RECENT ADVANCES

Recent advances in cystic fibrosis

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Abstract
The median life expectancy for cystic fibrosis is now over 30 years, and it is projected that in newborn infants it will become more than 40 years. The identification of the cystic fibrosis gene and its product, cystic fibrosis transmembrane conductance regulator (CFTR), has widened the spectrum of the disease from the classical case of the infant with cystic fibrosis to the elderly childless man with unexplained bronchiectasis. There is increasing evidence of the advantages of newborn screening for cystic fibrosis and subsequent specialist care. Management concentrates on optimising nutritional status and preventing lung infection and inflammation.
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While the prognosis of cystic fibrosis has improved beyond all recognition, in many ways the management of children with this disease lags behind that of other diseases, and it is useful to compare this annotation with a sister one on acute leukaemia in the same series.[1] In oncology there is an acceptance that all children should be referred to a specialist centre, their care is coordinated by appropriately trained specialists, and treatment is directed through large prospective trials that give a firm evidence base for management. In comparison, in some quarters there is a reluctance to accept the benefits of specialist care for cystic fibrosis, and there is a dearth of large prospective trials. It is salutary that this annotation refers to only three appropriately powered controlled studies. A comprehensive list of controlled trials in cystic fibrosis is available from the cystic fibrosis Cochrane database.

Prognosis

It is now over 60 years since the first description of cystic fibrosis, and over 10 years since the localisation of the genetic defect to the long arm of chromosome 7. Unquestionably the most important result of therapeutic advances is the continuing improvement in life expectancy, with the median life expectancy in the United Kingdom now being more than 30 years.[2] The acquisition of *Pseudomonas aeruginosa* is an important negative prognostic factor. The prognosis is continuing to improve, such that it is projected that a newborn infant with cystic fibrosis in the United Kingdom is likely to have a life expectancy well in excess of 40 years.[3] In individuals with adequate pancreatic function, life expectancy could be more than 50 years.[4]

Cystic fibrosis transmembrane conductance regulator

The cystic fibrosis gene product is a c-AMP mediated chloride channel known as cystic fibrosis transmembrane conductance regulator (CFTR). It has additional actions that may be relevant to the pathophysiology of cystic fibrosis, including transport of other ions and regulation of other ion channels (reviewed by Wine[5]). Despite an increasing understanding of CFTR function and the underlying processes of cystic fibrosis as a disease, the crucial step in how CFTR dysfunction leads to the clinical features of cystic fibrosis remains unclear. Current models concentrate on disturbances of the airway surface liquid[5] or dysregulation of the inflammatory process (see below).

Genotype/phenotype

Nearly 1000 mutations [http//:www.genet.sickkids.on.ca/cftr/] have been identified in the cystic fibrosis gene. The vast majority of these mutations are private (confined to one family), with a few common mutations causing disease in most subjects. Worldwide the most common mutation is ΔF508, where there is a deletion of phenylalanine at the 508th amino acid of CFTR. This accounts for around 70% of mutations in the white British population—thus in the United Kingdom approximately 50% of patients with cystic fibrosis are homozygous for ΔF508.

Mutations have been divided into five sequential classes, broadly based on the molecular fate of CFTR (reviewed by Zeitlin[6]).

- *Class I* mutations cause defects in CFTR synthesis resulting in absence of CFTR production.
- In *class II* mutations, which include ΔF508, there is production of abnormal CFTR, which then fails to escape the endoplasmic reticulum.
- *Class III* mutations have CFTR production and intracellular trafficking, but there is disruption of activation and regulation at the cell membrane.
- In *class IV* mutations CFTR is expressed at the cell membrane but chloride conductance is reduced.

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- *Class V* mutations decrease membrane CFTR function by decreasing splicing of normal CFTR.

To add further confusion, class V mutations may act concurrently with other mutations on the same allele. For example, the number of thymidine residues in intron 8 influences the splicing of exon 9, a functional section of mRNA. The class IV mutation R117H occurs with either five or seven thymidine residues (5T or 7T), and disease severity is increased with the 5T variant.

