Atypical cystic fibrosis—diagnostic and management dilemmas

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INTRODUCTION

For the majority of patients with cystic fibrosis (CF), the diagnosis is undisputed. A clinical suspicion of CF or a suggestive screening test is confirmed by a positive sweat test and further support is provided by the identification of two disease-causing mutations in the genes for CF transmembrane regulator (CFTR).[1] In classical CF, features may be present from birth or emerge over time with sinopulmonary disease, gastrointestinal manifestations, pancreatic insufficiency in 90% of individuals and male infertility all well documented and consistent features.[2]

The development of effective therapies over the last few decades has improved the long-term outcome for children with CF. Indeed, CF is now as much an adult disease as one of childhood and it is the adult services that are dealing with complications such as osteoporosis, diabetes, liver failure, issues of pregnancy and even the CF menopause—complications that are emerging as life expectancy increases.

The sweat test retains its position as a key diagnostic test for CF and, in most instances, sweat chloride levels above 60 mmol/L are diagnostic (with only a few exceptions that are readily distinguished clinically from CF) and levels below 40 mmol/L are normal (with a cautious interpretation in infancy and prematurity).[3] However, the sweat test has moments of weakness. Lying between the diagnostic and normal levels for sweat chloride lies an intermediate zone (40–60 mmol/L). Some clinically suspicious cases have intermediate or even fluctuating levels of sweat chloride and there is a well-described minority of patients in whom the sweat test is repeatedly normal.[4,5] Before the discovery of the gene for CF in 1989 clinicians had been aware of a small but diagnostically challenging group of children who presented with features of CF but in whom the sweat test was not reliably positive. For this group, it was always hoped that CF genotyping would provide diagnostic certainty. Two mutations within the CFTR gene would mean cystic fibrosis; normal genes would exclude the diagnosis. But it has not worked out that simply. Indeed, in the decade since the discovery of the CF gene, the situation is probably even more confusing and the number of 'atypical' or 'unusual' cases is growing.[4] The development of further sophisticated tests such as nasal mucosal potential difference (PD),[6] detailed radiological examination of the chest, sinuses or vas deferens, exocrine pancreatic function testing and rectal PD,[7] have failed to clarify the diagnostic dilemma of these atypical cases. Each test includes a grey zone of uncertainty where many of the atypical cases lie. No one test has emerged as the new gold standard.

Labels are important and the diagnosis of classical CF carries with it important implications. Failure to identify and label a patient with CF could lead to delays in effective therapies. But inappropriate categorization of a patient with an atypical form leads to an unnecessary burden of therapies and lifestyle restrictions. This article looks at the range of conditions that fall into the atypical category of CF, discusses the factors that influence the CF phenotype and explores an approach to management.

TOWARDS A DEFINITION FOR CF

Who has cystic fibrosis? A possible definition of CF could perhaps read as follows:

CF is a disease that

- usually arises from two disease-forming mutations in the gene for CFTR on chromosome 7
- that results in changes to the fluid and electrolytes on cell surfaces
- that leads to abnormal secretions and inflammatory response
- that predisposes to obstruction and infection
- that produces end organ disease to tubular structures such as the upper and lower airways, vas deferens, gut, liver and pancreas.

Most clinicians believe that the diagnosis of CF is a clinical decision—supported by biochemical and genetic tests. A CF Foundation consortium (USA) attempted to provide definitive diagnostic criteria and although their definition is imperfect it serves as a useful foundation.[8] The key features are summarized in Box 1 and discussed in detail in the referenced article.

It is clear, however, that although these diagnostic criteria hold true for the majority of cases, the path from genotype to phenotype is heavily influenced by a wide range

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Box 1 **A US consensus panel approach to diagnostic criteria for cystic fibrosis (CF)**

One or more clinical features consistent with CF:
- chronic sinopulmonary disease
- gastrointestinal and nutritional abnormalities
- salt loss syndromes
- male urogenital abnormalities resulting in obstructive azoospermia.

OR
A history of CF in a sibling
OR
A positive newborn screening test

AND an increased sweat chloride concentration by pilocarpine iontophoresis on two or more occasions
- or identification of two CF mutations
- or demonstration of abnormal nasal epithelial ion transport.

of modifying factors.[9] Just because you have two mutations in the gene does not necessarily mean that you will get the disease or that it will manifest in the classical way. Any definition based on genetic criteria alone could lead to two obvious shortcomings:

1 you may label a patient who is clinically normal as having a disease just because of their genotype—an inaccurate assumption and one with considerable life-changing consequences
2 the detection of a mutation from over a thousand possibilities is highly labour intensive and only available in selected centres. Just because you cannot find the gene mutation in a patient who has symptoms of CF and would benefit from therapy is clearly wrong.

