The Relevance of Sweat Testing for the Diagnosis of Cystic Fibrosis in the Genomic Era

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Abstract
Cystic fibrosis (CF) is the most common inherited disorder of childhood. The diagnosis of CF has traditionally been based on clinical features with confirmatory evidence by sweat electrolyte analysis. Since 1989 it has been possible to also use gene mutation analysis to aid the diagnosis. Cloning of the cystic fibrosis transmembrane conductance regulator (CFTR) gene has advanced our understanding of CF, in particular the molecular basis of an expanded CF phenotype. However, because there are over 1000 mutations and 200 polymorphisms, many without recognised effects on CFTR, the molecular diagnosis can be troublesome. This has necessitated measurement of CFTR function with renewed interest in the sweat test. This review provides an overview of the clinical features of CF, the diagnosis and complex genetics. We provide a detailed discussion of the structure and function of CFTR and the classification of CFTR mutations. Sweat electrolyte analysis is discussed, from the physiology of sweating to the rigours of a properly performed sweat test and its interpretation. With this information it is possible to understand the relevance of the sweat test in the genomic era.

Introduction
Cystic fibrosis (CF) has been recognised as a distinct clinical entity for more than 60 years.1 The earliest references include an adage from northern European folklore: ‘Woe to that child which when kissed on the forehead tastes salty. They are bewitched and soon will die’.2 The first comprehensive description of CF was provided in 1938 and the term ‘cystic fibrosis of the pancreas’ was coined.1 In 1948, a heat wave in New York City allowed di Sant Agnese to discover that patients with CF lose excess salt in their sweat.3 This landmark discovery led to the development of the sweat test for diagnosis in 1959.4 Building on these observations Quinton reported that CF sweat gland ducts are relatively impermeable to chloride.5 The genetic basis was confirmed by the discovery of the CF gene in 1989.6-8 The clinical disease of CF is a progressive multi-organ disorder principally characterised by suppurative lung disease and pancreatic insufficiency.9 CF is the most common inherited disorders of childhood with an incidence of 1:2500 and carrier frequency of 1:25 in Australia.10

The development of the Gibson and Cooke sweat test in 1959 was a milestone in the diagnosis of CF.4 Since then the measurement of sweat electrolytes by pilocarpine iontophoresis has served as the main diagnostic test for CF. Guidelines have been developed by many groups to standardise the sweat testing procedure and ensure the quality of the sweat test results.11-13 The sweat test is a reliable test for the diagnosis of CF in approximately 98% of patients with CF. However patients have been reported with characteristic clinical manifestations of CF yet normal or borderline sweat electrolyte values.14-16 This is an uncommon problem presenting in only 1 - 2% of cases.14,16,17 Available data suggest that healthy adults may have sweat electrolyte values in the range of patients with CF, but true normal data in adults has not been collected.18 Difficulties also arise in the use of the sweat test in infancy, due to insufficient sweat collection and a transient increase in sweat electrolyte levels in the first 24 hours of life.19,20 The traditionally recognised normal sweat test values may not apply in infancy, especially for those babies detected by newborn screening21,22

The discovery of the CF gene initiated a better understanding of the molecular processes causing CF and provided a new method of diagnosis.6-8 This gene was named the CFTR gene.7 The CFTR gene encodes a transmembrane glycoprotein, which acts as an electrolyte transporter at the apical membrane...
of epithelial cells. Since the discovery of the gene, over 1200 disease-associated mutations have been identified. As routine screening tests are not able to detect all CFTR gene mutations, a negative screening test does not ensure a normal CFTR genotype. Genotyping has also confirmed that some CF mutations are associated with milder disease phenotypes and normal or borderline abnormal concentration of sweat electrolytes. Gene mutation analysis is utilised for carrier identification, prenatal diagnosis in at-risk pregnancies and newborn screening programs for CF.

There is considerable debate regarding the diagnosis of CF and the relative role of genotype analysis and sweat electrolyte testing. The aim of this review is to examine the role of the sweat test for the diagnosis of CF in the genomic era.

**Cystic Fibrosis**

CF is the most common lethal genetic disease affecting Caucasians, with an incidence of 1 in 2500. The inheritance is autosomal recessive and the carrier frequency 1/25. At 31 December 2002 the Australasian Cystic Fibrosis Data Registry held records of 2394 people in Australia with CF. CF is uncommon among Asians (1 in 31,000 live births) and African Americans (1 in 15,000 live births).

**Clinical Features**

CF affects the epithelial cells of several organs, including the respiratory tract, exocrine pancreas, intestine, vas deferens, hepatobiliary system and the exocrine sweat gland. This results in multi-organ disease, characterised by suppurative lung disease, pancreatic insufficiency, multifocal biliary cirrhosis, male infertility and high sweat electrolyte loss. The full spectra of phenotypic characteristics of CF are listed in Table 1.

The pulmonary consequences are the most serious complications of CF. Abnormal epithelial electrolyte transport results in an altered airway surface fluid, the details of which are still being determined. The airway surface fluid is thought to become hypertonic with reduced depth of the periciliary fluid. Airway mucus is poorly hydrated, which in conjunction with the changes in airway surface fluid, impedes mucociliary clearance. This causes obstruction of small airways and promotes airway infection. Recurrent infections and the resulting inflammation leads to submucosal gland hypertrophy, excessive mucus secretion and airway damage. Early in life, CF patients become infected with a limited spectrum of bacteria, (most commonly *Staphylococcus aureus* and non-typable *Haemophilus influenzae*) and as the disease progresses *Pseudomonas aeruginosa* becomes the most common pathogen. The disease process is slowed with current therapies but inevitably progresses to end-stage lung disease. Patients with milder forms of CF usually have late onset or more slowly progressive lung disease.

Exocrine pancreatic insufficiency is present in 85% of patients with CF and is the only clinical feature well correlated with genotype. The clinical manifestations (such as steatorrhoea and poor nutrition) are mostly overcome with diet and pancreatic enzyme replacement. Pancreatic sufficient patients (15%) may present at an older age and usually have milder lung disease and normal or borderline sweat electrolyte values.

Meconium ileus (MI) occurs in 10 - 20% of newborns with CF, and has a range of presentations from delayed passage of meconium to frank bowel obstruction. It is caused by inspissated bowel secretions. Babies with MI should always be evaluated for CF as very few other conditions can cause this condition. Recurrent constipation and distal intestinal obstruction syndrome (previously known as MI equivalent) are features in older patients with CF by a similar mechanism to MI.

Approximately 97% of males with CF are infertile due to azoospermia attributed to congenital bilateral absence of the vas deferens (CBAVD). Infertility may be the initial presentation for some males with mild disease. Isolated CBAVD is also encountered in 1-2% of infertile males without CF but 80% of these men have one or two CFTR gene mutations. The diagnosis of CF in these men is problematic. Gene mutation analysis may not clarify whether these men have, or will develop clinical CF. The sweat test may be more discriminatory as a measure of CFTR function in this group.

