REVIEW ARTICLE/PRACA POGLĄDOWA

Professor John A. Dodge, CBE MD FRCP FRCPCH

A MILLENNIAL VIEW OF CYSTIC FIBROSIS

Department of Child Health, Swansea University, Swansea, Wales, United Kingdom

DEV PERIOD MED. 2015;XIX;1:9-13

LOOKING BACK

Although only identified as a distinct disease in the 1930s, it was soon apparent that Cystic Fibrosis (CF) had been present, but unrecognised, in European populations for many years – perhaps even centuries [1] . Within a decade of the early descriptions, the autosomal recessive nature of this genetic disease had been clarified, and its clinical features had been expanded. Secondary nutritional deficiencies complicated the underlying condition: the first clear description of CF as "a new disease", which included a speculation about its genetic basis (because there were 2 pairs of sibs in the case series) was published as Vitamin A deficiency in children [2]. The diagnosis was most often made at autopsy. When it was suspected in life, the diagnostic tests used included duodenal intubation to obtain fluid which would show impaired tryptic digestion of the coating of X-Ray film in CF children, and measurement of vitamin A in the blood. Some nutritional improvement could be expected with simple, rather inefficient pancreatic enzyme preparations, but it was not until mid-century that antibiotics began to treat pulmonary infections effectively. As a young doctor in the 1950s I soon became aware that the median age at death for affected children was about one year, and most died before reaching school age.

Diagnostic sweat tests were introduced after the observation by di Sant'Agnese in 1953 that children with CF who suffered severely from salt depletion in hot weather, lost excessive electrolytes in their sweat [3]. The standardised iontophoresis sweat test [4], and its more sophisticated variants, have remained the primary method of diagnosis until now. In 1983, Quinton showed that sweat duct cells are impermeable to chloride reabsorption in CF [5], bringing new possibilities for understanding the pathogenesis of the disease features in the many tissues in which the gene is expressed.

Identification of the CF gene, and characterisation of its protein product (Cystic Fibrosis Transmembrane Regulator, CFTR) followed in 1989 [6, 7]. The CFTR protein was shown to be, among its other functions, a chloride channel. By the end of the millennium, the large amount of money which had been invested in basic science and molecular genetics was beginning to produce results by opening horizons to the prospect of cure or control of the basic cause of CF.

Finding the gene also brought new insight into the antiquity of CF. Estimates for the origin of its commonest mutation, F508del, range from 3000 to more than 40,000 years ago. It is ancient, certainly pre-Neolithic, and may have been present before the emergence of anatomically modern humans [8]. Why have mutations with such a deleterious effect survived for millennia? Is there a heterozygote advantage? There is so far no absolutely convincing answer to these questions.

Although discovery of the CF gene and the CFTR protein was a key to understanding the pathogenesis of the disease, and opened up possibilities of definitive treatment, it also brought new problems for the clinician.

Firstly, there proved to be many different mutations of this gene. Formerly, we had naively assumed that once the gene was found, a simple diagnostic genetic test would replace the sweat test with its practical difficulties and sometimes inconsistent results. Unfortunately, so far at least 1500 disease-causing mutations have been identified, their relative frequency varying between different populations. The most frequent F508 del, accounts for up to 80% or more of all CF alleles in northern European populations but less than 50% in Mediterranean countries [9]. When genetic analysis is used to confirm the diagnosis in asymptomatic infants testing positive for a raised immuno-reactive trypsin (IRT) level in neonatal blood spots, it is essential to know the relative frequency of the most common mutations in that country, because it is unrealistic to test for all posssible mutations, most of which are rare.

Secondly, different mutations affect CFTR function by interfering with different intracellular processes. These mechanisms fall into 4 broad categories: primary failure to manufacture any or sufficient CFTR; altered assembly of mutant CFTR so that it is discarded and destroyed; failure of trafficking of CFTR to the cell membrane; and failure of correctly sited mutant CFTR to open and close appropriately, i.e to function as a channel.