For practical purposes, most clinicians who see children or adults with CF will have patients that fall into four groups: the first two are easy—those who have CF and fulfil established diagnostic criteria, and clearly normal individuals. But there are two further groups—admittedly small but disproportionately challenging:

1 the individual who has two CF mutations (and maybe evidence of biochemical or electrical changes associated with the mutation) but does not develop the classical phenotype and may not have any symptoms at all
2 the patient who has one or a few features traditionally associated with CF but in whom the sweat test, ancillary investigations and genetic tests are either equivocal or negative.

THE EXPANDING CLINICAL PHENOTYPE FOR TWO CFTR MUTATIONS

There are now over a thousand different mutations recorded for the CFTR gene. These mutations have been classified into five or six classes effectively creating severe (I, II, and III) and mild (classes IV, V and VI) mutations.[10] The final protein product may be incomplete, complete but incorrectly packaged and processed (the most common defect), or a final CFTR molecule that is unstable or incapable of reaching the cell surface in sufficient numbers to be physiologically effective.[11] Although this does provide some genotype–phenotype correlation, the predictive value is unreliable. In addition to disease forming mutations there are also recognized polymorphisms that do not necessarily result in a clinical phenotype. The situation becomes further confused when a combination of two mild or normal polymorphisms result in a protein product that produces clinical disease. The 5T thymidine run in intron 8 is a well-described example.

It has been a fascinating and unexpected discovery to find the wide range of clinical phenotype that two CF mutations can provide. In many instances the CF is not classical and can include a phenotype that previously would never have been considered within the CF spectrum. Examples are listed in Box 2 and a selection are discussed briefly below.

Congenital bilateral absence of the vas deferens (CBAVD)

Males with CF are almost all infertile with bilateral absence of the vas deferens. Infertility clinics are identifying a clinical syndrome of male infertility due to CBAVD in whom there is a high prevalence of CFTR mutations. Between 70% and 75% of males with CBAVD carry mutations in each CFTR gene—the most common being deltaF508/R117H (without the 5T thymidine run in intron 8).[12] A proportion of these patients who are homozygous for CFTR mutations also have sweat chlorides in the intermediate or abnormal range. Most have no other evidence of end-organ disease although, on careful questioning, a few reveal sinusitis or pulmonary symptoms.[13]

Box 2 **Examples from the range of clinical features that can be associated with two mutations in the cystic fibrosis (CF) gene**

- 'Classical CF' with pancreatic insufficiency
- Sinopulmonary disease, pancreatic sufficiency and positive sweat test
- Sinopulmonary disease and male fertility with normal sweat test
- Male infertility only
- Severe sinusitis and congenital bilateral absence of the vas deferens
- Chronic pancreatitis only
- Allergic bronchopulmonary aspergillosis
- Positive sweat test only
- No clinical features including normal sweat chloride

Disseminated bronchiectasis

Studies of disseminated bronchiectasis have determined a higher level of CFTR mutations than expected. Following a more detailed analysis of the CF gene, some have subsequently been determined to have two CF mutations and others have been found to carry the 5T variant.[14] It is likely that a number of these patients previously designated bronchiectasis of unknown origin have insufficient levels of functioning CFTR to maintain lung health and are in fact examples of atypical CF.

Allergic bronchopulmonary aspergillosis (ABPA)

ABPA is found in patients with asthma and is also known to complicate the clinical course in patients with CF.[15] In a small study, the CF gene showed a higher level of mutations than could be expected in patients with ABPA suggesting again that CFTR may play a role in a less classical lung phenotype.[16]

Acute and chronic pancreatitis

Studies looking at adults presenting with either acute or chronic pancreatitis have revealed that a proportion have an unusual form of CF (almost always pancreatic sufficient) but rarely demonstrate sinopulmonary disease or raised sweat chloride levels.[17,18]

Normal individuals

The most difficult group in terms of management are those individuals who have been discovered to have two CF mutations (usually as part of a family genetic study or through a screening programme) but no evidence of clinical disease even after detailed investigation. This group raises questions regarding the possibility that a CF phenotype will emerge with the passage of time. But how far should we investigate these patients? What surveillance is required to ensure that early changes are halted with therapy? And what is the chance that these individuals will not require any therapy let alone preventative treatment? We return to these dilemmas when considering the treatment options below.

CF-LIKE DISEASE WITH INCOMPLETE SUPPORTIVE TESTS

Many clinicians will have patients in their CF clinic who have one or more clinical features that are well recognized as part of the classical CF spectrum but in whom the sweat test, genotype or additional tests provide insufficient evidence for a CF label according to diagnostic criteria such as those tabulated (*see* Box 1). Not infrequently there is single organ disease, one identifiable CF mutation and perhaps an equivocal sweat test. Extensive screening of the CFTR genome either fails to identify a second mutation or, perhaps, highlights one or more polymorphisms that traditionally were not thought to be disease producing. Examples of conditions that have been described in this group are listed in Box 3. But how far should we take the search for mutation or CFTR dysfunction? Full screening of the CFTR gene is only available in a few specialized centres and detailed end organ examination for disease in other systems must be questionable in a patient who has no symptoms.

