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CYSTIC FIBROSIS IN THE UNITED ARAB EMIRATES

A spatial, medical and historical perspective

by

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“Children are a heritage from the Lord”

Psalm 127

Frontispiece

The ships of the desert Al Ain, UAE.

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Chapter 1

Acknowledgements

“No man is an Island, entire of itself”

Meditation XVII

John Donne (1571-1631)

Our studies into Cystic Fibrosis (CF) in the United Arab Emirates (UAE) have been supported by three research grants awarded to me as principle investigator, by the UAE University. These were received during the years 1994-5, 1995-6 and 1996-7 and have been used to cover the costs of the laboratory materials required in our DNA work. I am most grateful to the University for their support. This support has resulted in research publications in the fields of genetics and respiratory medicine in international journals.

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Chapter 2

Introduction

“The study of Cystic Fibrosis provides a fascinating insight into advances in medicine in the 20th Century.”

M. Super, 1992¹

Aim

The aim of this dissertation is to report our observations on the disease, Cystic Fibrosis (CF) as it presents in the United Arab Emirates (UAE). It contains the first full clinical reports of how the disease manifests itself in the UAE, it defines the molecular basis for the disease and establishes a population frequency of gene carriage. The observations are made in light of the new knowledge of CF gene mutations, the spatial and ethnic aspects revealed by these findings and the creation of a new geography related to cystic fibrosis. The intention is to further hypothesise as to the spread of CF to the UAE and postulate as to the prior and subsequent events in relation to the spread of gene mutations elsewhere.

Methods

A wide range of methodologies have been applied. The original observations as to the existence of CF in the Emirates were made in the Children's wards and outpatient clinics of Tawam Hospital, Al Ain, UAE. Subsequently, within the Faculty of

Medicine in the UAE University, were developed the techniques required for specific gene mutational analysis. These were developed in conjunction with, and validated by, the Laboratoire de Genetique Moleculaire in Paris, France. This was in collaboration with Dr Emmanuelle Girodon and her team. Individual methods are described in each chapter as appropriate.

Introduction and over- view

The development of human molecular genetics has permitted the study of diseases like CF from an evolutionary and geographical perspective. In rapidly developing countries like the UAE the recognition of “new” diseases can be aided by the application of these modern techniques thus allowing the establishment of gene frequencies.

Our interests and subsequent investigations into CF in the UAE were prompted and stimulated initially by my being asked to see an eight year old Emirati girl shortly after commencing work in the UAE. She was dying and had all the stigmata of chronic lung disease and respiratory failure and all the characteristics of CF. Subsequent laboratory studies and later molecular genetics confirmed the diagnosis and defined the underlying molecular defect which had brought about the manifestations of the disease process during her short life.

I established a clinic for children with chronic lung disease at Tawam Hospital, Al Ain and this resulted in the referral of many children with bronchiectasis, congenital lung disease or other lung pathology^{2,3,4}. Among these patients our studies defined a group who had a severe form of the disease CF. Later, we informed all paediatricians in the UAE that we were providing a clinic for those with CF specifically and would be willing to advise on the management of these children and help with the molecular diagnosis.

There was a steady referral of patients to our clinic and it became obvious that this was not a rare disease and that a number of children with CF were present in the country and were of UAE nationality. This was in spite of the dearth of published work regarding the disease in the UAE and indeed, it was clear that there was not a great deal published on the topic from the Middle East altogether. We described initially a group of children who were local Arabs⁵ and then realised subsequently, that the severity of their disease was greater than we had suspected. Further work defined the clinical spectrum and then we set out to study the molecular genetics of those under our care⁶. We were initially intrigued that we found children in the Bedouin population without the $\Delta F508$ mutation. However, later work did establish that the mutation we did find was located in such a position on the CF gene that it was likely to produce severe disease. With referral later of patients from the northern emirates we began to find children who were homozygous for the $\Delta F508$ mutation. Further clinical studies showed that these patients too had severe clinical disease⁷. We then established that the overwhelming majority of UAE patients had one of two mutations and they tended to be homozygous for their respective mutation. With the indigenous population of the Emirates being so small, the question of the role of

genetic drift or the importance and relevance of consanguinity in these findings is raised. However, with a limited number of mutations there is a possibility of screening for the disorder, or establishing the gene frequency in the general population⁸. However, it became increasingly apparent that within the UAE there was an ethnic, tribal or regional aspect to the inheritance of the disease and further study was necessary into the family background of those with CF. The results of these observations would permit a hypothesis to be constructed as to the origins, sources and spread of the CF genes, their spatial distribution and possibly reflect upon the world-wide pattern of population movements.

Chapter 3

Cystic Fibrosis: the historical perspective

“Das Kind stirbt 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 Switzerland⁹ circa 1600

Current evidence suggests that more than 52,000 years ago a sudden human gene mutation took place in people living in Asia which resulted in a major change in the CF gene¹⁰. This event took place in the Palaeolithic period which 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 gene mutation we now refer to as $\Delta F508$. It is hypothesised that the resultant admixture of these Indo-Europeans, Asians and North Africans led to biological and behavioural change. Among these was dietary change. 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 genes may have provided some advantage for the heterozygote carrier in the change of diet¹¹. 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 which 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 that 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 1606¹¹. 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 privilegios 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, scirrhou and enlarged pancreas which resulted in her death according to Pauw. 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 scirrhou 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 Vienna. He described a child who died from perforation of the bowel and meconium peritonitis. This is the classical course of untreated meconium ileus which is 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.

With the recognition that CF was not a new disease, interesting historical observations have been put forward. One of these is O'Shea's suggestion that Frederic Chopin's illness and subsequent death 150 years ago was as a result of CF and not tuberculosis as has previously been asserted¹². He cites the duration of the illness (24 years), Chopin's short stature and emaciation and his repeated respiratory illnesses as key

diagnostic features. The death of his sister, Emilia, at 14 years, possibly from CF too, his barrel-chested appearance, probable infertility and his final death from cor pulmonale are all supportive evidence. This diagnosis has been defended by Phelan¹³, but questioned by Kubba and Young¹⁴ due to the lack of finger clubbing present in the cast of Chopin's hands which was taken after his death. While now, 150 years later, it is impossible to be certain, what is important is that CF has all the clinical characteristics and protean forms to make it a strong possibility.

It is clear from this European folklore and the early medical records and descriptions that the disease which today we may recognise as cystic fibrosis (CF) must have existed for many centuries. It was only in the 20th century, however, that the first descriptions of the distinctive nature of CF were made. Landsteiner¹⁵ in 1905, provided the first detailed description of meconium ileus which later was recognised as part of the spectrum of CF. Five years later Garrod reported a consanguineous family with a number of children with steatorrhoea who had died from bronchopneumonia.¹⁶ However, it is to the Swiss Paediatrician, Fanconi (see Figure 1), that the credit is given for the first published account of the disease we now recognise as Cystic Fibrosis¹⁷. A most detailed description of the disease was published in 1938 by Dorothy Anderson from the United States¹⁸. She made an accurate and detailed description of the disorder discussing the relationship of meconium ileus to CF. She outlined further the approaches to treatment including the administration of pancreatic enzymes.

The sixty years subsequent to these early findings has seen rapid advances in our knowledge of the clinical aspects and management of CF, the pathophysiology and the

genetics of the disease. The important milestones are listed in Table 1. The genetic aspects of the disease are paramount in discussing the spatial aspects and spread of the disorder. Carter in 1952¹⁹ established that the condition was inherited in an autosomal recessive manner. It was the work of Lap-Chee Tsui and his colleagues²⁰ (see Figure 2) which identified chromosome 7 as the site for the CF gene. Subsequently, Tsui's group in Toronto, Canada in association with Collins in Michigan, USA, were able to identify and clone the CF gene^{21,22}. The gene was called the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR), and they termed the commonest known mutation (69% of Canadian and United States CF genes) as $\Delta F508$, indicating a missing phenylalanine at position 508 of the 1480 aminoacid protein.

Following the discovery of CFTR, Tsui quickly set up an international group to search for other gene mutations and to pool this knowledge.²³ This has led to the discovery of over 800 mutations. However, less than 20 occur with any frequency worldwide. These findings have opened a new chapter in ethnic studies and in the spatial distribution of variants and have raised interesting evolutionary considerations.

The last sixty years have thus seen a revolution in our knowledge of CF. The disease has been defined clinically and pathologically and the specific gene responsible has been cloned. The discovery of the vast number of mutations in the CFTR has raised new questions about the definition of the disease state. Kulczycki²⁴ has reviewed the highlights of the period 1938-88, stressing the pioneering work of Blackfan and May (1938). They demonstrated the important pathological changes which occur in the pancreas. Abnormal secretions, dilatation of the ducts and acini, atrophy and fibrosis

of the gland were the main findings in CF. Above all Kulczycki regarded the work of Dorothy Anderson in 1938 in defining the disease as truly important and that of Guido Fanconi in recognising CF as a separate disease entity. The development of the diagnostic test of sweat chloride analysis by Gibson and Cooke and the identification of the site of the CF gene were the other landmarks in this era.

The identification and cloning of the gene responsible for the CFTR and the disease state has far reaching consequences including the further development of gene engineering and the eventual eradication of the disease. The use of microsatellite molecular genetic techniques has given us the a glimpse of the past as far back as 50,000 years. The future looks brighter in helping those with this terrible disease.

Figure 1

Dr. G. Fanconi (left) who published the first clinical descriptions of Cystic Fibrosis.



