

Original Research

Cystic Fibrosis Transmembrane Conductance Regulator (*CFTR*) gene mutations in North Egyptian population: implications for the genetic diagnosis in Egypt

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Abstract: Cystic fibrosis (CF) occurrence in Arab populations is not common and still remains underidentified. Furthermore, the lack of disease awareness and diagnosis facilities have misled the identification of cystic fibrosis for decades. The knowledge about cystic fibrosis (CF) in Egypt is very limited, and a few reports have drawn attention to the existence of CF or *CFTR*-related disorders (*CFTR*-RDs) in the Egyptian population. Therefore a comprehensive genetic analysis of the *CFTR* gene was realized in patients of North Egypt. DNA samples of 56 Egyptian patients were screened for the *CFTR* gene mutations. The 27 exons and their flanking regions of the *CFTR* gene were amplified by PCR, using the published primer pairs, and were studied by automated direct DNA sequencing to detect disease-causing mutations. Moreover, large duplication/deletion was analysed by MLPA technique. *CFTR* screening revealed the identification of thirteen mutations including four novel ones: c.92G>A (p.Arg31His), c.2782G>C (p.Ala928Pro), c.3718-24G>A, c.4207A>G (p.Arg1403Gly) and nine previously reported mutations: c.454A>T (p.Met152Leu), c.902A>G (p.Tyr301Cys), c.1418delG, c.2620-15C>G, c.2997_3000delAATT, c.3154T>G (p.Phe1052Val), c.3872A>G (p.Gln1291Arg), c.3877G>A (p.Val1293Ile), c.4242+10T>C. Furthermore, eight polymorphisms were found: c.743+40A>G, c.869+11C>T, c.1408A>G, c.1584G>A, c.2562T>G, c.3870A>G, c.4272C>T, c.4389G>A. These mutations and polymorphisms were not previously described in the Egyptian population except for the c.1408A>G polymorphism. Here we demonstrate the importance of the newly discovered mutations in Egyptian patients and the presence of CF, whereas the p.Phe508del mutation is not detected. The identification of *CFTR* mutations will become increasingly important in undocumented populations. The current findings will help us expand the mutational spectrum of CF and establish the first panel of the *CFTR* gene mutations in the Egyptian population and design an appropriate strategy for future genetic diagnosis of CF.

Key words: Cystic fibrosis, *CFTR* mutations, Molecular diagnosis, pulmonary disease, Egyptian patients.

Introduction

Cystic fibrosis (CF) is the most frequent genetic lethal disease in the Caucasian populations. This autosomal recessive disorder is caused by mutations in the gene coding for the CF transmembrane conductance regulator (*CFTR*). This glycoprotein functions as a cAMP-regulated chloride (Cl⁻) channel in the apical membrane of epithelial cells (1). Mutations in the *CFTR* gene are responsible for both classical and atypical presentations of the disease, including pulmonary disease, pancreatic insufficiency, malabsorption, meconium ileus, failure to thrive, infertility, and elevated concentrations of chloride in sweat (2).

Despite the great diversity of CF-causing mutations (to date, almost 2009 have been documented; <http://www.genet.sickkids.on.ca>), most individuals with CF carry at least one allele with a deletion of a trinucleotide resulting in the loss of phenylalanine at position 508 (p.Phe508del) (3). Natural selection, allele flows and intra-community gene mixing are among the factors that modulate the *CFTR* mutational spectrum of each population. Moreover, the mutational spectrum varies according to the geographic and/or ethnic origin of each population (4). The highest allelic heterogeneity of the *CFTR* gene (5) has been detected in Mediterranean countries (6).

Molecular diagnosis studies of the *CFTR* gene mutations in the Arab populations revealed that even if the

mutation c.1521_1523delCTT (F508del, p.Phe508del) occurs here, it might not be the most frequent allele in this ethnic group. Another widespread mutation c.3909C>G (N1303K, p.Asn1303Lys) is very common in the Mediterranean countries (7-12). Furthermore, some Arabian patient carriers show alterations of the *CFTR* gene that have never been described in the Caucasians (13). In addition, little has been reported on its occurrence in Arab populations where mutation distribution seems to differ from that of Europeans (14-19). Many of the authors highlight the general lack of awareness of the CF or *CFTR*-related disorders (*CFTR*-RDs) in the Middle East and the lack of diagnostic facilities, such as sweat chloride determination (20).

