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Original Article

The relative frequency of *CFTR* mutation classes in European patients with cystic fibrosis

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Abstract

More than 1900 different mutations in the *CFTR* gene have been reported. These are grouped into classes according to their effect on the synthesis and/or function of the CFTR protein. CFTR repair therapies that are mutation or mutation class specific are under development. To progress efficiently in the clinical phase of drug development, knowledge of the relative frequency of *CFTR* mutation classes in different populations is useful. Therefore, we describe the mutation class spectrum in 25,394 subjects with CF from 23 European countries.

In 18/23 countries, 80% or more of the patients had at least one class II mutation, explained by F508del being by far the most frequent mutation. Overall 16.4% of European patients had at least one class I mutation but this varied from 3 countries with more than 30% to 4 countries with less than 10% of subjects. Overall only respectively 3.9, 3.3 and 3.0% of European subjects had at least one mutation of classes III, IV and V with again great variability: 14% of Irish patients had at least one class III mutation, 7% of Portuguese patients had at least one class IV mutation, and in 6 countries more than 5% of patients had at least one class V mutation.

Keywords: *CFTR* mutations; Cystic fibrosis; Genotype; Prevalence; *CFTR* mutation classes; Allele frequency

1. Introduction

Cystic fibrosis (CF) (MIM# 219700) is a life-shortening hereditary disease with relentless pulmonary infection and malabsorption as the main symptoms. The disease is caused by mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (*CFTR*) gene (MIM# 602421) coding for the CFTR protein that functions as an anion channel. For a concise description of the disease we refer to Proesmans et al. [1]. Considerable progress in CF care has been made in the past decades [2,3]. For patients with CF born in the United Kingdom (UK) in the years 2000 to 2003, the median life expectancy was estimated at 40 years [4]. However, as patients with CF live longer, for most of them the quality of life worsens [5] and the treatment burden [6] and cost [7] increase. More effective treatments are thus critically needed.

More than 1900 *CFTR* mutations have been reported, of which at least 1500 are considered potentially CF-causing since they are reported in subjects with symptoms characteristic of CF [8]. Many papers have pointed out regional differences in prevalence of specific mutations and much of that information was compiled and reported by Bobadilla et al. [9].

Grouping *CFTR* mutations into classes according to the molecular mechanism by which the mutation disrupts normal

Abbreviations: CF, Cystic Fibrosis; CFTR, Cystic Fibrosis Transmembrane Conductance Regulator; ECFSPR, European Cystic Fibrosis Society Patient Registry; PTC, premature termination codon.

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protein synthesis, traffic or function [10] is useful, especially in view of developing CFTR repair therapies. In addition, the type of mutation class can partially explain the wide variation in disease severity seen among patients with CF [10–13]. Under most functional classifications, *CFTR* mutations are divided into 5 classes [10,11]. Mutations that result in a truncated and mostly non-functional protein are categorized as class I. They include: nonsense *CFTR* mutations (also called stop or premature termination codon (PTC) mutations); frame-shift mutations; large deletions and insertions; splice-site mutations causing frame shifts which often introduce a PTC. Class II mutations (including the most common F508del mutation) lead to an aberrantly folded CFTR protein that is targeted by the cell quality control mechanism for degradation at the proteasome, resulting in the near-absence of mature CFTR protein at the apical cell membrane. Class III mutations lead to full-length CFTR protein incorporated into the cell membrane, but with a defective regulation (also called gating) so that no chloride ions flow through the CFTR channel. *CFTR* mutations leading to reduced conductance are grouped into class IV. Finally, class V mutations result in a markedly decreased amount of CFTR protein with normal function at the epithelial cell membrane and include splice site mutations that only partially disturb correct splicing (e.g. 3849+10KbC>T and 2789+5G>A) or mutant CFTR that only partially matures (e.g. A455E).

Since the discovery of the *CFTR* gene more than 2 decades ago, vast knowledge has been acquired in CFTR translational medicine. Therefore, the hope to interfere with the basic defect in the clinical setting and thus significantly alter the natural course of the disease is now perceived as a realistic goal [14,15]. Ivacaftor, a potent CFTR channel potentiator, is the first mutation specific drug on the market that improves the clinical status in patients with CF carrying at least one G551day mutation [16] or at least one of the other class III mutations [17]. Other mutation class specific treatments are in the pipeline [18]: stop codon read through drugs for patients with premature stop codon mutations, combination therapy with correctors and potentiators in subjects with class II mutations, evaluation of the efficacy of the CFTR potentiator ivacaftor in subjects with R117H class IV mutation or residual CFTR function.