An increased concentration of electrolytes in the sweat may result in hyponatremic/hypochloremic dehydration secondary to salt depletion, or hypokalemic metabolic alkalosis secondary to chronic salt loss. Because of the high surface area to volume ratio, infants with CF are prone to heat prostration. Once the diagnosis is known and regular salt replacement offered this problem is rarely seen.

Although CF is a multi-system disease, lung involvement is the major cause of morbidity and more than 90% of mortality. With a life expectancy of less than one year in 1940, the median survival has increased to 35 years in Australia. In spite of this progress, current therapies treat the symptoms rather than the underlying cause. Consequently, CF remains a life-threatening and lethal disease.

**Diagnosis**

Until the discovery of the CFTR gene in 1989 the diagnosis of CF was based on defined clinical criteria (Table 1) and
Sweat Testing in the Genomic Era

Table 1. Phenotypic features consistent with a diagnosis of CF.

1. Chronic sinopulmonary disease manifested by
   a. Persistent colonisation/infection with typical CF pathogens including *Staphylococcus aureus*, nontypeable *Haemophilus influenzae*, mucoid and nonmucoid *Pseudomonas aeruginosa*, and *Burkholderia cepacia*
   b. Chronic cough and sputum production
   c. Persistent chest radiograph abnormalities (e.g. bronchiectasis, atelectasis, infiltrates, hyperinflation)
   d. Airway obstruction manifested by wheezing and air trapping
   e. Nasal polyps; radiographic or computed tomographic abnormalities of the paranasal sinuses
   f. Digital clubbing
2. Gastrointestinal and nutritional abnormalities including
   a. Intestinal: meconium ileus, distal intestinal obstruction syndrome, rectal prolapse
   b. Pancreatic: pancreatic insufficiency, recurrent pancreatitis
   c. Hepatic: chronic hepatic disease manifested by clinical or histologic evidence of focal biliary cirrhosis or multilobular cirrhosis
   d. Nutritional: failure to thrive (protein-calorie malnutrition), hypoproteinemia and edema, complications secondary to fat-soluble vitamin deficiency
3. Salt loss syndromes: acute salt depletion, chronic metabolic alkalosis
4. Male urogenital abnormalities resulting in obstructive azoospernia (CBAVD)


accurate analysis of sweat electrolytes. The discovery of the CFTR gene and laboratory techniques to detect CFTR mutations has facilitated the diagnosis of CF, and greatly expanded the clinical spectrum of CF to include milder and atypical presentations. The diagnosis of CF has been well reviewed by the US CF Foundation consensus panel. This document takes into account the current tests used to aid in the diagnosis: clinical features (discussed earlier), CFTR gene mutations, measures of CFTR function (principally sweat electrolytes, but also nasal potential difference) and newborn screening results. In Australia, most children with CF are diagnosed following newborn screening.

Immunoreactive Trypsinogen (IRT) - Newborn Screening
The discovery of elevated serum levels of trypsinogen, a pancreatic enzyme precursor of trypsin, in infants with CF resulted in the first newborn screening (NBS) programs for CF in 1981 in New Zealand and New South Wales. Since then, other states in Australia have followed, Queensland in 1983, Victoria in 1989, South Australia (including Tasmania and Northern Territory) in 1990 and Western Australia in 2001. NBS for CF is not universal elsewhere in the world with only a few states in the United States and regions in Europe currently using a CF NBS program. CFTR gene mutation analysis with one or more mutations has been incorporated into existing NBS programs.

NBS for CF involves a heel prick blood specimen collected on a filter paper on day 2 to 4 of life to measure trypsinogen by an immunoassay as the primary screen. Babies with an IRT greater than a pre-defined cut-off (Australian centres use the 99th percentile of values) for that batch have CFTR mutation analysis from the same filter paper sample. Infants with two CFTR mutations are considered to have CF while those with one CFTR mutation are referred for sweat testing to determine whether they have CF (sweat chloride >60 mmol/L) or are carriers (sweat chloride <40 mmol/L) only. Although, the pathophysiology of hypertrypsinogenaemia is not completely understood, it is thought that pancreatic acini in infants with CF are capable of producing trypsinogen but ductules are blocked, preventing trypsinogen from reaching the small intestines to be converted to trypsin, hence leading to ‘spillage’ into the circulation. The IRT level decreases after 1-2 months, indicating that pancreatic acini are no longer functioning well enough to produce the enzyme and thereafter the IRT test becomes unreliable.

CF NBS programs have a false-negative rate that arises from either a falsely low neonatal IRT or the presence of CFTR
mutations missed by the panel of mutations selected. The NBS programs from Australia which have reported their results have consistently shown a false-negative rate of around 5% of unexpected cases of CF (i.e. those who do not have MI or older sibling with CF).10,45 This equates to about 5-10 children per year in Australia who are missed by screening, highlighting the need to request a sweat test if there is clinical suspicion of CF. There is also a ‘false-positive’ rate with an increased identification of healthy CF carriers.2,10 This is reported in the order of 1.5 - 1.8 times the expected rate and is likely a reflection of a skewed (higher) distribution of IRT amongst CF carriers.10,46 It is generally recommended that all infants detected by NBS (even with two CFTR mutations) have a sweat test to exclude clerical or laboratory errors with screening. The specific issues of the diagnosis of CF after NBS have been reviewed recently.22

Mutation Analysis
The availability of CFTR mutation analysis provided a new method of diagnosis while also facilitating accurate carrier testing for couples and prenatal testing.2,38,43 Molecular testing for CFTR mutations will be discussed in detail later.

Sweat Electrolyte Analysis
The quantitative measurement of sweat electrolytes (chloride and sodium) following stimulation by pilocarpine iontophoresis will be discussed in detail later.

Nasal Potential Difference
The impaired ion transport of CF respiratory epithelia can be studied in vivo by measuring the potential difference (NPD) in nasal mucosa.47 This is a significantly more complex test of CFTR function than measurement of sweat electrolytes. The protocol for NPD is well described and standardised but performed in only specialised (mostly adult) centres.48 Patients with CF have reduced chloride transport with sodium hyperabsorption, measured as a more negative basal potential difference. Perfusion with the sodium channel blocker amiloride results in a greater drop in NPD in CF patients, while perfusion with chloride-free solution and isoproterenol to stimulate CFTR function has no effect. NPD may complement sweat testing and CFTR mutation analysis. However, it may also produce indeterminate results, particularly in “borderline” cases.49,50 NPD measurement is technically difficult and requires extensive experience for proper conduct and interpretation.51 Only one centre has reported use of NPD in infants.52 The presence of nasal inflammation, as in allergic rhinitis or viral infection, can alter ion transport and result in false negative results.48 For patients in whom both the sweat test and mutation analysis are inconclusive, an abnormal nasal PD measurement can be used as evidence of CFTR dysfunction.28

Ancillary Tests
There are other measures of CFTR function, such as direct intestinal current measurements from rectal suction biopsies and pancreatic stimulation testing for pancreatic duct electrolyte secretion.46,53 These tests are only available in a few centres. It is possible to gather additional clinical information using tests for the evaluation of paranasal sinuses (X-ray or CT scanning), assessment of pancreatic exocrine function (e.g. faecal elastase, 3 day faecal fat analysis), and respiratory tract microbiology (sputum or bronchoalveolar lavage). Urogenital evaluation (semen analysis) can also be useful in the diagnosis of CF.37

The CF Foundation Consensus statement proposed diagnostic criteria (Table 2) which cover the vast majority of patients with CF.38 The diagnostic criteria include the presence of one or more characteristic clinical feature (or a history of CF in a sibling or a positive newborn screening test result) and then confirmed by laboratory evidence of CFTR dysfunction or identification of two CFTR mutations. Abnormal CFTR function is usually documented by elevated sweat chloride concentrations.