There are also reports of patients with classical CF symptoms and signs and a positive sweat test who do not appear to have any mutations in the CFTR gene—even when the entire gene has been sequenced using denaturing gradient gel electrophoresis to screen all 27 exons and the intron–exon boundaries.[19,20] These findings suggest that CF, on rare occasions, may be caused by mutations within the promotor region of the CFTR gene, in one of the introns or even in a distant controlling gene from an unrelated locus.[21] The route from genetic mutation to clinical disease is far more complex than originally thought.

WHY IS CF SOMETIMES ATYPICAL?

The complex route from two CFTR mutations to final clinical phenotype is influenced by many factors along the way. Figure 1 gives representation of the genetic factors, modifying genes and environmental influences that all interact in a multi-faceted, highly complex and competitive effort to create the final phenotype. A brief review of some of the central players that may influence the clinical outcome is summarized below.

A thousand CF mutations

The CFTR gene codes for a protein of 1480 amino acids. The most common mutation is the absence of a three base pair sequence resulting in the loss of a phenylalanine residue at position 508—designated deltaF508.[22] The remaining mutations are individually rare or unique, although some alleles tend to segregate within specific ethnic groups—for example, 36.2% of CF chromosomes in the Ashkenazi Jewish community carry the mutation W1282X, a gene with a frequency of $<1\%$ in the UK.[22]

Box 3 **Examples of disorders resembling cystic fibrosis (CF) and associated with an increased frequency of CF transmembrane conductance regulator mutations**

- Congenital bilateral absence of the vas deferens
- Chronic pancreatitis
- Chronic sinusitis
- Disseminated bronchiectasis
- Allergic bronchopulmonary aspergillosis
- Raised chymotrypsinogen in infancy

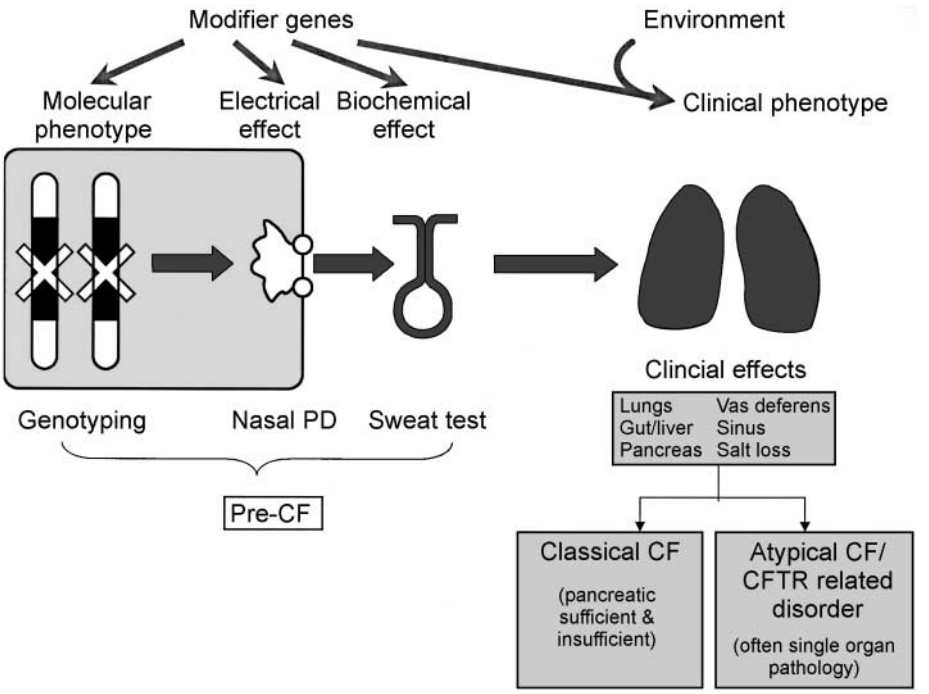

Figure 1 **A representation of the factors that influence the path from genotype to phenotype.** PD=potential difference; CF=cystic fibrosis. [Reprinted with permission from David T J, ed. *Recent Advances in Paediatrics*, Vol. 20. London: RSM Press, 2003]

The wide distribution of mutations supports the ancient origins of this disease. Scientists from the early years following the discovery of the gene predicted that there may be as many as 10 different mutations. Little could they have anticipated that by the end of that decade, over a 1000 different mutations would be described and the list continues to grow. Every month further 'novel' mutations are reported and polymorphisms, previously considered 'non-disease-causing', require re-evaluation.

