Demographic Characteristics, Clinical and Laboratory Features, and The Distribution of Pathogenic Variants In the Cftr Gene In the Cypriot Cystic Fibrosis (Cf) Population Demonstrate The Utility of a National Cf Patient Registry

Panayiotis K. Yiallouros (yiallouros.panayiotis@ucy.ac.cy)
Respiratory Physiology Laboratory, Medical School, University of Cyprus https://orcid.org/0000-0002-8339-9285
Andreas M. Matthaiou
Respiratory Physiology Laboratory, Medical School, University of Cyprus https://orcid.org/0000-0003-1941-0213
Pinelopi Anagnostopoulou
Respiratory Physiology Laboratory, Medical School, University of Cyprus
Panayiotis Kouis
Respiratory Physiology Laboratory, Medical School, University of Cyprus
Malgorzata Libik
Department of Biology and Medical Genetics, Charles University
Tonia Adamidi
Pulmonology Clinic, Nicosia General Hospital
Adonis Eleftheriou
Pulmonology Clinic, Paphos General Hospital
Artemios Demetriou
Private Practice
Phivos Ioannou
Respiratory Physiology Laboratory, Medical School, University of Cyprus
George A. Tanteles
Department of Clinical Genetics, The Cyprus Institute of Neurology and Genetics
Constantina Costi
Department of Molecular Genetics, Function and Therapy, The Cyprus Institute of Neurology and Genetics
Pavlos Fanis
Department of Molecular Genetics, Function and Therapy, The Cyprus Institute of Neurology and Genetics
Milan Macek
Department of Biology and Medical Genetics, Charles University
Vassos Neocleous
Department of Molecular Genetics, Function and Therapy, The Cyprus Institute of Neurology and Genetics
Leonidas A. Phylactou
Department of Molecular Genetics, Function and Therapy, The Cyprus Institute of Neurology and Genetics

Research

Keywords: CFTR gene, CFTR modulators, cystic fibrosis, next-generation sequencing, patient registry

Posted Date: April 13th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-413045/v1

Abstract

Background

Specialized clinical care for cystic fibrosis (CF) in Cyprus, a small island country, has been implemented since the 1990s. However, only recently, a national CF patient registry has been established for the systematic recording of patients' data. In this study, we aim to present data on the epidemiological, genotypic and phenotypic features of CF patients in the country from the most recent data collection in 2019, with particular emphasis on notable rare or unique cases.

Results

Overall, data from 52 patients are presented, 5 of whom have deceased and 13 have been lost to follow-up in previous years. The mean age at diagnosis was 7.2 ± 12.3 years, and the mean age of 34 alive patients by the end of 2019 was 22.6 ± 13.2 years. Patients most commonly presented at diagnosis with acute or persistent respiratory symptoms (46.2%), failure to thrive or malnutrition (40.4%), and dehydration or electrolyte imbalance (32.7%). Sweat chloride levels were diagnostic (above 60 mmol/L) in 81.8% of examined patients. The most common identified mutation was p.Phe508del (F508del) (45.2%), followed by p.Leu346Pro (L346P) (6.7%), a mutation detected solely in individuals of Cypriot descent. The mean BMI and FEV_1 z-scores were 0.2 ± 1.3 and − 2.1 ± 1.7 across all age groups, respectively, whereas chronic *Pseudomonas aeruginosa* colonization was noted in 26.9% of patients. The majority of patients (74.5%) were eligible to receive at least one of the available CFTR modulator therapies. In 25% of patients we recovered rare or unique genotypic profiles, including the endemic p.Leu346Pro (L346P), the rare CFTR-dup2, the co-segregated c.4200_4201delTG/c.489 + 3A > G, and the polymorphism p.Ser877Ala.

Conclusions

CF patient registries are particularly important in small or isolated populations, such as in Cyprus, with rare or unique disease cases. Their operation is necessary for the optimization of clinical care provided to CF patients, enabling their majority to benefit from evolving advances in precision medicine.

Background

Cystic Fibrosis (CF; MIM: 219700) is a multi-systemic autosomal recessive rare disease caused by pathogenic, 'CF-causing', variants (henceforward mutations) in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR; MIM: 602421; NM_000492.3) gene. More than 2,000 *CFTR* variants have been identified thus far as compiled by the CFTR1 database (www.genet.sickkids.on.ca), with their type and frequency being variable in different populations.

CF patient registries exist for decades in large countries in Europe, Australia, Canada, and the United States, facilitating comparative research in demographic characteristics, *CFTR* mutation distribution, clinical and laboratory features of CF, and based on comprehensive data analysis enable actual or longitudinal assessment of health care delivery in the field of CF and beyond (Schechter et al., 2014; Fink et al., 2017; Dasenbrook and Sawicki, 2018; Jackson and Goss, 2018). Nonetheless, the establishment of national CF patient registries in relatively 'small-sized' countries and their impact on the quality of care in respective CF populations has not received appropriate attention thus far.

In the Republic of Cyprus, a relatively small island nation located in the Eastern Mediterranean, CF care has been implemented since 1997, after the establishment of a tertiary Paediatric Pulmonology Unit in Nicosia. The first comprehensive report in Cyprus published in 2007 determined that the prevalence of CF at birth is significantly lower (1 in 8,000 live births) compared to other European countries (Bobadilla et al., 2002). This estimate was based on clinically diagnosed cases according to established diagnostic guidelines (Rosenstein and Cutting, 1998), using typical clinical and laboratory features, including sweat chloride testing (utilizing pilocarpine iontophoresis) (Gibson and Cooke, 1959) and limited *CFTR* genotyping and sequencing (Yiallouros et al., 2007).

The major CF-causing mutation in European-derived populations, the p.Phe508del (legacy nomenclature F508del), was also found to be the most common CF allele (50%) in the Greek-Cypriot CF population, while the second most common allele was the p.Leu346Pro (L346P) mutation (11.5%), which has been detected only in the Greek-Cypriot population (Boteva et al., 1994). This missense mutation is associated with an overall 'milder' course of the disease in our paediatric CF cohort and is likely due to an ancient founder effect (Yiallouros et al., 2007). Nevertheless, at that time a standard CF patient registry was not implemented and standard long-term monitoring of patients had not been available.

Since January 2017, the nation-wide CF patient registry has become operational and started to provide annual Cypriot data to the European Cystic Fibrosis Society Patient Registry (ECFSPR) (www.ecfs.eu/projects/ecfs-patient-registry/annual-reports). Hence, in the last three years, we accrued better understanding of demographic, clinical, laboratory and epidemiological characteristics of CF in Cyprus. At the same time, we have initiated academic collaboration with Charles University (Prague) which carried out an international *CFTR* genotyping project in under-tested CF populations, through a research project funded by an unrestricted charitable donation. This collaboration enabled us to sequence the entire *CFTR* gene by massively parallel sequencing, including intra-*CFTR* rearrangement analysis, in order to detect less common or even unique CF allele genotypes, and thus provide better insight into the distribution of variants in 12 cases where one or both CF-causing mutations remained unidentified with Sanger sequencing.

The main aim of this study is to outline the establishment of the CF patient registry in Cyprus and present a comprehensive analysis of the genotypic and phenotypic characteristics of Cypriot CF patients listed in the national registry until the end of 2019, with an emphasis on the clinical characteristics of rare or unique *CFTR* genotypes that are present at a relatively high frequency in our population. We hope that this study will provide a basis for improving diagnostics and clinical management, including the introduction of variant-specific CF therapies, in Cyprus.

Results

Demographic characteristics

As of December 31st, 2019, a total of 52 patients (57.7% males) aged 7.2 ± 12.3 years at diagnosis were registered in the national CF patient registry. Of these, 50 had classic CF and 2 CFTR-related disease. Five patients deceased and 13 were lost to follow-up in the last three years. The majority (57.7%) of patients were of Greek-Cypriot origin from both parents, while in 7.7% of patients one parent was Greek-Cypriot.

Clinical presentation at diagnosis

Most commonly, patients presented with acute or persistent respiratory symptoms (46.2%), failure to thrive or malnutrition (40.4%), and dehydration or electrolyte imbalance (32.7%), either isolated or in combination with other manifestations. Four patients (7.7%) presented with meconium ileus, and three of them were operated, shortly after birth. In 6 (11.5%) patients, clinical presentation was not defined, whereas in 48.1% patients, a combination of two or more manifestations led to further clinical and laboratory investigations which led to the diagnosis of CF (Table 1). Dehydration or electrolyte imbalance as initial manifestation was associated with younger age at diagnosis ($r=-0.36$, $p = 0.02$), and occurred primarily (82.4%) during the warm period of the year (approximately May-September) (chi-squared test 5.9; $p = 0.015$).