Nowadays, characterization of *CFTR* mutations will become increasingly important in undocumented populations like the Egyptian population where there is no available data on the nature and frequency of CF (21). Furthermore, knowledge about CF is very limited (22),

Received May 16, 2016; Accepted November 05, 2016; Published November 30, 2016

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and few reports have drawn attention to the existence of CF or CFTR-RDs in the Egyptian population (22-27). However, the spectrum of common *CFTR* gene mutations in the Egyptian population remain unclear, and the recent studies by Naguib *et al.* (22) and Shahin *et al.* (26) suggest that CF is more common in Egypt than previously anticipated and larger studies are needed to identify its incidence, molecular basis and clinical pattern.

Alexandria Governorate is located in the northern part of Egypt, directly on the Mediterranean Sea, making it one of the most important harbours in Egypt. The frequency of autosomal recessive disorders among patients attending the genetic clinic in Alexandria was estimated to be 33.6% (28). Recently, we have noticed an increased number of patients suspected to be carriers of CF and CFTR-RDs. Unfortunately, the molecular diagnosis of *CFTR* gene mutations for these patients is not available in the public hospitals but only in the private medical laboratories using commercial kits or a panel defined by the American College of Medical Genetics and Genomics (ACMG) and American College of Obstetricians and Gynecologists (ACOG) which is not suitable for the Egyptian population. Furthermore, there is only a single referral center for cystic fibrosis in Egypt, namely Cairo city, which deals with standardized operating procedures for CF diagnosis, involving sweat test and DNA analysis and only for the most severe mutations in the Caucasian population (25).

Identifying mutations in patients is important not only for the patients and their relatives, but it also enables more reliable carrier detection in the population-screening program (29). The need is therefore emphasized to consider the importance of extensive study of the CF mutations in the population of Alexandria city. Hence, it is the aim of this investigation to establish the mutation spectrum and to develop molecular screening program for CF in Egypt relying on a population-specific panel with all *CFTR* gene mutations previously and currently detected in our population. To our knowledge, this is the first study that describes the mutational spectrum of *CFTR* gene in the Egyptian patients with CFTR-RDs. The result of this framework will help us allude to the existence of CF disease in the Egyptian population and assist in genetic counseling, parental diagnosis and future screening of CF in Egypt.

Materials and Methods

Patient samples

A case control study that enrolled 56 unrelated patients, 33 of them were females and 23 were males, their ages ranged from 7 to 62 years, had been referred to us for diagnosis at the Chest Diseases Department, Alexandria Medical University Hospitals, Alexandria, Egypt between 2012 and 2014. The diagnosis of the patients was based on typical clinical phenotype. Clinical features of patients were enrolled in our investigation: recurrent or persistent respiratory symptoms such as chronic bronchitis ($n=14$), pneumonia ($n=4$), bronchiectasis ($n=15$), chronic respiratory insufficiency ($n=8$), bronchial asthma ($n=12$), and shortened breathing or sputum production or evidences of mal absorption such as poor growth or chronic diarrhea were determined ($n=$

1), patients with hepatomegaly were also examined ($n=2$). Family history, associated comorbidities, occupational history and detailed history of the current illness were determined. None of the patients had a familial history of classical cystic fibrosis symptoms, except for only one case that apparently had the severe form of this disease with high level of sweat chloride test. We had no more clinical data. All the patients underwent plain chest X-ray and oxygen saturation assessment. High resolution chest computed tomography (HRCT) was performed if indicated. Spirometry is available for some cases who can tolerate the test. Sweat chloride test was not performed routinely to our studied group. Healthy subjects ($n=25$) of the same region without apparently clinical respiratory or gastro-intestinal illness served as controls. The protocol of this CF program study was approved by the Institutional Ethics Committees and informed consent was obtained from all participating subjects.

DNA extraction and CFTR mutation analysis

Blood samples (2-5 ml) were collected from patients and control individuals. Genomic DNA was extracted from whole blood samples using QIAamp Blood Mini kit DNA extraction according to the manufacturer's instructions (QIAGEN, USA). In Egyptian medical genetic laboratories, the Cystic Fibrosis gene carrier screening is performed by commercial kits or a panel of *CFTR* mutations including only the most common mutations found in CF patients of European origin as defined by the American College of Medical Genetics (ACMG)/ American College of Obstetricians and Gynecologists (ACOG). Atypical CF phenotype, however, is caused by milder mutations; most of them are very rare or even not yet described, and thus not included in the panel of CF mutations usually screened. Furthermore, the sweat test as a diagnostic test for CF or CFTR-RDs is not readily available in large parts of Egypt. These facts lead us to undertake a complete analysis of the entire *CFTR* gene in patients with CF or CFTR-RD clinical symptoms. This will improve our knowledge about the type and the frequency of the *CFTR* mutations in Egypt.

