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The Impact of the Complexity of Cystic Fibrosis in Jordanian Patients on the Spectrum of Cystic Fibrosis Transmembrane Conductance Regulator Mutations

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Abstract

Contrary to earlier beliefs, cystic fibrosis (CF) is relatively common in Arab populations with an estimated incidence of about 1/2500 live births in Jordan. In order to identify the common mutations among CF Jordanian patients a total of 386 Jordanian CF patients (323 families) were followed up over a period of fifteen years from diagnosis and were screened for Cystic Fibrosis Transmembrane Conductance Regulator (*CFTR*) mutations. Furthermore, to characterize the spectrum of the *CFTR* mutations, DNA samples were obtained from sixty-eight patients and sixty-six parents and were subjected to complete *CFTR* gene screening by multiplex heteroduplex (mHET) analysis followed by direct sequencing. The screening included promoter, all exons with flanking intron sequence (including T-tract in intron 8) and resulted in the identification of twenty-six different mutations. The most prevalent mutation, p.Phe508del was found to account only for 7.4 % of the identified *CFTR* mutations. This low frequency of the p.Phe508del mutation among Jordanian patients is comparable with native Asians. In this study, seven *CFTR* mutations, which have not been previously reported, were identified (c.CFTR dele2 (ins186), c.296+9A>T, c.297-10T>G, p.Thr388Met, p.Thr760Met, c.3670delA and c.4006delA). The large number of mutations reflects the ethnic diversity of the Jordanian population and the complex history of the country. The obtained results will assist to improve the understanding of the molecular basis of the pathophysiology of cystic fibrosis, genetic counseling, and prenatal diagnosis in Jordan. Additionally, it will identify the correlation between the *CFTR* genotypes and the CF phenotypes in the Jordanian population, especially among the newly discovered mutations, which will, in turn, broaden the management of the disease in Jordan.

Keywords: Cystic fibrosis, Mutations, Genotypes, Jordan

1. Introduction

Cystic fibrosis (CF) is the most common lethal autosomal recessive disorder in Caucasians with the incidence of one in 2000 to 3000 live births in various populations (Tzetis *et al.,* 1996). The incidence rate of CF is considerably higher in certain ethnic groups such as Celts in Brittany (western France) where it is among the highest in the world with an estimated frequency of 1: 1983 (Scotet *et al.,* 2012) in contrast to Japan which has the low incidence rate of estimated frequencies of 1: 350,000 live births (Bois *et al.,* 1978; Rosenfeld *et al.,* 1997; Yamashiro *et al.,* 1997; Padoa *et al.,* 1999). Data from the annual report of US 'Cystic Fibrosis Foundation Registry' revealed an estimated 29497 CF patients in the United States and more than 70000 patients worldwide. In the Arab world, CF disease was initially thought to be a very rare disease; however, studies revealed that CF incidence rates are estimated at 1: 2500 live births in Jordan (Nazer, 1992; Kakish, 2001) followed by 1:5000 in Bahrain (Al-Mahroos, 1998) which is comparable with the incidence of CF among Caucasians ranging between 1:2000 and 1:4000 live births. (Dawson and Frossard, 2000; Nazer, 1992).

Cystic Fibrosis is caused by mutations in the cystic fibrosis transmembrane regulator (*CFTR*) gene encoding the c-AMP-activated chloride channel (Linsdell, 2006). The actual spectrum of *CFTR* mutations varies among different ethnic groups and geographical locations. More than 1900 mutations and variants in the *CFTR* gene have been reported from various populations (Jonsdottir *et al.,* 2008). The most frequently detected mutation in these populations is p.Phe508del. According to these reports, p.Phe508del mutation does not seem to account for more than 20 % of all Caucasian CF chromosomes (Antiñolo *et al.,* 1997; Lucotte *et al.,* 1995). In the Arab countries, several types of research have been investigating the

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mutational patterns of *CFTR* genes amongst patients from Oman (Frossard *et al.,* 1998), Bahrain (Al-Mahroos, 1998), Saudi Arabia (Nazer *et al.,* 1989; Kambouris *et al.,* 2000), United Arab Emirates (Saleheen *et al.,* 2006), Jordan (Al-Batsh *et al.,* 2013), Qatar (Rahman *et al.,* 2006), and Libya (Hadj Fredj *et al.,* 2011).