Table 1
Clinical presentation of Cypriot CF patients

Clinical presentation	Number of patients	Percentage of patients (%)
Acute or persistent respiratory symptoms	24	46.2
Failure to thrive or malnutrition	21	40.4
Dehydration and or electrolyte imbalance	17	32.7
Steatorrhea or other gastrointestinal symptoms	7	13.5
Meconium ileus or other intestinal obstruction	5	9.6
Prenatal screening (amniocentesis or CVS)	3	5.8
Family history and genotyping	3	5.8
Neonatal screening	2	3.9
Nasal polyposis and/or sinus disease	1	1.9
Unknown presenting symptoms	6	11.5

CFTR genotype

We found two CF-causing mutations (*in trans* on both parental *CFTR* loci) in 49 (94.2%) patients, one mutation in two (3.9%) patients, and no genotypic alterations by the technique applied in one case (1.9%). The three cases that were not fully genotyped are classic CF cases and their samples have not undergone massively parallel sequencing for logistic reasons. The overall population-specific mutation detection rate is 96.2% for the entire cohort of Cypriot cases including the classical form of the disease and the likely CFTR-related disorders (Table 2). The most common mutation was p.Phe508del (F508del) with a frequency of 45.2%, while the second most common allele, the p.Leu346Pro (L346P), was detected in 7 instances (6.7%) of all CF alleles. Another 28 individual *CFTR* mutations were found in patients' alleles but at very low frequencies (Fig. 1). Two novel CFTR variants (p.Gly178TrpfsX5/c.531dupT and p.Ser877Ala) were found and reported by us to the CFTR1 database (see further). Patients who had CFTR-related disorders had the following *CFTR* alleles p.Ser877Ala/p.Gly542X (Case 1) and cDNA. (TG)12(T5)/p.Lys684SerfsX38 (Case 2).

Table 2
Identified *CFTR* mutations

CFTR mutation			*CFTR* allelic number	*CFTR* allelic frequency (%)	Number of compound heterozygous / homozygous patients
Legacy name	cDNA name (NM_000492.3)	Protein name			
F508del	c.1521_1523delCTT	p.Phe508del	47	45.2	22 / 13
L346P	c.1037T > C	p.Leu346Pro	7	6.7	7 / 0
CFTR-dup2	NA	NA	4	3.8	0 / 2
CFTRdele2,3	c.54-5940_273 + 10250del21kb	p.Ser18ArgfsX16	4	3.8	4 / 0
R117C	c.349C > T	p.Arg117Cys	3	2.9	3 / 0
1677delTA	c.1545_1546delTA	p.Tyr515X	3	2.9	3 / 0
4332delTG + 621 + 3A->G	c.4200_4201delTG + c.489 + 3A > G	p.Cys1400X	3	2.9	3 / 0
S549N	c.1646G > A	p.Ser549Asn	2	1.9	2 / 0
W1282X	c.3846G > A	p.Trp1282X	2	1.9	2 / 0
2789 + 5G > A	c.2657 + 5G > A	NA	2	1.9	2 / 0
3601-65C > A	NA	NA	2	1.9	2 / 0
3849 + 10kbC->T	c.3717 + 12191C > T	NA	2	1.9	2 / 0
621 + 1G->T	c.489 + 1G > T	NA	2	1.9	2 / 0
CFTRdele4-11	NA	NA	1	1.0	1 / 0
D110H	c.328G > C	p.Asp110His	1	1.0	1 / 0
E379X	c.1135G > T	p.Glu379X	1	1.0	1 / 0
G542X	c.1624G > T	p.Gly542X	1	1.0	1 / 0
G551D	c.1652G > A	p.Gly551Asp	1	1.0	1 / 0
M348K	c.1043T > A	p.Met348Lys	1	1.0	1 / 0
N1303K	c.3909C > G	p.Asn1303Lys	1	1.0	1 / 0
N1303K + 3601-65C > A	c.3909C > G	p.Asn1303Lys	1	1.0	1 / 0
Q1476X	c.4426C > T	p.Gln1476X	1	1.0	1 / 0
R1066C	c.3196C > T	p.Arg1066Cys	1	1.0	1 / 0
R117H	c.350G > A	p.Arg117His	1	1.0	1 / 0
R347P	c.1040G > C	p.Arg347Pro	1	1.0	1 / 0
2183AA->G	c.2051_2052delAAinsG	p.Lys684SerfsX38	1	1.0	1 / 0
4382delA	c.4251delA	p.Glu1418ArgfsX14	1	1.0	1 / 0
NA	c.2629T > G*	p.Ser877Ala*	1	1.0	1 / 0

Legend: NA: not applicable; *novel alleles in this study.

CFTR mutation			*CFTR* allelic number	*CFTR* allelic frequency (%)	Number of compound heterozygous / homozygous patients
Legacy name	cDNA name (NM_000492.3)	Protein name			
NA	c.531dupT*	p.Gly178TrpfsX5*	1	1.0	1 / 0
*IVS8-T(n) 5T/5T (TG11)	NA	NA	1	1.0	1 / 0
Unidentified Mutations	NA	NA	4	3.8	2 / 1

Legend: NA: not applicable; *novel alleles in this study.

Sweat chloride concentrations

Sweat chloride concentrations were reported in 44 (84.6%) patients, with 18 (34.6%) patients having two serial measurements at least four weeks apart. Sweat chloride levels above 60 mmol/L in at least one measurement were found in 36 (81.8%) patients, between 30 and 60 mmol/L in a single and/or recurrent measurement in 9 (20.5%) patients, and below 30 mmol/L in a single and/or recurrent measurement in 3 (6.8%) patients. The sweat chloride concentrations of the two CFTR-related cases were 23.5 and 21 mmol/L (Case 1) and 54 mmol/L (Case 2).

Clinical and laboratory characteristics

From a total of 34 alive patients aged 22.6 ± 13.2 years by the end of 2019 (Fig. 2), 30 (85.3%) patients provided data during years 2018 and 2019.

Nutrition and lung function

The mean BMI z-score was 0.2 ± 1.3 across all age groups, -0.1 ± 1.6 in patients aged 2–17 years, and 0.3 ± 0.9 (mean BMI 23 ± 3.8) in adult patients. The mean FEV_1 z-score in patients who had never had a lung transplant was − 2.1 ± 1.7 across all age groups, -1.5 ± 1.4 in patients aged 6–17 years, and − 2.5 ± 1.7 in adults.

Overall, 23 (76.7%) patients underwent HRCT chest scan in previous years, and among them 19 (82.6%) patients were found to have bronchiectasis of variable severity. In 13 (43.3%) cases, digital clubbing was observed at physical examination. A 37-year-old male underwent lung transplantation at the age of 27 years in Vienna, Austria.

Airway microbiology

Twenty-six (86.7%) patients had at least one positive sputum sample or cough swab culture for 8 common pathogens. The most common pathogens were *Haemophilus influenzae* (19, 73.1%), methicillin-sensitive *Staphylococcus aureus* (13, 50%), and *Pseudomonas aeruginosa* (14, 53.9%). Only one (3.9%) patient was found to be positive for *Burkholderia cepacia* complex. Seven patients (26.9%) had chronic *Pseudomonas aeruginosa* colonization.

Symptomatic treatment

Pancreatic enzyme supplements were taken by 66.7% and multivitamins by 76.7% of patients. The majority of patients were taking recombinant human DNAse (63.3%) and regularly underwent chest physiotherapy (53.3%). Continuous inhaled antibiotic, i.e. *tobramycin*, was prescribed for 9 (30%) patients, including all seven patients with confirmed chronic *Pseudomonas aeruginosa* lung colonization. Six (20%) patients were hospitalized and received intravenous antibiotic therapy on one or more occasions during the years 2018–2019. CFTR modulator therapy was administered in 3 (10%) p.Phe508del (F508del) homozygous patients (see further). Details of the treatment modalities used during 2018 and 2019 appear in Table 3 in Supplement.

Table 3
Treatment modalities in 2019 or 2018 (Supplement)

Treatment modality	Number of patients	Percentage of patients (%)
Respiratory		
Continuous inhaled hypertonic saline	5	16.7
Continuous inhaled mannitol	0	0
Continuous inhaled antibiotics	9	30
Continuous inhaled bronchodilators	13	43.3
Oxygen therapy	0	0
Continuous NIPPV*	0	0
Recombinant human DNAse	19	63.3
Continuous inhaled steroids	11	36.7
Continuous oral steroids	0	0
Continuous azithromycin or other macrolides	12	40
Chest physiotherapy	16	53.3
Gastrointestinal, hepatobiliary and pancreatic substitution		
Ursodeoxycholic acid	2	6.7
Pancreatic enzymes	20	66.7
Proton pump inhibitors	2	6.7
Caloric fortification	6	23.3
Multivitamins	23	76.7
Electrolyte solutions	2	6.7
Targeted therapy		
CFTR modulators	3	10

Legend: 2018 data were used in cases which did not attend the CF centre during 2019; *NIPPV: Non-invasive positive pressure ventilation.