Genetics of CF
CFTR Gene
The identification of the gene responsible for CF was one of the early triumphs of positional cloning.68 The availability of a large number of families with two or more affected individuals enabled the use of linkage analysis to localise the gene to chromosome 7.54-56 Soon after, in 1989, cloning of the gene responsible for CF was reported, which was called the CFTR gene.6-4 The CFTR gene is located on chromosome 7q31.2 and spans approximately 250 kb of DNA with 27 exons which are separated by large introns.4,57

CFTR Protein
The CFTR gene encodes a transmembrane glycoprotein of 1480 amino acids and has a calculated molecular mass of 168kDa.6 CFTR resides on the apical membrane of epithelial cells lining the airways, biliary tree, intestines, vas deferens, sweat ducts and pancreatic ducts.57 CFTR is a member of the ATP binding cassette family of transporters.57 Figure 1 shows that the amino acids of CFTR form five domains: two membrane spanning domains each composed of six subunits; two nucleotide binding domains; and a cytoplasmic regulatory domain. The membrane spanning domains appear to contribute to the formation of a chloride channel pore, since mutation of specific residues within the first membrane spanning domain alters the anion selectivity of the channel.19,59 The nucleotide binding domain of CFTR is responsible for the binding and hydrolysis of ATP and provide the energy necessary for channel activity.2,57 The regulatory domain modulates the
The channel activity of CFTR and can have both inhibitory and stimulatory effects.\textsuperscript{2,60}

Accumulating evidence has demonstrated that CFTR is a cAMP dependent chloride channel.\textsuperscript{2,58} This was tested directly by Bear and colleagues in 1992 who showed that recombinant CFTR protein had the same biophysical and regulatory properties of the chloride channel found exclusively in CFTR-expressing cells.\textsuperscript{61} However, this function alone cannot explain the entire pathology of the disease. Numerous other functions have been described that do not relate directly to a disease mechanism based on a channelopathy.\textsuperscript{62}

The list of proteins with which CFTR interacts in its role as conductance regulator continues to grow and includes channels (sodium, potassium and calcium-activated chloride), transporters (ATP and glutathione), and proteins linked to the apical cytoskeleton scaffolding of epithelial cells.\textsuperscript{57,62} These regulatory roles cannot be easily related to one another, and suggest that the CFTR regulates multiple pathways.\textsuperscript{52}

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**Table 2.** A consensus statement: the diagnostic criteria for CF.

The diagnosis of CF will be suggested in individuals with:

1. One or more characteristic phenotypic features
   - or a history of CF in a sibling
   - or a positive NBS result

2. Laboratory evidence of CFTR dysfunction
   - Two abnormal quantitative pilocarpine iontophoresis sweat chloride concentrations
   - presence of two disease-causing mutations in the CFTR
   - demonstration of abnormal NPD


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**Figure 1.** Schematic diagram of the proposed structure of CFTR. A member of the ABC family, CFTR consists of a tandem repeat of the ABC motif. This motif comprises a membrane-spanning domain (composed of six transmembrane stretches of amino acids) followed by an nucleotide-binding domain (NBD). In CFTR, the two occurrences of this motif are separated by a regulatory (R) domain. Each NBD is able to bind and hydrolyse ATP to operate chloride (Cl-) channel function; hydrolysis of ATP by NBD-1 opens the chloride channel, while ATP hydrolysis by NBD-2 closes the channel. Channel function is further regulated by phosphorylation of serine residues in the R domain. Lyczak JB, Cannon CL. and Pier GB. Lung infections associated with cystic fibrosis. Clin Microbiol Rev. 2002 Apr;15:194-222. With permission from American Society for Microbiology Journals Department.

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CFTR co-regulates sodium transport through an epithelial sodium channel (ENaC). Wild type CFTR inhibits ENaC sodium transport, while mutant CFTR allows enhanced sodium transport.\(^{63}\) The exact mechanism of this regulation has not been elucidated. The amiloride-sensitive ENaC is responsible for the hyperpolarisation seen at respiratory epithelial surfaces and can be measured as an increased negative transepithelial potential in CF patients.\(^9\)

CFTR also stimulates an outwardly rectifying chloride conductance (ORCC) that may augment its own chloride transport activity.\(^{64,65}\) Yet how CFTR regulates ORCC is not clear. A current hypothesis suggests that CFTR facilitate release of ATP, which stimulates a purinergic receptor. The purinergic receptor then acts via a second messenger pathway to regulate ORCC.\(^2\)

In several tissues CFTR plays a key role in modulating or directly mediating bicarbonate secretion.\(^{2,9}\) An important role of bicarbonate in the function of secretory epithelia in CF was provided by Choi and colleagues in 2001, who found that CF patients with pancreatic insufficiency do not have measurable bicarbonate transport, and those associated with pancreatic sufficiency show reduced bicarbonate transport.\(^{66}\)

The epithelial apical membrane also contains another chloride channel that appears to be regulated by intracellular calcium, although the regulation and molecular identity remains to be established with certainty.\(^2\) This calcium-regulated chloride channel is present in the airways and sweat gland epithelial cells. In the secretory coil of the sweat gland, muscarinic agonists increase the intracellular calcium concentration and stimulate sweat production by opening calcium-activated apical membrane chloride channels.\(^2\)

From the initial finding that CFTR was principally a chloride channel we now realise that CFTR is a multifunctional protein complex that has many more functions. Future research will allow us to understand the complex functions of CFTR and how mutated CFTR causes the pathology and clinical manifestations of CF.

