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# Biochemical and Molecular Genetic Testing Used in the Diagnosis and Assessment of Cystic Fibrosis

Donovan McGrowder
*Department of Pathology, Faculty of Medical Sciences, University of the West Indies, Mona Campus, Kingston, Jamaica*

## 1. Introduction

### 1.1 Mutations in the CFTR gene

Cystic fibrosis (CF) is the most common life-threatening autosomal recessive genetic disease among the Caucasian population, with an estimated incidence throughout the world of between 0.25 - 5 per 10,000 live births (Lewis et al., 1995). It is caused by mutations on both CF transmembrane conductance regulator (CFTR) alleles, resulting in pancreatic exocrine insufficiency in 95% of patients, abnormal sweat electrolytes, sino-pulmonary disease and male infertility (MacCready, 1963). In its classic form, this multi-system disease is characterized by one or more of several features varying in severity, including a progressive decline of pulmonary function secondary to chronic lung infections, pancreatic exocrine insufficiency leading to malnutrition and growth impairment, liver disease, and decreased reabsorption of chloride ions from sweat (Zielenski et al., 2000). The disease is easily diagnosed early in life, through a combination of clinical evaluation and laboratory testing including sweat testing and CFTR mutation analysis (Ross, 2008). However, 7% of CF patients are not diagnosed until age 10 years, with a proportion not diagnosed until after age 15 years. Because the phenotype in these patients may vary widely some of these patients present a considerable challenge in establishing a diagnosis of CF (Wilcken et al., 1995; Hammond et al., 1991).

The heterogeneity of CF disease is partially explained by the identification of 1890 mutations in the CFTR gene (Cystic Fibrosis Mutation Database). The delta F508 (ΔF508) mutation, the most common CF allele is a 3-base pair deletion in exon 10 causing a loss of phenylalanine at the amino acid position 508 of the protein product (Kerem et al., 1989). The ΔF508 mutation reaches frequencies of 70% or more in northern European populations, with lower frequencies in southern European populations. In the United States of America (USA), two-thirds of patients carry at least one copy of the ΔF508 mutation, with approximately 50% of CF patients being homozygous for this mutation (Crossley et al., 1979). Other common mutations existing in most populations include G542X, G551D, R553X, W1282X and N1303K. These mutations have population frequencies of approximately 1 - 2% (De Boeck, 2006).

The CFTR gene consists of a TATA-less promoter and 27 exons spanning about 215 kb of genomic sequence (Zielenski et al., 1991). It encodes a transmembrane protein with a symmetrical, multi-domain structure, consisting of two nucleotide-binding domains (NBD1, NBD2), two membrane-spanning domains (MSD1, MSD2), and a central, highly charged regulatory domain (R) with multiple phosphorylation consensus sites (Riordan et al., 1989). The principal function of CFTR is that of cyclic adenosine-5'-monophosphate (cAMP)-regulated chloride transport at the apical membranes of epithelial cells. It has also been implicated in many other processes such as membrane trafficking regulation of other ion channels and pH, and apoptosis (Quinton, 1999; Sheppard & Welsh, 1999).

Mutations in CFTR may result in: (1) defective processing of CFTR, such as ΔF508 or G480C, where the mutant protein is not processed to its mature glycosylated form and is not correctly localised to the apical membrane, but is retained in the endoplasmic reticulum and degraded, (2) defective CFTR production, such as R553X, due to unstable messenger ribonucleic acid (mRNA) and/or premature protein truncation and/or (3) defective ion channel function, such as G551D or R117H, in which case some of the mutant protein becomes correctly localised but results in either very little residual function (in the case of G551D) or a substantially reduced level of ion transport (in the case of R117H). In each class of mutation the level of functional CFTR at the apical membrane of epithelial cells in CF patients falls below a critical level, resulting in the characteristic clinical abnormalities observed in the organs in which CFTR is expressed (Comeau et al., 2004).

Mutations in CFTR result in abnormalities in epithelial ion and water transport, which are associated with derangements in airway mucociliary clearance and other cellular functions related to normal cell biology (Sontag et al., 2005). Furthermore, mutations in the CFTR gene can alter the structure, function, or production of a cAMP-dependent trans-membrane chloride channel protein that is critical for normal functioning of multiple organs. The organs and systems that are affected in CF include: pancreas, liver, sweat glands, genitourinary and gastrointestinal tracts, the lungs and upper respiratory tract (Welsh et al., 2001). It is the involvement of the latter which leads to most morbidity and is the most common cause of death. A large retrospective cohort study of approximately 17,000 patients from the USA CF Foundation National Registry, confirmed that the CFTR genotype affects mortality (McKone et al., 2003).

Extensive genetic studies have produced both greater awareness of the spectrum of mutations in specific population groups (Alper et al., 2004) and increased the understanding of genotype-phenotype relationships (Groman et al., 2005; Mickle, 2000), illuminating distinctions between CFTR mutations with limited or no functional effects and those known or predicted to cause CF disease. Most classically diagnosed patients with CF carry severe loss-of-function mutations on both alleles and have evidence of pancreatic insufficiency (Kerem et al., 1999). Those with non-classic CF carry a mild CFTR gene mutation on at least one allele, and usually retain sufficient residual pancreatic function to confer pancreatic sufficiency (Cystic Fibrosis Genotype-Phenotype Consortium, 1993; Zielenski, 2000). Both pancreatic insufficiency and sufficiency are associated with specific CFTR mutations.

The CFTR gene mutations have been placed into five classes depending on their effect on the CFTR protein (Welsh et al., 2001). Classes I – III are associated with complete loss of cAMP-regulated chloride channel function and are identified as "severe" mutations. Class I

mutations lead to defective protein production, class II to defective protein maturation and processing, and class III to defective channel regulation/gating. Mutations in classes IV - V might allow for residual CFTR function, and lead to altered channel conductance in class IV and altered protein stability in class V. They are usually associated with milder phenotypes and pancreatic sufficiency (Welsh et al., 2001; Ahmed et al., 2003). Persons who have two mutations from within classes I, II, or III almost invariably experience pancreatic insufficiency, and those with < 2 mutations from classes IV or V usually maintain pancreatic insufficiency. The common ΔF508 mutation is a class II mutation that is associated with pancreatic insufficiency (Ahmed et al., 2003).

The sensitivity of a given DNA mutation panel for detecting persons with CF varies by race and ethnicity as different populations have different mutation frequencies. The inclusion of mutations specific to racial and ethnic minority populations can improve detection of CF among those populations (Bobadilla et al., 2002). Data from US newborn screening programs showed that birth prevalence is 1/2,500 - 3,500 births among non-Hispanic whites, 1/4,000 - 10,000 births among Hispanics, and 1/15,000 - 20,000 births among non-Hispanic Blacks (Comeau et al., 2004; Parad & Comeau, 2003). Non-Hispanic Whites constituted >90% of USA patients who received a diagnosis of CF (Cystic Fibrosis Foundation, 2001).