Genotype/phenotype correlations are strongest for pancreatic insufficiency and sweat chloride concentrations, but do not correlate particularly well with pulmonary function.[7] There is a broad hierarchy of disease phenotype/genotype relations. Subjects homozygous or compound heterozygotes for class I–III mutations have more severe disease than heterozygous subjects with a class IV or V mutation, suggesting that the mutations resulting in milder disease act in a dominant fashion. Thus almost all subjects homozygous for ΔF508 have pancreatic insufficiency and raised sweat chloride, and although most have pulmonary disease, there is wide intersubject variability.

The identification of CFTR has widened the spectrum of disease away from the classical cystic fibrosis phenotype of the infant with malabsorption, failure to thrive, and recurrent chest infections to encompass milder phenotypes. It is debatable whether some of these manifestations should be called cystic fibrosis, and increasingly they are labelled "CFTR related disease". Azoospermia has long been recognised as a feature of cystic fibrosis, but mutational analysis of otherwise healthy infertile males with congenital bilateral absence of the vas deferens has demonstrated two CFTR mutations in up to 50% of subjects.[8] Most subjects have either 5T splicing mutations or R117H, and although they are usually completely asymptomatic there is increasing evidence that they may still get respiratory complications. Idiopathic chronic pancreatitis—particularly in the absence of a history of chronic alcohol ingestion—is also associated with an increased frequency of CFTR mutations.[9 10] Between 37%[10] and 13%[9] of subjects had at least one CFTR mutation, with the 5T mutation being particularly common. Other diseases associated with an increased frequency of one or more CFTR mutations (box 1) include asthma, allergic bronchopulmonary aspergillosis (ABPA) in asthmatic individuals, disseminated bronchiectasis, neonatal transitory hypertrypsinaemia,[11] and possibly diffuse panbronchiolitis.

Box 1: Conditions associated with an increased frequency of one or more CFTR mutations

- Congenital bilateral absence of the vas deferens
- Chronic pancreatitis
- Asthma
- Allergic bronchopulmonary aspergillosis (ABPA) in asthmatics
- Disseminated bronchiectasis
- Neonatal transitory hypertrypsinaemia
- Diffuse panbronchiolitis

Newborn screening

Screening of newborn infants for cystic fibrosis is widely advocated by clinicians and patient groups such as the Cystic Fibrosis Trust, although there has been reluctance on the behalf of governments to introduce national screening programmes. Approximately 20% of newborn infants in the United Kingdom are screened for cystic fibrosis through measurement of immunoreactive trypsin in the dried blood spot taken for the Guthrie test. Observational studies comparing clinical indices in children detected by newborn screening with those presenting clinically show benefits of screening, but such studies may be confounded by improvements in morbidity and mortality of patients with cystic fibrosis[2] and by the effect of specialist centres.[12]

A recent randomised controlled investigation in over 650 000 American infants using immunoreactive trypsin measurement with or without DNA analysis has shown nutritional benefit from cystic fibrosis screening.[13] The study used rigorous randomisation protocols, but by chance the control (not screened) group had more patients who were pancreatic sufficient and fewer patients who carried the ΔF508 mutation (both positive prognostic factors). Despite this, the patients in the screened group were significantly less likely to have either height or weight below the 10th centile (an indication of severe malnutrition).

By allowing earlier diagnosis, newborn screening can decrease the incidence of further affected children within families,[14] and combining immunoreactive trypsin measurement with extended mutation analysis is extremely effective.[14]

Delivery of care

The recognition of the complex multisystem nature of cystic fibrosis has inevitably led to centres specialising in its management. The United Kingdom Cystic Fibrosis Trust currently advocates that all patients should have at least some of their care through specialist centres, arguing that patients receiving their care through such centres are in better condition,[12] live longer, and are more satisfied with their care. A cross sectional study of clinical outcome in adults with cystic fibrosis showed a stepwise improvement in nutritional status (body mass index), pulmonary function (forced expiratory volume in one second (FEV_1)), and chest *x* ray (Northern score), depending on whether the adult had received no cystic fibrosis centre care, only adult centre care, or both paediatric and adult centre care.[12] Patients receiving centre care were, however, significantly more likely to be colonised by *Pseudomonas aeruginosa*, though the authors ascribed this to superior surveillance and culturing techniques in the cystic fibrosis centres.