Complications

Among the 34 alive patients, one has developed CF-related diabetes mellitus, chronic pancreatitis, and mild hepatobiliary disease. In previous years, four patients succumbed to severe chest infections and respiratory failure, and one to biliary cirrhosis, portal hypertension, and liver failure. The mean age of death was 26.4 years.

Eligibility for CFTR modulator therapy

Overall, 47 patients, including 34 alive and 13 lost to follow-up, were assessed for their eligibility to receive at least one of the four currently available targeted CFTR modulator therapies. Seventeen (36.2%) patients are eligible to receive one of the three CFTR modulator and potentiator drugs that were available prior to 2020. The recently approved triple combination of *tezacaftor*, *ivacaftor* and *elexacaftor* (Wainwright et al., 2015; Keating et al., 2018; Middleton et al., 2019; Davies et al., 2020) significantly expanded the group of eligible patients and when the age limit for this combinations drops to 2 years of age this number will rise to a total of 31 (66% of the entire CF population). From the patients who are ineligible to receive the triple combination, three fulfil the criteria to take *ivacaftor* and one to take double combination of *tezacaftor* and *ivacaftor*. Thus, a total of 35 (74.5%) patients

in our centre are eligible to receive advanced variant-specific therapies. Table 4 shows numbers of eligible patients for treatment with the four CFTR modulator drugs in Cyprus.

Table 4
Eligibility for administration of CFTR modulators

CFTR modulator	Indications for use	Number (percentage, %) of currently eligible patients	Number (percentage, %) of futurely* eligible patients
Elexacaftor / Tezacaftor / Ivacaftor	At least one p.Phe508del	23 (48.9%)	8 (17%)
Lumacaftor / Ivacaftor	Two p.Phe508del	9 (19.2%)	0 (0%)
Tezacaftor / Ivacaftor	Two p.Phe508del or at least one *CFTR* mutation responsive to therapy based on clinical and/or in vitro assay data	11 (23.4%)	5 (10.6%)
Ivacaftor	At least one *CFTR* mutation responsive to therapy based on clinical and/or in vitro assay data	7 (14.9%)	0 (0%)

Legend: *Eligible in the future when indications will include children less than 12 years of age.

In 13 of the 52 (25%) patients in Cyprus we recovered rare or unique *CFTR* genotypes. Those rare cases are briefly presented below.

Cases with the p.Leu346Pro (L346P) mutation in compound heterozygosity

The p.Leu346Pro (L346P) mutation has been reported only in patients of Cypriot descent. It was identified in compound heterozygosity in seven patients, usually presenting in childhood with dehydration or electrolyte imbalance, followed by late-onset lung disease and bronchiectasis in adolescence and early adulthood (Table 5). A 48-year female patient, who also has Huntington's disease (MIM: 143100), demonstrated severe lung disease. The patient had chronic *Pseudomonas aeruginosa* airway colonization, very low lung function z-scores (FEV_1=-4.7 and FVC=-3.3), and diffuse severe bronchiectasis in the right lung, atelectatic left lung with multiple bullae and ipsilateral mediastinal shift.

Table 5
Diagnostic and clinical features of 7 cases with the 'Greek-Cypriot' p.Leu346Pro (L346P) mutation in compound heterozygosity

Features	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
Gender	Male	Female	Male	Female	Male	Female	Female
Age (years)	19.7	21	24.3	27.8	32.2	48.5	72.9
Age at diagnosis (years)	0	0.5	1	0.4	5.7	25.2	47.7
Other CFTR **mutation**	p.Phe508del	p.Phe508del	p.Phe508del	p.Tyr515X	p.Phe508del	CFTRdele2,3	p.Met348Lys
Sweat chloride (mmol/L)[1]	75	106	80	103.5	66.5	86	19
Clinical presentation	PS	RS; EI	EI; FTT	EI; FTT; GS	EI; FTT; GS	RS; FTT	FH
Digital clubbing	No	ND	No	ND	Yes	ND	ND
BMI z-score[2]	-0.2	0.1	1.2	ND	-0.2	ND	ND
FEV_1 z-score[3]	-0.9	-2.4	-0.2	ND	-4.6	ND	ND
Pancreatic insufficiency[4]	Yes	ND	No	ND	Yes	ND	ND
Chronic P. aeruginosa **col.**[5]	ND	ND	ND	ND	Yes	ND	ND
Bronchiectasis[6]	Moderate	ND	No	Moderate	Severe	ND	ND

Legend: [1]Mean value (mmol/L) of all performed sweat chloride tests; [2]BMI z-score measured on date of best FEV_1 z-score measurement in follow-up year; [3]Best FEV_1 z-score measured in follow-up year; [4]Pancreatic insufficiency defined as pancreatic enzyme supplementation requirement; [5]Chronic pulmonary colonization with *Pseudomonas aeruginosa* defined according to modified Leeds criteria; [6]Bronchiectasis revealed by chest CT scan. PS: prenatal screening. RS: respiratory symptoms. EI: electrolyte imbalance. FTT: failure to thrive. GS: gastrointestinal symptoms. FH: family history. ND: not defined.

Cases with the complex allele p.Cys1400X with **c.489 + 3A > G** in cis

Three young patients, a 1-year female and two 9- and 18-year males, were identified with the known p.Cys1400X (4326delTC) in compound heterozygosity with another known *CFTR* mutation. In all three cases, as shown by parental genetic testing, p.Cys1400Ter co-segregates with the rare splicing mutation c.489 + 3A > G (621 + 3A > G), which was originally detected in Greece and previously reported as having varying clinical consequence (Forzan et al., 2010). According to the CFTR2 database data, it is more likely to be associated with pancreatic sufficiency (www.cftr2.org/mutation/general/621%252B3A-%253EG/). The first case was diagnosed through neonatal screening when the index case´s family lived in the United Kingdom, while the other two cases presented in early childhood with respiratory symptoms and electrolyte imbalance. Spirometry in these two patients showed a mean best FEV_1 z-score of -1.8 and a mean FVC z-score of -0.9, while chest imaging revealed extensive bronchiectatic changes. The mutations in the other *CFTR* allele in these three cases are a large rearrangement c.54-5940_273 + 10250del21kb (CFTRdele2,3), the novel p.Gly178TrpfsX5 (c.531dupT), and the common p.Asn1303Lys (N1303K), respectively. In addition, one of the cases was found to have a benign intronic *CFTR* variant c.3469-65C > A (3601-65C/A), found previously in disseminated bronchiectasis, which is co-segregating with N1303K *in cis*.

Cases with homozygosity of the CFTR-dup2 intra-CFTR rearrangement

Two adult male siblings of Greek-Cypriot origin were found to bear a novel duplication of exon 2 on both parental *CFTR* alleles detected by massively parallel sequencing and confirmed independently by the MLPA technique. Although patients' parents come from the same small village, we could not ascertain parental consanguinity. The first case presented with persistent respiratory symptoms, pancreatic insufficiency and malnutrition at the age of 41 years and was diagnosed by two positive sweat chloride tests (75 and 117 mmol/L) and subsequent massively parallel sequencing revealed CFTR-dup2 in homozygosity. Patient had chronic *Pseudomonas aeruginosa* pulmonary colonization and mild bronchiectatic changes on chest HRCT scan. In 2019, his best FEV_1 and FVC z-scores were − 0.7 and 0.1, respectively, with a BMI z-score of -0.1. However, the patient developed severe hepatic involvement with biliary cirrhosis and eventually hepatic failure that led to his death at the age of 47 years.

The second case was diagnosed by a positive sweat chloride test (85 mmol/L) and identification of CFTR-dup2 mutation in homozygosity on massively parallel sequencing at the age of 49 years. The patient reported persistent respiratory and gastrointestinal symptoms since childhood. He was chronically colonized with *Pseudomonas aeruginosa* with moderate bronchiectatic changes and lung emphysema on chest CT scan in the presence of digital clubbing. He was a tobacco smoker for years in the past and his spirometric indices were extremely low with best FEV_1 z-score of -5.2, FVC z-score of -0.5, and BMI z-score of 0.3. Abdominal ultrasonography showed an enlarged and diffusely hyperechogenic liver, although liver function tests are to date within normal range. Increased plasma amylase and fasting glucose levels on serial measurements were indicative for chronic pancreatitis and CF-related diabetes mellitus, respectively.