The spectrum of CFTR mutation frequencies varies in populations of each ethnicity, and a large proportion of CFTR mutations is still unidentified in Hispanic and Black people. Heim and colleagues in their study used a 70- and 86-DNA mutation panel, and reported a detection rate of 62% in Black infants, 58% in Hispanic infants, 38% in Asian infants, and 81% in Native American infants in the USA compared with 85% in White infants and 95% in Ashkenazi Jewish infants (Heim et al., 2001). Identification of infants with CF can be enhanced by choosing an appropriate mutation panel. A 75% detection rate can be achieved in Black populations by screening for 16 "common white" mutations and 8 "common African" mutations (Macek et al., 1997).

This review will examine the advances in adult and newborn screening for CF that are reported in the literature such as the use of genetic testing techniques to identify CFTR gene mutations. It will also critically examine the use of biochemical tests capable of diagnosis, detecting and monitoring the end-organ disease processes in patients with CF. These tests include sweat test, immunoreactive trypsinogen (IRT), nasal potential difference (NPD), pancreatic associated protein (PAP) and intestinal current measurement (ICM).

## 2. Analysis of CFTR gene mutations for diagnostic purposes

### 2.1 Mutations within the CFTR gene

According to The Cystic Fibrosis Genetic Analysis Consortium (1994), the ΔF508 mutation, the most common CFTR defect identified among Caucasians accounted for 66% of 43,849 tested CF chromosomes (The Cystic Fibrosis Genetic Analysis Consortium, 1994). However its occurrence varies considerably between geographical locations and different populations with the lowest reported incidence in Tunisia (17.9%) and highest in Denmark (90%) (Messaoud et al., 1996; Schwartz et al., 1990). The spectrum of remaining CFTR mutations is highly variable and is represented by a large number of rare alleles. All types of mutations are represented (missense, frameshift, nonsense, splice, small and large in-frame deletions or

insertions), and are distributed throughout the entire gene (The Cystic Fibrosis Genetic Analysis Consortium, 1994).

A mutation detection rate of 90% in a specific population signifies that a mutation will be identified on both CFTR genes in 81% of the typical CF patients; a mutation will be found on only one CFTR gene in 18%; and no mutation will be found on either CFTR gene in 1% (De Boeck et al., 2006). Although currently available mutation screening panels can identify 90% of CFTR mutations, 9.7% of genotyped individuals in the Cystic Fibrosis Foundation Patient Registry have at least 1 un-identified mutation (Cystic Fibrosis Foundation Patient Registry, 2005).

### 2.2 Techniques used to analyze and detect CFTR gene mutations

The analysis and interpretation of CF genotype information requires the use of appropriate testing techniques to identify CFTR mutations, standardized criteria for defining a CF-causing mutation, and an understanding of the contribution of the genetic background to the phenotypic variability of CF. Rapid accurate identification of CFTR gene mutations is important for confirming the clinical diagnosis, for cascade screening in families at risk for CF, for understanding the correlation between genotype and phenotype, and moreover it is also the only means for prenatal diagnosis (Kolesár et al., 2008). The scanning of the whole coding region of the CFTR gene permits to identify about 90% of alleles from patients bearing CF and a lower percentage in patients bearing atypical CF. Several techniques such as allele specific oligonucleotide (ASO) dot-blot, reverse dot-blot, amplification refractory mutation (ARMS), and an oligo-ligation assay, are available to detect the most common mutations (Eshaque & Dixon, 2006). The ARMS is routinely used for the identification of specific mutations within genomes. This polymerase chain reaction (PCR)-based assay, although simple, is performed at a low-throughput scale, usually requiring gel-electrophoresis for the identification of specific mutations (Eaker et al., 2005). An extensive mutation screening of both CFTR genes may be required with assays such as single strand conformation polymorphism (SSCP) assay, sequencing, denaturing gradient gel electrophoresis (DGGE) and denaturing high pressure liquid chromatography (DHPLC) (Cuppens et al., 1993; Le Mare´chal et al., 2001). Sequencing approaches 100% sensitivity while the other techniques are indirect mutation scanning assays with sensitivities varying from close to 100% to as low as 90%. Other available commercial assays for CFTR mutation screening include the INNO-LiPA CFTR Assay (Innogenetics NV, Technologiepark, Gent, Belgium), oligonucleotide ligation assay (OLA) Cystic Fibrosis Assay (Abbott Laboratories, Abbott Park, Illinois, USA), and the Elucigene CF Assay (Tepnel Diagnostics Ltd, Oxon, UK). Most of these tests only screen for about 30 mutations, the majority of which are associated with classic CF (Dequeker et al., 2000).

A number of methods have been proposed for the detection of ΔF508. The multiplex ARMS analysis identified the ΔF508 mutation at an allele frequency of 24.0% in Indian CF cases (Ashavaid et al., 2005). Another study has reported that quantitative real-time PCR with melting curve analysis is a reliable and fast method for the detection of ΔF508 mutation. By using this method, the results are ready in 1 h following the DNA isolation. The applied primer-probe set with melting curve analysis gives additional information for the presence of other mutations in the ΔF508del region (Nagy et al., 2007). Furthermore, the ΔF508

mutation has been identified by PCR-SSCP. The appropriate 98 bp region of the CFTR gene was amplified by PCR and the reaction products were analysed by SSCP-electrophoresis using silver staining for band visualization. Single-strand DNA fragments gave a reproducible pattern of bands, characteristic for the ΔF508 mutation (Kakavas et al., 2006). However, detecting compound heterozygotes between ΔF508 and other mutations which are rare is difficult as some mutations are common only to particular ethnic groups. Therefore, diagnostic tests such as restriction enzyme assays and SSCP have been designed to recognize rare and population-specific mutations (Eshaque & Dixon, 2006).

### 2.3 Analysis, spectrum and frequency of CFTR mutations in different populations

One challenging aspect of genetic analysis as it relates to CF is the identification of CF mutations in some populations. There are a number of studies that have examined the spectrum and frequency of mutations in different countries. In a study conducted in Minas Gerais State, Brazil, the frequency of 8 mutations (ΔF508, G542X, R1162X, N1303K, W1282X, G85E, 3120+1G>A, and 711+1G>T) was analyzed using by ASO-PCR with specially designed primers in 111 newborn patients. An allele frequency of 48.2% was observed for the ΔF508 mutation, and allele frequencies of 5.41%, 4.50%, 4.05%, and 3.60% were found for the R1162X, G542X, 3120+1G>A, and G85E mutations, respectively (Perone et al., 2010). Mutational analysis of the CFTR gene was performed in 49 Lithuanian CF patients through a combined approach of ASO-PCR and DGGE analysis. A CFTR mutation was characterized in 62.2% of CF chromosomes and ΔF508 (52.0%) was the most frequent Lithuanian CF mutation. Seven CFTR mutations, N1303K (2.0%), R75Q (1.0%), G314R (1.0%), R553X (4.2%), W1282X (1.0%), and 3944delGT (1.0%), accounted for 10.1% of Lithuanian CF chromosomes (Giannattasio et al., 2006).

