

## Novel Mutation in the CFTR Gene of Cystic Fibrosis Patients in Oman

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### Abstract

**Introduction:** Cystic fibrosis (CF) is the most common lethal autosomal recessive disorder among Caucasians (1: 3,000). In CF, the *CFTR* gene is frequently mutated, with  $\Delta F508$ del being the largely common mutation in Caucasians. Our preliminary pilot study in Omani population revealed a CF prevalence of 1:2,738.

**Objective:** The objective of the present study was to determine the most common *CFTR* mutations in the Omani patients to establish a proper molecular genetics diagnostic basis of CF in Oman.

**Methods:** Blood Genomic DNA samples from Omani patients were examined by PCR and sequencing analyses for the entire coding sequence of the *CFTR* gene were performed.

**Results:** The innovative aspect of this study was the identification of a novel CF-causing mutation, L578delTA. Furthermore, in contrast to the west, p.S549R appears to be the most common mutation in Oman (65.2% for S549R and 13% for  $\Delta F508$ ).

**Conclusion:** The mutation spectrum of CF in Oman revealed six CF-causing mutations, p.S549R,  $\Delta F508$ , 3120+1G>A, L578delTA, p.A357T and 3849+10kbC->T. These findings will ultimately pave the way towards the development of molecular genetic tests in Oman to confirm the diagnosis of CF and serve the patient care and management.

**Keywords:** Cystic fibrosis; CFTR; Mutations; Oman

### Introduction

Cystic fibrosis (CF) is an autosomal recessive disease occurring in 1 per 3,500 newborns [1]. It is the most common lethal genetic disease in the Caucasian population [2]. CF results from mutation in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene [3], located on the long arm of human chromosome 7 at the q31.2 locus, with 27 exons. When both alleles fail to produce the *CFTR* protein, an individual is prone to develop CF. On both *CFTR* alleles, a CF patient can carry, either the same or two dissimilar *CFTR* mutation. Mutations in *CFTR* gene affect the protein folding responsible for coding the chloride ion channels. Consequently, it causes a defect in water and chloride balance, which is essential in maintaining mucus layer in the airways and digestive tract. It will ultimately lead to multisystem deregulation ranging from pancreatic insufficiency to poor lung function, chronic pseudomonas colonization and high mortality [4].

The *CFTR* gene was first identified in 1989 [5], and since the cloning of the gene, more than 1300 mutations have been reported. The delta F508 ( $\Delta F508$ ) is the most prevalent mutation worldwide, accounting for 66-70% and 90% cases in the Western population [6]. It involves deletion of three nucleotides from exon 10, resulting in a loss of the amino acid phenylalanine at position 508 [6]. However, in the Middle Eastern countries, including Oman and UAE, p.S549R is more prevalent [7].

In order to establish the mutation spectrum and pave the way towards the development of molecular genetic tests for CF in Oman, we sequenced the entire *CFTR* gene of CF patients representing the Omani population, and identified one novel CF-causing mutation amongst 6 other known mutations.

### Methods

#### Subject and sample collection

This research was approved by the local Research Ethics Committee at the Sultan Qaboos University (SQU) and the consent was obtained from patients for blood sample collection. The samples were obtained from 46 patients seen in the Pediatrics clinic at the two tertiary hospitals in Oman (in Collaboration with Dr. Qasim Al-Salmi; Royal Hospital and SQU Hospital) between 2000 and 2011. All children less than 13 years of age, presenting indicative clinical features of CF, were involved in this study. The diagnosis was based on typical clinical presentation in addition to positive sweat tests.

#### PCR amplification and sequencing

Blood samples (2-5 ml) were collected from patients and genomic DNA was isolated using QIAamp Blood Maxi kit DNA extraction according to the manufacturer's instructions (QIAGEN, USA). The full coding sequence of the *CFTR* gene was amplified using primers specific for each of the 27 exons. Amplification included a 4-min denaturation set up at 94°C, followed by 35 cycles each consisting of 30s denaturation at 94°C, 30s of annealing at temperatures ranging from 58 to 65°C

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depending on each exon's (melting temperature), and 30s extension at 72°C. After the last cycle, the samples were incubated for 10 min at 72°C for final extension. The PCR product from each exon was visualized using 2% agarose gel electrophoresis.