Inflammation

The cystic fibrosis lung is characterised by an excessive yet ineffective inflammatory response. The cystic fibrosis airway becomes chronically infected with specific bacterial pathogens with a neutrophil predominant inflammatory response.[15] Thus bronchoalveolar fluid from individuals with cystic fibrosis contains increased concentrations of interleukin-8 (IL-8), the major neutrophil chemoattractant in the lung,[15] neutrophils, and free neutrophil elastase (reviewed by Konstan and Berger[16]). Bacterial infection of the airway in cystic fibrosis can be detected from as early as eight weeks,[15] but intriguingly the excessive inflammatory response may occur independently of infection. Bronchoalveolar lavage in infants as young as four weeks shows increased concentrations of IL-8 and activated neutrophils in the absence of bacterial infection.[17] Supporting evidence has come from a bacteria-free cystic fibrosis mouse model, where similar inflammation is seen.[18] A possible mechanism derives from the observation that airway cells in cystic fibrosis have constitutively upregulated production of IL-8[19] and downregulated production of the anti-inflammatory cytokine IL-10.

Anti-inflammatory treatment

The side effects of oral glucocorticosteroids preclude their long term use. The largest controlled study of their use in cystic fibrosis was discontinued owing to unacceptable side effects, and although there were short term benefits for pulmonary function they did not last once treatment was discontinued.[20] Although substantial numbers of patients with cystic fibrosis receive inhaled glucocorticosteroids, controlled trials to date have been underpowered and have shown little benefit.

Diffuse panbronchiolitis is a disease of middle aged Japanese men with pulmonary features similar to cystic fibrosis, including *Pseudomonas aeruginosa* infection. The serendipitous observation that macrolide antibiotics were beneficial has altered the survival from diffuse panbronchiolitis from 10% to 90%. Macrolides have significant anti-inflammatory actions and it is these rather than their antimicrobial effects that are likely to be important in diffuse panbronchiolitis. An observational study of regular azithromycin in seven boys with cystic fibrosis showed significant improvements of approximately 10% in FEV_1 and forced vital capacity over six months.[21] There are currently at least three large controlled studies of macrolides in cystic fibrosis underway worldwide.

DNAse and hypertonic saline

Aerosolised recombinant human deoxyribonuclease (rhDNase) decreases sputum viscosity and clinically results in decreased sputum tenacity, easier expectoration of sputum, decreased chest congestion, and decreased dyspnoea, although it is unclear at present which patients benefit most from rhDNase treatment.[22] A potential alternative is nebulised hypertonic saline. This increases mucociliary clearance, measured by 90 minute isotope clearance, in a dose dependent manner in adults with cystic fibrosis.[23] Short term trials of 6% hypertonic saline over two to three weeks in patients with mild to moderate disease show a subjective improvement in feeling of chest clearance and an improvement in FEV_1 of 15% compared with isotonic saline.[24] Care must be taken as some subjects have paradoxical bronchospasm following inhalation of hypertonic saline. Clearly hypertonic saline is substantially less expensive than rhDNase, but longer term studies are required before its widespread use can be recommended.

Microbiology and antimicrobial treatment

The bacterial pathogens infecting the cystic fibrosis airway change with age. *Staphylococcus aureus* is the commonest pathogen early in life and the prophylactic use of regular antistaphylococcal treatment in young children appears beneficial.[25] At some stage *Pseudomonas aeruginosa* will become established. *Burkholderia cepacia* came to prominence in the 1990s owing to its transmissibility between patients and its ability to trigger a rapidly progressive decline to death in previously healthy patients. Preventative measures to decrease transmission between patients, such as separate clinics and ward areas, have reduced this risk.

Chronic airway *Pseudomonas aeruginosa* infection is associated with a more rapid decline in pulmonary function. A meta-analysis of the use of continuous nebulised antibiotics (gentamicin, tobramycin, colistin, or ceftazidime) in chronic *Pseudomonas aeruginosa* infection concluded that they decreased the bacterial load, decreased the frequency of respiratory exacerbations requiring supplemental antibiotics, and decreased the decline in pulmonary function.[26] The small studies that contributed to the meta-analysis have been dwarfed by the recent controlled study of intermittent high dose preservative-free tobramycin in 520 American patients.[27] Patients were randomised to receive, for six months, either 300 mg of nebulised tobramycin twice daily (in one month long treatment cycles, alternating with one month without treatment) or placebo. At the end of three treatment cycles (20 weeks) the patients randomised to receive tobramycin had a 10% increase in FEV_1 compared to a 2% decrease in controls ($p < 0.001$). Patients randomised to receive tobramycin had a decreased sputum bacterial load and were significantly less likely to be admitted to hospital or to receive intravenous antibiotics. The greatest benefit was seen in patients aged 13–17 years, while benefit was least clear in those less than 12 years of age. Despite the unquestionably rigorous design of the study, the role of nebulised tobramycin in Britain has been less clear, as most chronically infected patients in the United Kingdom already receive nebulised antibiotics (usually colistin). It is possible that nebulised tobramycin will be beneficial even in patients already receiving colistin. Like rhDNase, the cost of nebulised tobramycin is not

inconsiderable, and similar battles with purchasers are likely.

