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THE SPECTRUM AND FREQUENCY OF CYSTIC FIBROSIS MUTATIONS IN ALBANIAN PATIENTS

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ABSTRACT

BACKGROUND

Cystic fibrosis (CF) is a genetic disease characterized by a wide spectrum of severity, resulting from the inheritance of a mutant allele of the gene for cystic fibrosis transmembrane conductance regulator (CFTR). The aim of the study was to present a CFTR mutation analysis among the Albanian population and to identify rare variants.

METHODS

We identified CFTR mutations in a representative cohort of CF patients comprising of Albanian patients and some Kosovo patients followed up by the Department of Pediatrics at the University Hospital Center "Mother Theresa" (UHCMT). Compiled clinical and genotypic data include 133 previously analyzed patients, of whom 116 have two identified mutations, 6 have only one known mutation, and 11 are unexamined.

RESULTS

The most frequent mutation is F508del (83.19%), followed by 621+1G>T (2.45%). Other mutations identified in decrease order are E822X, G85E, G542X, R1066C, R1070Q, R1158X, G1349D, N1303K, S466X, 1811+1G->C, E831X, CFTRdele2,3(21kb).

CONCLUSIONS

The data suggest that most of these patients can benefit from new modulatory therapies targeting CFTR mutations, translating to very hopeful prospects for these patients.

The Albanian population would benefit from Cystic Fibrosis neonatal screening, since outcomes can be improved through early diagnosis.

Keywords: CFTR, cohort, variants, modulatory, neonatal.

INTRODUCTION

Cystic fibrosis (CF) is a genetic multisystem disease resulting from the inheritance of a mutant allele of the gene for cystic fibrosis transmembrane conductance regulator (CFTR) from each parent. Cystic fibrosis (CF) is the most common life shortening condition in Caucasians [1]. About 162,428 people are estimated to be living with CF worldwide, of which 37,002 are estimated to be diagnosed in North America and more than 47,650 in Europe, while an estimated 57,076 CF patients are undiagnosed. [2]. Whilst prevalence is broadly similar in populations which had their origin from northern Europe, there are considerable variations through Europe from as high as 1 in 1,400 live births in Ireland, 1 in 4,200 in Italy and 1 in 25,000 in Finland [3]. Prevalence rates are much lower among non-Caucasian populations [4,5].

A major step forward was achieved by grouping CFTR mutations with a similar effect on CFTR protein synthesis or function in the same mutation class [3]. In view of drug development and drug distribution, it is therefore also useful to know the relative prevalence of these mutation classes [3].

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In Albania since 1992, cystic fibrosis patients are followed up by the Department of Pediatrics, in UHCMT. The CF diagnosis is based predominantly on clinical criteria and two positive sweat test results for patients suspected of the disease. The diagnosis is confirmed by the identification of two CF causal mutations. Genetic assessment has been in practice since 2004, when the search of the genetic mutations in patients with cystic fibrosis began to be recorded.

Unfortunately, there was no national registry for Cystic Fibrosis in Albania until 2017. However, every pediatric patient diagnosed in the Department of Pediatrics in the UHCMT has been documented throughout the course of the disease. Practically, this is the only center which diagnoses and follows cystic fibrosis patients in our country. Since 2017 Albania is part of the European Cystic Fibrosis Society Patient Registry (ECFSPR) which collects anonymized demographic and clinical data from consenting people in Europe with CF [6].

The aim of this paper is to present a summary of the distribution of the CFTR mutations in 133 CF patients diagnosed in the Pediatrics Department in the UHCMT. Furthermore, we aim to compare our data with other countries in Balkan Peninsula and Europe.

MATERIALS AND METHODS

This retrospective study takes into consideration 133 pediatric patients diagnosed with Cystic Fibrosis followed in the Department of Pediatrics in the UHCMT, during the year (2019) of follow up and who have been included in the European Patient Registry of Cystic Fibrosis.

The examination of the CF mutations is conducted over a 25-year period from 1994-2019 for 116 patients at the Center of Molecular Diagnosis and Genetic Research in the University Hospital of Obstetrics and Gynecology "Queen Geraldine".