To detect *CFTR* gene mutations, an extensive *CFTR* screening was carried out in all DNA samples by direct gene sequencing as there is neither panel nor data about the frequent *CFTR* gene mutations in the Egyptian population. The 27 exons and their flanking sequences of CFTR were amplified by polymerase chain reactions (PCR) using *CFTR* gene specific primers as described by Zielenski *et al.* (30) and Le Maréchal *et al.* (31). The PCR products were purified with an ExoSAP-IT reagent (Amersham Biosciences). The PCR was carried out in a total volume of 25 μ l containing 100ng DNA, 20 pmol of each primer, 200mmol/L dNTPs, 1x reaction buffer, 1.5 mmol/L MgCl₂ and 1U *Prime Taq* DNA polymerase (Promega, USA). The conditions used for amplification were as follows: an initial denaturation (94°C, 5min), followed by 25 cycles of denaturation (94°C, 30s), annealing temperature (ranging from 50-59°C, depending on each primer melting temperature 30s) and extension (72°C, 1min 30s), and a single final extension (72°C, 10min). Direct nucleotide sequencing was carried out using Big Dye terminator cycle sequencing ready reaction kit (Applied Biosystem, New

Jersey, USA). Sequence analysis was performed on an ABI Prism 3100 DNA sequencer (Applied Biosystem, New Jersey, USA). The novel mutations were submitted to the Cystic Fibrosis Mutation Database (<http://www.genet.sickkids.on.ca/cftr/>). The 5-thymidine (5T) variant of the intron 9 polythymidine tract was also documented by direct DNA sequencing. The same patients were also analyzed for CFTR large duplication/deletion using the multiplex ligation-dependent probe amplification (MLPA) test Kit (SALSA® P091-B CFTR MLPA kit, MRC-Holland, Amsterdam, The Netherlands).

Bioinformatics Analysis

Novel and previously reported mutations detected in this study were analyzed *in Silico* using computer programs to predict the effect on protein. Polymorphism Phenotyping v2 (PolyPhen-2) is an automatic tool for prediction of possible impact of an amino acid substitution on the structure and function of a human protein available at <http://coot.embl.de/PolyPhen/>. This prediction is based on straightforward empirical rules which are applied to the sequence, phylogenetic and structural information characterizing the substitution: input options for PolyPhen server (32). Human Splicing Finder (HSF), a splice site prediction analysis, is used to investigate the impact of mutations on the splicing process (33). In addition, we have used Swiss-model program

for predicting the functional motifs and structure of proteins (34).

Results

Spectrum of variations

A comprehensive analysis by direct gene sequencing for 27 exons and their flanking regions of the *CFTR* gene was performed in 56 unrelated patients of Egyptian origin with pulmonary disease. Bronchiectasis was the commonest presentation in our cohort followed by chronic bronchitis (26.8% and 25%, respectively). The sweat chloride test was negative for 35 subjects examined; 20 subjects did not undergo the test and only one had positive values (111mmol/L and 126mmol/L). A total of 13 variations were identified on 22 of the 112 alleles, as reported in Table 1. Two mutant alleles were identified in four patients (7.14%). Among them, one CF patient was a compound heterozygote for c.1418delG/c.2997_3000delAATT mutations, described in 1997 by Dörk et al. in CFTR data base (<http://www.genet.sickkids.on.ca>). The analysis of his parents confirms that these mutations are in trans in the child genome. Furthermore, one mutant allele was identified in 17 patients (30.35%), of whom two carried clearly pathogenic mutations (c.2782G>C and c.4207A>G, respectively). Genotypes of 56 patients from this study were shown

Table 1. CFTR genotypes in 56 Egyptian patients.

