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Detection of *CFTR* mutations using PCR/ARMS in a sample of Algerian population

Détection des mutations CFTR par PCR/ARMS dans un échantillon de la population algérienne

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Abstract. Cystic fibrosis (CF) is the most common autosomal recessive disease in Caucasians. Wrongly considered as a European disease, CF is found in Algeria; but the literature data on the clinical profile and the spectrum of *CFTR* gene mutations are poor. In this study we investigate twenty-four unrelated Algerian families, with at least one child with CF. DNA extracts from blood samples of patients and parents were screened for *CFTR* gene mutations using Elucigene CF30 Kit which is based on a PCR/ARMS technique. Only five different mutations were identified. On the 48 alleles studied, most common mutations were: c.1521_1523delCTT (F508del) 18.75%, c.579+1G>T (711+1G>T) 12.5%, c.1624G>T (G542X) 10.41%, c.3909C>G (N1303K) 4%, and c.1652G>A (G551D) 2%. The Elucigene CF30 kit highlights a portion of *CFTR* mutations in the Algerian population. It remains important for a first screening as it reveals the most common mutations. All this information is of interest for genetic testing and genetic counseling in Algeria and in European countries where immigration from the Maghreb is common.

Key words: *cystic fibrosis, PCR/ARMS, Elucigene CF30 Kit, Algeria*

Résumé. La mucoviscidose est la maladie autosomique récessive la plus fréquente chez les Caucasiens. Considéré à tort comme une pathologie européenne, la mucoviscidose est rapportée en Algérie, mais les données de la littérature sur le profil clinique et le spectre des mutations du gène CFTR sont pauvres. Dans cette étude, nous avons exploré vingt-quatre familles algériennes non apparentées, avec au moins un enfant atteint de mucoviscidose. L'ADN a été extrait à partir d'échantillons de sang. Les patients et les parents ont été testés pour les mutations du gène CFTR en utilisant le Kit Elucigene CF30 qui est basé sur une technique de PCR/ARMS. Seulement cinq mutations ont été identifiées. Sur les 48 allèles étudiés, les mutations les plus fréquentes étaient: c.1521_1523delCTT (F508del) 18,75%, c.579+1G>T (711+1G>T) 12,5%, c.1624G>T (G542X) 10,41%, c .3909C > G (N1303K) 4 %, et c.1652G >A (G551D) 2%. Le kit Elucigene CF30 met en évidence une partie des mutations CFTR dans la population algérienne. Il demeure important pour un premier dépistage, car il révèle les mutations les plus communes. Toutes ces informations sont d'intérêt pour le test génétique et le conseil génétique en Algérie, mais aussi pour les pays européens où l'immigration en provenance du Maghreb est importante.

Mots clés : *mucoviscidose, PCR/ARMS, Kit Elucigene CF30, Algérie*

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Cystic fibrosis (CF) [MIM # 219700] is the most common autosomal recessive genetic disease, in Caucasian populations. It is due to the alteration of the *CFTR* (cystic fibrosis transmembrane conductance regulator) gene [MIM # 602421] which encodes a chloride channel protein essential for ion transport and epithelial cells homeostasis [1]. Alteration or loss of function of this protein leads to an imbalance of ion exchange, resulting in dehydration of the mucous secretions of many organs [2].

To date, more than 1900 mutations in the *CFTR* gene have been reported to the cystic fibrosis genetic analysis consortium [3]. The frequency of these mutations varies among different populations according to the geographical and ethnic origin of patients [4]. In Europe, the incidence of the CF is average 1/2000 to 1/4300 births. However, it is marked by local and regional variation [4].

Wrongly considered as a European disease, cystic fibrosis is found in the North African population. Studies have been done, in Algeria by Loumi O *et al.* and Cabet F *et al.* [5, 6] in Tunisia by Messaoud T *et al.* [7] and Morocco by Ratbi I *et al.* [8] confirmed the presence of this disease in these respective populations. However, no information is available about the incidence of CF in the Maghreb.

In Algeria, the literature data on the clinical profile of CF and the spectrum of *CFTR* gene mutations are poor. This study has two aims: the first one is to detect *CFTR* mutations in a sample of CF patients using a PCR/ARMS (polymerase chain reaction/ amplification refractory mutation system) technique and the second to evaluate the effectiveness of the Elucigene CF30 Kit (Tepnel Diagnostics, Oxon, United Kingdom) in the molecular diagnosis of CF in the Algerian population. This information is important in order to provide and improve the appropriate genetic services for CF patients and their families.