Whole blood was collected into EDTA anti-coagulant tubes. The genomic DNA was isolated from peripheral blood using Qiagen extraction kit (DNA Blood Isolation Kit, Qiagen, Valencia, California, USA). CFTR gene mutations were analyzed by PCR/OLA protocol (Abbott Applied Biosystems), testing for 33 mutations (Cystic Fibrosis V3 Genotyping Assay).

Undetermined samples of 15 cases were sent to the University Hospital Motol, in Prague, Czech Republic as part of the project: GENOTYPING IN UNDERTESTED / UNTESTED CYSTIC FIBROSIS POPULATIONS IN MIDDLE EAST, TRANSCAUCASIA, TURKEY & EASTERN NORTH AFRICA (2015-2019; Vertex grant scheme - CG-2015-104643).

Firstly, the most common mutations of the CFTR gene were examined using the commercial Elucigene CF-EU2 kit, where 50 mutations are simultaneously tested (CFTRdele2,3 / 21kb /, I507del, 2789 + 5G> A, E60X, F508del, Q890X, P67L, 1677delTA, 3120 + 1G > A, G85E, V520F, 3272-26A> G, 394delTT, 1717-1G, A, R1066C, 444delA, G542X, Y1092X (C> A), R117C, S549N, M1101K, R117H, S549R (T> G), D1152H , Y122X, G551D, R1158X, 621 + 1G, T, R553X, R1162X, 711 + 1G, T, R560T, 3659delC, L206W, 1811 + 1.6kbA, G, 3849 + 10kbC, T, 1078delT, 1898 + 1G> A S1251N, R334W, 2143delT, 3905insT, R347P, 2184delA, W1282X, R347H, 2347delG, N1303K, A455E, W846X, including IVS8-T variants (5/7/9) .

If just one mutation was detected or both causal mutations are not detected, but the patient shows clear clinical and laboratory signs, next generation sequencing of the whole coding region of CFTR (NGS CFTR Devyser) was provided. This ensures maximum capture of causal mutations within the diagnostic process we provide. All pathogenic variants were confirmed by Sanger sequencing or MLPA protocol https://www.mlpa.com/.

Also, the databases CFTR1 http://www.genet.sickkids.on.ca/, CFTR2 https://cftr2.org and CLIN Var https://www.ncbi.nlm.nih.gov/clinvar, were used for clinical interpretation.

RESULTS

Based on the number of pediatric patients diagnosed with CF every year from 1992 to 2017, and the birth rate during the same period [7], the incidence of CF in Albania is 1:4041. It is important to note that this incidence is not entirely correct since in the UHCMT, there are also CF patients from Kosovo, or Albanian children born in other countries.

Table 1. Incidence of Cystic fibrosis in Albania

Time Period	Birth Rate in Albania	CF cases diagnosed at UHCMT	Incidence
1992-2017	1144138	285	1:4014

We have presented in Table 2 an overview of the demographic as well as the clinical and laboratory characteristics of Albanian patients diagnosed at UHCMT who were included in the study, although data on some births are missing.

Allele Frequencies.

Among the studied group of patients, 116 of 133 patients have two CFTR alleles identified, and 6 patients have only one mutation determined. Although genetic examination was made possible, 11 patients remained unexamined due to their lack of interest in performing this analysis. As shown in Table 3, 14 CFTR mutations were identified from 122 patients, 116 of whom with two

Table 2. Overview data about patient with CF in Albania*

Year of follow up 2019	**Number (n=)**	**Percentage (%)**
No. of patients	133	100
Male	72	54.14
Female	61	45.86
Mean Age (years) at follow up. (Min-max)	10.5 0.5-28.0	
Mean Age (years) at diagnosis. (Min-max)	0.75 0.0-16.0	
No. of patients <18 years	115	86.47
No. of patients > 18 years	18	13.53
Homozygote for F508del	87	65.41
Compound heterozygous mutation F508del	23	17.29
Non- F508 mutation patients	6	4.51
One determined mutation	6	4.51
Unexamined	11	8.27
Mean BMI Z-score: ± SD. >18 years old <18years old	 -1.2 -0.7	
Mean FEV1 (% predicted)	89.7	
Chronic *S.aureus*	39	29.32
Chronic *P.aeruginosae*	33	24.8
CF liver disease	54	40.60
CF-related diabetes	6	4.51
Pancreatic sufficient cases	3	2.2

*Note data from ECFSPR 2019

Table 3. Allele frequencies in 122 Albanian patients and Classes according to their effect on the synthesis and/or function of the CFTR protein.