Thirdly, as might be expected, the severity of the effect of such a wide range of mutations differs between patients and between affected organs in individuals, and these variations are not always predictable. Clinical features such as pancreatic insufficiency may be manifest at birth or appear later, or not at all. Similarly, the onset of lung disease may be in early infancy or sometimes delayed into adult life. Intestinal dysfunction may cause the CF neonate to present at birth with meconium ileus, or it may not be an important feature until distal intestinal obstruction occurs in an adult. Liver disease may be evident in children or may never be recognised until it is seen as an incidental finding in an adult autopsy. This clinical variation is not only a consequence of the CFTR mutation, but is undoubtedly influenced by modifier genes, and, importantly, by environmental factors, and also by treatment. The course and severity of CF may vary considerably between patients with the same mutations, occasionally even between identical twins.

Fourthly, the spectrum of disease and the recognition that not all CF is the same raises many questions. What constitutes a CF diagnosis? Even with all the tools at our disposal in the 21st century, CF remains a clinical diagnosis which may be supported by laboratory tests. Making a CF diagnosis has enormous implications for the patient and her family; for the health services; for employers and insurers; for the pharmaceutical industry, and there are now far more, rather than fewer, ways in which people with a CFTR mutation can be mis-classified. With experience we have come to accept an intermediate range of sweat chloride values, rather than just a positive or negative judgement according to an arbitrary cut-off point. People within that broad intermediate range may be heterozygotes, but some have 2 identified mutations, at least one of which is "mild", but they may have few or no symptoms in early adult life. Will they develop lung disease later, as some do (and when they do it often progresses rapidly) or perhaps recurrent pancreatitis but no other CF features? If they are male, their only problem may be infertility due to atresia of the vas deferens, but can – or should - that be called CF? Many meetings and workshops have resulted in guidelines and algorithms to help arrive a firm diagnosis one way or the other [10]. There are significant transatlantic differences in diagnostic criteria which may be depend more on differences in the funding of health services than on the science. Although we knew so much more about the cause and the pathophysiology of CF, at the end of the millennium we still had no drugs targeted at cure or control of its underlying process. However, the prognosis had improved dramatically through good medical practice. Clearly, the genes had not changed, but mean survival of patients increased from less than a decade in 1960 to about 40 years by the end of the 20th century in countries such as the USA, UK, and Denmark where it has been monitored [11]. There now more affects adults than children. CF was no longer an important cause of death in childhood [12]. This transformation in life expectancy has been attributed to a variety of factors. The greatest effect from a single intervention was the virtual elimination of death from meconium ileus, which affects up to 20% of CF newborns, by new surgical techniques introduced in the 1960s. Even if they did not die in the neonatal period, most survivors died within the first year, but successful surgery and immediate care by CF paediatricians has given those babies a much better The importance of nutritional intervention when CF children fail to thrive was emphasised by Canadian colleagues [13] while the Copenhagen clinic showed that aggressive management of respiratory infection is effective in patients recently or chronically colonised with Pseudomonas aeruginosa [14]. Provision of expert care in special CF centres has a major impact [15] and the quality of the surveillance and interventions used at those centres partly accounts for differences in survival [16]. The importance of the CF care package as a whole, rather than its individual components, was emphasised in a clinical review from Israel [17]. It must be remembered that standards of living and health care for the general population improved considerably in the last 50 years, and CF patients may have disproportionately benefited. A persuasive case has been made for socio-economic factors being the most important determinant of an individual CF patient's course and Survival [18].

With all our new knowledge, and new technical skills, what lies ahead?

LOOKING FORWARD

35 years ago I gave a lecture entitled "Cystic Fibrosis in the year 2000". Although I am not a prophet, most of my predictions were broadly correct, because they were based on research and development which were already in progress. We have found the gene, enabled prenatal diagnosis, introduced newborn screening programmes, established specialist CF centres, and seen survival steadily increase so that there are now more adults than children with CF. Although there is more CF research than ever, it is difficult to foresee which will be the most fruitful directions, and some will render others obsolete. I did not foresee the current development of new drugs capable of controlling the basic defect arriving so soon. In another 35 years I will not be around to find out how inaccurate I am now!

