CYSTIC FIBROSIS IN CHILDREN OF THE EASTERN ARABIAN PENINSULA

A clinical, spatial and genetic study

By

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Submitted in fulfilment of the requirements for the Degree of Doctor of Health Sciences,
Deakin University, 2003
Desert Scene,
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Figure 1

Location of the UAE and Oman in the Arab World
Figure 2

The Location of the UAE and Oman in the Arabian Peninsula
Figure 3

The Location of the United Arab Emirates and the Sultanate of Oman on the Eastern Arabian Peninsula
Signed Statements

1. Candidate’s Own Work

I hereby certify that the formation of this thesis is my work alone. However, the wider body of study to which it refers and those who contributed to the study programme are duly acknowledged in the Acknowledgement Section. Each chapter contains references to the material that has been published by others and is relevant to this dissertation. Similar references are made to the papers that have been published jointly by our group.

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2. Other Degree Material

I hereby certify that the material in one chapter that has been included in another thesis is duly indicated and acknowledged in the text.

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“Dans les champs de l’observation le hasard ne favorise que les esprits préparé.”

“Where observation is concerned. Chance favours only the prepared mind.”

Louis Pasteur 1822-1895

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“I keep six honest serving men,  
(They have taught me all I knew);  
Their names are What and Why and When  
And How and Where and Who”  
Rudyard Kipling

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Dawson KP, Frossard PM, Girodon E, Goossens M. Cystic Fibrosis in Arab Communities: experience in the United Arab Emirates. Middle East Paediatrics In press


Dawson KP, Frossard PM. Cystic fibrosis in the UAE. Clinical and molecular genetic studies. XXIInd Congress of the Union of Middle-Eastern and Mediterranean Paediatric Societies. Abu Dhabi, United Arab Emirates, November 1994


Frossard PM, Dawson KP. Molecular genetics of Paediatric diseases in the UAE. Fourth Paediatric Conference, Dubai, United Arab Emirates, January 1995


Frossard PM, Hertecant J, Bossaert Y, Lestringent GG, Dawson KP. Molecular aetiology, prevalence and clinical severity of cystic fibrosis in the UAE. 6th UAE Paediatric Conference, February 1999, Dubai, UAE

Dawson KP, Frossard PM. The clinical severity of cystic fibrosis mutation S549R (T→G) in the United Arab Emirates. 25th Congress of the Union of Middle East and Mediterranean Paediatric Societies. April 1999, Beirut, Lebanon.


Dawson KP, Frossard PM Cystic Fibrosis in Asian communities: experience from the United Arab Emirates. The 10th Asian Congress of Pediatrics, March 2000, Taipei, Taiwan

Dawson KP. The Genetics of Cystic Fibrosis. The 2nd Qatari International Pediatric Conference, April 2000, Doha, Qatar
Figure 4

Rural Scene Al Ain, United Arab Emirates
Statement on Personal Contribution

The work described in this thesis has involved many colleagues in the care of the children, the research aspects and the writing and presenting of reports.

My principle role has been in the provision of professional leadership. I have been the principle investigator for three major research grants and principle co-investigator of a fourth grant that supported equipment purchase and the development of a molecular genetics laboratory. Above all I have been responsible for the identification of the children with cystic fibrosis and their clinical management. My other role has been also, in the planning of the research, asking the research questions and planning how to answer them. My colleague Dr. Phillipe Frossard, a molecular geneticist, has supported me throughout. Phillipe has been responsible for the setting up of the molecular genetics laboratory, its staffing and the technical expertise required. Professor Emmanuelle Goosens and her team in Paris provided peer review. Hence I was not responsible form the processing of blood specimens and the obtaining of the molecular results. I did make it my business to learn how this process was carried out. I did actually draw the blood specimens myself from the UAE children in the clinical studies.

In summary, I developed and designed the studies after consultation with colleagues. I wrote the reports in whole with input from the interest group. I carried out the clinical examinations and investigations and managed all the UAE patients. I collected the data and have made the analysis with technical advice from Phillipe Frossard. The major role of the laboratory is recognised in our joint publication of papers. The sequence of names in papers is by agreed rotation with a tendency on my part, as Head of Department, not to appear to preclude the junior members and place my name later. The sequence does not in general reflect the
individual contribution to each paper. The hypotheses presented are wholly mine and are based upon the data we had collected.

As a result of the paradox between the clinical findings in our patients and those in French patients, we entered discussions with the French group. It was as a result of these discussions that the project leading to the discovery of the mutation in the promoter region came about.
Acknowledgements

“No man is an Island, entire of itself”
Meditation XVII
John Donne (1571-1631)

The major supporter of the investigations described in this dissertation has been the United Arab Emirates University. To this organisation and hence the United Arab Emirates government I wish to give sincere thanks for the four research grants that have been awarded to our group. The actual grants are listed at the end of this section. The funds provided have gone a long way to help in the establishment of the molecular genetics laboratory and to develop the specific genetics tests involved in cystic fibrosis research.

On an individual basis I must acknowledge, above all, the contribution and support of my colleague Dr. Phillipe Frossard. Phillipe who was then an Associate Professor in the Department of Pathology in the Faculty of Medicine and Health Sciences of the United Arab Emirates University. Subsequently, he has become Professor and Chairman of the Basic Sciences Department in the Aga Khan University Medical School in Pakistan. He has been a stalwart supporter of our research and has encouraged me to develop the clinical services and subsequent research projects. Dr. Frossard, a molecular geneticist, has been the guiding force in the establishment of the laboratory-based aspects of the studies. Through his good offices we were able to establish good working relations with Professor Emmanuelle Girodon and her team in Paris. I am grateful to their support and for the peer review they have provided over our activities in the UAE. Ms Annie John, senior laboratory technician, has carried out the bulk of the routine laboratory work such as the extraction of DNA and the other time
consuming preparatory work. She has always carried out her duties skilfully and with good grace. I thank her for her contribution.

The dissertation has been written, in the main, using the first person plural. This is not intended to infer that there has been a lack of originality on my part but to stress and recognise that any health related activity is a team effort and should be thus recognised. Our team consisted of many people. They ranged from my clinical colleagues who referred patients to me to those who helped manage the patients and share their acute care. Of the latter group I must pay tribute to Dr. Joseph Hertecant and the late Dr. Yves Bossaert. While their contribution has been acknowledged in co-authorship of some of the clinical papers, they were always supportive and encouraging of our efforts. Dr. Bossaert was tragically killed in a desert motor vehicle accident in the UAE towards the midpoint of our clinical investigations, but his contribution is fondly remembered. Dr. Ghazala Bal Haj was of great help in making sure that the parents understood about the disease and what we were trying to achieve. This help was not simply a reflection on her fluent English language and Arabic skills but her talents as a caring doctor and human being.

The actual text has been typed and prepared by the author. However, I am grateful to Fiona White and Nikki Rush for professional advice and help when I got into trouble and difficulty with the word processor and when I needed guidance on layout.

Finally, but most importantly, Professor Madeleine Ball has been a great source of encouragement, help and advice over the period of the development of this thesis. Subsequently, Professor Julian Mercer has guided me in the production of the dissertation and I am grateful to him for undertaking this onerous task and his continuing support.
Relevant Research Grants

1. Identification of the genetic mutations responsible for cystic fibrosis in the UAE. (1)
   Dawson KP, Frossard PM  1994-5

2. Identification of cystic fibrosis mutations in the UAE. (2) Dawson KP, Frossard PM.
   1995-6


4. Cystic fibrosis in the Gulf: mutations in the cystic fibrosis gene, clinical phenotype and
   genetic testing. Frossard PM, Dawson KP, John A, Kochiyil J. Three Year Project Grant

Competitive research grants awarded by the United Arab Emirates University.
Figure 5

An Omani Mother and her Child with Cystic Fibrosis
Cystic Fibrosis: an Historical Preamble

“Das Kind sterbt bald wieder, dessen Stirne beim Kussen salzig schmeckt”
“The child will die soon, whose forehead tastes salty when kissed”

German Children’s Songs and Games of Switzerland circa 1600

Current evidence suggests that more than 52,000 years ago a sudden human gene mutation took place in people living in Asia that resulted in a major change in the CF gene\(^1\). This event took place in the Palaeolithic period that corresponded to the post glacial warm epoch. It appears that at least three different mutations arose in humans living in the same geographical area. The circumstances under which these changes took place is unknown as are the actual people in which it arose but they were genetically distinct from any present European group. Subsequent emigration of people from the geographic regions of southern Russia, North Africa and the Middle East into Europe brought the gene mutations with them. Of particular importance was the transfer of the specific gene mutation we now refer to as ΔF508. It is hypothesised that the resultant admixture of these Indo-Europeans, Asians and North Africans led to biological and behavioural change. Among these changes was an alteration in diet. The consumption of grains and the introduction of cows’ milk into the diet required an ability to break down lactose. Interestingly, it has been suggested that the mutant CF alleles may have provided some advantage for the heterozygote carrier when these dietary changes occurred\(^2\). This advantage may have helped to secure the survival of these new settlers in Europe and their subsequent spread across the European continent.

The descendants of these Neolithic people became strongly influenced by the supernatural. There was a belief that demons, magic powers and the evil eye were all intimately responsible
for the causation of the diseases to which they fell victim. Thus children who developed restlessness, vomiting or failure to thrive were considered bewitched (hexed) and this in turn led to the belief in later times that such children should not be baptised. The practise of licking the forehead of the children in a crosswise fashion was introduced. The idea behind this was to prevent or treat the bewitched state of the child. It is thought that this practise, in turn, had derived from the cleansing ceremonies that had been in vogue as a form of medicinal ritual to cure disease. When mothers perceived a salty taste on kissing or licking their child’s forehead, the children were then regarded as bewitched and therefore likely to die in the very near future. The relationship to CF is thus very interesting with it well established now those CF children have a very high sweat sodium and chloride content. Even to this day, some children are recognised early as having a ”salty taste” when kissed and this may lead to the seeking of medical advice and diagnosis prior to the onset of symptoms.

The evidence of the superstition regarding the salty taste is drawn from numerous documents, the earliest dating from 1606. Interestingly, these documents are derived from sources in twelve of the modern European states. Especially prominent are the German speaking countries of Germany, Austria and Switzerland, but reports also emanate from Spain, Russia and Italy. No source data is available from the British Isles or Sweden. The oldest of the above documents dating from 1606 was written by Juan Alonso y de los Ruyzes de Fontecha, Professor of Medicine in the University of Acala de Henares. The references are contained within his book “Diez previlegios para mugeres prenadas”.

It is well established that in CF, the pancreas of the patients can be macroscopically (naked-eye) abnormal, being firmer, lobulated and having multiple cysts present. In 1595 the Professor of Anatomy in Leiden, Pieter Pauw (1564-1617), dissected the body of an 11-year-
old girl. The patient had been ill for eight years with failure to thrive and repeated fevers. At the post mortem examination she was found to have a swollen, scirrhouus and enlarged pancreas which resulted in her death according to Pauw5. Was this the first ever medical report of cystic fibrosis of the pancreas? Subsequently, Georg Seger (1629-1698), in Germany, treated a girl for three years who had failed to thrive, had diarrhoea, fever and vomiting. Following her death an autopsy revealed an enlarged indurated and scirrhouus pancreas, which he attributed to the cause of her illness and subsequent death. Another important early report was that of Carl von Rokitansky (1838) from Vienna1. He described a child who died from perforation of the bowel and meconium peritonitis. This is the classical course of untreated meconium ileus that can be a neonatal presentation in 10-20% of infants with cystic fibrosis. It seems reasonable to accept this as very strong evidence of the existence of CF in earlier times.
References

Abstract

“Her cabin’d ample Spirit,
it flutter’d and fail’d for breath
To-night it doth inherit
The vasty hall of death”

The Scholar Gypsy
Mathew Arnold 1822-1888

Aim:
The aim of this thesis is to describe the process by which the inherited disease, cystic fibrosis, (CF) was recognised as an important clinical entity in the United Arab Emirates (UAE) and the Sultanate of Oman (Oman). It examines the clinical presentation of the first patients and assesses their degree of severity. Further, it describes the first studies carried out to determine the underlying CF mutations associated with the disease in the UAE and Oman. An estimate is offered of the birth frequency of the condition. Overall, the cultural, geographical and historical aspect of the societies in which the disease occurs is stressed.

Methods:
An initial literature search was carried out using Medline of any literature pertaining to the Arab World and CF. This was read and classified into the relevance to Arabs in general, the Middle East and then specifically the Arab (Persian) Gulf societies.

Thereafter, a clinic was established at Tawam Hospital, Al Ain, UAE, for children presenting with chronic respiratory disease that could serve as a national referral centre. It was run by the author as a service of the Paediatric Department of the UAE University Medical School. I sent a letter to every Paediatrician working in the UAE informing them of our clinic and offering our services for the diagnosis and management of chronic respiratory disease in children. This was based on the author's experience as a respiratory paediatrician in Australia and New
Zealand and as the Professor of Paediatrics in the UAE. No such service then existed in the UAE.

Funding was sought to establish a research programme and develop a molecular genetics laboratory in the UAE Medical School. A series of successful research applications provided the grants to commence the investigations. Once a small number of children had been identified as having CF from those referred to the respiratory clinic, the initial project was to assess and report their clinical presentation. Following this an early start was made on the identification of the mutations responsible. Once these were established an attempt was made to estimate the frequency of the condition at birth.

Additional clinical studies revolved around assessing the severity of the condition that was associated with the main mutations that were identified. A clinical comparison was made with those with the mutation ΔF508 and the other main mutation, despite the obvious limitation of small numbers then available. Radiological assessment was made to evaluate the progression of the disease.

The final aspect of the study was to assess patients from Oman and compare their findings and mutations with the neighbouring UAE. Based on information gained hypotheses are proposed regarding the spread of the gene mutation by population drift.

Thesis outline:
A literature review is presented in the form of a critique on the disease and a résumé of the relevant aspects of the genetics of CF. Additionally, facts about the two countries' geography and history are presented. Finally, knowledge about CF mutations and population origins from other areas is presented.
The second main section deals with the clinical features of the disorder as it presents in the UAE. Molecular findings are then presented and details of the common mutation found in Bedouin Arabs. Hypotheses are then presented based on the information gathered.

Results:

CF is not a rare disease in the Arab children of the UAE and Oman. These findings refute previous reports of CF being a rare or non-existent disease in Arabs. The condition presents with a severe clinical picture, with early colonisation of the respiratory tract with staphylococcus, haemophilus and pseudomonas organisms, even with conventional CF management practices in place.

The CF mutation S549R is prevalent in Arabs of Bedouin stock, while ∆F508 is found in those of Baluch origin. The former may be descendants of Arabs who left southern Arabia and travelled to the Trucial Coast at the time of the destruction of the great dam at Marib. The origins of this mutation may lie in the area that corresponds to the modern Republic of Yemen. The latter groups are descendants of those who came originally from Baluchistan. It is hypothesised also that the ancestral home of the ∆F508 mutation may be in the geographical area now known as Baluchistan, that spans three separate modern political territories. The evidence presented supports the concept that the S549R mutation may be associated with a severe, if not the severest, clinical pattern recognised. It equates with that seen with the homozygous ∆F508 genotype. The absence of an additional mutation in the promoter region accounts for the different clinical pattern seen in previously described patients.

Conclusions:
There needs to be a major awareness of the presence of CF as a severe clinical disease in the children of the Gulf States. The clinical presentation and findings support the concept of under recognition of the disease. Climatic conditions put the children at special risk of hyponatraemia and electrolyte imbalance. The absence of surviving adults with the disease suggests premature deaths have occurred, but the high fertility rates have maintained the gene pool for this recessive disorder.
Chapter 1

Introduction

“There are two things which I am confidant I can do well:
One is the introduction to any work, stating what it is to
Contain, and how it should be executed, in the most perfect manner”

Life of Johnson vol. 1 p 292 (1755)
Samuel Johnson (1709-1784)

The Arab world, in general, and the Eastern Arabian Peninsula, in particular, is characterised by a rich history and culture and has a strong mixture of religious and traditional beliefs. While the Bedouin population of the region tends to be homogeneous, especially in the United Arab Emirates (UAE), the people of the Sultanate of Oman (Oman) tend to be more heterogeneous. They possess a rich admixture of genes from Bedouin Arabs, Persians, Turks, Asian sub-continentals, Africans and Europeans. Indeed, in one small area of the Musandam Peninsula, the local “Arabs” claim direct descent from the Portuguese who invaded the area in the 18th Century. In addition, the region has a “majority” population of “guest workers”, originating, in the main, from India and Pakistan, but has drawn people from every corner of the globe. Despite many of these people having lived in the Gulf countries for decades, they are not integrated into society and have no rights of ownership or permanent residence. Citizenship of the Gulf States is very rarely given to anyone born outside of the country and almost never to anyone who is not a Moslem. Thus the term “National” refers almost exclusively to Arabs who are Moslems born in the Arab states of Oman or the UAE. It is,
however, with the indigenous people of these two countries that our studies have been concerned with and directed at solely and not with the ex-patriate residents.

It is frequently stated that the inherited disease, cystic fibrosis (CF) is rare in those of African and Asian descent. Similarly, there has been a belief that the disease is rare or non-existent in Arab populations. Teebi has regarded this assumption as surprising as Arabs are of Caucasian descent in the main, and CF is regarded as the most common lethal genetic disease in Caucasian populations.

My interest in the existence of CF in Arab children was initiated and stimulated by my being asked to see an eight year old Emirati girl on the day I commenced duties as Professor of Paediatrics in the Tawam Hospital and the Medical School in Al Ain, UAE. The child was clearly moribund and had all the clinical stigmata of chronic lung disease and respiratory failure. She subsequently died of cor pulmonale. However, blood samples obtained prior to her death permitted laboratory studies and a molecular genetic analysis to later confirm that she had, indeed, died as a result of CF and some of its many complications and these had resulted in her premature death. Autopsy examinations are not carried out in the Arab States on the basis of religious belief, except where there may be a forensic indication. Thus a post-mortem examination was not an option open to us to assess further the cause of death and its associated pathology.

The question was then raised as to whether such events were commonplace or not in the Emirates. No record existed in this specific hospital of similar children, or more correctly, children given a similar diagnosis. Tawam Hospital acted as the unofficial tertiary referral hospital for children due to its University affiliation status. Review of the medical literature in
the UAE, revealed that there had been two case reports of children suspected clinically of having CF and who were later confirmed to have the condition by having elevated sweat chloride levels on testing by the pilocarpine iontophoresis method (sweat test)\textsuperscript{2, 3}. The children involved were all UAE nationals. The first report in 1987 described an undernourished four-year-old with diarrhoea. Investigations revealed an elevated sweat chloride, but there was no evidence of chronic lung disease. The second report, in 1991, described two children, one with meconium ileus and the second with malabsorption characterised by bulky stools and failure to thrive and frequent chest wheezes. Several important points were raised in the reports. The authors noted there was consanguinity in the marriages, Baluch descent and origins in the Northern Emirates (Ras-al-Khaimah and Sharjah). In addition, the patient with meconium ileus may be, in retrospect, the only UAE National to have been observed to have had this neonatal complication of CF.

This thesis is intended, therefore, to describe the process of establishing the fact that CF was an important disease in the Eastern Arabian Peninsula. It describes the Arab children affected, the molecular genetics of the condition and the severity of the clinical presentation. The approach I have taken is to report each step as it was achieved over a number of years. It does involve, therefore, some repetition and duplication within the text as consolidation of knowledge occurred and the increased patient pool was established. This method has the advantage of “real time” as the jigsaw was completed. To fit the pieces together, I have drawn directly from many of the original research papers and material. Again, the disadvantage of this is some repetition within the text, but it does demonstrate evolution of the research plan. To make it easier for the reader, I have placed the appropriate references at the end of each chapter.
The research plan was “dictated”, in part, by our need and ability to satisfy the research funding organisation and its grants committee that our intentions were logical, practical and were directed at the needs of the local population. Health Science research funding in the United Arab Emirates (UAE), at that time had a directive to be relevant to the needs of the national Arab population and have an immediate practical application to the UAE society.

The establishment of a hospital based clinical service for CF, permitted the acquisition of information about the probable patients and permitted the subsequent practical application of any knowledge gained from the research. Thus, rapid diagnosis and management of the disease could take place. No such facility existed in the UAE at that time and any patients with chronic respiratory disease were sent overseas if the family could secure funding.

This thesis thus covers the investigations that have led to the identification of CF in eastern part of Arabia. It provides information that is leading to the establishment of the molecular genetic basis of the disease in Arabs. While the UAE and Oman are two of the most rapidly developing countries in the world, the background of genetic disease in the area has not been investigated. It is strongly influenced by history and the cultural habit of consanguineous marriage. Despite its economic strength, the UAE has yet to achieve excellence in medical care, while Oman with lesser revenue is leading the region by its progressive and enlightened approach.

I report herewith the unravelling of some of the background to CF in the region, which has been achieved over several years and despite the above remarks has been funded by the UAE government through the University system.
References

Chapter 2

The Clinical Disease

“For death and life, in ceaseless strife,
Beat wild on this world’s shore
And all our calm is in that balm
Not lost but gone before”

Not Lost But Gone Before
Caroline Norton (1808-1877)

CF results in progressive lung disease, pancreatic dysfunction and elevated sweat electrolytes and in males, infertility. There are wide variations, however, in the clinical expression and the mode of presentation of the disorder. These variations are dependent on the time at which clinical signs are manifest and this time can vary from birth to middle adult life.

The purpose of this chapter is to explore what is relevant regarding the clinical presentation of CF to patients emanating from the Arabian Peninsula. It is not intended to reiterate the vast literature on CF and all the details of its clinical manifestation and presentation. There are numerous good reviews and texts that have performed this function\(^1\)-\(^3\). These are invariably orientated from a Western viewpoint or more specifically to patients of European descent.

Life expectancy of CF patients in the Western World has increased considerably in recent years and the current predicted median life expectancy in the United Kingdom is forty years\(^4\). This is attributed to the advent of the newer therapies and regular contact with CF centres\(^5\). There is evidence also, that early diagnosis and the introduction of newborn screening is an important additional factor\(^6\), \(^7\). There is certainly no evidence available regarding an
improvement in the prognosis and better outcomes for children from non-Western countries. The only previous attempt in an Arab country to screen a population and to establish a frequency of the disease was in Jordan. Nazer used the method of the detection of albumin in meconium as his screening test. He did identify three children by this method but did acknowledge the severe limitations of the technique. Importantly he did stress that atypical presentations were likely in his population\(^8\). Until the last decade, there have been no attempts to define the severity of the disease in Arabia, its clinical presentation and prognosis.

Wallis\(^9\) has observed that over the last 30 years suggestive clinical symptoms or a family history and a positive sweat test have been used for confirmation of the clinician’s suspicions of CF. The disease presentation was characterised by the clinical features of chronic obstructive pulmonary disease, pancreatic enzyme deficiency and small intestinal obstruction. The diagnosis was then confirmed by the classic sweat test\(^10\). It had been recognised, however, that there could be borderline sweat test results or negative sweat tests in the face of convincing clinical evidence. These concepts did cause confusion and the technique and quality of the sweat test performed on the individual patient was often questioned\(^9\).

CF was known to be an autosomal recessive disorder. It was not until the discovery of the gene for the cystic fibrosis transmembrane conductance regulator (CFTR), however, that it was demonstrated that in Western Countries approximately 70\% of CF clinical disease was due to the presence of the gene mutation referred to as ΔF508 (see later Chapter). Importantly, the presence of homozygosity for the mutation appeared to impart pancreatic insufficiency to the individual. However, there appeared to be little phenotype–genotype correlation with regard to the other clinical presentations of the disease.
Two events are of particular relevance to the clinical phenotype of CF in Arabs. First, was the recognition that while the ΔF508 mutation was the commonest mutation, there were other CFTR gene mutations? Subsequently, over 1,000 mutations and sequence changes have been described which can be associated with clinical disease\textsuperscript{11}. Indeed, the prior conundrum of negative sweat tests in CF\textsuperscript{12} is explained by the presence of differing mutations at different sites on the gene. The concept of the spectrum of clinical disease being associated with different mutations is now increasingly accepted. However, phenotype-genotype correlation is highly complex and there is now recognition that there are many factors involved. Three main factors are recognised which contribute to variability in the CF phenotype. These include the specific pair of CFTR gene mutations, variations in the remainder of the genome and the possibility that other genes may compensate for the chloride ion channel dysfunction\textsuperscript{13}. Finally, environmental and therapeutic intervention may be relevant. Reported later are our findings in relationship to rare mutations of the CF gene. Further, the finding that one newly described additional gene mutation can influence the clinical severity of another pair of mutations is presented later.

Pancreatic insufficiency is present in about 90% of patients with CF. It is associated with several mutations within three of the five classes of mutations (see later Chapter). Examples of these mutations include ΔF508, G542X and R334W. Congenital bilateral absence of the vas deferens (CBAVD) in CF compounds the confusion. This condition leads to infertility in about 99% of male patients and is almost universal in classical CF. In some individuals it can also be the presenting symptom of milder and rarer mutational variants. They may form the very mild end of the clinical spectrum of the disease. It appears that in the embryonic development of the vas deferens there is a requirement for a higher level of CFTR activity, greater than 10\%\textsuperscript{14}. Thus some of the "minor" mutations may reduce the CFTR activity to less
than this amount but may allow sufficient activity to result in pancreatic sufficiency (see Figure 11, Chapter 3). The sweat chloride levels, while helpful in diagnosis, appear to be unhelpful in terms of quantifying the severity of the disease and the higher levels do not necessarily equate with severe clinical disease\textsuperscript{15}.