Mutation genotypes	IVS8-TGmTn	GATTn	1001+11	M470V	Other variants	No. of patients (%) [§]
Two mutations detected						
1548delG/3129del4	TG10T7/TG11T7	7/7	C	G	c.2562T>G, c.4389G>A	1(1.78)
One mutation detected						
1548delG/-	TG11T7/TG11T7	7/7	C	G		1(1.78)
3129del4/-	TG10T7/TG11T7	7/7	C	G/G	c.2562T>G, c.4389G>A	2(3.57)
R31H*/-	TG11T7/TG11T7	7/7	C	G	c.2562T>G, c.2620-15C>G	1(1.78)
M152L/-	TG12T5/TG10T9	7/7	T	G		1(1.78)
Y301C/-	TG11T7/TG11T7	7/7	C	G/G		1(1.78)
2752-15C>G/-	TG10T9/TG11T7	6/7	T	G		4(7.14)
A928P*/-	TG10T7/TG11T7	7/7	C	G	c.2562T>G, c.4389G>A	2(3.57)
F1052V/-	TG10T7/TG11T7	7/7	C	G	c.2562T>G, c.4389G>A, c.743+40A>G	2(3.57)
3850-24G>A*/-	TG10T7/TG11T7	7/7	C	G	c.4389G>A	1(1.78)
Q1291R/-	TG12T5/TG11T7	6/7	T	A		1(1.78)
V1293I/-	TG10T7/TG11T7	6/7	T	G		1(1.78)
4374+10T>C/-	TG10T9/TG11T7	7/7	C	A/A	c.2562T>G	2(3.57)
R1403G*/-	TG11T7/TG11T7	7/7	C	G/G		1(1.78)
No mutation detected						
-/-	TG10T7/TG11T7	7/7	C	G	c.2562T>G	14(26.78)
-/-	TG10T7/TG11T7	7/7	C	G	c.4389A/A	3(5.35)
-/-	TG10T7/TG11T7	7/7	C	G	c.2562T>G c.4272 C>T c.4389 G>A	1(1.78)
-/-	TG10T7/TG10T7	7/7	C	G	c.2562G/G c.3870A>G,	5(8.92)
-/-	TG11T7/TG11T7	7/7	C	G/G	c.3870A>G, c.4389G>A	5(8.92)
-/-	TG11T5/TG11T5	7/7	C	G	c.4389G>A	7(12.50)

*Novel mutation identified in the *CFTR* gene.

Minus signs (-) indicate the absence of *CFTR* gene mutations after complete screening by direct sequencing.

Mutation genotypes were designated following the recommended nomenclature (Beaudet and Tsui, 1993). The IVS8-TGmTn haplotypes were named by the number of upstream TG dinucleotide followed by the number of consecutive thymidines (Dörk et al., 1994b; Costes et al. 1995). M470V alleles were designated M for nucleotide A at position 1540 and V for nucleotide G at position 1540 (Dayangac et al. 2004).

[§]Genotype frequencies are given as numbers and, in brackets, as percentages of the total studied patients.

in Table 1.

The detected variations contain four novel mutations (Fig. 1) in five heterozygous patients. They are all homozygous for 7T in intron 9. These novel mutations include c.92G>A (p.Arg31His), c.2782G>C (p.Ala928Pro), c.3718-24G>A, c.4207A>G (p.Arg1403Gly). Besides, nine previously reported mutations [c.902A>G (p.Tyr301Cys); c.454A>T (p.Met152Leu); c.1418delG; c.2620-15C>G; c.2997_3000delAATT; c.3154T>G (p.Phe1052Val); c.3872A>G (p.Gln1291Arg); c.3877G>A (p.Val1293Ile); c.4242+10T>C] were detected in our group of patients.

Furthermore, eight polymorphisms were found: c.1408A>G (p.Met470Val), c.3870A>G (p.Pro1290Pro), c.2562T>G (p.Thr854Thr), c.1584G>A (p.Glu528Glu), c.4389G>A (p.Gln1463Gln), c.869+11C>T, c.743+40A>G, c.4272C>T (p.Tyr1424Tyr). These mutations and polymorphisms were not previously observed in the Egyptian population except for the p.Met470Val polymorphism. Additionally, the MLPA analysis did not reveal any abnormal profile.

Interestingly, no F508del (p.Phe508del) and N1303K (p.Asn1303Lys) mutations, known to be the most common widespread mutations, were detected in this study. No variations were found in control samples of 25 healthy individuals (50 alleles).

Novel mutations

Three missense mutations, including two being located in highly conserved residues, and one intronic mutation were first described in this work (Fig. 1).