Figure 2

**Dr. Lap-Chee Tsui who with John Riardon and Collins discovered
the CF gene**



Table 1**Important milestones in cystic fibrosis - definitions and discoveries**

Based upon Super, 1992¹

Researcher	Year	Discovery
Fanconi	1936	Clinical description
Anderson	1938	Clinical and pathological definition
Di Sant'Agnese	1946	<u>S. aureus</u> as an important pathogen
Carter	1952	Autosomal recessive nature of CF
Shwachman	1956	CF without pancreatic involvement
Holsclaw and Shwachman	1968	Involvement of the vas deferens and seminiferous tubules
Quinton	1983	The basic defect in chloride secretion
Yacoub	1984	Heart - lung transplant for CF
Tsui	1985	The gene for CF on chromosome 7
Tsui, Riordan, Collins	1989	Discovery of the CF gene (CFTR)
Karter	1991	CFTR is a chloride channel
Crystal	1992	Potential of the adenovirus for gene transfer of CFTR

Chapter 4

The Disease and its Clinical Presentation

“Her cabin’d ample spirit

It flutter’ and fail’ for breath

Tonight it doth inherit

The vasty hall of death”

The Scholar Gypsy

Mathew Arnold (1822-1888)

A diagnosis of CF has lifelong implications and repercussions for the affected individual, their family and the many people they will encounter during their lives²⁵. It remains, however, the most common life-shortening inherited disease in white populations and its geographical distribution in other ethnic and racial groups has only been recognised in recent years.

In pathological terms, the disease is as a result of dysfunctional ion transport across epithelial surfaces. Impermeable chloride channels and overactive sodium pumps of these epithelial cells lead to biochemical and bioelectrical abnormalities within organ lumens including relative dehydration of luminal secretions²⁶. The resultant abnormal secretions cause blockage of ducts and air passages. These molecular and cellular defects are manifest by the development of obstruction in the lungs and subsequent

infection. In addition, gastrointestinal disease due to exocrine pancreatic insufficiency, liver disease with focal biliary cirrhosis, vas deferens abnormalities giving rise to obstructive azoospermia, excessive loss of salt via sweat glands can all occur in various combinations.

CF is inherited in an autosomal recessive manner, thus parents heterozygous for a CFTR mutation have a 1 in 4 risk of having an affected child in each pregnancy. The gene for CFTR is located on the long arm of chromosome 7 (7q31). To date over 800 mutations of the gene have been recognised. Those who share the same gene mutation (genotypes) do not necessarily have the same disease pattern (phenotype). The clinical progression of CF is highly variable, children in the same family may have different presentations, manifestations and degrees of severity. However, pancreatic insufficiency is strongly associated with the $\Delta F508$ mutation.

Children with CF usually present with a combination of diarrhoea, recurrent respiratory infections and failure to thrive. However, 10-15% may present with the condition called meconium ileus, usually on the first or second day of life. Intestinal obstruction occurs due to thick inspissated meconium. The infants present with abdominal distension, bile-stained vomiting and delayed passage of meconium. There are a range of other possible presentations and combinations. These include rectal prolapse, nasal polyps, prolonged neonatal jaundice, hypochloraemic alkalosis (Pseudo-Bartter's Syndrome), hepatomegaly and oedema and hypoproteinaemia.

The lung disease of CF starts early in life, the lungs tending to take the brunt of the disease process and this accounts for the majority of the morbidity and mortality

associated with the condition. While generalised dysfunction of the small airways results from defective hydration it is made worse by a susceptibility to infection and inflammation. Again, there is a particular susceptibility to infection by the gram negative organism Pseudomonas aeruginosa. The combination of abnormal secretions, infection and inflammation leads to necrotizing bronchitis and bronchiolitis. This in turn leads to bronchiectasis and eventual respiratory failure.

The gastrointestinal disease is usually as a result of exocrine pancreatic insufficiency with 90% of the patients developing this complication by nine years of age²⁷. The insufficiency is manifest by maldigestion of fats and protein leading to malabsorption, steatorrhoea and failure to thrive. Malabsorption of fat soluble vitamins A, D and E is frequent. Bowel obstruction (distal intestinal obstruction syndrome) due to thick mucus and pancreatic insufficiency can occur in 20-25%²⁸. Liver disease including focal biliary fibrosis is extremely serious but less common. It may present with cirrhosis, hepatic failure and portal hypertension.

Most male patients develop atresia of the vas deferens and consequent obstructive azoospermia and sterility. Females have thick cervical mucus which may result in decreased fertility. Chronic illness and malnutrition lead to delayed puberty. Bone disease, clubbing and hypertrophic pulmonary osteoarthropathy are all well defined complications of the disorder.

CF was regarded formerly as being universally fatal with most affected children dying before or during the school years. In the USA, mean national survival had reached 28 years by 1990. Survival depends upon the inherited severity of the disease (genotype),

aggressiveness of the treatment programme, physical fitness, male sex, pulmonary function and probably early diagnosis²⁹.

Chapter 5

The relevant human geography and demography of the United Arab Emirates

“The cares that infest the day

Shall fold their tents like the Arabs

And as silently steal away”

The Day is done

Henry Wadsworth Longfellow (1807-1882)

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 moment³⁰.

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 whose traditions dominate the new states of the UAE, Qatar and Bahrain³⁰.

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. The first came via Oman and through the Hajar Mountains, reaching the Al Ain area around the second century AD; the other seems 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 marriages³¹. 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. The northern emirates were dominated by the Qawasim tribe whose interests lay in the sea. Similarly, immigration from Baluchistan and Persia occurred for this reason³².

The total population of the UAE stood at 2.624 million in 1997. The annual increase has been in the order of 100,00, 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 population³³. 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 determine whether the country can still afford to import labour for unskilled jobs.

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.

Chapter 6

Cystic fibrosis in the middle east: the historical perspective

"Lives of great men all remind us

We can make our lives sublime

And, departing leave behind us

Footprints on the sands of time"

A Psalm of Life

Henry Wadsworth Longfellow (1807-1882)

Twenty years after the definition of cystic fibrosis (CF) as a specific disease, the first report of an affected Arab child was made in 1958³⁴. 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 patient³⁵. Pedigree analysis in one child at least, showed that the family had been of pure Arabic origin for four generations.

There was a dearth of information and reports for the next twenty years until sporadic reports began to appear from Iraq, Israel and Kuwait^{36,37}. In 1981, Al Uwhare from Kuwait commented that in his two patients, the condition was not suspected before

death and the disease was not considered to occur in the Middle East³⁸. This prompted a brisk response from Katznelson³⁹, 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 cross-roads of the Middle East, has experienced an influx of people from many different areas. Desgeorges et al⁴⁰ have identified twenty families living in Lebanon for several generations, who have had at least one child with CF. They have reported the religious and community backgrounds of the affected families (Maronite, Greek Catholic, Shiite or Sunni) 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.

In Jordan, CF was first documented in 1984⁴¹ when twelve patients were collected retrospectively from the period 1976 to 1980. Some of these patients had been diagnosed only at post-mortem examination. Commenting on the situation in Jordan, Nazer⁴² stated that in 1985, there were eight children known to have CF in the country (population three million). The prevalence figure may, however, have been underestimated because of the high rate of consanguineous marriages and the lack of facilities to make a definite diagnosis in centres other than the Jordan University Hospital.

In 1992, Nazer⁴³ attempted to define the prevalence and incidence of CF in Jordan. He studied prospectively 7,682 neonates from ten different hospitals in Jordan and screened them for raised levels of albumin in the meconium. Final confirmation of CF in three children was made by sweat tests. He concluded that the incidence of CF in Jordan was 1 in 2560 live births, acknowledging the limitations of the method for screening, but stressing the importance of awareness of the disease in their communities.

Following the initial observations in Kuwait that CF existed there³⁸, Kollberg reported that 17 patients of assorted nationalities who had been seen in Kuwait in a fifteen year period⁴⁴. 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 50,000 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 in 3500 births per annum. Three siblings with CF were reported from Kuwait in 1987⁴⁵. While being Arab, however, the patients were the children of a Jordanian family. The diagnosis was confirmed by very high levels of sweat chloride, well over 100 mmol/L. Another Jordanian child living in Kuwait, presented with metabolic alkalosis and electrolyte disturbance and was reported by Issa et al⁴⁶. 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^{47,48}. Only one child was a Kuwaiti National and the others were Palestinian Arabs. The high summer temperatures, around 45°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 Mohammad⁴⁹ described eight children with proven CF in 1985. 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 is in 1998 when 25 patients with proven CF were described⁵⁰. 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 of the population. In 80% of cases, the patients had been products of consanguineous marriage - in contrast to the normal consanguinity rate of 39% in Bahrain. They reported further, that the clinical disease spectrum is severe in its presentation in Bahrain. 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

presented with the classical features of recurrent respiratory infection, diarrhoea and failure to thrive⁵¹. 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 colleagues⁵² 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. He speculated that the majority of patients with CF in Saudi Arabia are under-diagnosed 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 CF⁵³. 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 children⁵⁴. 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 Rahbeeni⁵⁵ 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 mutations which give rise to hepatic manifestations may be different to others

previously described. A clinical description of a further ten Saudi children was published in 1995⁵⁶. 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) or vitamin E deficiency or gall-stones or nasal polyps. In keeping with the changing knowledge of CF brought about by molecular genetics, the two most recent publications from Saudi Arabia^{57,58} 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 novel⁵⁷. 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 al⁵⁷ was drawn from the population of the Eastern Region of the country.

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 repeated respiratory illnesses⁵⁹. The second account, again from a Northern Emirate, involved a new-born 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 the clinical suspicion of CF⁵⁹. Subsequently, further clinical reports and molecular studies occurred in the Emirates^{59,60}. Later, the common CF mutations in the UAE were identified⁶¹.

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 the general lack of diagnostic facilities, mainly the sweat electrolyte test. Despite this, pioneers such as Nazer in Jordan and subsequently in Saudi Arabia, were able to screen certain populations and give some form of incidence. With the burgeoning new knowledge of the CF gene and its mutations from the mid-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 $\Delta F508$ did not characterise all mutations and that, in most of the region, other presentations may 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, and many of these being novel mutations, reflects on the characteristics of these societies. The Lebanese findings reflect the numerous communities, who tend to have their own range of mutations, showing little mixing between the groups. Consanguinity in all of these Arab countries must be a major factor in perpetuating many of the rare mutations. Consanguinity was found in 80% of families with CF members in Bahrain. The ethno-history of CF is just becoming unravelled and the tracing of these various mutations in diverse populations gives a fascinating insight into the patterns of population migrations throughout history.

Chapter 7

The cystic fibrosis gene and some of its mutations.

Does this mutation offer any advantages?