***CFTR* gene mutations NM_00492.3** **Legacy name, coding DNA, protein name**	**Mutation Type**	**Class of Mutation**	**All Alleles N= 244(%)**
F508del, c.1521_1523delCTT, p. Phe508del	deletion	II	203(83.19)
621+1G>T, c.489+1G>T	splicing	I	6 (2.45)
E822X, c.2464G>T, p. Glu822Ter	nonsense	I	5 (2.04)
G85E, c.254G>A, p. Gly85Glu	missense	II	5 (2.04)
G542X (c.1624G>T) p. Gly542Ter	nonsense	I	4 (1.63)
R1066C, c.3196C>T, p. Arg1066Cys	missense	II	3 (1.22)
**R1070Q, c.3209G>A, p. Arg1070Gln	missense	Unclassified	3 (1.22)
R1158X, c.3472C>T, p. Arg1158Ter	nonsense	I	2 (0.81)
G1349D, c.4046G>A p. Gly1349Asp	missense	III	2 (0.81)
N1303K, (c.3909C>G), p. Asn1303Lys	missense	II	2 (0.81)
**S466X, c.1397C>G, p. Ser466Ter	nonsense	I	2 (0.81)
1811+1G->C, c.1679+1G>C	splicing	V	1 (0.40)
E831X, c.2491G>T, p. Glu831X	nonsense	I	1 (0.40)
CFTRdele2-3(21kb), c.54-5940_273 + 10250 del, p. Ser18Argfs*16	deletion	I	1 (0.40)

The new mutations for the Albanian population detected by University Hospital Motol are bolded.
**2.0 complex alleles S466X-R1070Q
N: number of alleles; %: percentage rounded up to max. 2 digits after the full stop (thus may not add up exactly to 100%).
HGVS – Human Genome Variation Society nomenclature (www.hgvs.org/mutnomen/);
Legacy nomenclature according to the Cystic Fibrosis Mutation Database (www.genet.sickkids.on.ca/app)

CFTR mutations determined and 6 patients with only one mutation determined.

The most frequent mutation is F508del with 83.19% (203/244 alleles). F508del mutation is present in 87 homozygous patients, 23 patients with heterozygous mutations, and in all 6 patients who have only one known mutation. The second most frequent mutation is 621+1G>T with 2.45% (6/244 alleles), followed by G85E and E822X with 2.04% (5/244 alleles), G542X (1.63%), R1066C (1.22%), R1070Q (1.22%).

The mutations R1158X (0.81 %), S466X (0.81%), G1349G (0.81%), N1303K (0.81%), and CFTRdele2,3(21kb)

Table 4. Genotype variants and their frequency

Genotype variants	Frequency number (N=)	Percentage %
F508del/F508del	87	75.00
F508del/non F508del	23	19.82
F508del/G85E	4	3.44
F508del/E822X	3	2.58
F508del/621+1G>T	3	2.58
F508del/R1066C	3	2.58
F508del/G1349D	2	1.72
F508del/G542X	2	1.72
F508del/ N1303K	2	1.72
F508del/R1070Q	1	0.86
F508del/ S466X-R1070Q - in cis	1	0.86
F508del/ R1158X	1	0.86
F508del /E831X	1	0.86
non F508del/non F508del	6	5.17
G542X/621+1G>T	1	0.86
G85E/ R1158X	1	0.86
G542X/ E822X	1	0.86
621+1G>T/621+1G>T	1	0.86
CFTR dele 2.3/ 1811+1G->C	1	0.86
S466X-R1070Q in cis / E822X	1	0.86

N: number of genotypes; %: percentage rounded up to max. 2 digits after the full stop (thus may not add up exactly to 100%).

(0.40 %), E831X (0.40 %), do not surpass 1% of the CF patients included.

According to their effect on the synthesis and/or function of the CFTR protein, 87.29 % (213/244 alleles) of the mutations pertain to class II with four different mutations, explained by F508del being by far the most frequent mutation in this cohort.

Following class II, the second most frequent mutation class was class I with 8.60 % (21/244 alleles) and eight different mutations. Three mutations are unclassified 1.22% (3/244 alleles).Two mutations belong to class III 0.81% (2/244 alleles). One mutation belongs to class V 0.40% (1/244 alleles). Mutations belonging to class IV and VI were not present in our patients. The mutation classes are presented in Table 3. Only one mutation (1/244 alleles) has a varying clinical expression (consequences) [8].