The c.92G>A (R31H, p.Arg31His) mutation is located in exon 2 of *CFTR*. The mutation was detected in a patient with chronic bronchitis associated with hyperinflation detected in the plain chest X-ray. The mutation on the other chromosome is unknown. The novel missense p.Arg31His variation is predicted to be a mild change by PolyPhen-2 program (<http://coot.embl.de/PolyPhen/>), whereas according to HSF program (<http://www.umd.be/HSF/>), this modification could enhance a new splice site inducing a RNA with a deletion of 37 nucleotides. Therefore it is not clear if it is a disease causing mutation, but it could be a mutation of class V or a mutation of class I if the aberrant splicing is severe.

The c.2782G>C (A928P, p.Ala928Pro) mutation located in exon 17 induces the substitution of alanine to proline in the transmembrane domain 2 of *CFTR*. It occurs in the seventh putative hydrophobic transmembrane segment (MSD7) of *CFTR* protein and results in the addition of a positive charge and bulky side chain (35) program PolyPhen-2 predicted that this amino acid substitution was “probably damaging” with a score of

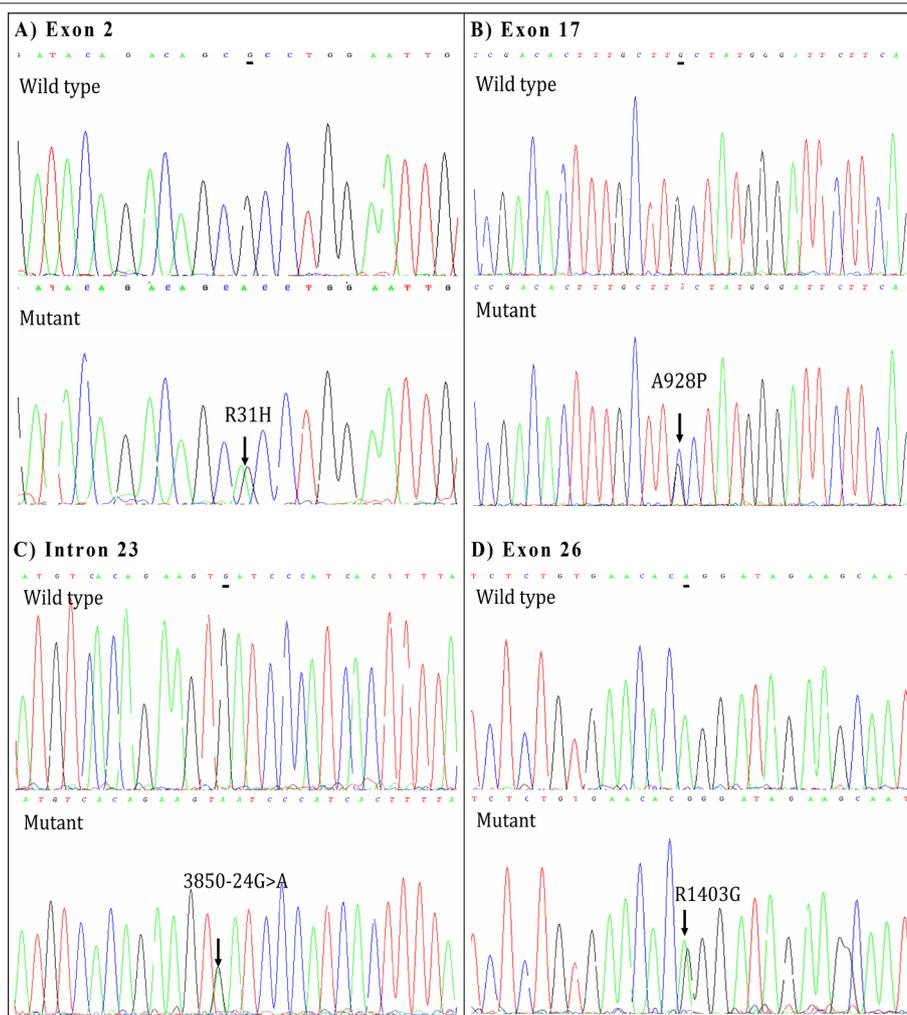


Figure 1. Sequence electropherograms showing the novel mutations detected in the *CFTR* gene by direct sequencing. (A) Sequence analysis revealed a G (underlined in the wild type) to A nucleotide transversion at position 92, as indicated by the arrow. (B) Sequence analysis revealed a G (underlined in the wild type) to C nucleotide transversion at position 2782, as indicated by the arrow. (C) Sequence analysis revealed a G (underlined in the wild type) to A nucleotide transversion at position 3718-24, as indicated by the arrow. (D) Sequence analysis revealed a G (underlined in the wild type) to G nucleotide transversion at position 4207, as indicated by the arrow.