CFTR—a complex protein product

The principal function of the CFTR appears to be as a cAMP-dependent chloride channel situated at the apical epithelial cell membrane. It is expressed in a wide range of tissues such as the airway, salivary and sweat glands, gastrointestinal tract, liver, pancreas and epidydimis. Studies of the impact of a defective CFTR protein product have shown that the influence of this protein to maintain a stable salt and water milieu on the cell surface extends beyond regulation of chloride alone. Indeed, CFTR appears to have influence on amiloride sensitive epithelial sodium channels, other chloride channels within the cell wall, and endocytosis and exocytosis in the CF pancreas.[23]

Not only are the gene mutations proving a conundrum but the final protein product behaves unpredictably and does not necessarily correlate with the final disease phenotype. It appears that when the genotype consists of two different mutations, some mutations are phenotypically dominant, sometimes to positive effect for the final protein product.[24]

Defective CFTR—a range of biochemical effects

The path from defective CFTR to end organ disease is still unknown. Several possibilities are currently being explored in an attempt to explain the lung disease.[25]

(A) CFTR may lead to a dehydrated airway surface liquid with:
- the altered synthesis of surface proteins responsible for intrinsic mucosal defence such as defensins and lysosymes
- a tendency to increased proinflammatory cytokines
- an alteration of mucins further impairing mucociliary clearance
- an increase in the binding sites for *Pseudomonas aeruginosa* or a mucus that favours entrapment of bacteria such as *Pseudomonas*

(B) Defective CFTR may reduce the ability of the epithelial cells to ingest pseudomonads

(C) Abnormal CFTR interferes with normal cell apoptosis and thus leads to a release of potentially toxic enzymes that play a role in disease pathogenesis.

An immense orchestra of modifying genes

It is now recognized that modifying genes have significant influence on the behaviour of the CFTR gene, as well as the final protein product, the cell surface liquid and the final phenotype. Modifying genes are likely to be numerous, unique for each individual with CF and represent an

understudied but up-and-coming area in the understanding of the pathophysiology of CF[23,26]. Consider them in two groups: (i) polymorphisms with influence that fall within the CFTR gene and (ii) genetic influences from elsewhere in the genome.

Modifying genes co-inherited within the CFTR gene

The mutation R117H is associated with CBAVD but does not cause lung disease—indeed it is commonly found in otherwise healthy males attending infertility clinics. If, however, the patient inherits a 5T variant in intron 8 (instead of the commoner 7T variant) then the likelihood of lung disease increases. This 5T variant reduces the splicing efficiency with lower levels of functioning CFTR and thus greater clinical impact.[27]

The A455E mutation is mostly associated with mild pancreatic disease. This mutation appears to have an interesting effect on the deltaF508 mutation. If co-inherited, it appears to act in a dominant fashion over deltaF508, causing less severe lung disease.[28]

The clinical significance of polymorphisms such as the missense mutation S1235R is also generating interest. This allele appears at a frequency that is significantly higher than that of many other CF mutations. When combined with a second CF mutation the combination may cause disease. The question as to whether this mutation (and others like it) are rare polymorphisms or a disease-forming mutation when combined with a known mutation leads to difficulties with genetic counselling, especially in prenatal cases.[29]

Modifying genes elsewhere in the genome

A gene that predisposes to meconium ileus

Meconium ileus (MI) is a presenting feature *in utero* or the early neonatal period in 10–15% of CF patients. There is a higher than expected rate of MI in siblings suggesting an inherited factor. Some investigators had postulated an association with the gene for haemochromatosis showing an increase in the carrier frequency of the haemochromatosis gene in children with MI and CF.[30] More recently, the detection of a CF modifier locus for MI on human chromosome 19q13 has provided further evidence for a modifying gene that predisposes the carrier to this particular presentation of CF.[31]

Genetic variants of inflammation

Inflammation is a major player in the pathogenesis of lung disease in CF. There is a complex and highly individual soup of pro-inflammatory cytokines and protection factors that temper the final inflammatory response. Studies have looked at the impact of naturally occurring variants of a pro-inflammatory cytokine, tumour necrosis factor alpha (TNF-α)[26] and the detoxifying enzyme glutathione S-transferase M1 (GSTM1).[32] A small study showed that the X-ray changes were significantly worse in those patients who were homozygous for the GSTM1 null allele and the absence of the TNF-α promotor polymorphism. We all have our own genetically determined and individual inflammatory system that could influence the pulmonary phenotype for those who also have CF.[26]

Other modifying proteins

Researchers have recently focused on the role that other modifying proteins may have on the biochemical environment of the cell surface that could influence the CF phenotype and contribute to the spectrum of unusual forms. Some examples include the following:

Alternative chloride channels and protein kinases The cell surface is known to have alternative chloride channels, the best studied of which carries the acronym ORDIC or ORCC (Outward Rectification of the current voltage relationship and increased open probability Induced by Depolarization Chloride Channel).[25] In addition, there are a number of second-messenger pathways to cAMP (cGMP, calcium and protein kinase C). These alternative pathways are controlled to varying degrees by CFTR and provide a complicated orchestra of players contributing to the final electrophysiological environment on the cell surface of the CF epithelium.