Materials and methods

Population study

A total of 24 unrelated Algerian families with at least one CF affected child were studied. In one family we have no information about the parents. The patients were referred to us by Pneumology and allergology department of the specialized hospital center, in Canastel, Oran (Algeria). CF diagnosis was based on clinical findings and repeated positive sweat chloride tests (>60 mmol/L). A written consent to the genetic study was obtained from all subjects.

DNA extraction

Blood samples were collected from the affected children, and their parents. Genomic DNA was extracted from peripheral blood leucocytes using the salting out method [9].

Mutation detection strategy

Mutations were detected as the following procedures. All samples were analyzed for 30 mutations using the Elucigene CF30 Kit (Tepnel Diagnostics, Oxon, United Kingdom) which detects point mutations or small deletions in deoxyribonucleic acid, using a method based on ARMS (amplification refractory mutation system) allele specific amplification technology.

The Elucigene CF30 Kit can identify the 30 most common mutations among Caucasian population at the same time: c.3276C>A (Y1092X), c.1585-1G>A (1717-1G>A), c.1624G>T(G542X), c.3846G>A (W1282X), c.3909C>G (N1303K), c.1521_1523delCTT (F508del), c.3718-2477C>T (3849+10kbC>T), c.262_263delTT (394delTT), c.489+1G>T(621+1G>T), c.1657C>T (R553X), c.1652G>A (G551D), c.350G>A(R117H), c.3484C>T (R1162X), c.1000C>T (R334W), c.1364C>A (A455E), c.2051_2052delinsG (2183AA>G), c.3528delC (3659delC), c.948delT (1078delT), c.1519_1521delATC (FI507), c.1040G>C (R347P), c.3752G>A (S1251N), c.178G>T (E60X), c.1680-877FG>T (1811+1.6kbG>A), c.3140-26A>G (3272-26A>G), c.2988+1G>A (3120+1G>A), c.2657+5G>A (2789+5G>A), c.579+1G>T (711+1G>T), c.254G>A (G85E), c.366T>A (Y122X) and c.2538G>A (W846X) [10].

DNA was amplified following the amplification program from Elucigene CF30 work protocol. It consists on AmpliTaq Gold polymerase activation at 94°C for 20 minutes, followed by 35 cycles consisting of denaturation at 94°C for 30 seconds, primer attachment stage at 58°C for 2 minutes and extension at 72°C for 1 minute. At the end of the amplification program, the extension stage of the last cycle, at 72°C, was programmed to last 20 minutes [10].

PCR products were carried out on agarose gel 3% (NuSieve 3:1). Samples with c.1521_1523delCTT (F508del) mutation were validated on a non-denaturing 12% polyacrylamide gel [11].

Results

A total of 24 Algerian CF families were screened 16 of them were consanguineous (66.66%). Five mutations were identified. These mutations were: c.1521_1523delCTT (F508del), c.579+1G>T (711+1G>T), c.1624G>T (G542X), c.3909C>G (N1303K), and c.1652G>A (G551D).

In 8 families (33%), the children were homozygous for their mutations and 5 (17.85%) were compound heterozygous. All mutations and family details are summarized in *table 1*.

Table 1. CFTR mutations detected in a sample of 24 Algerian CF families.