EPIDEMIOLOGY

Knowledge of the worldwide distribution of CFTR mutations, and of CF itself, is still incomplete [9]. In Eastern Asia, a different disease, panbronchiolitis, is recognised and often associated with a CFTR mutation but its clinical features are distinctive and there is no involvement of other organs such as the pancreas or sweat glands [19]. In India, where typical CF occurs but is often missed, small populations have been screened for CFTR mutations including F508 del, and some calculations estimate that the number of infants born with CF may be greater than in the whole of Europe. We are well aware of it among Indian immigrants to the UK. Where CF is known to be present in socio-economically deprived communities it is likely to be underestimated, for many reasons. Eventually, through universal application of rapidly developing molecular genetic techniques, the worldwide distribution of CFTR mutations and their frequency in different populations will be known.

SCREENING FOR CF

Neonatal screening is currently based on a combination of a demonstrable abnormality of cell physiology – a raised level of immunoreactive trypsin in a blood spot

– and a confirmatory DNA analysis showing that the infant carries at least one CFTR mutation. This is a cumbersome system which sometimes leads to errors when the clinical picture does not correspond with the prediction. Difficult cases may need sequencing the whole gene if the mutations are rare. Sometimes only one, or even no mutations may be found, in spite of a clinical diagnosis of CF. As our experience of matching genotype with phenotype increases, these anomalies will become even less frequent and our confidence in genetic analysis will improve, perhaps taking in new knowledge of the effects of modifying genes, or of other genes which can cause diseases closely mimicking CF. With rapidly developing technology, more possibilities will appear and costs per test will eventually fall, making them applicable and affordable worldwide.

In future, a confident diagnosis of CF will be made on the basis of genetic analysis alone, based on blood taken from the umbilical cord at birth, or perhaps even on a sample of maternal blood taken in early pregnancy, from which the fetal cells have been isolated. Of course, such screening will not be set up only for CF: ultimately the infant's whole genome will be screened for multiple common and rare diseases. Similarly, children who have been identified as healthy heterozygote carriers will be able, or perhaps required, to access that knowledge in later life. This will increase preconceptual and early pregnancy options. Armed with this knowledge, will parents want to bring CF children into the world? Ethical, moral, cultural, political, social and religious factors may be more important than purely scientific and medical ones in determining the answers to the questions which will be raised, and will vary from one society to another.

SURVIVAL

The mean survival of males with CF in the UK today is about the same as that of males in the general population prior to the First World War. That is a measure of how far we have come in management of CF in the last half-century. However, we have also seen an enormous increase in longevity in the general populations of all industrialised countries. It is probably coincidental that the difference in life expectancy remains much the same. If we assume in very approximate terms a mean survival of 40 years for men in 1910 and 80 years in 2014, this would compare with less than I year in 1910 and 40 years in 2014 for males with CF. Of course, this comparison masks the fact that the improvement in survival did not begin until almost mid-century. It also ignores the slight but significant differences in females: non-CF women live longer than men, but the opposite is true in CF. None of these survival data has any relevance for an individual with CF, but they do raise important questions about relationships between biological factors which could help us to understand and possibly modify the pathophysiology of CF so that life expectancy will continue to move closer towards the population norm. Will that goal ever be reached?