Mannose binding lectin Mannose binding lectin (MBL) is a key factor in innate immunity.[33] This protein is the product of a single gene that has been mapped to chromosome 10.[34] Investigators have shown interest in MBL variant alleles in CF addressing the possibility that they may predispose CF patients to recurrent infections and worsening clinical course. Lung function may be reduced in carriers of MBL variant alleles when compared with normal homozygotes and there may be correlation with the propensity to pseudomonas infection. According to some authors,[35] the presence of MBL variant alleles (and consequently low levels of serum MBL) is therefore associated with poor prognosis and early death in patients with CF.

Environmental factors

The environment in which a patient with CF lives and grows has central bearing on the outcome of their disease. Indeed it could be said that the single most important factor in determining the phenotype is the access that a patient has to CF care! Studies of siblings and twins (including identical twins) have confirmed that, although your genetic make-up is important in determining the course of your CF disease, environmental factors account for the considerable differences in outcome between closely similar genotypes.

Although there are many reports of phenotypic heterogeneity in twins including monozygotic twins,[36] studies of large families demonstrate the striking variations present. In a highly consanguineous Bedouin tribe consisting of 29 subjects with CF all homozygous for the mutation 11234V in exon 9, there was a wide variation of pulmonary disease, pancreatic insufficiency and electrolyte imbalance.[37] Almost any external encounter, including psychological factors can influence the clinical course. Examples include treatment, infections, passage of time and diet.

Treatment and adherence to therapy

Treatment improves outcome. Failure to adhere to therapies could result in a deteriorating phenotype irrespective of the genetic background. Unfortunately, strict adherence does not guarantee good health, which understandably, is a cause of considerable distress for children and their families. The timing of therapy is also important. Recent studies show that lung disease starts early, even before symptoms and signs are clinically evident.[38] A child growing up in a healthcare system where early intervention is available is likely to have an improved long-term outcome. Access to CF services improves outcome and alters the phenotype.

Infections

Pulmonary outcome in CF is influenced primarily by mucoid *P. aeruginosa* infection and only modestly by genotype. It is well known that the acquisition of pseudomonas is associated with deterioration in lung function although the rate of progression of the lung disease is less predictable.[39] Viral lower respiratory tract infections in infancy can also produce a sustained negative impact and subsequent deterioration in lung function.[40]

The passage of time

The clinical condition of CF is dependent on the effects of abnormal functioning CFTR protein on end organs—often tubular structures. But these effects can take time. Some, such as pancreatic insufficiency, are present at birth whilst lung disease can emerge with time (Figure 2). Absence of the vas deferens could go undetected throughout life if no demands were made on its patency.

Dietary influences

The associations between malnutrition, decreased vitamin and trace element absorption and a deteriorating lung function is described.[41] The use of additional vitamin supplementation and enteric enzymes has a strongly positive impact on nutritional status.[42] Social circumstances are clearly central to effective dietary interventions.

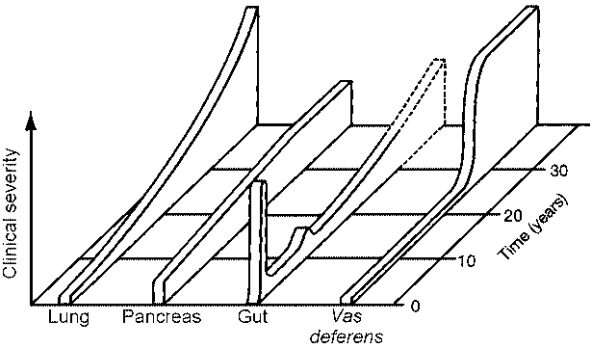

Figure 2 **A graphic representation of the possible interactions of single organ involvement, severity and time in the evolution of the cystic fibrosis phenotype**

PHENOTYPE–GENOTYPE CORRELATIONS

The discovery of the CF gene led to the hope that phenotype–genotype correlation would be robust. By obtaining a genotype, one would be able to predict the type and perhaps even the course that your CF would take. It is disappointing to find that even though CF is a single gene disorder, there has been no convincing evidence that the risk of lung disease, liver disease, nasal polyposis or recurrent intestinal obstructive syndrome is related to any specific mutation.[43] There may be 'mild' mutations and 'severe' mutations and mild mutations may be dominant over severe ones, but the final effect on the phenotype is far more complex.[23]

There are a few possible exceptions to the general disappointment:

- pancreatic sufficiency has been linked to certain mutations such as R117H and A445E although insufficiency may emerge with time
- patients homozygous for the deltaF508 mutation generally (but not exclusively) have a more severe phenotype in all affected organs when compared with CF patients with one deltaF508 mutation or none
- mutations carrying a milder pancreatic phenotype may be associated with less abnormal sweat tests and perhaps a similarly mild effect on nasal potential difference responses
- the male reproductive tract appears to have a high need for functioning CFTR but splice mutations such as 3840+10kbC>T allows for the production of sufficient CFTR that fertility is possible (although not guaranteed).

Perhaps one might imagine that future generations will have access to a genetic blueprint that identifies a modifier-gene-profile documenting the summated genetic influences that will fashion the phenotype and provide a printout of risks for end organ involvement. This would enable the patient to alter their environment to lessen

these risks without imposing unnecessary restrictions in order to forestall complications that are not in their destiny. Time will tell whether this futuristic concept becomes a reality.