A large group of Arab CF patients including 202 patients with a follow up of more than nine years have been reported (Rawashdeh and Manal, 2000). Around 73 % of the patients were reported with classical CF clinical manifestations (growth failure, malabsorption, and pulmonary involvement). Some of the less common CF-associated clinical features, such as liver disease and pseudo-Bartter syndrome were significantly more prevalent among Jordanian patients than Caucasian populations (13.6 % vs. 3-5 % and 7.4% vs. few cases report respectively). These clinical differences could be attributed to both genetic and environmental factors. Some mutations are associated with certain clinical features and sweat chloride concentrations (Beck *et al.,* 1999; Bienvenu *et al.,* 1996; di Sant'Agnese *et al.,* 1953; Romey *et al.,* 1999; Southernl and Peckham, 2004; Wilschanski *et al.,* 1995; Braun *et al.,* 2006; Chaudry *et al.,* 2006; Hamosh *et al.,* 1998).

Genetic investigations of CF have not been conducted on Jordanian population, which is considered as a population of interest for many reasons. The territory constituting modern Jordan was the site of some of the earliest settlements known to the world (Ammonites and the kingdoms of Edom, Gilead, and Moab). The population of Jordan consists almost entirely of Arabs along with some racial minorities of Circassians and Armenians (Al-Kindy *et al.,* 2014). Furthermore, the consanguinity rate in Jordan varies from 50 % to 64 %; therefore, it is an important feature for the occurrence of genetic diseases among this population (Al-Salem and Rawashdeh, 1993).

Accurate information on the incidence and the spectrum of mutations are considered essential for health care planning and predicting the risk for prenatal diagnosis in Arab countries. Therefore, this study is aimed at identifying the spectrum of the *CFTR* mutations and polymorphisms among the Jordanian population.

2. Materials and Methods

2.1. Patients

A total of 386 patients from 232 families were diagnosed with CF. The diagnosis of CF was based on either a suggestive or consistent clinical picture with CF as well as concentrations of two sweat sodium and chloride tests with 60mmol^{-1} or higher (Doull *et al.,* 2001; LeGrys, 1996). The presence of other affected family members, the death of a sibling with a similar condition, the degree of parent's consanguinity, family pedigrees, sweat chloride concentration and relevant clinical information were collected at diagnosis and during follow ups.

2.2. Blood Sampling and DNA Extraction

Informed consents were signed by all participants in the study. EDTA blood samples were collected from 150 patients and their parents for mutation screening. Plasma was separated by centrifugation, and stored at -80^{o}C. Genomic DNA was extracted from blood by a standard method and was subjected to *CFTR* mutation screening analysis.

2.3. Screening for CFTR Mutations

Genomic DNA from patients was subjected to mutation detection analysis using *CFTR* -specific primers previously described (Zielenski *et al.,* 2002) applying polymerase chain reaction (PCR) and multiplex heteroduplex (mHET) analysis on the Hydrolink gel matrix and direct sequencing using the Thermo Sequenase radiolabeled terminator cycle sequencing kit (USB) which has been refined by (Zielenski *et al.,* 2002). In addition, the thymidine tract (T-tract) in the acceptor splice site in intron 8 was evaluated to detect the RNA splice variant c.IVS8-5T (Mak *et al.,* 1997).

3. Results

The diagnosis of CF was conducted on 386 (220 boys and 166 girls) patients coming from 232 families. Consanguineous marriage was present in 263 patients (68 %); the parents were first or second-degree cousins in 205 patients (53 %). Complete clinical data were available on 294 patients (Table 1). DNA samples from sixty-eight patients and sixty-six parents were obtained for the study and screening was completed for sixty-eight CF patients. The selected sixty-eight patients were born from healthy non-consanguineous parents.

Twenty-six different *CFTR* mutations/variants accounting for 45.7 % of the CF chromosomes were identified. The spectrum of *CFTR* mutations identified is shown in Table 2. The p.Phe508del mutation accounted for only 7.4 % in this cohort. Only four patients were homozygotes for p.Phe508del mutation with sweat chloride concentration being above 100 mmol/l, pancreatic insufficiency (PI) and severe lung disease. Two patients died; one patient died at the age of three years from respiratory failure, and the second was aged thirteen years and died from liver failure.

Four patients were p.Phe508del heterozygotes. They had a very variable clinical presentation and disease severity probably due to the effect of the second mutation, despite the fact that all were diagnosed with pancreatic insufficiency. Among the mutations detected, seven alleles were found for the first time [c. CFTR del2 (ins186), c.296+9A>T, c.297-10T>G, p.Thr388Met, p.Thr760Met, c.3670delA and c.4006delA]. These mutations, as well as short clinical characteristics of the patients carrying them, are shown in Table 3.