The increasing knowledge of the molecular basis of CF has, in fact, confused the concept of a “clinical disease” rather than clarifying the issue. The dilemma is as to what actually constitutes clinical disease. Wallis\textsuperscript{9} has outlined this in a diagrammatic form (Figure 8) and has asked the question as to “what constitutes a CF diagnosis?” One extreme is a healthy male with CBAVD and two CF mutations as compared to the girl with nasal polyps and a normal sweat test, recurrent chest infections and the detection of one CF mutation. Which has CF? Wallis\textsuperscript{9} stresses the consequences of such a diagnosis on educational opportunities, insurance status and the workplace. He cites the dilemma of homogeneity for the mutation 3849+10kbC→T. It is associated with severe bronchiectasis, but with a normal sweat chloride level and male infertility.

Kerem and Kerem\textsuperscript{16} have classified phenotypes into (a) severe (b) mild (c) extremely variable. The severe phenotype is characterised by early onset disease, high sweat chloride levels and pancreatic insufficiency. While ΔF508 mutations are associated with severe disease presentation, it is being recognised increasingly that other mutations are associated with severe disease. Kerem and Kerem\textsuperscript{16} have listed the characteristics of CF patients having mild and severe phenotypes (Table 1). They have gone further and classified mutations according to the severity of the phenotype (Table 2). The group described as having variable phenotypes poses difficulty. Pulmonary involvement varies considerably in those carrying the same mutations. Several mutations are associated with such variability. The G85E mutation is one
such example. Many of the clinical measures seem to vary in different individuals such as the
time of onset, age at diagnosis, respiratory involvement, sweat electrolyte levels and
pancreatic sufficiency or insufficiency. Similarly, the severity of lung disease varies
considerably, but to add to the confusion, the disease manifestations differ between siblings.
Some may develop liver disease while others do not.

To provide a sense of balance, however, the possession of a positive sweat test and two CF
mutations and clinical disease does provide evidence of CF. However, those with an unusual
phenotype and borderline sweat electrolyte levels do require genotyping for the less common
mutations. This should be followed by a thorough evaluation of the respiratory tract e.g.
microbiology of the sputum, radiological imaging, evaluation of pancreatic function and
urogenital assessment e.g. by ultrasound. Thus, “classical” disease is characterised by chronic
obstructive pulmonary disease, pancreatic enzyme deficiency, small intestine obstruction and
male infertility. Untreated disease results in death from pulmonary infection.

The second important event in relation to CF in the Arab world was the steady recognition
that delays in diagnosis of CF were occurring in children of ethnic minorities and those of
mixed origins living in the UK. It was being recognised further that the disease was present
in those from cultures previously considered free of the disorder. Teebi and Farag in their
monograph on the Genetic Disorders among Arab Populations quote the old belief that CF is
rare or non-existent in Arabs. They comment that this view is surprising because of the
Caucasian descent of many of them. Thus, there has been a steady increase in the
documentation of CF patients of African, Japanese and Chinese descent. Spencer reported
patients of Indian, Pakistani and Bangladeshi origins. Studies from the United States have
given an estimated incidence in Native Americans as 1:80,000, while in the Black population
It became apparent that as new and different genotypes were becoming recognised that there
was often a relationship between the phenotype and the geographical area as well as historical
events. Examples of such occurrences are the association of the G551D mutation and its
distribution in areas of Europe, which correspond to the past populations of Celtic descent.
Similarly the R553X mutation reflects those of Germanic origin, while 1717,1G→A is found
in Switzerland and Northern Italy.

the figure has been put at 1:17,000.
Figure 8

Spectrum of Phenotype manifestations
### Table 1

**Clinical characteristics of CF patients having mild and severe phenotypes**

<table>
<thead>
<tr>
<th></th>
<th>Severe phenotype</th>
<th>Milder phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type of mutation</strong></td>
<td>Two severe mutations</td>
<td>At least one mild mutation</td>
</tr>
<tr>
<td><strong>Age of diagnosis</strong></td>
<td>Early (usually &lt; 1 year)</td>
<td>Late (usually &gt; 10 years)</td>
</tr>
<tr>
<td><strong>Pancreatic function</strong></td>
<td>Pancreatic insufficient (&gt;95%)</td>
<td>Pancreatic sufficient (70-80%)</td>
</tr>
<tr>
<td></td>
<td>Poor</td>
<td>Good</td>
</tr>
<tr>
<td><strong>Nutritional status</strong></td>
<td>High incidence</td>
<td>Not present</td>
</tr>
<tr>
<td><strong>Meconium ileus</strong></td>
<td>High (&gt; 80mmol/L)</td>
<td>Usually 40-80 mmol/L</td>
</tr>
<tr>
<td><strong>Sweat chloride levels</strong></td>
<td>Variable</td>
<td>Variable</td>
</tr>
<tr>
<td><strong>Pulmonary function</strong></td>
<td>None</td>
<td>Possible</td>
</tr>
<tr>
<td><strong>Male fertility</strong></td>
<td>None</td>
<td>Possible</td>
</tr>
</tbody>
</table>

### Table 2

**Classification of mutations according to phenotype severity**

<table>
<thead>
<tr>
<th>Severe</th>
<th>Milder</th>
<th>Substantial variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1078delT</td>
<td>R117H</td>
<td>G85E</td>
</tr>
<tr>
<td>ΔF508</td>
<td>A455E</td>
<td>R334W</td>
</tr>
<tr>
<td>1717-1G→A</td>
<td>3849+10kbC→T</td>
<td>5T</td>
</tr>
<tr>
<td>G542X</td>
<td>R334W</td>
<td>R347P</td>
</tr>
<tr>
<td>G551D</td>
<td>R347H</td>
<td></td>
</tr>
<tr>
<td>R553X</td>
<td>R352Q</td>
<td></td>
</tr>
<tr>
<td>621+1G→A</td>
<td>2789+5G→A</td>
<td></td>
</tr>
<tr>
<td>W1282X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N1303K</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1811+1.6kbA→G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1677delTA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R347P</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

References

Chapter 3

The “New Genetics” and Cystic Fibrosis

“Pale despair and cold tranquillity
Nature’s vast frame, the web of human things,
Birth and the grave, that are not as they were.”

Alastor 1, 718
Percy Bysshe Shelley (1792-1822)

The last twenty years have seen a spectacular change in our knowledge of and ability to manipulate deoxyribonucleic acid (DNA). The first recombinant DNA molecules were generated in 1972 and twenty years later; DNA diagnosis had become the mainstay in such conditions as CF and Duchenne muscular dystrophy. While there may be up to 50,000 human genes encoded in the DNA, we are concerned here with the gene relating to CF and its gene product.

The rapid accumulation of this knowledge has been dependent upon the major progress made in techniques allowing the manipulation and study of DNA. An important early step was the improved methods of extraction of DNA from blood and tissue. The development of hybridization methods by which heat applied to double stranded DNA permitted comparison of DNA strands. The use of heat and cooling led to the development of polymerase chain reaction (PCR) techniques. The discovery that bacteria produced restriction enzymes that cleaved specific DNA sequences permitted the recognition of heritable restriction sites and the subdivision of long chains of DNA into manageable fragments. PCR technology allowed the amplification and copying of regions of the genome demarcated by primers1.
It was established in the early 1980s that the basic physiological defect in CF was a failure of regulation of chloride transport\(^1\). However, in 1989 the gene responsible was located on chromosome 7, region q21-22. The gene is approximately 250,000 base pairs in length, contains 27 exons and encodes a 1480 amino acid polypeptide, with a molecular weight of 170,000 daltons. It has been named the cystic fibrosis transmembrane conductance regulator (CFTR)\(^3\)\(^4\)\(^5\). It consists of two repeated motifs, each consisting of a domain capable of spanning the membrane six times and a domain called a nucleotide binding fold, which binds and hydrolyses ATP. It has been classified as a member of the ATP binding-cassette super family. While the evolutionary basis and interaction of CFTR with other ion channels is not fully understood, the abnormal CFTR is related to a lack of chloride recapture leading to an osmotic effect. In the pancreas the effect is obstruction due to the clogging of its ducts by abnormally viscid secretions. Thus in these tissues CFTR normally mediates chloride secretions, not reabsorption\(^6\). Study of the aminoacid sequence of the CFTR has led to a prediction of the structure of CFTR. This is outlined in Figures 9 and 10.

Gene expression is highly tissue specific. The CF gene is detectable in the epithelial lining of certain organs only. The majority of the sites are ductal – pancreatic ducts, sweat glands, male genital ducts and the proximal and distal kidney tubule. It is also found in the lung epithelium, jejunum and colon\(^7\).

The promoter region of the gene, which is normally involved with transcription, is as yet not fully understood in the CF gene. In the flanking region 5’ to this area there is a lack of a TATA box, but it does include a GC-rich region. There are several putative regulating signals including two consensus GC boxes (GGGCGG) which may be sites of regulation of phorbol esters\(^8\). Reference to this promoter region will be made in a later chapter.
Figure 9

Organisation of CFTR in relation to functional domains
Figure 10

Predicted structure of the CFTR
The aspect of particular importance in relation to this dissertation was the discovery of the array of mutations that can occur in the CF gene. At molecular level these mutations include missense mutations, premature stop codons, small insertions and deletions causing frameshifts, in-frame deletions and mutations that effect splicing. However, no large deletions have been demonstrated and early reviews stated that no mutations occur in the promoter region. Important evidence is included later to refute this statement and to demonstrate that promoter region mutations may be important in the development and manifestations of clinical disease.

The Cystic Fibrosis Genetic Analysis Consortium has been pivotal in the international collaboration that has occurred in the documentation of novel mutations of the CF gene and the population genetics of the different mutations. At the time of writing over 1,000 mutations and sequence variations have been recorded. It soon became apparent that the three base pair deletion called ΔF508 was the most common deletion. It is a result of a shortening of the CFTR by one aminoacid, phenyalanine (F), at position 508 on the protein. In a worldwide survey of 43,849 CF chromosomes it accounted for 66% of all CF mutations.

The CFTR gene (Figure 9) and the model of the protein (Figure 10) indicate that mutations occurring in certain exons are more likely to produce major biological effects. The first membrane-spanning domain (MSD1) is coded for by parts of exons 3, 4, 6a and 7. One well-recognised example of a MSD1 change results from a mutation in exon 4, due to the 621+1G→T mutation, which is relatively common. Mutations occurring in the nucleotide-binding fold (NBF1, NBF2) representing exons 9-12 and 19-22 provide the largest cluster and are responsible for 70% of disease associated mutations of the CFTR in Western Countries. The ΔF508 mutation is the common example of NBF1 mutations. Conversely, exon 13, which
codes for the regulatory domain (R), contains relatively few mutations compared to the rest of the CFTR. All are rare, however.

Mutations in the second membrane-spanning domain (MSD2) are rare except for those in exon 17b, but these do not equate to the cluster in the equivalent position in the MSD1. Finally, the NBF2 (19-22) is also a site of disease associated mutations. Those localised to exons 19 and 20 are particularly common. Of special note is a stop codon due to mutation W1282X. Similarly, N1303K on exon 21 is relevant to future clinical observation in this text.

It would appear that disease-causing alterations that are compatible with survival are situated within the two nucleotide-binding folds of the protein. This observation does indicate the functional importance of these regions of the CFTR. Interestingly, the disease related mutations are thus spread in a non-random fashion. There are twice as many in NBF1 than in NBF2 and twice as many in the first part of the protein as to the second part.

In the nomenclature of the mutations, those named by a position plus or minus a second number occur in introns and interfere with messenger RNA splicing (e.g. 3849+10kbC→T). Those ending in “X” create a premature stop codon (e.g. G542X). Those labelled “del” involve loss of a single nucleotide, causing a frameshift in the gene’s code (e.g. 2184delA).

There has been recent progress in the understanding of how CF-associated mutations cause a loss of CFTR chloride channel function. The location of the mutations in the exons of the gene and the DNA and their relation to sites on the protein has already been outlined. In Chapter 2 the degree of disease severity was discussed in relation to recognised specific mutations. Here, the effects of the mutation are discussed in terms of cellular action. There
have been five mechanisms described by which the mutations cause a loss of function\textsuperscript{10,11}.

Table 3 shows the classes, the involved domain and the resultant effect upon pancreatic
# Table 3

## Classes of CFTR Mutations that cause CF

<table>
<thead>
<tr>
<th>Class</th>
<th>Defect</th>
<th>Example</th>
<th>Domain</th>
<th>Clinical effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Protein production</td>
<td>Nonsense G542X</td>
<td>NBD1</td>
<td>PI</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Frameshift 3905 insT</td>
<td>NBD2</td>
<td>PI</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Splice 621+ G→T</td>
<td>MSD1</td>
<td>PI</td>
</tr>
<tr>
<td>II</td>
<td>Processing</td>
<td>∆F508</td>
<td>NBD1</td>
<td>PI</td>
</tr>
<tr>
<td>III</td>
<td>Regulation</td>
<td>G551D</td>
<td>NBD1</td>
<td>PI</td>
</tr>
<tr>
<td>IV</td>
<td>Conduction</td>
<td>R117H</td>
<td>MSD1</td>
<td>PS</td>
</tr>
<tr>
<td>V</td>
<td>Synthesis</td>
<td>3849 + 10kb C→T</td>
<td>Splice</td>
<td>PI/PS</td>
</tr>
</tbody>
</table>

Key: PI = Pancreatic insufficiency, PS = Pancreatic sufficiency

MSD = Membrane spanning domain, NBD = nucleotide-binding domain

function. It should be appreciated that any specific mutation may result in more than one type of dysfunction such as defective processing and defective regulation\textsuperscript{12}. Class 1 mutations are found throughout the gene and result in premature termination signals because of splice site abnormalities, frame shift due to insertions or deletions or nonsense mutations. The mutation R553X results in unstable mRNA and no detectable protein. All class 1 mutations produce little or no full-length protein; thus they should cause a loss of CFTR chloride channel function in affected epithelia.

In class II, the several mutations in this group fail to traffic the protein to the correct cellular location. These mutations include the most common mutation, ΔF508. The failure of the CFTR to mature to the fully glycosylated form results in it being degraded. Thus, the protein cannot be detected at the cell surface, or it is present in very reduced quantities in the apical membrane. The degradation process involves the CFTR being relocated (or mislocated). This process is considered to be due to the failure of the CFTR to fold correctly and thus it is prevented from taking its position in the membrane. Denning et al.\textsuperscript{12} showed that for CFTR\textsubscript{ΔF508} when incubated at 23-30ºC, some of the mutant protein escapes from the endoplasmic reticulum, is fully glycosylated in the Golgi complex and is delivered to the cell membrane. It appears to retain some function, the level of which may be one third of the wild-type of CFTR.

Class III mutations are those which result in defective regulation. Intra-cellular ATP regulates the opening of CFTR chloride channels through direct interactions with nucleotide binding domains. Many of this type of mutation occur in the nucleotide binding domains and hence it is likely that they will result in alterations in channel function. There is a wide variation, from the minimum function associated with mutation G551D to the good function associated with
mutation G1244E. CFTR also regulates phosphorylation of the regulatory domain. Welsh and Smith\textsuperscript{10} speculate that this function may be redundant and mutations in this area are not likely to produce CF.

Finally, mutations in the membrane-spanning domains are now thought to be involved with the channel pore. It appears that when the mutants are expressed, the membrane-spanning sequences are correctly processed and present in the apical membranes. The resultant regulation appears similar to that of the wild type but the current is reduced. There appears to be a gradation in effect with wild-type CFTR> R347P >R117H >R334W. The explanation of this finding may be in the time duration that the channel is open and hence the rate of ion flow\textsuperscript{10}. The classes of mutation can be represented in a diagram to show the biosynthesis and function of CFTR in an epithelial cell (figure 11).

Riordan\textsuperscript{13} has proposed that CF is a disease of the misprocessing of CFTR glycoprotein. The mutant CFTR protein fails to mature or to proceed beyond the endoplasmic reticulum within the cell. The ΔF508 variant results in local misfolding of the protein and the loss of its normal shape. This improper folding is detected within the cell and the protein produced is selected for degradation. The basis for this is a form of biosynthetic quality control. Failure of this type of control results in a disease process of its own in other biological situations. Examples of these are amyloid disease and α-1-antitrypsin deficiency where cellular function is disrupted by the accumulation of such aggregates. The fate of the mutant CFTR molecule is illustrated in figure 12. As stated before, there is experimental work indicating that temperature manipulation of cell culture systems promotes maturation of the protein and allows transportation of the variants to the cell surface. How the selection process for altered proteins occurs is unknown. However, wild-type and variant CFTR interacts with multiple molecular
chaperones on both sides of the endoplasmic reticulum (ER) membrane. The ΔF508 variant is potentially functional and treatments to provide the maturation of the misfolded protein could prove a useful tool in the management of CF\textsuperscript{13}. Any method that could prevent the initial misfolding of the protein could perform this function and serve the purpose of channel survival. Certain in vitro manoeuvres including the use of glycerol can influence protein folding, but as yet none have an in vivo potential. Another approach may be to promote the action of chaperones and hence stimulate maturation of the CFTR that way\textsuperscript{13}.

The expanding knowledge of genes, their polymorphisms and mutations has given rise to the study of their geographic distribution. This distribution provides insight into population history and the evolution of inherited diseases. When this knowledge and technique is applied to CF, like many other aspects of the disease, we end up with more questions than answers. Barbujoni\textsuperscript{13} has summarised many of the questions we need to ask in assessing CF mutations: What is the overall pattern of geographic variation? Is variation continuous in space suggesting geographic variation or isolation by distance? Are there gradients of variation? Etc. Study of the ΔF508 CF mutation indicates a frequency cline from 30% in Turkey to 90% in Denmark. Currently this finding is explained by Neolithic expansion from Southern Europe to Northwest Europe. The gene mutation may have been retained by selective advantage or by isolation or by increased diversity and population change at the point of introduction in the Levant or Middle East. Further chapters are devoted to the geography of CF (q.v.). Information that has been gathered permits a table to be constructed, which reflects world-wide frequencies of the more common CF mutations. Table 3 lists this information. It will be seen that the ΔF508 mutation represents 66% of mutations world-wide and hence is the most frequently found mutation. Table 4 indicates the specific frequencies in various ethnic groups. Most mutations are rare, however\textsuperscript{15}.
Figure 11

Biosynthesis and function of CFTR in an epithelial cell
Figure 12

The fate of the CFTR molecules synthesised on endoplasmic reticulum
Table 4

Frequency of common CFTR mutations world-wide

<table>
<thead>
<tr>
<th>Mutation</th>
<th>% Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔF508</td>
<td>66</td>
</tr>
<tr>
<td>G542X</td>
<td>2.4</td>
</tr>
<tr>
<td>G551D</td>
<td>1.8</td>
</tr>
<tr>
<td>W1282X</td>
<td>1.5</td>
</tr>
<tr>
<td>N1303K</td>
<td>1.2</td>
</tr>
<tr>
<td>R553X</td>
<td>0.9</td>
</tr>
<tr>
<td>3849+10kbC→T, 621+1G→T</td>
<td>0.6</td>
</tr>
<tr>
<td>1717-1G→T, R1162X, 1898+1G→A</td>
<td>0.4</td>
</tr>
<tr>
<td>2789+5G→A, R117H</td>
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<tr>
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<tr>
<td>A455E, R334W, s549N, G551S</td>
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</table>

After: Knowles MR, Friedman KJ, Silverman LM. 1999\textsuperscript{15}
Table 5

Frequency of the ΔF508 mutation in different ethnic groups

<table>
<thead>
<tr>
<th>Group</th>
<th>% of CF chromosomes</th>
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<tbody>
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<td>White (world-wide)</td>
<td>66</td>
</tr>
<tr>
<td>White (Northern Europe)</td>
<td>70-80</td>
</tr>
<tr>
<td>White (Southern Europe)</td>
<td>50-55</td>
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<td>Jews (Ashkenazi)</td>
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<tr>
<td>African Americans</td>
<td>48</td>
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<tr>
<td>Native Americans</td>
<td>&lt;5</td>
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</tbody>
</table>

After: Knowles MR, Friedman KJ, Silverman. 1999\textsuperscript{15}
We have now come full circle. The initial descriptions of the clinical disease can be matched with specific organ involvement and specific CF mutations. Davis et al.⁸ have proposed a specific hierarchy of organ sensitivity to deficits in functional CFTR (see figure 13). The organ that has the greatest requirement for normal CFTR is the vas deferens. It requires CFTR of levels of 10% or more of normal for proper development. The sweat duct is the next most sensitive, requiring up to 10% of normal. This is highly relevant to those presenting with CF disease and normal sweat electrolytes, as discussed in Chapter 2. Normal sweat duct function appears to require more functional CFTR than does normal pancreatic function. The position in regard to lung function is more complex. Some patients have lung disease and normal sweat electrolytes, while a significant number have lung disease and normal exocrine pancreatic function. Severe deficiency (<1%) of CFTR leads to pulmonary, pancreatic and sweat abnormalities⁸. Normal individuals will produce 95-100% full-length CFTR transcripts in place in the airway cells. Conversely, those with a severe mutation in one allele and a mild mutation such as R117H/5T in the other may end up with 0.75% of normal CFTR activity. However, here again this does not always hold true. Massie et al. have shown that compound heterozygotes may be pancreatic insufficient but in general²⁴ they are pancreatic sufficient. This mutation is rare and does not feature in the common 20 mutations as illustrated in the table. Various permutations can be found, therefore. About 8% of A455E CFTR reaches the cell surface with full activity, but a person with ΔF508/A455E will have only 4% of normal CFTR function. They will have pancreatic sufficiency, high sweat chloride levels and delayed pulmonary disease⁸.

CF is one of the most frequent autosomal recessive and lethal genes in Europeans and populations of European descent. Thus, the question is posed as to why the gene remains so frequent in these populations, as it would be assumed that those with the disease are at a
reproductive disadvantage. In terms of the ΔF508 mutation, about one individual in twenty-two (1:22) is a healthy carrier of a recessive gene for the disorder. Romeo et al.\textsuperscript{16} suggested five differing hypotheses to explain this phenomenon viz. (1) genetic heterogeneity (2) high mutation rate (3) meiotic drive (4) heterozygote advantage (5) genetic drift. The genetic heterogeneity theory, namely that two or more genes may be responsible for the same clinical phenotype, was soon dismissed. This was on the basis of the results of studies on consanguinity and linkage studies. Other work has not supported the concept that there is a preferential transmission of the CF gene. Thus the idea of meiotic advantage especially via fathers has not been sustained nor has a high mutation rate been established.

Wright and Morton\textsuperscript{17} estimated that with a lethal recessive gene like CF, the probability of reaching the incidence observed by chance, in European populations, was in the order of 0.001. However, for a frequent disease state like CF, there is no direct evidence to support the hypothesis that the gene incidence has occurred by chance. The evidence to date points to the existence of a specific heterozygote advantage associated with the CF mutation. This suggests that the heterozygotes show a slight increase in biological fitness compared to unaffected homozygotes. The best understood example of this, to date being the sickle cell trait, where relative immunity to falciparum malaria occurs in those who carry the gene and are not affected by the full sickle cell disease.

In relation to heterozygotes for CF, one suggestion was that non-carriers were three times more likely to develop asthma than carriers\textsuperscript{18} but this finding could not be substantiated in a United Kingdom study of heterozygotes\textsuperscript{19}. For a disease that has a high frequency only in the 20\textsuperscript{th} century, it seems most unlikely that this would be the basis of genetic advantage.
However, further evidence has been produced to support the asthma relationship without establishing any benefit to the individual\textsuperscript{20}.

Until recently, functional evidence supported the hypothesis that CF carriers can withstand secretory diarrhoea better than normal persons can. This has been studied using a mouse CF model, with the concept that cholera was in the past the most likely disease to produce such a situation. However, results from testing with a variety of secretagogues, including cholera toxin exposure, have been conflicting\textsuperscript{21, 22}. The concept was based upon the idea that mice lacking CFTR protein would not secrete fluid in response to the stimulus, while heterozygotes would secrete about 50% of the normal fluid and chloride ion. The reduced fluid loss would protect them from death due to the toxic effects of cholera. However, no significant difference between heterozygote and homozygote mice could be demonstrated\textsuperscript{22}. Recently, Pier et al.\textsuperscript{23} have produced evidence that in the heterozygous state CFTR mutations increase resistance to the infectious disease, typhoid fever, due to infection with the organism \textit{Salmonella typhi}. The reason for this is that the organism uses CFTR to enter into the intestinal epithelial cells. Thus in the heterozygote, with diminished levels of CFTR, there may be a decreased susceptibility to typhoid fever. Interestingly, the group demonstrated that this was organism type-specific and that \textit{Salmonella enterica} or \textit{S. typhimurium} did not enter the cells in a similar manner. The authors further support their argument by saying that despite previous statements, cholera did not enter Europe from India until 1832, so it is unlikely to have selected for mutant alleles of CFTR. Thus, resistance to typhoid fever could serve as the selective factor for heterozygote advantage conferred by the $\Delta F508$ CFTR allele\textsuperscript{23}.
Despite the vast new information revealed about the molecular basis of CF, there are many unanswered questions. These are highly relevant in finding treatments for the disorder if molecular manipulation is to become a practical treatment for the disease.
Figure 13

CFTR activity and tissue manifestations of CF
In the discussion of mutations, the following terms are defined as below.

**Missense mutations:** are single-base changes that result in substitution of one aminoacid for another in the protein product of the gene.

**Nonsense mutations:** are single-base changes that create one of three termination codons in the genetic code (UAA, UAG or UGA) resulting in a shortened, dysfunctional protein product of the gene.

**Insertions and deletions:** are the addition or the loss of one or more bases can be as much as the deletion or duplication of an entire gene. The impact of an insertion or deletion in the coding sequence of a gene produces a frameshift. Codons consist of three bases, hence adding or subtracting a base changes the coding of all the codons that follow.