Cases with compound heterozygosity of the novel p.Ser877Ala variant

A 16-year old male was diagnosed at the age of 14 years after presenting with persistent respiratory symptoms. He is compound heterozygous for the common p.Gly542X (G542X) mutation and the novel *CFTR* variant p.Ser877Ala and had two negative sweat chloride tests (24 and 21 mmol/L). According to the ACMG.net classification this variant is Class 3 – variant of unknown significance (Harrison et al., 2019). His best FEV_1 z-score was 0.2, and FVC z-score − 0.8, and he is a high-performing athlete. Although several sputum cultures were positive for *Pseudomonas aeruginosa*, the modified Leeds criteria for chronic colonization were not fulfilled. Chest HRCT scan revealed localized mild bronchiectatic changes. Therefore, this case is classified as a CFTR-related (Bombieri et al., 2011) disorder and the patient will be continuously monitored for the eventual development of CF symptoms in adulthood.

Discussion

In this report, we present a comprehensive description of the demographic, genotypic, clinical and laboratory features of CF patients and CFTR-related disorders in Cyprus from the recently established national registry. Furthermore, we characterized rare or unique *CFTR* genotypes in 25% of cases, underscoring the importance of operating national patient registries in small and/or isolated populations. Since the introduction of the first CF patient registry in the United States in 1966, an increasing number of registries have been established all over the world, with most of them operating at a national level (Jackson and Goss, 2018). In parallel, the multinational ECFSPR combines contributions from local registries, concentrating data from 48,000 patients across 35 countries in Europe (Zolin et al., 2019). Cyprus national registry was developed in 2017, aiming to systematically record characteristics of CF patients in the country, and longitudinally follow-up their clinical outcomes, in order to optimize specialized healthcare to patients.

Comparing our data with the latest published ECFSPR Annual Data Report of 2017 (Zolin et al., 2019), the nutritional indices (mean BMI z-score) of our patients are among the higher in Europe, and could be included in the top 5 and 2 countries' values for patients below and above 18 years of age, respectively. In contrast, the mean FEV_1% predicted in Cyprus is among the 8 countries with the lowest values for children, whereas it is close to the median value of all 34 participating countries for adults. The percentage of our patients proven to be chronically colonized with *Pseudomonas aeruginosa* is also close to the median value of the other European countries.

The broad eligibility criteria of the recently EMA approved triple combination *elexafactor-tezacaftor-ivacaftor* (Keating et al., 2018; Middleton et al., 2019), significantly expanded the group of eligible patients after the age of 12 years for CFTR modulator therapy to up to 66% of the total CF population in Cyprus. In addition, several other patients bear at least one *CFTR* mutation which has

been shown to be responsive to either *ivacaftor* (Ramsey et al., 2011) or *ivacaftor-tezacaftor* combination (Davies et al., 2020), raising the total percentage of patients who were eligible for any type of CFTR modulator therapy to 75%. However, these orphan medicinal products have a high economic cost and national health authorities need to receive high quality data for their compensation to eligible CF patients. Currently, only 10% of CF patients in Cyprus have been receiving any kind of CFTR modulator therapy.

A neonatal screening program for CF has not been established in the Republic of Cyprus and the majority of patients are diagnosed after presenting with one or more CF-related manifestations (Yiallouros et al., 2007; Neocleous et al., 2014). The most common clinical manifestations at presentation are respiratory symptoms, observed in slightly less than half of the cases, followed by failure to thrive or malnutrition. Due to the temperate dry climate of the country, dehydration or electrolyte imbalance is the third most common presenting manifestation, mainly during the warm period of the year from mid-spring to mid-fall, as it has been also reported from other countries with very warm climates (Dogru et al., 2020; Yalçin et al., 2005; Dahabreh & Najada, 2013; Al-Mobaireek & Abdullah, 1995).

We have assessed *CFTR* genotype using commercial assays (Camajova et al., 2009) comprising common *CFTR* mutations in European-derived populations, complemented with limited DNA sequencing, which led to the diagnosis of many cases, but also failed to identify pathogenic mutations in 29% of cases. By using massively parallel sequencing and MLPA technique for intra-*CFTR* rearrangement analysis additional rare CF alleles and large rearrangements in the *CFTR* gene were detected in incompletely genotyped cases, three of which are novel. Despite the lower prevalence of CF in Greek-Cypriots in comparison to the majority of European countries, p.Phe508del (F508del) is the most common mutation (44.4%) on the island, which is compatible to the observed northwest-to-southeast gradient in Europe (Yiallouros et al., 2007). Quite interestingly, we recovered rare or unique genotypes in 25% of Cypriot CF patients primarily due to the utilization of massively parallel sequencing and MLPA demonstrating their diagnostic robustness and utility by achieving a population specific detection rate of 96.3% for the entire patient population listed in the national registry.

In agreement with our previous report (Yiallouros et al., 2007), the indigenous variant p.Leu346Pro (L346P), which is encountered only in individuals of Cypriot descent, is the second most common mutation (6.5%), detected in seven compound heterozygous cases. The p.Leu346Pro (L346P) missense mutation is generally associated with a milder disease phenotype, i.e. dominating over more severe mutations in compound heterozygous cases (Boteva et al., 1994). Nevertheless, the seven patients bearing this mutation demonstrated significant phenotypic variability, which could be attributed to ion channel disorders affecting CFTR function (Mall and Galietta, 2015; Mall, 2020), or other comorbidities (Girodon et al., 1997; Wang et al., 2000; Boyle, 2003). A profound example is the severe case with Huntington's comorbidity and motor symptoms (Reyes et al., 2014) that could have contributed to deterioration of chronic lung disease, underlining the possibility of combined effects of CF with concurrent other rare genetic diseases affecting the respiratory system.

The c.489 + 3A > G (621 + 3A > G) splicing mutation was identified *in cis* with p.Cys1400X (4326delTC) mutation in three compound heterozygous young patients with moderate clinical phenotype. Interestingly, co-segregation of these *CFTR* variants was previously reported in an Algerian patient (Loumi et al., 2008) and possibly in four Greek patients (Tzetis et al., 2001; Forzan et al., 2010). The c.489 + 3A > G mutation was initially considered as a severe mutation affecting the splicing of *CFTR* transcripts, reducing the amounts of normal mRNA and functional protein, and eventually leading to a severe disease phenotype (Tzetis et al., 2001). However, its pathogenicity was later disputed based on more advanced epidemiological and molecular evidence (Forzan et al., 2010), as it was found to be frequent among healthy individuals, and associated with the production of adequate amounts of correctly spliced mRNA and functional protein, despite its putative effect on *CFTR* transcript processing. The severe disease phenotype previously described in four Greek patients was probably attributed to another undetected mutation, possibly p.Cys1400X, which is commonly co-inherited with c.489 + 3A > G *in cis* as a complex allele. The cluster of complex alleles where c.489 + 3A > G co-segregates *in cis* with p.Cys1400X in Eastern Mediterranean and North Africa indicates that it is likely of common origin in the region.

Rearrangements within the *CFTR* gene, including large duplications and deletions, account according to CFTR1 for approximately 2% of the > 2,000 known *CFTR* variants, although their frequency in specific ethnic groups has not been comprehensively and systematically assessed thus far. Interestingly, two large rearrangements, novel CFTR-dup2 found by us and the Slavic large

deletion c.54-5940_273 + 10250del21kb (CFTRdele2,3(21kb)) cumulatively account for 7.4% of all mutations in Cypriot CF patients. The frequency of this rare group of *CFTR* mutations is significantly higher than in other Mediterranean CF populations, such as the Italians (Tomaiuolo et al., 2008) and Spaniards (Ramos et al., 2010), where it is reported to be roughly 2.5% and 1.3%, respectively. The rare CFTR-dup2 mutation was previously reported to cause a mild disease phenotype when *in trans* with another common mutation (Taulan et al., 2012). However, CFTR-dup2 homozygosity is reported for the first time in our two cases. These patients were characterized by severe hepatobiliary disease, eventually leading to lethal liver failure in one of them, indicating its association with a more severe CF phenotype. Further data are warranted for the determination of the exact phenotype and appropriate treatment approaches in patients bearing this rare allele. Based on our experience, we recommend that in patients bearing two CFTR-dup2 mutations, prophylactic administration of ursodeoxycholic acid is started at an early age, and hepatic function is regularly assessed.

The *CFTR* variant p.Ser877Ala is thus far considered variant of uncertain significance. However, in compound heterozygosity with a common mutation in a young patient from Cyprus, p.Ser877Ala was associated with normal sweat chloride levels, normal lung function, but also with chronic respiratory symptoms, mild bronchiectatic changes, and recurrent *Pseudomonas aeruginosa* isolation in sputa cultures. Based on these observations, p.Ser877Ala could potentially be associated with CFTR-related disorders, when *in trans* with another CF-causing mutation.