“Damnosa hereditas”

“Ruinous inheritance”

Institutes ii 163

Gaius (2nd Century AD)

The gene and the Cystic Fibrosis Transmembrane Regulator

Since the isolation of the CF gene in 1989^{22, 23, 66}, fundamental information has been gained about the gene and the gene product. The CF gene is located at the position 7q31, spanning 230 Kb of genomic DNA. The coding region is made up of 27 exons (see Table 2⁶⁷), ranging from 50 to 250 base pairs⁶⁷. The product of the gene has been named the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR).

The promoter of the gene, which controls transcriptions, is not yet clearly identified. Most studies have focused on about 3,700 base pairs immediately 5' to the initiation

of transcription. This region does contain consensus sequences for binding sites of a variety of known transcription factors such as Ap1 and Sp1. Ap1 sites suggest the regulation of the promoter by various kinase pathways, whereas Sp1 sites appear to increase transcriptional activity by RNA polymerase 2. Ap1 sites may down regulate CFTR transcription^{67,68}. These findings are highly relevant in relation to our findings in CF mutations in the UAE which are discussed later.

The product of the cDNA sequence of the CF gene is a polypeptide of 1480 amino acids designated the CFTR. It functions as a chloride channel in the apical membrane of the epithelial cells. It has the appearance of a typical ATP binding cassette super family of transporters⁶⁸. The molecule has several distinct regions, the majority of which span the cell membrane (Figure 3⁶⁷). The protein consists of two repeated motifs made up of six membrane spanning segments and a nucleotide-binding domain separated by a regulatory domain (R domain) with multiple potential phosphorylation sites. The clinical problems in cystic fibrosis reflect the expression of the CFTR gene being highly expressed in the epithelial cells of the sweat ducts, the pancreatic ducts, digestive ducts (small intestine and Brunner's glands), biliary tract, reproductive organs and the lungs (serous cells of the submucosal glands).

Mutations of the CF Gene

Since the isolation of the CFTR gene there have been 832 different mutations identified (March 1999)⁷⁰. However, world-wide 68%, of all mutant alleles are represented by the $\Delta F508$ mutation, a three base pair deletion in exon 10 that removes phenylalanine 508 from the first nucleotide binding domain. Current evidence suggests that abnormalities in CFTR protein results in a failure to regulate apical

membrane ion channels. The CFTR channel fails to open correctly or is incorrectly sited, failing to respond to the hormones and neurotransmitters acting on the surface of epithelial cells. The end product therefore, is an abnormality in ion transport causing an altered composition of the epithelial secretions which in turn leads to the clinical manifestations of the disease⁷¹. The classes of CFTR dysfunction have now been elucidated and named. Class i mutations account for 54% of the total and lead to an absence of protein production. This class of abnormalities can be due to “nonsense” mutations or may result from “frameshift” or “splice-site” changes. Class ii, processing mutations, prevent the protein reaching the epithelial membrane. The $\Delta F508$ mutation is an example of this type of defect. Class iii, regulation defects, can be caused by “missense” mutations which affect the regulation of the chloride channel. Class iv, represent abnormalities in the conduction of the chloride current and are described as being due to “missense” mutations. Class v are defects in synthesis of normal CFTR and are usually due to mutations such as “splice” mutations.

Does this mutation offer any advantage?

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 $\Delta F508$ mutation, about one in twenty-two individuals are healthy carriers of the disorder. Romeo et al⁷² 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 meiotic rate been established.

Wright and Morton⁷³ 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 carriers⁷⁴, but this finding could not be substantiated in a United Kingdom study of heterozygotes⁷⁵. For a disease which has a high frequency only in the 20th century, it seems most unlikely that this would be the basis of genetic advantage. However, further evidence has been produced to support the asthma relationship⁷⁶.

Until recently, functional evidence supported the hypothesis that CF carriers can withstand secretory diarrhoea better than normal persons. 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^{77,78}. 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 demonstrated⁷⁸. Recently, Pier et al⁷⁹ have produced evidence that in the heterozygous state CFTR mutations increase resistance to the infectious disease, typhoid fever, due to infection with the organism 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 Salmonella enterica or 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 allele⁷⁹.

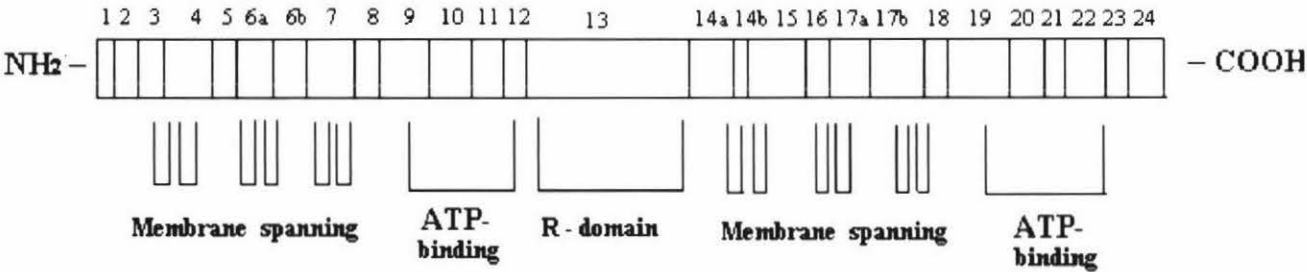
Table 2

CFTR Exon Sizes

Exon	Start	Size of Base Pairs
1	121	65
2	186	111
3	297	109
4	406	116
5	622	90
6a	712	164
6b	876	126
7	1002	247
8	1249	93
9	1342	182
10	1524	193
11	1717	95
12	1812	87
13	1899	723
14a	2622	130
14b	2752	37
15	2789	52
16	3041	80
17a	3121	150
17b	3271	229

Exon	Size	Size of Base Pairs
18	3599	99
19	3599	151
20	3850	156
21	3599	90
22	3850	173
23	4006	106
24	4375	198

Figure 3



Organization of CFTR cDNA to show location of exons relative to predicted functional domains

Chapter 8

Towards a geography of cystic fibrosis mutations

“The art of Biography

Is different from Geography

Geography is about maps

But, Biography is about chaps”

Biography for Beginners

Edmund Bentley (1875-1956)

Over 800 mutations and sequence variations have been reported in the CFTR gene since the identification of the gene itself in 1989. Reports from the Cystic Fibrosis Genetic Analysis Consortium^{80,81} have shown a striking difference in the distribution of CFTR mutations in different populations. Many of the mutations are rare or unique, but a worldwide survey has indicated that the $\Delta F508$ mutation has an overall frequency proportion of 0.68. There are marked variations, however, in this proportion in different geographic populations⁸⁰. Two other mutations account for the major share of the remaining reported mutations worldwide, namely G542X and

N1303K⁸¹. Information gained nationally, regionally and worldwide has permitted a geographic (synthetic) map to be produced for many countries. Such maps have been useful in shedding light and making inferences about natural selection gradients, local gene flow, recent and historical migration patterns and the characteristics of human populations.

To date the most closely studied populations have been in Europe in relation to the Δ F508 mutation, but also interesting information is emerging with regard to the other mutations. New data is appearing with regard to populations of African descent and casts new light on whether there is a single origin for a commonly shared mutation.

$\Delta F508$

Morral et al¹⁰ studied microsatellite haplotypes for $\Delta F508$ and normal chromosomes and demonstrated that they are genetically distinct and that $\Delta F508$ arose in a population with a different genetic background to that of the present European population. By calculating the number of mutations necessary to generate the differences observed between chromosomes, the group calculated that the $\Delta F508$ mutation occurred at least 52,000 years ago. This event took place in an ancestral population that expanded and spread the $\Delta F508$ 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. The current European population are closely related to these people and resembled them in appearance⁸². 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 $\Delta F508$. The theory of an advance through Europe in a wave formation has been promoted with the concept that there has been genetic replacement in the area of Central Europe at a later period by relatively recent immigrants of a different genetic background¹⁰.

To establish the frequency distribution of $\Delta F508$ in Europe, the European Working Group on CF Genetics collected data on 6,000 CF and 4,000 normal chromosomes for many European countries and included Turkey and Israel⁸³. They were able to construct a synthetic map of the gene frequency. The results showed considerable

variation in the frequency with minimum values of 30% of all CF mutations in Turkey to over 88% observed in Denmark. There was, throughout Europe, a south-east to north-west gradient of frequency for the $\Delta F508$ 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 Europe^{84,85} (see Table 3). Once again the gradient was confirmed and reinforced by data from several towns or regions within each country. In so doing the data permits rebuttal of the argument that these maps are inaccurate if too few samples are taken within each country⁸⁶. Denmark was confirmed as having a frequency of the mutation of 87%, while the Faeroe Islands had 100% of mutations that were $\Delta F508$. Istanbul, Turkey, had the lowest frequency at 27%. This distribution was based on the analysis of 17,886 CF chromosomes. Table 3 outlines the frequency and incidence of CF in selected European countries and cities while Figure 4 indicates the gradient for $\Delta F508$.

The concept proposed is that the lower frequencies of mutation observed in the southern European populations are as a result of a greater heterogeneity being present in the southerners. In addition, other markers on the chromosomes suggest that the $\Delta F508$ 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 as a result of migration of early farmers during the Neolithic Period (ie Indo-Europeans). However, a puzzling aspect to this is that there is a very high frequency of $\Delta F508$ mutations within the Basque Region between France and Spain and frequency rates as high as 87% are recorded. 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 which has survived despite successive waves of colonisation of neighbouring areas. This hypothesis was tested by Casals et al⁸⁷ who studied intragenic markers which indicated that, indeed, the $\Delta F508$ 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 $\Delta F508$ 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 $\Delta 508$ 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 community⁸⁸. However, Casals et al⁸⁷ 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 $\Delta F508$ mutation in some populations by bringing other mutations into Europe⁸⁷. 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 Brittany⁸⁹. 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 $\Delta F508$ 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 is described as a nonsense mutation being the second most common mutation after $\Delta F508$. It results in a failure of CFTR protein production. Evidence suggests that it was as a result of a single mutational event⁸⁹. The mutation accounts for 2.4% of CF mutations worldwide, but as with other mutations, its frequency varies geographically⁸¹. Karem et al described the G542X in their major paper on the identification of the CF gene⁶⁶. The distribution of this mutation throughout Europe has been described by Lucotte and Hazout⁹⁰ (see Table 4⁹⁰ and Figure 5). 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 al⁹¹ 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. Figure 6 indicates the frequency of G542X mutations in Europe which may relate to the sites of the ancient Carthaginian occupation and areas of subsequent expansion and spread to Europe. 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 geography⁹¹.