There is a reported high incidence of the CFTR mutations 3272-26A-->G and L927P in Belgian CF patients. The technique DGGE was used to extensively analyse the CFTR gene in those patients with at least one unknown mutation after preliminary screening. There was also the identification of three new CFTR mutations (186-2A-->G, E588V, and 1671insTATCA). The mutation, 3272-26A-->G has a frequency of 3.8%, while L927P, 2.4% (Storm et al., 2007). In another study, different methods, such as ARMS-PCR, SSCP analysis, restriction enzyme digestion analysis, direct sequencing, and MLPA (Multiplex Ligation-mediated Probe Amplification) were used to analyse mutations in the complete coding region, and its exon/intron junctions, of the CFTR gene in 69 Iranian CF patients. CFTR mutation analysis revealed the identification of 37 mutations with a CFTR mutation detection rate of 81.9% (Alibakhsh et al., 2007). The most common mutations were ΔF508 (18.1%), 2183AA>G (6.5%), S466X (5.8%), N1303K (4.3%), 2789+5G>A (4.3%), G542X (3.6%), 3120+1G>A (3.6%), R334W (2.9%) and 3130delA (2.9%). These 9 types of mutant CFTR genes accounted for 52.0% of all CFTR genes derived from the Iranian CF patients (Alibakhsh et al., 2007).

Extensive CFTR gene sequencing can detect rare mutations which are not found with other screening and diagnostic tests, and can thus establish a definitive diagnosis in symptomatic patients with previously negative results. This enables carrier detection and prenatal diagnosis in additional family members (McGinniss et al., 2005). Prenatal diagnosis and carrier screening of relatives can be performed by segregation analysis of polymorphisms within or linked to the CFTR gene. Most commercial tests screen for the T5 allele, a splicing error in intron 8 that is

considered to be a mild mutation with an incomplete penetrance (Rave-Harel et al., 1997). The T5 polymorphism is found on about 5% of the CFTR genes in the general White ethnic population, but on about 21% of the CFTR genes derived from patients with congenital bilateral absence of the vas deferens (CBAVD) (Chillo'n et al., 1995) and it may even confer non-classic CF (Cuppens et al., 1998; Noone et al., 2000). In most cases the partial penetrance is explained by the polymorphic TGm locus (11, 12 or 13 TG repeats) in front of the T5 allele. Analysis of the TGm locus can be accurately determined by sequencing (De Boeck et al., 2006). A higher number of TG repeats also results in less efficient splicing of CFTR transcripts (Cuppens et al., 1998). In patients with CBAVD and non-classic CF, the milder TG11-T5 allele is infrequent while the TG12-T5 allele is most frequently found (Cuppens et al., 1998). The TG13-T5 is rarer but also more frequently found in patients with CBAVD and non-classic CF (Cuppens et al., 1998; Groman et al., 2004).

Mutation analysis of the CFTR gene in Slovak CF patients by DHPLC and subsequent sequencing resulted in the identified four novel mutations (G437D, H954P, H1375N, and 3120+33G>T). This was done by the gene scanning approach using DHPLC system for analysing specifically all CFTR exons. There was the identification of a total of 28 different mutations in Slovak CF patients, and 17 different polymorphisms (Kolesár et al., 2008). Elce et al. (2009) reported three novel CFTR polymorphic repeats (IVS3polyA, IVS4polyA, and IVS10CA repeats) which improve segregation analysis for CF. They also developed and validated a procedure based on PCR followed by capillary electrophoresis (CE) for large-scale analysis of these polymorphisms. The allelic distribution and heterozygosity results suggest that the 3 novel intragenic polymorphic repeats strongly contribute to carrier and prenatal diagnosis of CF in families in which 1 or both causal mutations have not been identified (Elce et al., 2009). A universal array-based multiplexed test for CF carrier screening using the Tag-It multiplex mutation platform and the Cystic Fibrosis Mutation Detection Kit have been introduced. The Tag-It CF assay is a multiplexed genotyping assay that detects a panel of 40 CFTR mutations including the 23 mutations recommended by the American College of Medical Genetics (ACMG) and American College of Obstetricians and Gynecologists (ACOG) for population screening. A total of 16 additional mutations detected by the Tag-It CF assay may also be common (Amos et al., 2006).

Methods that include genetic testing can be done using a single sample. The controversy is the appropriate number of mutations to include in the genetic test. The answer depends in part on the heterogeneity of the population. The ΔF508 mutation is found in 72% of the US Non-Hispanic Caucasian CF population, but in much lower percentages of patients with CF from other ethnicities (Hispanic Caucasian, 54%; African American, 44%; Asian American, 39%; Ashekenazi Jewish, 31%) (Watson et al., 2004). In 2001, the ACMG Cystic Fibrosis Carrier Screening Working Group recommended a panel of 25 mutations which would account for > 80% of CF alleles in the pan-ethnic US population with CF (Grody et al., 2001). This panel was updated in 2004 based on a larger more pan-ethnic CF data-base that finds six additional mutations with a frequency > 0.10% and another 14 that occurred at slightly lower frequency but would be useful for specific ethnic minority communities (Watson et al., 2004).

### 2.4 Detection of rearrangements in CFTR gene

Large rearrangements (deletions, duplications, or insertion/deletion mutations) have recently been reported to constitute 1-2% of CFTR mutations (Svensson et al., 2010). The

developments in quantitative PCR technologies have greatly improved our ability to detect large genome rearrangements. In particular oligonucleotide-based array comparative genomic hybridisation has become a useful tool for appropriate and rapid detection of breakpoints (Ramos et al., 2010). Using quantitative PCR analysis of all coding regions, the occurrence of CFTR rearrangements in 130 alleles from classic CF patients bearing unidentified mutations after the scanning of CFTR were assessed in the Italian population. Seven rearrangements (i.e. dele1, dele2, dele23, dele 14b17b, dele17a18, dele2223, and dele2224) were identified in 26.0% of CF alleles bearing undetected mutations (Tomaiuolo et al., 2008).

Ramos et al. (2010) analysed 80 samples (42 unknown CF alleles) applying three quantitative technologies (MLPA, quantitative PCR and array-comparative genomic hybridization) to detect recurrent as well as novel large rearrangements in the Spanish CF population. They identified three deletions and one duplication in five alleles. The new duplication in this cohort, CFTRdupProm-3 mutation spans 35.7 kb involving the 5'-end of the CFTR gene. Additionally, RNA analysis revealed a cryptic sequence with a premature termination codon leading to a disrupted protein (Ramos et al. 2010). In another study, de Becdelièvre et al. determined the contribution of large CFTR gene rearrangements in fetuses with bowel anomalies using a semi-quantitative fluorescent multiplex PCR (QFM-PCR) assay. Deletions were found in 5/70 cases in which QFM-PCR was applied, dele19, dele22_23, dele2_6b, dele14b_15 and dele6a_6b, of which the last three remain un-described (de Becdelièvre et al. 2010).