In terms of population genetics, the spectrum of non-p.Phe508del mutations observed in Cypriot CF population is generally distinct from neighbouring CF populations in countries surrounding the Eastern Mediterranean basin. However, some of these mutations observed at lower frequencies in Cypriots are relatively more common in Turkish (1677delTA, G542X and 2183AA > G) (Dogru et al., 2020), Israeli Ashkenazi Jewish (W1282X, N1303K, G542X, 3849 + 10kbC > T) (Shoshani et al., 1992), Lebanese (N1303K, W1282X, S549N) (Farra et al., 2010), Greek (621 + 1G > T, G542X, N1303, 2789 + 5G > A, 2183AA > G), and Egyptian (2183AA > G, N1303K, W1282X, G155D, CFTRdele23 (21kb)(Shahin et al., 2016) CF populations in decreasing order of their relative frequencies. In fact, many of the *CFTR* rare alleles were also previously detected (as per CFTR1 database) in other CF populations residing around the Mediterranean basin, such as. Q1476X (in Tunisia) (Messaoud et al., 2005), R1066C (in Southern Italy) (Chamayou et al., 2020), 4382delA (in Southern France) (des Georges et al., 2004) and E379X (in Greece) (Kanavakis et al., 2003), or reported in a Greek-Cypriot patient (M348K) (Deltas et al., 1996).

Conclusions

In this study, we demonstrated that the contribution of CF patient registries is particularly important in small or isolated populations, such as in Cyprus, where unique genotypic (e.g. documented founder effect in p.Leu346Pro (L346P), presence of complex allele p.Cys1400X with c.489 + 3A > G *in cis*, relatively common intra-*CFTR* rearrangement CFTR-dup2, including to a large degree a distinct mutation spectrum from neighbouring populations in the Eastern Mediterranean basin) and particular phenotypic profiles can be found. These observations together with comorbidity with other common recessive disorders in the region shed light on thus far less explored aspects of CF in specific populations. This study also corroborates the utility of massively parallel sequencing of the *CFTR* locus together with the use of the MLPA technique in terms of robust and accurate ascertainment of common and rare variants. The recent establishment of a national patient registry in the country has documented similarities between local CF population with other European countries, but also several notable distinct features, which are attributed to either regional environmental factors, such as the warm climate, or impact of rare *CFTR* variants on the course of CF. Finally, our data provide a strong basis for the improvement of CF genetic diagnostics, the eventual introduction of a multi-tier strategy using *CFTR* genotyping, and the introduction of CFTR-modulator therapies in the Cypriot CF population.

Methods

National CF patient registry

The national CF patient registry in Cyprus was established in 2017 based on the operating procedures and definitions of the ECFSPR. Initial approval from the Cyprus National Bioethics Committee (ΕΕΒΚ ΕΠ 2017.01.117) was limited to retrospective collection of demographic, clinical and laboratory data. Subsequent approval covered prospective data collection and CFTR

genotyping. A written informed consent was provided by the patients or their guardians. To protect patient confidentiality, pseudonymisation was used for their identification within the collaborative genetic testing scheme.

Patient selection

The patient status was defined according to the diagnostic inclusion criteria of the ECFSPR (Zolin et al., 2019): (a) two sweat chloride test values of at least 60 mmol/L, or (b) one sweat chloride test value of at least 60 mmol/L and two disease-causing *CFTR* mutations, or (c) typical CF features at clinical presentation and two disease-causing *CFTR* mutations if sweat chloride test value was less than 60 mmol/L or not reported. Patients bearing one CF mutation and one CFTR-related disease mutation or two CFTR-related disease mutations and a sweat chloride of less than 60 mmol/L were classified as CFTR-related disease (Bombieri et al., 2011).

Genotyping was performed in all of our patients, whereas most of them underwent quantitative measurement of sweat chloride with the pilocarpine iontophoresis stimulation of a localized skin area (Gibson & Cooke, 1959) method. The earliest date when at least one of the above criteria was fulfilled was considered as the date of diagnosis.

Sanger DNA sequencing / fragment analysis and CFTR genotyping (Cyprus)

Genomic DNA was isolated from peripheral blood leukocytes using the QIAamp Blood Midi Kit™ (QIAGEN.com, GmbH D-40724, Hilden, Germany). We have utilized a 'cascade cost saving approach' by initially testing for the most common mutation p.Phe508del (F508del) by amplifying a 79 bp DNA fragment containing exon 11 of the *CFTR* gene with the following primers: CF10 forward 5'- GTT TTC CTG GAT TAT GCC TGG C -3', and CF10 reverse 5'- GTT GGC ATG CTT TGA CGC TTC – 3'. The 20 μL PCR reaction mixture contained 100 to 200 ng of genomic DNA, 1x PCR buffer, 200 μM of each dNTP, 5 pmol of each primer and 0.5 unit of AmpliTaq Gold™. The reaction mixture was subjected to one cycle of denaturation at 95°C for 5 min followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 58°C for 1 min, extension at 68°C for 1 min and a final extension at 68°C for 6 min. An aliquot of the PCR product was subjected to cycle sequencing using the BigDye Termimator™ and was electrophoresed and sized on the automated ABI 3130xl Genetic Analyser™ (all supplied Thermo Fisher Scientific; USA www.thermofisher.com). Subsequently, in cases where one or both CF alleles remained unidentified, we initially used the ElucigeneCF29 v.2™ assay. This assay has been replaced since 2019 with its more advanced ElucigeneCF-EU2v1™ version (both Elucigene Diagnostics, United Kingdom; www.elucigene.com) that has higher non-p.Phe508del (F508del) detection rate in our geographic region.

MLPA-based intra-CFTR rearrangement analysis (Cyprus and Czechia)

DNA from all individuals in this study was also examined. MLPA was employed to investigate any possible large intra-*CFTR* rearrangements using probe mixture P091-D2 *CFTR* according to the manufacturer's recommendation. The Coffalyser.net software was used for graphical and statistical analyses (MRC. Holland, www.mlpa.com).

Massively parallel sequencing of the CFTR locus (Czechia)

Analysis of the entire *CFTR* coding region, adjacent splice site junctions and several introns using a locus-specific library preparation assay (CFTR NGS assay™; Devyser, Sweden) while massively parallel sequencing was performed on the MiSeq System™ in 12 cases (Illumina; USA). Bioinformatic analysis was carried out using the SOPHiA Platform for Hereditary Disorders™ (SophiaGenetics; Switzerland). Positive cases were confirmed by targeted Sanger DNA sequencing on ABI 3130xl DNA Analyser™ (ThermoFisher Scientific; USA). MLPA was carried out in the same manner as listed above on a selected subset of patients not tested by this methodology in Cyprus. Where applicable (e.g. in further discussed complex *CFTR* alleles with two variants *in cis*), the linkage phase of detected mutations was confirmed by their re-analysis in patients' parents.

CFTR variant nomenclature and classification of CF-causing mutations

For the *CFTR* variant nomenclature we used the recommendation of the Cystic Fibrosis Mutation Database (CFTR1; Toronto, Canada; www.gene.sickkids.on.ca). However, in particular in the Discussion section we resorted to the still more widely

understood ‘legacy nomenclature’ indicated in parentheses. Variant pathogenicity was assessed according to the ‘Clinical and Functional Translation of CFTR’ database (CFTR2; Baltimore, USA; www.cftr2.org) where applicable.

Demographic and clinical data

Patients were categorized into three main groups: (a) living patients who were seen by a CF specialist at least once during the last three years (2017–2019), (b) patients who were lost to follow-up, i.e. not seen for more than three consecutive years, and (c) deceased patients.

Patients’ data were distinguished to (a) baseline data, including demographics, age and clinical presentation at diagnosis, sweat chloride concentrations (in mmol/L), and *CFTR* genotype, and (b) annual follow-up data from both scheduled clinical visits and hospital admissions during the last three years, including clinical manifestations, anthropometrics, spirometry, airway microbiology, medical imaging, multi-systemic complications, and treatment modalities. For the purpose of this study, annual follow-up data from the last completed year, i.e. 2019, were used, while most recent data from 2018 were used for the cases that had not attended the CF clinic during 2019. Patients’ age at follow up is generally reported as of December 31, 2019.

Clinical data were retrieved from patients’ medical records. Spirometry (Vitalograph Pneumotrac, Vitalograph Inc.; USA) was routinely performed in every clinical visit in patients above 6 years of age, according to the American Thoracic Society (ATS) and European Respiratory Society (ERS) guidelines (Graham et al., 2019). The best FEV_1 and FVC values per year were recorded. The z-scores for BMI and FEV_1 were calculated using the WHO AnthroPlus® (WHO, 2009) and the GLI Online Calculator® (Quanjer et al., 2012) software systems, respectively. Sputum or cough swab microbiology testing was performed routinely in patients during each clinical follow-up. Chronic lung colonization with *Pseudomonas aeruginosa* was defined according to the modified Leeds criteria, i.e. when more than 50% of the cultures were positive for the pathogen, provided at least 4 cultures were performed in the previous year (Zolin et al., 2019). High-resolution chest computed tomography (HRCT) scans were performed in the majority of patients.