Source: http://intouchlive.com/cancergenetics/mutypes.htm
References

9. Cystic Fibrosis Genetic Analysis Consortium [http://www.genet.sickkids.on.ca/cftr/rpt/Table1Full.html](http://www.genet.sickkids.on.ca/cftr/rpt/Table1Full.html)


Chapter 4

The United Arab Emirates and the Sultanate of Oman

“And the night shall be filled with music
And the cares that infest the day
Shall fold their tents like Arabs
And as silently steel away.”

The Day is Done
Henry Longfellow (1807-1882)

The people of the UAE, like those in the rest of the Eastern Arabian Peninsula, are of Arab stock. Their antecedents spread eastwards across the peninsula in successive waves of migration 2,000 – 3,000 years ago, carrying with them their language and survival skills into this new and harsh environment. On arrival at the northern seaboard they mingled with and eventually merged with the people already living in the area. These residents were, too, of Semitic stock. Inscriptions in their now extinct Semitic language can be found to this day in Sharjah (Al Dur) and in Umm al-Qaiwain. However, by the seventh century AD both populations had become a homogeneous society with a common Moslem faith.

The physical geography of the UAE is immensely varied and ranges from rugged mountains to a low mangrove-fringed coastline and from the vast arid desert of the Rub al Khali (Empty Quarter) to fertile oases packed with date palms and fruit. The country lies between latitude 22°30 north and longitude 51° and 56°30 east. To the north and north-west the country is bounded by the Arabian Gulf, the Musanden Peninsula enclave of Oman and the Gulf of Oman; to the south by Saudi Arabia and Oman; and to the west by Qatar and Saudi Arabia.
The Gulf coast consists of salt marshes (sabkha) that give way to inland to desert and gravel plain. The Hajar mountains rise to 2,134 metres and form a barrier between the east and west coasts. The continental plate upheaval has left brown jagged folds and fissures of limestone and igneous rock, which in certain places are rich in fossils, revealing the land’s earlier submarine existence. Only 5.5% of the land area is said to be cultivable and only 0.2% is actually being cultivated at the moment².

Prior to their independence and federation in December 1971, the UAE was known as the Trucial States, a loosely defined affiliation of the main seven emirates. Six of the federation’s seven states share the Arabian Gulf coast, extending east from the base of the Qatar’s peninsula for 700 kilometres to the Musanden Peninsula. These emirates from west to east are Abu Dhabi, Dubai, Sharjah, Ajman, Umm al Quaiwain and Ras al Khaimah. The seventh emirate Fujairah, lies on the Gulf of Oman coast with no direct access to the Arabian Gulf. While the current political map of the Gulf has taken shape in recent years, their origins go back to the beginning of the 18th century. The people are descendants of the maritime tribal groups from the Arabian Peninsula, as discussed earlier, whose traditions dominate the new states of the UAE, Qatar and Bahrain².

Trade and seafaring were the two main occupations of Gulf inhabitants from ancient times. The Gulf formed the link between east and west with Gulf traders carrying eastern commodities from India and China to the mouth of the Shatt al Arab and from there by caravan routes to the Mediterranean. The oldest excavated settlements of the UAE are in Al Ain at Jebel Hafit which date back to the 4th millennium BC. Excellently constructed graves have been found on Abu Dhabi Island and the contents of these graves contain pottery and copper daggers, which strongly indicate a link with the Indus valley and Baluchistan.
Similarly, the concept of the falaj system of bringing water over considerable distances is not only found in Arabia but in Iran and Baluchistan. These links with other cultures outside of the region remain important to the present population of the Emirates. They have been reinforced by tribalism, Islam and current political structures like the Gulf Co-operative Council.

The tribal structure is important and remains strong. The Bedouin tribe (al qabilah) is composed of clans (al ashirah) which in turn are divided into families (al ailah). The tribal pattern of the UAE was probably established in the 2nd century AD. Two major migrations into the area seem to have originated in South Arabia, probably in the Yemen. They first came via Oman and through the Hajar Mountains, reaching the Al Ain area around the second century AD. Other groups seem to have come via the Nejd and eastern Saudi Arabia, attracted also to the well-watered and strategic oases of Al Ain which controlled the passes to and from the coast. Later, most of the population of the emirates became settled with the true nomadic element of the population being about 10%. The tribal structure was tightly knit by ties of marriage characterised by substantial bridal dowries kept within the family. Even today consanguineous marriage accounts for over 50% of all marriages. There are four main tribal groups in the emirate of Abu Dhabi of which the Bani Yas dominates. Over many generations the Bani Yas extended its territory into the interior. However, a breakaway branch of the Bani Yas, Al Bu Falasah established itself in Dubai. Dubai presents a different picture with a larger foreign element within its population. The position of Dubai on one of the best harbours in the Gulf attracted a cosmopolitan population of Baluchis, Persians and Indians. People of the Qawasim tribe politically dominated the Northern Emirates. Their interests and power base lay in the sea. Immigration from Baluchistan and Persia also took place for the same reasons of geography and trade.
The total population of the UAE stood at 2.624 million in 1997. The annual increase has been in the order of 100,000 with 41,893 births occurring in 1997. The additional numbers being made up by those on residential permits for work and their dependants. Abu Dhabi Emirate accounts for 1.017 million and Dubai Emirate 757,000 of the population with Sharjah (439,000) having the only other significant population centre. The population pyramid indicates an excess of young adult males. This is accounted for by the importation of young men without their families on short-term work-related visas. They are in the main from Afghanistan and the Indian subcontinent. Young people below the age of 14 years account for 26.3% of the total population\(^4\). There is a normal sex distribution in this age range reflecting that, in the main, they represent UAE nationals. The critical statistic is the proportion of the total population, which is of UAE nationality. This figure is not officially available nor is it available in any publication or from the census data, although the information is collected in each census. The stated reason for this is security. The information gained from unofficial samples and observations is that the ex-patriate workforce represents about 75% of the population. There have been no major efforts to “Emiratise” the workforce in the immediate past, unlike countries such as the neighbour, Oman. The mean size of UAE families is seven children, thus in the immediate future major demographic forces will be in play. The other important factor will be economic and oil prices this determines whether the country can still afford to import labourers for unskilled jobs\(^5\).

Thus, when we are discussing the gene pool in relation to CF we have been dealing with approximately 750,000 people, of whom the overwhelming majority are of true Bedouin origin. There has been little naturalisation, as the rules require a fluent knowledge of the Arabic language, to be of the Moslem faith and not to represent a political threat.
The Omani culture has its roots in the Islamic religion. Oman developed, however, its own form of Islam which is called Ibadhism. It is named after its founder Abdullah ibn Iba who lived during the 7th century AD. Oman is a tolerant state and unlike some if its neighbours permits others to practice their religion. Like the UAE, the history of Oman dates back many thousands of years. In biblical times, the country was the hub of the frankincense trade. Its strong seafaring traditions led to the development of an empire ruled over by the Sultans of Oman that spread from Zanzibar to the tip of the Indian sub-continent. This empire reached its heyday in the late 16th century. Subsequently the country's fortunes declined, reaching a nadir in the 1950s. At this time the country was impoverished and the then Sultan Said bin Tamur adopted an isolationist policy. After further political trouble and the incursions of Marxist forces from South Yemen, Sultan Qaboos, the Sultan’s son, overthrew his father in a bloodless coup. The country was rebuilt, the rebels suppressed with the aid of British forces and the economy restructured using the countries oil revenue. Subsequently, the country has been opened up and became a member of the Gulf Cooperative Council. The current population of the country is 2.325 million.

The Sultanate occupies the south-eastern tip of the Arabian Peninsula. Oman is bounded by sea on two sides, the Gulf of Oman to the northeast and the Indian Ocean to the southeast, resulting in a coastline of 1062 miles. The total land area is approximately 309,500 square kilometres and is the third largest in the Arabian Peninsula. The land boundaries are with the Kingdom of Saudi Arabia and the Republic of Yemen to the west and the UAE to the north. The country is predominantly open desert with gravel plains and sand. There are two large mountain ranges. The Hajar range dominates the north with the highest peaks reaching 3075 metres. Dry river valleys (wadis) heavily dissect the upland region. However, these easily flood after winter storms. Between the Hajar Mountains and the sea lies a narrow fertile strip.
It is within this strip that the majority of the country’s population live. The southern province of Dhofar is also dominated by the coastal mountains which help capture moisture from the summer monsoons. A fertile strip of land lies adjacent to the region's capital, Salalah.

The climate is hot and humid at the coast in summer and inland it is hot and dry. The mountains enjoy a moderate climate throughout the year. The north has an average temperature in the summer of 32-48°C. In the south, the temperature, all year round, ranges between 30-35°C.

In relation to specific health matters, at the time of the accession of Sultan Qaboos, the country was in a sad state. There was one 23 bedded Mission Hospital and only one Omani trained doctor. Over the next twenty-five years, 51 hospitals had been established and over 115 health centres. Two tertiary level hospitals had been established in Muscat and the health system has expanded has become increasingly more sophisticated. There is an increasing proportion of the health workforce of Omani nationality. This has been achieved with the continued rapid growth and success of the health system in Oman. To educate the indigenous population and to be self-sufficient in professional staff a medical school had been established in Muscat at the Sultan Qaboos University. Currently, about 80 new doctors graduate annually. Major inroads have been made into the health problems of the country. Mass immunisation schemes have been successful with a 95% coverage and a tuberculosis control programme is in place. The health services have improved to the extent that acute infection and malnutrition have been overcome and are no longer a major problem, such that the presence and management of inherited disease becomes a worthwhile future goal in Oman.
References

1. [Http://emirates.org/traditions/tribes.htm](Http://emirates.org/traditions/tribes.htm)
8. [Http://ww.inforamp.net/-emous/oman/health2.htm](Http://ww.inforamp.net/-emous/oman/health2.htm)
Figure 14

Near Nizwa, Oman
Chapter 5

Cystic fibrosis in the Middle East: the Historical Perspective

“Lives of great men remind us
We can make our lives sublime
And, departing leave behind us
Footprints in the sands of time”

A Psalm of Life
Henry Wadsworth Longfellow (1807-1822)

Twenty years after the definition of cystic fibrosis (CF) as a specific disease, the first report of an affected Arab child was made in 1958\(^1\). This report came from the Lebanon and, four years later, a further three Arab children were described from the same centre in a paper that included an up-date on the condition of the original patient\(^2\). Pedigree analysis, in one child at least, indicated that the family has been of pure Arabic origin for the previous four generations.

The recent discovery of over 1000 mutations of the cystic fibrosis transmembrane regulator gene (CFTR) has cast new light on the patterns of recognition of CF in the Middle East. I have reviewed, but not cited all, the indexed medical literature and some non-indexed papers to gain an historical perspective and insight into the recognition and definition of this important disease as it affects Arab communities.

Following the initial report, there was a dearth of information and reports for the next twenty years until sporadic reports began to appear from Iraq, Israel and Kuwait\(^3\)-\(^5\). In 1981, Al
Uwihare from Kuwait commented that, in two patients, the condition was not suspected before death and the disease was not considered to occur in the Middle East\textsuperscript{5}. This prompted a brisk response from Katzenelson\textsuperscript{6}, suggesting that CF was not rare among the Jewish population of Israel and that it was very common among the Arabs of Israel. His contention about the Arab population has not been sustained in the subsequent literature. While the original report of CF in an Arab child came from the Lebanon, the most recent report from that country has thrown considerable light on the situation there. Lebanon, being the crossroads of the Middle East, has experienced an influx of people from many different areas. Desgeorges et al\textsuperscript{7} have identified twenty families living in Lebanon for several generations, who have had at least one child with CF. They reported on the religious and community backgrounds of the affected families (Maronite, Greek Catholic, Shiite and Sunnite) and showed that ten different DNA alterations - including two novel mutations - accounted for 88% of the CF alleles there. Delta F508 was found in 37.5% of alleles. Four mutations remained unidentified.

CF was first reported in Jordan in 1984\textsuperscript{8}, when 12 patients were reviewed retrospectively, some having been diagnosed only at post-mortem. Nazer\textsuperscript{9} in 1985 felt the prevalence was underestimated due to lack of awareness, consanguineous marriage and poor diagnostic facilities. He attempted to define the prevalence rate by studying 7,682 neonates from ten different hospitals by the meconium albumin method. Three infants were confirmed positive, by sweat test analysis, which resulted in an incidence of 1 to 2,560 live births.

Following the initial observations in Kuwait that CF existed in that country\textsuperscript{5}, Kollberg reported that 17 patients of assorted nationalities had been seen in Kuwait in a fifteen-year period\textsuperscript{11}. Eight were Kuwaiti and three were from other Arab countries. Kollberg made
similar comments to those of Nazer from Jordan, in that the diagnostic facilities for CF were not easily available, the diagnosis was not often considered because of a low frequency of the condition and that the clinical presentation may be atypical. Despite this, he commented that with a birth rate of 50000 infants per year (population 1.3 million) and based upon the number of infants with meconium ileus, the incidence in Kuwait should be around 1 to 3500 births per annum. Three siblings with CF were reported from Kuwait in 1987\textsuperscript{12}. While being Arab, however, the patients were the children of a Jordanian family. The diagnosis was confirmed by very high levels of sweat chloride, with results that were well over 100 mmol/L. Another Jordanian child living in Kuwait presented with metabolic alkalosis and electrolyte disturbance and was reported by Issa et al\textsuperscript{13}. The authors further supported the contention that the classical disease presentation may be different in very warm climates where an unexplained metabolic alkalosis and prolonged neonatal jaundice should raise the suspicion of CF. Finally, three further children with an atypical presentation and metabolic alkalosis were reported from Kuwait\textsuperscript{14}. Only one child was a Kuwaiti National and the others were Palestinian Arabs. The high summer temperatures, (around 45\textdegree C) with high humidity and insufficient salt intake were felt to be the key factors in the summer presentation of these children.

In the first report from Bahrain, Khan and Mohammad\textsuperscript{15} described, in 1985, eight children with proven CF from Bahrain. They suggested that it was the largest number, to date, of CF patients reported from an Arabian Gulf state. Five of the children were products of consanguineous marriages, there being two brother and sister pairs, and two of the families were closely related. It is not specifically stated that the patients were Bahraini nationals, however. The most recent documentation of CF from Bahrain was in 1998 when 25 patients with proven CF were described\textsuperscript{16}. The authors attempted to define the incidence, phenotype
and outcome of the disorder in Bahrain. The reported patients were drawn from an 18-year period and the authors concluded that the incidence is 1 in 5,800 and the prevalence is 3 in 100,000. In 80% of cases, the patients had been products of consanguineous marriages - in contrast to the normal consanguinity rate of 39% in Bahrain. They reported further that the spectrum of clinical presentation of the disease is severe in that country. Meconium ileus was present in about 20% of these patients. It was noted, however, that there has been a steady fall in the age-related mortality from CF there, which was attributed to improved living standards and medical care in Bahrain. Increased awareness among medical professionals about the disease and its management was advocated as an additional beneficial factor.

Saudi Arabia, a large and populous Arab state, has produced, as one would expect, more literature on CF in Arabs. Surprisingly, the first identification did not occur until 1985, when a seven month old Saudi child was shown to present with the classical features of recurrent respiratory infection, diarrhoea and failure to thrive\textsuperscript{17}. It was noted that the child came from the ‘Unooz’ tribe, who are located in northern Saudi Arabia (near to the border of Jordan and Iraq), an area already established as one in which CF was known to exist with some frequency. Nazer and colleagues\textsuperscript{18} in 1989 commented that the CF gene was believed to be rare, or non-existent, in Saudi Arabia. They, however, were able to find thirteen Saudi children who had elevated sweat chloride levels and typical clinical histories suggesting CF. The principal author, Professor Nazer, had previously described CF in Jordan\textsuperscript{9}. He speculated that the majority of patients with CF in Saudi Arabia are undiagnosed and die in infancy or early childhood. To support this argument he showed that in the seven families reported by his group, four siblings had died of CF-like symptoms within their first year of life. Their contentions are well supported by the subsequent flow of information about CF from Saudi Arabia. Three further patients from the Eastern Province were presented with intestinal
obstruction, intussusception and meconium ileus, secondary to CF. Again, a further ten children from the Eastern Province were documented as having CF in 1991 and Mathew et al suggested an incidence of 1 in 4,243 for Saudi children. Again, as previously described in Kuwait, vomiting and metabolic alkalosis associated with the high ambient temperatures in the summer months were associated with the presentation in these patients. A review by Nazer and Rahbeeni of their experiences of CF included 36 patients seen by them over the period 1986 to 1992. Emphasis was placed on the hepatic presentation of the disease and on the fact that CF was relatively common there and associated with serious sequelae. Hepatomegaly, jaundice and possible glycogen storage disease were the main referral diagnoses. It was postulated that the CF mutations, which give rise to hepatic disease manifestations, might be different to others that have previously been described. A clinical description of a further ten Saudi children was published in 1995. It was suggested that while the common presentations of the disease occurred in Saudi, rarer forms had to be considered. Eight of the children had the complex of metabolic alkalosis and hyponatraemia (Pseudo-Bartter Syndrome), while vitamin E deficiency or gallstones or nasal polyps were present among the others. In keeping with the changing knowledge of CF brought about by molecular genetics research, the two most recent publications from Saudi Arabia concentrated attention upon the CFTR mutations, which may occur in Saudi children. Over 85 children have been identified and attempts have been made to determine the responsible mutations. In one group of 15 patients, six different mutations were identified, of which two were novel. In the larger series of 70 patients, mutations were identified in 42 patients. Six novel and six known mutations were reported. The difference between these two studies is in that the study of El Harith et al was drawn from the population of the Eastern Region of the country. Banjar and Mogarri, who provided the largest series of Arab CF patients, published
the most recent clinical overview of Saudi CF patients. An important finding was that median survival was 10.9 years compared to 32 years in North America\textsuperscript{24}.

The pattern of sporadic case reports followed by more detailed studies also applies to CF in the United Arab Emirates (UAE). The first patient described, in 1987, was a UAE citizen of Baluch descent, who presented with diarrhoea, cough and repeat respiratory illnesses\textsuperscript{26}. The second account, again from a Northern Emirate, involved a newborn with meconium ileus and a five-year-old child who developed an intussusception. The latter patient, however, had no confirmatory tests performed and the diagnosis was based on clinical suspicion of CF\textsuperscript{27}. Subsequently, further clinical reports and molecular studies occurred in the Emirates\textsuperscript{28, 29, 30}. Later, the common CF mutations in the UAE were identified\textsuperscript{31}. It was reported that two mutations, $\Delta F508$ and S549R (T$\rightarrow$G) account for 46 out of 52 (88\%) CF alleles and characterise 95\% (18 out of 19) of the affected families that were investigated. Furthermore, these two mutations are segregated in the UAE population according to ethnic background: 16 CF patients (who were of true Bedouin origin) were S549R (T$\rightarrow$G) homozygotes and 7 patients (who were of Baluch lineage) were homozygous for $\Delta F508$\textsuperscript{31}.

Review of the history of CF in the Middle East provides recognition and development patterns. The general recognition of the disease in the area came some 40 years after the original clinical definition in Switzerland and the pathological definition in the United States. Many of the authors allude to the general lack of awareness of the disease in the Middle East and to the lack of diagnostic facilities, such as sweat chloride determinations. Despite this, pioneers such as Nazer in Jordan\textsuperscript{9} and subsequently in Saudi Arabia\textsuperscript{18}, were able to screen some populations and provide some information about CF incidence and prevalence. With the burgeoning new knowledge of the CF gene and its mutations from the late 1980s, together
with the mounting prosperity in the Gulf Region and the upgrading of medical facilities, a new era has dawned. It became clear that the classical presentation of the common mutation ΔF508 did not characterise all mutations and that, in most of the region, other presentations occur. We have noted the predominance of hepatic disease in Saudi, electrolyte disturbances from Kuwait and the rarity of meconium ileus associated with CF in countries such as the UAE. The ever-increasing number of mutations being recognised from Saudi Arabia and the Lebanon (many of these being novel mutations) reflects on the characteristics of these societies. The Lebanese findings reflect the coexistence of numerous communities, who tend to have their own range of mutations, showing little mixing between the groups (Table 6). Consanguinity in all of these Arab countries (consanguinity was found in 80% of families with CF members in Bahrain) must be a major factor in perpetuating many of the rare mutations, as clearly illustrated with the example of mutation S549R (T→G) in the UAE. The ethno-history of CF is just becoming unravelled and the tracing of these various mutations in diverse ethnic groups gives a fascinating insight into the patterns of population migrations throughout history.
Table 6

Important milestones in clinical and mutational findings in CF in the Middle East

<table>
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<tr>
<th>COUNTRY</th>
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References


Chapter 6

The Geographic Distribution of Cystic Fibrosis Mutations Gives Clues about Population Origins

“The dust of creeds outworn”
Prometheus Unbound, 1,697

Percy Bysshe Shelly (1792-1822)

The founding of the CF Genetic Analysis Consortium in 1989 has fostered extraordinary advances in the molecular genetics of CF. At this time, the Consortium has compiled over 1000 DNA variations (mutations and other sequence alterations) scattered throughout the CFTR gene and responsible for the various forms of CF\(^1\). The most common alteration that leads to abnormal CFTR is that resulting in the loss of a phenylalanine at position 508 of the CFTR protein; hence its designation as ΔF508\(^2\). ΔF508 frequencies vary from 87% in Northern Europe to 28% in Asia (Middle East), while its combined world-wide frequency is 66%\(^1\).

Other mutations are rarer but their frequencies are quite variable amongst different ethnic and geographically located populations\(^1\). In some populations, a few mutations only (three to six) account for 60-90% of all CF chromosomes; many more rare mutations, however, have to be detected to characterise all CF chromosomes.

This information is presented to support the hypothesis that this knowledge is important and has direct applications include genetic counselling, prenatal diagnosis, and genotype-to-
phenotype correlations allowing one to evaluate an individual’s prognosis and give appropriate patient management. Information gained nationally, regionally and world-wide has also permitted geographic (synthetic) maps to be produced for many countries. Such maps are useful in shedding light and making inferences about natural selection gradients, local gene flow, recent and historical population migration patterns, and about the characteristics of human populations and ethnicities.

I present here population genetics data that has been gathered about the ΔF508 mutation and information with regards to two other common mutations, namely G542X and N1303K. Furthermore, mutation -3120+1GA casts new light on populations of African descent and allows us to probe whether there is a single origin for a commonly shared mutation.

ΔF508

Morral et al\textsuperscript{3} studied microsatellite haplotypes for ΔF508 and normal chromosomes and demonstrated that the ΔF508 mutation occurred at least 52,000 years ago. This event took place in an ancestral population that expanded and spread the ΔF508 mutation. Other genetic markers suggest that the mutation was present in the humans who entered Europe commencing some 40,000 years ago bringing with them the culture and life of the Upper Palaeolithic period\textsuperscript{4}. Following the initial introduction, further expansions occurred at differing periods and it is thought that the spread into Europe was in a wave pattern. Evidence for this is based upon the differing geographical frequencies of different haplotypes associated with ΔF508. The theory of an advance through Europe in a wave formation has been promoted and supported as a concept by the finding that there has been genetic
replacement in the area of Central Europe, at a later period, by relatively recent immigrants of a different genetic background³.

There is throughout Europe a south-east to north-west gradient of frequency for the ΔF508 in keeping with the concept of human spread across Europe starting from the Middle East. Further work was carried out to establish a more detailed map of the mutation and its frequencies in Europe⁵.⁶. Once again the gradient was confirmed and reinforced by data from several towns or regions within each country. Denmark was confirmed as having a frequency of the mutation of 87%, while in the Faeroe Islands ΔF508 accounts for 100% of CF mutations. Istanbul, Turkey had the lowest frequency at 27%. This distribution was based on the analysis of 17,886 CF chromosomes.

The concept proposed is that the lower frequencies of mutation observed in the southern European populations are the result of a greater genetic heterogeneity in the southerners. In addition, other markers on the chromosomes suggest that the ΔF508 mutation was introduced more recently into northern Europe than into the southern part of the continent. Thus, as stated earlier, the spread throughout Europe was a result of migration of early farmers during the Neolithic Period (i.e. Indo-Europeans). However, a puzzling aspect to this is that there is a very high frequency of ΔF508 mutations within the Basque Region between France and Spain, where frequency rates are as high as 87%. The Basque people are one of the oldest populations in Europe and it is suggested that they are derived from people who became established in the late Palaeolithic period. Their language (Euskera) is a pre Indo-European language that has survived despite successive waves of colonisation of neighbouring areas. This hypothesis was tested by Casals et al⁷ who studied intragenic markers which indicated that, indeed, the ΔF508 mutation was not spread by Indo-Europeans to the Basque Region,
but was already present in Europe during the Palaeolithic Period and prior to the arrival of the Indo-Europeans. Thus the Basque population probably represents the oldest settlement in Europe carrying the ∆F508 mutation and probably comes from a different population root. When further expansion and migration of people into Europe occurred there was little introduction of other CF mutations into the community and hence there occurred an increased frequency of ∆F508 within the Basques compared to other nearby communities. An alternative hypothesis is that the Basque population acquired the mutation from the Indo-European migrants and the high gene frequency was due to genetic drift and selective homozygote advantage in this close and formerly isolated community. However, Casals et al findings favour the fact that the mutation was already in Europe in the Palaeolithic population of which the Basques are the most homogeneous relic population. Further, the subsequent Neolithic migrations diluted the frequency of ∆F508 mutation in some populations by bringing other mutations into Europe. In the Basques this probably did not occur due to factors such as isolation, linguistic cohesion, climate and in that their territory was not deemed attractive for the agricultural aspirations of the new migrants.

