related disorder, and prenatal diagnosis. When testing is requested to investigate the possibility of CF, it may be useful to indicate to the laboratory how strong is the evidence so far collected in favour of CF. Usually, the lower is the mean sweat chloride concentration, the less likely it is to find any mutation in the *CFTR* gene. However, a single CF-causing mutation found by a standard panel together with CF clinical features are highly correlated with the presence of a second deleterious mutation, detectable by more extensive analysis [140].

Conclusion

Integration of clinical and molecular findings is of paramount importance. Laboratories should request information about race/ethnicity, family history, and reason for testing.

4.2. From the molecular analysis laboratory to the clinician

A complete written report should always conclude the testing [137]. The person tested should also receive either a copy of the original laboratory report, or the report will be included in a more comprehensive report of the genetic counselling session or of the hospitalization.

Test results should be reported using the *terminology* described above: disease-causing mutations, mutations of uncertain clinical relevance, and mutations of unknown clinical relevance. Polymorphisms or mutations with no clinical relevance may be misinterpreted by the clinician or the patient, and should not be mentioned in the report. A statement to this effect should be placed in every report.

The *clinical relevance* and the *consequences* of the molecular findings for the individual, and for his/her family should be explained. The information provided should be clear and concise, as too much information may confuse the exact meaning of the result. Referral to a physician belonging to a CF centre or with experience in the care of CF and/or to a genetic counsellor for additional information as well as for further diagnostic work up may be more appropriate.

Laboratories should provide estimated clinical sensitivity and specificity. *Clinical sensitivity* is defined as the proportion of individuals who have CF and who also have a positive CF test with two identifiable CF-causing mutations. Currently, most laboratories will merely rely on published reports. For example, the panel of 23 mutations proposed by the ACMG will identify about 88% of carriers in Caucasians. Thus, about 77% (88% × 88%) of Caucasians with CF (or the same proportion of carrier couples) will have a positive test result (two mutations identified). In case one or no mutation is identified in a CF patient, laboratories should comment on the feasibility of further testing. Laboratories should also be able to provide estimated clinical sensitivities for other defined racial/ethnic groups that may be tested. *Clinical specificity* can be defined as the proportion of negative test results among individuals who do not have CF. Analytical error or variable expressivity of certain mutations can reduce the clinical specificity of the test. Although the clinical expression of most of the commonly tested mutations is known to be highly consistent with a classic CF phenotype, there may be some exceptions. For example, the R117H mutation may produce a quite variable clinical phenotype.

Large intragenic *CFTR* gene deletions and benign variants can lead to incorrect assignment of homozygosity when a mutation is present at the same site on the second allele.

Parental testing to confirm homozygosity is always recommended.

For neonatal screening results, the report will be made available according to the procedures established regionally or nationally for neonatal screening.

Conclusion

A standard molecular analysis laboratory report should be provided for all diagnostic analysis for CF. A check-list for a standard molecular analysis laboratory report and an example of a typical report are shown in Table 7 and Table 8.

4.3. Mutation nomenclature

Mutation nomenclature needs to be accurate and unambiguous. However, the effort of producing a reliable nomenclature has led, in recent years, to wide disparities in the naming of DNA sequence variants. The traditional mutation nomenclature is currently used in the vast majority of research papers, and in the CFGAC Mutation Database. More recently, a completely different nomenclature has been recommended by the Human Genome Variation

Society (HGVS) (Human Genome Variation Society. Nomenclature for the description of sequence variations) [141]. HGVS is an international body for defining gene variation nomenclature under the umbrella of the Human Genome Organization (HUGO) and the International Federation of Human Genetics Societies (IFHGS). HGVS nomenclature is still evolving. The simultaneous use of 'traditional' versus 'HGVS' nomenclature is a potential source of confusion, both in the laboratory and the clinical setting. In this regard, the Cystic Fibrosis European Network has produced the following statement: "It is recommended that the symbol Δ is not used, but F508del and I507del are used instead. At present, we advise using the mutation names of the routinely and widely used *CFTR* mutation database [4]. If HGVS nomenclature is used, reference should be made to the 'traditional' mutation names, to avoid confusion for the referring clinician. HGVS nomenclature can be included in a footnote, if a laboratory considers it essential." [34]. Presently, the parallel HGVS nomenclature will be incorporated into the CFGAC Mutation Database.

Conclusion

Many frequently observed mutations have historical names which do not comply with the HGVS recommendations. Currently most clinicians are familiar with the historical names. Therefore, at present adoption of the standardised system will inevitable result in confusion. It is recommended that laboratory reports include HGVS nomenclature in addition to the traditional name.