CFTR Mutations

Over 1000 mutations and 200 polymorphisms have been defined throughout the CFTR gene.\(^{23}\) There are regions where mutations are more common, such as the nucleotide-binding domain and regulatory domain.\(^{67}\) Types of mutations include missense (44%), frameshift (22%), splice site (16%), nonsense (14%) and in frame deletions (2%). Most of these mutations (83%) are associated with clinical CF disease, while the remainder are classified as polymorphisms,\(^2\) indicating that they are ‘non-disease’ causing mutations. One such example is ΔF508C (T – C conversion at position 508), which may have the same electrophoretic appearance as ΔF508.\(^{68}\) Other polymorphisms may have mild dysfunction that in some circumstances can alter CFTR function enough to cause disease e.g. M470V.\(^{69}\)

Alterations in the CFTR gene should fulfill at least one of the criteria suggested in the US CF Foundation consensus statement to be designated as CF-causing mutations.\(^{78}\) Mutations in the CFTR gene were initially classified into five groups according to the mechanisms of which they disrupt CFTR function (Figure 2) and recently a sixth group has been included.\(^{59,67,70}\) However, these classes are not mutually exclusive and specific mutations may have characteristics of more than one class. These mechanisms of CFTR dysfunction are intended to provide a framework for understanding the molecular basis of epithelial cell abnormalities in CF.\(^{71}\)

Class I: Defective Protein Synthesis

Mutations in this class include the most severe CF phenotypes resulting in no protein being synthesised.\(^{2,59,67}\) The most common Class I mutation is G542X, which either prevents the synthesis of a stable protein or results in the production of a truncated protein due to the creation of a premature termination codon.\(^{67}\) The aminoglycoside antibiotics can suppress premature termination codons by permitting translation to continue to the normal termination of the transcript.\(^{72}\) This has shown to be promising in in vitro and in clinical trials but further studies need to be performed to make it a safer compound that may be administered to children with this class of mutation from the time of diagnosis.\(^{72}\)

Class II: Defective Protein Processing

Mutations in this class result in a CFTR protein that fails to traffic to the correct cellular localisation due to mis-folding of the protein. This includes the most common and the first recognised mutation, ΔF508 (deletion of phenylalanine at position 508). Other missense mutations associated with misfolding are found throughout CFTR.\(^2\) In recombinant cells, CFTR containing the ΔF508 mutation fails to mature to the fully glycosylated form or to proceed beyond the endoplasmic reticulum. The mis-formed CFTR is destroyed by the ‘cellular quality control machinery’, the details of which are only just being elucidated.\(^{73}\) There is also good evidence indicating that CFTR ΔF508 is similarly mislocalised in CF airway epithelial cells and in sweat gland duct cells. Thus, the protein is either missing or present in reduced amounts in the apical membrane. In intestinal, respiratory and hepatobiliary epithelia of ΔF508 homozygous CF patients, a proportion of the CFTR protein has been localised to the apical membranes. Some mutations, such as P574H have a defect in folding that is less severe than ΔF508, and as a result the protein reaches the plasma membrane and retains some function.\(^{74}\)
Class III: Defective Regulation
Mutations in this class produce the CFTR protein which is trafficked to the cell membrane but does not respond to cAMP stimulation. To date Class III mutations have been located within the nucleotide binding domain. As intracellular ATP regulates the opening of CFTR chloride channels through direct interactions with the nucleotide-binding domain, it is not surprising that mutations in these domains could alter channel function. In some mutations, e.g. G551D, there is minimal function and in some, e.g. S1255P, ATP is less potent at stimulating activity. CFTR is also regulated by phosphorylation of the regulatory domain but there appear to be fewer mutations in this domain than in other parts.

Class IV: Defective Conduction
Many missense mutations have been identified in the membrane spanning domains, where the CFTR gene encodes a protein that is correctly trafficked to the cell membrane and responds to stimuli but generates a reduced chloride current. Some examples include mutations in which arginine is replaced by histidine at residue at 117 (R117H), tryptophan at 334 (R334W), or proline at 347 (R347P). When these mutant CFTRs are expressed in heterologous epithelial cells, all three are correctly processed, are present in the apical membrane, but generate reduced current. This is due to the reduced rate of ion flow through a single open channel. In addition, at least for R117H, the amount of time that the channel is open is also reduced. R117H is a particularly interesting mutation as the affected CFTR function is also determined by the M470V polymorphism and the amount of protein produced. The clinical effects vary from pancreatic insufficient CF through to no clinical disease depending on the combination of these factors.

Class V: Reduced Abundance
Mutations in this group include missense, e.g. A455E (substitution of glutamic acid for alanine) and aberrant exon splicing, e.g. 3849 10kbC→T and the intron 8 polythymidine and TG repeat sequences that regulate exon 9 splicing. These mutations produce a reduced amount of CFTR transcript and low levels of functional protein that is translocated to the cell membrane. As a small amount of full length mRNA is often still synthesised in these cases, they would be predicted to result in a mild phenotype. Exon 9 of the CFTR gene encodes the first 21% of nucleotide binding domain and is critical for CFTR function. However, the fact that up to 92% of CFTR mRNA transcripts can lack exon 9 without causing CF defined a threshold level for the amount of functional CFTR.
mRNA needed to maintain a clinically normal phenotype. Predicted mutations in the CFTR promoter can have similar effects by reducing the level of transcription.²,⁶⁷

**Class VI: Reduced Protein Stability**

Mutations in this novel class include protein stability mutants which cause lability of the CFTR protein, such as mutations resulting in absence of the 70-98 residues of the CFTR C-terminus.⁷⁰,⁷⁹ Although the C-terminus is not required for the biogenesis and chloride channel function of CFTR, it is indispensable for maintaining the stability of complex-glycosylated CFTR.² The shortest truncation reported that caused CF with pancreatic insufficiency and recurrent pulmonary infection is Q1412X which lacks 70 amino acids.⁸⁷

**CFTR Mutations**

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CFTR Mutations are classified as severe or mild, depending on the effect on the functional protein and clinical effect.⁷⁰ Generally, severe mutations result in no synthesis or blocked processing (Class I, II, or III), whereas mild mutations show altered conductance or reduced synthesis (Class IV, V and VI).

The geographical distribution of CFTR mutations varies worldwide. The ΔF508 mutation, the first CF mutation identified, accounts for about 70% CF alleles in European-derived Caucasian populations.² The frequency of ΔF508 varies with an apparent gradient of increasing frequency from south-east to north-west Europe,²,⁶⁷ with the highest frequency of ΔF508 in Denmark (90%) and the lowest frequency in Turkey (18.8%).²¹ The high frequency of the ΔF508 mutation indicates that around 90% of CF patients will have at least one copy of ΔF508; 50-60% of patients will be homozygous, while 30-35% will be heterozygous.²⁷ It has been proposed that this high frequency of CF in Caucasians could be due to an advantage of the CF heterozygote such as resistance to secretory diarrhea from cholera and typhoid fever, higher fertility, reduced rates of asthma and resistance to a variety of infectious agents.²,⁶⁷,⁸⁰ However, these theories lack confirmatory evidence. Other mutations are often found in higher frequency in particular ethnic populations, such as the W1282X mutation in Ashkenazi Jewish populations and G551D in French Canadians.⁷¹ Such information is useful in designing mutation panels suitable for screening programs in different populations.

**Genotype Analysis**

**Diagnostic Testing**

Molecular testing for CFTR mutations is part of the routine diagnostic evaluation for CF or carrier testing. In selecting a range of mutations to test, consideration must be given to ethnicity, since the carrier rate, mutation frequencies and detection rate for the panel vary for different populations.