Further evidence to support the ancestral population concept comes from the studies of de Braekeleer et al who studied the Celtic population of Brittany. The population in Brittany has one of the highest CF rates (1 in 1600 live births) and is characterised by low immigration, high consanguinity and cultural and linguistic isolation. The original Celtic people settled in Brittany in the 4th century having sailed from Ireland. The study of microsatellite haplotypes in this population would be expected to be similar to that of Ireland, but different to that of Spain and Italy. However, the findings of de Braekeleer et al showed that the three most frequent haplotypes of ∆F508 chromosomes are the same as those found in
Ireland, Spain and Italy. This then provides further evidence that these haplotypes were associated with an ancestral population from which all four populations are descended.

**G542X**

G542X, a nonsense mutation, is the second most common mutation after ΔF508. It results in failure of CFTR protein production. Evidence suggests that it arose as a result of a single mutational event. The mutation accounts for 2.4% of CF mutations worldwide, but as with other mutations, its frequency varies geographically. Kerem et al described the G542X in their major paper on the identification of the CF gene. Lucotte and Hazout have described the distribution of this mutation throughout Europe. The highest frequency was found in Spain (8.8%) and Italy (10.9%) with a high percentage of all CF mutations being found in Macedonia and the Slovak Republic. Loirat et al have developed further the frequency distribution pattern of the mutation showing it to be lower in north-eastern Europe compared with south-western Europe and very high in Turkey, the Canary Islands and with the highest frequency of all in Tunisia. They have produced a fascinating hypothesis that the areas with an elevated frequency of the G542X mutation correspond to ancient sites of occupation by the occidental Phoenicians. They postulate that the mutation was introduced into Spain by Phoenicians (from Carthage), hence the relatively high frequencies observed in Tunisia (Carthage), the Canary Islands, Sardinia and Sicily. The initial intrusion by the Phoenicians occurred between 2,500 and 3,000 years ago. Thus the evidence to date suggests that the G542X mutation may provide another link in the story of the spread of the CF gene mutations and in the definition of their geography.

**N1303K**
This is a missense mutation, which results in the CFTR being prevented from reaching the epithelial membrane. The frequency of the N1303K allele varies significantly between countries and ethnic groups. It has a higher relative frequency in the Mediterranean region and in the north of Africa and the south of Spain suggesting that it was introduced into Europe through the Iberian Peninsula. Microsatellite markers indicate that the mutation is about 35,000 years old (similar to G542X) and again diffusion through Europe from an Asian origin is suggested by these recent findings. Further, it is one of the six frequent mutations found among Ashkenazi Jews and results in a severe form of CF. Severe disease is associated with the mutation and in particular, pancreatic disease. No correlation could be found between the mutation in heterozygous or homozygous states and the severity of the lung disease.

“Out of Africa” - 3120+1G→A Mutation

Cystic fibrosis has been regarded as rare in the black population of Africa. CF mutation studies were carried out on three patients in South Africa in 1996. These studies revealed that one patient was homozygous for the 3120+1G→A mutation and the other two were compound heterozygotes for 3120+1G→A/G1249E and 3120+1G→A/3196 del 54. The mutation 3120+1G→A was first described in three African-American CF patients and subsequently found in 12% of African-American CF chromosomes, but if mutations found in white populations were excluded the figure rose to 53%. The mutation was found in the father of one patient who originated from Cameroon and proved to be a carrier for 3120+1G→A. Again, this mutation was found to be the predominant CF mutation in the Eastern Oasis population of Saudi Arabia and in addition three Greek CF families have been found to have the mutation. Dork et al have attempted to find out if the CF mutation in these three diverse populations has a common origin. They analysed DNA samples from African-Americans, Greeks and native Africans (South Africa and Cameroon). All three
groups carried $3120+1G \rightarrow A$ mutations as confirmed by sequencing. Three highly informative CFTR microsatellites in intron 8 and 17b were examined. The analysis indicated that the mutation was most likely to have been derived from a common ancestor. In the case of African-Americans, this is not surprising as the group originated from West Africa between the 16th and 19th centuries. The Saudi patients are less easy to explain, as the authors state that the families were not anthropomorphologically of African descent\textsuperscript{19}. However, a continuous gene flow between Arabia and Africa has been present for many centuries in association with trade and the spread of the Islamic religion.

The findings in the Greeks also were difficult to explain, as they are the only Caucasian population who have had the $3120+1G \rightarrow A$ mutation identified. There are, however, rare mutations shared between Saudi Arabs and Greeks such as a polyadenylation-signal mutation in the $\alpha$-globin gene in thalassaemia patients. It is postulated that historic contacts between the Greeks and Saudis such as that of Alexander the Great or the ancient Minoan civilisation may be the source of contact, which linked these populations with the ancient CF mutation of Africa\textsuperscript{15}.

**Conclusion**

The commonest CF mutations in Europe and one rare African mutation arose as the result of initial founder effects that seem to have occurred between 30-50,000 years ago. The current spatial distribution of these mutations appears to reflect some of the history of population migrations. The survival and spread of these mutations adds weight to the “gene advantage” hypothesis, whereby CF mutations may have conferred selective advantages be they against cancer, typhoid or against any other diseases that yet remain to be identified.
References

11. Lucotte G, Hazout S. Geographic and ethnic distributions of the more frequent cystic fibrosis mutations in Europe show that a founder effect is apparent for several mutant alleles. *Human Biology* 1995; 67: 561-76.
Chapter 7

Cystic Fibrosis in the United Arab Emirates: Initial Clinical Observations

“When sorrow come, they come not single spies
But in battalions”

Hamlet IV v.78
William Shakespeare (1564-1616)

The paucity of information on CF in the UAE must be viewed in the light of the health services development in the country during the last century. Indeed, at the beginning of the 20th Century, the then Trucial Coast/Trucial States or Trucial Oman as the country was variously known, was dependent on locally practised folk medicine. Early in the 20th Century visits were made by missionaries who provided the initial contact with Western medicine. The lack of organised medical care in the region has been attributed to the hesitant or suspicious attitudes of the people, the erratic nature of contact and the probable disinterest and neglect by the British Government. In due course the British agent in Bahrain was able to provide some medical services for the people of the Gulf Emirates. An outbreak of smallpox in the 1930s, followed by a cholera epidemic encouraged some vaccination against smallpox, the cost of which was underwritten by the British Government. It was, however, the impact of the smallpox deaths (about 600) which finally was the incentive for the British authorities to introduce some form of medical services and the development of health awareness among the local inhabitants. Since independence there have been great efforts made to establish modern and sophisticated health facilities, with modern hospitals and the latest technical advances. In
1993 the culmination of these efforts saw the first graduates from the UAE University Medical School take their place in the medical workforce.

Section I
As outlined in the Introduction, the identification of an eight-year old Emirati girl with CF prompted the eventual establishment a tertiary level consulting clinic for children with chronic respiratory disease in 1993. This was run as part of my role as Professor and Chairman of the Paediatric Department of the UAE Medical School, and the clinic was based at Tawam Hospital, Al Ain, a University associated hospital. It was open, however, for referral of national children from any of the seven Emirates. It formed part of the Medical Services offered by the staff of the Medical School to the whole UAE. All practising Paediatricians in the country had been informed of this service.

The steady referral of children with chronic respiratory disease permitted the study of the causes of such disease. Early recognition that bronchiectasis in children was the commonest cause of referral took place\textsuperscript{2,3}. It was clear, however, that several children who were Bedouin Arabs had the features of cystic fibrosis. The disease had never been recognised to occur in those of Bedouin descent before in the UAE. The clinical details of the first eight Bedouin children diagnosed as having CF are presented below.

**Patients and Methods**

Eight children who had been referred to the Respiratory Clinic at Tawam Hospital, Al Ain, UAE with a history of recurrent respiratory illness and or failure to thrive and have been fully assessed and investigated are described. Data regarding birth history, demographic information, prior illnesses, ethnic and tribal background and recent clinical features were obtained for each patient from the parents and the notes of the referring doctors.
The following investigations were carried out: sweat chloride electrolyte levels, serum electrolytes, stool chymotryptic activity, full blood count, vitamin E, A and D measurements and venous blood for DNA analysis. The sweat electrolyte analysis was measured in each patient by the pilocarpine iontophoresis method. Radiological assessment was by chest film and subsequent computed tomography as indicated by the initial chest films. Respiratory function tests were carried out on the one patient who was old enough to perform the tests. The normal clinic weight and height measures and sputum or cough swab collections were performed. Based upon the above findings and a full clinical examination, an assessment was made of the degree of pulmonary or intestinal disease present in each patient.

Results

A summary of the main details of the patients is given in Table 7 and an overall clinical assessment. All the children had strikingly elevated sweat test results with a mean sweat chloride level of 110 mmol/L (sd ± 25.95, range 80-155 mmol/L). Meconium ileus had not been present in any of the patients, nor were mucus plugs reported in the newborn period. All families were of Bedouin descent and belonged to the tribal groups of the Abu Dhabi Emirate of the UAE.

Pulmonary disease was variable, with one child presenting with gross cor pulmonale (patient 5), while patient 6 presented early and had no clinical lung disease and a normal chest radiograph. Patients 3 and 4 are siblings, as are patients 5 and 8. All patients had malabsorption and pancreatic disease. Subsequent to diagnosis, all patients received pancreatic enzyme supplements, vitamins, nutritional advice and support in the form of a high calorie diet. Appropriate antibiotic regimens were commenced based upon the degree of lung disease and the organisms isolated.
Comment

All these patients have severe disease. The youngest had no chronic lung disease but marked malabsorption. All had the raised sweat chloride levels and malabsorption with very low or absent stool chymotryptic activity. None of the eight children had a history of meconium ileus at birth.

As in many Islamic countries, the UAE society is a very private one and personal details about families and their background are hard to acquire. In many towns, streets do not carry names or numbers and the follow up of patients is difficult. Thanks to her personal charm and local nationality, one of our staff was able to interview the families and learn about their tribal background. In addition, the full name of each patient helped to identify which of the families of the tribe they belong to. Thus it is felt that beyond doubt that we had demonstrated the presence of CF in the Bedouin Arabs of the UAE. This is, therefore, the first report of such an event and suggested that the disease presentation was not mild. ^

^ This section formed the basis of the paper: Cystic Fibrosis in the United Arab Emirates: I Clinical presentation"
Table 7

Summary of Patients’ Clinical Details

<table>
<thead>
<tr>
<th>Number</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Pulmonary Disease</th>
<th>Intestinal Disease</th>
<th>Sweat Chloride mmol/L</th>
<th>Meconium Ileus</th>
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<tr>
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<td>5</td>
<td>Female</td>
<td>6</td>
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<td>++</td>
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<td>++</td>
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<td>7</td>
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<td>+</td>
<td>++</td>
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<tr>
<td>8</td>
<td>Female</td>
<td>9</td>
<td>+</td>
<td>++</td>
<td>110</td>
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</tbody>
</table>

0 = absent
+ = present
++ = severe
+++ = very severe
Section II

Corresponding to the initial detection of eight Bedouin children with CF, there was a rapid expansion of the numbers of children referred to the respiratory service from outside our base in Al Ain. Within a one year period a further 12 children were given a diagnosis of CF. The clinical assessment and presentation of these additional children is documented in this second section. This data and their diagnosis formed the basis of a paper to alert Paediatricians in the UAE and Gulf States to the presence of the disease and to reflect upon its severe clinical presentation. It is considered that CF was an under-recognised condition in the UAE.

Methods

The study involved all patients of UAE nationality who were seen at the Paediatric Respiratory Clinic or the children’s wards of Tawam Hospital, Al Ain, UAE and to whom a new diagnosis of CF was given. The review period was from the 1st August 1993 to 31st July 1994. All patients were seen and reviewed by me personally and had sweat tests performed by the pilocarpine iontophoresis method. Demographic and clinical data were abstracted from the medical records. All the children had similar investigations to those children described in section I. An assessment was made as to their clinical severity based upon the examination and laboratory and radiological tests. Stool chymotryptic activity was measured by the method of Kaspar et al.\textsuperscript{5}. The mode of presentation for each child was recorded.

Results

Twelve children had a diagnosis of CF made within the one-year period. There were 10 females and 2 males. Their mean age was 3.7 years (SD ± 2.4 years, range 6 months - 8 years). All families were of Bedouin Arab decent. Their clinical details are summarised in Table 8. Sweat test analysis gave a mean sweat chloride level of 107 mmol/L (SD ± 27.33
mmol/L, range 75-150 mmol/L). Three pairs of siblings were identified from index patients, but all had serious undiagnosed disease by the time of their identification.

Eleven of the children had significant lung involvement. The second youngest patient, who presented at one year of age, had at that time no major lung involvement, however. Conversely, the youngest child had extensive lung disease at the time of identification.

All patients had marked growth retardation and were malnourished. Weight percentiles were all below the fifth percentile with the majority below the third percentile. No patient had a history of meconium ileus, rectal prolapse or meconium ileus equivalent. None had presented with heat exhaustion or salt depletion in the past. In all the children, at the time of their initial clinic review, the levels of stool chymotrypsin were all below 2.65 units/G⁵. Molecular genetic studies have been performed and are reported in Chapter 8.
Table 8

### Summary of Clinical Information

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Mode of presentation</th>
<th>Pulmonary disease</th>
<th>Gastrointestinal disease</th>
<th>Sweat chloride mmol/L</th>
<th>Sex</th>
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<td>++</td>
<td>+</td>
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<td>M</td>
</tr>
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<td>++</td>
<td>95</td>
<td>M</td>
</tr>
<tr>
<td>11</td>
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<td>++</td>
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<td>F</td>
</tr>
<tr>
<td>12</td>
<td>2</td>
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<td>++</td>
<td>150</td>
<td>F</td>
</tr>
<tr>
<td>13</td>
<td>6</td>
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<td>++</td>
<td>155</td>
<td>F</td>
</tr>
<tr>
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<td>120</td>
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<tr>
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<td>++</td>
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<td>F</td>
</tr>
<tr>
<td>16</td>
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<td>FTT+RRI</td>
<td>+</td>
<td>++</td>
<td>110</td>
<td>F</td>
</tr>
<tr>
<td>17</td>
<td>2</td>
<td>FTT</td>
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<td>++</td>
<td>90</td>
<td>F</td>
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<tr>
<td>18</td>
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<td>FTT</td>
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<td>++</td>
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<td>F</td>
</tr>
<tr>
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<td>++</td>
<td>75</td>
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<tr>
<td>20</td>
<td>0.6</td>
<td>RRI/SS/FTT</td>
<td>++</td>
<td>++</td>
<td>90</td>
<td>F</td>
</tr>
</tbody>
</table>

**RRI = repeated respiratory infection**  
**FTT = failure to thrive**  
**SS = sibling screening**
Comment

No estimate of the extent of the disease nationally can be made from this referral pattern. Some of the children of wealthier or more influential families are treated abroad for their chronic lung disease. Personal communication with Paediatricians in the Hospital for Sick Children, Great Ormond Street, London, UK has confirmed this. Therefore, it is clear that not all affected children are being referred to our clinic.

What is certain is that the patients we have seen suffer from severe disease with marked pulmonary disease in the main and all have malabsorption and pancreatic insufficiency. Sweat chloride levels were all markedly elevated. This elevation is far in excess of that seen in malnutrition alone. Thus, our patients present with the cardinal features of CF. It does not exclude the fact that less severe disease may also exist in this community and as yet has not been recognised locally or seen in our clinic.

It is surprising that there have been only two reports of CF occurring in UAE Arab patients with neither of those being a UAE Bedouin child. The establishment of a medical school in the Al Ain district has drawn doctors who have trained in Western countries in which a diagnosis of CF is a common differential. It may be that this factor has influenced the referral and diagnosis of these patients. The provision of accurate and easily accessible sweat tests is, of course, an essential factor in the establishment of the diagnosis and the ease in which it can be made.

The conclusion to be drawn from this study is that CF exists in our national Emirati population as a major clinical disease. It is not a rare condition and should be
considered as part of the differential diagnosis of those with malabsorption with or without lung disease in childhood. The extent of less severe disease, the incidence and overall prevalence of the condition are all important subjects for continuing study. B

Chapter summary

Twenty children of Bedouin Arab decent from the UAE are described. They all have severe clinical manifestations of cystic fibrosis. They form the second largest series of Arab children with the disorder that have been reported in the medical literature.

B This section forms the basis of the paper: Cystic Fibrosis in the United Arab Emirates: an Under-Recognized Condition?
References


Figure 15

A Wadi in the Hajjar Mountains
Maha, a three-year-old Emirate girl with cystic fibrosis
Chapter 8

Cystic Fibrosis in the United Arab Emirates: Initial Molecular Genetic Studies

“Children sweeten labours, but they make misfortunes more bitter”

Of Parents and Children
Essay 7
Francis Bacon (1561-1626)

The establishment of the respiratory disease clinic and a cystic fibrosis clinic subsequently brought about the recognition that CF and other chronic lung diseases existed in the UAE childhood population\textsuperscript{1, 2}. We then wished to offer a diagnostic service that would incorporate molecular genetic diagnosis. This in turn would permit us to investigate the background of CF in the UAE and perhaps later the Gulf Region. Part of this strategy involved obtaining funding to establish a molecular genetics laboratory in the Faculty of Medicine as the hospitals in the city did neither provide nor were willing to provide such facilities. However, with the establishment of the clinic, I considered it essential that we did offer a molecular diagnostic service as well as clinical advice on patient management. Research funds were obtained to establish the laboratory under the aegis of Dr. Philippe Frossard. Thus, we were in the position to gather more information about the individual patients and develop a broader view of the disease as it presented in the UAE as a whole. Described below are the methods used and the results of the first molecular genetic studies on CF in the region.
Subjects and Methods

Subjects

Mutational analysis was performed on children drawn from 17 families and comprised 23 children with CF. Fifteen children from ten families were of UAE Bedouin descent. They comprised 15 of the 20 children described in Chapter 7. Four families were UAE nationals of Baluch origin (five children). Three additional families were included (three children) who were not UAE nationals but were Pakistani citizens of Baluch origin working in the UAE.

The ages ranged from one month to eight years. The CF diagnosis in non-Bedouin children was based in the same criteria as described in Chapter 7 i.e. elevated sweat chloride levels, malabsorption and/or respiratory infection.

Methods

The children's DNA was extracted from leucocytes isolated from 2-5 mL of venous blood collected in EDTA tubes according to standard methods\(^3\).

DNA Analysis

Detection of ΔF508 was done by denaturing gradient gel electrophoresis (DGGE) according to conditions described by Fanen et al.\(^4\).

Denaturing gradient gel electrophoresis strategy. The CFTR gene coding regions were amplified in 32 fragments suited to DGGE analysis using PCR primers and conditions previously described\(^4\), in a multiplex format when possible\(^5\). Mutations detected by DGGE analysis were identified either by endonuclease restriction analysis or direct DNA sequencing\(^6\) using a Sequenase DNA sequencing kit and \([S^{35}]dATP\) (Amersham International plc).
Detection of S549R by restriction endonuclease analysis. The mutation S549R localised in exon 11 (T→G at nucleotide 1779) alters a Dra III restriction site. Five microlitres (500 ng) of exon 11 PCR products, amplified between CF11 and GCCF 11 without GC-tail4 were digested overnight at 37°C with 6 units of Dra III (Sigma); DNA fragments were visualised by 6.5% non-denaturing polyacrylamide gel electrophoresis.

Results

In the 15 UAE national patients of Bedouin descent, all CF patients were homozygous for the same DGGE mutant pattern in exon 11 whereas their parents were heterozygous. This shift in mobility was identified by DNA sequencing as the S549R mutation (T to G transversion at nucleotide 1779). This mutation abolishes a Dra III restriction site: S549 alleles, on which the Dra II site has been abolished, are detected as 189 bp fragments; R549 alleles are evidenced as 107 and 82 bp fragments. All of these 15 CF patients were S549 R homozygous (see Figure 17).

In six families out of seven of Baluch ethnicity, CF patients were ΔF508 homozygotes (Table 9). In the seventh family of Pakistani Baluch ethnicity, the elder of two sisters, aged 8 and 5 years, presented was included. They had presented with milder forms of CF, sweat chloride concentrations were in the 50-60 mmol/L range, as opposed to 100-160 mmol/L in the other patients (i.e those homozygous for ΔF508).

Comments
We approached this study with the expectation that the majority of patients would carry at least one allele with the mutation ΔF508. It came as a surprise to find that none of the UAE Bedouin children in our sample carried such a mutation. Similarly, it was an unexpected finding that all but one of the Baluch UAE patients were homozygous for the ΔF508 mutation. The remaining Baluch child still presents difficulties in defining her mutation even after gene sequencing. The three "control" Pakistani patients were similar to the UAE Baluch children, being homozygous for the same mutation.

We have identified the main CF-causing mutation in the Bedouin as the S549R (T→G) mutation with all the patients examined to date being homozygous for this mutation. In the sample of 30 Bedouin alleles examined none carried the ΔF508 mutation. The ΔF508 has a worldwide frequency of 70% in all mutated CF alleles. The lowest community values reported are in the range of 25-30% among Southeast Asians and patients from the Indian Subcontinent\textsuperscript{7,8,9}. Paradoxically, it was those of Indian Subcontinental descent who exhibited this mutation in our series.

We have to consider, of course, the limited size of the CF sample analysed and whether the patient group studied is representative of the UAE CF population at large. However, the absence of the ΔF508 mutation in the 30 alleles examined from Bedouin patients is far fewer than the 25% minimum that one could expect. In addition, one has to consider the fact that there may be other mutations present that we have not yet identified. As mutation frequencies vary among different ethnic groups and geographic locations, it does seem important to make an inventory of all the CF mutations and their frequencies in the UAE society. It may be that if there are a
limited number of mutations, that population screening may be appropriate and feasible.

Until very recently, the indigenous population of the UAE was organised into a small number of Bedouin tribes that often consisted of individual, although extended, families. The UAE society is thus the epitome of an ethnic population that is characterised by a rapid expansion a small number of families. The S549R (T→G) mutation accounts for 100% of the 30 CF alleles in families of Bedouin descent. It is thus tempting thus to speculate that this high rate of one single mutation is the result of a founder effect in a common ancestral family. C

C This work has given rise to two papers: Cystic Fibrosis Mutations in the United Arab Emirates: 2. Molecular Genetic Analysis and Identification of Cystic Fibrosis Mutations in the United Arab Emirates.
Table 9

Distribution by ethnicity and by ethnic origins of the CF patients and their families and the number of alleles carrying the two mutations, S549R (T→G) and ΔF508.

<table>
<thead>
<tr>
<th>Ethnic Origin</th>
<th>Number of Families</th>
<th>Number of Patients</th>
<th>Mutation</th>
<th>Number of CF Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>UAE Nationals</td>
<td>10</td>
<td>15</td>
<td>S549R</td>
<td>30 of 30</td>
</tr>
<tr>
<td>Bedouin descent</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UAE Nationals</td>
<td>4</td>
<td>5</td>
<td>ΔF508</td>
<td>8 of 10</td>
</tr>
<tr>
<td>Baluch descent</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pakistani Baluch</td>
<td>3</td>
<td>3</td>
<td>ΔF508</td>
<td>6 of 6</td>
</tr>
</tbody>
</table>
Figure 17
References

Figure 24

Othman: an eleven year old
Chapter 9

The Determination of the Frequency of Two Cystic Fibrosis Mutations by Carrier Screening

“If you can look into the seeds of time
And say which grain will grow and which will not”

MacBeth 1.3, 58
William Shakespeare (1564-1616)

Clearly the prevalence of CF in the UAE is unknown. Examination of all referred patients indicated that two mutations, ΔF508 and S549R (T→G), accounted for 54 out of 56 (96%) of CF alleles studied. All patients that we have investigated were homozygous for either of the two mutations: 20 CF patients (who were of Bedouin origin) were S549R (T→G) homozygotes and seven patients of eight studied (who were of Baluch lineage) were ΔF508 homozygotes. In light of these observations, we designed a study aimed at screening CFTR genes of a random sample of the indigenous UAE population for asymptomatic S549R (T→G) and ΔF508 carrier status. The aim was gain information about the frequency of these genes in the population at large. An obvious fundamental difficulty was in obtaining a true random sample that would reflect the population as a whole and contained unrelated subjects. As discussed earlier, information pertaining to ethnicity and family relationships is difficult to obtain for cultural and political reasons.

Methods
The sample population for the study comprised 400 unrelated UAE nationals (200 males and 200 females). These subjects were part of another separate investigation into a range of inherited skin disorders. Local Arabic speakers employed for the dermatological study documented details of family histories and tribal backgrounds. Information so obtained confirmed the lack of close family ties to other members of the sample. The sample appeared to be one drawn from the majority of tribes found in the UAE. As best one could judge by the data, it represented as good as sample as was practical in light of the cultural difficulties. The actual volunteers were recruited from general practice attendees at the specific practices nominated for national patients. All attendees had trivial medical problems or were simply accompanying persons. Full ethical approval had been obtained for this process and there was informed volunteer participation.

Detection of ΔF508 was carried out by non-denaturing polyacrylamide gel electrophoresis (PAGE). PCR reactions were performed on 500ng DNA samples using primers and conditions previously described in Chapter 8. Mutation S549R is localised to exon 11 (T→G at nucleotide 1779) and alters a Dra III restriction site. Detection of the mutation was carried out routinely by Dra III restriction endonuclease analysis of exon 11 PCR products, which were generated using CGCF113 without a GC-tail as the sense primer and CF113 as the anti-sense primer. Five hundred nanograms of PCR products were digested overnight at 37°C with 5U of Dra III (New England Biolabs) into a final volume of 20µL. Restriction digests were then dried down to 8µL and loaded on 8 cm long, 6.6% non-denaturing polyacrylamide gels (Hoefer Scientific Instruments), San Francisco, CA: electrophoreses were carried out at 75V for 1.5 hours. Presence of suspected ΔF508
and S549R (T→G) mutants alleles was confirmed by sequencing analyses according to protocols that have been described in Chapter 8.