4.4. Carrier testing and carrier screening

CF carrier testing is advised in relatives of a person with CF or in the partner of a CF carrier who is planning to have children [142]. Carrier testing of children should be deferred until the child is old enough to make an informed decision about the implications of genetic analysis [143]. In several countries, population carrier testing is also offered to individuals or couples with no family history of CF. For over the counter or direct to consumer testing, pre-test information should be given to the individual being tested and genetic counselling should be made available to him/her to review the exact meaning of the result. In this regard, more information about CF testing should be made available to the general public to prepare them to better understand the relevance and impact of testing. Software programs that teach people about the potential value and limitations of the CF carrier testing may offer some useful insights. Population screening for CF carriers should conform to the same basic quality principles as those which apply for the testing of individuals.

Glossary

Abbreviations

ACMG	American College of Medical Genetics (http://www.acmg.net//AM/)
CBAVD	Congenital Bilateral Absence Of The Vas Deferens
CF	Cystic Fibrosis
CF EQA	Cystic Fibrosis External Quality Assessment
CFGAC	Cystic Fibrosis Genetic Analysis Consortium (http://www.genet.sickkids.on.ca)
CFTR	Cystic Fibrosis Transmembrane Conductance Regulator
DGGE	Denaturing Gradient Gel Electrophoresis

DHPLC	Denaturing High Performance Liquid Chromatography
DIOS	Distal Intestinal Obstruction Syndrome
ENaC	Epithelial Sodium Channel
HGVS	Human Genome Variation Society (http://www.hgvs.org/)
HRMCA	High-Resolution Melting Curve Analysis
HUGO	Human Genome Organization (http://www.hugo-international.org/)
IFHGS	International Federation of Human Genetics Societies (http://www.ifhgs.org/)
MI	Meconium Ileus
MLPA	Multiplex Ligation-dependent Probe Amplification
OECD	Organization for Economic Co-operation and Development (http://www.oecd.org)

Glossary

Allele	alternative forms of a gene at a given locus.
CF-causing mutation	a DNA sequence alteration causing CF, if in *trans* with another CF-causing mutation.
CFTR-related disorders	clinical entities associated with *CFTR* mutations, but where a diagnosis of CF cannot be made by the current standard diagnostic criteria.
Complex allele	more than two functional DNA alterations on a single allele.
Exon	region of DNA within a gene that is transcribed to the final mRNA and that usually contains coding information.
Frameshift mutation	a mutation involving a deletion or insertion that is not an exact multiple of three base pairs, which changes the reading frame of the gene.
Gene scanning	analysis of DNA by indirect means, as opposed to direct identification by sequencing, aimed at identifying sequence alterations.
Gene sequencing	process of recording the exact sequence of nucleotides in a given gene fragment.
In cis	two mutations on the same parental *CFTR* gene.
In trans	two mutations on different parental *CFTR* genes.
Haplotype	a series of alleles found at near loci on the same chromosome.
Intron	a non-coding sequence of DNA that is initially copied into RNA but is cut out of the final RNA transcript.
Missense mutation	a single DNA base substitution resulting in a codon specifying a different amino acid.
Modifier gene	a gene that affects the phenotypic expression of another gene.
Mutation	an alteration in DNA sequence, whose molecular and clinical consequences are not established, and could range from having no consequences to deleterious or beneficial.

Nonsense mutation	a single DNA base substitution resulting in a stop (termination) codon.
Polymorphism	in population genetics, a DNA sequence alteration which has a frequency of at least 1% in the general population. In medical genetics, the term polymorphism is often incorrectly used as a synonym for non disease-causing variant.
Promoter	a DNA sequence located upstream the coding sequence of the gene, which contains information for temporal and spatial activation of the gene.
Segregation analysis	a procedure for investigating the pattern of inheritance of a character within a family.
Splicing	removal of introns in the generation of mature RNA.
Uniparental disomy	situation in which both copies of a chromosome pair have originated from one parent.