When molecular analysis is used to confirm a diagnosis based on clinical concerns and elevated sweat electrolytes, as many mutations as possible should be tested. In Australia, the maximum available mutation panel includes 17 mutations, which determine 83.5% of known mutations. Commercially available panels used overseas include up to 70 mutations. However there is a diminishing chance of detecting a mutation after ΔF508 which has an allele frequency in CF of 70%, when one considers that the next most common mutations (in Australia) are G551D (5%), G542X (2.4%), N1303K (1.3%) and most other mutations have an allele frequency significantly less than 0.5%. The phenotype of many rarer mutations is poorly understood, so the US CF consensus statement recommends that only mutations known to be associated with clinical CF be tested.³⁸ In patients with atypical disease, including CBAVD the widest possible mutation search may be helpful. Some centres are able to offer complete CFTR exon sequencing. Technological advances may make sequencing of CFTR routinely possible.

The situation with regard to screening programs, such as NBS or community carrier testing is a little more complex. In this situation, healthy people are being tested so that most screening services would accept that it is best to test for mutations associated with severe disease only (i.e. mutations in class I, II, or III).³¹

**Carrier Screening**

Carrier testing is most commonly offered to individuals with a family history of CF.³² In Australia this most commonly follows NBS when affected infants or healthy carriers are detected. Carrier testing is done in combination with genetic counselling. It is facilitated when the CFTR gene mutation in the affected person is known. The number of mutations chosen to screen for will depend on ethnic and economic considerations. CFTR mutation screening cannot detect all mutations so that it is important for the concept of residual risk to be understood before testing is offered. When mutation-based tests are inconclusive, linkage analysis can be performed using DNA markers that flank, or are within the CFTR gene.² Linkage analysis can predict carrier status with high accuracy in almost all families but incurs greater effort and expense than mutation analysis.²

Population based screening for CFTR gene mutation carriers is recommended by the American Academy of Obstetricians and Gynaecologists and the National Institutes of Health (NIH). The only successful program to date has been in Edinburgh. Using analysis for ΔF508 alone, the incidence of CF in that community has been halved.³³ No such program exists in Australia.
Prenatal Testing
Another implication of CFTR mutation analysis is the ability to implement prenatal diagnosis in order to allow a person to make informed reproductive decisions. Prenatal diagnosis may be performed by chorionic villus sampling in the first trimester or by amniocentesis in the second or third trimester. Such testing is usually carried out in a family that has had a previously affected child or because of the detection of foetal echogenic bowel on routine ultrasonography.

Genotype-Phenotype Correlation
The correlation between CFTR genotype and CF-phenotype is extremely variable. It is best characterised for the pancreatic status and less well for other manifestations, in particular for pulmonary disease. In general, patients with two severe mutations (class I-III) can expect childhood onset suppurative lung disease and pancreatic insufficiency, while patients with at least one mild mutation (class IV-VI) are usually pancreatic sufficient with later onset suppurative lung disease. This paradigm is understood on the basis of epithelial cell apical membrane CFTR activity, a combination of CFTR product and function as determined by CFTR gene mutations. However, the outcome of individuals with the same CFTR mutations, even from the same family can be significantly different. This suggests an influence of environmental (inhaled pollutants, infectious pathogens and smoking) and perhaps secondary genetic factors which may act as modifiers of CFTR function. However, there are no non-CFTR genetic modifiers that have been discovered to influence pulmonary function. Gene mutations for cytokines associated with pulmonary inflammation have been studied with no associations found. The only well described CFTR modifier gene is one associated with MI.

Another variable to consider with regard to the effect of CFTR mutations on phenotype is that CFTR mRNA splicing may be different between tissues, with some tissues such as the vas deferens being more sensitive to CFTR mutations than other tissues such as airway epithelia. What emerges from the association of specific organ involvement with specific CFTR mutations, some of which have significant residual CFTR activity, is that there exists a hierarchy of tissue responsiveness to deficits in functional CFTR (Figure 3). This pattern became clear from Chillon’s study in 1995 of infertile men and the interaction of intron 8 splice variants and exonic mutations, which was further clarified by Davis and colleagues. The CF phenotype is a complex interaction of: CFTR gene mutations, modifier genes, CFTR chloride transport and interaction with other ion channels, intracellular CFTR functions, tissue expression of CFTR and tissue responsiveness to CFTR mutations in combination with environmental exposure to a range of possible agents.

Pitfalls of Genotype Analysis
There are many cases described where clinical features, CFTR genotype and electrophysiological measurements are

![Figure 3](image-url)

**Figure 3.** Relation between CFTR activity and the clinical manifestations of CF. Left panel shows estimate of CFTR activity from published data in the literature. Right panel shows descending order of tissue sensitivity to CFTR deficit. Decreased levels of normal CFTR activity may be involved in various clinical phenotypes, ranging from the normal phenotype to the phenotypes of CBAVD, cystic fibrosis with pancreatic sufficiency, and cystic fibrosis with pancreatic insufficiency. CBAVD = congenital bilateral absence of vas deferens.
contradictory with respect to the diagnosis of CF. Warren and colleagues described a patient in whom NBS and mutation analysis suggested a diagnosis of cystic fibrosis, however the clinical course and sweat test results were not consistent with the diagnosis. Direct sequencing of the patient’s genomic DNA showed compound heterozygosity for ΔF508 and ΔF508C, a polymorphism not associated with clinical disease. A report from Chmiel and colleagues presented a case where an asymptomatic female infant (3 weeks of age) was given the diagnosis of CF solely based on DNA analysis from cord blood which was positive for both the ΔF508 and R117H mutations. Despite any other presentations and a normal sweat chloride, she received pancreatic enzyme supplements. At 2 months of age, she was evaluated including sweat chloride, NPD and bronchoscopy and bronchoalveolar lavage measurements, all of which were consistent with findings expected from an individual without CF. This initial diagnosis of CF adversely affected the family’s emotional, employment and financial status. These cases illustrate potential pitfalls of using mutation analysis as a sole diagnostic criterion of CF. The full evaluation should include a thorough clinical assessment and measurement of CFTR function (by sweat test or NPD) before making the diagnosis and intervention.

At the other end of the spectrum, there are patients with clinical CF but no CFTR mutations, despite complete gene sequencing. The diagnosis of CF was based on highly elevated sweat chloride in the presence of CF-like pulmonary symptoms but the CFTR genetics and NPD data provided no evidence for defective CFTR and no molecular lesion was identified. Recently a group of patients with symptoms of CF have been described, in whom the complete gene sequence for CFTR has been determined and found to be normal. Taken together these data suggest that factors other than CFTR dysfunction can cause a non-classic phenotype. Of particular interest is whether the defective pathways in these patients involve CFTR or act independently of CFTR. Further study of these patients may reveal additional pathways contributing to CF phenotypes.

**Sweat Electrolyte Analysis**

The association of sweat electrolyte abnormalities with cystic fibrosis was established during the 1948 heat wave in New York. In 1956 Shwachman and Gahm used sweat electrolyte analysis for diagnosis in their clinic, but it was not until Gibson and Cooke described their method of pilocarpine iontophoresis with pad collection of sweat in 1959 that a standardised test was accepted. Since then there have been significant advances in our understanding of sweat electrolyte analysis as a measure of CFTR function and refinements in the use of sweat testing for the diagnosis of CF.