Up till now, papers that analyzed data from CF registries have mainly explored the association between mutation class and disease severity: indeed, having at least one mutation of class IV or V is associated with a lower risk of being pancreatic insufficient, and on average a better lung function and survival [12,13]. These papers do not discuss the relative frequency of mutation classes in different countries. CF registry reports also do not report on relative frequency of mutation classes but present demographic data and clinical data as well as relative frequencies of specific *CFTR* mutations in individual countries. In recent years however, knowing the geographic distribution of mutation classes has become of interest, because drug development proceeds to a large extent in a mutation class specific way [18]. The clinical phases of drug development require testing new compounds in a group of subjects with a specific mutation or mutation class. These phases can only be efficient if clinical trials are preferentially directed to countries or regions where these mutation classes are prevalent. The current paper therefore reports the spectrum of the *CFTR* mutation classes in more than 25,000 European patients with CF.

2. Methods

The 2009 (latest data update at time of writing) the European Cystic Fibrosis Society Patient Registry (ECFSPR) data were analyzed. The ECFSPR contains data from the national registries of Belgium, Czech Republic, Germany, Denmark, France, Hungary, Ireland, Israel, Moldova, The Netherlands, Sweden, UK and from individual CF centers in Austria, Switzerland, Greece, Spain, Italy, Latvia, Portugal, Serbia, Slovenia, Bulgaria, and the Republic of Belarus. To evaluate the representativeness of this data collection, we compared the number of patients reported in the ECFSPR to the number of patients with CF as reported by Farrell for several European countries [19] and as estimated from the paper by Efrati et al. [20] for Israeli patients.

We used *CFTR* mutation legacy names and converted all other ways to refer to the same mutation into this nomenclature. Obvious spelling mistakes were corrected according to the database provided by the CFTR1 project available on-line [8]. *CFTR* mutations were then grouped into 5 mutation classes as outlined in Table 1. We modified the classification proposed by McKone et al. [21] based on new insights: G85E was reassigned to class II [22], and recently recognized gating mutations with proof of CFTR potentiator responsiveness were added to class III [23]. Like McKone, we assigned all PTC mutations to class I. All other mutations were grouped as mutation class unclassified.

The percentage of patients homozygous or heterozygous for mutation classes I to V was then calculated on the European and on the national level. Ninety five percent confidence intervals were determined. If these did not overlap, differences in occurrence were considered statistically significant. Since for class I, only the subgroup with nonsense mutations is currently targeted with specific drug therapy [18], we also reported this

Table 1
List of mutations by mutation class.

Class	Type of defect	List of mutations attributed to this class
Class I	Defective protein production	Nonsense mutations Large deletions and insertions 1078delT; 1717-1G→A; 3659delC; 621+1G→T
Class II	Defective protein processing	G85E, F508del, I507del, R560T, N1303K
Class III	Defective protein regulation ('gating')	G178R, S549N, S549R, G551D, G551S, G970R, G1244E, S1251N, S1255P, G1349D
Class IV	Defective protein conductance	R117H, R334W, R347P
Class V	Reduced amount of functioning protein	2789+5G→A, 3849+10KbC→T, A455E
Unclassified		All other mutations, including those unknown.

subgroup separately. When computing these percentages, patients who do not carry 2 mutations belonging to the same class, are represented twice. Therefore, the number of patients with specific combinations of mutation classes are also reported.

3. Results

In Table 2 we list the number of patients reported in the ECFSPR in participating countries and compare them to the number of patients with CF as reported in several European countries [19,20]. There are obvious differences in representativeness between countries. In Table 2 data are put in italics and in Fig. 1 country names are put in a shaded area when less than 70% of estimated patients in that country were included in the ECFSPR or when the number of patients was lower than 100.

CFTR genotype information was available for 25,394 of 26,685 (95.2%) patients. Therefore, 25,394 was used as denominator when reporting the % of patients with at least one mutation of a specific mutation class. In total 50,788 alleles were thus examined (Table 2). In 46,441 alleles (91.4%), a *CFTR* mutation was identified and 42,388 of these (91.3% of the identified mutations) were classified into one of the 5 mutation classes.

The percentage of alleles with mutations unidentified after DNA analysis varied between countries: overall, 8.6% of mutations remained unknown, but the proportion of undetected mutations ranged from 1% in Denmark to 46.9% in Hungary. However, in 14/23 countries less than 10% of alleles remained unknown (Table 2).