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Table 1

Geographical distribution of the most common mutations

E60X	Southern European	S549N	Indian
CFTR	Slavic — Eastern European	G551D	United Kingdom, Central Europe
R75X	Southern European, US-Hispanic	Q552X	Southern European, Italian
394delTT	Nordic — Baltic sea region	R553X	Central European
G85E	Southern Europe	A559T	African-American
406-1G>A	US-Hispanic	R560T	Northern Irish
R117H	European-derived populations	1811+1.6kbA>G	Spanish, US-Hispanic
R117C	Northern European	1898+1G>A	United Kingdom, Central Europe
621+1G>T	Southern European	1898+5G>T	East Asian populations
711+1G>T	French, French Canadian	2143delT	Slavic — Eastern European
711+5G>A	US-Hispanic	2183delAA>G	Southern Europe, Middle Eastern, Iranian, Latin American
L206W	Spanish and US-Hispanic	2184delA	European-derived populations
V232D	Spanish and US-Hispanic	2789+5G>A	European-derived populations
1078delT	French Brittany	Q890X	Southern European
R334W	Southern European, Latin American	3120+1G>A	African, Arabian, African-American, Southern Europe
1161delC	Indian	3272–26A>G	European-derived populations
R347P	European-derived, Latin America	3659delC	Scandinavian
R347H	Turkish	3849+10kbC>T	Ashkenazi-Jewish, Southern European, Middle Eastern, Iranian, Indian
A455E	Dutch	R1066C	Southern European
1609delCA	Spanish, US-Hispanic	Y1092X (C>A)	Southern European
I506T	Southern European, Spanish	M1101K	US-Hutterite
I507del	European-derived populations	3905insT	Swiss
F508del	European-derived populations	D1152H	European-derived populations
1677delTA	Southern European, Middle Eastern	R1158X	Southern European
1717–G>A	European-derived populations	R1162X	Italian, Amerindian, Latin America
V520F	Irish	S1251N	European-derived populations
G542X	Southern European, Mediterranean	W1282X	Ashkenazi-Jewish, Middle Eastern
S549R(T>G)	Middle Eastern	N1303K	Southern European, Middle Eastern

Legend: these alleles occur with a frequency superior to 0.1% in selected populations. Limitations due to small numbers are discussed, elsewhere [144]; “European-derived” indicates presence in Europe, and due to immigration also in America, Australia, etc.

References: [4,15,11,30,144–150].

Table 2

CFTR mutations in selected populations

Regional European		**Middle Eastern**	
2184insA	Central European	I1234V	Bahrain
I336K	Central European	Q359K/T360K	Georgian-Jewish
574delA	Central European, French	3130delA	Iranian
4374+1G>T	Eastern European	H139L	Saudi
S1196X	Estonian, Finish, Russian	1548delG	Saudi
3732delA	Estonian, Finish, Russian	S466X	Turkish, Greek, Iranian, Indian
W846X1	French Brittany	Y569D	UK-Pakistani
Q220X	French, Bulgarian		
S945L	French, Central European	East Asian	
L1065P	French, Italian	1898+5G>T	Chinese, Thai
L1077P	French, Italian	3121-2 A>G	Japanese
4016insT	French, Italian, Swiss	K166E	Korean
Y122X	French, Reunion Island		
2711delT	French, Spanish	Hispanic	
S1235R	French, Spanish	Q98R	
1525–1G>A	German (Thuringia)	663delT	
L346P	Greek Cypriot	H199Y	
E822X	Greek, Turkish	P205S	
R352Q	Irish, Italian	935delA	
852del22	Italian (Apulia)	1288insTA	
T338I	Italian (Sardinia)	2055del9>A	
A561E	Portuguese	2105del13insAGAAA	
45TAT>G	Northern Balkans	3171delC	
P67L	Scottish	3199del6	
1154insTC	Southern European	3876delA	
4005+1G>A	Southern European		
405+1G>A	Southern European	US African-American	
D110H	Southern European	405+3A>C	
E585X	Southern European	444delA	
L558S	Southern European	F311del	
Q493X	Southern European	G330X	
R1066H	Southern European	G480C	
G1244E	Southern European	A559T	
G178R	Southern European	2307insA	
E92K	Southern European, Turkish	3196del54	

Regional European		**Middle Eastern**
R1070Q	Southern European, Turkish	3791delC
W1089X(TAG)	Southern European, Turkish	S1255X
W1204X	Southern European, US-Hispanic	D1270N
1812-1G>A	Southern European, US-Hispanic	
S492F	Southern European, US-Hispanic	Native American
K710X	Southern French, Spanish	D648V
V232D	Spanish	L1093P
712–1G>T	Spanish	R1162X
A1006E	Spanish	
Q890X	Spanish, US- Hispanic	References
2869insG	Spanish-Catalan	[11,15,30,144–150]
2043delG	Turkish, Greek	

CF-causing and CFTR-related disorders associated mutations are included. Some mutations may be found in more than one column.