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 Peninsula⁹³. 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 findings⁹³. Further, it is one of the six frequent mutations found among Ashkenazi

Jews and results in a severe form of CF⁸⁵ . Figure 6 outlines the frequency of the mutation in various populations. 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 disease⁹⁴ .

“Out of Africa” - 3120+1G→A Mutation

Cystic fibrosis has been regarded as rare in the black population of Africa⁹⁵. CF mutation studies were carried out on three patients in South Africa in 1966. 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 54⁹⁶. 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→A⁹⁷. Again, this mutation was found to be the predominant CF mutation in the Eastern Oasis population of Saudi Arabia⁹⁸ 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→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 descent⁹⁸. 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 is less easy to explain, as they are the only Caucasian population who have had the 3120+1G→A mutation identified. There are, however, rare mutations shared between Saudi Arabs and Greeks such as a polyadenylation-signal mutation in the α -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.

Conclusion

Study of three of the commonest CF mutations in Europe and one rare African mutation indicates an initial founder effect between 30-50,000 years ago. The current spatial distribution of these mutations appears to reflect some of the history of mankind. Particularly interesting is the situation of the Basques, the possible spread of genes by the Phoenicians and the story of the one African mutation. The survival of these mutations adds weight to the “gene advantage” hypothesis. The story is still unfolding, however. The G551D mutation distribution corresponds to areas with large past populations of Celtic descent. R553X mutation reflects those of Germanic origin, while there is a preponderance of 1717.1G→A in Switzerland and Northern Italy⁸⁵. Further studies with microsatellite markers promise to reveal more of mankind’s geographical and cultural spread throughout the world.

Table 3

Geographic distribution of the $\Delta F508$ mutation in European populations.

Country	Incidence of CF at birth	Frequency of all CF mutations (%)
Finland (Helsinki)	1:2,500	45
Sweden (Stockholm)	1:7,700	63
Denmark (Copenhagen)	1:4,700	87
UK (Aberdeen)	1:2,400	80
Germany (Berlin)	1:3,300	60
France (Paris)	1:2,900	73
Spain (Barcelona)	1:3,500	50
Spain (Basque)	1:3,500	87
Greece (Athens)	1:3,500	55

After Lucotte et al 1995⁸⁵

Table 4

Geographic distribution of mutation G542X in selected European and North African populations.

Country	Region/Town	Frequency (percentage)
Finland	Helsinki	2.5
Denmark	Copenhagen	0.7
Northern Ireland	Belfast	2.1
Scotland	Edinburgh	3.4
England	Manchester	1.1
Wales	Cardiff	2.7
Czech Republic	General	2.2
Slovak Republic	General	7.2
France	Paris	3
Italy	Rome	0.9
	Sicily	10.9
	Sardinia	5.8
Slovenia	General	3.5
Macedonia	General	6.5
Greece	Athens	4.6
Belgium	Leuven	5.5
Algeria	Algiers	7.8

Tunisia	Tunis and Djerba	21.4
Turkey	Istanbul	15.3
Canary Islands	General	14.3

After Loirat et al⁹¹ 1997

Table 5

Geographic distribution of mutation N1303K in Southern Europeans

Country	Town	Frequency (Percentage)
France	Montpellier	1.9
Switzerland	Basel	1
Austria	Vienna	1
Hungary	Budapest	1.2
Spain	Barcelona	2.2
	Madrid	3
Italy	Milan	3.6
	Verona	3.9
	Genoa	0.5
	Sardinia	3.9
Slovenia	General	0
Albania	Tirana	0
Bulgaria	Sofia	5.9
Macedonia	General	0.8
Greece	Athens	2.3

After Lucotte and Hazout⁹⁰ 1995

Figure 4

Distribution of **CF** mutation $\Delta F508$ in Europe.
Percentage frequency of all mutations

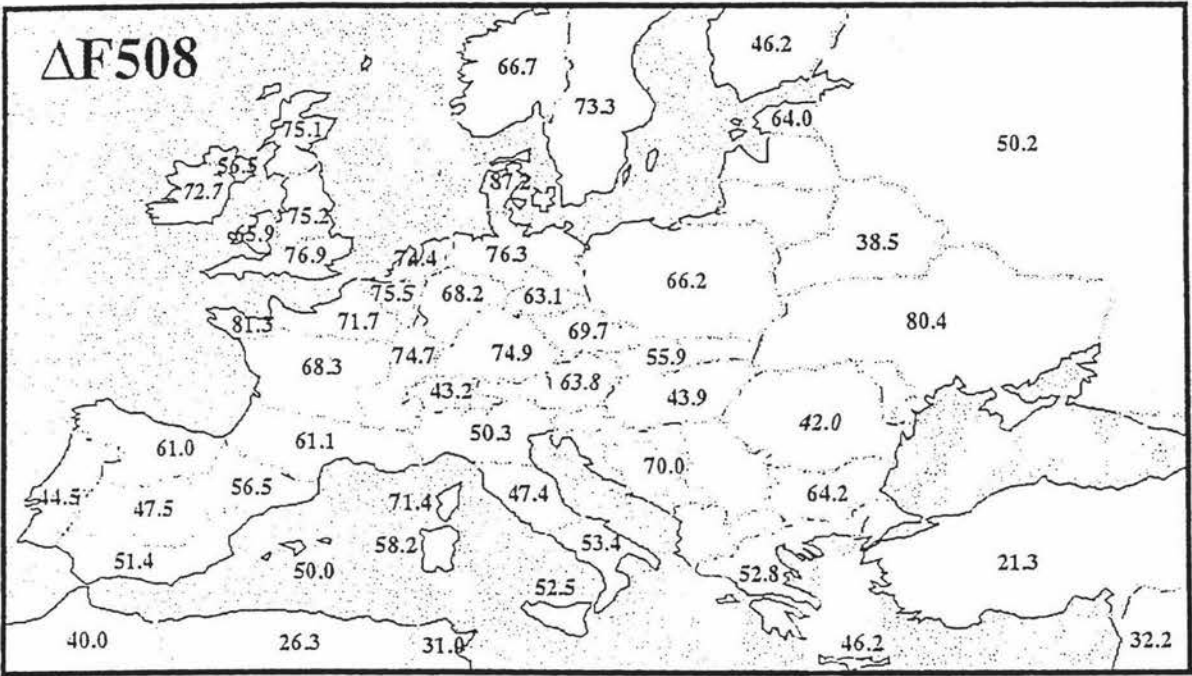
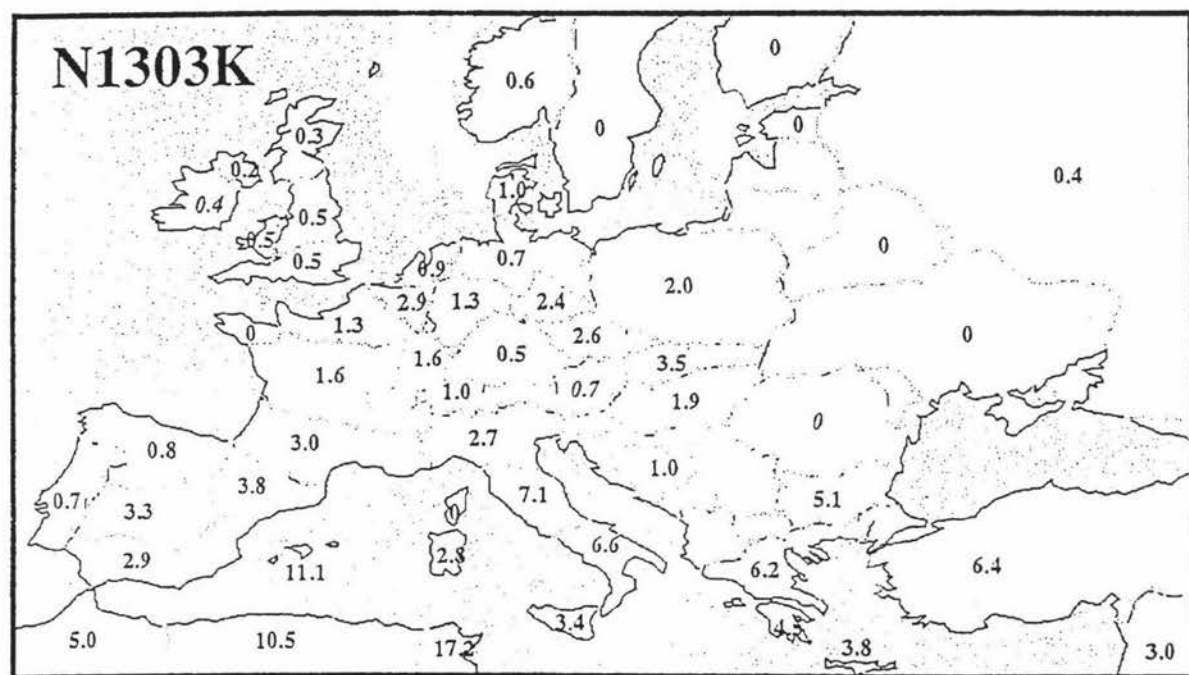


Figure 5

Distribution of **CF** mutation N1303K in Europe.
Percentage frequency of all mutations



Distribution of **CF** mutation G542X in Europe.
Percentage frequency of all mutations



Chapter 9

Clinical and genetic studies on Cystic Fibrosis in the United Arab Emirates

“In research the horizon recedes as we advance.....and research is always incomplete”

Isaac Casaban

Mark Pattison (1613-1884)

Introduction

The information presented in this chapter is drawn overwhelmingly from our own studies in CF in the UAE. In order that the discourse adheres to the main themes, much of the technical (molecular genetic), clinical and therapeutic details have been eliminated. The interested reader is directed to the original papers which contain such technical information and these are freely referred to within the text.