Schneider et al. (2007) used the CFTR MLPA Kit (MRC-Holland, Amsterdam, Netherlands) that allows the exact detection of copy numbers from all 27 exons in the CFTR gene, to screen 50 patients with only one identified mutation for large deletions in the CFTR gene. Detected deletion in the CFTR gene was confirmed using real-time PCR assay and deletion-specific PCR reactions using junction fragment primers. Large deletions were detected in eight CF alleles belonging to four different deletion types (CFTRindel2, CFTRdele14b-17b, CFTRdele17a-17b and CFTRdele 2-9) (Schneider et al., 2006). The LightCycler assay allows reliable and rapid screening for large deletions in the CFTR gene and detects the copy number of all 27 exons (Schneider et al., 2007).

## 3. Diagnosis of CF using sweat test

### 3.1 Methods used for assessing sweat chloride

The report of the Consensus Conference initiated by the CF Foundation in the USA stated that the criteria for the diagnosis of CF should include the following: (1) one or more characteristic phenotypic features, or a history of CF in a sibling, or positive newborn screening test results; and (2) an elevated sweat chloride concentration by pilocarpine iontophoresis (>60 mmol/L) on two or more occasions, or identification of two CF mutations (Rosen & Cutting, 1998), or 3) *in vivo* demonstration of characteristic abnormalities in ion transport across the nasal epithelium (Welsh et al., 2001; Rosenstein & Cutting, 1998).

The chloride ion is most directly related to CFTR dysfunction. Chloride concentration measurement is the analysis of choice because the chloride ion concentration shows the greatest discrimination between normal individuals and CF subjects. Concurrent

measurement of sodium acts as a quality control. The sweat test is based on the observation in 1953 by Darting et al. (1953) that stimulated sweat of CF patients contains elevated levels of sodium and chloride ions. The development of the quantitative pilocarpine iontophoresis by Gibson and Cooke dates from 1959 and is preferred method of sweat stimulation (Gibson & Cooke, 1959). The sweat test involves transdermal administration of pilocarpine by iontophoresis to stimulate sweat gland secretion, followed by collection and quantitation of sweat onto gauze or filter paper or into a Macroduct coil (Wescor Inc, Logan, Utah) and analysis of chloride concentration as described by Clinical Laboratory Standards Institute (2000). If carried out properly and with considerable care, this method is still the most specific biochemical test for CF (Shwachman, 1979). There is documentation in the literature of a semi-quantitative test, based on die production of a white silver chloride precipitate ring on a brown silver chromate background that was originally proposed by Shwachman and Gahm (1956) and adapted to die so-called paper patch test (Yeung et al., 1984). Although intended for the non-specialist centre, die method is very subjective and liable to misinterpretation and has not gained popularity.

One of the major consequences of mutations in the CFTR gene is a dysfunction of ion channels resulting in elevated sweat chloride concentrations, progressive lung disease and pancreatic insufficiency (Pilewski & Frizzell, 1999; Bals et al., 1999). In CF subjects the sweat chloride is usually higher than the sweat sodium, but the converse is true in normal persons. Normal sweat contains less than 60 mmol/L chloride and sodium (Association of Clinical Biochemistry, 2002). The 60 mmol/L value of sweat chloride concentrations has been used for a long time to discriminate between the populations of patients with CF and without CF (LeGrys, 1996). An elevated sweat chloride level has been the "gold standard" for diagnosis of CF (Gibson & Cooke, 1959). All patients with a sweat chloride level above 60 mmol/L and a clinical phenotype compatible with CF have a diagnosis of classic CF. However patients have been reported with characteristic manifestations of CF, and chloride levels, below 60 mmol/L (Highsmith et al., 1994; Cystic Fibrosis Genotype-Phenotype Consortium, 1993). Most of the studies exploring these patients with equivocal sweat tests have focused on the chloride range 40 - 60 mmol/L (Desmarquest et al., 2000). In the UK guidelines on sweat testing (Association of Clinical Biochemistry, 2000), 40 mmol/L is considered as the lower limit for equivocal sweat tests because this value represents the mean +2SD in carriers.

### 3.2 Intermediate sweat test results

The evidence that a proportion of CF patients with chloride concentrations of 30 - 60 mmol/L with two CFTR mutations following testing is documented in the literature (Lebecque et al., 2002). Sweat chloride concentrations of 30 - 60 mmol/L are seen in about 4% of sweat tests; 23% of these patients will subsequently be found to have two CFTR mutations. CF affected patients occur with similar frequency in the 30 - 40 mmol/L range as in the 40 - 60 mmol/L range (Lebecque et al., 2002). Furthermore, in the 2005 Cystic Fibrosis Foundation Patient Registry, only 3.5% of patients with a diagnosis of CF had a sweat chloride value <60 mmol/L, and only 1.2% had a value <40 mmol/L (Cystic Fibrosis Foundation Patient Registry, 2005). A Canadian study reported that sweat chloride values <60 mmol/L were observed in 21% with pancreatic-sufficient CF (Wilschanski et al., 2006).

Increasing recognition of the wide range of CF phenotypic variability (Nick & Rodman, 2005; Bishop et al., 2005) should lead to increasing diagnosis of CF in individuals with intermediate sweat chloride values. Farrell et al. (2008) recommends that sweat chloride values ≥40 mmol/L in individuals over age 6 months should be considered beyond the normal range and merit further evaluation, to include repeat sweat chloride testing and DNA analysis for CFTR mutations. A sweat chloride level above 60 mmol/L in the absence of CF is rare, although it has been reported in a number of unusual clinical conditions that can usually be readily distinguished from CF (Rosenstein, 2000). In patients with a sweat chloride level below 30 mmol/L the diagnosis of CF becomes very unlikely.

### 3.3 Sweat tests in infants

New born screening (NBS) of CF identifies only newborns at risk for CF. A positive screening result, indicating persistent hypertrypsinogenemia, should be followed by referral for direct diagnostic testing (i.e. sweat chloride test) to confirm a diagnosis of CF. With sufficient experience, sweat testing can be performed adequately in infants, but interpreting the results can be problematic. Studies of sweat chloride testing in infants have demonstrated most infants identified by NBS will undergo sweat testing after 2 weeks of age. Earlier testing could lead to misleading results, because sweat chloride concentrations in healthy newborns gradually decrease over the first weeks of life (Parad et al., 2005). A study in 103 infants without CF found a mean sweat chloride value of 23.3 ± 5.7 mmol/L at age 3 to 7 days, decreasing to 17.6 ± 5.6 mmol/L by age 8 to 14 days and then to 13.1 ± 7.4 mmol/L after age 6 weeks (Eng et al., 2005). This gradual early decline in sweat chloride values suggests that sweat test results are less likely to be difficult to interpret after age 2 weeks (Eng et al., 2005).