Table 3

Examples of *CFTR* mutations with regard to their clinical consequences

Mutation group	Examples
A. CF-causing	F508del, R553X, R1162X, R1158X, 2184delA, 2184insA, 3120+1G>A, I507del, 1677delTA, G542X, G551D, W1282X, N1303K, 621+1G>T, 1717-1G>A, A455E, R560T, G85E, R334W, R347P, 711+1G>T, 711+3A>G*, 1898+1G>A, S549N, 3849 +10kbC>T, E822X, 1078delT, 2789+5G>A, 3659delC, R117H-T5 (*),R117H-T7 (*), D1152H (*), L206W (*), TG13-T5 (*)
B. CFTR-related disorders associated	R117H-T7(*), TG12-T5(*), R117H-T5 (*), D1152H (*), TG13-T5 (*), S997F, R297Q*, L997F, M952I, D565G*, G576A*, TG11-T5**, R668C-G576A-D443Y, R74W-D1270N
C. No clinical consequences	I148T, R75Q, 875+40A/G, M470V, E528E, T854T, P1290P, 2752-15G/C, I807M, I521F, F508C, I506V, TG11-T5**
D. Unknown or uncertain clinical relevance	Mainly missense mutations***

* mutations which may belong either to Group A or to Group B.

** mutations which may belong either to Group B or to Group C.

*** certain common sequence (missense) variants with subclinical molecular consequences (e.g. M470V) may co-segregate on the same chromosome and exert more potent, cumulative phenotypic effect. Such polyvariant haplotypes could be potentially disease causing [56].

Only a fraction of mutation and patients have been characterized in detail, and, with the exception of frequent mutations, only limited sample numbers have been available for the study of most mutations. Data shown here have to be interpreted with caution.

Table 4

Splicing mutations generating both correctly and aberrantly spliced transcripts in CF patients

Mutation	Affected exon/intron	Correctly spliced transcripts (%)	Reference
3849+10kbC->T	Intron 19	1–50	[43]
			[151]
IVS8-T5	Intron 8	6–37	[50]
			[61]
2789+5G>A	Intron 14b	4	[152]
3272–26A>G	Intron 17a	5	[47]
1811+1.6kbA>G	Intron 11	1–3	[153]
621+3A>G	Intron 4	~50% (semiquantitative)	[154]
711+3A>G	Intron 5	~70% (semiquantitative)	[154]
2751+2T>A	Intron 14a	30–50% (semiquantitative)	[154]
296+1G>A	Intron 2	~30% (semiquantitative)	[154]
D565G	Exon 12	15–30%	[28]
G576A	Exon 12	22%	[28]
D579Y	Exon 12	>75%? (semiquantitative)	Al-Baba, pers. comm. 2007

Table 5

Classes of *CFTR* Mutations

Class of mutation	Molecular defect of the CFTR protein	Type of mutation	Functional consequence
I	Defective synthesis	Nonsense Frameshift Splice junction	*CFTR* function abolished
II	Defective processing and maturation	Missense	*CFTR* function abolished
III	Defective regulation	Missense	*CFTR* function abolished
IV	Defective conductance	Missense	Residual expression and function
V	Reduced function/synthesis	Splicing defects Missense mutations (e.g. A455E)	Residual expression and function

Examples of mutations classified as class I, II or III:
G542X, R553X, W1282X, R1162X, E822X, 621+1G>T, 1717-1G>A, 1078delT, 711+1G>T, 1525-1G>A, 2751+2T>A, 296+1G>C, 1717-9T>C, 3659delC, F508del, I507del, N1303K, S549N, G551D, R560T, S549I, S549R, S945L, H1054D, G1061R, L1065P, R1066C, R1066M, L1077P, H1085R, V520F, R560S, Y569D.

Examples of mutations classified as class IV or V:
R117H, R334W, R347P, 3849+10kbC>T, 2789+5G>A, A455E, R117C, R117P, R117L, D1152H, L88S, G91R, E92K, Q98R, P205S, 3272-26A>G, IVS8-T5, D565G, G576A, 4006-1G>A, 621+3A>G, 711+3A>G.