Genotype Frequencies

Table 4 shows the genotypes of the 116 patients for whom the two mutations were identified, as well as their frequency expressed in numbers and percentages.

As it is noted from the frequency of genotypes in the Table 3, the most frequent genotype was F508del/F508del (75%; 87/116) followed by F508del/non F508del genotype (19.82%; 23/116) and non F508del/non F508del (5.17%; 6/116).

From 6 patient non F508del/non F508del genotypes resulted six different ones as follows: G542X/621+1G>T (0.86%); G85E/R1158X (0.86%); G542X/ E822X (0.86%); 621+1G>T/621+1G>T (0.86%); CFTR dele 2.3/1811+1G->C (0.86%); S466X-R1070Q in cis / E822X (0.86%).

DISCUSSION

This study aims to shed some light on the current situation about CF mutation pattern, presenting data from patients with CF followed during 2019 in the Department of Pediatrics in the UHCMT. Practically, UHMCT is the only center which diagnoses and follows cystic fibrosis patients in Albania. It is important to note that in Albania no CF national neonatal screening has been applied until now. It is assumed that the incidence would be higher if neonatal screening program would take place.

The Albanian population is composed of an ethnic group originating in the Balkan peninsula. There are approximately five million Albanians in this region, with roughly half living in Albania and the other half in Kosovo, Northern Macedonia, Montenegro and smaller populations in Croatia and Serbia. Significant numbers of the Albanian population are also found in Greece and smaller insignificant communities in Bulgaria and Romania as well. Albanians also make up a significant diaspora, spread all over the world, especially in North America, Europe and Oceania.

According to the latest census of the National Institute of Statistics Albania (INSTAT), the current population of Albania is 2,876,591 inhabitants [7].

The most common CFTR mutation is F508del, either in homozygous or heterozygous form. In Europe, this mutation is met in 82% of patients with CF (41% homozygous and 41% heterozygous) [9]. Among European countries, there are different variations of this CFTR mutation prevalence, from Denmark 83.2% [10], to a minimal prevalence in Turkey of only 24.5% [11]. This confirms the northwest to southeast gradient in the F508del distribution in Europe [11]. The Albanian population expresses a high prevalence of F508del mutation, in comparison to other populations in the region. Thus, in neighboring Italy is 43.9% [12], in North Macedonia is 75.9% [13], in Serbia and Montenegro it is 72.28 % [14].

In Albania, there is a high prevalence of this mutation which is characteristic of Central, Northern and Northeastern Europe. This fact is well known and previously explained due to early migrations of the Caucasian population towards Northwestern Europe and Southeastern Europe, and thereafter the migration through the Mediterranean routes from Middle East and Africa towards Europe [11]. The second migration seems to have left the Albanian population unaffected.

The second most prevalent mutation found in the Albanian population is 621+1G>T (2,56%). This mutation is most prevalent in Southern Europe, found more frequently in our neighboring country Greece (6.4%) [10], while in North Macedonia this mutation is under 2% [15].

The third most prevalent mutations found in the Albanian population are G85E (2.13%) and E822X (2.13%). The G85E mutation, is most frequent in Israel (2.6%) [10].

E822X, R1066C, G1349D, S466X, 1811+1G->C, E831X, CFTRdele2-3(21kb) mutations were newly found in the patients of our study. The latter, the CFTRdele2-3(21kb) mutation, was found in only one patient in this study, is found mainly in Slavic and Eastern and Central European populations [16]. The most frequent result of this mutation is in Belarus (11.2%) [10]

There are 14 CFTR mutations and 18 confirmed genotypes. To define the optimal treatment for an individual patient, genotyping of CFTR is clearly the first step [17].

CONCLUSIONS

This information suggests that most of these patients can benefit from new modulatory therapies targeting CFTR mutations, which translates to very hopeful prospects for these patients [18, 19]. The Albanian population would benefit from Cystic Fibrosis neonatal screening, since outcomes can be improved by early diagnosis.

Despite a lack of discussion on the topic of a neonatal screening program, it is crucial to improve diagnosis and allowing optimal treatment of patients with CF before overt disease progression sets in.

Declaration of Interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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