**Physiology of the Sweat Gland**

The sweat gland is composed of two different regions; the secretory coil and the reabsorptive duct. The primary sweat is made in the secretory coil and is isotonic with serum. As the isotonic secretions travel from the acini of the sweat gland through the water-impermeable duct, sodium and chloride are absorbed resulting in hypotonic sweat. Sodium transport establishes the ion concentration and voltage gradients that drive passive chloride absorption. Chloride is transported from the duct lumen by CFTR and a distinct calcium-activated chloride channel (CaCC). Sodium is transported through the epithelial sodium channel, ENaC which is also down-regulated by CFTR via a mechanism that is not completely understood. Sweat duct electrolyte and water transport is shown in Figure 4.

Sweat glands in CF patients do not show any histological abnormalities but have pronounced abnormalities in sodium-chloride homeostasis due to defective CFTR function. This was first studied in detail by Quinton in 1983 who used isolated sweat ducts from control subjects and cystic fibrosis patients and determined that there was abnormally low chloride permeability in cystic fibrosis sweat duct epithelium which led to poor reabsorption of chloride and sodium. Subsequent research, after identification of CFTR, showed that the absence of functioning CFTR is responsible for inhibited reabsorption of chloride. Despite the pathways for sodium absorption, in the absence of a co-ion, sodium is also poorly reabsorbed. The consequences of this are: 1) the resultant sweat has a relatively elevated concentration of chloride and sodium compared with normal sweat 2) high chloride/sodium ratio (>1) is often seen in CF patients compared to the normal population 3) the transepithelial potential difference between the extracellular fluid and sweat at the opening of the sweat duct of CF glands is approximately twice as negative as the normal sweat glands. In CF patients, CaCC are normal so that there is some modification of the primary, isotonic sweat. The values of sweat chloride and sodium are usually above 60 mmol/L in patients with CF, and can be as high as 120 mmol/L. This compares with values for normal subjects varying from 10-50 mmol/L. The subject of normal sweat electrolyte values will be discussed in more detail later.

**The Sweat Test**

Sweat testing is a general term referring to the quantitative or qualitative analysis of sweat to determine electrolyte concentration, conductivity, or osmolality for the diagnosis of CF. The principle indications for performing a sweat test include: positive newborn screening for CF (elevated IRT followed by CFTR mutation analysis); clinical signs suggestive of CF (Table 1); or a family history of CF.
Guidelines for appropriate performance of the sweat test procedure, which are complementary to each other, have been published by a number of organizations including: 1) the National Committee for Clinical Laboratory Standards (NCCLS): Sweat Testing: Sample collection and quantitative analysis; 2) Association of Clinical Biochemists: UK guidelines; and 3) Australasian Association of Clinical Biochemists: Australian guidelines for the performance of sweat test for diagnosis of CF. Sweat testing should be carried out in accordance with these guidelines; alternative procedures are no longer acceptable for the diagnosis of CF.

The sweat test collection based on the Gibson and Cooke method is either performed by an 'in-house' system or by the commercial Wescor system. In both, the sweat test generally has three technical parts: sweat stimulation, collection and analysis. The sweat analysis is then followed by the interpretation of the results.

Sweat Stimulation
The preferred site for sweat collection is the flexor surface of the forearm. Other sites used successfully include the upper arm, thigh and calf. Localised sweating is produced by iontophoresis of the cholinergic drug, pilocarpine nitrate, into the selected area of skin. Aqueous solutions or Wescor gel discs containing pilocarpine nitrate at 2-5 g/L is recommended for use at the positive electrode. The negative electrode may either contain the same or alternative dilute electrolyte solution (e.g. magnesium sulphate or potassium sulphate). The electrodes are made of copper or stainless steel. A current of 0.5 mA is applied and is gradually increased to a maximum of 4 mA. With the applied current the positively charged pilocarpine ions move away from the positive electrode and into the skin where they increase the intracellular calcium concentration and stimulate sweat production by opening the calcium-activated chloride channel. The sweat volume in response to muscarinic agonists is not altered in CF, allowing adequate collection of sweat for biochemical analysis. Once 4 mA is attained, the current should be applied for no more than 5 minutes. The electrodes are then removed and the skin is cleaned with distilled water.

The chance of urticaria or burn to the patients skin after iontophoresis is rare (<1%). A skin burn can occur if the current is greater than 4 mA, bare metal of the electrode touches the skin, reagent interface is not sufficiently moist or if the electrode is damaged or oxidised. Localised urticaria may occur if the patient has a reaction to pilocarpine or to the phenomenon of electrical stimulation. In both circumstances, the sweat should not be collected over the affected site.

Sweat Collection
Immediately following stimulation a pre-weighed gauze/filter paper (in-house equipment) or a Wescor collection device (Figure 5) is placed directly over the site of the positive electrode. Critical issues in sweat collection include avoiding evaporation or contamination of the sample and ensuring an adequate sweat rate. At the end of the collection the gauze/filter or Wescor collector is removed and the weight or volume is determined respectively.

To ensure accurate results a minimum sweat rate of 1g/m²/min is required. Since the sweat rate is related to sweat electrolyte...
concentration, minimum acceptable weight/volume is necessary. The minimum acceptable weight/volume can be calculated using the formula shown in Figure 6.

In the Australian guidelines, if the laboratory uses 2x2-inch gauze or filter paper, the minimum sweat weight should be 75 mg collected in 30 minutes. Using the Macroduct system, the electrodes and stimulation area are smaller and the minimum acceptable sample is 15 μL collected in 30 minutes. Thus, the minimum acceptable sweat volume/weight depend on the size of the electrode used, the type and size of the collecting material used and duration of sweat collection.

Sweat collection time should not be more than 30 minutes and not less than 20 minutes. Insufficient samples must not be pooled, rather the whole test must be repeated.

Sweat Analysis

Sweat analysis can be performed in two ways: indirect method based on the colligative physical chemical properties of ions or solutes in sweat such as conductivity and osmolality; and the direct measurement of the electrolytes chloride and sodium.

1. Indirect measurement

On theoretical grounds osmolality and conductivity measurement might be expected to provide very good discrimination between normal and CF sweat electrolyte concentrations. Sodium, potassium and chloride, the predominant electrolyte constituents of sweat, are all increased in CF and the incremental contribution of each would be additive by virtue of the colligative nature of the measurement. The contribution by other solutes and electrolytes present in sweat, in variable concentration and not specifically increased in CF, would predictably diminish the discriminating power of these indirect measurements.

A. Osmolality

Osmolality of sweat reflects the total solute concentration of sweat measuring the total cations and anions along with other solutes such as urea and amino acids. Thus osmolality has a poor discriminatory power compared to chloride in distinguishing between CF and normal individuals and as such is not recommended for analysis of sweat for the diagnosis of CF.