To identify *CFTR* gene mutations, PCR products of *CFTR* gene were sequenced using the ABI BigDye® Terminator v3.1 Cycle Sequencing Kit. The conditions of the sequencing reaction included 25 cycles at 96°C (10s), 60°C (5s), 60 (4min), 4°C (holding temperature). Sequencing data analysis was performed using the Chromas Pro version 1.41 software, a bioinformatics tool to interpret the sequencing results by comparing the normal sequence of the targeted gene *CFTR* to the tested sequence.

## Results

Our results identified a *CFTR* mutation spectrum that encompasses 6 CF-causing mutations and 1 polymorphism (Table 1). The S549R was identified as the most common *CFTR* mutation in the Omani CF patients with a frequency of 65.2%, followed by the ΔF508 with frequency of 13 %. More interestingly, we identified, and for the first time, one novel unreported mutation L578delTA (Figure 1). Another rare mutation p.A375T was identified in one of our patients, and we

MutTation	Substitution nucleotide	Number of Patients	%
p.S549R	AGT→AGG at 549	30	65.2
p.ΔF508	Deletion of Phe at AA 508	6	13
3120+1G>A	G→A- Splice mutation	4	8.7
<b>p.L578delTA</b>	<b>TA is deleted from CCTAGAT-Frameshift mutation</b>	<b>2</b>	<b>4.3</b>
<b>p.A357T</b>	<b>Ala→Thr at AA 357</b>	<b>1</b>	<b>2.2</b>
3849+10kbC->T	C→T, 10kb insertion in intron 19	1	2.2
p.S549R/p.ΔF508	Heterozygous	1	2.2
p.ΔF508/3120+1G>A	Heterozygous	1	2.2
		<b>46</b>	<b>100</b>

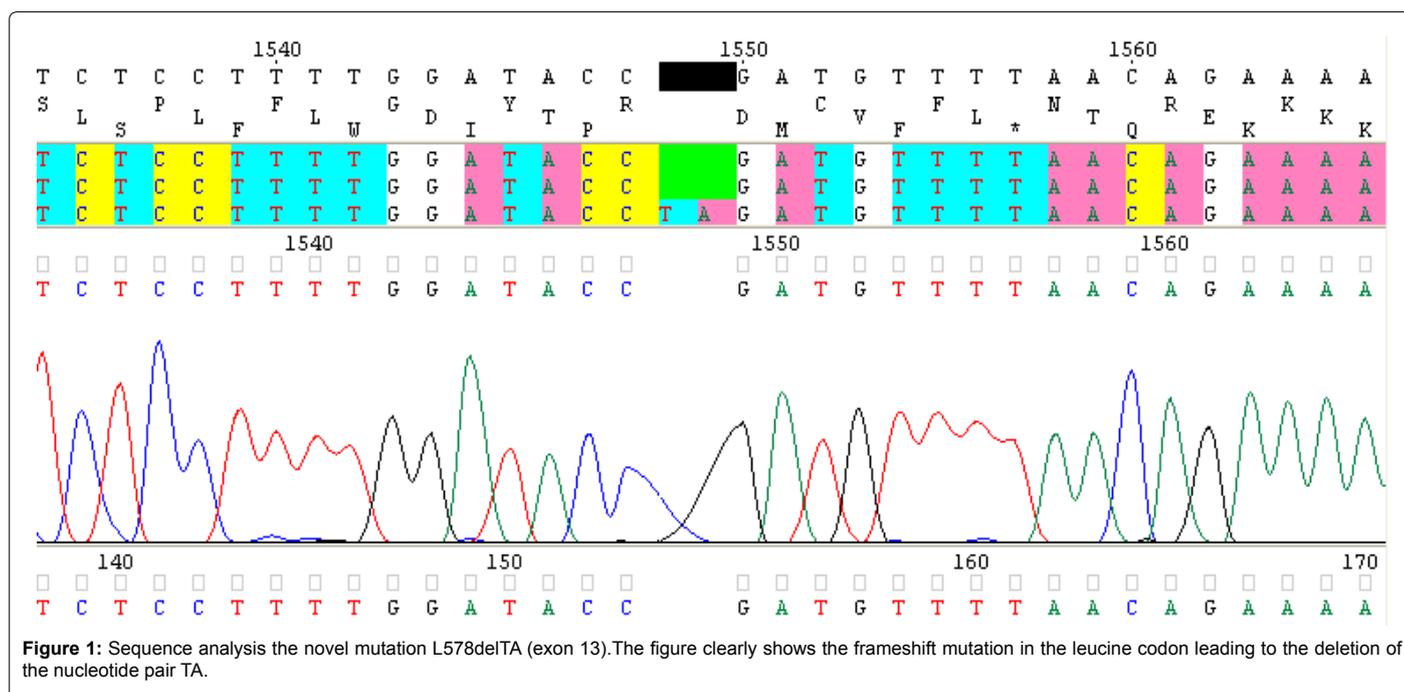
Table 1: The *CFTR* mutation spectrum in Omani population.