**Results**

Screening of the 800 chromosomes led to the detection of six carriers: four individuals were S549R (T→G) heterozygotes and two were ΔF508 heterozygotes (Table 10). The estimated frequencies of carriers of each of the two mutations in the population are thus: S549R (T→G) 1:100, ΔF508 1:200. Given that ΔF508 and S549R (T→G) mutations characterise 96% of CF alleles, however, the estimated carrier frequency of any CF mutation in the population of the UAE is [6 x (100/96)]/ 400 = 1:64. To estimate the 95% confidence interval for this number, the formula M - tsM < μ > M + tsM was applied. (M = sample size, sM estimate of population standard deviation, t = t distribution and degrees of freedom). The confidence interval obtained was 6 ±0.5.

We cannot assume that genotype frequencies occur in Hardy-Weinberg proportion. The high rates of consanguinity at 53%, require an adjustment factor (F) to allow for this. The correction for consanguinity used is for recessive alleles i.e. aa genotype frequency = q²(1-F) + qF. A consanguinity factor of 0.5 (F = 0.022) was used. Thus the corrected equation is 1/128 x 1/128 (1-0.022) + 1/128 x 0.022.

The result of the calculation for the combined estimated frequency of individuals homozygous for the mutant CF alleles gives a figure of 1:4318. A birth frequency for CF is therefore, at least, one in 4,318 births.

**Discussion**

Within a period of two years, a total of 30 children of UAE nationality were attending the CF Clinic. Additional children of various other Arab and non-Arab ethnic groups...
were also being seen. I inferred previously that we might not be seeing all children with the disorder due to under diagnosis and patient management elsewhere. If by some chance we were seeing all children with CF, then with a National population of 750,000 people, the population prevalence would in the order of 1:25,000 (minimum de facto prevalence).

It was considered that one could gain a more informed estimate of the potential birth frequency of the gene mutations from a random sample of the UAE population. A major difficulty, of course, was in obtaining a true population sample. This was in part due to the secretive nature of this society and to the fact that "degrees" of citizenship occur depending upon one's ancestry. Thus, in general, this type of information is not released. I attempted to address this specific problem by the selection of the dermatology study group, rather than duplicate the interview methods.

The second difficulty lay in the structure of the society. As alluded to earlier, UAE society has evolved by the expansion of a small number of families. Consanguinity has reached levels of 53% and is only reducing in the youngest and highly educated sector of the society. Family size remains large with an average of seven children per family. These factors will reduce the frequency of heterozygotes and increase the frequency of homozygotes present. Hence the Hardy-Weinberg law does not apply in these circumstances. The consanguinity factor has been applied. The selected consanguinity factor has been based upon the work of Al-Awadi et al who found that the rate of consanguineous mating in Kuwait was 54.3% and the average inbreeding coefficient was 0.0219.
The estimates rely on small numbers –30 CF patients and 6 asymptomatic carriers in a sample size of 400. Clearly one has to remain cautious with their interpretation. Furthermore, a population bias is possibly introduced by the organisation of the UAE indigenous society into tribes, several members of which still live in remote desert areas. In the sample population recruited for this study, we could therefore have missed out isolated affected families. The random sample was not analysed for the its distribution of Baluch and Bedouin and no information exists as to the degree of interbreeding between the groups. Personal observation suggests that this is rare. Hence calculations for the individual ethnic groups would not be valid. For these reasons, the predicted CF incidence of 1:4318 among Emirati is probably a conservative estimate.

CF until recently has been a fatal and under-recognised condition in the UAE. Population prevalence will, therefore be different to birth frequency. In our studies to date we have not found a patient with a history of meconium ileus. It could be argued that many of the infants might have died from this condition which is usually fatal soon after birth if untreated and not recognised. However, the study of 10,000 births at the Tawam Hospital, identified only two infants with the condition. Neither of these infants were Arab children and both survived.

Although the value of genetic testing is clear, the perception of genetic screening varies but there now seems to be general agreement across countries that the pendulum has swung towards the side of seeking information on CF carrier status. The goals of carrier screening projects are two-fold: 1) to provide informed reproductive choices; and 2) to identify couples at risk in order to give them the opportunity to reduce the “phenotype load” in affected families. At this point,
introducing a large-scale carrier-screening programme for a disease such as CF (despite a high prevalence at birth) is considered premature at best in this rapidly developing society. In the UAE, however, genetic testing for CF could certainly be offered to adult individuals with a positive family history of CF, to couples planning a pregnancy and to couples seeking prenatal care.

From this study it is suggested that the social structure and high consanguinity rates have increased the rates of the relatively rare disease, CF. The lethal nature of the condition when untreated has to date masked the true situation. It may be expected with increased medical vigilance and the eventual advent of screening that the true nature and frequency of the condition will be recognised more widely.
Table 10

Heterozygote frequencies of the two common cystic fibrosis mutations in 400 random, unrelated UAE individuals

<table>
<thead>
<tr>
<th></th>
<th>S549R</th>
<th>ΔF508</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of</td>
<td>4</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>heterozygotes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% in the population</td>
<td>1</td>
<td>0.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Frequency in the population</td>
<td>1:100</td>
<td>1:200</td>
<td>1:64</td>
</tr>
</tbody>
</table>
References


Chapter 10

The S549R (T→G) Cystic Fibrosis Mutation

“The young disease, that must subdue at length, 
Grows with his growth, and strengthens with his strength”

An Essay on Man 1,135  
Alexander Pope (1688-1744)

CF in UAE Bedouin children is associated with the mutation S549R (T→G) [S549R] in the children so far identified. This mutation has been rarely reported (see summary at the end of this chapter) and hence several questions needed to be addressed. Could we confirm that there was a severe clinical phenotype associated with this genotype? Was it possible to compare the clinical phenotype produced in patients who are homozygous for S549R with those homozygous for ΔF508 mutations that are resident in the UAE. Is there a practical value in this society in having a rapid diagnostic service for CF mutations?

This chapter reports several studies aimed at answering these questions or providing at least some new information.

Is the clinical severity of mutation S549R (T→G) extreme?

Having acquired the knowledge that thirty of our patients had CF as a result of S549R mutations, fifteen of the earlier patients have been reviewed specifically to address the severity of their clinical disease.
Subjects and methods

Patients: The study group consisted of 15 CF patients (9 girls and 6 boys) who were all UAE nationals of Bedouin origin. They were drawn from thirty patients diagnosed as having CF due to the mutation S549R. Ten of the patients are from the group briefly described in Chapter 9. All patients were referred directly to me at the Cystic Fibrosis Clinic of Tawam Hospital (Al Ain, Abu Dhabi Emirate, United Arab Emirates) from 1993. The Research Ethics Committee of the Faculty of Medicine and Health Sciences, UAE University, Al Ain, UAE approved the protocol for this CF programme.

Initial clinical investigations:

General outcome variables that were evaluated in this investigation included current age, age at diagnosis, sweat chloride values (measured by quantitative pilocarpine iontophoresis), height, weight and weight for height percentiles (from most recent clinic visits), and Shwachman-Kulczycki scores (Table 11). All results are presented as means and standard deviations as determined for the 15 patients.

The presence or absence of the following presenting clinical problems was recorded (Table 11): meconium ileus, pulmonary problems, failure to thrive, diarrhoea. The presence of pancreatic insufficiency was determined on the basis of requirement for pancreatic enzyme supplementation to control steatorrhea and the measurement of stool chymotrypsin activity. Associated complications included nasal polyps, sinusitis, pancreatitis, diabetes mellitus, and rectal prolapse (Table 11).
Respiratory bacterial infections (*Pseudomonas aeruginosa* and *Staphylococcus aureus*) were determined as the first positive culture on record, and cultures of tracheal aspirates were performed in the case of children who were too young to provide sputum or cough swab samples (Table 11).

Due to the young age of the subjects, regular pulmonary function tests on all subjects could not be performed. Radiological analyses as appropriate were carried out to evaluate both the extent and the severity of lung disease.

**Treatment and management**

All patients were commenced on pancreatic enzymes, dietary and nutritional supplements including multivitamins from the time of diagnosis. Selected individuals had nasogastric night feeds and total parenteral nutrition. No patient had a gastrostomy feeding port. This was on the basis of a cultural bias against such devices. Aggressive antibiotic therapy as guided by sputum culture, cough swabs or tracheal aspirates was practised. Nebulised hypertonic saline or pulmozyme was used for those with extensive lung disease on an individual basis. Regular inhalation of tobramycin via a nebuliser was prescribed for those with pseudomonas colonisation. Inhaled steroids and bronchodilator therapy was prescribed again on an individual basis.

**Results**

The clinical presentation and details of the 15 CF children (6 boys and 9 girls) is given as pooled data in Table 11. Mean current age was 5.4±3.5 years, mean age at diagnosis was 1.0±1.1 year. Sweat chloride concentrations were 120±21 mmol/L.
(range: 105-155 mmol/L), and Shwachman scores (45.5±7.0) were in keeping with an extremely severe, overall clinical manifestation.

No child had presented with meconium ileus at birth, but all were pancreatic insufficient (PI) and had severe pulmonary disease. None of the 15 CF patients had associated complications (Table 11). *Pseudomonas* and *Staphylococcus* colonisation were frequently observed (9 and 7 cases out of 15, respectively).

**Discussion**

The clinical data does support the concept that S549R is a very severe mutational allele. First of all, the age at diagnosis was low (1.0±1.1 year, see Table 11) supporting an early and severe presentation of clinical effects. Sweat chloride concentrations associated with S549R homozygosity were high, even at very young ages (mean sweat chloride levels in the group of 15 patients were 120±21 mmol/L). All 15 patients exhibited the PI phenotype. Chronic infections with *Pseudomonas aeruginosa* and *Staphylococcus aureus* were present in 9 and 7 patients, respectively, and were found unusually at very young ages. Overall Shwachman-Kulczycki scores, as well as height and weight percentiles, were poor (see Table 11) and support a severe clinical phenotype.

A remarkable feature of the clinical presentation associated with S549R in the UAE is that none of the 15 homozygous patients had presented with meconium ileus at birth. This contrasts with mutation ΔF508, which is frequently associated with meconium ileus². Furthermore, ΔF508 subjects who present with meconium ileus are more likely
to be colonised earlier with *Pseudomonas*³. In R549 homozygotes, the rate of serious chronic infections (including *Pseudomonas*) is high and severe pathogenic colonisation occurs early in life. Early colonisation by *Pseudomonas* has also been reported in CF patients from Saudi Arabia⁴, which tends to indicate that genetic as well as non-genetic factors play an aggravating role in the course of CF in this part of the world.

It is also interesting to note that, besides meconium ileus, none of the 15 patients has showed evidence of classically associated complications such as rectal prolapse or nasal polyps (Table 11). All 15 patients, however, have had severe pulmonary complications. Even though we were unable to perform pulmonary function tests on the patients in this study, the severity of radiological findings (see below) on 12 of these patients lead us to conclude that the pulmonary phenotype was extremely severe in all 15 R549 homozygous patients.

Although there is usually good correlation between CFTR genotype and pancreatic function status, genotypes have failed to predict pulmonary severity so that the pathophysiology of lung complications remains elusive⁵-¹². Our results also point to the fact that the pulmonary phenotype associated with R549 mutations is indeed fairly heterogeneous, although it indicates a marked overall severity. Despite this severity the oldest surviving patient, however, who has complied well with therapeutic interventions, is now 12 years old and may well have a reasonable prognosis.

In conclusion, genotype-phenotype analysis in CF patients from the UAE shows that the R549 allele is associated with an extremely severe clinical presentation. Detection
of the S549R mutation is thus imperative in these CF children, as aggressive treatment and management modalities should be instigated as soon as possible in order to increase patient chances of survival.
# Table 11

## Clinical description of CF patients with CFTR mutation S549R (T→G).

<table>
<thead>
<tr>
<th>Variable</th>
<th>R549/R549</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (n)</td>
<td>15 (6M, 9F)</td>
</tr>
<tr>
<td>Mean current age (years)</td>
<td>5.4 ± 3.5</td>
</tr>
<tr>
<td>Mean age at diagnosis (years)</td>
<td>1.0 ± 1.1</td>
</tr>
<tr>
<td>Sweat chloride mmol/L</td>
<td>120.0 ± 21.0</td>
</tr>
<tr>
<td>Shwachman score</td>
<td>45 ± 7.0</td>
</tr>
<tr>
<td>Height percentile</td>
<td>&lt;3rd - 10th</td>
</tr>
<tr>
<td>Weight percentile</td>
<td>&lt;3rd - 5th</td>
</tr>
<tr>
<td>Presentation (n)</td>
<td></td>
</tr>
<tr>
<td>Meconium ileus</td>
<td>0</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>14</td>
</tr>
<tr>
<td>Failure to thrive</td>
<td>13</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>10</td>
</tr>
<tr>
<td>Absent chymotrypsin activity in stool</td>
<td>7</td>
</tr>
<tr>
<td>Pancreatic insufficiency</td>
<td>15</td>
</tr>
<tr>
<td>Associated complications*</td>
<td>0</td>
</tr>
<tr>
<td>Pseudomonas colonisation</td>
<td>9</td>
</tr>
<tr>
<td>Staphylococcus colonisation</td>
<td>7</td>
</tr>
</tbody>
</table>

Values are mean ± SD: M Male, F Female
*Associated complications: nasal polyps, sinusitis, pancreatitis, diabetes mellitus, rectal prolapse.
To obtain further information about the pulmonary status of the children described above an analysis of their radiological findings over time was carried out. The patients were drawn from the group described above, but only those with full radiological assessment were included.

**Patients and Methods**

**Patients**

The study group consisted of 12 CF patients who were all UAE nationals of Bedouin origin.

The CF group included eight girls and four boys. The mean age of the group was 4.1 ± 3.3 years, and the age at diagnosis had been 10 ± 9 months. All 12 patients were pancreatic insufficient (PI) and the mean sweat chloride value (as demonstrated by quantitative pilocarpine iontophoresis) was 119 ± 17mmol/L. None of these patients had presented with meconium ileus at birth, but they all had experienced pulmonary complications at the time of diagnosis.

**Clinical investigations**

Detailed radiological analyses were carried out in the 12 patients by a single observer in order to evaluate both the severity and the extent of the lung disease. Thus, all 12 children had a series of chest radiographs (PA and lateral). Radiological features of CF were recorded from the start of the disease until the last chest radiograph, which enabled comparisons and facilitated the evaluation of the progression shown on sequential chest films. Ultrasound examinations of the abdomen were carried out in four children and one child had high-resolution computerised radiography (HRCT) of the lungs.
Results

To evaluate the lung pathology on chest radiographs, the 12 CF children were placed into two groups – below and above two years of age.

In the group of six children who were two years old or younger, a picture of hyperinflation was dominant. Due to bronchoconstriction in peripheral airways, the lungs appeared extremely hyperinflated, with associated depression of the diaphragm and sternal bowing. Five children had bronchial line shadows that were due to thickening of the peribronchial interstitial tissues resulting from inflammation caused by proven respiratory syncytial virus infections. This triggered very early, severe and persistent obstruction in the youngest patients.

Ring shadows representing bronchiolectatic cavities were seen in three of the infants below one year of age. These were mainly localised in the superior and posterior segments of the lower lobes. One six month–old baby presented with persistent collapse of a segment of the middle lobe, which was due to mucous plugs obstruction. One other patient of this younger group presented with radiological features of pneumonia.

The six CF patients who were older than two years presented with mottled and ring shadows of bronchiolectatic dilatation of the airways. Mottled shadows were round shadows with ill-defined edges caused by abscesses with a lobular distribution. In one patient, these were persistently found on all chest radiographs during follow-up. Lobular infections were diffusely distributed throughout all lobes.
Ring shadows of dilated bronchioles with thick walls were present in all five patients who were five years or older. These were mainly distributed in the upper lobes and posterior segments of the lower lobes. Ring shadows were the dominant component of the lung pathology in these children and their severity gradually increased with age.

One five-year old patient had a very prominent interstitium with noticeable peribronchial and perivascular bundle branches and reticular shadows of lung fibrosis. Lung fibrosis was diagnosed on HRCT in one 9-year old patient. This was the only child followed by CT in the overall group of patients. A mosaic perfusion pattern and air trapping with peribronchial thickening was the dominant radiological finding. Four children of this group showed tracheomegaly that was most probably due to chronic recurrent inflammation. This led to enlargement of the major airways and structural abnormalities in the tracheal walls, which became flaccid.

There was no evidence of honeycombing and bullous change in any of the 12 CF patients who were included in this analysis. However, inflammatory mucosal oedema and accumulation of mucopurulent secretions were the main reason for the hyperinflation that was observed in children of all age groups.

Two patients had enlargement of the liver with hyperechogenic parenchyma suggestive of fatty infiltration. One child showed a hyperechogenic pancreas due to fatty infiltration. One other patient had gross hepatomegaly and fatty infiltration.
Changes in cardiac configuration occurred in all patients with CF in early infancy. Due to severe obstruction, which contributed to increased lung volume, the heart shape became elongated. Early features of cor pulmonale were seen in three children. Enlargement of the main pulmonary artery was seen in four children at age 3-7 years. Enlargements of main pulmonary artery shadows in the hila occurred in two children in the early stages. These two patients later developed heart failure.

Discussion

The information that we obtained from chest radiographs in the cohort of 12 CF patients revealed a high degree of complexity of radiological findings which required subsequent detailed analysis. Diversity of pulmonary changes and associated radiological expressions showed remarkably severe hyperinflation in early infancy in conjunction with inflammation of the interstitium. The main findings consisted of depressed diaphragms and sternal bowing with reduction of vascular shadows on the periphery. These infants also presented with early signs of liver involvement, as assessed by ultrasound examination.

In the older age group (i.e. above two years), signs of central airways involvement occurred in association with early signs of pulmonary hypertension. This indicates that aggressive patient management is required in these children.

These combined findings clearly indicate that the phenotypic expression of lung disease due to CFTR mutation S549R (T→G) is extremely severe and that there is a rapid decline in the pulmonary status of these patients. In this respect, the pulmonary
phenotype expression of mutation S549R (T→G) appears to be at least as severe as that of mutation ΔF508.

Summary
In answer to the question regarding the degree of clinical severity associated with the S549R mutation, the overall clinical presentation of CF in these R549 homozygous patients is extreme. Furthermore, *Pseudomonas aeruginosa* and *Staphylococcus aureus* colonisation was frequently observed (in, respectively, eight and six cases) and occurred at an unusually early age (at least four patients had been recorded as having presented with *Pseudomonas* or *Staphylococcal* infections by three months of age).\(^\text{D}\)

\(^\text{D}\) The above findings gave rise to two papers: Genotype-phenotype correlations in cystic fibrosis\(^\text{13}\) and Radiological analysis of children with cystic fibrosis who are homozygous for the CFTR mutation S549R\(^\text{14}\).
Is it possible in the UAE to compare disease severity associated with Cystic Fibrosis mutations ΔF08 and S549R (T→G)?

To date the children with the S549R mutation all have had severe disease. Indeed, it appeared to me to be as severe as I had seen before elsewhere. The clinical phenotype associated with the ΔF508 mutation is usually regarded as the "gold standard" for comparison. Clearly, as outlined in the literature review other mutations around exons 10 and 11 produce severe disease also. The purpose of this section is an attempt to compare the disease severity associated with each of these two mutations as they present in the UAE. In our situation, the environmental factors and health care access are uniform and controlled and the patients are homozygous for their respective mutations. While there may be ethnic differences between groups, these are not apparent in day to day living and there are no obvious socio-economic differences. Indeed, those of Baluch and Iranian descent are more than likely to be represented in the professional groups.

Methods

Subjects
The study subjects consisted of two groups of 5 children who were age- and sex-matched and were homozygous for the mutation S549R (T→G) or ΔF508 (Table 12). There were two males and three females in each group. I also compare the group of 5 CF, ΔF508 homozygote patients with a non-matched group of 15 CF children who are S549R (T→G) homozygotes. This latter group, that I have described earlier, includes the 5 patients constituting the matched S549R (T→G) group.
The overall number of CF patients in this study may appear as relatively small. I have shown previously, however, that these subjects constitute the pool of most of the CF patients found in the UAE, and the overall pool of 20 patients investigated here has been actively recruited over the past few years.

All children, as UAE citizens, have free access to hospital care and the costs of their inpatient care, drugs and investigations are met by the State. Management plans were instigated and supervised by me, but were individualised.

**Clinical investigation**

Data was collected from the matched children with regard to age, age at diagnosis, current height and weight percentiles. A Shwachman score was calculated for each child\(^1\). Data was expressed as means for the separate CF mutation groups with one standard deviation. Information was collected also with regard to the following: history of meconium ileus, pulmonary infection, evidence of pancreatic insufficiency (determined on the basis of enzyme replacement and/or chymotryptic activity in the stool), associated problems viz. nasal polyps, sinusitis, pancreatitis, diabetes mellitus or rectal prolapse. The presence of respiratory tract colonisation was noted and the presence of specific organisms was based upon positive sputum cultures or tracheal aspirate results.

**Results**

The comparative pooled data for both groups of children are listed in Table 12. Included for comparison is the findings from 15 children with mutation S549R (T→G) previously described. In the matched groups the main difference is the earlier
diagnosis in the ΔF508 group, while Shwachman scores show no difference and sweat electrolyte levels are marginally higher in the S549R (T→G) group. The mean sweat chloride levels were higher in both the matched and unmatched S549R (T→G) groups. This did not reach statistical significance (p = 0.34). Similarly, the age at diagnosis for the ΔF508 patients was earlier than both other groups, but again did not reach a significant level (p = 0.57).

Discussion

These findings support the view that the S549R (T→G) CF mutation is associated with a clinically severe form of the disease. Further, the results are in keeping with our further contention that the clinical disease associated with the mutation in the homozygous state is as severe as that produced by the ΔF508 mutation. The major difference found between the age- and sex-matched patients was that the diagnosis was established earlier in the group of ΔF508 homozygotes. However, despite this, there was no difference in the Shwachman Scores between groups. One of the unique features of this study is that all the patients are homozygous for their particular mutation, live in the same harsh climatic and physical environment and have free access to health care. I have included another group of patients with S549R (T→G) in Table 12 whose details have been given. The clinical details from this larger group suggest that our age- and sex-matched sample do reflect the general pattern of disease found in our society resulting from the mutation. However, it is of interest to note that in general the diagnosis was made earlier than in the matched group and was nearer to that of the group of ΔF508 homozygotes.
The severity of CF mutations has usually been based upon whether there is pancreatic sufficiency or not. ΔF508 is regarded as a severe mutation conferring marked pancreatic insufficiency and the presence of meconium ileus in 10-15% of the affected neonates\textsuperscript{14}. In addition, the mutation is usually associated with lung disease. This mutation remains the common severe mutation worldwide and results in major CFTR chloride channel disruption and produces little channel activity. It has been labelled as a class II mutation (defective protein processing) which results in the failure of the protein to reach the epithelial membrane\textsuperscript{8}. Further, the R549 mutant has also been shown to fail to yield the fully glycosylated form of the protein. This in turn leads to its failure to reach its correct cellular location (as occurs with ΔF508). The S549R (T→G) mutation is thus also a class II mutation\textsuperscript{15, 16} Hence our clinical findings are in keeping with the molecular changes.

The patients we have described match the classical homozygote pattern for ΔF508, chronic obstruction and infection of the respiratory tract, pancreatic insufficiency and elevated levels of sweat electrolytes\textsuperscript{10}. However, we have not seen a patient, homozygous for either of the studied mutations, in the UAE who presented with meconium ileus. As the patients studied here were all pancreatic insufficient, and if we take into account the range of 10-15%\textsuperscript{38}, we would have expected to observe two to three neonates with meconium ileus in the overall cohort of 20 subjects. Whether the small sample size accounts for the lack of significance or whether additional factors play a role in the absence of meconium ileus among UAE patients remain to be elucidated. Similarly, the higher sweat chloride levels in the S549R (T→G) patients may reach significance if and when additional ΔF508 patients are found. The apparent, but statistically insignificant, earlier diagnosis age of ΔF508 patients is
unlikely to be to a referral bias, but may reflect a cultural difference and hence different social expectations. My experience of this group suggests that it is unlikely.

I conclude from this study that S549R (T→G) homozygote patients have severe clinical disease and that this disease severity equates with the clinical severity of ΔF508 homozygote patients. These clinical findings are in keeping with the known molecular modifications affecting the CFTR gene.
Figure 27

Clubbing
Table 12

Clinical data associated with CFTR mutations S549R (T→G) and ∆F508

<table>
<thead>
<tr>
<th>Variable</th>
<th>Matched ∆F508/∆F508 (n = 5)</th>
<th>Matched S549R/S549R (n = 5)</th>
<th>Non-matched S549R/S549R (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current age range (months)</td>
<td>36-120</td>
<td>36-120</td>
<td>1-144</td>
</tr>
<tr>
<td>Mean age (months) at diagnosis ± SD*</td>
<td>6.8 ± 4.2 (range 2-12)</td>
<td>22.0 ± 25.6 (range 1-60)</td>
<td>12.0 ± 13.0 (range 2-36)</td>
</tr>
<tr>
<td>Mean sweat chloride level (mmol/L) ±SD**</td>
<td>97.8 ± 19.1</td>
<td>113.0 ± 23.3</td>
<td>120.0 ± 21.0</td>
</tr>
<tr>
<td>Shwachman score ± S.D.</td>
<td>45.0 ± 5.7</td>
<td>45.4 ± 5.1</td>
<td>45.5 ± 7.0</td>
</tr>
<tr>
<td>Pancreatic insufficiency</td>
<td>4/5</td>
<td>5/5</td>
<td>15/15</td>
</tr>
<tr>
<td>Pseudomonas colonisation</td>
<td>4/5</td>
<td>2/5</td>
<td>9/15</td>
</tr>
<tr>
<td>Meconium ileus</td>
<td>0/5</td>
<td>0/5</td>
<td>0/15</td>
</tr>
</tbody>
</table>

- p = 0.57 (ns)
- p = 0.34 (ns)
Is there a practical value in having a rapid diagnostic service for CF mutations?