Seven different *CFTR* mutations were identified in nine chromosomes of twelve patients with overt liver disease (p.Phe508del, c.CFTR del2, c.296+9A>T and c.297-10T>G, c.1716G>A, including c.1677delT and c.IVS8-5T). The liver disease was not associated with any specific genotype. *CFTR* mutations were identified in six alleles of four patients in the cohort who had hypotonic dehydration associated with hyponatremia, hypochloremia, hypokalemia, and metabolic alkalosis (Pseudo-Bartter syndrome). One patient was homozygote for p.Gln1100Pro, and a second was homozygote for *CFTR* del2. Other mutations detected in these patients were p.Phe508del and p.Gly1244Glu. None of them carried the p.Thr338Ile and p.Ser1455X mutations, previously associated with these manifestations (Padoan *et al.,* 1996; Epaud *et al.,* 2005).

Table1. Major presenting symptoms in 294 Jordanian children with cystic fibrosis

CFTR mutation/variant	Locationexon/intron	Number of chromosomes	Proportion (%)
p.Phe508del	10	10	7.4
c.1677delTA	intron 10	6	4.4
c.IVS8-5T	intron 8	5	3.7
c.CFTR del17a-18	17a-18	4	3.0
*c.3670delA	19	4	3.0
c.CFTR dele2	2	4	3.0
p.Arg75Gln	3	2	1.5
c.1716G>A	10	2	1.5
p.Arg1066Cys	19	2	1.5
p. Gln1100Pro	19	2	1.5
p.Trp1282X	20	2	1.5
c.3849+5G>A	intron 19	2	1.5
*c.CFTR del2(ins186)	2	2	1.5
*c.296+9A>T	intron 2	2	1.5
*c.297-10T>G	Intron 3	2	1.5
p.Ala120Thr	4	1	0.7
p.Ile148Thr	4	1	0.7
*p.Thr388Met	8	1	0.7
*p.Thr760Met	13	1	0.7
p.Asp1152His	18	1	0.7
p.Ser1235Arg	19	1	0.7
p.Gly1244Glu	20	1	0.7
c.3849+10kbC>T	Intron 19	1	0.7
p.Asn1303Lys	21	1	0.7
*c.4006delA	21	1	0.7
p.Gly1244Asp	20	1	0.7
Total identified		62	45.7
Unknown		74	54.3
Number of tested chromosomes		136	100

*NOT previously reported mutation

Table2. The spectrum of *CFTR* Mutations among Jordanian CF population

Clinical manifestations	Number of patients	Percentage (%)
Respiratory	54	18.4
Malabsorption	53	18
Gastrointestinal & Respiratory	108	36.6
Meconium ileus	18	6
Liver disease	40	13.6
Pseudo-Bartter syndrome	21	7.4
Total	294	100

Table 3. Genotypic and phenotypic matching of new *CFTR* mutations in Jordanian population.

Mutation	Nucleotide Change	Exon/ Intron	Consequence	Second mutation	Age at DX (mo)	Lung Disease	Pancreatic Insufficiency/ Pancreatic sufficiency/ (PI/PS)	Sweat Cl Mmol/L	Other Symptoms
c.CFTR del2(ins186)	Complex	2	Large del.	Unknown	15	Moderate	PI	65	
c.296+9A>T	IVS 2	Missplicing	splicing	c.296-10T>G	72	Severe	PI	65	Hepatomegaly
c.297-10T>G	IVS 2	Missplicing	splicing	c.296+9A>T	72	Severe	PI	65	Hepatomegaly
p.Thr388Met	T to ?/ a 1295	8	Missense	p.Trp1282X	11	Moderate	PI	80, 90	Normal growth
p.Thr760Met	C to T at 2411	13	Missense	p.Phe508del /c.1677delTA	60	Moderate	PI	110	
c.3670delA		19	Frameshift	c.3670delA	4	Severe	PI	112	Severe
c.4006delA		21	Frameshift	p.Phe508del	14	Mild	PI	95	Malnutrition

4. Discussion

A large number of CF patients points to the high prevalence of this disease in Jordan. The incidence rate of CF in Jordan is 1: 2500 live births; this is close to the reported rate of Caucasian patients (Singh *et al.,* 2015; Nazer, 1992). The high consanguinity rate, the large (average of 6.7 individuals) and the extended families in the Jordanian community can explain the segregation of many cases in relatively few pedigrees. The higher number of boys over girls in families in Jordan (1.3:1) may reflect the higher mortality rate among the affected females according to the observed gender gap in the CF survival rate (Rosenfeld *et al.,* 1997; Schneiderman-Walker *et al.,* 2005).