Family	CM	Sex	CF mutations
1	Yes	M F M	* * *
2	Yes	M F M	* * *
3	No information	M	c. [1521_1523delCTT];[579+1G>T] (F508del/711+1G>T)
4	No	M F M	c.[1652G>A];[=] (G551D/-) * c. [1652G>A];[?] (G551D/*)
5	No	M F F	* * *
6	Yes	M F F	c.[3909C>G];[=] (N1303K/-) c.[3909C>G];[=] (N1303K/-) c.[3909C>G];[3909C>G] (N1303K/N1303K)
7	Yes	M F M	* c. [1624G>T];[=] (G542X/-) c. [1624G>T];[?] (G542X/*)
8	Yes	M F M	c. [579+1 G>T];[=] (711+1G>T/-) c. [579+1G>T];[=] (711+1G>T/-) c. [579+1G>T];[579+1G>T] (711+1G>T/711+1G>T)
9	No	M F M	c. [579+1G>T];[=] (711+1G>T/-) c. [1521_1523delCTT];[=] (F508del/-) c. [1521_1523delCTT];[579+1G>T] (F508del/711+1G>T)
10	Yes	M F F	* * *
11	Yes	M F M	c. [579+1G>T];[=] (711+1G>T/-) c. [579+1G>T];[=] (711+1G>T/-) c. [579+1G>T];[579+1G>T) (711+1G>T/711+1G>T)
12	No	M F F	* c. [1521_1523delCTT];[?] (F508del/*) c. [1521_1523delCTT];[?] (F508del/*)
13	Yes	M F M	c. [1624G>T];[=] (G542X/-) c. [1624G>T];[=] (G542X/-) c. [1624G>T];[1624G>T] (G542X/G542X)
14	Yes	M F F	c. [1624G>T];[=] (G542X/-) c. [1624G>T];[=] (G542X/-) c. [1624G>T];[1624G>T] (G542X/G542X)
15	No	M F M	* * *
16	No	M F M	c.[1521_1523delCTT];[=] (F508del/-) c.[1521_1523delCTT];[=] (F508del/-) c.[1521_1523delCTT];[1521_1523delCTT](F508del/F508del)
17	Yes	M F M	* * *
18	No	M F M	* * *
19	Yes	M F F	c.[1521_1523delCTT];[=] (F508del/-) c.[1521_1523delCTT];[=] (F508del/-) c.[1521_1523delCTT];[1521_1523delCTT] (F508del/F508del)
20	Yes	M F F	* * *

Table 1. (Continued).

Family	CM	Sex	CF mutations
21	Yes	M F F	* * *
22	Yes	M F M	* * *
23	Yes	M F M	c.[1521_1523delCTT];[=] (F508del/-) c.[1521_1523delCTT];[=] (F508del/-) c.[1521_1523delCTT];[1521_1523delCTT](F508del/F508del)
24	Yes	M F M	* * *

CM: consanguineous marriage ; *: Mutation none detected by the Elucigen CF30 Kit. Genotypes are described as: "[change allele 1];[change allele 2]" according to: Den Dunnen JT and Antonarakis SE. *Hum.Mutat* 2000; 15: 7-12.

Table 2. Mutation identification results in a sample of 24 CF Algerian patients *(N=48* CF chromosomes).

Mutations	E/I[1]	Substitution nucleotide	Substitution Amino acid	N. of chromosomes	Frequency (%)
c.1521_1523delCTT (F508del)	Exon 11	delCTT	del phe 507/508	9	18.75
c.579+1G>T (711+1G>T)	Intron 5	G→T711+1	RNA splicing defect	6	12.5
c.1624G>T (G542X)	Exon 12	G→T 1756	Gly→Stop 542	5	10.41
c.3909C>G (N1303K)	Exon 24	C→G4041	Asn→Lys 1303	2	4
c.1652G>A (G551D)	Exon 12	C→A 1652	Gly→Asp 542	1	2
Total				23/48	47.66

[1]: exon/intron

Table 3. Frequency of CFTR mutations in our sample compared to previous Algerian studies.

Mutations detected in the Algerian population	Present study (2013)	Loumi *et al.* (1999)	Boukari *et al.* (2005)	Loumi *et al.* (2008)	Cabet *et al.* (2010)
c.1521_1523delCTT (F508del)	18.75%	20%	18%	16.70%	20%
c.579+1G>T (711+1 G>T)	12.50%	10%	10%	8.30%	19%
c.1624G>T (G542X)	10.41%	-	-	1.40%	-
c.3909C>G (N1303K)	4%	20%	15%	8.30%	-
c.1652G>A (G551D)	2%	-	-	-	-

In screening the twenty four CF patients (48 chromosomes) for 30 mutations available in Elucigene kit. Only five mutations were found. These mutations were, as reported in *table 2*: c.1521_1523delCTT (F508del) in 9 alleles (9/48), c.579+1G>T (711+1G>T) in 6 alleles (6/48), c.1624G>T (G542X) in 5 alleles (5/48), c.3909C>G (N1303K) in 2 alleles (2/48) and c.1652G>A (G551D) in only 1 allele (1/48).

Our results were validated in the molecular endocrinology and rare diseases department, Femme Mere Enfant Hospital, Bron, Lyon (France), except for the c.1521_1523delCTT (F508del) mutation which was done in our laboratory.