To support the argument that it is of value to have this service available, I present below two clinical examples. The knowledge of the CFTR mutation aided in the diagnosis of one child and was helpful in the management in another. Both examples formed case reports which were published in the Gulf Region medical journals. The purpose of this was to draw attention to the condition locally and to stress that CF should be considered in the differential diagnosis of ill Arab children. Both patients were referred to me for diagnosis and the referring paediatricians were included as co-authors. The reports are presented below in an abridged version of the originals.

Illustrative Case Reports

Post-mortem confirmation of a rare cystic fibrosis mutation

AK, a five-month-old infant, was the first child of healthy UAE nationals who were first cousins. His birth weight was 3.2 kg. The pregnancy, delivery and neonatal period were normal.

He was first admitted to hospital at the age of four months because of failure to thrive and diarrhoea. He was anaemic and wasted (weight 3.4 kg.) with hepatosplenomegaly, hypoalbuminaemia and a prolonged blood coagulation time. CF was suspected but sweat tests were unsuccessful because of an inadequate weight of sweat. Before repeat tests could be performed the child was removed from hospital against medical advice.

At aged five months he was readmitted in extremis, shocked, dehydrated, hypoxic and jaundiced. Chest radiology revealed extensive atelectasis and widespread opacities. He was intubated and ventilated. Pseudomonas aeruginosa was grown from the...
tracheal aspirate and was shown to be sensitive to amikacin and aztreonam. Despite aggressive antibiotic therapy, ventilation and other supportive therapy, there was progressive extension of the pneumonia. He died from hypoxia, despite maximal ventilatory settings.

Prior to death, blood had been taken for numerous investigations, including DNA analysis. Permission to perform a post-mortem examination was withheld. DNA analysis revealed a homozygous mutation S549R on exon 11.

**Discussion**

An infant of UAE nationality and Bedouin origin is described in whom there was a very strong suspicion of cystic fibrosis. Parental consanguinity, the patient’s diarrhoea, severe failure to thrive and *Pseudomonas* pneumonia are all strong pointers to this diagnosis. CF is generally considered a rare disease in Arab countries. We have recently drawn attention to the fact that in the UAE it appears more common than previously considered and that it presents with serious disease. Associated molecular genetic studies found a mutation in close proximity to the ATP-binding domains in patients of Bedouin origin. All these patients have been shown to be homozygous for an S549R mutation due to a T→G transversion at nucleotide position 1770.

The prior knowledge of CF mutations in the population permitted rapid analysis of the DNA sample obtained from our patient. This confirmed our clinical diagnosis that the patient was homozygous for the S549R mutation and therefore had cystic fibrosis. Our patient was the first child of the family and this confirmation of the diagnosis made genetic counselling possible. The mutation described is rare, except in the UAE where, along with ∆F508 mutation it accounts for over 90% of cases of CF. Prior
knowledge of the frequency of this mutation in this specific population thus permitted us to establish a definite diagnosis after the death of the child.
A metabolic alkalosis associated with low serum electrolytes is an uncommon finding in infancy and is usually associated with pyloric stenosis, chloride-loosing nephropathy or Bartter’s Syndrome.

Herewith presented are details of an Omani child who developed alkalosis and electrolyte disturbance. She had an unusual set of circumstances that produced a pseudo-Bartter’s syndrome.

**Case Report:**

A four-month-old Omani female was admitted to Al Ain Hospital, Al Ain, United Arab Emirates with a history of failure to thrive and diarrhoea. She was one of nine children (five males and four females) of parents who were first cousins. All the siblings were apparently well and the patient was born after a normal pregnancy and delivery.

On admission she was wasted and miserable, weighing 2.06 kg. (birth weight 2.4 kg.). Her blood pressure at that time was 70/50 mm Hg. Laboratory findings are as follows:

Serum Na =118 mmol/L, potassium = 2.42 mmol/L, chloride = 70mmol/L, CO₂ = 35.7, base excess – 24.2, serum osmolality = 270 mOsm/kg., urine osmolality = 72 mOsm/kg., sweat chloride = 35 mmol/L.

Initial treatment was by oral electrolyte solution with added potassium, which resulted in a rapid restoration of normal electrolyte levels. However, she failed to gain weight and developed an irritating cough and persistent fever. A chest radiograph was in keeping with the clinical finding of a bronchopneumonia with marked changes in the right lung.
Further investigations revealed the absence of urinary sodium loss over 24 hours, normal plasma renin and aldosterone levels. The plasma electrolytes remained normal without extra potassium while she remained in hospital. The pneumonia was treated with antibiotics and, following resolution, further sweat tests were performed. Sweat chloride levels in excess of 70 mmol/L were detected. Stool chymotryptic activity was 1 U/g (normal > 6 U/g. Examination of 7 exon 11 of the CFTR gene localised on chromosome 7 revealed a homozygous mutation S549R. The patient was started on a regimen that included pancreatic enzymes, vitamin supplements and supplemental feeding, after which a 1.6 Kg. weight gain occurred within 14 days. A rapid decrease in stool frequency and improved consistency was noted thereafter. On family screening, the parents and four siblings were found to be carriers of the S549R CF mutation.

Discussion

On admission our patient was given a provisional diagnosis of Bartter’s syndrome. This was based on the fact that she had “normal” sweat electrolyte levels, a metabolic alkalosis, low plasma electrolytes and normal blood pressure. However, the finding of normal plasma renin and angiotensin and the absence of urinary salt wasting refuted that diagnosis. After restoration of normal serum electrolyte balance and removal of supplements, repeat sweat tests confirmed elevated sweat chloride levels, all in excess of 70 mmol/L. It was of interest that despite an adequate weight of sweat, the initial sweat test result was within the normal laboratory range. Paradoxically it is known that sweat electrolytes can be elevated in malabsorption19. I presume that the false sweat test originally was as a result of the profound electrolyte disturbance that the child was experiencing. The diagnosis of cystic fibrosis was, however, confirmed
beyond doubt by the demonstration of the CF gene mutation S549R. We have previously reported this mutation as the main CF-causing mutation in the United Arab Emirates population\textsuperscript{20} and have indicated that the disease caused by it is severe and we suspect that the disorder is underdiagnosed\textsuperscript{23}.

The association of pseudo-Bartter’s syndrome and CF has been described before in a small number of children from North America\textsuperscript{24} and the United Kingdom\textsuperscript{22}. Cystic fibrosis has been regarded as a rare disease in the Arab and Al-Mabairreek and Abdullah described patients in Saudi Arabia with findings consistent with pseudo-Bartter’s syndrome in many of the twelve children they had seen with CF\textsuperscript{25}. Their patients had disturbed electrolyte balance associated with vomiting and failure to thrive.

We believe our patient had experienced marked electrolyte loss due to high environmental temperatures, aggravated by intercurrent illness and diarrhoea as has been described before\textsuperscript{24}. She had a low salt intake and an inadequate solute feed. This patient is the first Omani patient to have a defined genetic mutation demonstrated in cystic fibrosis. She also typified the problems resulting from the disease and exacerbated by high environmental temperatures reaching 45ºC. Her illness should alert paediatricians in the Arab world to the possibility of cystic fibrosis in patients with a metabolic alkalosis and electrolyte depletion.
**Brief overview of the S549R (T→G) Cystic Fibrosis Gene Mutation**

Cystic fibrosis (CF) is due to variations in DNA which result in modifications in the sequence, structure, function and/or expression of the cystic fibrosis transmembrane conductance regulator (CFTR) gene situated on chromosome 7\(^{26}\). Over 1000 CF-causing mutations have been identified worldwide in the CFTR gene\(^{27}\). While the ΔF508 mutation accounts for over 60% of these alterations there are a number of important mutations being recognised among differing ethnic groups\(^{26, 27}\). Among these are the several mutations that have been described in exon 11 of the CFTR. The mutation designated S549R (T→G) is a missense mutation occurring at nucleotide 1779 that is the result of a G (guanine) to T (thymine) transversion. The consequence of this mutation, in codon 549, is the replacement of a serine by an arginine in the first nucleotide binding domain of the CFTR. Serine is a highly conserved amino acid in this important binding domain and its replacement by an arginine residue is expected to result in a severe chloride dysfunction and hence severe clinical disease\(^{28}\). S549R is defined as a Class II mutation\(^{28-32}\). These are associated with defective protein processing. The ab normally processed protein fails to progress through the biosynthetic pathway and is degraded. The protein is thus either missing or present in reduced amounts in the apical membrane. It is thought that the mutation prevents the protein from being properly post-translationally glycosylated. This results in incorrect folding, so that the protein is recognised as abnormal and degraded\(^{28-32}\). These events appear to be similar to those seen with the ΔF508 mutation in that the misfolded protein is trapped in the endoplasmic reticulum prior to its degradation\(^{31, 32}\).

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\(^{E}\) This overview gave rise to the paper: The S549R (T→G) Cystic Fibrosis Mutation\(^{39}\)
The existence of the mutation S549R (T→G), was first reported to the Cystic Fibrosis Genetic Analysis Consortium on the 9th of February 1990 by Kerem and Tsui. The individual who carried the CF chromosome was a non-Ashkenazi Jewish patient from Morocco who also carried the ΔF508 mutation on the other allele. Further reference to this and one other Moroccan patient was made by Kerem et al. who studied cystic fibrosis mutations in Jewish communities. It was reported that CF mutations vary within each Jewish ethnic group, each one having its own repertoire of mutations. S549R was found only in these two patients from Morocco and it thus accounted for 6% of the identified Jewish CF patients from that country.

There have been four separate mutations described in codon 549, viz. S549R (A→C), S549I, S549R (T→G), and S549N. A study of 27,177 CF chromosomes from 29 European and 3 North African countries showed that of the 272 mutations examined, S549R occurred on 12 occasions (0.04%) and the S549N on 16 occasions (0.059%). Interestingly, despite its general rarity, the S549R mutation accounted for 2.6% of all mutations in Algeria. However, a recent detailed study of 10 Algerian CF families revealed no patients with a mutation on exon 11.

Another important report in the literature regarding the S549R mutation is from Saudi Arabia. Banjar et al. described a single individual who was homozygous for the mutation. The patient was a Saudi native who came from the Eastern Province of Saudi Arabia. The affected individual had pancreatic sufficiency and mild chest disease. However, El-Harith et al. investigated 15 Arab children from 12 families who had CF. No examples of S549R were detected and it is of significance that the
families studied were all from the Eastern Region from where the one previous Saudi patient with S549R had been identified.

The data from Eastern Arabia points to S549R (T→G) being a mutation causing severe clinical disease and probably as severe as found in CF. The founding mutation may have originated in Arabia. However, there is considerable ethnic variation between the UAE and Oman, and there is little evidence of the mutation in Bedouin Saudis. The reports of sporadic alleles from Israel, North Africa and France confuse the issue further. Current evidence suggests the origins lie in Bedouin of Eastern Arabia.

Study of what appeared at first to be a rare or private mutation is contributing to our understanding of genotype-phenotype correlation. To date the distribution of the mutation does not fit patterns of historical population migration such as that associated with the ΔF508 mutation\textsuperscript{28} but it is found mainly in the Bedouin Arab people of Oman and the UAE. Disease severity in the homozygous patient appears to be very severe. It can be postulated that this severity, in itself, probably accounts for the early demise of those with the disease in the early times. With the introduction of the medical services in the Gulf Region recognition has occurred of the importance of this mutation in the causation of CF in the region.
Chapter summary

I have presented data in this chapter to support my contention that CF due to mutation S549R (T→G) results in severe clinical disease. My prior experience with severe CF had been with children having the mutation ΔF508. I felt that it would, therefore be important to establish a factual basis to my feeling that the clinical disease produced by S549R (T→G) mutation was more severe than that produced by ΔF508. I have accumulated what evidence I could gather to support this hypothesis. In order to control such factors as health care standards, climate, diet and culture I should have acquired a larger matched group of children with CF due to ΔF508. However, the sex/age matched patients presented are the largest group to be identified in the UAE. Thus, I do not have strong evidence to support my claim that the S549R mutation produces a more severe clinical disease. There is sufficient evidence to state that the mutation creates a clinical disease, which is at least equal in severity to that of ΔF508.

The clinical cases are presented to reinforce the practical use of mutational knowledge in the clinical setting. The harsh climate of the region does cause severe salt depletion and dehydration and infants with CF are particularly vulnerable. The true cause of their dehydration can be missed easily. The finding of an Omani child, later shown to have CF, from the neighbouring town of Al-Buraimi, stimulated the idea of looking further at the clinical and genetic pattern of CF in the neighbouring country of Oman. Information about these investigations is presented in a later chapter.
Finally, the rarity of reports about the S549R mutation prompted a summary of the known facts. To reduce repetition, the overview has been markedly abridged and the remaining information presented in the next chapter.
References


Chapter 11

Does the Cystic Fibrosis Mutation S549R (T→G) Always Produce Severe Clinical Disease?

“Since ‘tis Nature’s law to change, Constancy alone is strange”

Works
A dialogue between Strephon and Daphne, I I
John Wilmot
Earl of Rochester (1647-1680)

The information that has been presented has supported the view that all patients who carry the CF mutation S549R (T→G) have severe clinical disease. However, to date I have reported only on patients who are homozygous for the mutation. With increasing access to patients throughout the UAE and from the ever-expanding patient pool, we encountered one CF patient who had mild clinical disease. At this time he proved to be a unique patient and family in the UAE. Hence, the clinical details of this older child are presented in this chapter (Section I) along with the relevant molecular genetic findings. These findings indicated that in the heterozygote, severe disease does not always ensue.

A close relationship had been established, by this time, with the Paris-based group led by Professor Emmanuelle Girodon. They had acted as a peer review group for us and had provided additional and separate laboratory confirmation of our results. Following the publication of a joint paper with the group on the clinical severity and mutation
identification of CF in the UAE, we were approached by another French molecular
genetic group. Professor Mireille Claustres from Montpellier had been working on CF
mutations at a molecular and cellular level. Her team had observed some patients in
Europe, of North African descent, who carried the S549R CF mutation, yet appeared
to have mild clinical disease. The resultant cooperation produced a paper with a very
interesting finding. I have included the full text of this paper later in this Chapter
(Section II) as it was published in 1999.

Section I

Mild Clinical Phenotype Associated with R1158X/S549R (T→G) CFTR
Genotype

CF is due to variations that modify the sequence, structure, function and/or
expression of the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Besides
ΔF508 (which accounts for 66% of all CF chromosomes studied so far), more
than 1000 other CF-causing mutations have been identified worldwide in the CFTR
gene. This allelic heterogeneity partly explains the remarkable clinical heterogeneity
of CF. Specific mutations, however, have been also reported to be associated with a
wide spectrum of clinical manifestations. Genotype-phenotype correlation analyses
thus contribute to providing unique information on the unravelling of CFTR function,
prognostic evaluations and optimal patient management. We have shown that in the
UAE, two mutations, ΔF508 and S549R (T→G), account for 88% of CF alleles and
characterise 95% of the affected CF families referred to us. All patients that we
investigated were homozygous for either of the two mutations. Those of Bedouin
origin were S549R (T→G) homozygotes while those of Baluch lineage were ΔF508
Moreover, the clinical presentation associated with S549R homozygosity is quite homogeneous and shows an extreme degree and course of CF severity – and may be comparable to that associated with ΔF508<sup>6</sup>–<sup>7</sup>. Reported here is the identification of the first compound heterozygote patient in the UAE, who harbours mutations S549R/R1158X in his CFTR gene. This patient presents with a surprisingly atypical, mild form of CF.

**Clinical presentation**

AM, a UAE national (Emirati), was born in 1984 by a normal vaginal delivery; his birth weight was 4.5 kg. He was first referred to a Paediatric clinic at eight days of age because of constipation. At 3.5 months of age, he presented with an attack of wheezing, a condition which became recurrent. He was at this stage treated as a child with bronchial asthma. At the age of three years, he experienced episodic bouts of diarrhoea and his stools were reported as greasy and offensive. A sweat test was performed at this stage and it revealed a sweat chloride concentration of 105 mEq/L. He was treated with pancreatic extract granules at this time. He continued to have mild generalised wheezing but there was no evidence of lower respiratory tract infection, especially of a bacterial nature. Additionally, no finger clubbing was present and radiological examination of his chest was reported as normal. At the age of three and a half years he developed a rectal prolapse. This was managed by a medical regimen, but subsequently recurred at the age of five years and was accompanied by bouts of abdominal pain. Four months later he developed a meconium ileus-like syndrome and intestinal obstruction. He underwent surgical treatment but the details of this are not obtainable. One sibling was reported to have
died from severe lower respiratory infection but a diagnosis of CF was not sought or excluded.

The patient’s family are reported to be very non-compliant with prescribed treatment. The patient has attended follow-up clinics infrequently over many years and failed numerous appointments. Overall, he receives only occasional treatment for bouts of lower respiratory tract infection. He has been prescribed pancreatic enzyme supplements on the basis of clinical evaluation only based on the presence of frequent, greasy and offensive stools. No formal exocrine pancreatic function tests have been performed to establish pancreatic sufficiency or otherwise. His pancreatic extract capsules are taken irregularly.

The patient, who is now 16 years old, is in relatively good health, well nourished and has developed normal puberty changes. His height was 166 cms. (55%) and his weight was 52 kg. (25%). His weight, however, had remained around the 5th percentile for the first nine years of his life and had gradually increased to the 25% over the last six years. His peak flow has been measured at 400 L/second, but more formal spirometry had not been performed by the supervising doctor. Current physical examination revealed a youth without finger clubbing, with a few scattered crepitations in his chest and an occasional cough but who was otherwise generally well.

**CFTR mutations**

A blood sample was taken for CFTR genotype determination at age 15 years. The patient’s DNA sample was extracted from leukocytes isolated from 5 ml of venous
blood collected in EDTA tubes. DNA extraction and detection of CFTR mutations were carried out according to the experimental protocols that we have described previously. Results indicated that AM is a compound heterozygote harbouring mutation S549R (T→G) in exon 11 of one CF allele and the nonsense mutation R1158X in exon 19 of the other allele.

Discussion
We had to date observed that, in the UAE, mutations ΔF508 and S549R (T→G) accounted for 88% of CF chromosomes and that CF patients were homozygous for either of the two mutations. Moreover, the clinical phenotypes associated with both mutations are quite homogeneous and extremely severe, including dramatic losses of pulmonary function.

The patient described here is the first compound heterozygote that we have characterised as part of our programme on CF in the UAE. Moreover, his R1158X/S549R (T→G) CFTR genotype is associated with an atypically mild course of the disease.

R1158X had originally been reported as a rare mutation with, for example, one allele reported in Spain and Portugal, and two alleles in southern France and Canada. Recently, it has been observed that its frequency is higher in Greece, where it is found to constitute 0.8% of CF mutations and in southern Italy, where it forms 1.3% of the total CF mutations. Genotype-phenotype correlations for this mutation have indicated that R1158X homozygosity underlies a very severe expression of CF and a patient with ΔF508/R1158X genotype had experienced several complications of the disease. Thus, although a patient with R1158X/S549R (T→G) genotype could have
been expected to present with severe clinical manifestations, we find that this was not the case. The fact that the Emirati patient investigated lives in and has experienced the same environmental and medical conditions as the subjects that we have studied before\textsuperscript{5-7} prompts us to believe there must be attenuating genetic factors. Indeed, Duarte et al.\textsuperscript{10} have reported that a complex allele containing two mutations, R334W and R1158X, is associated with reduced levels of correctly processed mRNA and also that a patient with the complex genotype R334W-R1158X/ΔF508 presented with pancreatic sufficiency and an atypical course of CF. It has also been shown that the sequence change –102(T→A) in the CFTR minimal promoter attenuates the clinical severity of mutation S549R (T→G), so that the complex allele [-102(T→A) + S549R (T→G)] is associated with milder forms of CF than allele S549R (T→G) alone\textsuperscript{15}. Yet, AM’s DNA does not have mutation –102(T→A), Moreover, we have so far, failed to identify any additional mutation after screening both parents’ DNA for any of the 31 most frequent mutations detected by the OLA CF kit (Perkin-Elmer) and scanning CFTR exons 3-6a, 7-15 and 17b-23 by polymerase chain reaction/ denaturing gradient electrophoresis method described by Costes et al.\textsuperscript{16}. Nonetheless, we cannot exclude the possibility of an additional DNA variation that could contribute to the observed phenotype effect.

Although almost all of the previously analysed nonsense CFTR mutations have been associated with either considerable transcript reduction or production of aberrant transcripts (skipping)\textsuperscript{17}, an explanation for the mild phenotype might be that the transcript corresponding to the R1158X allele is stable and correctly spliced. Such a mechanism may be supported by the fact that there was no exon skipping or instability in the case of mutation R1162X as described in three patients who were unrelated\textsuperscript{17}. It has, therefore, been suggested that a non-standard decoding mechanism
(e.g. readthrough) may contribute to alleviate the molecular consequences of the mutation (CF patients carrying R1162X have, in fact, milder pulmonary presentations). As the R1158X mutation is located four codons upstream from codon 1162, it could be associated with the same non-standard decoding mechanism that rescues the message and thus produces milder symptoms. The extent of the rescue may depend on CFTR-independent transacting elements and account for inter- and intra- familial phenotype variations. It will be very informative to examine CFTR transcripts in this patient.
Section II

The paper below is a reproduction of that published in Human Genetics. It is included to illustrate another modifying mechanism altering clinical severity.

**Complex allele [-102T→A + S549R (T→G)] is associated with milder forms of cystic fibrosis than allele S549R (T→G) alone**\(^{15}\).

CF is a generalised exocrine disorder characterised by a highly variable clinical presentation involving the pulmonary, digestive and reproductive systems\(^2\). Both severity of the disease and rate of progression of CF show an extreme heterogeneity; some variation may result from the type of mutations of the CFTR gene. Besides the existence of over 800 different CFTR gene defects\(^3\), the complex clinical heterogeneity in CF might be further explained by possible interactions between mutations, as well as with environmental and other genetic modifiers. A number of studies, however, have now demonstrated a strong (although not absolute) correlation between pancreatic status and CF genotype. Indeed, pancreatic insufficiency is associated with one or two “mild” mutations (of classes 4 or 5) and a milder phenotype\(^{18,19}\). The involvement of specific CFTR genotypes in the variability of lung disease is still poorly understood, as a wide range of severity is observed even in patients with identical CFTR genotypes. Discrepancies between anticipated and observed phenotypes are commonly attributed to “modifier genes” but the possibility of multiple sequence variations on the same allele has received little attention\(^20\). Indeed, in almost all laboratories, genetic analysis is usually considered complete when two mutations have been found. Improved strategies and techniques for scanning the CFTR sequences, however, have contributed to identify several alleles
containing more than one mutation. Such additional second site alterations can modulate the effects of the main mutation\textsuperscript{21, 22}.

Recently, we evidenced a novel combined CF allele containing a previously described missense mutation S549R (T→G) in exon 11 and a first putative regulatory mutation in the minimal CFTR promoter region (-102T→A)\textsuperscript{23}. This complex allele [-102T→A +S549R (T→G)] was identified in two unrelated patients from Southern France, both considered as having a mild form of CF. Mutation S549R (T→G) alone, first described by Kerem et al\textsuperscript{24} in a Moroccan-Jewish patient, has since been shown to be associated with very severe clinical phenotypes in all children reported to date\textsuperscript{5, 6, 24}.

In order to better understand the discrepancy between the phenotypes presented by patients carrying mutation S549R, we have conducted a collaborative study aimed at comparing ethnic, clinical and genetic data of patients with this mutation. We report a genotype-phenotype correlation analysis in six patients carrying the [-102T→A + S549R (T→G)] complex allele and in 16 patients homozygous for S549R (T→G) alone.

**Methods and materials**

**Recruitment of CF Patients**

A total of 22 CF patients from the Mediterranean basin (France, Spain) [4 females and 2 males] and Arab countries (Oman, UAE) [10 females and 6 males] were recruited for the study. Mutation S549R (T→G), which accounts for less than 0.5% of 27,177 CF European chromosomes, has been reported to have a relatively high frequency in Algeria (2.6%)\textsuperscript{27}. Recently, Frossard et al.\textsuperscript{5} showed that it had a very high frequency
in the United Arab Emirates, where it appears as the major CF mutation and accounts so far for 100% of 20 CF chromosomes from unrelated Bedouin families.

Clinical variables taken into account in this study included gender, current age, age at diagnosis, first clinical symptoms, sweat chloride concentrations, weight, height, Schwachman-Kulczcki general status score (100 is the best score), forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC), lung colonisation with bacterial pathogens and age at onset, history of meconium ileus and pancreatic status.

**DNA analysis**

Specific analysis of sequence (-102T→A) was performed in all 22 patients by scanning the minimal CFTR promoter region using a DGGE assay from genomic DNA. PCR products that gave abnormal migration were subsequently sequenced on an ABI 377 automated sequencer with specific primers\(^2\). This allowed the detection of alteration (-102T→A) in 6 unrelated CF patients. In all cases, careful familial segregation studies showed that (-102T→A) was associated *in cis* (on the same allele) with mutation S549R (T→G).