Table 6

Main *CFTR* mutations as related to pancreatic status

Usually PI associated mutations	Usually PS associated mutations
F508del	R117H
G542X	R347P**
G551D	3849+10kbC>T
N1303K	A455E
W1282X	R334W**
R553X	G178R
621+1G>T	R352Q
1717-1G>A	R117C
R1162X	3272-26A>G
I507del	711+3A>G
394delTT	D110H
G85E*	D565G
R560T	G576A
1078delT	D1152H
3659delC	L206W
1898+1G>T	V232D
711+1G>T	D1270N
2183AA>G	
3905insT	
S549N	
2184delA	
Y122X	
1898+5G>T	
3120+1G>A	
E822X	
2751+2T>A	
296+1G>C	
R1070Q-S466X*	
R1158X	
W496X	
2789+5G>A*	
2184insA	
1811+1.6kbA>G	
1898+1G>A	
2143delT	
1811+1,6kbA>G	
R1066C	
Q890X	
2869insG	

Usually PI associated mutations	Usually PS associated mutations
K710X	
1609delCA	

PS/PI classification is based on an apparent consensus from literature or from unpublished reports.

* may also be associated with PS.

** may also be associated with PI.

Table 7

Check-list for a standard report

- Laboratory identifiers; (ISO accreditation/certification number if allowed and applicable)
- Title, date
- Name of patient (at least two identifiers; hospital number, sample number, etc)
- Date of birth, gender
- Place of birth/ethnic origin
- Nature of the sample and arrival date

  If the DNA is already extracted, name and address of the lab that extracted the DNA
- Name and address of the referring physician
- Indication for testing or question to be answered
- Test performed, list of mutations tested, sensitivity (mutation detection rate), methods used (in brief)
- Result

  Formal genotype

  Interpretation of result in simple language, including:

    Whether the diagnosis has been confirmed

    Whether counselling and/or further testing is required

    Implications for family members
- Need for further testing for additional mutations based on clinical or genetic motifs
- References to scientific publications
- Signature of lab director, visa/signature of second person (can be electronic)
- Laboratory's standard phrase concerning reproduction of report and scope of results.
- Numbering of pages (1 of 1 …)

Adapted from the recommendations of the Swiss Society of medical genetics (Best practice guidelines on reporting in molecular genetic diagnostic laboratories in Switzerland, 2003) and the quality criteria for reporting of the CF European Network [31].

Table 8

An example of a molecular CF report (modified from a CF network EQA form http://www.cfnetwork.be/)

XX-0X-200X

Doctor X

Street X

City, Country X

Molecular genetic analysis for cystic fibrosis

Name:	Gary BRAUN
Date of birth:	20-06-2006
Gender:	Male
Place of birth:	Hamburg, Germany
Ethnic origin:	Caucasian: mother from Brittany, father from Germany
Reason for referral:	Failure to thrive, chronic diarrhea, two episodes of bronchiolitis and a positive sweat test
Sample received:	3-06-2007
Sample type:	DNA
Sample number:	MUCO-412
Result	Compound heterozygote G551D/R553X

Interpretation

This results shows that Gary Braun is a compound heterozygote for two different cystic fibrosis mutations. This result confirms the diagnosis of cystic fibrosis.

Comments

Cystic fibrosis is an autosomal recessive disorder and therefore both parents of the affected child should be carriers of the disease. It is thus suggested that the parents' DNA should be tested to establish carrier status and origin of each mutation. Once carrier detection is confirmed, this couple has a 25% risk to have an affected child in each pregnancy. Prenatal diagnosis is therefore feasible in this pregnancy and in every subsequent pregnancy of this couple. Since this is an inherited disease, screening for carrier status of other members of their families may also be offered.

The parents of the patient and their families should consult with the referring doctor and a genetic counselor.

Molecular biologist Director

Mutations have been classified according to GenBank Accession Number NM_000492. Nucleotide 1 has been counted as the first nucleotide of transcription leading to nucleotide 133 being the first nucleotide of the translation initiation codon.

The method used: INNO-LiPA CFTR19 and 17+Tn update (reverse dot blot)

Mutations screened for: F508del, I507del, G542X, 1717-1G>A, G551D, R553X, R560T, Q552X, W1282X, S1251N, 3905insT, N1303K, CFTRdele2,3, 711+1G>T, 3272-26A>G, 1898+1G>A, I148T*, 3199del6, 3120 +1G>A, 394delTT, G85E, E60X, 621+1G>T, R117H, 1078delT, R347P, R334W, 2143delT, 2183AA>G, 2184delA, 711+5G>A, R1162X, 3659delC, 3849+10kbC>A, A455E, T5, T7, T9 (*I148T is a neutral polymorphism and does not cause CF disease)

The mutation detection rate is about 90% for the North European Caucasian population and ≤70% for South European Caucasians.

Page 1/1