B. Conductivity

The U.S. CF Foundation has approved the use of the Wescor Macroduct Sweat Chek to measure conductivity as a screening test. When evaluating sweat conductivity results, physicians should be aware that sweat conductivity is approximately 15 mmol/L higher than sweat chloride because of the presence of unmeasured anions such as lactate and bicarbonate. According to the U.S. CF Foundation, sweat conductivity of ≥50 mmol/L should be referred to an accredited CF care centre for measurement of sweat chloride by quantitative pilocarpine iontophoresis. Several studies have shown conductivity to be as effective as chloride measurement in the laboratory diagnosis of CF and could be used alone as a confirmatory test. Recently, a new point of care conductivity analyser Nanoduct (“new system”) has been developed for use especially in the neonatal population as it requires only 3 μL of sweat. However, the accuracy of the new system in comparison to the quantitative sweat chloride test in discriminating between CF and normal individuals still needs to be investigated. To date current published guidelines do not support the use of the conductivity as a confirmatory test.

2. Direct measurement

A. Chloride

Sweat chloride is the measured analyte most directly related to the abnormal function of CFTR and shows greater discrimination when compared to sodium. The acceptable measurement techniques to measure chloride are colorimetry, coulometry or ion selective electrode.

B. Sodium

The acceptable measurement techniques to measure sodium are flame photometry, atomic absorption spectrophotometry or ion selective electrode. Sweat sodium is elevated in CF but is less discriminatory when compared to chloride for diagnosis and so should not to be used alone for the diagnosis of CF.
For quality control purposes, a significant discordance between the two electrolyte concentrations can indicate technical error in collection, analysis or both and the test should be repeated. Generally, sweat chloride and sodium concentrations agree within 15 mmol/L.12

Interpretation
The sweat chloride concentration must be interpreted with regard to the patient’s clinical presentation, family history, age and the knowledge that some mutations of the CFTR gene are associated with a borderline or negative sweat chloride concentration.99

1. Reference Intervals
The reference ranges of sweat chloride that are currently accepted for the diagnosis of CF in children are chloride <39 mmol/L negative/normal; 40-59 mmol/L borderline (could be CF); and >60 mmol/L positive for CF. These values, however, may not accurately represent those in adults or infants.

There appears to be a normal increase of sweat electrolyte values from childhood to adulthood and healthy adults can have sweat chloride concentrations greater than 60 mmol/L.17,32 So there is some cause for caution when interpreting sweat test results in older patients.

A few studies have tried to address the issue of age-related reference ranges, but have been flawed. Most did not recruit true healthy controls but relied on values generated by sweat test laboratories from patients referred for sweat testing who were later considered not to have CF. The earlier studies only reported sweat sodium which is much less discriminatory than chloride measurement.18,100,101 Many of these studies were carried out prior to the availability of CFTR mutation analysis when there was less awareness of a broader CF phenotype. In the absence of CFTR mutation analysis, it is possible that some of the control individuals could have had CF or CF related disorders or been carriers.

With regard to sweat test values in infants, the Australasian Paediatric Respiratory Group recommends sweat chloride values in infants following NBS to be: chloride ≥ 60 mmol/L positive for CF; 30-59 mmol/L, borderline and ≤ 29 mmol/L normal.22 Lowering the cut-off to 30 mmol/L in infants following NBS screening was also supported by Farrell who showed that the mean sweat chloride in healthy infants (without ΔF508) was 10.6 mmol/L (± 5.2 sd) and ΔF508 carriers 14.9 mmol/L (± 8.4 sd), making a sweat chloride of 30 mmol/L four standard deviations above the mean.21 Similar values were reported from the NSW NBS program.19

2. Electrolyte Ratio
Diagnostically, several studies have evaluated the use of sweat chloride/sodium ratios, which is usually >1 in CF.93 In Augarten’s study of sweat chloride/sodium ratio, the mean ratio was higher in patients with two severe mutations (mean 1.2 mmol/L ± 0.1 SD) than patients with a mild mutation (mean 0.94 mmol/L ± 0.1 SD), which in turn was higher than controls (mean 0.7mmol/L ± 0.4 SD). The results were significantly different but the spread of results included patients above, below and equal to 1.0, making it less useful for individual diagnosis. In addition, of patients classified as having mild mutations, only 20% had higher sweat chloride than sodium levels. This is the group of patients who may have minimal symptoms and borderline sweat chloride and for whom additional information is required. While a chloride/sodium ratio of >1 supports the diagnosis, a ratio of <1 does not exclude CF and thus is not recommended for interpretation.

3. Sweat Chloride in Carriers
Some studies have proposed that sweat chloride values in obligate carriers (parents and siblings of CF) are higher compared to the random population.102 Farrell and colleagues demonstrated that CF heterozygote carriers with a ΔF508 mutation have significantly increased sweat electrolyte concentrations, although they are not high enough to be in the range diagnostic of the disease. In this study, the control patients mean sweat chloride was 10.6 mmol/L (± 5.2 SD) while CF heterozygote carriers had a statistically different mean sweat chloride of 14.9 mmol/L (± 8.4 SD).21 In addition, it was also demonstrated that CF heterozygote carriers

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\text{The average sweat rate} = \frac{10000 \times \text{weight (mg/\mu L)} \times 1}{\text{area (cm}^2) \times 1000 \times \text{collection time (min)}}
\]

\[
= \frac{10 \times \text{weight (mg/\mu L)}}{\text{collection area (cm}^2) \times \text{time (min)}}
\]

Figure 6. Calculation of average sweat rate and the minimum acceptable weight/volume.

Sweat Testing in the Genomic Era
have transient hypertrypsinogenaemia during the neonatal period and this may be regarded as another phenotypic manifestation. These abnormalities are probably attributable to minor degrees of impaired chloride channel function in the carrier and the secondary consequences in electrolyte and water transport. These changes in sweat electrolytes are not clinically significant but are supported by similar findings in pancreatic electrolyte secretion of carrier infants.

4. Normal and ‘Borderline’ Sweat Chloride

Although the vast majority of patients with CF have elevated sweat chloride concentrations, there are many reports of patients with clinical symptoms suggestive of CF but with normal or borderline electrolyte values. These patients often maintain pancreatic sufficiency and have a normal nutritional status, absent vas deferens in males and a later age of diagnosis. It was not until the CF gene was cloned and the CFTR mutation analysis became available that the concept of a ‘normal’ sweat test became understood. Certain mutations were found to be associated with a ‘normal’ range sweat test and are discussed later.

Borderline range sweat chloride (40-59 mmol/L) can also pose a diagnostic dilemma. It should be noted that during infancy, a sweat chloride of 40 mmol/L or greater has a low probability of being a true normal, and a sweat chloride level between 40-59 mmol/L is likely to be diagnostic for CF. Therefore, infants with borderline sweat chloride values need follow-up, careful observation, repeat sweat testing and extended CFTR mutation analysis.