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 was attributed to the hesitant or suspicious attitudes of the people, the erratic nature of contact and the probable disinterest and neglect by the British

Government⁹⁹. 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.

Cystic Fibrosis in the United Arab Emirates: 1 - Clinical Presentation⁵

The two case reports published in 1987 and 1991 drew attention to two children and possibly a third who had developed CF and who were nationals of the UAE^{59,60}. There had been no previous references to the disorder in the UAE in the medical literature of the UAE or elsewhere prior to this period. The clinical presentation of eight UAE national children is herewith reported.

Patients and Methods

Eight children who had been referred to the CF and Respiratory Clinic at Tawam Hospital, Al Ain, UAE and had been fully assessed and investigated were included in the study group. Data regarding historical details, laboratory findings and clinical features was obtained for each patient. Sweat electrolyte levels were measured in each patient by the pilocarpine iontophoresis method.

Results

A summary of the main details of the patients is given in Table 6. All the children had strikingly elevated sweat test results with a mean sweat chloride level of 110 mmol/L (sd \pm 25.95). Meconium ileus had not been present in any of the patients, nor were mucus plugs reported in the newborn period.

All patients had malabsorption and pancreatic disease and were receiving pancreatic enzyme supplements, vitamins and nutritional support in the form of a high calorie diet. Pulmonary disease was variable, with one child presenting with gross cor pulmonale (patient 5), while patient 6 presented early and has, to date, no clinical lung disease and a normal chest radiograph. Patients 3 and 4 are siblings, as are patients 5 and 8.

Discussion

All these patients have severe disease. Bowler reported CF in nine Asian children born in the United Kingdom of Pakistani parents. He concluded that the patients had a more severe clinical course than matched controls and genetic and environmental factors may be a contributing reasons¹⁰⁰. The children from Saudi Arabia described

by Nazer et al⁵² certainly had severe disease with a wide spectrum of clinical manifestations. One of their patients presented with meconium ileus, as did one of the three patients previously reported from the UAE. None of our eight patients had meconium ileus.

Much debate has occurred about the relationship of phenotype to genotype in CF. The only clear correlation is in pancreatic function¹⁰¹. Ten to thirteen percent of patients have sufficient pancreatic function to prevent steatorrhoea and these patients tend to have lower sweat electrolyte concentrations and a slower decline in pulmonary function and a better prognosis. All of our patients have pancreatic insufficiency and all but the youngest has marked lung disease. All have high sweat chloride levels.

Karem et al¹⁰² reported an association between the possession of the $\Delta F508$ allele and pancreatic insufficiency and postulated that certain alleles which confer more severe disease may be recessive to those which produce a milder clinical expression. However, it appears that the clinical expression of the disease with each genotype is wide. Our patients all had pancreatic disease and this suggests, therefore, that the mutant alleles that our patients do carry should be regarded as severe and that the patients are likely to be homozygous for these as milder mutations would be dominant.

In summary, the main clinical features of the largest group of CF patients described in the UAE and the second largest series from the Arab world (1994) is presented. It is clear that the disease is not rare in this country and we are aware of at least nine other patients with a diagnosis of CF.

Table 6

Summary of Patients' Clinical Details

Number	Sex	Age (years)	Pulmonary disease	Intestinal disease	Sweat Chloride mmol/L	Meconium ileus
1	Male	3.5	++	+	133	absent
2	Male	6	++	++	95	absent
3	Female	2	++	++	150	absent
4	Female	6	+	++	110	absent
5	Female	6	+++	++	155	absent
6	Female	1	0	++	120	absent
7	Female	2	+	++	80	absent
8	Female	9	+	++	110	absent

0 = absent

+ = present

++ = severe

+++ = very severe

Cystic Fibrosis in the UAE: 2 - Molecular Genetic Analysis⁶

The clinical assessment of the first eight patients who presented to us and had been clinically investigated, provided some interesting data, if not contradictory findings. We appeared to have severe disease as is found in those with $\Delta F508$ mutations yet, we had to date not encountered a patient presenting with meconium ileus. Those with pancreatic and lung involvement to this degree have usually the $\Delta F508$ mutation, so it was mandatory that we carried out a genetic analysis of those under our care.

We designed therefore, a pilot study of CF among UAE nationals with four goals in mind. First, to determine the frequency of $\Delta F508$ mutation; second, to uncover other mutations that may be common in the UAE; third, to identify genetic polymorphisms that would allow the co-segregation of disease alleles by linkage analysis to be followed in all affected families (including those in which the CF-causing mutations remain unknown); and fourth, to correlate altered genotypes with clinical phenotypes.

Materials and Methods

Subject population: mutation analysis was performed on eight families with one child suffering from CF. The families were drawn from Abu Dhabi, Dubai and Ras Al Khaima and thus were fairly representative of the country. Polymorphic frequencies were determined on a sample of 30 random (unrelated) UAE Nationals.

Seventeen CFTR exons and their surrounding intronic sequences were analysed in all patients. The technical details of these procedures are as outlined by Frossard et al⁵, Dawson and Frossard⁶.

Results

None of the 16 chromosomes studied carried the $\Delta F508$ mutation. Investigation was then carried out on the whole of exon 10 and its neighbouring intronic sequences. A mutation was revealed in codon 470 which was due to the replacement of a serine by an arginine at the position 549 of the protein (S549R) and accounted for 12 out of the 16 chromosomes that were screened. In addition several polymorphisms were identified that will be useful in the future study of the co-segregation of the deleterious alleles in most of the families affected by cystic fibrosis in the UAE.

Discussion

We had no idea what the outcome would be as we were unaware of any similar work being carried out in a Gulf State at that time. We chose a strategy allowing us to scan a large part of the CFTR gene in a reasonable time and to detect any sequence change, including single-point mutations. Using this approach we were able to analyse the 17 exons of the CFTR gene which have been reported to contain the majority of CF-causing mutations. We have, thus identified a missense mutation in exon 11 that affects the sequence of NBF1 and accounts for 75% of the CF chromosomes screened in this initial study.

It came as a surprise to find out that the $\Delta F508$ mutation was absent in this sample of CF patients. Indeed, worldwide frequency of this DNA variation accounts for 70% of all CF chromosomes⁸⁰, and the lowest values reported to date have been in the range of 25-30% among Southeast Asians and patients from the Indian Subcontinent^{80,100}. Of course, we have to consider the limited size of the CF samples analysed in this pilot project, but even if the frequency is not zero, it will most certainly turn out to be

lower than reported elsewhere. Indeed, the patient group studied here is representative of the UAE population at large.

We have, however, identified the main CF-causing mutation in this group of CF patients - S549R - which had previously been reported as a rare mutation in one instance only⁸⁰. Genotype-to- phenotype correlations can be inferred. Indeed, this mutation occurs in exon 11 of the CFTR gene, one of the three exons encoding NBF1. S549R, as does $\Delta F508$, may disrupt the function of NBF1 and therefore explain the severity of CF among the patients.

The feature that will be directly applicable to genetic screening programmes is that the S549R mutation affects 75% of the CF chromosomes screened in this study. It is now known from the plethora of reports in this field since 1989, that ideally, the combination of a few mutations (3 to 4) only allows the detection of over 90% of CF mutations in most populations. This is the case in European countries, where the $\Delta F508$ accounts for 50-90% of CF chromosomes^{63,64,80}. There are social and ethnic subpopulation differences so that each population has its particular set of mutations. Specific inventories of CF mutations together with their relative frequencies are indispensable before deciding whether direct DNA detection is possible in massive screening programmes. In the USA, screening programmes are much more difficult to implement because genetic heterogeneity leads to the co-existence of many causative mutations. If the high frequency of the S549R mutation is confirmed in a larger-scale study, it will be an invaluable tool for genetic and population screening in the UAE. We have also identified two polymorphisms, M470V and E528E, which will also be of value in following co-segregation of CF alleles within affected family members, so that prenatal detection and genetic counselling are now feasible in most, if not all, UAE families.

Cystic Fibrosis in the United Arab Emirates: an under-recognised condition?⁷

Among the vast array of health problems occurring within tropical zones there may be some disorders which are more prevalent than previously suspected. CF may be one of these. While CF is well recognised in those of European descent, it is less common in non-European populations. Bowler et al¹⁰⁰ reported CF in Asian residents in the UK, but the condition has been shown to exist also in Negro and Mongoloid races^{103,104}. The historical basis of CF in the UAE and the Middle East has been discussed in Chapter 6. Thus, in an attempt to identify the true extent of the disease within the UAE, this report details our experience over a one year period.

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 diagnosis of CF was given. The review period was 1 August 1993 to July 1994. All patients were seen and reviewed by the author and had sweat tests performed by the pilocarpine iontophoresis method. Demographic and clinical data were abstracted from the medical records. Stool chymotryptic activity was measured by the method of Kasper et al¹⁰⁵.

Results

Twelve children were seen within the one year period, 10 females and 2 males. Their clinical details are summarised in Table 7. Their mean age was 3.7 years (SD \pm 2.4 years). Sweat test analysis gave a mean sweat chloride level of 107 mmol/L (SD \pm 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, with one child dying during the study period of severe cor pulmonale. The second youngest patient, who presented at one year of age, has to date no lung involvement, however. 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 the three children who were not receiving pancreatic enzyme replacement at the time of clinic review, all had levels of stool chymotrypsin below 2.65 units/G¹⁰⁵.

Molecular genetic studies have been performed and 17 of 27 exons of the CFTR gene have been screened. Seventy-five per cent of the CF chromosomes studied have a serine replaced by an arginine at the position 549 of the protein (S549R)⁶.

Discussion

The Al Ain district has a population of approximately 300,000, of whom more than half are non-UAE nationals. It lies 130 kilometres from the main population centres of Dubai and Abu Dhabi city. Thus, in this relatively isolated area, in a one year period, we have seen 12 national patients with the disease from a population of 150,000. This does not in any way suggest that we have seen all the patients who may be affected as they may be treated elsewhere in the Emirates or overseas.