1.0 as this alanine is highly conserved even in rodents. Also, the Swiss-model program revealed an impact on the structure of CFTR protein and functional motifs. So, it could affect the biosynthesis and maturation of CFTR protein and this mutation could be a mutation of class II. No other classical *CFTR* mutation was found, but a 5T allele was identified in intron 9 (IVS9-5T) and 9T in trans. These patients also carry in exon 11 a heterozygous c.1408A>G (p.Met470Val, M470V) polymorphism.

The c.4207A>G (R1403G, p.Arg1403Gly) was found in exon 26 of the *CFTR* gene and has not been reported elsewhere. This arginine at the C-terminal of CFTR is highly conserved in mammalian species and the experimental program PolyPhen-2 predicted that this amino acid substitution was “probably damaging” with a score of 0.99. The potential functional effect of p.Arg1403Gly was also viewed using the Swiss-model program and the amino acid substitution should modify the folding of the CFTR protein and substantially alter CFTR function. It might therefore cause defective protein function and could be classified in class II. This mutation was detected in a 14 years old female referred to us for diagnosis at the Chest Diseases Department, Alexandria Main University Hospital, complaining of recurrent respiratory symptoms since the age of ten. She had neither a family history of cystic fibrosis nor of similar condition. Clinically, the patient presented with evidence of Kartagener syndrome and hyperinflation by the plain chest X-ray. Classic bilateral extensive bronchiectatic changes were detected in the high resolution chest computed tomography (CT). She had normal growth and no gastrointestinal symptoms.

The c.3718-24G>A was located in intron 22 of the *CFTR* gene and has not been reported elsewhere. The change is predicted to enhance abnormal splicing using Human Splicing Finder (HSF) prediction tools. Indeed, an alternative cryptic acceptor splice site could compete with the normal acceptor splice site during RNA processing reducing splicing from the correct site. Splicing studies should be performed to confirm this prediction. It was found in a patient with chronic bronchitis. The chest radiograph revealed widespread bronchiectatic changes with interstitial markings.

Discussion

Cystic fibrosis (CF) is rare in non-Caucasian populations, and in such populations little is known about the spectrum of mutations and polymorphisms in the *CFTR* gene (36). The identification of the *CFTR* mutations facilitates molecular investigation of the disease and better understanding of its pathophysiology in Egyptian patients. CF mutation frequencies vary widely among different populations, and haplotype heterogeneity has been found to be greater in populations with lower F508del frequencies (37). In addition, the distribution of *CFTR* mutations has important implications for understanding the complexity of the *CFTR* functions and predicting the phenotypic effects of particular mutation (38). The knowledge of the spectrum of mutations causing CF in any specific geographic region provides useful information to design the best approach in pre and postnatal diagnosis of CF, could facilitate the screening

of mutations in the affected population, and estimate the genetic risk. Moreover, the response to different therapeutic approaches could vary depending on the CF mutations. For these reasons, every single patient should be typed in as detailed a way as possible (39).

Our present framework as well as a few other studies has provided evidence for the existence of Cystic Fibrosis in the Egyptian population with atypical form of this disease. This is the first study to describe the mutation spectrum of the *CFTR* gene in Egyptian patients with CFTR-related disease. The distribution of CF mutations detected in the Egyptian population is different from the one seen in the Caucasian populations.

Firstly, the CF detected child carries two deletions in trans previously described (c.1418delG and c.2997_3000delAATT). The first frameshift mutation was detected in a Saudi-Arabian CF family by El Harith *et al.* (40). This mutation is the most frequent (17%) among Arabs (18). However, the second frameshift mutation was rare and only detected once in Denmark, described in 1993 by Schwartz *et al.* in CFTR data base (<http://www.genet.sickkids.on.ca>).

Moreover, the *CFTR* gene mutations have been found to be associated with other CFTR-related diseases like bronchiectasis, chronic pancreatitis, pneumonia, and asthma bronchiale. The 10 most common disease-causing mutations reported in the Caucasians was not observed in North Egyptian patients tested in this study. These mutations are c.350G>A (R117H, exon 4), c.489+1G>T (621+1G>T, intron 4), c.1521_1523delCTT (F508del, exon 11), c.1585-1G>A (1717-1G>A, intron 11), c.1624G>T (G542X, exon 12), c.1652G>A (G551D, exon 12), c.1657C>T (R553X, exon 12), c.3484C>T (R1162X, exon 22), c.3846G>A (W1282X, exon 23) and c.3909C>G (N1303K, exon 24). This may have implications in the testing process for local patients suspected to have diseases related to mutations in the *CFTR* gene (41). The process will either need to involve sequencing the entire *CFTR* gene in all patients or testing with a panel of the most common mutations found in our population (41).