Genotype-Phenotype Correlation: Sweat Electrolytes

The correlation between sweat electrolytes and genotype was initially explored by Witt and colleagues. Later, with better classification of the various gene mutations, sweat chloride was correlated to some groups of mutations. Wilshanski and colleagues found no differences between patients bearing class I, class II, class III and class V mutations. The only statistical difference was in the class IV mutations, which had a significantly lower mean sweat chloride level than those homozygous for AF508 and nearer to normal than those in class I, II and III. However, one would expect class V to have reduced sweat chloride values, and class IV and V to have similar sweat chloride values. This discrepancy could have been due to the limited number of patients in Class IV (17 patients) and V (11 patients).

Highsmith and colleagues (1994) studied 23 patients with pulmonary disease characteristic of CF but with a normal sweat test and identified a point mutation in intron 19 of the CFTR gene, termed 3849+10kb C-T. This mutation produces an alternative splicing site and decreased amounts of CFTR mRNA can be detected. Thus, according to the classification of the CFTR mutations, this mutation falls into Class V. Other mutations associated with normal or borderline sweat electrolytes are R117H, D1152H, A455E, G551S and 2789+5G – A.

An interesting phenotype, presenting with elevated sweat chloride concentration in the absence of other CF symptoms, has been described in a patient with a nonsense mutation, S1455X. This mutation truncates 26 amino acids from the C-terminus of the protein product. On detailed clinical evaluation, this patient had normal pulmonary function, normal sputum flora and no manifestations of exocrine pancreatic disease and the serum IRT was not suggestive of CF. CFTR mRNA transcripts bearing the S1455X mutation were normally processed and functional, which therefore suggests that the truncated stretch C-terminal amino acid plays some role in the sweat gland only.

Pitfalls of the Sweat Analysis

Most errors relating to sweat tests are caused by the use of unreliable methodology, inadequate sweat collection, technical errors and, occasionally, misinterpretation of the results. The technical aspects of performing a sweat test are demanding and these errors occur more often in institutions doing relatively few tests, usually not in accordance with published guidelines.

False Positive Sweat Test

Approximately 98% of patients with CF have sweat chloride concentrations greater than 60 mmol/L. There are a variety of well described, although rare conditions, which are associated with elevation of sweat electrolytes. These conditions are generally easy to differentiate from CF and should not be considered true false positives. They include atopic eczema, untreated Addison’s disease, ectodermal dysplasia, some types of glycogen storage diseases, and untreated hypothyroidism. Sweat electrolytes measured within the first 24 hours after birth may also be transiently elevated. Up to 25% of normal newborns show a sweat sodium concentration greater than 65 mmol/L on day 1 but this rapidly declines on the second day after birth.

There have also been reports of patients with intermittent elevations of sweat electrolyte concentrations. Shaw and Littlewood found 4% of a clinic of 179 to have been falsely diagnosed with CF on the basis of a sweat test. Rosenstein and Langbaum described eight patients in which the diagnosis of CF was initially made on the basis of a suggestive clinical picture and at least two positive quantitative pilocarpine iontophoresis sweat test results but who were subsequently documented to have normal sweat electrolyte concentrations.
Sweat Testing in the Genomic Era

The misdiagnoses resulted from poor quality sweat tests, which had been performed by inexperienced laboratories. Patients who do not follow a typical course should have their diagnostic criteria re-assessed, including reviewing the original sweat test and given consideration for a repeat test.

A sweat chloride concentration of more than 160 mmol/L is physiologically impossible and suggests laboratory error or Munchausen syndrome (including by proxy). Technical errors such as evaporation and contamination, except for the misdilution of sample, tend to produce false-positive values. The development of the Wescor Macroduct system overcame many of the previous known errors. Being a closed system, its advantages include no condensate error, an easily visible sample, complete recovery of the sweat (no dead space), virtually no loss by evaporation, no contamination by surrounding dried sweat, standardised electrical current area, and pilocarpine concentration.

False Negative Sweat Test
The most important pathophysiological cause of a false negative sweat test is oedema. Oedema is commonly seen in infants with hypoproteinaemia, which can be secondary to pancreatic exocrine insufficiency, before diagnosis and treatment with replacement pancreatic enzymes. The use of mineralocorticoids can also decrease sweat electrolyte concentrations.

From a technical point of view, the rate of sweating is important in achieving accurate results as the sweat electrolyte concentration is related to sweat rate. At low sweat rates, sweat electrolyte concentration decreases and the opportunity for sample evaporation is increased. The average sweat rate should exceed 1g/m²/min.

Insufficient samples can be due to several factors such as age, skin condition, hydration status and collection system. Though it may seem to be more difficult to obtain an adequate sweat sample in early infancy, studies have shown the sweat test to be performed successfully in infants a few weeks old. Data obtained following the introduction of newborn screening in Colorado reported that 99.2% of infants have an adequate collection of sweat at a mean age of 6.2 weeks. A recent study demonstrated that 74% of overall infants ≤ 6 weeks of age can be successfully sweat tested, 87% in non-African-American infants ≥ 36 weeks of gestational age and that maturational factors have a mild impact on sweat chloride concentration. As a general guideline, sweat tests can reliably be performed after 2 weeks of age in infants greater than 3 kg and occasionally the sweat test can be attempted in term infants after 7 days of age. Collection systems vary with regard to insufficient samples, for example, there is a 0.7% failure rate associated with collection onto gauze or filter paper compared with a 6.1% failure rate associated with the use of Wescor Macroduct coils although Heeley reported only 1.4% with the latter.

Conclusion
The genomic era for CF which began with the cloning the CFTR gene and continues with an expanding understanding of the many functions of CFTR has solved many of the diagnostic dilemmas confronting clinicians. At the same time the complexity of the diagnosis has increased with the recognition of milder phenotypes and patients with no clinical manifestation detected by screening programs waiting to see if their mutations will be clinically relevant. At the other end of the spectrum, there are patients with CF phenotypes in the absence of CFTR mutations.

Mutation analysis informs us about the gene, but not the gene product (mRNA or protein) or its function. Mutation analysis is often least helpful when the diagnosis is in greatest doubt. Diagnosis that remains unclear after sweat testing and mutation analysis may be confirmed by other tests of CFTR function such as NPD. However, NPD can be inconvenient to perform on infants and its use as a diagnostic tool requires further evaluation.

What has become clearer is that CF is a clinical diagnosis, supported by genetic testing and the demonstration of abnormalities of CFTR function. A sweat test performed in a reliable laboratory is still a good measure of CFTR function in most cases.

Future Directions
A good deal of clinical research remains to be done to understand the role of the sweat test for the diagnosis of CF in the genomic era. Basic information such as age related normal values in infants and adults are needed. There are no data on serial sweat electrolyte values in unaffected subjects and in particular, subjects with initial borderline sweat test results. The apparent association of pulmonary disease with sweat electrolyte values needs to be elucidated and whether this can be relied on to inform clinicians and patients. Continued vigilance in the maintenance of laboratory standards is essential, especially as more centres take on newborn screening and laboratories are asked to perform sweat tests on infants.

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