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. 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 so few reports of CF occurring in Arab patients, and especially in the UAE. 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.

Confirmatory molecular genetic studies have been performed on the patients reported here. While the $\Delta F508$ mutation has not been detected so far, the mutation we have found is in close proximity and at the ATP-binding domains. The conclusion to be drawn from our studies is that CF exists in our national Emirati population as a major clinical disease, and that 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 and the carrier rates for the various gene mutations are all subjects for continuing study. The observations of Nazer et al⁵² that many patients in Saudi Arabia are undiagnosed and die in infancy or early childhood may have held true for the UAE until recent changes in the health care system came about. The lessons drawn from our studies here may be transferable to other tropical areas, as an expansion of medical facilities and research into child health problems takes place.

Table 7**Summary of Clinical Information**

Patient	Age (years)	Mode of presentation	Pulmonary disease	Gastrointestinal disease	Sweat chloride mmol/L	Sex
1	4	RRI	++	+	133	M
2	6	RRI+FTT	++	++	95	M
3	6	FTT	+	++	110	F
4	2	SS	++	++	150	F
5	6	RRI+FTT	++++	++	155	F
6	1.2	FTT		++	120	F
7	2	FTT	+	++	80	F
8	2	FTT+RRI	+	++	110	F
9	2	FTT	+	++	90	F
10	8	FTT	++	++	80	F
11	5	SS/RRI/FTT	+++	++	75	F
12	0.6	RRI/SS/FTT	++	++	90	F

RRI = repeated respiratory infection

FTT = failure to thrive

SS = sibling screening

Identification of Cystic Fibrosis Mutations in the United Arab Emirates⁶⁵

Introduction

CF is the most common, potentially lethal, recessive disease in populations of European origin, in which it occurs in an estimated 1/2500 births¹⁰⁶. It has been found in all ethnic groups investigated, and worldwide estimates of the incidence of the disorder range from 1/500 in Ohio Amish to 1/90,000 in Hawaiian Orientals¹⁰⁷.

Following the identification of the CFTR gene^{21,22,65}, the founding of the CF Genetic Analysis Consortium in 1989 has fostered extraordinary advances in the molecular genetics of CF⁸⁰. Besides $\Delta F508$, more than 800 other putative disease-causing mutations have been reported so far, scattered throughout the CFTR gene and responsible for the various forms of CF⁸⁰.

Mutation frequencies are variable among different ethnic groups and geographically located populations^{80,81}. It is thus of the utmost importance to make an inventory of CF mutations and of their frequencies in different parts of the world in order to determine the extent to which DNA detection is feasible in screening programmes. We have thus designed a study aimed at characterising CF alleles among nationals from the UAE.

Subjects

Mutation analysis was performed on 17 families including a total of 23 children suffering from CF. Family names (corresponding to the original tribal names) and detailed family histories allowed us to establish that 10 of the 17 families originally belong to true UAE Bedouin tribes. Four other families were UAE nationals of Baluch (Pakistani) origin and the last three families were from Pakistani Baluch families that

are part of the expatriate population living in the UAE (Table 8). Patient ages ranged from newborn to 8 years.

CF diagnosis in all patients was based on the usual typical clinical presentation including high sweat chloride concentrations. Sweat chloride levels were measured by the pilocarpine iontophoresis method. All patients, except for two UAE national sisters of Baluch origin, presented with extremely severe forms of CF, including sweat chloride concentrations between 100 and 160 mmol/L⁵.

Subjects DNA was extracted from leukocytes isolated from 2 to 5 mL of venous blood collected in EDTA tubes according to standard methods¹⁰⁸.

DNA Analysis

1. Detection of $\Delta F508$ was done by denaturing gel electrophoresis (DGGE) according to conditions described by Fanem et al¹⁰⁹.
2. Denaturing gradient gel electrophoresis (DGGE) strategy. The CFTR gene coding regions were amplified in 32 fragments suited for DGGE analysis using PCR primers and conditions previously described¹⁰⁸, in a multiplex format when possible. Mutations detected by DGGE analysis were identified either by endonuclease restriction analysis or direct DNA sequencing using a Sequence DNA sequencing kit and [355] dATP (Amersham International plc).
3. Detection of S549R by restriction endonuclease analysis. The mutation S549R localised in exon 11 (T→G at nucleotide 17779) alters a Dra 111 restriction site. Five mL (500ng) of exon 11 PCR products, amplified between CF11 and GCCF 11 without a GC-tail, were digested overnight at 37 degrees C with 6 units of Dra 111 (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 111 restriction site: S549 alleles, on which the Dra 111 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 S549R homozygous (Table 8).

In 6 families out of 7 of Baluch ethnicity, CF patients were $\Delta F508$ homozygotes (Table 6). In the 7th family of Pakistani Baluch origin, two sisters, aged 8 and 5, presented with milder forms of CF, which contrasted with the very severe clinical presentations of all other affected children (their sweat chloride concentrations were in the 50-60 mmol/L range, as opposed to 100-160 mmol/L in the other patients). In this case, screening of the 27 exons of the CFTR gene failed to identify any CF-causing DNA abnormality.

Discussion

The most extensive reports on CF in the Gulf countries have come from Saudi Arabia^{51,53,110} and Kuwait¹¹¹. Some authors have suggested that many Saudi and Kuwaiti patients have been undiagnosed and have died in infancy^{51,111}. These observations seem to hold true in the UAE as well⁷, where only two prior clinical reports have presented three cases^{59,60}. Until very recently, anecdotal comments from local physicians hinted at a very low incidence of CF in the UAE.

The commencement of the CF and Respiratory clinics at Tawam Hospital (Al Ain) three years ago has raised the level of awareness for CF among local paediatricians in

this country⁷. This report is based on the study of 23 children. This does not represent, by far, all CF cases in this region, as our estimates suggest that we have an overall indigenous population of about 300,000 individuals, the number of 20 UAE national patients indicates that the most conservative prevalence estimate of CF is 1/15,000.

Mutation S549R

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 characterised by rapid expansion of a small number of families. The S549R (T→G) mutation accounts so far for 100% of the 20 CF chromosomes in the unrelated families that are of Bedouin descent (Table 8). It is thus tempting to speculate that the extraordinary high rate of one single mutation, S549R (T→G), could be the result of a founder effect in a common ancestral family. Haplotype analysis should help to resolve this issue¹⁰.

Mutation ΔF508 in the Emirates

Our results suggest that ΔF508 could have been brought to the UAE by Baluchistanis and that its frequency is 86% in this ethnic group. Of course, the limited number of alleles studied here should introduce a word of caution. Screening among CF patients from different ethnic groups in Pakistan, Iran, Afghanistan and India should help to establish whether Baluchistanis do indeed, exhibit higher than expected rates of mutation ΔF508. Several authors have reported this mutation in affected Pakistani children living in Europe and in the USA, with a frequency of 30% to 54%^{100,112,113,114}, although no mention was made of the precise ethnic origins. Spencer

et al¹¹³ wondered whether the detected $\Delta F508$ mutations among these minorities were derived from North European stock or occurred de novo. It is tempting to hypothesise that

$\Delta F508$ could have originated from ancestors of Baluchistani people.

Table 8

**Distribution by ethnicity and by ethnic origins of the CF patients and families,
the number of alleles carrying S549R and Δ F508**

Ethnic origin	Number of Families	Number of Patients	Mutation	Number of CF alleles
Bedouin¹	10	15	S549R	30/30
UAE Baluch²	4	5	ΔF508	8/10
Pakistani³	3	<u>3</u>	ΔF508	6/6

1.United Arab Emirates national of Bedouin descent

2.United Arab Emirates national of Baluch descent

3. Pakistani of Baluch descent

The determination of the prevalence of cystic fibrosis in the United Arab Emirates by genetic carrier screening¹¹⁵

Introduction

The prevalence of cystic fibrosis in the UAE is unknown. Our previous work indicated that two mutations, $\Delta F508$ and S549R (T→G), account for 46 out of 52 (88%) of CF alleles and characterise 95% (18 out of 19) of the affected families that have been referred to us⁶⁵. All patients that we investigated were homozygous for either of the two mutations: 16 CF patients (who were of Baluch origin) were S549R (T→G) homozygotes and seven patients (who were of Baluch lineage) were $\Delta F508$ homozygotes⁶⁵. In light of these observations, we designed a pilot study aimed at screening CFTR genes of a random sample of the indigenous UAE population for asymptomatic S549R (T→G) and $\Delta F508$ carrier status, with a view to estimating the prevalence of CF in the UAE.

Methods

The sample population for this study comprised 400 unrelated UAE nationals (200 male and 200 female), who were out-patients of Tawam Hospital, Al Ain (Abu Dhabi Emirate) who had consulted because of minor ailments. Great care was taken to ensure that all subjects in this study were unrelated.

Detection of $\Delta F508$ was carried out by non-denaturing polyacrylamide gel electrophoresis. PCR reactions were performed on 500ng DNA samples using primers and conditions previously described⁶⁵. Mutation S549R detection was carried out routinely by Dra 111 restriction endonuclease analysis of exon 11 PCR products.

The presence of suspected $\Delta F508$ and S549R (T→G) mutant alleles was confirmed by sequencing analysis according to protocols that have already been described¹⁰⁸.

Results

Screening of the 800 chromosomes led to the detection of six carriers: four individuals were S549R (T→G) heterozygotes and two were $\Delta F508$ heterozygotes (see Table 9). The estimated frequencies of carriers of each of the two mutations in the population are thus: S549R (T→G), 1:100, $\Delta F508$, 1:200. Given that $\Delta F508$ and S549R (T→G) mutations characterise 95% of families⁶⁴, however, the estimated carrier frequency of any CF mutation in the population of UAE nationals is $[6 \times (100/95)] / 400 = 1: 63$. If we assume that genotype frequencies occur in Hardy-Weinberg proportions, the estimated frequency of affected CF subjects in the Emirate population is 1:15876.