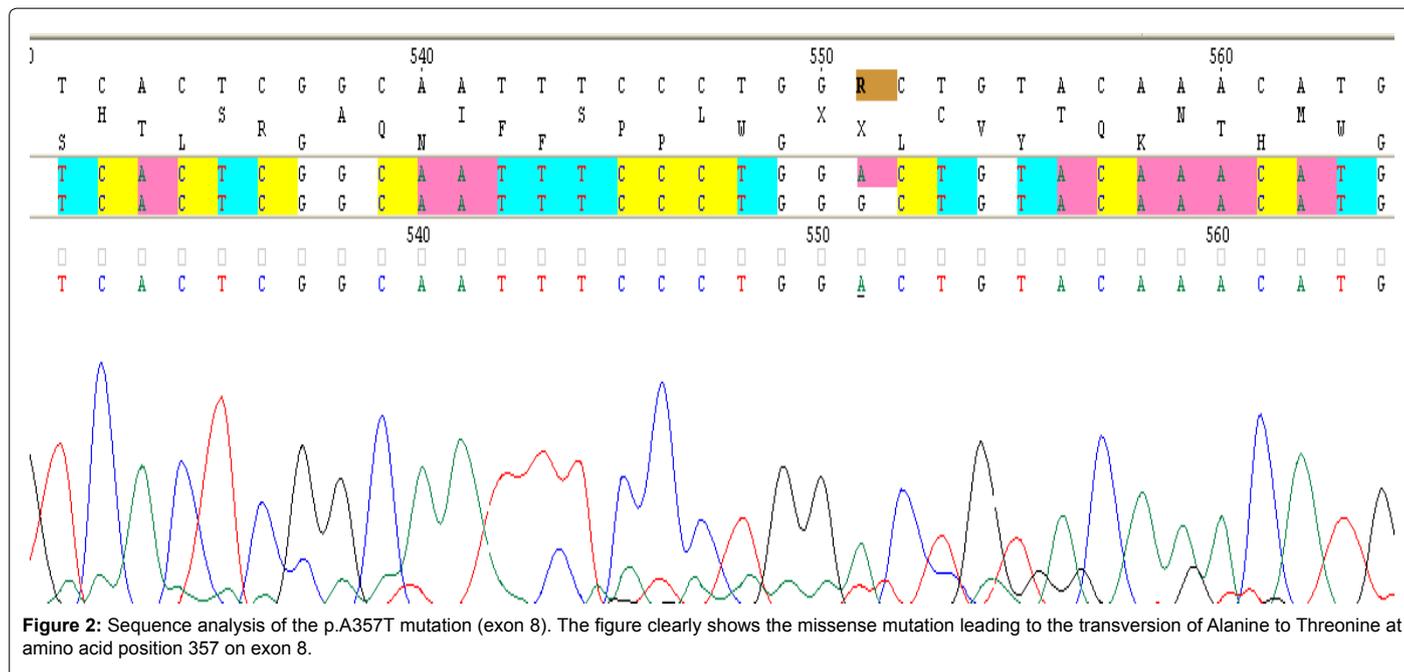
are the second group to detect this mutation (Figure 2) since it was reported last year in Chinese population [8]. The other two mutations found in our patients, were c.3120+1G>A with a frequency of 8.7% and c.3849+10kbC->T (2.2%) as it has been previously reported in the UAE. Out of two patients, one patient was heterozygous for both p.S549R and p.ΔF508 mutations and the other one was heterozygous for p.ΔF508 and c.3120+1G>A. Interestingly, 4 of the patients also displayed c.Q1463Q variant located on exon 24.