There is debate over the role of regular intravenous antibiotics in patients chronically infected with *Pseudomonas aeruginosa*, as a retrospective study from Denmark had suggested benefits in pulmonary function and survival. In a recent United Kingdom multicentre study 60 patients were randomised to receive either elective (three monthly) or symptomatic (as required) courses of intravenous antibiotics over a four year period.[28] No benefits of elective treatment could be shown. The study's findings were weakened by there being no marked differences in the mean number of courses of antibiotics each group received in a year.

Nutrition

Worldwide to date there have been 85 reported cases of fibrosing colonopathy, including 18 in the United Kingdom (Bakowski M, personal communication). There has been debate over the role of high strength enzyme preparations or the use of high doses of enzymes in the aetiology of this condition, but it now seems likely that a methacrylic acid co-polymer (Eudragit) in the enteric coating of some preparations is the culprit.[29] The risk of fibrosing colonopathy in patients receiving non-Eudragit-containing products, even at higher doses, appears extremely small.

Lung transplantation

Lung transplantation in cystic fibrosis is not curative, but may prolong life. Of those children who are accepted for transplant, most will die before a donor becomes available.[30] In patients accepted for transplant, receiving a lung or heart-lung transplantation decreases the risk of death by 69%.[30] However, the results of lung transplantation are markedly poorer than for other solid organs, with international registry reports of 70–80% one year survival and 30–45% five year survival.[31] This situation is unlikely to change without increased donor availability and improved immunosuppression.

New pharmacological treatments

Newer treatments are likely to be aimed at specific classes of CFTR mutation (see above). For example, class I CFTR mutations are commonly non-sense mutations and cause the formation of truncated or unstable protein. The aminoglycoside antibiotics increase the frequency of erroneous insertion of the non-sense mutation, permitting protein production to continue. In patients with non-sense mutations, 14 days of gentamicin nasal drops resulted in a significant change in nasal potential difference, with increased repolarisation of the nasal epithelium representing chloride transport.[32] Newer treatments for class II mutations undergoing phase I or II trials include the butyrate compounds (phenylbutyrate and biphenyl), which increase CFTR trafficking by impairing the molecular chaperones that target mutant CFTR for destruction; CPX, an adenosine receptor antagonist that binds directly to ΔF508; and the flavonoid genistein, which has no effect on CFTR trafficking but rather activates the small amounts of ΔF508 that reach the apical membrane (reviewed by Zeitlin[6]). Consequently genistein may also have a role in class III mutations.

Gene therapy

Gene therapy aims to deliver copies of normal CFTR to the airway epithelium using viral or non-viral vectors. Viral vectors include adenoviruses, adeno-associated viruses, or sendai viruses; non-viral vectors include cationic liposomes or cationic polymers (reviewed by Boucher[33]). Both viral and cationic liposome delivered gene treatment in the nose can produce some gene transfer (detectable CFTR mRNA) and some degree of correction in chloride transport. The limited studies of the lower airway have again shown some detectable CFTR mRNA with viral vectors, and both detectable CFTR mRNA and mild changes in chloride transport with a liposomal vector.[34] It should be stressed that the changes observed are not close to values in subjects without cystic fibrosis, and it is unlikely that any phase III trials will start within the next five years.

The future

The improvements in cystic fibrosis survival to date are not the result of major technological breakthroughs, but rather of the central tenets of cystic fibrosis management—optimising nutritional status and preventing pulmonary damage. Various potentially useful pharmacological agents are in development and look likely to extend survival even further. The tantalising hope of a cure through gene treatment seems far off. Our role as paediatricians must be to guide our patients to reach their maximum physical and developmental potential, so that they will be well enough to benefit from gene treatment should it become available.

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