Statistical methods

Categorical variables are presented as frequencies (%), while continuous variables are presented as mean (standard deviation). Categorical comparisons were calculated using the chi-squared test. All summary statistics and statistical comparisons were calculated using STATA 12 (Version 12, StataCorp, College Station; USA).

Abbreviations

ATS: American Thoracic Society

BMI: body mass index

CF: cystic fibrosis

CFTR: cystic fibrosis transmembrane conductance regulator

DNA: deoxyribonucleic acid

dNTP: deoxynucleoside triphosphate

ECFSPR: European Cystic Fibrosis Society Patient Registry

ERS: European Respiratory Society

FEV_1: forced expiratory volume in 1 second

FVC: forced vital capacity

GLI: Global Lung Function Initiative

HRCT: high-resolution computed tomography

MIM: mendelian inheritance in man

MLPA: multiplex ligation-dependent probe amplification

PCR: polymerase chain reaction

WHO: World Health Organisation

Declarations

Ethics approval and consent to participate

The establishment and operation of the Cyprus National Cystic Fibrosis Patient Registry, including the potential for use of all data from the Registry records for the purposes of epidemiological studies, has been approved by the Cyprus National Bioethics Committee (ΕΕΒΚ ΕΠ 2017.01.117). All cystic fibrosis patients in Cyprus, or their parents or legal guardians in the cases of patients below the age of 18 years, have given their written informed consent for their participation in the Registry and the potential for use of their data from the Registry records for the purposes of epidemiological studies.

Consent for publication

All cystic fibrosis patients in Cyprus, or their parents or legal guardians in the cases of patients below the age of 18 years, for whom separate case reports are included in the current study, have given their written informed consent for the publication of their individual data.

Availability of data and materials

The data that support the findings of this study are available from the Cyprus National Cystic Fibrosis Patient Registry, but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of the cystic fibrosis patients participating in the Registry.

Competing interests

The authors declare that they have no competing interests or financial relationships that might have influenced the present work.

Funding

The study received funding from A.G. Leventis Foundation (LAP), the Vertex Pharmaceuticals Grant Scheme (CG-2015-1046430), the Czech Ministry of Health (IP00064203/6003), and the Czech Ministry of Youth Education and Sports (LM2018132; CZ.02.1.01/0.0/0.0/18_046/0015515) to MM. The funders were not involved in the design of the study, the collection, analysis, and interpretation of data, the writing of the manuscript or the decision to submit it for publication.

Authors' contributions

PKY conceived, designed, and supervised the study. AMM searched the literature, collected and analysed data with the support of TA, AE, AD and PI. *CFTR* genotyping (MLPA-based intra-gene rearrangement analysis and massively parallel sequencing) was carried out by ML under the supervision of MM, while CC and PF performed *CFTR* genotyping (Sanger sequencing and MLPA-based intra-gene rearrangement analysis) under the supervision of VN and LP. PKY, AMM, PA and PK prepared the first draft of the manuscript and developed figures and tables. All other authors contributed towards the interpretation of findings and critically revised the manuscript.

Acknowledgments

We are grateful to all Cypriot cystic fibrosis families who participated in the current study. We would like to thank for technical assistance to Ms. Jana Hrbková from Motol University Hospital in Prague.

References

Bobadilla, J.L., Macek, M., Jr., Fine, J.P. and Farrell, P.M., 2002. Cystic fibrosis: a worldwide analysis of CFTR mutations--correlation with incidence data and application to screening. Hum Mutat 19, 575-606.

Bombieri, C., Claustres, M., De Boeck, K., Derichs, N., Dodge, J., Girodon, E., Sermet, I., Schwarz, M., Tzetis, M., Wilschanski, M., Bareil, C., Bilton, D., Castellani, C., Cuppens, H., Cutting, G.R., Drevinek, P., Farrell, P., Elborn, J.S., Jarvi, K., Kerem, B., Kerem, E., Knowles, M., Macek, M., Jr., Munck, A., Radojkovic, D., Seia, M., Sheppard, D.N., Southern, K.W., Stuhrmann, M., Tullis, E., Zielenski, J., Pignatti, P.F. and Ferec, C., 2011. Recommendations for the classification of diseases as CFTR-related disorders. J Cyst Fibros 10 Suppl 2, S86-102.

Boteva, K., Papageorgiou, E., Georgiou, C., Angastiniotis, M., Middleton, L.T. and Constantinou-Deltas, C.D., 1994. Novel cystic fibrosis mutation associated with mild disease in Cypriot patients. Hum Genet 93, 529-32.

Boyle, M.P., 2003. Nonclassic cystic fibrosis and CFTR-related diseases. Curr Opin Pulm Med 9, 498-503.

Camajova, J., Berwouts, S., Matthijs, G., Macek, M., Jr. and Dequeker, E., 2009. Variability in the use of CE-marked assays for in vitro diagnostics of CFTR gene mutations in European genetic testing laboratories. Eur J Hum Genet 17, 537-40.

Chamayou, S., Sicali, M., Lombardo, D., Maglia, E., Liprino, A., Cardea, C., Fichera, M., Venti, E. and Guglielmino, A., 2020. The true panel of cystic fibrosis mutations in the Sicilian population. BMC Med Genet 21, 89.

Dasenbrook, E.C. and Sawicki, G.S., 2018. Cystic fibrosis patient registries: A valuable source for clinical research. J Cyst Fibros 17, 433-440.

Davies, J.C., Sermet-Gaudelus, I., Naehrlich, L., Harris, R.S., Campbell, D., Ahluwalia, N., Short, C., Haseltine, E., Panorchan, P., Saunders, C., Owen, C.A., Wainwright, C.E. and Group, V.X.I., 2020. A phase 3, double-blind, parallel-group study to evaluate the efficacy and safety of tezacaftor in combination with ivacaftor in participants 6 through 11 years of age with cystic fibrosis homozygous for F508del or heterozygous for the F508del-CFTR mutation and a residual function mutation. J Cyst Fibros.

Deltas, C.C., Boteva, K., Georgiou, A., Papageorgiou, E. and Georgiou, C., 1996. Description of a symptomless cystic fibrosis L346P/M348K compound heterozygous Cypriot individual. Mol Cell Probes 10, 315-8.

des Georges, M., Guittard, C., Altieri, J.P., Templin, C., Sarles, J., Sarda, P. and Claustres, M., 2004. High heterogeneity of CFTR mutations and unexpected low incidence of cystic fibrosis in the Mediterranean France. J Cyst Fibros 3, 265-72.

Dogru, D., Cakir, E., Sismanlar, T., Cobanoglu, N., Pekcan, S., Cinel, G., Yalcin, E., Kiper, N., Sen, V., H, S.S., Ercan, O., Keskin, O., S, B.E., Al Shadfan, L.M., Yazan, H., Altintas, D.U., Sasihuseyinoglu, S., Sapan, N., Cekic, S., Cokugras, H., A, A.K., T, R.G., Aslan, A.T., Bingol, A., Basaran, A.E., Ozdemir, A., Kose, M., Hangul, M., Emiralioglu, N., Tugcu, G., Yuksel, H., Yilmaz, O., Orhan, F., Gayretli Aydin, Z.G., Topal, E., Tamay, Z., Suleyman, A., Can, D., Bal, C.M., Caltepe, G. and Ozcelik, U., 2020. Cystic fibrosis in Turkey: First data from the national registry. Pediatr Pulmonol 55, 541-548.

Farra, C., Menassa, R., Awwad, J., Morel, Y., Salameh, P., Yazbeck, N., Majdalani, M., Wakim, R., Yunis, K., Mroueh, S. and Cabet, F., 2010. Mutational spectrum of cystic fibrosis in the Lebanese population. J Cyst Fibros 9, 406-10.

Fink, A.K., Loeffler, D.R., Marshall, B.C., Goss, C.H. and Morgan, W.J., 2017. Data that empower: The success and promise of CF patient registries. Pediatr Pulmonol 52, S44-S51.

Forzan, M., Salviati, L., Pertegato, V., Casarin, A., Bruson, A., Trevisson, E., Di Gianantonio, E. and Clementi, M., 2010. Is CFTR 621+3 A>G a cystic fibrosis causing mutation? J Hum Genet 55, 23-6.

Gibson, L.E. and Cooke, R.E., 1959. A test for concentration of electrolytes in sweat in cystic fibrosis of the pancreas utilizing pilocarpine by iontophoresis. Pediatrics 23, 545-9.