## Discussion

Cystic Fibrosis is the most common potentially lethal genetic disorder in the Caucasian population, and the incidence rate is steadily increasing. While around more than 1200 *CFTR* mutations have been reported since 1989 [3], reports in the Arab populations are limited. The current study was carried out because of lack of genetic studies on CF in Oman. Therefore, the entire *CFTR* gene was sequenced to identify the most common mutations in the CF Omani patients. Trivial heterogeneity in the *CFTR* mutations was observed in the Omani population. The *CFTR* mutation spectrum in Oman encompasses Six disease-causing mutations (p.S549R, ΔF508, 3120+1G>A, L578delTA, p.A357T, 3849+10kbC->T) and one polymorphism, p.Q1463Q. Of the six disease-causing mutations, our present study identified two novel mutations; p.A357T and L578delTA. Furthermore, two other mutations, ΔF508 and p.S549R, which are prevalent in different regions of the world as well as in the Middle Eastern populations [9]. However, while ΔF508 is the most common mutation in the *CFTR* gene in the west, p.S549R is considered the most common mutation in the UAE [10].

The mutation spectrum in Oman tends to overlap with those mutations observed in different regions of the Arab world and is less common to that of Europe. As described for Caucasians, a study in Tunisia (a Mediterranean country) showed that ΔF508 mutation was the most common *CFTR* mutation, which was identified among 17 different mutations in 390 Tunisian CF patients [4]. In contrast, other studies in the Maghreb revealed 18% of the CF patients to be carriers of ΔF508 mutation [11]. A study in Lebanon revealed 34% of the CF





patients showing the  $\Delta F508$  mutation [12]. In Bahrain however, only 8% of the CF patients examined carried  $\Delta F508$  mutation [13]. Similarly, studies in the UAE showed that 15 out of 17 people from Bedouin descent had the p.S549R mutations. Curiously, amongst a distinct population of Baluch origin, 6 out of 7 affected families were  $\Delta F508$  homozygotes, clearly indicating that Bedouin people had the p.S549R mutation while the Baluch origin people had  $\Delta F508$  mutation [10]. More interestingly, a previous study including UAE and Oman populations showed that all patients were homozygous for the single mutation p.S549R. Another study conducted for only 16 Omani patients revealed that 69% from Bedouin descent were found to have the p.S549R (T-G) mutation, while 12.5% (two), from Baluch descent had the  $\Delta F508$  mutation, and the remaining patients were negative for the mutations [4,10]. All these studies are in agreement with the findings of our present study. The majority of the cases showed p.S549R T->G mutation (63.2%) as previously reported by Frossad et al. (69%) [10].

More interestingly, our present study identified one novel mutation L578delTA (Leucine CCTAGAT>>>CC\_GAT) was a frameshift mutation leading to the deletion of 2 base pairs on exon 13. Leucine (L) is the 578<sup>th</sup> amino acid encoded by the codon CTA at position 1737. The base pair TA is deleted from CTA resulting in a frameshift mutation, which could lead to the onset of CF. Moreover, our study is the second to identify another mutation p.A357T, which results in the substitution of the amino acid alanine to threonine at amino acid position 357 on the peptide encoded by exon 8.