DIAGNOSING AND CLASSIFYING ATYPICAL CF

The US diagnostic criteria for CF[8] (*see* Box 1) hold true for many cases but there are shortcomings as admitted by its members.[9] Clinicians who care for patients with CF are keen to provide diagnostic labels that benefit the patient rather than satisfy the needs of geneticists or biochemists. In addition to the labels for 'normal' and 'classical CF', perhaps two further categories of CF are required:

1 *A category for atypical cases*: The World Health Organization has recognized the need for a category that incorporates patients with atypical (often single organ disease) who may or may not yield evidence for CFTR dysfunction (sweat test, nasal potential difference) or two CFTR mutations.[44] It is now likely that this additional category will appear in future editions of the International Classification of Diseases.
2 *A category for patients who have two CF mutations but have no evidence of clinical disease*: These patients may or may not have evidence for abnormal CFTR dysfunction. They may only show very subtle end organ changes when subjected to detailed and sophisticated testing. For these individuals it could be considered appropriate that a well-established oncological concept is adopted.[45] Premalignant disease (e.g. carcinoma *in situ* of the cervix), which may never progress to cancer and which does not require immediate treatment has long been recognized. In a similar light, two abnormalities in the CFTR region do not make the disease CF—although they may influence how we follow up their owner.

A classification that includes the concept of 'pre-CF' relieves the clinician from the falseness of 'all-or-nothing' decisions. No longer do we need to decide whether someone is either normal or has a life threatening disease of CF. There may be a slow evolution of CF-related complications in some individuals whereas others may never proceed beyond the 'pre-CF' phase.

MANAGEMENT OF ATYPICAL CF

The establishment of an extended classification of CF to accommodate the atypical forms assists carers in the design and evaluation of appropriate management plans. The following three illustrative cases highlight some of the management principles:

Case 1: When the signs are suspicious but the tests are non-confirmatory

A 15-year-old girl had a long history of recurrent chest infections during the first 5 years of life. She had an intermediate sweat chloride on numerous occasions ranging from 400 mm/L to 50 mmol/L. Her respiratory symptoms were much improved over the last 10 years but she now presented with nasal polyps. Her chest examination was normal (including high resolution CT) and she had no evidence for pancreatic insufficiency. Analysis for CF mutations using a routine panel of 30 common mutations for her ethnic origin showed a single deltaF508 mutation.

Management principles:

1 This girl has mild single organ disease and would best be labelled 'atypical CF'
2 There is incomplete corroborative testing to confirm or exclude a diagnosis of CF. A label of CF carries life-long consequences and must be reserved for those with the classical form. A diagnostic label of atypical CF does not imply the same burden of morbidity and mortality associated with the classical CF label
3 Extensive analysis of the CFTR genome in search for a second mild mutation or polymorphism would be of academic interest but is probably not indicated, as it is unlikely to alter her clinical care
4 Treatment for atypical CF must be individualized. Deal with the symptoms, anticipate the early development of further complications and introduce therapy as appropriate. Do not impose therapeutic regimens and protocols that have been designed for the child with classical CF. They are inappropriate and burdensome.

Case 2: When the genes are abnormal but the child is well

A 10-year-old girl had been tested in utero *for CF as her older brother had been diagnosed with the disease. She was found to have a disease-associated mutation in each CFTR gene (N1303K and R117H associated with the 7T variant in intron 8). Sweat chloride levels have been repeatedly in the normal range. The patient is clinically asymptomatic and there is no evidence of lung, liver or pancreatic dysfunction.*

Management points:

1 This patient could be considered as having 'pre-CF' and at this stage, no therapy is required. Genotype alone is an insufficient basis for the diagnosis of CF and a label as important as CF must be confined to someone with disease
2 A clinical phenotype could emerge in time but it is possible that she may never develop disease

3 The need and frequency for long-term follow up needs to be customized for the individual in conjunction with their paediatrician or physician to monitor for potential complications and intervene early to halt disease progression.

Case 3: When the child looks like 'CF' but the tests are non-confirmatory

A 15-year-old girl was diagnosed with CF at the age of 2 years following a history of chronic cough, asthma, foul smelling stools and failure to thrive. She is pancreatic insufficient and has sweat chloride levels that fluctuate between 50 mmol/L and 70 mmol/L. Her height and weight are below the 5% and lung function tests reveal an FEV1 of 87%. She has grown Staphylococcus *on cough swabs in the past and there is bronchial wall thickening on a CT scan of her chest. Extensive analysis of both CFTR genes in this patient (sequencing of all coding regions, flanking introns and the promotor, and southern analysis for deletion/insertions) has failed to identify a mutation in either gene.*

Management points:

1 Ensure that other conditions that can masquerade as CF have been excluded. These would include some of the immunodeficiency states, ciliary abnormalities, rarer conditions such as Schwachman syndrome and certain allergic disorders that may present with diarrhoea, respiratory symptoms and nasal polyps
2 This patient is likely to benefit from aggressive CF therapy and it is appropriate to give her the classical CF label on the basis of clinical findings—irrespective of the lack of confirmatory genetic information. CF is a clinical diagnosis and if it looks like CF and responds to CF therapy, it should be treated as if CF
3 The role of genes other than those responsible for the CFTR protein could be candidates in the clinical phenotype of this girl. Geneticists are not yet in the position to say with certainty that, because the mutations cannot be detected, the disease is excluded.