The Consensus Committee recommends based on the available data on sweat chloride test results in healthy and CF-affected infants, the following sweat chloride reference ranges for infants up to age 6 months: ≤29 mmol/L, CF unlikely; 30 to 59 mmol/L, intermediate; ≥60 mmol/L, indicative of CF (Farrell et al., 2008). A study of 725 infants identified as being at risk through NBS or based on clinical presentation who carried 0, 1, or 2 copies of the common CFTR gene mutation ΔF508, showed that all of the ΔF508 homozygous infants had sweat chloride concentrations >60 mmol/L. The findings from this study are in accordance with other studies from Australia (Massie et al., 2000; Parad et al., 2005). Although sweat chloride values are generally ≥60 mmol/L in infants with CF, lower values also can occur (Taceetti et al., 2004; Rock et al., 2005). In a 4-year cohort of infants in the Massachusetts NBS program in the USA who had clinician-diagnosed CF, 8.2% had a sweat chloride concentration of 30 to 59 mmol/L and 2.7% had a concentration <30 mmol/L (Parad & Comeau, 2005).

There are studies which support the recommendation that a sweat chloride value ≥ 30 mmol/L in infants <age 6 months should be considered abnormal and trigger further patient evaluation (Eng et al., 2005; Barben et al., 2005). A sweat chloride value ≤39 mmol/L after age 6 months generally is not consistent with a diagnosis of CF, although CF can occur in this group in rare cases (Lebecque et al., 2002; O'Sullivan et al., 2006). Some infants have been particularly difficult to classify, such as those with 2 CF mutations and a sweat chloride value <40 mmol/L and those with only 1 CF mutation and a slightly elevated sweat chloride value. Although such infants represent only a small fraction of patients, they may

be at risk for developing complications of CF and thus should be identified and followed (Farrell et al., 2008). Sweat testing can be performed accurately on the majority of infants at age 2-3 weeks; however, not all infants have sufficient quantities of sweat for reliable testing (Boyle, 2003).

### 3.4 Limitations, advantages and disadvantages of sweat test

The sweat test is cheap and, in nearly all populations, will result in a greater diagnostic yield than a standard CFTR deoxyribonucleic acid (DNA) screening test. Sweat should be collected for 30 minutes onto pre-weighed gauze or filter paper low in sodium chloride. A minimum sweat rate of 1 g/m$^2$ body surface area/min is required; thus a sweat volume of 50 -100 mL is adequate. Testing can be carried out after the first 2 weeks of life in infants weighing more than 3 kg who are normally hydrated and without significant illness. Testing should be delayed in infants who are acutely ill or dehydrated, who have eczema or oedema, or who are receiving supplemental oxygen. Raised sweat electrolyte concentrations can be found in infants who are underweight or dehydrated.

Sweat electrolyte concentrations can be lowered by systemic steroids and oedema. Sweat electrolytes are not affected by administration of intravenous fluids, diuretics or intake of flucloxacillin (Association of Clinical Biochemistry, 2002; National Committee for Clinical Laboratory Standards, 2000). False negative results have been reported (LeGrys & Wood, 1988**)** as well as consistently borderline values (Canciam et al., 1988). False positive results can also occur (Smalley et al., 1979**)** often due to lack of care during sweat collection, resulting in evaporation of collected sweat prior to analysis. Consequently the test which is time-consuming should be done only by properly trained personnel. An additional problem is that in the very young and those with dry skin, sweat collection volumes may be too small for analysis.

As the appropriate performance of the sweat test is crucial for the accurate diagnosis of CF, the Cystic Fibrosis Foundation (2007) requires that sweat testing conducted at accredited CF care centers adheres to the standards recommended by a Cystic Fibrosis Foundation Committee comprising CF center directors (LeGrys et al., 2007). Laboratories accredited by the College of American Pathologists must follow the protocols and procedures outlined in the College's Laboratory Accreditation Program Inspection Checklist (College of American Pathologists, 2007). Because of the additional technical challenges involved in obtaining sweat from newborns, it is often recommended that NBS-positive newborns undergo sweat testing only at a Cystic Fibrosis Foundation certified laboratory.

## 4. Immunoreactive trypsinogen (IRT) in CF neonatal screening

### 4.1 The sensitivity and specificity of IRT

The purpose of CF newborn screening is identification of CF-affected infants. Strategies used by CF newborn screening programs have included measuring for elevated levels of IRT which is relatively inexpensive and adaptable to large numbers (Crossley et al., 1981). The IRT is an indirect measure of pancreatic injury that is present at birth in most newborns who have CF on serial dried blood spot specimens (Hammond et al., 1991) or measuring for elevated IRT followed by assaying for ΔF508 on the same dried blood spot (2-tier algorithm)

(Gregg et al., 1993). Increased IRT concentrations at birth are characteristic of newborns affected by CF, but can also be found in healthy infants. In 1979, Crossley et al. reported a two to threefold increase in IRT in blood from CF neonates, compared with normal (non-CF) infants. The test was based on a radioimmunoassay for serum trypsin, and was adapted for use on dried blood-spots.

The IRT levels tend to remain elevated for several months in newborns with CF, because pancreatic trypsinogen leaks back through interstitial fluid due to partial obstruction of pancreatic ducts (Crossley et al., 1979). The lack of specificity of IRT means, however, that they may also be false positives (Wilcken et al., 1983). The 'falsely' elevated IRT levels usually return to normal within the first weeks of life of the child's birth. This presents a diagnostic dilemma. CF should be confirmed or ruled out as quickly as possible in these situations to alleviate parental distress and allow earlier therapeutic intervention and genetic counseling. In most older CF children IRT levels are subnormal and there is considerable child to child variation (Chatfield et al., 1991). To improve the specificity of neonatal screening, a second blood sample is obtained in neonates with raised levels of IRT at birth, and only infants with persistently raised IRT values progress to a sweat test. Furthermore, standard diagnostic strategy calls for extensive analysis of the CFTR gene and repetition of the sweat $Cl^-$ measurement (De Boeck et al., 2006; Rosenstein & Cutting, 1998).

Prospective studies have shown false positive incidence of 0.5% (from first blood spots) but the false negative incidence was very low if infants presenting with meconium ileus were excluded (Crossley et al., 1981; Heeley et al., 1982). The false positive incidence could be significantly reduced by repeating the test on a second blood spot (Travert, 1988). The results of routine screening from 16 centres around the world were correlated and it was found that false positive rate ranged from 0.2 - 0.5% although this was higher in those laboratories where a lower cut-off point was taken (Travert, 1988). There have been reports concerning improvement in sensitivity and specificity for the IRT test, by including the use of complementary tests (Pederzini et al., 1990) have suggested a combination of meconium screening by measurement of lactase, on those infants who are IRT positive. Sweat tests are done on those patients who test positive, either by the lactase test or where blood spots are above a certain value by IRT test. This approach achieved a marked drop in false negative incidence but it seems likely that the extra work and expense will be unacceptable (Pederzini et al., 1990).