Our estimate relies on small numbers - 26 CF patients and 6 asymptomatic carriers in a sample size of 400 - and we remain cautious with their interpretation. Furthermore, a population bias is possibly introduced by the organisation of the UAE society into tribes, several members of which live in remote desert areas. In the sample population recruited for this study, we could therefore have missed out isolated affected families. For this reason, the predicted CF prevalence of 1 : 15876 among Emirati is probably a conservative estimate.

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 switched towards the side of seeking information on CF carrier status^{115,116}. 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 families¹¹⁷. The introduction of a large scale carrier screening programme for a disease such as CF (with a low prevalence at birth) is considered premature at best. 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.

Table 9

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

	S549R	ΔF508	Total
Number of heterozygotes	4	2	6
% in the population	1.0	0.5	1.5
Frequency in the population	1:100	1:200	1.67

Summary of Chapter 9

The initial studies presented here, document that, indeed, CF is present in the UAE in a severe clinical form. This contrasts with the prior lack of studies and data about the disease not only in the UAE but throughout the Arab world in general. With the increased referral of new patients to our CF clinic we have been able to gain more information about the clinical presentation and of the disease manifestations in the UAE patients.

Initially the severity of the clinical profile in the absence of reported meconium ileus, appeared as a paradox. Severe CF is usually associated with $\Delta F508$ and we were unaware of a common mutation which resulted in such severe disease. Thus increased recognition of the disease and its severity supported the concepts of others, such as Nazer⁵⁵ that the condition is underrecognised among the Arabs and that many children die in infancy from the disease without the true cause of their demise being defined. However, in retrospect it would appear that the concept of genetic homogeneity among Arabs was, in fact, an incorrect concept¹¹⁸.

When we had acquired sufficient patients and families at the clinic we were able to embark upon genetic studies. Our original premise was that the disease severity was likely to be due to the $\Delta F508$ mutation despite the lack of a history of meconium ileus in any of the patients. As only 10-20% of children develop meconium ileus who have the $\Delta F508$ mutation, then it could be a reflection on the small numbers of subjects in our study group. We were particularly surprised, then, to find that not one of these initial Bedouin patients carried the $\Delta F508$ mutation. The clue to the ultimate definition of the genetic defect lay again with the issue of severity. It was clear that the

mutation lay near the very important nucleotide binding domain (NBD). Thus, the discovery of a mutation in exon 11 was well in keeping with the clinical findings (see Chapter 7). We were puzzled that all the initial patients from different families shared the S549R (T→G) mutation, but did note that they were all of pure Bedouin descent. Referrals increased from other parts of the UAE and we found that, indeed, the $\Delta F508$ mutation was present within the national population and, as expected, in some non-nationals living in the UAE. Further analysis indicated that nationals of Baluch descent were likely to be homozygous for $\Delta F508$ mutation and that between the two mutations 95% of our patients could be identified in terms of the genetic cause of their disease.

The final part of the puzzle was to assess the frequency of the two mutations and hence determine a prevalence rate for CF in the UAE. The prevalence rate established by the examination of a random sample of the UAE population was 1:15876. However, this could be a conservative estimate in that we may not have been sampling from some of the remote desert populations in whom the gene mutations may or may not be more frequent, and are a closely inbred group and who do not readily seek medical help. Our findings suggest that CF could be screened for in the UAE. However, the cost and the medical need for this would make it rank very low in terms of national priorities. We can advise, of course, individuals as to their carrier status and offer genetic counselling to known CF families.

We have established that there is an interesting ethnic and spatial distribution of the two major mutations in the UAE. The northern Emirates tend to have more people of Baluch (Iranian and Pakistani) descent and hence the $\Delta F508$ mutation while in the Bedouin of the Abu Dhabi and Dubai Emirates S549R is dominant. We have not, to

date encountered a patient who is a heterozygote with one $\Delta F508$ gene or S549R heterozygote or an admixture of both.

Chapter 10

The S549R (T→G) Cystic Fibrosis Mutation

“Let us then, be up and doing

With a heart for any fate

Still achieving, still pursuing

Learn to labour and to wait.”

Song of Hiawatha iii (1855)

Henry Wadsworth Longfellow (1807-1882)

The mutation named S549R (T→G) was first reported to the Cystic Fibrosis Genetic Analysis Consortium in February 1990 by B Kerem and L-C Tsui⁷⁰. The CF chromosome with this mutation was carried by a non-Ashkenazie Jewish patient from Morocco. Information on the severe clinical presentation was subsequently published and further reports from this group suggested the disease manifestation included pancreatic insufficiency^{119, 120}. Overall, the S549R (T→G) mutation accounted for less than 0.5% of 27,177 European CF chromosomes, but was reported to have a relatively high frequency in Algeria (2.6%)⁹³.

The mutation is as a result of a G (glycine) to T (threonine) transversion at CFTR nucleotide 1779, located in exon 11. The mutation results in the replacement of a

serine with an arginine in the first nucleotide binding domain of the protein and thus would be expected to produce a severe clinical presentation.

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 more than one mutation on the same allele has received little attention. Indeed, in almost all laboratories, genetic analysis is usually considered complete when two mutations are found.

A novel combined CF allele containing the missense mutation S549R (T→G) and a mutation the CFTR promoter region -102 >A + S549R (T→G) was identified¹²¹. This complex allele was identified in two unrelated patients from Southern France, both considered as having a mild form of the CF.

Our group entered a collaborative study which was conducted with the aim of comparing the ethnic, clinical and genetic data of patients with this mutation with or without the combined promoter mutation. Our patients were included in a group of 22 CF patients from the Mediterranean basin (France and Spain) and 16 of them were from the UAE. All of our group had the S549R (T→G) mutation alone and the other patients had the combination. A summary of the major findings is given in the next paragraph and the text of the full paper is published in the journal, Human Genetics¹²¹.

Summary

We compared the main clinical features of six patients with CF carrying the complex allele [-102T>A+S549R(T→G)] with that of 16 CF patients homozygous for the mutation S549R(T→G) alone. The age at diagnosis was higher, and the current age was significantly higher ($p = 0.0032$) in the group with the complex allele, compared with the S549R group alone. Although the proportion of patients with lung colonisation was similar in both groups, the age at onset was significantly higher in the group with the complex allele ($p=0.0022$). Patients with the complex allele also had significantly lower sweat test chloride levels ($p = 0.0028$) and better overall clinical scores ($p = 0.004$). None of the 22 patients had had meconium ileus. All 16 patients who were homozygous for the mutation S549R alone, however, had pancreatic insufficiency, as compared with 50% of patients with complex mutations ($p = 0.013$). Moreover, a patient homozygous for the complex mutation did not present until 34 years of age with mild disease. These observations strongly suggest that the sequence change (-102T>A) in the CFTR minimal promoter could attenuate the severe clinical phenotype associated with mutation S549R (T→G).

Comment

The detection of the mutation S549R (T→G) in our Arab population has been an important finding. However, the clinical features and absence of the (-102T>A) promoter mutation have been important factors in suggesting that additional mutations need to be searched for and may be important in modifying the expression of these severe mutations.

Chapter 11

An hypothesis regarding the spread of the cystic fibrosis mutation $\Delta F508$ and the United Arab Emirates

“Delightful task! To rear the tender thought

To teach the young idea to shoot”

The Seasons: Spring

James Thomson (1700-1748)

Information presented in Chapter 8, indicated that the $\Delta F508$ mutation arose in a population genetically distinct from the present European population¹⁰. Further, data plotted on synthetic maps indicates a marked frequency gradient from south-west to north-west Europe eg 100% presence of $\Delta F508$ in CF mutations in the Faeroe Islands compared with 27% of all mutations in the Turkish CF population. 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 $\Delta F508$ 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 Calafell¹²³ have summarised the basis of the estimates of the age of the $\Delta F508$ mutation. Mutations accumulate in an inexorable fashion along lineages and the number of mutations that would have accumulated in the individuals follows a Poisson distribution with a mean $\lambda = \mu t$ (μ = the mutation rate, t = time). For the 46 haplotypes derived from the original haplotype in which the $\Delta F508$ mutation occurred, the mean number of mutations may be computed by considering the fraction of individuals that have each haplotype and the number of mutations that this haplotype has accumulated. The 1705 chromosomes studied carried 1477 microsatellite mutations, corresponding to a mean $\lambda = 0.866$. With a mutation rate of 3.3×10^{-4} , this gives a total time ($t = \lambda / \mu$) of 2,625 generations, which for a generation of 20 years is equivalent to 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 arose some 50,000 years ago and this mutation provided some biological advantage to those who were carriers. Currently, this advantage is considered to be a protection from typhoid fever rather than cholera which had formerly been proposed⁷⁹.

In an attempt to determine where in Asia this mutation may have arisen the study of the presence of $\Delta F508$ 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 $\Delta F508$). However, an affected Pakistani child born to consanguineous parents was shown to be homozygous for the mutation S549N (G→A)¹²⁴. Schwartz et al reported six affected

Pakistani children, of whom three were homozygous for $\Delta F508$ mutation¹²⁵. It was not stated which ethnic group within Pakistan that the children belonged to. Further study of Asians with CF was reported by Bowler et al¹⁰⁰ who outlined the clinical course in nine Pakistani Asians. Four of the nine were homozygous carriers for the $\Delta F508$ mutation. A comparison was made with a group of 18 Caucasians, 17 of whom carried $\Delta F508$, 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 clinics¹²⁶, seven patients carried the $\Delta F508$ mutation of whom 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 UK¹²⁷. The only study which gives some information about ethnicity within the Indian subcontinent is that of Spencer et al¹²⁸ who reported on 13 CF patients. Seven patients were from Mirpur, Kashmir, one of these was homozygous for the $\Delta F508$ mutation. Four patients were Sikhs from the Punjab, India, two of whom had the $\Delta F508$ 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.

If the founder mutation leading to $\Delta F508$ 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 descent 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 10th Century AD, they had migrated to their present location as described in the Arabic Chronicles of that time, with migrations continuing into the 14th Century AD. Their eventual settlement area is an arid region surrounded by the daunting mountain regions of Bag-e Band and Bampusht. This region is regarded as one of the most isolated in the world¹²⁹.

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 to Oman, the present UAE and into the Punjab of India.

