CLEARING THE WAY

ADVANCES IN OUR UNDERSTANDING OF CYSTIC FIBROSIS HAVE SEEN GREAT LEAPS IN OUTCOMES FOR PATIENTS, BUT A CURE REMAINS ELUSIVE. **MATT JOHNSON** REPORTS.

Embedded in the walls of nearly every cell in your body are small pumps.

Made of protein, they are constantly working to maintain the delicate chemical balance required for cells to function normally.

Depending on the protein and cell they inhabit, they can permit, assist or reject the passage of sodium, chloride, potassium and a host of other electrolytes.

Suffer a mutation of the gene that instructs these pumps and, for the sake of a few electrolytes, your lungs can become congested and inflamed, your pancreas will struggle to provide the enzymes necessary to digest your meals, your liver can fail, and your life expectancy plummets.

Cystic fibrosis (CF) occupies an uncomfortable place in modern medicine. The genetic cause of the disease was established as early as 1949, when researchers investigating the disease's pattern of inheritance suggested it was produced by a single defective gene. It took another 35 years to localise that gene, and during that time sufferers would rarely reach adolescence before their lungs failed.

With knowledge comes hope, however, and identification of the gene sequence in 1989 offered the possibility of curing one of medicine's more cruel conditions. Indeed, life expectancy has more than doubled in the past 40 years to a median age of 37.

But there is still no cure. While one school of research has discovered how a faulty protein causes the disease and tested a number of potential cures, the improvements in morbidity and mortality have come from another camp of researchers, which has been working to identify the disease as early as possible, isolate the pathogens that accelerate its progression, and develop better nutrition and physical therapy. The latter group has improved the length and quality of life of people with CF, but with the knowledge their patients will ultimately succumb to the disease.

The genetic component

The CF gene lies on the long arm of chromosome 7 and produces a protein that determines a cell's ability to move chloride and other ions across the cell membrane. Early attempts to identify this protein found high levels of salt in the sweat of CF patients, whose glands, it was discovered, are impermeable to chloride.

Subsequent studies of epithelial cells from the airways of these patients also provided evidence of a chloride permeability defect in the lungs.

The protein was eventually identified and named the cystic fibrosis transmembrane conductance regulator (CFTR). It's found in membranes of cells that line the lungs, liver, pancreas and reproductive tract.

In patients with CF, the dysfunctional (or absent) CFTR protein causes the lungs to produce a thick, extremely viscous mucus. Unable to clear this mucus, the CF lung is susceptible to chronic infection with pathogens such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* that are nearly impossible to eradicate once established. Inflammation from the dysfunctional CFTR and chronic infections create a cycle of tissue destruction and airway obstruction that, over a long period of exacerbations and partial recovery, eventually leads to respiratory failure.

In the pancreas, the large quantities of thick, viscous mucus block the flow of digestive enzymes through the ducts, food cannot be properly absorbed and weight gain is limited. Eventually the blockages can cause cysts and scar tissue to develop that ultimately may cause diabetes.

Mucus can also block the bile ducts, and 98% of men with CF are infertile because of blocked seminal vesicles.

The discovery of elevated serum levels of immunoreactive trypsinogen (IRT) in

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infants with CF resulted in the first community-wide newborn screening programs for the condition in NSW in 1981. Since then, all Australian states have introduced newborn screening.

The initial screening programs conducted an IRT test on day four, with a second test at six to eight weeks for infants who returned a positive result on the first test. A second positive result would prompt a sweat test, in which the amount of sodium in the child's sweat was analysed.

The discovery of the gene responsible for CF added genetic analysis to the screening program, and all states in Australia now use a combined IRT/DNA-based screening regime. An IRT is now performed between 48 and 72 hours after birth, and infants with an elevated IRT have a CFTR gene mutation analysis performed from the same blood sample.

Screening struggle

Professor Bridget Wilcken is the Clinical Director of NSW Biochemical Genetics and Newborn Screening at The Children's Hospital at Westmead in Sydney and an Honorary Fellow of the RCPA.

"Different parts of the world have responded differently to newborn screening, with some more enthusiastic than others," she says.

"In some places it's been a struggle." Here, she is referring to an ad hoc committee of North American paediatricians who suggested that the testing wouldn't be sensitive enough to pick up pancreatic insufficiency and that identification would stigmatise the child and interrupt mother/child bonding.

Professor Wilcken struggles to understand this continued reluctance.

"The test has proved both accurate and sensitive, and it's now quite clear there is a benefit in early diagnosis of CF: there's a lot of data that children diagnosed at birth don't get as sick early in life, they spend less time in hospital, they get better nutrition earlier and there are fewer childhood deaths.

"There have also been some studies linking late diagnosis to poor nutrition and subsequent poor intellectual development," she adds.

"You get improved lung function with early diagnosis – and while it's a big shock for most parents, you have to remember the alternative is a prolonged period of various illnesses while they search for a diagnosis."

While genetic analysis has made testing for CF more accurate, the nature of the mutation means not every newborn with the disease is identified.

"The common deltaF508 mutation represents 75% of all mutations," Professor Wilcken explains, "and as

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94–95% of babies who have CF will have at least one copy of this mutation, we only test for that common mutation in NSW."

Funding at present doesn't allow Professor Wilcken's service to routinely test for the other mutations, a process that would require an extra $34,000 a year to identify just one extra case.

"But mutation testing will become easier as technology develops, and if we were able to test for six or seven mutations we could cover half the babies we don't find at the moment. We're constantly considering it and it will be included eventually."

While the genetic component of the test has added a degree of certainty to the diagnosis, it has also raised issues for parents. Part of Professor Wilcken's program directs parents to genetic counselling.This includes parents of children with the disease, as well as those who are carriers.