The 3849+10kbC->T mutation was also identified in our patients; it was first described in 1994 in patients that suffered from chronic pulmonary disease, but displayed normal sweat chloride values. This mutation is a result of a 10kb insertion in intron 19 along with the replacement of Cytosine (C) with Thymine (T) that leads to the development of an alternative splice acceptor. It is a rare mutation accounting for 0.15% in Europe, 2% among Hispanics and Native American population and a bit higher rates in Jews, Eastern Europe and Polish populations (4%). This is a splice mutation that leads to the

development of an atypical mRNA. Patients with 3849+10kbC->T have a milder form of CF and a better prognosis, because of the presence of small amounts of normal spliced transcripts [14].

However, the splicing mutation 3120+1G>A involving transition from Guanine (G) to Adenosine (A) on intron 16 of the *CFTR* gene, which is common amongst Africans was also found in our patients. Oman has been known as a trading nation involving immigration and passage through the country of people from other continents, particularly Africa, and a significant number of families originated from Africa; this might explain the presence of such African-specific mutation in Oman and other neighboring countries in the Middle East. The frequency of this mutation in Jordan, Oman, Saudi Arabia, Qatar, and UAE is 0.107%. A study carried out in Saudi Arabia including 16 patients, identified two novel mutations in the *CFTR* gene; c.1548delG and c.406-2A-G [7]. The most prevalent mutations included 3120+1G>A, p.N1303K and c.1548delG [7]. In Kuwaiti population, 55% of the CF patients exhibited the delF508, G542N and p.W1282X while 24% of cases showed c.1548delG, c.I1234V and 3120+1G>A [15]. In another study, the 3120+1G>A was a rare mutation, been detected in 3 patients along with another patient having an additional mutation  $\Delta F508$  on the other allele [16]. Our results showed 4 patients with the 3120+1G>A mutation only and one patient carrying both the 3120+1G>A and  $\Delta F508$  mutations. Being a rare mutation, it could be either due to Uniparental Disomy for chromosome 7 (UPD7) or to the deletion of a major portion of the *CFTR* gene on the other chromosome that spans the same region as previously reported [16]. Last but not least, 5 of 46 patients also displayed a variant p.Q1463Q mutation. It is an exonic polymorphism associated with CF, where Guanine is substituted by Alanine at position 4521 on exon 24, leading to a sequence variation that has little or no effect on the protein function [17].

It has to be noted that in the Arab world, the incidence of CF is believed to be high, but the rates reported appear to be low because of under-diagnosis. In few isolated Arab tribes/populations, where

consanguineous unions are common, there is a chance of higher rates of CF incidence [18]. Consequently, compared to the Western world, CF is likely to be prevalent in the Arab world, but with a clear difference in the mutation spectrum.

The strength of this study was the use of standardized, reliable and validated set of experiments and tools to identify the mutation within the CFTR gene of CF patients representative of Omani population. In spite of these strengths, this study has few limitations. Although, only one tertiary hospital was chosen, the patients (46 patients), involved in this study were referred to our hospital from all the regions and represent the Omani population. In addition, we are the only hospital performing molecular genetic testing with standardized operating procedures and quality control for CF diagnosis, including sweat test, and DNA analysis.

In conclusion, this study examined the genetic and the clinical aspects of CF in Oman. The mutation spectrum of CF in Oman is quite similar to the one in neighboring countries with two major mutations, the p.S549R, which is the most common,  $\Delta F508$ , 3120+1G>A, L578delTA, p.A357T, and 3849+10kbC->T. The innovation of this study was the identification of two novel CF-causing mutations, L578delTA and p.A357T. The establishment of this CF-mutation spectrum in Omani patients will now pave the way towards the development of molecular genetic tests based on these six mutations to support the diagnosis of CF, which will serve patient care, including genetic counseling and management of the disease. Although the sweat test remains the vital diagnostic test for CF, it does not eliminate CF. Combined with the clinical presentation; genetic sequencing of the CFTR gene is the most common diagnostic tool currently available for CF.

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