Figure 7 illustrates the geographical location of Baluchistan in relation to the UAE and indicates the likely direction of transfer of the $\Delta F508$ mutation. 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 $\Delta F508$ ⁶⁵. Similarly, population screening has indicated that UAE Nationals of Baluch descent and Pakistanis from Baluchistan living in the UAE were similar in their carrier state ie $\Delta F508$ only. Indirect supporting evidence for this hypothesis may be found in the study of Karjoo et al¹³⁰. 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 nor 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 population¹³¹.

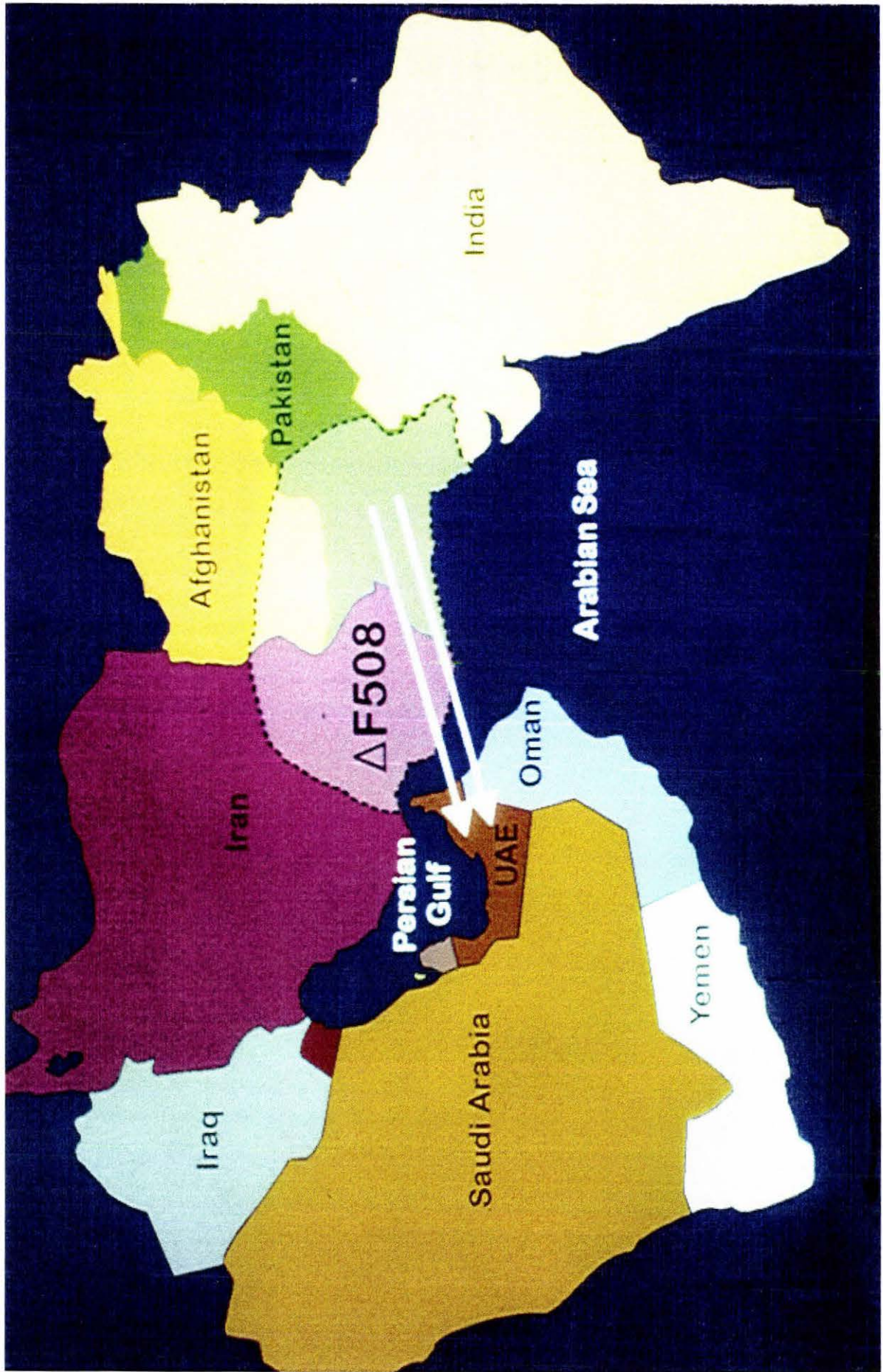
To gain genetic information about the relation of the early settlers in Europe and the present day residents in the UAE of Baluch descent and Pakistani Baluch people a study of microsatellite markers on the respective alleles is necessary. This is currently in progress but will take some time before any information is available.

The hypothesis proposed here is that the original founder mutation of $\Delta F508$ 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 $\Delta F508$ mutation has joined the S549R mutation as the common CF mutation in this region.

Figure 7

The map shows the location of the UAE in relation to other Gulf countries, Iran and the Indian sub-continent.

The dotted lines represent the approximate boundaries of the Baluch ethnic group. The solid arrows indicate the spread of the $\Delta F508$ mutation from the Baluchistan Province of Pakistan.



Chapter 12

Summary and Conclusions

“Roma locuta est: causa finita est”

“Rome has spoken: the case is concluded”

Sermons Book 1

St. Augustine (354-430)

The major advances in medical knowledge over the last thirty years have resulted in an expansion in the field of Medical Geography and have added to its relevance and importance. This is particularly well illustrated in the discipline of human molecular biology which has experienced an exponential growth in knowledge and an explosion in technical achievements. This has occurred over the last twenty years and dates particularly from the discovery of DNA.

Although there is a vast range of genetic disease to which humankind is prone, CF stands out as a condition which illustrates the application of this new molecular genetic and biological knowledge. Instead of simplifying and confirming our prior concepts it has revealed a complex, confusing and ever expanding source of new information about the disease. The landmark event in CF work was the cloning of the

CF gene and the subsequent determination of its function. A mass of new knowledge has accrued which has revealed our prior ignorance about a condition that we assumed to be one disease or abnormality which, in fact, may have up 1,000 varieties and modifications.

The dissertation has been concerned with what may be regarded as a “new” geography that has resulted from the recent knowledge gained about CF and the many genetic mutations that have been revealed by the recently discovered molecular genetic techniques. Not only are there important spatial aspects to these findings, but exciting glimpses of mankind’s history are being revealed. To use CF as an example of how a genetic disease reveals spatial, historical and cultural information, a modicum of knowledge about the disease is required. I have presented information about the clinical aspects of the disorder and the historical background. I have stressed that within half a century, we have seen a definition of the disease developed and its cause and effects at molecular level unravelled. Unfortunately, we still lack a cure for the disease. Further information to gain an understanding of a gene defect and the concept of numerous mutations resulting in different patterns of disease is presented. Specific mutations such as the $\Delta F508$ are dealt with at length.

Rapidly developing countries like the UAE, provide a unique opportunity to apply the new technologies to a previously untapped and unexplored source. The removal of the major health problems such as undernutrition, infection and the provision of sanitation, health service structures and a stable economy have permitted a search for other conditions which result in ill health in a community. In this regard recent information indicates that the UAE is now the leading Middle East country in terms of the lowest infant and child mortality rates and the highest immunisation coverage¹³². The high fertility rate combined with consanguinity and a small gene pool, make the

UAE an ideal place to study genetic diseases. The inbreeding between relatives is deeply entrenched in Arab populations and the consanguinity rate is among the highest in the world.

Our interest in CF among Arab people developed following contact with several patients referred to my outpatient clinic with puzzling chest disorders. Initial assessment indicated that they had CF in a severe form. The previous medical literature suggested that CF was a very rare condition in Arab communities and that the disease was mild. Molecular studies indicated that the common mutation giving rise to CF, namely, $\Delta F508$ was not found among the Bedouin population of the Emirates. $\Delta F508$ occurs in about 80% of affected patients with severe disease in Europe and the distribution has an interesting spatial frequency distribution. It is associated with severe disease in the homozygous state (ie the same mutation on each of the two chromosomes). We eventually determined that another mutation, S549R (T→G), was responsible for CF in the Emirates of Abu Dhabi, Dubai and Fujairah. However, the situation was more complex in that in Northern Emirates such as Sharjah and Ras al Khaimah, many people were of Iranian (Baluch) descent. In addition, many non-Nationals of Pakistani Baluch descent live and work in the UAE. A search for children with CF from these ethnic groups revealed that indeed, the $\Delta F508$ mutation was present in all patients seen. Further, in all patients seen there was a homozygous state as in the S549R group. This finding suggests close family or ethnic group interbreeding. Having identified the two major mutations present in the country, we were able to carry out a population screening for the prevalence of the carrier state (i.e. unaffected by the disease but having one mutation). This indicated that at least 1: 15,000 Emiratis carry a cystic fibrosis gene mutation. It would therefore

be possible to detect the carriers at birth if it were deemed medically, culturally or financially indicated.

The CF mutation $\Delta F508$ founder effect is believed to have arisen spontaneously about 50,000 years ago in a population said to have originated in the Middle East and migrated to Europe carrying the mutation with them. We have postulated that based upon evidence from the UAE and other circumstantial evidence that the origins of these people may have been in the Iranian Plateau. Subsequent emigration suggests that the current Baluchi people from the geographic Baluchistan represent the modern Eastern descendants of the people who migrated to Europe after the ice age.

Conclusion

CF is present in the Arab population of the UAE. It exists in two major forms resulting from the effects of two different gene mutations. There is a north-south distribution, based upon the ethnic background. Those of Baluch descent carry $\Delta F508$ mutation while those of Bedouin descent (Saudi and Yemeni) have the mutation S549R (G \rightarrow T). We have thus uncovered the largest number of people with CF due to the latter mutation reported in the literature and all are homozygous for the mutation. Observations are made on the geographical distribution of CF mutations and a hypothesis is proposed that $\Delta F508$ mutation arose in the forebears of the Baluchi people.

Chapter 13

References

“One of the greatest pains to human nature

Is the pain of a new idea”

Physics and Politics No V

Walter Bagehot (1826-1877)

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