Complete *CFTR* mutation screening in the cohort of sixty-eight CF patients led to the identification of twenty-six different mutations/variants accounting for 45.5 % of tested CF chromosomes. Relatively the large number of different mutations found in less than half of the analyzed chromosomes reflects a complex history with a considerable ethnic diversity of the Jordanian population that goes back several centuries. Being at the crossroads of the Middle East, the location of the lands of Jordan have served as a strategic point connecting Asia, Africa, and Europe.

More than 1900 mutations have already been reported in the *CFTR* gene (US Cystic Fibrosis Foundation, 2010; Jonsdottir *et al.,* 2008). The frequencies of each mutation in a population vary according to the geographical and ethnic origin of the population. Reports on these mutations in Arab populations have so far been very limited. Recently, a panel of eleven common mutations accounting overall for 70 % of all Arab CF chromosomes have been reported: p.Phe508del, c.3120+1G> A, p.Asn1303Lys, p.Trp1282X, p.Gly115X, c.711+1G>A, p.Ser549Arg, p.Ile1234Val, c.1548delG, p.His139Leu and c.4010del4 (Bois *et al.,* 1978; Kakish, 2001). The latter three mutations are believed to have originated in the Arab native populations since they were never described in Caucasian CF patients (Kerem *et al.,* 1995; Kambouris *et al.,* 2000; El-Harith *et al.,* 1997; Angelicheva *et al.,* 1994).

The spectrum of mutations detected so far in Jordanian CF population is different from that reported in many countries across Europe and North America (Guilloud-Bataille *et al.,* 2000; Rock *et al.,* 2005; Tzetis *et al.,* 1996). The frequency of most common Caucasian mutation (p.Phe508del) was present only in 7.4 % of Jordanian patients. This is the lowest p.Phe508del mutation ever reported in any ethnic group (Elahi *et al.,* 2006; Kakish, 2001; Lao *et al.,* 2003). The unusual low frequency of the p.Phe508del mutation in the current study matches the same low incidence of a former study in Jordan (Al-Batsh *et al.,* 2013), in Asia (12-31 %) compared to the 66 % high frequency worldwide (Singh et al., 2015). The rarity of the p.Phe508del mutation can be attributed partly to the genetic heterogeneity among Jordanian population due to the genetic drift and gene flow that play a major role in reshaping the genetic structure of Jordanians. Patients with one p.Phe508del mutation had a wide range of clinical presentation and a variable course of the disease, although all were PI. In adult CF patients, p.Phe508del was associated with PI in only19 % of cases (Modolell *et al.,* 2001). This variability is partially related to the presence of another mutation (Antiñolo *et al.,* 1997). The second most common (4.4 %) Jordanian *CFTR* allele, is c.1677delTA deletion, which is of high prevalence in the Turkish population (Onay *et al.,* 1998). This may be associated with the four centuries of Ottoman rule (1516-1918 CE). Another ethnic-specific mutation relatively frequent (3.0%) among the Jordanian *CFTR* mutations is multi-exonic deletion spanning exons 17 to 18 (c.CFTR del17a-18) previously reported in Palestinian CF patients (Lerer *et al.,* 1999). This can be explained by the presence of the large Palestinian community in the country of Jordan. There are also other mutations including [p.Asp1152His, p.Gly1244Glu, c.3849+5GA, c.CFTR del2 (186kb) and c.CFTR del2 (5kb)] that appear to be more widely spread throughout the Near and Middle East, but are rarely observed elsewhere. Seven mutations were found for the first time in the Jordanian population [c.CFTR del2 (ins186), c.296+9A>T, c.297-10T>G, p.Thr388Met, P.Thr760Met, c.3670delA and c.4006delA]. The most frequent mutation was the c.3670delA frameshift mutation which was diagnosed at a younger age with PI, severe lung disease, and a correspondingly worse prognosis. Finally, some mutations in Jordan are

compatible with mutations in many other regions of the world, such as p.Asn1303Lys, p.Trp1282X, p.Ile148Thr, p.Ser1235Arg, c.3849+10kbCT, and p.Arg1066Cys.

The relatively common liver disease in Jordanian CF patients was not associated with a specific *CFTR* mutation (genotype). No specific genetic abnormality has been linked with more frequent or more advanced involvement of the liver and biliary tree in CF patients (Fagundes *et al.*, 2005). However, there is evidence that hepatobiliary involvement in CF correlates with pancreatic insufficiency and specific human leukocyte antigen (HLA) loci A2, B7, DR2 (DR15), and DQ6 (Fagundes *et al.*, 2005).

This large diversity in the *CFTR* mutations makes the technical feasibility of a large-scale CF carrier screening extremely difficult (Grody *et al.*, 1997). However, these results can help improve genetic counseling and prenatal diagnosis of CF as well as the understanding of the molecular pathophysiology of the disease.

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