CONCLUSION

CF is a highly complex and heterogeneous disorder and although it is still considered a single gene disorder, the influences that determine the phenotype resemble a polygenic scenario. The absence of a tight definition for CF and the failure of a reliable genotype–phenotype correlation need not necessarily present as a problem to the practising clinician. Patients are individuals and require individualization of their therapy. Monitor for anticipated problems and intervene early to halt disease progression. Ensure that all is done to halt progression of the pathology but nothing is done that is superfluous and could impact negatively or impose unnecessary burden. Finally, the effects of mutant CFTR may accumulate over time. Regular review of labels and management will best serve our patients with atypical CF.

REFERENCES

1 Wallis C. Diagnosing cystic fibrosis: blood, sweat and tears. *Arch Dis Child* 1997;**76**:85–91

2 Davis P, Drumm M, Konstan MW. Cystic fibrosis. *Am J Respir Crit Care* 1996;**154**:1229–56

3 Littlewood JM. The sweat test. *Arch Dis Child* 1986;**61**:1041–3

4 Massie J, Robinson P. Cystic fibrosis: the twilight zone. *Pediatr Pulmonol* 1999;**28**:222–4

5 Knowles MR, Durie PR. What is cystic fibrosis? *N Engl J Med* 2002;**347**:439–442

6 Wilson DC, Ellis L, Zielenski J, *et al*. Uncertainty in the diagnosis of cystic fibrosis: possible role of *in vivo* nasal potential difference measurements. *J Pediatr* 1998;**132**:596n9

7 Goldstein JL, Shapiro AB, Rao MC, Layden TJ. *In vivo* evidence of altered chloride but not potassium secretion in cystic fibrosis rectal mucosa. *Gastroenterology* 1991;**101**:1012–19

8 Rosenstein BJ, Cutting GR. The diagnosis of cystic fibrosis: a consensus statement. Cystic Fibrosis Foundation Consensus Panel. *J Pediatr* 1998;**132**:589–95

9 Rosenstein BJ. Cystic fibrosis diagnosis: new dilemmas for an old disorder. *Pediatr Pulmonol* 2002;**33**:83–4

10 Welsh MJ, Smith AE. Molecular mechanisms of CFTR chloride channel dysfunction in cystic fibrosis. *Cell* 1993;**73**:1251–4

11 Zeitlin P. Novel pharmacologic therapies for cystic fibrosis. *J Clin Invest* 1999;**103**:447–52

12 Dork T, Dworniczak B, Aulehla-Scholz C, *et al*. Distinct spectrum of CFTR gene mutations in congenital absence of vas deferens. *Hum Genet* 1997;**100**:365–77

13 Dohle GR, Veeze HJ, Overbeek SE, *et al*. The complex relationships between cystic fibrosis and congenital bilateral absence of the vas deferens: clinical electrophysiological and genetic data. *Hum Reprod* 1999;**14**:371–4

14 Girodon E, Cazeneuve C, Lebargy F, *et al*. CFTR gene mutations in adults with disseminated bronchiectasis. *Eur J Hum Genet* 1997;**5**:149–55

15 Milla C. Allergic bronchopulmonary aspergillosis and cystic fibrosis. *Pediatr Pulmonol* 1999;**27**:71–3

16 Miller P, Hamosh A, Macek M, *et al*. Cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations in allergic bronchopulmonary aspergillosis. *Am J Hum Genet* 1996;**59**:45–51

17 Choudari CP, Lehman GA, Sherman S. Pancreatitis and cystic fibrosis gene mutations. *Gastroenterol Clin North Am* 1999;**28**:543–9

18 Sharer N, Schwartz M, Malone G, *et al*. Mutations of the cystic fibrosis gene in patients with chronic pancreatitis. *N Engl J Med* 1998;**339**:645–52

19 Mekus F, Ballmann M, Bronsveld I, *et al*. Cystic-fibrosis-like disease unrelated to the cystic fibrosis transmenbrane conductance regulator. *Hum Genetics* 1998;**102**:582–6

20 Groman JD, Meyer ME, Wilmott RW, Zeitlin PL, Cutting GR. Variant cystic fibrosis phenotypes in the absence of CFTR mutations. *N Engl J Med* 2002;**347**:401–7