Fig. 1 shows the percentage of CF patients who are homozygous (darker color) or heterozygous (lighter color) for a specific mutation class. In Fig. 1A percentages are given for all class I mutations. In Europe overall, 3195 patients (12.6%) were reported to be compound heterozygous or homozygous for a nonsense mutation (Fig. 1B). The percentage of these patients was remarkably higher in Israel (45.5%, 95%CI: 41.2; 49.8), Italy (32.4%, 95%CI: 28.4; 36.6) and Slovenia (27.3%, 95%CI: 17.0; 39.6). Overall 22,144 European patients (87.2%) carried at least one class II mutation (Fig. 1C): 9147 in heterozygosity and 12,997 in homozygosity. Their proportion was higher in Denmark (97.1%, 95%CI: 95.1; 98.5), Latvia (96.5%, 95%CI: 82.2; 99.9), Ireland (94.6%, 95%CI: 93.1; 95.9), Serbia (94.2%, 95%CI: 87.9; 97.8), and lower in Israel (46.8%, 95%CI: 42.5; 51.1), Hungary (57.30%, 95%CI: 53.1, 61.4) and Italy (73.0%, 95%CI: 69.0; 76.8). Since the frequency of F508del greatly outnumbered the frequency of all other class II mutations, the proportion of patients carrying at least one class II mutation was almost identical to the proportion of patients carrying F508del on at least one allele (Fig. 1C and d). Jointly all ten gating mutations were found in only 990 patients in Europe (3.9%). There was a higher prevalence of patients carrying at least one class III mutation in Ireland (13.9%, 95%CI: 11.9; 16.2), UK (6.2%,

Table 2
Number of patients and data contribution by country.

Country	Number of patients reported in the literature	Number of patients reported to the ECFSPR	Number of patients with DNA analysis performed (%)[a]	Number of patients with 2 mutations identified (%)[b]	Number of patients with 1 mutation identified (%)[b]	Number of alleles unknown (%)[c]	Number of alleles classified into classes I–V (%)[c]
Total		26,685	25,394 (95.16)	21,958 (86.47)	2525 (9.94)	4347 (8.56)	42,388 (83.46)
Austria (*AT*)	*686*	*352*	*352* (*100.0*)	*312* (*88.64*)	*28* (*7.95*)	*52* (*7.39*)	*602* (*85.51*)
Belgium (BE)	1065	1129	1107 (98.0)	987 (89.16)	91 (8.22)	149 (6.73)	1874 (84.64)
Bulgaria (*BG*)	*170*	*95*	*95* (*100.0*)	*84* (*88.42*)	*11* (*11.58*)	*11* (*5.79*)	*153* (*80.53*)
Republic of Belarus (BY)	–	145	141 (97.2)	97 (68.79)	44 (31.21)	44 (15.60)	222 (78.72)
Switzerland (CH)	–	190	189 (99.5)	181 (95.77)	7 (3.7)	9 (2.38)	323 (85.45)
Czech Republic (CZ)	570	507	507 (100.0)	483 (95.27)	17 (3.35)	31 (3.06)	882 (86.98)
Germany (DE)	6835	5048	4534 (89.8)	3397 (74.92)	710 (15.66)	1564 (17.25)	7080 (70.08)
Denmark (DK)	412	451	451 (100.0)	443 (98.23)	7 (1.55)	9 (1.00)	833 (92.35)
Spain (*ES*)	*2200*	*740*	*739* (*99.9*)	*575* (*77.81*)	*143* (*19.35*)	*185* (*12.52*)	*1095* (*74.09*)
France (FR)	4533	5640	5473 (97.0)	5292 (96.69)	181 (3.31)	181 (1.65)	9331 (85.24)
Greece (*GR*)	*555*	*92*	*89* (*96.7*)	*67* (*75.28*)	*18* (*20.22*)	*26* (*14.61*)	*141* (*79.21*)
Hungary (HU)	410	555	555 (100.0)	241 (43.42)	108 (19.46)	520 (46.85)	565 (50.90)
Ireland (IE)	1182	1090	1042 (95.6)	975 (93.57)	65 (6.24)	69 (3.31)	1914 (91.84)
Israel (IL)	507	533	530 (99.4)	372 (70.19)	78 (14.72)	238 (22.45)	692 (65.28)
Italy (*IT*)	*5064*	*539*	*519* (*96.3*)	*469* (*90.37*)	*46* (*8.86*)	*54* (*5.20*)	*833* (*80.25*)
Latvia (*LV*)	*24*	*29*	*29* (*100.0*)	*18* (*62.07*)	*11* (*37.93*)	*11* (*18.97*)	*45* (*77.59*)
Moldova (*MD*)	–	*41*	*41* (*100.0*)	*33* (*80.49*)	*6* (*14.63*)	*10* (*12.20*)	*60* (*73.17*)
The Netherlands (NL)	1275	1249	1179 (94.4)	1112 (94.32)	52 (4.41)	82 (3.48)	2188 (92.79)
Portugal (*PT*)	*285*	*117*	*115* (*98.3*)	*110* (*95.65*)	*5* (*4.35*)	*5* (*2.17*)	*193* (*83.91*)
Serbia (RS)	–	122	104 (85.2)	84 (80.77)	18 (17.31)	22 (10.58)	178 (85.58)
Sweden (SE)	362	578	578 (100.0)	559 (96.71)	14 (2.42)	24 (2.08)	931 (80.54)
Slovenia (*SI*)	*66*	*66*	*66* (*100.0*)	*59* (*89.39*)	*6* (*9.09*)	*8* (*6.06*)	*112* (*84.85*)
United Kingdom (UK)	8284	7377	6959 (94.3)	6008 (86.33)	859 (12.34)	1043 (7.49)	12,141 (87.23)