Girodon, E., Cazeneuve, C., Lebargy, F., Chinet, T., Costes, B., Ghanem, N., Martin, J., Lemay, S., Scheid, P., Housset, B., Bignon, J. and Goossens, M., 1997. CFTR gene mutations in adults with disseminated bronchiectasis. Eur J Hum Genet 5, 149-55.

Graham, B.L., Steenbruggen, I., Miller, M.R., Barjaktarevic, I.Z., Cooper, B.G., Hall, G.L., Hallstrand, T.S., Kaminsky, D.A., McCarthy, K., McCormack, M.C., Oropez, C.E., Rosenfeld, M., Stanojevic, S., Swanney, M.P. and Thompson, B.R., 2019. Standardization of Spirometry 2019 Update. An Official American Thoracic Society and European Respiratory Society Technical Statement. Am J Respir Crit Care Med 200, e70-e88.

Harrison, S.M., Biesecker, L.G. and Rehm, H.L., 2019. Overview of Specifications to the ACMG/AMP Variant Interpretation Guidelines. Curr Protoc Hum Genet 103, e93.

Jackson, A.D. and Goss, C.H., 2018. Epidemiology of CF: How registries can be used to advance our understanding of the CF population. J Cyst Fibros 17, 297-305.

Kanavakis, E., Efthymiadou, A., Strofalis, S., Doudounakis, S., Traeger-Synodinos, J. and Tzetis, M., 2003. Cystic fibrosis in Greece: molecular diagnosis, haplotypes, prenatal diagnosis and carrier identification amongst high-risk individuals. Clin Genet 63, 400-9.

Keating, D., Marigowda, G., Burr, L., Daines, C., Mall, M.A., McKone, E.F., Ramsey, B.W., Rowe, S.M., Sass, L.A., Tullis, E., McKee, C.M., Moskowitz, S.M., Robertson, S., Savage, J., Simard, C., Van Goor, F., Waltz, D., Xuan, F., Young, T., Taylor-Cousar, J.L. and Group, V.X.S., 2018. VX-445-Tezacaftor-Ivacaftor in Patients with Cystic Fibrosis and One or Two Phe508del Alleles. N Engl J Med 379, 1612-1620.

Loumi, O., Ferec, C., Mercier, B., Creff, J., Fercot, B., Denine, R. and Grangaud, J.P., 2008. CFTR mutations in the Algerian population. J Cyst Fibros 7, 54-9.

Mall, M.A., 2020. ENaC inhibition in cystic fibrosis: potential role in the new era of CFTR modulator therapies. Eur Respir J.

Mall, M.A. and Galietta, L.J., 2015. Targeting ion channels in cystic fibrosis. J Cyst Fibros 14, 561-70.

Messaoud, T., Bel Haj Fredj, S., Bibi, A., Elion, J., Ferec, C. and Fattoum, S., 2005. [Molecular epidemiology of cystic fibrosis in Tunisia]. Ann Biol Clin (Paris) 63, 627-30.

Middleton, P.G., Mall, M.A., Drevinek, P., Lands, L.C., McKone, E.F., Polineni, D., Ramsey, B.W., Taylor-Cousar, J.L., Tullis, E., Vermeulen, F., Marigowda, G., McKee, C.M., Moskowitz, S.M., Nair, N., Savage, J., Simard, C., Tian, S., Waltz, D., Xuan, F., Rowe, S.M., Jain, R. and Group, V.X.S., 2019. Elexacaftor-Tezacaftor-Ivacaftor for Cystic Fibrosis with a Single Phe508del Allele. N Engl J Med 381, 1809-1819.

Neocleous, V., Yiallouros, P.K., Tanteles, G.A., Costi, C., Moutafi, M., Ioannou, P., Patsalis, P.C., Sismani, C. and Phylactou, L.A., 2014. Apparent Homozygosity of p.Phe508del in CFTR due to a Large Gene Deletion of Exons 4-11. Case Rep Genet 2014, 613863.

Ramos, M.D., Masvidal, L., Gimenez, J., Bieth, E., Seia, M., des Georges, M., Armengol, L. and Casals, T., 2010. CFTR rearrangements in Spanish cystic fibrosis patients: first new duplication (35kb) characterised in the Mediterranean countries. Ann Hum Genet 74, 463-9.

Ramsey, B.W., Davies, J., McElvaney, N.G., Tullis, E., Bell, S.C., Drevinek, P., Griese, M., McKone, E.F., Wainwright, C.E., Konstan, M.W., Moss, R., Ratjen, F., Sermet-Gaudelus, I., Rowe, S.M., Dong, Q., Rodriguez, S., Yen, K., Ordonez, C., Elborn, J.S. and Group, V.X.S., 2011. A CFTR potentiator in patients with cystic fibrosis and the G551D mutation. N Engl J Med 365, 1663-72.

Reyes, A., Cruickshank, T., Ziman, M. and Nosaka, K., 2014. Pulmonary function in patients with Huntington's disease. BMC Pulm Med 14, 89.

Rosenstein, B.J. and Cutting, G.R., 1998. The diagnosis of cystic fibrosis: a consensus statement. Cystic Fibrosis Foundation Consensus Panel. J Pediatr 132, 589-95.

Schechter, M.S., Fink, A.K., Homa, K. and Goss, C.H., 2014. The Cystic Fibrosis Foundation Patient Registry as a tool for use in quality improvement. BMJ Qual Saf 23 Suppl 1, i9-14.

Shahin, W.A., Mehaney, D.A. and El-Falaki, M.M., 2016. Mutation spectrum of Egyptian children with cystic fibrosis. Springerplus 5, 686.

Shoshani, T., Augarten, A., Gazit, E., Bashan, N., Yahav, Y., Rivlin, Y., Tal, A., Seret, H., Yaar, L., Kerem, E. and et al., 1992. Association of a nonsense mutation (W1282X), the most common mutation in the Ashkenazi Jewish cystic fibrosis patients in Israel, with presentation of severe disease. Am J Hum Genet 50, 222-8.

Taulan, M., Viart, V., Theze, C., Guittard, C., Altieri, J.P., Templin, C., Mely, L., Claustres, M. and des Georges, M., 2012. Identification of a novel duplication CFTRdup2 and functional impact of large rearrangements identified in the CFTR gene. Gene 500, 194-8.

Tomaiuolo, R., Sangiuolo, F., Bombieri, C., Bonizzato, A., Cardillo, G., Raia, V., D'Apice, M.R., Bettin, M.D., Pignatti, P.F., Castaldo, G. and Novelli, G., 2008. Epidemiology and a novel procedure for large scale analysis of CFTR rearrangements in classic and atypical CF patients: a multicentric Italian study. J Cyst Fibros 7, 347-51.

Tzetis, M., Efthymiadou, A., Doudounakis, S. and Kanavakis, E., 2001. Qualitative and quantitative analysis of mRNA associated with four putative splicing mutations (621+3A–>G, 2751+2T–>A, 296+1G–>C, 1717-9T–>C-D565G) and one nonsense mutation (E822X) in the CFTR gene. Hum Genet 109, 592-601.

Wainwright, C.E., Elborn, J.S., Ramsey, B.W., Marigowda, G., Huang, X., Cipolli, M., Colombo, C., Davies, J.C., De Boeck, K., Flume, P.A., Konstan, M.W., McColley, S.A., McCoy, K., McKone, E.F., Munck, A., Ratjen, F., Rowe, S.M., Waltz, D., Boyle, M.P., Group, T.S. and Group, T.S., 2015. Lumacaftor-Ivacaftor in Patients with Cystic Fibrosis Homozygous for Phe508del CFTR. N Engl J Med 373, 220-31.

Wang, X., Moylan, B., Leopold, D.A., Kim, J., Rubenstein, R.C., Togias, A., Proud, D., Zeitlin, P.L. and Cutting, G.R., 2000. Mutation in the gene responsible for cystic fibrosis and predisposition to chronic rhinosinusitis in the general population. JAMA 284, 1814-9.

Yiallouros, P.K., Neocleous, V., Zeniou, M., Adamidou, T., Costi, C., Christophi, C., Tzetis, M., Kanavakis, E. and Deltas, C., 2007. Cystic fibrosis mutational spectrum and genotypic/phenotypic features in Greek-Cypriots, with emphasis on dehydration as presenting symptom. Clin Genet 71, 290-2.

Al-Mobaireek K.F., Abdullah A.M., 1995. Cystic fibrosis in Saudi Arabia: common and rare presentations. Annals of tropical paediatrics. 1;15(4):269-72.

Dahabreh M.M., Najada A.S., 2013. Pseudo-bartter syndrome, pattern and correlation with other cystic fibrosis features. Saudi Journal of Kidney Diseases and Transplantation. 1;24(2):292.