21 Rozmahel R, Wilschanski M, Matin A, *et al*. Modulation of disease severity in cystic fibrosis transmembrane conductance regulator deficient mice by a secondary genetic factor. *Nat Genet* 1996;**12**:280–7

22 Santis G. Basic molecular genetics. In: Hodson M, Geddes D, eds. *Cystic Fibrosis*. London: Arnold, 2000:27–48

23 Cutting G. Phenotype–genotype relationships. In: Hodson M, Geddes D, eds. *Cystic Fibrosis*. London: Arnold, 2000:49–60

24 Gan KH, Veeze HJ, van den Ouweland AM, *et al*. A cystic fibrosis mutation associated with mild lung disease. *N Engl J Med* 1995;**333**:95–9

25 Alton E, Smith S. Applied cell biology. In: Hodson M, Geddes DM, eds. *Cystic Fibrosis*. London: Arnold, 2000:61–82

26 Acton JD, Wilmott RW. Phenotype of CF and the effects of possible modifier genes. *Paediatr Respir Rev* 2001;**2**:332–9

27 Chu CS, Trapnell BC, Curristin S, Cutting GR, Crystal RG. Genetic basis of variable exon 9 skipping in cystic fibrosis transmembrane conductance regulator mRNA. *Nat Genet* 1993;**3**:151–6

28 Gan KH, Veeze HJ, van den Ouweland AM, *et al*. A cystic fibrosis mutation associated with mild lung disease. *N Engl J Med* 1995;**333**:95–9

29 Monaghan KG, Feldman GL, Barbarotto GM, Manji S, Desai TK, Snow K. Frequency and clinical significance of the S1235R mutation in the cystic fibrosis transmembrane conductance regulator gene: results from a collaborative study. *Am J Med Genet* 2000;**95**:361–5

30 Rohlfs EM, Shaheen NJ, Silverman LM. Is the hemochromatosis gene a modifier locus for cystic fibrosis? *Genet Test* 1998;**2**:85–8

31 Zielenski J, Corey M, Rozmahel R, *et al*. Detection of a cystic fibrosis modifier locus for meconium ileus on human chromosome 19q13. *Nat Genet* 1999;**22**:128–9

32 Hull J, Thomson AH. Contribution of genetic factors other than CFTR to disease severity in cystic fibrosis. *Thorax* 1998;**53**:1018–21

33 Garred P, Madsen HO, Hofmann B, Svejgaard A. Increased frequency of homozygosity of abnormal mannan-binding-protein alleles in patients with suspected immunodeficiency. *Lancet* 1995;**346**:941–3

34 Taylor ME, Brickell PM, Craig RK, Summerfield JA. Structure and evolutionary origin of the gene encoding a human serum mannose-binding protein. *Biochem J* 1989;**262**:763–71

35 Garred P, Pressler T, Madsen HO, *et al*. Association of mannose-binding lectin gene heterogeneity with severity of lung disease and survival in cystic fibrosis. *J Clin Invest* 1999;**104**:431–7

36 Mekus N, Ballman M, Bronsveld I, Bijman J, Veeze H, Tummler B. Categories of deltaF508 homozygous cystic fibrosis twin and sibling pairs with distinct phenotypic characteristics. *Twin Res* 2000;**3**:277–93

37 Abdul Wahab A, Al Thani G, Dawod ST, Kambouris M, Al Hamed M. Heterogeneity of the cystic fibrosis phenotype in a large kindred family in Qatar with cystic fibrosis mutation (I1234V). *J Trop Pediatr* 2001;**47**:110–12

38 Ranganathan SC, Dezateux C, Bush A, *et al*. Airway function in infants newly diagnosed with cystic fibrosis. *Lancet* 2001;**358**:1964–5

39 Kosorok M, Zeng L, West S, *et al*. Acceleration of lung disease in children with cystic fibrosis after *Pseudomonas aeruginosa* acquisition. *Pediatr Pulmonol* 2001;**32**:277–87

40 Hiatt PW, Grace SC, Kozinetz CA, *et al*. Effects of viral lower respiratory tract infection on lung function in infants with cystic fibrosis. *Pediatrics* 1999;**103**:619–26

41 Dodge JA. Nutritional requirements in cystic fibrosis: a review. *J Pediatr Nutr Gast* 1988;**7**:S8–11

42 Bell SC, Bowerman AR, Davies CA, Campbell IA, Shale DJ, Elborn JS. Nutrition in adults with cystic fibrosis. *Clin Nutr* 1998;**17**:211–15

43 Kerem E, Corey M, Kerem BS, *et al*. The relationship between genotype and phenotype in cystic fibrosis—analysis of the most common mutation (deltaF508). *N Engl J Med* 1990;**323**:1517–22

44 World Health Organization. *Classification of Cystic Fibrosis and Related Disorders*, WHO/ICF(M)A/ECFS/ECFTN. Geneva: WHO, 2000

45 Bush A, Wallis C. Time to think again: cystic fibrosis is not an 'all or nothing' disease. *Pediatr Pulmonol* 2000;**30**:139–44