AGEING WITH CF

Survival of CF patients into adult life has raised questions about possible late complications. We know that CF-related diabetes (CFRD) often develops by the third decade, and liver disease even earlier, while male infertility is predicable from birth. Because until recently few clinics have cared for more than a handful of patients in their 40s or beyond, sharing of experience with older patients is essential if we are to monitor and advise them concerning previously unknown problems which may be encountered as they become older. How do the ageing process and CF interact? Is normal ageing accelerated or even conceivably retarded by CF? Some disorders associated with ageing include cancers, type 2 diabetes, cataracts, deafness, dementias including Alzheimer disease, osteoarthritis, osteoporosis and Parkinson disease. From this list we already know that CF patients are at higher risk of deafness, for example, but this could be related to high doses of aminoglycosides, while osteoporosis may at least in part be secondary to low vitamin D status. There are now several observations that they may also be at increased risk of colon cancer [20], but is this because they have CF or is it also related to long term treatment, perhaps with pancreatic enzymes? T he overall cancer risk is probably not greater than that for age-matched controls [21]. Clearly, these studies need to be continued and updated as more patients enter their fifties and beyond.

CONTROL OF INFECTION

The management of pulmonary infection has always been central to CF care. Respiratory failure secondary to lung damage is the usual cause of death, but since the 1960s it has been clear that the pathogens likely to be present at the time of death have changed with age and over time. When survival was measured in months or a few years, Staphylococcus aureus was usually found, but later Pseudomonas aeruginosa, later becoming mucoid, was the dominant pathogen. Resistance to antibiotics develops easily and the pharmaceutical industry has been active in finding new antibiotics to try to keep up with or overtake the organisms' evolution. New pathogens have emerged, such as Stenotrophomonas maltophilia, and perhaps most feared, Burkholderia cepacia and atypical mycobacteria. Immunisation against Ps aeruginosa has so far not been very successful, and even if more effective vaccines are developed, I have little doubt that sooner or later patients would become colonised with other possibly more deadly pathogens. Much of the damage is caused by inflammation rather than by direct action of bacteria, and no doubt more efficient anti-inflammatory treatment will be devised. The medium-term future for frantic development of new antibacterial agents seems secure; but protecting CF lungs against colonisation, chronic infection and inflammation will only become a reality when infants diagnosed at or before birth start lifelong treatment to control the underlying genetic or intracellular abnormality.

TRANSPLANTS

Many of the adult patients now alive owe their continued survival to transplanted lungs and, occasionally, livers. Surgical techniques and immunosuppressive regimens have developed, and post-transplant survival in CF patients tends to be longer than that of non-CF transplant subjects. This progress will continue until the need for transplanted organs disappears.

There are possibilities for pancreas transplants, which if carried out early enough probably also prevent the development of CF-related diabetes, but the technical difficulties are considerable. Islet transplants may be possible for CFRD alone, but if the islets were transplanted into a CF pancreas they would be vulnerable to the same process which destroyed the original ones.

GENE THERAPY

Inserting a normal CFTR gene into a human CF genome is possible. The individual will then have three CFTR genes, two of which are mutated, and the normal one would effectively transform such a person into a CF carrier. One normal gene should suffice for chloride transport and other CFTR cellular functions. However, there are legal and ethical objections to giving the gene in such a way that it enters the germline and can be transmitted to the next generation. We do not know the possible long term adverse effects of such a radical intervention into human biology: there may be none, but at the present time society is not willing to take the risk. This means that gene therapy must be precisely targeted at the organs where CFTR is expressed, particularly the lungs, but could also include the pancreas, liver and intestines. Thus, gene therapy for CF has come to mean inhalation of the gene to the respiratory epithelium using either a virus or liposomes as the vector. Viruses are more effective at entering the cells, but also more pathogenic than liposomes. Early trials with adenoviruses were abandoned because of unacceptable side effects. Studies with liposomes have so far been limited to proof of concept by applying them to nasal mucosa and measuring the effect on electrolyte transport. Problems which can be anticipated include possible attenuation of effect with repeated administration, and unpredictable uptake when the aerosol is delivered to and via airways covered with mucopurulent secretions. Although limited studies continue, gene therapy in its present form is a long way from clinical application, and will probably be superseded either by a more radical approach which would mean whole-body uptake of the gene, or by the alternative of pharmacological correction of the basic CF defect.