Counselling includes advice on prenatal testing for parents prior to subsequent pregnancies, with studies showing about two-thirds of parents would alter their reproductive activity if they were aware of their genetic status.

Ensuring access

"It's not an aim of newborn screening to identify carriers but can be useful for parents, and we need to ensure they have access to the counselling they need," Professor Wilcken says.

And while the move towards prenatal testing is probably unavoidable, it shouldn't be at the cost of newborn screening, she argues.

Despite advances in screening, children with CF will still be born and, until a cure is discovered, their quality and length of life will depend on treatment of the disease.

Once identified, many of these children will find themselves patients of Professor Peter van Asperen, head of the Department of Respiratory Medicine at The Children's Hospital at Westmead.

"The expectations for patients with CF are so much better today, and probably the main reason is because we have much more coordinated care," he says.

"Sure, we don't have a cure, but we're developing better treatments and we're delivering those treatments much more effectively."

The thick mucus produced in the lungs of CF sufferers obstructs airflow, but also allows viruses and bacteria to colonise the lungs. These infective exacerbations and the inflammation they cause accelerate the respiratory failure. But they also offer a potential avenue for therapeutic control.

"While there was a lot of expectation when the gene was discovered, there unfortunately hasn't been a lot of progress – and until there's a cure, treatment is really aimed at slowing the progression of the disease," Professor van Asperen says.

"The principles are basically: mobilise the thick secretions and reduce the infections. The most recent area to emerge is using new therapies to treat the inflammation."

Oral steroids have been shown to improve outcomes in CF, but they also carry significant side effects. The search for better anti-inflammatory drugs has recently uncovered azithromycin: an antibiotic that also appears to have anti-inflammatory properties.

"The control trials have been good in patients with advancing lung disease, but there are also promising results coming from trials in patients with early lung disease."

The other area of focus is the removal of secretions and there are several therapies Professor van Asperen says can be effective.

"Inhaled hypertonic saline softens the mucus and makes it easier to clear and, given with a bronchodilator, it's an inexpensive, safe and effective therapy. We're currently also assessing mannitol to see if it has a similar effect."

CF: incidence and inheritance

CF is one of the most common life-threatening autosomal recessive conditions affecting Caucasians. The incidence is 1/2500 to 1/90,000, depending on the population. It is uncommon in Asians and Africans.

Individuals who become symptomatic will have two copies of a mutated CFTR gene, one from each parent.

Carriers will have one normal and one mutated CFTR gene and their health will not be affected. However, carriers have the potential to pass on the gene to their offspring. Brothers and sisters of affected individuals are at increased risk (one in four) of having CF because both parents will be carriers.

If two carriers of the mutated CFTR gene have children, there is a:

- **one in four chance their baby will have CF**
- **one in four chance their baby will not have CF or carry a CFTR mutated gene**
- **two in four chance the baby will not have CF, but will carry one CFTR mutated gene.**


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- Professor Bridget Wilcken

Perhaps the most significant recent breakthrough occurred when researchers at the University of Queensland (UQ) discovered that common bacterial pathogens in CF, *Pseudomonas aeruginosa*, glued themselves together with a 'biofilm' to protect themselves from antibiotics.

Until then, experts had thought a sugar called alginate thickened the secretions, but when UQ researchers analysed the substance they found far more DNA than alginate. This has led to the development of a new type of drug that breaks down the DNA in the film, reducing the tenacity of the mucus and allowing antibiotics to attack the pathogen.

Controlling the colonisation of *P. aeruginosa* is a major focus for Professor van Asperen as studies show that eradicating early infections dramatically slows the disease.

"We're trying to learn more about the organism, more about its colonisation, about the possibility of vaccination – but we're also taking a very different approach to infection control," he says.

Within his hospital, CF patients with *Pseudomonas* (and other common infections) are segregated from those who remain unexposed to the organism.

"The practicality varies with different hospitals, but we've set up age-based clinics where preschool-age patients start and remain until they are colonised with *Pseudomonas*. We then have school-age and adolescent clinics, where the majority of patients have *Pseudomonas*."

Another change has been to develop chest physiotherapies and breathing techniques to assist lung function that can be performed by the patient without needing to visit a clinic.

"It not only allows patients to be more independent, but it leads to later colonisation."

And when the treatments are no longer effective, Professor van Asperen now has the option of using bi-level positive airway pressure and continuous positive airway pressure – devices to assist with breathing - that can bridge patients with end-stage disease to a lung transplant.

"Outcomes from transplants are improving all the time and they are now a serious option for end-stage patients. Until we find a cure, they are our only opportunity to improve the eventual outcome of the disease."

Towards a cure

Nearby, clinical geneticist and genetic pathologist Professor John Christodoulou, Director of the Western Sydney Genetics Program, will not put a time frame on a cure, but he is confident it will one day emerge.

"Understanding of the genetic basis of CF has improved our understanding of the disease and how other treatments could be effective, but despite a lot of work and hype, gene therapy is struggling to provide a cure," he notes.

"The problem for gene therapy is delivering the corrected gene to the target tissue. Some researchers have tried using genetically modified viruses, others have used lipid capsules, and others are trying to get naked DNA into the cells.

"They all have their positives and negatives, but the major issue is the consistency and efficiency of the delivery."

Far from being despondent, Professor Christodoulou (who recently became an RCPA fellow) is confident the techniques will eventually be found, and until then, learning more about the hundreds of gene mutations that contribute to CF will allow researchers to provide a more accurate prognosis and effectively treat the disease.

"Since we've discovered the mutation we've identified a much wider spectrum of CF, and we now find patients with milder forms of the disease, like men presenting at infertility clinics. It's these people that may give us a better understanding of how to treat – and then ultimately, cure – the disease."

GPs NOTE: This article is available for patients at http://pathway.rcpa.edu.au