Quanjer, P.H., Stanojevic, S., Cole, T.J., Baur, X., Hall, G.L., Culver, B.H., Enright, P.L., Hankinson, J.L., Ip, M.S., Zheng, J., Stocks, J., 2012. Multi-ethnic reference values for spirometry for the 3–95-yr age range: the global lung function 2012 equations. Eur Respir J 40, 1324-43.

World Health Organization, 2009. WHO AnthroPlus for personal computers manual. Software for assessing growth of the world's children and adolescents. www.who.int/growthref/tools/who_anthroplus_manual.pdf?ua=1

Yalçin E., Kiper N., Doğru D., Özçelik U., Aslan A.T., 2005. Clinical features and treatment approaches in cystic fibrosis with pseudo-Bartter syndrome. Annals of tropical paediatrics. 1;25(2):119-24.

Zolin, A., Orenti, A., Naehrlich, L., van Rens, J. et al., 2019. ECFSPR Annual Report 2017.

Figures

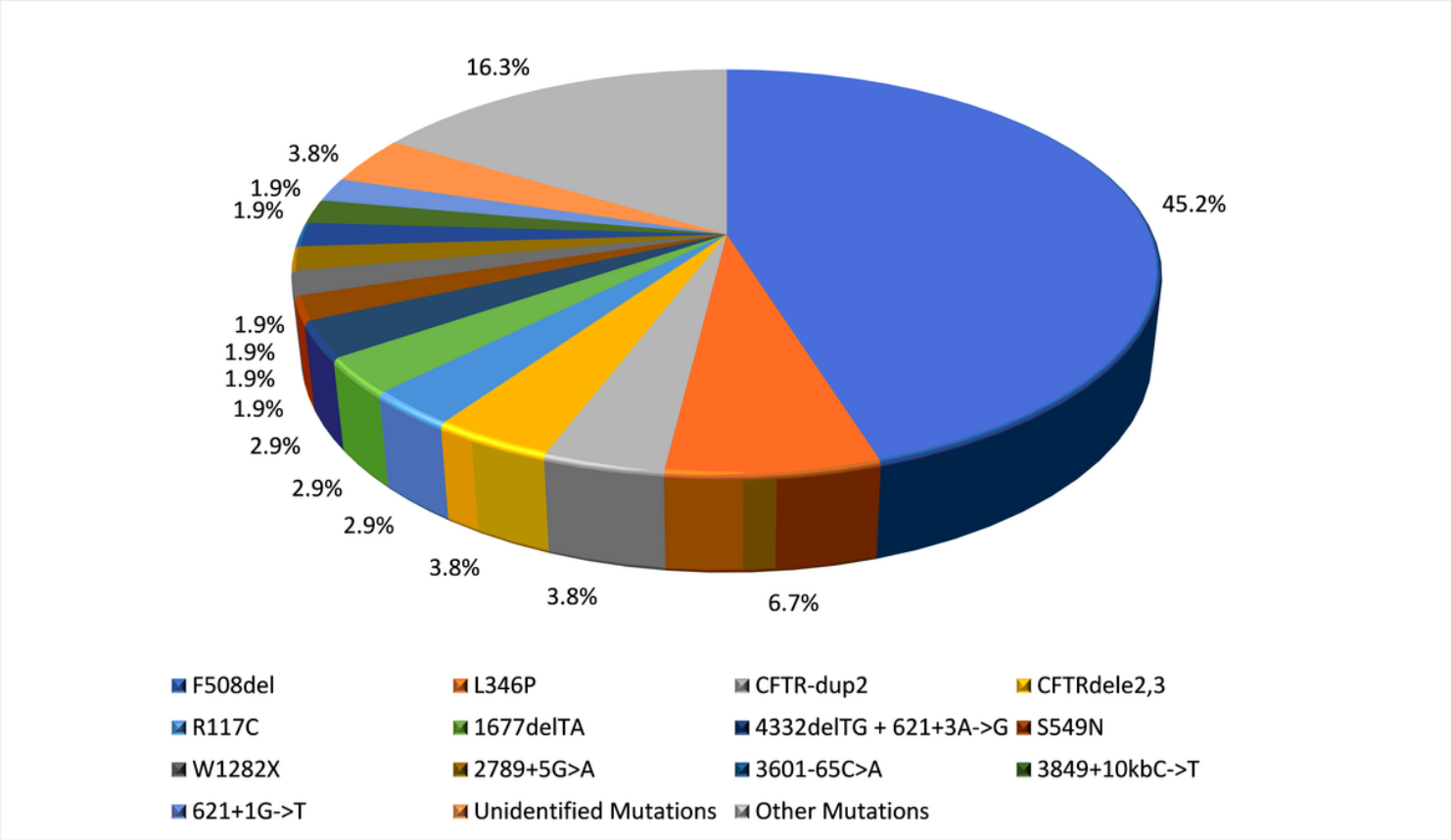

Figure 1

Frequency of CFTR mutations in on all CF alleles in the Cypriot population. Legend. CFTR mutations are presented in legacy nomenclature; the listed variant frequencies are present both in the classical forms of the disease and cases with CFTR-related disorders.

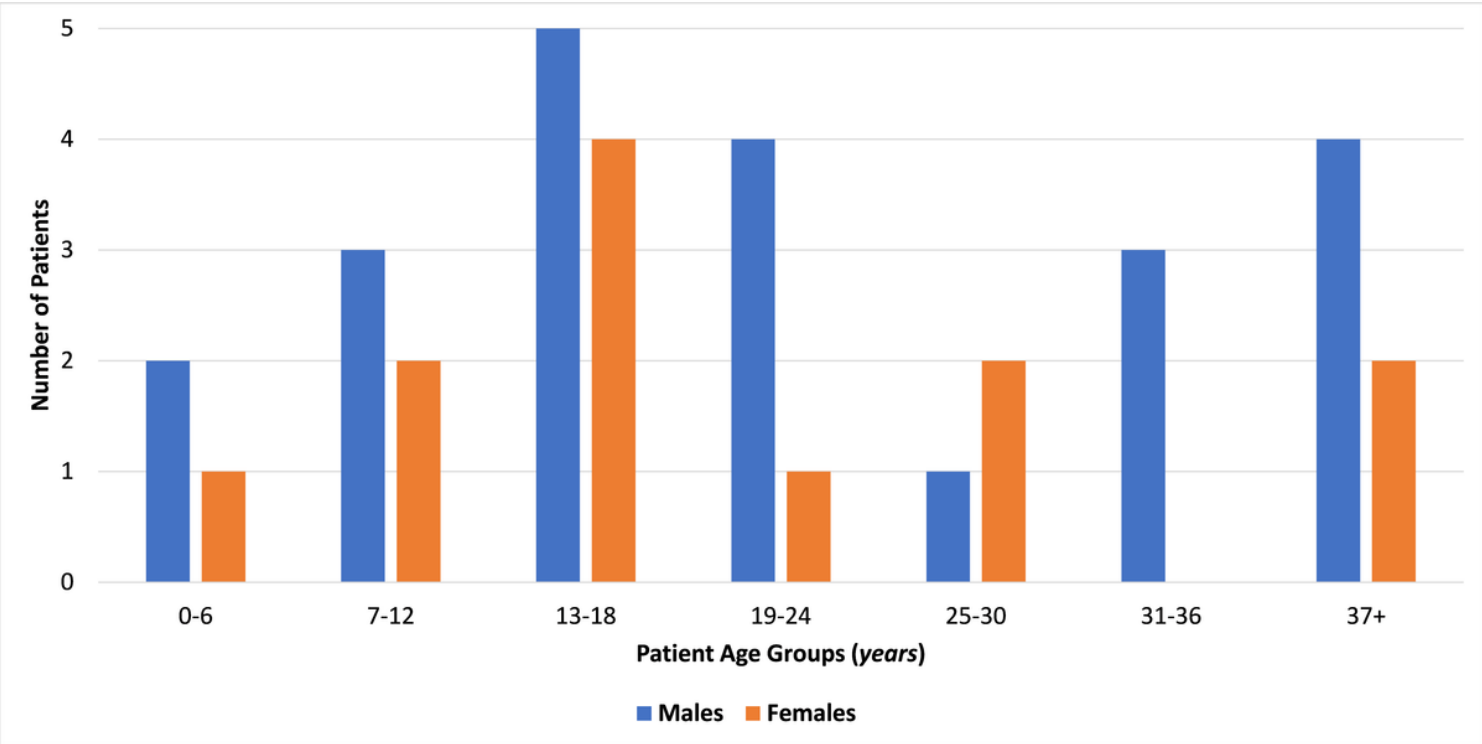

Figure 2

Gender and age distribution of living Cypriot CF patients by the end of 2019.