All human history testifies to gradual changes in the definition of ethics. In the early 21st century, abortion which was formerly widely denounced as unethical, and therefore illegal, has been legalised in many countries. Its application is often but not universally restricted, but the conditions and regulations vary widely, and it remains anathema to different societies and religions. Attitudes to gene therapy likely to affect the germline will probably evolve in a similar way, but are unpredictable.

CF-SPECIFIC DRUG THERAPY

This holds the most exciting prospect of "cure" in the sense of control of the clinical features of CF, short of modifying the genome. We have seen that clinical CF may result from at least four distinct intracellular malfunctions, according to the type of mutation. Possible drugs may work by suppressing premature stop codons (PTC) which terminate synthesis of CFTR (Type1); stabilising protein structure (Type 2) thus promoting promote folding of mutant CFTR so that it is trafficked ("chaperoned") to the cell membrane (Correctors); or enhancing the activity of depleted normal or abnormal CFTR in situ at the cell membrane Types (3-5), so-called Potentiators [22].These quite different functional activities are likely to be mediated by different drugs, and this has proved to be the case. Unless targeted correctly, they are unlikely to work. The first clinical trials demonstrating clinical effects were carried out in Israel, where a high proportion of CFTR mutations are Type 1, and gentamicin, already in the market as an antibiotic, was used. Soon after, a specific drug called Ataluren, which reads through PTCs but has no antibiotic effect, was developed. The most frequent CF mutation in the world, F508del, is a type 2 mutation and modest chaperone activity has been demonstrated by several drugs including genistein and VX 661, but clinical benefits have yet to be demonstrated. Kalydeco (VX770), targeted at mutations in classes 3-5, has been very effective and is already marketed in some countries. It also has some additional effect on Class 2 mutations, and perhaps in the future combinations of drugs will be employed. We do not know anything about possible long term side effects [23].We do know, however, that such products emerge after huge investment in research, and have a very small potential market. Their cost to the consumer – the patient, insurance company or health service – is breathtaking. They are barely affordable in prosperous countries, so what is the prospect of bringing their benefits to CF patients in the wider world where CF occurs but even now is missed or untreated? There are, however, other drugs already in the marketplace which have also been shown to be effective correctors and potentiators [24]. these studies employed high doses which may rule them out for use in CF, but their molecules might potentially be modified to make them suitable, at lower cost. We will be able to find CF patients worldwide through screening programmes, but should we do so if we cannot afford to treat them? How will different societies deal with CF? What will be the structure of those societies, and their ethnic mix, 100 years from now? Will CF still be around on the Earth in another 1000 years? Probably not, but who knows?

REFERENCES

1. Busch R. On the history of Cystic Fibrosis. Acta Univ Carol Med. (Praha) 1990;36:13-15.
2. Blackfan KD, Wolbach SD. Vitamin A deficiency in infants. J Pediatr. 1933;3:679-706.
3. di Sant'Agnese PA, Darling MD, Perera G, Shea E. Abnormal electrolyte composition of sweat in cystic fibrosis of the pancreas. Clinical significance and relationship to the disease. Pediatrics 1953;12:549-563.