In most neonatal screening protocols, IRT retesting in infants with an initially raised value has been replaced by analysis of a panel of CF causing mutations in the neonatal blood sample (Ranieri, 1994). Comeau et al. (2004) implemented statewide CF newborn screening in Massachusetts, USA using a 2-tier algorithm in which all specimens were assayed for IRT. Those with elevated IRT then had multiple- CFTR-mutation testing. Infants who screened positive by detection of 1 or 2 mutations or extremely elevated IRT (>99.8%; failsafe protocol) were then referred for definitive diagnosis by sweat testing**.** The authors reported that by using the multiple-CFTR-mutation panel, a screening result with a genetic "diagnosis" of CF was made in 75% of screened-positive CF-affected infants, compared with 50% had they used ΔF508 alone, thus facilitating more rapid referral and intervention (Comeau et al., 2004).

### 4.2 The use of the IRT/IRT method in neonatal screening

Multiple protocols and algorithms are used to screen newborns for CF. All protocols begin with a first-tier phenotypic test that measures IRT in dried blood spots. Different laboratory kits for IRT produce varying distributions of IRT measures, and screening programs set cut-offs on the basis of evaluations of specimens from their own populations and the screening protocols and algorithms used. Screening programs in five states in the USA (Colorado, Connecticut, Montana, New Jersey, and Wyoming) have set absolute cut-offs for a normal IRT value on the first newborn blood spot (range: 90 - 105 ng/mL) (Wilfond et al., 2003).

The IRT-IRT algorithm involves measurement of IRT during the first week on the Guthrie blood spot and repeating the measurement at 3 - 4 weeks in those with initial high levels. The sensitivity of a raised 3 - 5 day IRT is high, but the positive predictive value is low. Because blood levels of IRT decay slowly in CF infants, a second IRT at 3 - 4 weeks increases the specificity, but about 1 in 200 newborn infants progress to the second blood test (Price, 2006).

In the USA, because normal IRT reference values vary slightly, the individual NBS program in the state in which the newborn is being tested sets the specific cut-off value that defines an elevated IRT. After an abnormal IRT value is identified, most NBS programs perform DNA testing to identify known CFTR gene mutations (IRT/DNA strategy), while other programs repeat the IRT measurement in a second blood sample obtained from the infant at age approximately 2 weeks (IRT/IRT strategy) (Comeau et al., 2007). These strategies have been reported to provide approximately 90% to 95% sensitivity (Wilcken et al., 1995) and have identified newborns at risk for a wide spectrum of disease severity (Farrell et al., 1997). However, there are studies which have shown that IRT/DNA screening suggested better sensitivity than IRT/IRT (Gregg et al., 1997; Padoan et al., 2002), but relatively small populations were previously studied.

In the IRT/IRT algorithm, both the first and the second IRT values must be above the fixed cut-offs to recommend a sweat test; therefore, the initial IRT is the more crucial step. In the USA, a first IRT value of 100 or 105 ng/mL, and a second value of 70 ng/mL are used. These values have been set in an attempt to maximize sensitivity and positive predictive value. Although the initial cut-off for IRT/IRT algorithm decreased over time from 140 ng/mL (Hammond et al., 1991) to 100 or 105 ng/mL (Sontag et al., 2005) in attempts to decrease false negative results in the IRT/IRT, there are concerns regarding sub-optimal sensitivity and observations which have revealed that the second specimens of some patients with CF showed precipitous decreases which have led Wisconsin in the USA (Rock et al., 1990) and Australia (Gregg et al., 1993) to develop the 2-tier IRT/DNA (ΔF508) method.

### 4.3 IRT/DNA and IRT/DNA/IRT protocols in neonatal screening

According to Price (2006), IRT/DNA employs DNA analysis instead of a second IRT at 3 - 4 weeks. Infants with very high IRT in the first week undergo DNA analysis and those with at least one mutation have a sweat test. The advantage of the IRT/DNA protocol is that both tests can be done on the initial blood spot sample (Price, 2006). However, the sensitivity of the IRT/DNA protocol is, however, dependent on the gene frequency of common CFTR mutations in the population. Many programs that use an IRT/DNA methodology also recommend sweat testing on children with a very high IRT level without mutations in an

attempt to capture children who have rare mutations. This safeguard will reduce the number of false negatives (Benhardtet et al., 1987).

If an IRT/DNA method is used, the number of carriers detected will depend on the number of mutations included in the screening test. The more mutations included, the more children will be identified with one common mutation. The screening panel should include more rather than less mutations to avoid disproportionate number of missed screened cases (false negatives) in USA ethnic minorities. In order to capture a high percentage of cases involving ethnic minorities, full sequencing of the CFTR gene is required (Ross, 2008). Kammesheidt et al. (2006) have shown the feasibility of temporal temperature gradient electrophoresis-based full sequence analysis and targeted sequencing from DNA in newborn blood specimens which can increase the identification of mutations in ethnic minorities. This method allowed a more comprehensive diagnosis on one blood sample because only children with two mutations and/or variants would need to undergo sweat testing. It should reduce the overall number of cases referred for sweat tests, unless questionable variants are more common than previously anticipated (Kammesheidt et al., 2006).

The IRT/DNA/IRT protocol use 2 IRT measurements and DNA testing. This method, applies a mutation panel to primary samples with an elevated IRT. Children whose sample has at least one mutation or whose sample has a very high initial IRT measurement are asked to provide a second sample for a second IRT measurement. Only those with an elevated IRT levels on the second sample undergo sweat testing (Ross, 2008). Corbetta et al. (2002) assessed the performance of IRT/DNA/IRT based on IRT followed by direct CFTR gene analysis (based on a panel of up to 31 mutations) in hypertrypsinaemic newborn infants in Italy. The screening strategy consisted of an IRT assay from dried blood spots, a PCR followed by an OLA (PCR-OLA), and a sequence code separation. The researchers reported that the IRT/DNA/IRT protocol with an OLA showed the identification of 94% of infants with CF. They concluded that PCR-OLA assay was a reliable, robust method to apply to the neonatal screening programme (Corbetta et al., 2002).

In the UK approximately 4% of children diagnosed with CF are non-Caucasian in origin. A DNA panel comprising the most common 31 CF mutations will detect 97% of mutations in a Caucasian population, but only just over 65% of mutations in a non-Caucasian population. The gene frequency of ΔF508 in the UK Indian sub-continent CF population is less than half that in the UK Caucasian CF population (McCormick et al., 2002). A three stage IRT/DNA/IRT protocol is reported to likely increase the chances of detecting CF in non-Caucasian infants (McCormick et al., 2002).