**Haplotype analysis**

Sufficient DNA samples from individuals carrying the complex allele only were available for further genotyping. Six intragenic CFTR polymorphic sites (IVS8 (T)n, M470V, 3601-65C/A and microsatellites IVS8CA, IVS17BTA, IVS17BCA) and four extragenic restriction fragment length polymorphisms (RFLPs) (XV-2c, KM.19, J44, J3.11) were analysed by appropriate methods, after amplification by PCR. Whenever possible, haplotypes were determined by familial segregation analysis. In two cases, microsatellite haplotypes were derived from previous studies.
**Statistical analysis**

All quantitative variables were expressed as mean +/- standard deviation (SD) and/or as median (with associated ranges). Data from patients homozygous, or compound heterozygous, for the [-102T→A + S549R (T→G)] complex allele were pooled (n = 6) and compared with all 16 matched patients homozygous for mutation S549R (T→G) alone. In the case of nonparametric variables with non-normal distribution (current age, age at diagnosis, sweat chloride values and Shwachman-Kulczycki score), Mann-Whitney tests were used to assess differences among both groups. Categorical variables (age at onset of lung colonisation by *Pseudomonas aeruginosa* and pancreatic status) were analysed by Chi-squared and Fisher exact tests, respectively. All statistical analyses were performed with an SAS statistical software package.

**Results**

Sixteen patients from Oman and the UAE were found to be homozygous for mutation S549R (T→G) alone (Table 13). None of the children had presented with meconium ileus at birth, but all were pancreatic insufficient and had a very severe lung disease with a high rate of *Pseudomonas aeruginosa* and *Staphylococcus aureus* infections occurring at a very early age and a rapid pulmonary decline. Three of them died of pulmonary failure, two at five months and one at 6.5 years of age. Due to the young age of the patients, FEV1 and FVC could not be performed and the severity of the lung disease was confirmed by radiological analysis.

In six CF patients from France and Spain, an additional sequence variation (-102T→A) was identified on S549R (T→G) alleles (Table 14). Five patients were compound heterozygotes for the complex allele and another mutation, two with ΔF508 and three with, respectively R334W, G542X or S945L, one patient was
homozygous for the [-102T→A + S549R (T→G)] complex allele. Almost all the patients carrying the double mutant allele who were investigated so far have a mild to moderate cystic fibrosis presentation (Table 14). Both markers of pulmonary function, FEV1 and FVC indicate that lung disease among these patients is relatively attenuated except for patients P2 in whom FEV1 and FVC are 19.3% and 38.7% respectively. This patient was genotyped for CFTR mutations at 34 years of age because of dyspnoea and bilateral bronchiectasis but had normal sweat tests. Interesting also is the case of patient P4, who was diagnosed with CF at two months of age because of recurrent airways infections. She had no further medical follow-up for CF for more than 22 years, until a sudden deterioration of pulmonary function lead to a pulmonary transplantation at age 23.

The overall CF status of most patients carrying [-102T→A + S549R (T→G)] has been considered as mild by their physicians. In agreement with this observation is the case of the unique patient who is homozygous for the complex allele. This patient, diagnosed at six years of age for episodic bronchitis, is the product of a consanguineous mating between first cousins from Algeria. She presents with a very mild disease at the age of 34 years and is considered as almost pancreatic sufficient by her physician.

Clinical features of the six patients carrying [-102T→A + S549R (T→G)] were compared with those of the sixteen children homozygous for S549R (T→G) alone (Table15). Age at diagnosis was higher in the first group, although the difference was not statistically significant, most probably due to the small size of the cohorts. Current age of the patients was higher in the group with the complex allele (p = 0.0032). No significant differences were found when comparing Pseudomonas aeruginosa
colonisation in both groups. A statistically significant lower age of the onset of Pseudomonas colonisation, however, was found in the group with the [-102T→A + S549R (T→G) complex allele (p = 0.0022). Fifty percent of patients carrying the (-102T→A) sequence alteration were pancreatic insufficient as opposed to 100% of S549R (T→G)/S549R (T→G) patients (p = 0.013). Sweat chloride concentrations were significantly lower (p = 0.028) and Shwachman-Kucycki scores were significantly higher (p = 0.004) in the [-102T→A + S549R (T→G)] double mutant group.

A common extended [-102T→A + S549R (T→G)] haplotype could be derived in almost all CF patients who were studied (Table16).

**Discussion**

Mutation S549R is a serine to arginine change resulting from a T-to-G substitution at nucleotide 1779, a site included in a six-amino acid region defined as “C consensus”, which appears to be a unique feature of the ABC transporter superfamily. As serine 549 is a highly conserved amino acid in the first nucleotide-binding domain of the CFTR protein, its alteration is expected to cause a severe clinical presentation. Previous functional *in vitro* assays for CFTR activity have shown that the R549 mutant failed to yield the fully glycosylated form of the protein, which subsequently failed to traffic to the correct cellular location, as also occurs with the ΔF508 mutant. S549R was originally considered as a “severe” mutation with respect to pancreatic insufficiency. It has been classified as a class II mutation (defective protein processing). Three other changes occurring at the same residue, S549R (A→C), S549N (G→A) and S549I (G→T), have all been reported as severe. In addition, recent studies have evidenced that clinical features associated with S549N (G→A)
and S549R (T→G) homozygosity (in Pakistan and the UAE respectively) were quite homogeneous and showed an extreme degree and course of CF severity. To date, the nucleotide change (-102T→A) has only been found to occur in combination with the mutation S549R (T→G), and only on CF chromosomes. Investigations of haplotypes with both intragenic and extragenic markers suggests that allele [-102T→A + S549R (T→G)] has a single origin in the families analysed here.

Most CF patients carrying the complex allele were diagnosed at a much later age, had pancreatic function relatively well preserved, a milder lung disease and a better overall clinical course, compared with the patients carrying S549R (T→G) alone. AS no other sequence alteration was found in the coding sequence of the CFTR, these observations suggest that the promoter change (102T→A) may lead to milder CF phenotypes in patients carrying the severe mutation S549R (T→G).

A few reports have drawn attention to the existence of complex genotypes and to their possible involvement in the phenotypic expression of disease-causing mutations. They provide a unique opportunity to study the effects of two in cis-interacting gene defects on gene expression. For instance, the missense mutation R553Q when carried on a ΔF508 allele, has been shown to be associated with almost normal sweat electrolyte values in a patient with severe CF, suggesting that R553Q may partially suppress the ΔF508 chloride transport defect in sweat glands of this patient. It was later demonstrated in a yeast expression system model that the abnormal folding produced by Δ508 could be partially corrected by the additional, “revertant” mutation R553Q. Because the majority of maturation-incompetent mutants (such as ΔF508 or R549)
retain at least some residual function, procedures that would allow a proportion of CFTR mutant proteins to escape the quality control machinery at the endoplasmic reticulum, could form the basis for therapeutic strategies. Importantly, Brown et al.\textsuperscript{31} showed that “chemical chaperones” that stabilise protein structure, such as glycerol, have the ability to restore normal trafficking behaviour of the $\Delta$F508-CFTR protein. As suggested by Seibert et al.\textsuperscript{32}, another way that enables some proteins to escape from the endoplasmic reticulum is the flooding of the control machinery by stimulating over-expression. Chemical agents such as sodium butyrate have been reported to promote $\Delta$F508-CFTR movement to the cell surface at levels that are sub-wild-type\textsuperscript{33}. Consistent with this latter approach, it is tempting to speculate that the (-102T→A) sequence alteration in the minimal CFTR promoter could stimulate the CFTR expression at sufficient level to partially compensate the S549R (T→G) molecular defect. To test this hypothesis, \textit{in vitro} and \textit{in vivo} expression studies are now in progress.

In summary, the combined allele [-102T→A + S549R (T→G)] appears to be associated with a better prognosis of CF disease, suggesting that the (-102T→A) modification in the CFTR promoter may, at least partially, compensate the severity of mutation S549R (T→G). In agreement with previous studies, our findings further support the idea that double mutant alleles may be more common than expected and could influence the status of clinical phenotypes. Such complex alleles would thus have important implications for molecular diagnosis and genetic counselling.
### Table 13

Clinical characteristics of 16 patients homozygous for the S549R (T→G) mutation

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patient</th>
<th>Genotype</th>
<th>Geographic background</th>
<th>Sex</th>
<th>Current age</th>
<th>Age at death</th>
<th>Sweat Cl (mmol/l)</th>
<th>Height %</th>
<th>Shwachman Kulczycki Score</th>
<th>Pseudomonas aer.</th>
<th>Age at onset</th>
<th>Meconium ileus</th>
<th>Pancreatic insufficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S549R</td>
<td>D1</td>
<td>S549R</td>
<td>Oman</td>
<td>F</td>
<td>6 months</td>
<td>5 months</td>
<td>ND</td>
<td>&gt;10</td>
<td>ND</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>S549R</td>
<td>D2</td>
<td>S549R</td>
<td>UAE</td>
<td>F</td>
<td>5 months</td>
<td>5 months</td>
<td>120</td>
<td>10</td>
<td>60</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>S549R</td>
<td>D3</td>
<td>S549R</td>
<td>UAE</td>
<td>M</td>
<td>2 years</td>
<td>2 years</td>
<td>133</td>
<td>5</td>
<td>55</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>S549R</td>
<td>D4</td>
<td>S549R</td>
<td>UAE</td>
<td>F</td>
<td>1 year</td>
<td>1 year</td>
<td>100</td>
<td>5</td>
<td>55</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>S549R</td>
<td>D5</td>
<td>S549R</td>
<td>UAE</td>
<td>F</td>
<td>4 months</td>
<td>4 months</td>
<td>150</td>
<td>5</td>
<td>55</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>S549R</td>
<td>D6</td>
<td>S549R</td>
<td>UAE</td>
<td>F</td>
<td>8 months</td>
<td>8 months</td>
<td>150</td>
<td>10</td>
<td>55</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>S549R</td>
<td>D7</td>
<td>S549R</td>
<td>UAE</td>
<td>F</td>
<td>8 months</td>
<td>8 months</td>
<td>150</td>
<td>10</td>
<td>55</td>
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<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>S549R</td>
<td>D8</td>
<td>S549R</td>
<td>UAE</td>
<td>M</td>
<td>3 months</td>
<td>3 months</td>
<td>150</td>
<td>10</td>
<td>55</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>S549R</td>
<td>D9</td>
<td>S549R</td>
<td>UAE</td>
<td>F</td>
<td>3.5 years</td>
<td>3.5 years</td>
<td>150</td>
<td>10</td>
<td>55</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>S549R</td>
<td>D10</td>
<td>S549R</td>
<td>UAE</td>
<td>F</td>
<td>3 years</td>
<td>3 years</td>
<td>150</td>
<td>10</td>
<td>55</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>S549R</td>
<td>D11</td>
<td>S549R</td>
<td>UAE</td>
<td>M</td>
<td>1 year</td>
<td>1 year</td>
<td>150</td>
<td>10</td>
<td>55</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>S549R</td>
<td>D12</td>
<td>S549R</td>
<td>UAE</td>
<td>F</td>
<td>3 months</td>
<td>3 months</td>
<td>150</td>
<td>10</td>
<td>55</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>S549R</td>
<td>D13</td>
<td>S549R</td>
<td>UAE</td>
<td>F</td>
<td>3 months</td>
<td>3 months</td>
<td>150</td>
<td>10</td>
<td>55</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>S549R</td>
<td>D14</td>
<td>S549R</td>
<td>UAE</td>
<td>F</td>
<td>3 months</td>
<td>3 months</td>
<td>150</td>
<td>10</td>
<td>55</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>S549R</td>
<td>D15</td>
<td>S549R</td>
<td>UAE</td>
<td>M</td>
<td>1 month</td>
<td>1 month</td>
<td>150</td>
<td>10</td>
<td>55</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>S549R</td>
<td>D16</td>
<td>S549R</td>
<td>UAE</td>
<td>M</td>
<td>1 month</td>
<td>1 month</td>
<td>150</td>
<td>10</td>
<td>55</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>
# Table 14

**Clinical characteristics of six patients carrying the complex allele [\(-102T\rightarrow A + S549R (T\rightarrow G)\)]**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patient P1</th>
<th>Patient P2</th>
<th>Patient P3</th>
<th>Patient P4</th>
<th>Patient P5</th>
<th>Patient P6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genotype</strong></td>
<td>-102T→A + S549R (T→G)/ 102T→A + S549R (T→G)</td>
<td>102T→A + S549R (T→G)</td>
<td>102T→A + S549R (T→G)/R334W</td>
<td>102T→A + S549R (T→G)/ΔF508</td>
<td>102T→A + S549R (T→G)/G542X</td>
<td>102T→A + S549R (T→G)/S945L</td>
</tr>
<tr>
<td><strong>Geographic background</strong></td>
<td>South of France</td>
<td>South of Spain</td>
<td>South of France</td>
<td>North of France</td>
<td>North of France</td>
<td>South of France</td>
</tr>
<tr>
<td><strong>Ethnic background</strong></td>
<td>Jews of Algeria</td>
<td>Unknown</td>
<td>Jewish</td>
<td>Unknown</td>
<td>North African</td>
<td>Jewish</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td><strong>Current age (years)</strong></td>
<td>34</td>
<td>38</td>
<td>6</td>
<td>31</td>
<td>23</td>
<td>18</td>
</tr>
<tr>
<td><strong>Age at diagnosis</strong></td>
<td>6</td>
<td>35</td>
<td>3.5</td>
<td>2 months</td>
<td>4 months</td>
<td>9</td>
</tr>
<tr>
<td><strong>Age at first clinical symptoms</strong></td>
<td>Unknown</td>
<td>3</td>
<td>2 months</td>
<td>4 months</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td><strong>Sweat Cl- (mmol/l)</strong></td>
<td>Unknown</td>
<td>&lt;60</td>
<td>122</td>
<td>66</td>
<td>72</td>
<td>85</td>
</tr>
<tr>
<td><strong>Height %</strong></td>
<td>60</td>
<td>50</td>
<td>Unknown</td>
<td>60</td>
<td>26</td>
<td>30</td>
</tr>
<tr>
<td><strong>Weight %</strong></td>
<td>60</td>
<td>50</td>
<td>Unknown</td>
<td>40</td>
<td>12</td>
<td>&lt;25</td>
</tr>
<tr>
<td><strong>Shwachman</strong></td>
<td>85</td>
<td>Unknown</td>
<td>85</td>
<td>Unknown</td>
<td>60</td>
<td>85</td>
</tr>
<tr>
<td><strong>Kazolewski score</strong></td>
<td>30</td>
<td>19.3</td>
<td>89</td>
<td>60</td>
<td>59</td>
<td>89</td>
</tr>
<tr>
<td><strong>FEV1 %</strong></td>
<td>50</td>
<td>38.7</td>
<td>96</td>
<td>100</td>
<td>76</td>
<td>115</td>
</tr>
<tr>
<td><strong>Lung colonisation</strong></td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>(age of onset)</strong></td>
<td>12</td>
<td>-</td>
<td>3.5</td>
<td>22</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td><strong>Meconium ileus</strong></td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td><strong>Pancreatic insufficiency</strong></td>
<td>Yes (minimal)</td>
<td>No</td>
<td>No</td>
<td>Yes (minimal)</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>
### Table 15

Comparison of clinical features between CF patients carrying the complex allele and those carrying S549R (T→G) alone

<table>
<thead>
<tr>
<th>Feature</th>
<th>Patients with[-102T→A + S549R (T→G) n = 6]</th>
<th>Patients with S549R (T→G) n = 16</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean +/- SD</td>
<td>Mean +/- SD</td>
<td>Median (5&lt;sup&gt;th&lt;/sup&gt; – 95&lt;sup&gt;th&lt;/sup&gt; %)</td>
</tr>
<tr>
<td>Current age (years)</td>
<td>25 +/- 11.8</td>
<td>27 (6-38)</td>
<td>5 +/- 3.35</td>
</tr>
<tr>
<td>Age at diagnosis (years)</td>
<td>9 +/- 13.2</td>
<td>4.75 (0.16-35)</td>
<td>0.88 +/- 1</td>
</tr>
<tr>
<td>Sweat Cl⁻ (mmol/l)</td>
<td>79 +/- 27.13</td>
<td>72 (50-120)</td>
<td>117.2 +/- 25.1</td>
</tr>
<tr>
<td>Shwachman-Kulczcki score</td>
<td>78.8 +/- 12.5</td>
<td>85 (60-85)</td>
<td>50 +/- 7.3</td>
</tr>
<tr>
<td>Age at onset of lung colonisation</td>
<td>11.5 +/- 6.7</td>
<td>11 (3.5-22)</td>
<td>1 +/- 1.43</td>
</tr>
<tr>
<td>Lung colonisation (number positive/number studied) %</td>
<td>5/6 83</td>
<td>13/16 81</td>
<td>NS</td>
</tr>
<tr>
<td>Pancreatic insufficiency (number positive/number studied) %</td>
<td>3/6 50</td>
<td>16/16 100</td>
<td>0.013</td>
</tr>
</tbody>
</table>
Table 16

CFTR haplotypes for seven [-102T→A + S549R (T→G)] chromosomes

<table>
<thead>
<tr>
<th>Patient</th>
<th>Genotype</th>
<th>XV2c</th>
<th>KM9</th>
<th>J44</th>
<th>IVS8CA</th>
<th>IVS8(T)n</th>
<th>M470V</th>
<th>IVS17BTA</th>
<th>IVS17BC</th>
<th>360165C/A</th>
<th>J3.11</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>[-102T→A + S549R (T→G)]</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>23</td>
<td>7</td>
<td>1</td>
<td>34</td>
<td>13</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>[-102T→A + S549R (T→G)]</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>23</td>
<td>7</td>
<td>1</td>
<td>34</td>
<td>13</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>P2</td>
<td>[-102T→A + S549R (T→G)]</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>23</td>
<td>7</td>
<td>1</td>
<td>34</td>
<td>13</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>R334W</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>17</td>
<td>7</td>
<td>2</td>
<td>46</td>
<td>13</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>P3</td>
<td>[-102T→A + S549R (T→G)]</td>
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<td>2</td>
<td>1</td>
<td>23</td>
<td>7</td>
<td>1</td>
<td>34</td>
<td>13</td>
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<td>1</td>
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<td></td>
<td>∆F508</td>
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<td>2</td>
<td>1</td>
<td>23</td>
<td>9</td>
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<td>31</td>
<td>13</td>
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<td>1</td>
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<tr>
<td>P4</td>
<td>[-102T→A + S549R (T→G)]</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>23</td>
<td>7</td>
<td>1</td>
<td>34</td>
<td>13</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>∆F508</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>23</td>
<td>9</td>
<td>1</td>
<td>32</td>
<td>13</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>P5</td>
<td>[-102T→A + S549R (T→G)]</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>23</td>
<td>7</td>
<td>1</td>
<td>34</td>
<td>13</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>G542X</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>22</td>
<td>9</td>
<td>1</td>
<td>33</td>
<td>13</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>P6</td>
<td>[-102T→A + S549R (T→G)]</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>23</td>
<td>7</td>
<td>1</td>
<td>34</td>
<td>13</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>S945L</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>16</td>
<td>7</td>
<td>2</td>
<td>29</td>
<td>13</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
Overview

From the information presented in the two research papers it is clear that the CF mutation S549R (T→G) is not invariably associated with a severe clinical disease. Even in the homozygous state it is not always so, but to date, we have not encountered a patient in the UAE with the additional promoter mutation which can alter biological events and eventually end in the production of a milder clinical disease. All patients have been tested in France, however, for this possibility. It is of interest that the “European” patients with the additional complex were of North African origin in the main and one was of Arab descent. It is of further interest that three patients were of Jewish background and that the original patient with S549R (T→G) mutation, described by Kerem and Kerem, was of North African Jewish descent like these patients. I later postulate that the origins of the S549R (T→G) may have been from the Yemen. At one time this country had a large and well integrated Jewish population who have subsequently moved to Israel. This former close integration may have resulted in some interesting genetic alterations. Unfortunately there is no information available about CF and its mutations from the Yemen. My own observations there revealed continuing evidence of the former Jewish population who had lived closely with the Moslem people. Many homes still carry Jewish symbols and evidence exists of the famous Jewish silversmiths and their products. Former synagogues are converted to Mosques.

The strict control of permanent resident visas and citizenship in the UAE makes it highly unlikely that further studies will reveal a large pool of heterozygote combinations within the UAE. However, we have encountered a ΔF508 homozygote
CF child who is an offspring of Syrian parents who have been given UAE citizenship.

Similarly, a UAE citizen who is the child of an Arab father and a Pakistani mother has CF due to the mutation 3849 + 10Kb C→T/G628R.
References


3. Cystic Fibrosis genetic analysis consortium, CFGAC, Http//genet.sickkids.on.ca.


Cystic fibrosis in Omani Children

“The only trouble is, we can not understand what is happening to our neighbours”

Speech, Smethwick, 18th January 1906
Joseph Chamberlain (1836-1914)

In Chapter 10, I drew attention to the observation of cystic fibrosis in an Omani child, the first that we had encountered. While visiting Muscat, Oman to take part in a Paediatric Conference and to present data on CF in the UAE, I discussed the CF situation in Oman with colleagues there. Most Omani Specialist Paediatricians had trained in Western countries and were familiar with CF as it presents in the West. Further, the well-organised health system in Oman meant that complex patients with conditions like CF will be referred to one of the two Teaching Hospitals in Muscat, the capital. While the two countries, which form the Eastern Arabian Peninsula, have much in common there are sufficient ethnic and racial differences to make a close study of CF there of interest. It was agreed among the small group of interested clinicians that we would undertake an investigation into the presence of CF in Oman using the same protocols that had been used in the UAE. In addition all genetic studies would be performed in Al Ain, UAE in the University Department, using the same methods as described in this text. This Chapter therefore, contains the first observations on, CF, as it presents in Oman, and have lead to the first publications on the topic to appear in the medical literature.
We set out to answer two questions initially. The first was whether CF mutations in Oman were different to those in the UAE, and if not which were the common mutations associated with the disease in the Sultanate (Section I). The second related to the assessment of the clinical presentation and the severity of the condition in Omani children (Section II) with confirmed disease.

Section I
Identification of cystic fibrosis mutations in Oman

Introduction
Table 6 supports the view that each Arab country has an individual range of CF-causing mutations. Oman is one of the UAE’s neighbouring countries and shares some common genetic influences. The indigenous population of Oman is principally composed of Omani nationals who belong to different tribes that are all of Bedouin origin. Other genetic influences, however, have come from many diverse places. These include Yemen, East Africa, Iran and especially Baluchistan.

Subjects and methods
Mutational analysis was performed on 15 Omani families, which included 16 children suffering from CF (Table 17). Family names (corresponding to original tribal names) and detailed family histories allowed us to establish that 12 of the 15 families (including 13 patients) originally belonged to true Omani Bedouin tribes. Three other families were Omani nationals of Baluch (Pakistani) origin. Patient ages ranged from infancy to 8 years.
CF diagnosis was based, in all patients, on the typical clinical presentations including high sweat chloride concentrations. All patients had sweat chloride levels ranging from 90 to 130 mmol/l.

Patient DNA samples were extracted from leucocytes isolated from 2 ml of venous blood collected in EDTA tubes. DNA extraction and detection of mutations ΔF508 and S549R (T→G) were carried out according to the experimental protocols that were described previously.

**Results and Discussion**

The data presented in Table 17, indicates that, of the 16 CF Omani patients, 11 (two affected siblings and nine CF children from nine other families: age range: 4 months to 11 years) were homozygous for mutation S549R (T→G). All of these 11 patients were of Omani Bedouin descent and their sweat chloride concentrations ranged from 121 to 130 mmol/l. Two unrelated CF patients, who were of Baluch descent, were homozygous for the ΔF508: their sweat chloride levels were 109 (a one-year-old) and 127 (1.5-year-old) mmol/l. At this point, three patients (two Omani Bedouins and one Omani of Baluch descent) have other CFTR mutations that have not yet been identified. Their sweat chloride levels, however, were lower (90-117 mmol/l) and their ages ranged between three and eight years.

Considering the relatively small number of cases in this study, one has to remain cautious with the data interpretation. We have shown here, however, that the two
mutations ΔF508 and S549R (T→G) allow the characterisation of, respectively, 13% and 73% of CF families (12.5% and 69% of patients). Therefore, the combination of both mutations allows the detection of 87% of CF families and 81% of CF patients. The pattern of distribution of CF mutations found in this study from Oman is thus very similar to that observed in the UAE. Anecdotal observations from colleagues, however, seem to indicate that there is a lesser degree of genetic homogeneity in Oman.

The relationship of ΔF508 mutation in the homozygous state to Baluch origin appears to hold true for Oman as well as the UAE. The close ties of Oman to Makram in Baluchistan are discussed in the next chapter.
Table 17

Distribution by ethnicity and by ethnic origins of CF patients and families that were recruited and the number of alleles carrying either of the two mutations S549R (T→G) and ΔF508

<table>
<thead>
<tr>
<th>Ethnic origin</th>
<th>Number of families</th>
<th>Number of patients</th>
<th>Mutation</th>
<th>Number of CF alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bedouin descent</td>
<td>12</td>
<td>13</td>
<td>S549R (T→G)</td>
<td>22/26</td>
</tr>
<tr>
<td>Baluch origin</td>
<td>3</td>
<td>3</td>
<td>ΔF508</td>
<td>4/6</td>
</tr>
</tbody>
</table>
Section II

The finding that the mutational pattern in the sample of Omani patients is similar to that of the UAE patients that have been assessed poses the question as to whether the clinical presentations are similar. It should be remembered, however, that all the patients seen in the Emirates where under my clinical care. Thus, the protocols for management are standardised and based upon the currently published CF management guidelines used in the Western countries. The Symptomatic therapy used is founded on the current published knowledge such as that reviewed recently by Ratjen and Döring\(^3\). Similar standard treatment plans were not in place in Oman, hence a comparative outcome study could not be carried out.