Discussion

The c.1521_1523delCTT (F508del) was the most common mutation detected in the current study. Its frequency in our sample was 18.75%, which is comparable to the one reported by Boukari R *et al.* (18%), and not so far from the one reported by Cabet F *et al.* (20% in a sample of 27 CF patients) [12], [6] on *table 3*. Loumi O *et al.* [5] showed a frequency of 16.7%, which is less than our finding. This small variation can be explained by the limited size of our population sample. In comparing the present results to the ones obtained by Messaoud T *et al.* [7] in the Tunisian population, we notice that the frequency of this mutation

in our sample is low (18.75% vs. 50.4%). However, it is also comparable to reported data for Iranian populations [13]. In Europe, c.1521_1523delCTT (F508del) shows a northwest-to-southeast gradient, with a maximum (100%) of all CF chromosomes in the Faroe Islands of Denmark and a minimum (24.5%) in Turkey [14].

Beside the c.1521_1523delCTT (F508del), the c.579+1G>T (711+1G>T) was observed on two homozygous and two heterozygous, corresponding to 12.5% of CF chromosomes. This mutation has been first described with a high frequency in CF families living in Quebec. It had already been found in previous work of Loumi O *et al.* in 1999 and in 2008, in the Algerian population, respectively with a frequency of 10 and 8.3% [5, 15].

Cabet F *et al*., reported a frequency of 19% in a sample of 27 Algerian CF patients; it was the second most frequent after the c.1521_1523delCTT (F508del) in their study [6].

Finding this mutation in our sample is further evidence that this splicing mutation is indeed present, in the Maghreb. It seems to be rare in European countries; it has been reported in Italy (1.3%), and Spain (1.3%) [16, 17]. The c.1624G>T (G542X) mutation was found on three CF patients, two of them were homozygous of which were from consanguine families. This mutation is considered as the most common mutation, in the Mediterranean regions of Europe and Africa [12]. It is supposed to be introduced into the Mediterranean region by the migration of Phoenicians [18].

The c.3909C>G (N1303K) was found only in one patient who was homozygous for this mutation. It has been reported as the second most frequent mutation after the c.1521_1523delCTT (F508del) in a sample of classic CF in Loumi O *et al*. work [5].

The c.3909C>G (N1303K) was known as a common mutation in the Mediterranean region [4]. It is described as the fourth mutation with a percentage of 5.92% in the Tunisian population [7].

However, the frequency of this mutation seems to be higher in Lebanese and Palestinian populations, respectively with a frequency of 20 and 21% [19, 20].

The c.1652G>A (G551D) known as the Celtic mutation was found in only one CF patient. It is more common in north-west, central Europe and in particular, the UK (3.1%) and also in Northern Ireland (3.7%), the Czech Republic (3.8%), and Ireland (5.7%) [14].

The Elucigene CF30 detected 5 mutations in our sample, which is about 16.66%.

Although, the detection rate seems low compared to European populations.

The ARMS-PCR method used by Elucigene CF30 Kit offers the possibility of a fast diagnosis; it allows having the results in one day. The simple protocol and minimal equipment requirement (thermocycler and electrophoresis system) makes CF DNA analysis available to most laboratories with experience in PCR. The kit is sensitive even if the amount of DNA is small. This may be interesting for Guthrie cards or buccal cells.

One limitation of this kit is the inability to distinguish homozygous/heterozygous except for the c.1521_1523delCTT (F508del). However, this constraint is quickly exceeded if a family study is undertaken, and this is what we did in this study.

The kit detects some common mutations in the Mediterranean edges and does not detect specific mutations in the Algerian population as the c.422C>A (A141D), c.680T>G (L227R), and c.3907A>C (N1303H) [21, 22].

Even if the kit covers only 16.66% of the CF chromosomes, it remains interesting in first instance, the sequencing remains indispensable to the complete genetic study.

The most interesting is to develop in collaboration with Elucigene one specific Maghreb kit and include mutations which frequency is higher than 2% to improve coverage (current kit provides coverage for 85% of mutations involved in classic cystic fibrosis forms in France).

Conclusion

In the present study, we detected five mutations (c.1521_1523delCTT (F508del), c.579G+1>T (711+1G>T), c.1624G>T (G542X), c.3909C>G (N1303K), c.1652G>A (G551D) accounting for 47.66% of alleles, by the Elucigene CF30 kit. However, some of mutations remain uncharacterized in this case sequencing is required. To our knowledge, this is the first time that PCR/ARMS using Elucigene CF30 Kit is performed in Algeria, this contributed to an evaluation of this Kit in a sample of CF patients. All this information is of interest in designing an appropriate strategy for genetic testing of patients in Algeria and is interest for genetic counseling, a carrier screening program and eventually prenatal diagnosis.

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