4. Gibson LE, Cooke RE. Test for concentration of electrolytes in sweat in cystic fibrosis of the pancreas utilizing pilocarpine by iontophoresis. Pediatrics 1959;23:545-549.
5. Quinton PM, Bijman J. Higher bioelectric potentials due to decreased chloride absorption in the sweat glands of patients with cystic fibrosis. New Eng J Med 1985;(19)308: 1185-1189.
6. Kerem B-S, Rommens JM, Buchanan JA, *et al.* Identification of the cystic fibrosis gene: genetic analysis. Science 1989; 245:1073-1080.
7. Riordan JR, Rommens JM, Kerem B-S, *et al.* Identification of the cystic fibrosis gene: cloning and characterisation of complementary DNA. Science. 1989;245:1066-1073.
8. Mateu E, Calafell F, Ramos MD, *et al.* Can a place of origin of the main cystic fibrosis mutations be identified? American Journal of Human Genetics 2002;70:257-264.
9. Cuppens H, Macek M. The Molecular Genetic epidemiology of Cystic Fibrosis. Report of a joint meeting of WHO/ ECFTN/ICF(M)A?ECFS. WHO/HGN/CF/WG/04.02 Geneva, WHO 2004.
10. Bombieri C, Claustres M, de Boeck K, Derichs N, *et al.* Recommendations for the classification of diseases as CFTR-related disorders. Journal of Cystic Fibrosis 2011; 10(Suppl 2):S86-S102.
11. Dodge JA, Lewis PA, Stanton M, Wilsher J. Cystic Fibrosis mortality and survival in the United Kingdom 1947-2003. Eur Respir J. 2007;29:522-526.
12. Dodge JA, Lewis PA. Cystic fibrosis is no longer an important cause of childhood death in the United Kingdom. Arch Dis Child. 2005;90:547.
13. Corey M, McLaughlin FJ, Williams M, *et al.* A comparison of survival, growth, and pulmonary function in patients with cystic fibrosis in Boston and Toronto. J Clin Epidemiol. 1988;41(6):583-591.
14. Frederiksen B, Koch C, Høiby N. Antibiotic treatment of initial colonisation with Pseudomonas aeruginosa postpones chronic infection and prevents deterioration of pulmonary function in cystic fibrosis. Pediatric Pulmonology. 1997;23: 330-335.
15. Mahadeva R, Webb K, Westerbeek RC, Carroll NR, *et al.* Clinical outcome in relation to care in centres specialising in cystic fibrosis: cross-sectional study. Brit Med J. 1998; 316:1771-1775.
16. Gawande A. The Bell Curve. What happens when patients find out how good their doctors really are? (Based on comparative survival data between US CF centres held by the Cystic Fibrosis Foundation). The New Yorker Nov. 23, 2004.
17. Cohen-Cymberknoh M, Shoseyov D, Kerem E. Managing Cystic Fibrosis: Strategies that increase life expectancy and improve quality of life. Am J Respir Crit Care Med. 2011; 183:1463-1471.
18. Schechter MS, Shelton BJ, Margolis PA, *et al.* The association of socioeconomic status with outcomes in cystic fibrosis patients in the United States. Am J Respir Crit Care Med. 2001;163(6):1331-1337.
19. Yoshimura K, Iizuka S, Anzai C, et al. Diffuse panbronchiolitis is closely associated with mutations of the CFTR gene. Am J Respir Crit Care Med. 2000;161:A77.
20. Hernadez-Jimenez I, Fischman D. Colon cancer in cystic fibrosis patients: Is this a growing problem? Journal of Cystic Fibrosis 2008;7:343-346.
21. Maisonneuve P, Marshall BC, Knapp EA, Lowenfels AB. Cancer risk in cystic fibrosis: a 20-year nationwide study from the United States. J Natl Cancer Inst. 2013;105:122-129.
22. Amaral MD, Kunzelmann K. Molecular targetting of CFTR as a therapeutic approach to cystic fibrosis. Trends in Pharmacological Sciences. 2007;28:334-341.
23. Wilschanski M. Novel therapeutic approaches for cystic fibrosis. Discovery Medicine. 2013;15:127-133.
24. Lubamba B, Lecourt H, Lebeque P, *et al.* Preclinical evidence that sildenafil and tadalafil activate chloride transport in cystic fibrosis. Am J Respir Crit Care Med. 2008;177: 506-515.

Received: 10.09.2014 r.
Accepted: 24.09.2014 r.

Address for correspondence:
John A. Dodge
Department of Child Health
Swansea University, Swansea, Wales,
SA8 8QA, United Kingdom
e-mail: j.a.dodge@btinternet.com