Country data in italic means that the data from that country cover less than 70% of the estimated total patient number or concerns less than 100 patients.

[a] Percentage was computed using as denominator the number of patients reported to the ECFSPR.

[b] Percentage was computed using as denominator the number of patients for whom DNA analysis was performed.

[c] Percentage was computed using as denominator the number of alleles for which DNA analysis was performed.

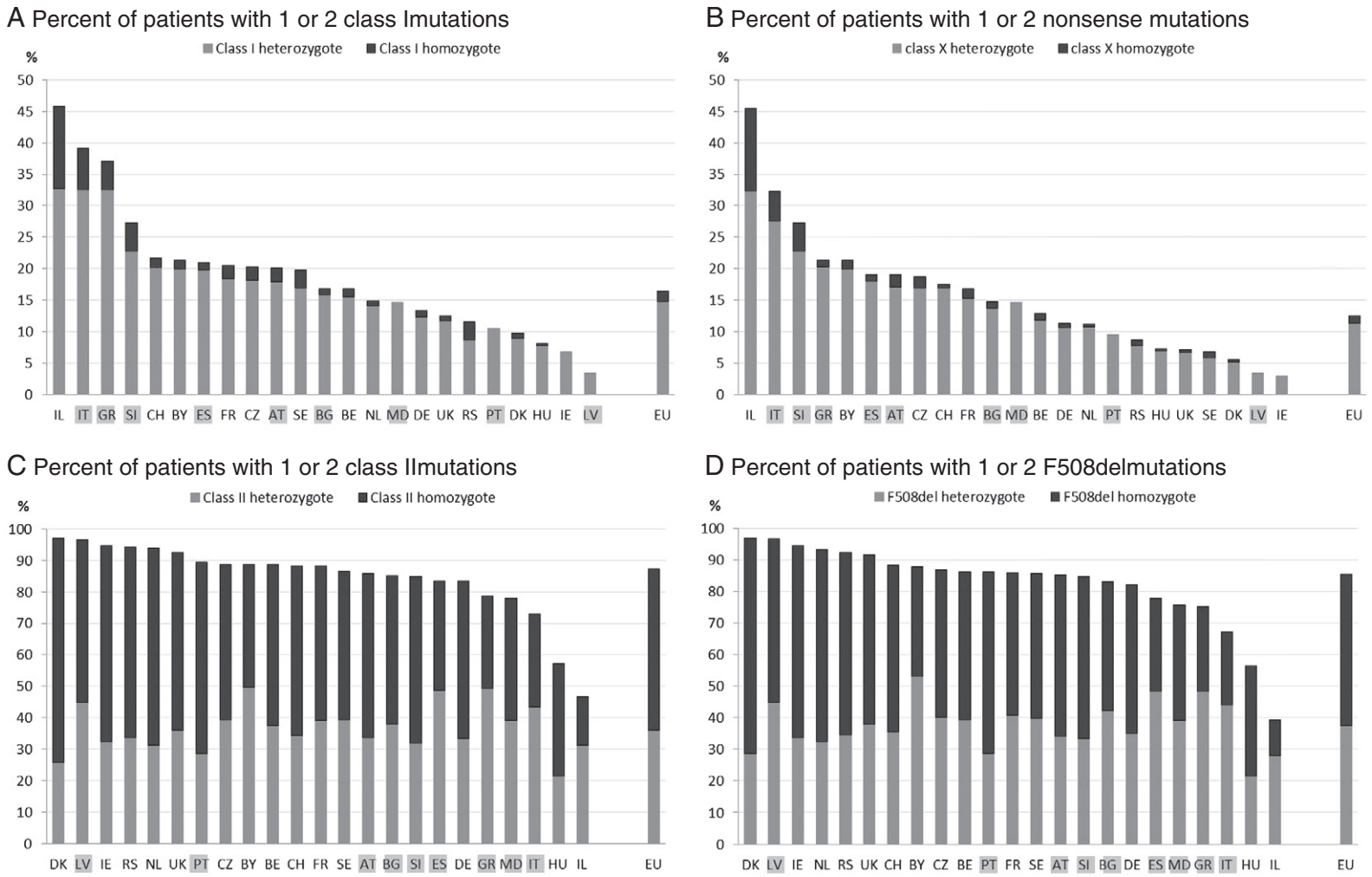
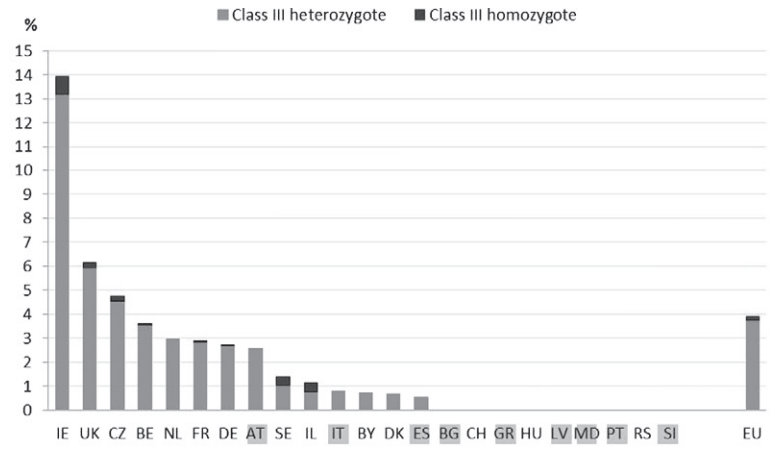
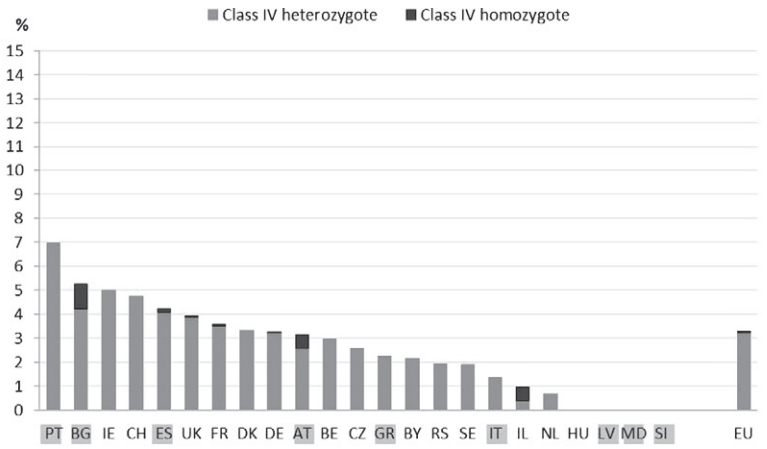


Fig. 1. Percentage of European patients (N = 25,394) carrying one (lighter shade) or two (darker shade) mutations of a specific mutation class, by country and in total cohort. Panel A shows class I, panel B shows nonsense mutations, panel C shows class II, panel D shows mutation F508del, panel E shows class III, panel F shows class IV, panel G shows class V. *AT*: *Austria*, BE: Belgium, BY: Republic of Belarus, *BG*: *Bulgaria*, CH: Switzerland, CZ: Czech Republic, DE: Germany, DK: Denmark, *ES*: *Spain*, FR: France, *GR*: *Greece*, HU: Hungary, IE: Ireland, IL: Israel, *IT*: *Italy*, *LV*: *Latvia*, *MD*: *Republic of Moldova*, NL: The Netherlands, *PT*: *Portugal*, RS: Serbia, SE: Sweden, *SI*: *Slovenia*, UK: United Kingdom. Country name in italics in the legend above and in a shaded box in the figure means that the data from that country cover less than 70% of the estimated total patient number or concerns less than 100 patients.