The main benefits of the IRT/DNA/IRT protocol over a single IRT/DNA methodology is that they reduce the number of children who need to undergo sweat testing, and the number of parents who are informed of their child's carrier status and need genetic counselling (Ross, 2008). However, the main disadvantage of the IRT/DNA/IRT protocol is its complexity and the anxiety generated for families who have to wait for the result of a second IRT (McCormick et al., 2002). Both the IRT/DNA/IRT and IRT/DNA protocols involve DNA testing, and may fail to detect ethnic minorities with rare mutations. Some ethnic minority children with rare mutations may still be detected to the extent that the IRT/DNA/IRT method employs the safeguard of recommending sweat testing of children with a very high IRT measurement even if no mutations are detected. Modeling in different

ethnic communities using different DNA panels would be necessary to determine whether the costs of the extra laboratory testing are outweighed by the benefits achieved by reducing the number of children who need to undergo sweat testing and genetic counselling (Ross, 2008).

## 5. The use of the nasal potential difference (NPD) in aiding the diagnosis of CF

Genetic studies sometimes take several weeks and may find no useful information, neither confirming nor ruling out CF, such as when one or both mutant alleles remain unidentified or when the CF-causing nature of the mutations cannot be proven (Castellani et al., 2001). In addition, there are ancillary tests currently used by clinicians to clarify the diagnostic status of individuals with less CF-specific gastrointestinal or pulmonary symptomatology. The NPD test, which has been used in CF research for decades, has been introduced to clinical practice to aid diagnosis (Knowles et al., 1995). It may be particularly helpful in individuals with inconclusive sweat chloride values (Wilson et al., 1998). In *vivo* demonstration of abnormal CFTR-related ion transport across nasal epithelium could serve as an important diagnostic tool in these difficult situations.

Measurements of trans-epithelial NPD in adults accurately characterize CFTR-related ion transport. Nasal PD is determined by standard criteria as described by Knowles et al (1995). The PD is measured between a fluid filled exploring bridge on the nasal mucosa and a reference bridge on the skin of the forearm. The reference bridge may be applied to the skin by a thin needle inserted subcutaneously or placed directly on the skin after performing a small abrasion (Gelrud et al., 2004). After consistent baseline PD measurements have been obtained, the effect of amiloride superfusion through a second tube overriding the exploring catheter is evaluated. To study nasal chloride permeability and cAMP activation of chloride permeability, a large chloride chemical gradient is generated across the apical membrane by superfusion of the nasal mucosa for 3 minutes with a chloride free solution containing $10^{-4}$ M amiloride in Ringer's solution with gluconate substituted for chloride at a rate of 5 ml/min. Sodium ($Na^+$) transport and CFTR-related $Cl^-$ transport is measured electrically by recording the changes in the nasal transepithelial PD (Knowles et al., 1995).

The nasal PD of a patient with classic CF is remarkably different from controls. The profile of classic CF patients is characterized by hyperpolarization of basal PD, increased $Na^+$ channel activity, an amiloride response that is exaggerated, and there is very little or no response to chloride free and isoproterenol solutions. In non-classic CF the nasal PD may be borderline and there is not yet a total consensus as to what exactly constitutes an abnormal result, but a formula which takes into account both sodium and chloride transport has been proposed by Wilschanski and colleagues (Wilschanski et al., 2001).

There have been several reports on the usefulness of NPD measurements for diagnosing CF (Hubert et al., 2004; Schüler et al., 2004). Nasal PD measurement has been widely validated in adults (Knowles et al., 1995) and provides an easy, quick and painless tool to discriminate between adults with atypical CF and those presenting some CF symptoms without CF (Delmarco et al., 1997; Wilschanski et al., 2001). Therefore a NPD test showing a significant response to zero-chloride perfusate containing isoproterenol may be useful in ruling out a diagnosis of CF. But the quantitative aspects of NPD results that are clearly indicative of CF

are not defined consistently across all CF-testing centers. Moreover, some overlap likely occurs between CF and non-CF values for both the basal PD and response to zero-chloride and isoproterenol, analogous to the overlap in sweat chloride values. The NPD test's predictive capability improves somewhat when analyses of sodium and chloride channel abnormalities are combined (Standaert et al., 1997).

The NPD measurements in infants reported so far come mostly from case reports (Barker et al., 1997; Southern et al., 2001). These few studies used either the equipment already validated in adults, or specially designed one-of-a-kind devices. In a study, Sermet-Gaudelus et al. (2006) sought to validate NPD testing as a diagnostic tool for children with borderline results in neonatal screening. They adapted the standard NPD protocol for young children, designed a special catheter for them, used a slower perfusion rate, and shortened the protocol to include only measurement of basal PD, transepithelial sodium ($Na^+$) transport in response to amiloride, and CFTR-mediated $Cl^-$ secretion in response to isoproterenol. The authors reported that the new protocol was well tolerated and produced NPD measurements that did not differ significantly from those obtained with the standard protocol. They conclude that this preliminary study will provide a basis for interpreting NPD measurements in patients with suspected CF after neonatal screening (Sermet-Gaudelus et al., 2005).

It is important however to standardize the protocol and to verify that the reference data and patterns in infants are similar to the values previously validated in adults. It is not yet known, for example, whether airway epithelium undergoes maturation during the first months of life, as renal and sweat gland epithelia do (Wilken and Travert, 1999). Therefore, before this test is implemented as a diagnostic tool for cases with borderline observations in neonatal screening, there is an urgent need to obtain and validate reference data for NPD measurements in infants and very young children with CF, and in healthy controls of the same age (Wilken and Travert, 1999). Properly conducted NPD testing at a research center can provide valuable information for diagnosis when clinical evidence is not clear-cut; however, access to the test is limited. Because there are no clear reference values, validation studies, or standardized technical protocols for NPD testing for diagnostic purposes, the test should be used only to provide contributory evidence in a diagnostic evaluation (Standaert et al., 2004).

## 6. The value of pancreatic associated protein (PAP) as a screening test for CF

Genetic analysis has certain drawbacks, the most important of which being the management of heterozygotes, and in France the requirement by law of previous informed consent (Barthellemy et al., 2001). In cases of CF, pancreatic alterations are already present in utero. Previous studies have demonstrated the value of PAP as a screening test for CF, and has indicated that a feasible two-stage strategy could involve the selection of infants with elevated PAP levels, and in this group of infants, subsequent detection of those with elevated IRT levels for direct CF diagnosis by the sweat test thereby avoiding the use of genetic analysis (Sarles et al., 1999; Sarles et al., 2005).

The IRT/PAP protocol can be done on one sample and preliminary data show comparable sensitivity and specificity with the other methods using the Guthrie cards (Sarles et al.,

2005). Barthellemy et al. (2001) evaluated PAP levels in a prospective study involving 47, 213 infants in the Provence region of France. In infants with a PAP > 7.5 ng/mL, 1.28% had an elevated IRT level > 700 ng/mL (0.37%). In this limited population sample (0.37% of the total), the sweat test diagnosed five cases of CF. The authors concluded that the PAP/IRT technique for CF detection seems to be suitable for mass screening, without the drawbacks of genetic testing (Barthellemy et al., 2001).