Nine previously reported mutations (c.1418delG; c.2997_3000delAATT; p.Tyr301Cys; c.2620-15C>G; p.Val1293Ile; p.Met152Leu; p.Phe1052Val; c.4242+10T>C) and four previously unreported variations (p.Arg31His; p.Ala928Pro; c.3718-24G>A; p.Arg1403Gly) were detected. Furthermore, eight polymorphisms were found: c.1408A>G, c.3870A>G, c.2562T>G, c.1584G>A, c.4389G>A, c.869+11C>T, c.743+40A>G, c.4272C>T.

The most frequent found variation was c.2620-15C>G in intron 15 (3.6%), this transversion was observed in a single CF family of Turkish/Syrian descent, described in 1994 by Dörk *et al.* in CFTR data base (<http://www.genet.sickkids.on.ca>). The patient carries in cis c.204A>T (p.Lys68Asn, K68N) and the authors suggest that the variation has no phenotypic effect even it is located within the branch/acceptor splice side.

Four mutations were also found in equal proportion (1.8% each): a new variation c.2782G>C (p.Ala928Pro), two deletions c.1418delG, c.2997_3000delAATT, and c.3154T>G (p.Phe1052Val). The p.Phe1052Val mutation has been reported to have low frequencies in some other ethnics including neighboring Greek population

(42). Moreover the c.902A>G (p.Tyr301Cys) mutation detected in this study was also found in Cyprus population (43). These mutations are described here for the first time among Egyptian population. Additionally, the existence of some *CFTR* gene mutations identified in this study in different populations either European or non-European populations may be explained by the occurrence of gene flow to Egyptian population (Pharaonic origin) from Ethiopian, Greco-Roman, Arab, Turkish, French and English settlers. This is due to the strategic position of Egypt in Mediterranean Sea which attracted many invaders throughout its history (44).

More interestingly, our current study also report four novel variations identified in patients from this ethnic group, and were not found in 50 normal chromosomes. These variations are (c.92G>A; c.2782G>C; c.4207A>G; c.3718-24G>A), which appear to be specific to Egyptian CF patients and could have a pharaonic origin. Further functional studies are required for its pathological significance and to determine the impact of these variations on splicing or on CFTR process.

Interestingly, we detect one Egyptian patient carrying c.[744-33GATT(6);869+11C] whereas we always detect in Lebanese patients the complex allele c.[744-33GATT(6);869+11T] or c.[744-33GATT(7);869+11C] considered as wild type (45). It would be interesting to obtain Egyptian patients carrying N1303K to determine their haplotypes and the difference between complex alleles present in these two Arabic countries.

The mutation c.1521_1523delCTT (F508del, p.Phe508del), the most common CFTR mutation in Caucasian population was not detected in this study. However, recent investigations in the Egyptian population revealed the high incidence of F508del mutation either in CBVAD (27) or CF patients (26), as well as in other Arab countries such as Palestine (46), Jordan, Lebanon, and areas of Arab Gulf (19). These data emphasize the importance of extensive CFTR analysis in Egyptian patients in order to give them a better genetic counselling.

As only a single mutation or none was detected in the CFTR gene in the majority of Egyptian patients showing CFTR-RD symptoms, the cis-regulator regions could be sequenced in these patients to identify possible mutations far from the gene, which may lead to its dysfunction by modifying the chromatin conformation. Blackledge *et al.* (47) and Gheldof *et al.* (48) had respectively proposed looping models of the *CFTR* locus organization in epididymis cells and intestinal cells. More recently, Moisan *et al.* (49) identified new cooperative *CFTR* regulatory elements flanking the gene in primary human nasal epithelial cells and suggested a similar conformation in airway cells via the formation of chromatin loop with CTCF-CTCF binding around the *CFTR* gene. It is interesting to note that no variation was detected in control samples, suggesting that CFTR mutations found in our Egyptian patient cohort are essential for CF or CFTR-RDs phenotypes.