This section then reports the clinical features and available mutational information on a larger group of Omani children with CF.

Cystic fibrosis in Omani children: clinical features and genotypes\(^2\)

Subjects and methods:

Omani children in whom a diagnosis of cystic fibrosis has been established were included in the study group. They were attending, either the Royal Hospital, Muscat, the Sultan Qaboos University Hospital, Muscat after referral from elsewhere in Oman, or they had been referred to the CF clinic at Tawam Hospital, Al Ain, United Arab Emirates (UAE) from neighbouring parts of Oman. Included were the group of 16 children described in Section I who had been studied in relation to their CF mutations.

Clinical data was collected on age at diagnosis, demographic details, sweat electrolyte levels, measures of clinical severity (Shwachman Score\(^14\)), Pseudomonas colonisation rates, lung disease, pancreatic function and evidence of previous meconium ileus,
genotype information and details of any parental consanguinity. The patients' DNA was screened for the 31 most frequent mutations detected by the OLA CF kit (Perkin-Elmer).

**Results:**

Thirty children with cystic fibrosis were identified, (14 males and 16 females). Nine families gave a family history compatible with CF. The thirty children were drawn from 26 unrelated families. All families were Omani nationals but four families were of Baluch descent and the remainder was of Bedouin descent.

Table 18 outlines the clinical details of the patients indicating that overall they have a mean sweat chloride level of 105 mmol/L. Table 19 shows that the S549R (T>G) mutation was the commonest CF mutation identified. The families of seven children declined to have mutational analysis performed.

**Discussion:**

The Hospitals in Muscat serve as the tertiary referral centres for the Sultanate. The patients were drawn from a wide geographic area within Oman. Consanguinity was present in 42% of the families, and this was at first cousin level.

The clinical disease manifestations indicated that the disease process is severe in Oman, with one third of the patients having established chronic lung disease with *Pseudomonas* colonisation being present in most of these. This is despite early diagnosis and ready access to free treatment within the country. Pancreatic insufficiency was present in all but one of the patients in whom the genotype has been
established, and in all the seven patients in whom we have not as yet identified the 
actual mutations. It is of interest that three patients have had a clearly documented 
episode of meconium ileus, which lead to an early diagnosis. Two of these patients 
were homozygous for the S549R (T→G) mutation and are the first that have been 
documented with meconium ileus associated with this mutation⁵.

Sweat chloride levels are very high in all but two patients. These two belong to the 
unidentified mutation group but they do have pancreatic insufficiency. The mean 
Schwachman Scores⁴ are within the moderate range of disease severity but within this 
group there are several severe patients. More importantly the mean age of the group 
was only 4.4 years, thus despite early diagnosis and treatment disease progression 
continues.

The identified genotype distribution is very similar to that found in the neighbouring 
United Arab Emirates with mutation S549R (T→G) being found in the majority of 
patients, but contrasts dramatically with that reported from Saudi Arabia⁶,⁷. Again, as 
in the UAE, the three patients homozygous for ΔF508 mutation were both OMANIs of 
Baluch descent. It is clear that there are major variations within the Arabian Peninsula 
in terms of genotypes. Within the UAE and Oman the S549R (T→G) mutation 
dominate, the I1234V is universal in Qatar⁸ and a different and wide range is found 
in Saudi Arabia with at least 17 different CFTR mutations having been described, 
none being dominant however⁶,⁷.

CF children are widely distributed throughout Oman. The disease pattern in the 
sample of patients presented is severe. Meconium ileus associated with S549R
(T→G) mutation has been recognised for the first time in two patients. Genotypes recognised to date are similar to the UAE, but there may be novel mutations still to be identified.
Table 18

The Clinical Details of 30 Omani Patients

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweat chloride mmol/L</td>
<td>105</td>
<td>29.8</td>
<td>27-140</td>
</tr>
<tr>
<td>Schwachman Score</td>
<td>64</td>
<td>12.5</td>
<td>41-90</td>
</tr>
<tr>
<td>Current age (in years)</td>
<td>4.4</td>
<td>3.5</td>
<td>1-14</td>
</tr>
<tr>
<td>Age at diagnosis (months)</td>
<td>11.8</td>
<td>10.7</td>
<td>3-48</td>
</tr>
</tbody>
</table>

n = 30
### Table 19

**Genotype and Phenotype distribution in 30 CF patients**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>R549/ R549</th>
<th>ΔF508/ ΔF508</th>
<th>Not Identified</th>
<th>Not Performed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Patients</td>
<td>13</td>
<td>3</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Chronic lung disease</td>
<td>5</td>
<td>0</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Pseudomonas colonisation</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Meconium ileus</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Pancreatic insufficiency</td>
<td>12</td>
<td>3</td>
<td>7</td>
<td>2</td>
</tr>
</tbody>
</table>
Overview

It is clear from the studies reported above, that the pattern of identified mutations is similar in the UAE and Oman. However, seven patients to date, have not had their specific mutation defined. This may well be explained by there being novel mutations present or that we simply have not been able to define them as yet. From the data presented from the UAE, the longer we have studied patients and the increasing number identified the more interesting and unusual are the findings that emerged. I suspect that this holds good for Oman also.

The pattern of immigration to Oman from the Baluchistan geographic area is again similar to the UAE. All patients had the ∆F508 mutation and were homozygous. This raises some interesting possibilities and has led to an hypothesis about the origins of the mutation. These ideas are presented in Chapter 13.
Figure 26 Three children
References


Chapter 13

Hypotheses regarding the origin and spread of the cystic fibrosis mutations S549R (T→G) and ΔF508

“Read no history: nothing but biography, 
For what is life without theory”

Contarini Fleming Part 1 Chapter 7
Benjamin Disraeli (1804-1881)

In this section I present two hypotheses. The first concerns the S549R mutation, which is speculation, based on some historical facts. My input was to visit the relevant areas in the Yemen and discuss what the traditional feelings were about the links between the Yemen and the UAE. The second is a published hypothesis about the ΔF508 and is reproduced in part below.

The mounting information that had been gathered about the once rare or “private” mutation S549R (T→G), permitted me to review what was known of its origins and clinical phenotype. From the single initial report in 1990, we have identified over sixty alleles carrying the mutation. The location in the Eastern Arabian Peninsula of those carrying the mutation does not fit any widely publicised pattern of human migration. It does seem to be found mainly in the Bedouin people of Oman and the UAE. An historical link does exist between these people and those in the Yemen, particularly in the Marib region. Indeed, a dormitory village of Al Ain, UAE has a population of purely Yemeni people of many years standing. Marib has been one of
the most famous areas in the Republic of Yemen since ancient times. It was well known historically as “Arabia Felix” or the “Land of the two Paradises”. A great civilisation flourished there under the famous Queen of Sheeba. It was also known for the massive and famous Marib Dam. This dam and its irrigation canals suggest that the Yemenis were the pioneers of irrigation systems. The presence of these canals allowed agriculture to flourish and wealth to accumulate and a large population to be supported. History and tradition state that in this region the collapse of the dam by a major earthquake, about one thousand years ago led to a subsequent famine and this caused a major disaster. Subsequent resultant emigration of the population occurred. To emigrate east involved crossing the Arid Quarter (Rhub al Khali) of the Arabian Desert, one of the most treacherous places on earth. To the North lay the Gulf coast a sparsely populated area. Certainly, today on visiting Marib, there is an acceptance of these close links and of this tradition. I visited also the site of the ruined dam and saw the inscriptions in pre-Arabic script. His Excellency Shaik Zayed, the President of the UAE, funded the restoration of the Marib Dam and lake. When I visited the region and the site of the new dam, I found Shaik Zayed’s portrait and that of the President of Yemen dominating the visitors area at the edge of the lake and testifying to the strong traditional links between the two countries (see Figures).

I am therefore proposing that the ancestral home of this mutation may be the Yemen. The mutation was carried to what is now Abu Dhabi and then on to the remainder of Oman by the tribal links of that Emirate to the tribes of northern Oman. There is no literature on CF in the Yemen. However, it must be remembered that Yemen is the least developed of the Arab countries. Medical care is minimal for those in the desert regions and the high mountains of central Yemen make transportation and contact
nigh on impossible for the scattered tribes. Political unrest made it necessary for me to travel in the Marib region with an army escort and in a military convoy.

A personal communication with Professor El Harith in Saudi Arabia who informed me that his group had only identified one patient with the S549R on a single allele. Interestingly, this family had originated in the UAE. Thus unlike the UAE the mutation is apparently not present in those of Saudi Arabian stock. This adds indirect evidence that the migrations from Yemen may have been targeted at the Trucial Coast and not the Hejaz or Nejad regions. Ideally, a search for the mutation in Yemen would support my hypothesis. Unfortunately this was not possible at that time.
Figure

The Lake and Dam at Marib, the Yemen.

The ancestral home of the S549R (T→G) mutation?
Figure

Mother and Child in Marib, the Yemen
An hypothesis regarding the origin and spread of the cystic fibrosis mutation ΔF508

Morral et al.¹ have presented strong evidence that the ΔF508 mutation arose in a population genetically distinct from the present European population. Further, data plotted on synthetic maps indicates a marked frequency gradient from south-west to north-west Europe e.g. 100% presence of ΔF508 in CF mutations in the Faroe Islands compared with 27% of all mutations in the Turkish CF population². The explanation offered for this phenomenon is that there has been a greater mixing and heterogeneity in the southern populations and relative isolation in the northern. The current view is that ΔF508 was not spread by the Indo-Europeans but by a group that preceded them and had originated in the “Middle-East” or the ”East”. Bertranpetit and Calafell³ have summarised the basis of the estimates of the age of the ΔF508 mutation. The 1705 chromosomes studied carried 1477 microsatellite mutations (corresponding to a mean λ = 0.866). A mutation rate of 3.3 × 10⁻⁴, gave a total time of 2,625 generations, which gives an estimate of 52,000 years. This is a mean estimate and its standard error may be large.

We are thus considering a population based in the East, in which a mutation arising some 50,000 years ago, provided some biological advantage to those who were carriers. Currently, some authors have hypothesised that this advantage could be considered as a protection from typhoid fever rather than cholera as had formerly been suggested.⁴

In an attempt to determine where in Asia this mutation may have arisen, the study of the presence of ΔF508 in Asians living in Western countries has not been very
rewarding. The lack of information from Asian countries has made this method necessary. In a large Asian population, the study of almost 900 chromosomes revealed the absence of carriers of the common Caucasian-related mutations (including ΔF508). However, an affected Pakistani child born to consanguineous parents was shown to be homozygous for mutation S549N (G→A)⁵. Schwartz et al.⁶ reported six affected Pakistani children, of who three were homozygous for the ΔF508 mutation. It was not stated to which ethnic group within Pakistan the children belonged. Further study of Asians with CF was reported by Bowler et al.⁷, who outlined the clinical course in nine Pakistani Asians. Four of the nine were homozygous carriers for the ΔF508 mutation. A comparison was made with a group of 18 Caucasians, 17 of whom carried ΔF508, of which 12 were homozygous. A high degree of consanguinity was reported and a more severe clinical course, which it is suggested, may have been influenced by genetic and environmental factors.

In a study from the USA of Asians with CF, 20 patients were identified in US CF clinics⁸. Seven patients carried the ΔF508 mutation, of which four were homozygotes. Pakistanis represented 10 (probably 11) of these patients, Indians 8, and one Palestinian. Again, no information was given as to the ethnic sub-group of the families. The authors calculated that the incidence of CF in Asians was 1: 40,750 but noted a figure of 1: 10,000 proposed from the UK⁹. The only study, which gives some information about ethnicity within the Indian subcontinent, is that of Spencer et al ¹⁰, who reported on 13 CF patients. Seven patients were from Mirpur (Kashmir) and one of them was homozygous for ΔF508. Four patients were Sikhs from the Punjab (India) and two of them carried the ΔF508 mutation. The remaining patients were from Bangladesh and a Moslem Punjabi, but their specific mutations were
unknown. Overall, most reports about CF have focused upon Pakistanis and only rare reports come from others such as Sikhs and Bangladeshis. In about half of these, the $\Delta F508$ mutation is implicated and is usually found in a homozygous pattern, which reflects the extensive consanguinity amongst these ethnicities.

If the founder $\Delta F508$ mutation occurred in Asia some 52,000 years ago and bestowed some benefit on that early population, where are the descendants of these early people? It is highly unlikely that they all migrated to Europe, so there should be a remnant population, which carries evidence of this genetic descent.

It is proposed that the most likely ethnic group who could provide evidence of this decent are the Baluchi people. They number about 5 million and are located in Iran (20%), Pakistan (Baluchistan Province 70%) and Afghanistan (10%). The original Baluchi homeland was said to be the Iranian Plateau and by the 10$^{th}$ Century AD, they had migrated to their present location as described in the Arabic Chronicles of that time, with migrations continuing into the 14$^{th}$ Century AD $^{11}$. Their eventual settlement area is an arid region surrounded by the daunting mountain regions of Baghe Band and Bampusht. This region is regarded as one of the most isolated in the world$^{11}$.

The Iranian Baluchi territory provided a land route to the Indus Valley and the Babylonian civilisation. This route was exploited by Alexander the Great who marched through Baluchistan in 326 BC. Thus the area was in a key position in relation to South-West Afghanistan, the Indus Valley, Iran, Iraq and the Levant. Later emigration occurred from Baluchistan into the Punjab of India, as well as to Oman and the present UAE.
The close geographical location of Baluchistan to the UAE makes it very likely direct transfer of the ΔF508 mutation occurred by the well-established sea links. The Baluchi people have remained an isolated group living in a large area (347,190 square kilometres) and have practised traditional arranged consanguineous marriages. It is not surprising, therefore, that all the patients we have seen of Baluch descent are homozygous for the mutation ΔF508. Similarly, population screening has indicated those UAE Nationals of Baluch descent and Pakistanis from Baluchistan living in the UAE were similar in their carrier state, i.e. ΔF508 only. Indirect supporting evidence for this hypothesis may be found in the study of Karjoo et al. He investigated prospectively and retrospectively all suspected cases of CF in Southern Iran seen in the three University hospitals of Shiraz University. The people studied were living in Fars Province adjacent to the Baluchi areas of Iran, but are ethnically different. The investigators could find no previous reports of CF in a 20-year retrospective study or a patient with a firm diagnosis of CF. In the prospective study of 125 suspected individuals, only three patients were considered to have the disease on clinical grounds. No genetic studies were undertaken. Thus is an area close to Baluchistan, CF appears to be a very rare disease, yet it is now found regularly in Baluchis.

Quaife et al. studied the spectrum of the disease beta thalassaemia in the UAE national population. They concluded that specific mutations were introduced into the UAE by immigrants from Baluchistan and matched those found in that region. One mutation in particular, the β+ IVSI-5 (G → C), has rarely been found in Arab populations elsewhere and appears to have arisen in the Baluchi population.

To gain genetic information about the relation of the early settlers in Europe and the present day residents in the UAE of Baluch descent and Pakistani Baluch people a study of microsatellite markers on the respective alleles is necessary.
The hypothesis proposed here is that the original founder ΔF508 mutation giving rise to cystic fibrosis occurred in those inhabiting the Iranian Plateau and travelled from there eventually to Europe in the first wave of emigrants. Their descendants moved to the area of Baluchistan bringing the mutation with them. During the last 150 years, further migration has occurred into the Gulf Region and the ΔF508 mutation has joined the pool of CF mutations that are common in this region12,15.

2 This hypothesis was presented initially in a Master of Science (Geography) thesis that was accepted by Massey University and also published in the Quarterly Journal of Medicine
Epilogue

It has been proposed in the hypothesis above that the transfer of the $\Delta F508$ mutation to Oman and the UAE could have been by the established sea routes across the Arabian Sea and Indian Ocean. A very relevant trade network was that between Oman and Gwandar. This settlement is situated in the present day Baluchistan area of Pakistan (see map Figure 3) and was originally an Omani enclave. It played an important role within the slave, ivory and spice trades coming from the Arabian Peninsula and East Africa and linking with Central Asia. This coastal strip had also been the main route between the Middle East and the sub-continent.

In the last quarter of the 18th Century, Gwadar and the area of Makran were succeeded to the "Masnad" of Muscat in 1783. Prior to this the Portuguese had captured several places along the 600 kilometres of Gwadar and Makran coast. Gwadar remained an Omani possession until September 1958, when it was transferred (sold) to Pakistan.

Thus it is possible that this important area provided the ideal route to the Middle East, Iran, and Pakistan from and to the Eastern Arabian Peninsula and permitted the "transfer" of the $\Delta F508$ mutation to the Arab world from Iran (Baluchistan).
Figure 21 Map of Baluchistan
References


18. Figure 28

Saleem
Chapter 14

Summary and Conclusions

“Rome has spoken: the case is concluded”

Sermons, Book 1
St. Augustine (354-430)

The content of this dissertation developed as a result of the observation that a dying Arab girl had the clinical features of cystic fibrosis. More importantly, her subsequent confirmed diagnosis stimulated the development of a clinical and laboratory service designed to serve those with cystic fibrosis.

The clinical observation raised many questions. If this was truly CF, is it an important disease among Arabs in the Gulf Region and if so how common is it? Certainly, the prior medical literature was unhelpful and suggested that the condition was either absent or very rare among Arab communities. The overwhelming majority of citizens in the UAE are Arabs of Bedouin origin and there were simply no reports of the disease occurring in this group of people. However, there were two reports of probable CF in two children from the Northern Emirates that had been published in the local medical journal. These children, while UAE citizens, were of the Baluch ethnic group and were thus not Bedouin Arabs.

This thesis, therefore, supplies the answer to many of the questions that were raised. What has been learned in addressing these questions? Probably the most important advance has been in establishing beyond doubt that the disease CF does affect Arab
children in the UAE and Oman and throughout both countries. It is an important cause of serious illness and is much more common than previously appreciated, causing much morbidity and almost certainly premature death. With this knowledge I established a centre for the diagnosis and management of the condition. This was open for referral of children from all areas in the UAE. My colleague, Dr. Phillipe Frossard, developed the technical facilities to define the majority of known mutations found in the CFTR gene. Thus, in a combination, we were able to provide a basis to answer more questions about the background of CF and provide a better clinical service.

Clearly, having the information that the condition is more prevalent than previously appreciated serves no purpose if it is not acted upon. Our aim was to achieve an increased level of awareness and to establish early diagnosis and instigate treatment. Certainly experience from Western countries indicates that this is worthwhile in terms of quality of life and longevity for the affected children. To raise the level of awareness about the condition, I wrote to every Paediatrician in the country informing them of our interest, the establishment of a clinic and the facilities in the Medical School for mutational analysis. Further, our early results were published in the local medical journal and subsequently in international journals. Presentations were made at the national Paediatric Society scientific meeting and regional Paediatric meetings. Eventually, I made presentations in Lebanon (Middle East and Eastern Mediterranean Paediatric Society), Qatar, Bahrain and Oman. Finally, an even wider scope for publicity was achieved by a presentation to the Congress of Asian Paediatric Societies at its meeting in Taiwan.
Not only was CF being recognised in the local Emirati population, there was an increasing number of children in total, being referred to the clinic and an increase in the numbers being diagnosed as having CF. Referral of children of other national groups was taking place. Indeed, we established and confirmed the diagnosis of CF in Arab children from Egypt, Palestine, Syria and the Sudan. In addition, children from the UK and Pakistan were investigated for CF. However, their clinical details are not included in this thesis. However, an example of a delightful non-Emirati Arab boy with CF is to be seen in the end figure.

It appeared that the clinical presentation was severe. The prior treatment received by individuals obviously influences the disease presentation overall. However, we were assessing and confirming the diagnosis in young children and noting early colonisation with pseudomonas organisms in many. Information was thus collated to support the clinical impression that we were dealing with children with severe disease.

If the disease exists and in such a severe form then why is it only now that it is being recognised? I have only indirect evidence to answer this question. No UAE adults with the disease have been recognised in our area (total population of 400,000). Recognition was in terms of hospital records and those attending the adult respiratory service. This seems a reasonable assumption in that surviving adults with the disease would require hospital facilities and treatments for their management. It can only be assumed that those with the disease have died prior to reaching adulthood. I have described elsewhere that non-CF bronchiectasis is common in the children of the UAE. This situation is well recognised and the adult Respiratory Physicians treat such
patients regularly. I can only postulate that many deaths previously have been attributed to bronchiectasis and pneumonia. Post mortem examinations are not carried out in the UAE for cultural reasons. Only in cases of suspicious death are autopsies performed. The other important factor is the harsh environment and climate of the area. I have given an example of an infant who could have died from dehydration, hyponatraemia and hypochloraemia. She is one of many infants who could have died in former times without the underlying disorder having been recognised. CF children are, of course, at particular risk of salt loss. Those living in the Gulf States who do not have CF are at great risk of salt depletion when ambient summer temperatures can reach 48ºC. Above all the medical services in the UAE and Oman have only recently reached a minimum standard. This was not due to a lack of funds, but to a lack of qualified personnel and a suitable infrastructure and service planning. I have indicated that we had to obtain research funding to establish the molecular genetics laboratory.

The absolute diagnosis of CF is based on the determination that the individuals carry mutations in the CFTR gene and on each allele. Our feeling was that the severity of the clinical disease was similar to that associated with the mutation ΔF508. However, it was with some surprise that the rarely found mutation S549R (T→G) was associated with CF in the majority of our patients. Indeed, all the patients initially seen were homozygotes for this mutation. Our next important finding was that a few children were, in fact, homozygotes for the ΔF508 mutation. However, these children belonged to the minority group of UAE citizens who belong to the Baluch ethnic group. From the study of patients to date, it appeared that 96% of CF children had one of these two mutations. An attempt was made to compare the clinical severity of an age and sex matched group of children who were homozygous for the two mutations.
The small number of children available to age and sex match frustrated these attempts to confirm or refute my feeling that the S549R mutation had an even more severe clinical outcome than that of the ΔF508. Enough information was available to add further evidence; however, that S549R is associated with severe disease and it probably at least equates with that of the ΔF508 mutation phenotype.

The next area that was addressed was the question of how common CF was in the UAE. To determine the frequency of carriage of the two mutations identified, a random sample of the population was required. This has been achieved as well as possible in the Emirati society. The combined estimated frequency for individuals homozygous for a mutant CF allele is 1 in 64 of the national population. Taken that the Emirati national population was 750,000 and we were aware of at least 50 children with CF, an extremely crude prevalence rate of 1 in 15,000 could be assumed. Using our CF mutation frequency of 1 in 64, then a frequency of 1 in 15,000 could be obtained. This calculation is not applicable in a population in which over 50% of the marriages are consanguineous. Further, there has probably been a major "loss" of CF patients by attrition. The birth incidence figure, thus calculated, using a formula allowing for consanguinity is one of 1 in 4318 births for all nationals.

No information has been published about CF in Oman. I had identified one child who was an Omani national. The extension of our studies to Oman confirmed the presence of CF in that country. Again we were surprised that the major mutation associated with CF was S549R in a homozygous state. As in the UAE, those of Baluch descent were homozygous for the ΔF508 CFTR mutation. Data collected from thirty diagnosed patients pointed to the disease being of a severe nature there also.
The accumulated knowledge of CF in the Eastern Arabian Peninsula allowed some speculation as to the likely original sources of the mutations. It is suggested that the founding mutation of the S549R mutation arose in what is the present day Republic of Yemen. The ΔF508 may have arisen in the ancestral people of the geographical area Baluchistan, spanning Pakistan, Iran and parts of Afghanistan.

Overall, CF produces a significant health problem for children in the Eastern Arabian Peninsula. The gene mutational findings are different for the major ethnic groups. Evidence points to the fact that children have and are dying from the disease due to its non-recognition or suspicion. Increased awareness of the condition is required and aggressive treatment regimens are essential. The combination of climate, geography and genetic factors has made CF in the UAE and Oman a lethal disease. This situation can be changed by increased education about the condition for doctors and the public at large. Therapeutic regimens must include allowances for the climatic extremes in the region.

**Concluding Summary**

The contribution made to new knowledge about CF in Eastern Arabia as described in this thesis and the input into disease management to can be summarised as follows:

1. Recognition of the disease as being present throughout both countries and it not being as rare as previously thought.

2. Raising the level of awareness of the condition in the UAE of Paediatricians and in the public in general by publications, presentations and seminars.
3. Obtaining funding to develop a laboratory to define the CF mutations present in the population.

4. Outlining the molecular genetics of CF in the UAE and Oman. Establishing that the S549R mutation is found almost always in a homozygous state in Bedouin UAE and Omani nationals.

5. Recognising that the mutation ΔF508 is frequently found in those of Baluch descent and usually in a homozygous state.

6. Providing the first reports of CF in the UAE Bedouin population and the first reports of its mutational basis.

7. Providing the first reports of CF in Omani nationals and the associated CF mutations.

8. Defining CF in these populations as having a severe clinical presentation and equating the phenotype to the genotypes.

9. Presenting the largest series of patients with the S549R mutation ever reported.

10. Providing an estimate of birth frequency for CF in the UAE.

11. Contributing to the new knowledge that a mutation in the promoter region of the CFTR can modify the clinical phenotype of the S549R mutation.

12. Presentation of hypotheses regarding the two gene mutations and their ethno-geography.

13. Establishing a service for CF children in the UAE.
Figure Edward from Palestine