A recent study Sommerburg et al. (2010) used a prospective and sequential IRT/PAP strategy, and validated this biochemical approach against the widely used IRT/DNA protocol in a population-based NBS study in southwest Germany. The study involved the prospective quantitation of PAP and genetic analysis for the presence of four mutations in the CFTR gene most prevalent in southwest Germany (ΔF508, R553X, G551D, G542X) on all newborns with IRT >99.0$^{th}$ percentile. New born screening was rated positive when either PAP was ≥1.0 ng/mL and/or at least one CFTR mutation was detected. The results showed that out of 73,759 newborns tested, 0.13% were positive with IRT/PAP and 0.08% with IRT/DNA. In addition, after sweat testing of 135 CF NBS-positive infants, 13 were diagnosed with CF. The authors reported that the detection rates were similar for both IRT/PAP and IRT/DNA protocols (Sommerburg et al., 2010).

Sequential measurement of IRT/PAP provides good sensitivity and specificity and allows reliable and cost-effective CF NBS which circumvents the necessity of genetic testing with its inherent ethical problems. However, to-date it has not been tested outside of Europe and its benefits and harms in a pan-ethnic community have not been clarified.

## 7. Intestinal current measurement (ICM) as a diagnostic tool for CF

As many intestinal ion transport processes are electrogenic, measuring the electrical current that they generate (ICM) can be used to monitor their activity. Intestinal current measurements on rectal suction biopsies are a tool for the *ex vivo* diagnosis of classical and atypical CF. The ICM technique allows the registration of CF-induced changes in electrogenic transepithelial ion transport ($Cl^-$, $HCO_3^-$, $K^+$) in a $Cl^-$ secretory epithelium, and on the basis of pharmacological criteria, is able to discriminate between CFTR-mediated $Cl^-$ secretion, and secretion through alternative anion channels. In CF, intestinal chloride secretion is impaired while absorptive processes remain unchanged and may even be enhanced. Furthermore, ICM is particularly useful for the classification of individuals with CF-like clinical features with equivocal sweat test values (De Jonge et al., 2004).

There is a clear difference between ICM measurement in classic CF and in normal individuals. There is information in the literature about the use of ICM as a clinical diagnostic tool (De Jonge et al., 2004; Hug et al., 2004). Derichs et al. (2010) described reference values and validated ICM for the diagnostic classification of questionable CF at all patient ages. The ICM method was performed in 309 rectal biopsies from 130 infants, children and adults including patients with known pancreatic-insufficient, pancreatic-sufficient, patients with an unclear diagnosis with mild CF symptoms, intermediate sweat test and/or *CFTR* mutation screening and healthy controls. The researchers found that the cumulative chloride secretory response of $\Delta I_{sc,carbachol}$, $\Delta I_{sc,cAMP/forskolin}$ and $\Delta I_{sc,histamine}$ was the best diagnostic ICM parameter, differentiating patients with questionable CF into

pancreatic-sufficient-CF and 'CF unlikely' groups. The study underlines the diagnostic value of ICM, especially for confirmation of CF in the absence of two disease-causing *CFTR* mutations, exclusion of CF despite intermediate sweat test and age groups unsuitable for NPD measurements (Derichs et al., 2010). They conclude that ICM is an important tool for functional assessment in *CFTR* mutations of unknown clinical relevance (Derichs et al., 2010). However at present the technique has remained mainly in the research setting, so it is not yet included in the diagnostic algorithms.

## 8. Conclusion

Cystic fibrosis, a recessively inherited condition caused by mutation of the CFTR gene is a disease with the complex, multi-faceted clinical phenotype and is one of the most investigated monogenic disorders. More than 1800 different disease-causing mutations within the CFTR gene have been described. Mutations affect CFTR through a variety of molecular mechanisms, which can produce little or no functional gene product at the apical membrane. This results in abnormal viscous mucoid secretions in multiple organs and the main clinical features are chronic infection and progressive obstruction of the respiratory tract, pancreatic insufficiency and intestinal disease. Disease severity, to some extent, correlates with organ sensitivity to CFTR dysfunction and to the amount of functional protein, which is influenced by the type of mutation.

CFTR gene studies are now one of the most frequent activities in clinical molecular genetics laboratories and with advances in DNA analysis there is an increased knowledge of the mutational spectrum for cystic fibrosis. 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. A growing number of tests capable of simultaneously detecting several frequent CF mutations are being developed, and commercial kits are now available.

The sweat chloride test remains the gold standard for CF diagnosis but does not always give a clear answer. For patients in whom sweat chloride concentrations are normal or borderline and in whom two CF mutations are not identified, an abnormal NPD measurement recorded on 2 separate days can be used as evidence of CFTR dysfunction. Newborn infants with CF have raised levels of IRT in their serum. Measurement of IRT in the first week of life has enabled CF to be incorporated into existing NBS blood spot protocols. The IRT detection test is practical, adaptable to large scale screening of dried neonatal blood spots, relatively inexpensive, and promising for the detection of newborns with CF who have pancreatic insufficiency. However, IRT is not a specific test for CF and NBS therefore requires a further tier of tests to avoid unnecessary referral for diagnostic testing. DNA analysis for common CF-associated mutations has been increasingly used as a second tier test. The sequential measurement of IRT/PAP provides good sensitivity and specificity and allows reliable and cost-effective CF newborn screening which circumvents the necessity of genetic testing. ICM is particularly useful for the classification of individuals with CF-like clinical features with equivocal sweat test values However, standardization of international programs for newborns has not yet been achieved. The significant advances in our understanding of CF and the development of new technologies now allow prenatal diagnosis. However, despite steady

improvements in prenatal diagnosis, NBS and adult, CF remains a serious disease which places a heavy burden on affected families.

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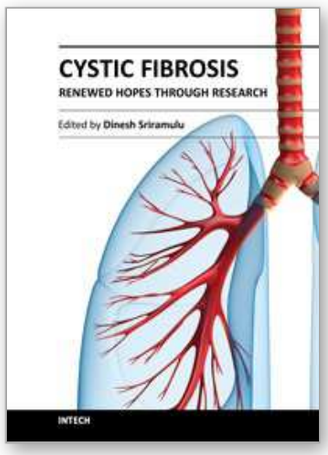


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Living healthy is all one wants, but the genetics behind creation of every human is different. As a curse or human agony, some are born with congenital defects in their menu of the genome. Just one has to live with that! The complexity of cystic fibrosis condition, which is rather a slow-killer, affects various organ systems of the human body complicating further with secondary infections. That's what makes the disease so puzzling for which scientists around the world are trying to understand better and to find a cure. Though they narrowed down to a single target gene, the tentacles of the disease reach many unknown corners of the human body. Decades of scientific research in the field of chronic illnesses like this one surely increased the level of life expectancy. This book is the compilation of interesting chapters contributed by eminent interdisciplinary scientists around the world trying to make the life of cystic fibrosis patients better.

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Unit 405, Office Block, Hotel Equatorial Shanghai
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