Although several reports indicating the presence of cystic fibrosis in the Egyptian population (21-27), the geographic distribution of *CFTR* mutations in Egypt has not previously been described and then the disease is considered very rare in this population. Moreover, our study is the first to identify novel mutations and

to detect certain numbers of previously reported mutations in the Cystic Fibrosis Genetic Analysis Consortium (CFGAC) database in the Egyptian population. Screening of the *CFTR* gene mutations in the Egyptian population is difficult due to numerous reasons: first, a significant proportion of infant morbidity and mortality was caused by respiratory infections, diarrheal disease and malnutrition (50, 51). A second concern is the increased neonatal mortality rate which may be due to more severe disease or neonatal complications (22). In addition, limited attention has been devoted to pregnant women due to poor medical facilities available to them. Moreover, lack of awareness of the public and medical community in Egypt, concerning the presentation and diagnosis of CF and CFTR-RDs may be a contributing factor to underdiagnosis of this illness (22). Thus, screening of the Egyptian people for *CFTR* gene mutations is quite important to set up a reliable diagnostic program for CF in this population.

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 risk of higher rates of CF incidence (52). 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 (53) and reports on these mutations in Arab populations have so far been very limited (54).

It would be desirable to have more population-specific mutation panels which could help to overcome the underdiagnosis of cystic fibrosis and other CFTR-related diseases in those countries where the mutational distribution does not match that of Central Europe (55). In this regard, a population-specific mutation panel for Egyptian population including: c.92G>A (exon 2), c.454A>T (exon 4), c.902A>G (exon 8), c.1418delG (exon 11), c.2620-15C>G (intron 15), c.2782G>C (exon 17), c.2997_3000delAATT (exon 19), c.3154T>G (exon 20), c.3718-24G>A (intron 23), c.3872A>G (exon 23), c.3877G>A (exon 24), c.4207A>G (exon 26), c.4242+10T>C (intron 26) mutations identified in this study, and all previously reported mutations in this population [c.1766+3A>C (intron 13), c.1993A>T (exon 14), c.3909C>G (exon21), c.1521_1523delCTT (exon11), (22); c.54-5940_273+10250del21kb (intron 1 - exon 3), c.443T>C (exon 4), c.1040G>C (exon8), c.1364C>A (exon10), c.1652G>A (exon12), c.2051_2052delAAinsG (exon 14), c.3067_3072delATAGTG (exon19), c.3484C>T (exon 22), c.3846G>A (exon 23), (26); c.1040G>C (exon 8), c.1364C>A (exon 10), c.3484C>T (exon 22), c.3752G>A (exon 23), c.3846G>A (exon 23), (27)] should be highly recommended for Egyptian patients. However, full clinical manifestations of additional CF and CFTR-RDs patients could lead to clarify the pathogenicity of these mutants in this population.

Our current data provide the significance for introducing the CFTR molecular screening programs to our Hospitals for patients suspected of having the disease. This requires a special attention of paediatricians, gastroenterologists and pulmonary medicine practitioners to include CFTR mutation screening in their diagnostic procedure and positive cases should be addressed to ge-

neticians for further counselling (56).

In conclusion, Our data show that detected mutations in the *CFTR* gene are strongly associated with CFTR-RD and only two disease causing mutations were identified. In addition, eight mutations characterized in this study are thought to be disease causing in CF or CFTR-RDs. Four of these mutations (c.92G>A; c.2782G>C; c.3718-24G>A; c.4207A>G) are described here for the first time. This result provides definitive evidence of links between novel genetic mutations of the *CFTR* gene in Egyptian patients and CF or CFTR-RDs. So, further investigations on large numbers of cases with different genotypes are required to provide more insight into underlying mutations. The present findings represent a major contribution to the molecular profile of CF in the Egyptian population. The data obtained here can be combined with other previous investigations in this population to establish the first panel of *CFTR* gene mutations in Egypt which could be used in DNA diagnostics in the entire Egyptian population. However, these results will serve as a basis for improving the future genetic diagnosis of CF or CFTR-RDs and assist in genetic counselling, parental diagnosis in Egypt. Further biochemical and physiological studies remain to be done for evaluating the pathological effect of detected mutations.

Acknowledgment

We thank subjects and controls who accepted to participate in this study. We also thank Mohamed EL-Komy for excellent technical support for sample selection. Finally, we are appreciated the valuable support of French Embassy in Cairo and Dr. Louis Moreau 'attaché scientifique' for their kind help and encouragement the fellowship to Dr. Ayman EL-SEEDY. This work is also supported by Poitiers University Hospital and University of Poitiers, France.

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