E Percent of patients with 1 or 2 class IIImutations



F Percent of patients with 1 or 2 Class IVmutations



G Percent of patients with 1 or 2 Class Vmutations

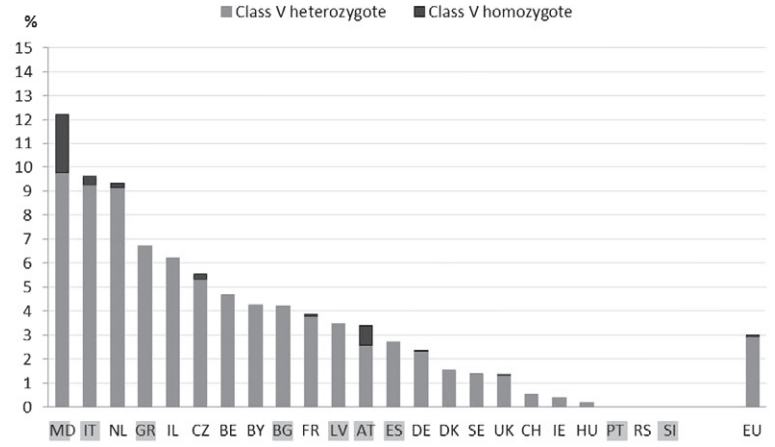


Fig. 1 (*continued*).

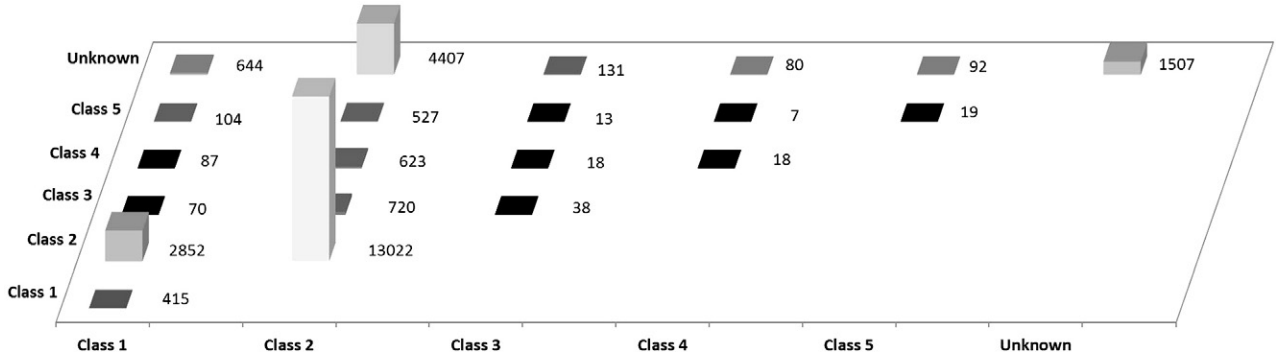


Fig. 2. Distribution of *CFTR* mutation classes in European patients with CF. Each bar represents the number of CF patients with a specific mutation class combination.

95%CI: 5.6; 6.8), the Czech Republic (4.7%, 95%CI: 3.1; 7.0) and Belgium (3.6%, 95%CI: 2.6; 4.9) when compared with the other countries, where the prevalence was below 3% (Fig. 1E). Patients carrying at least one class IV mutation were also uncommon: 833 were registered in the ECFSPR (3.3%), but their proportion within countries was variable (Fig. 1F). Similarly, patients carrying at least one class V mutation were uncommon: 762 (3.0%) were reported to the ECFSPR again concentrated in specific countries (Fig. 1G).

The combination of mutation classes within patients is shown in Fig. 2. The most frequent combination was by far class II/class II (51.3%), followed by class I/class II (11.2%).

4. Discussion

We describe the *CFTR* mutation class frequency in a large cohort of European patients with CF and show marked heterogeneity between countries. The variability in *CFTR* mutations between countries has been documented before [9,24] and many reports describe the increased frequency of mutations in specific countries, but this report is the first to document the relative frequency of mutation classes in a European population as well as in specific countries. This is relevant for the development of *CFTR* mutation class specific therapies, a new form of individualized treatment aimed at correcting the basic CF defect.

Although patients with CF carry a wide spectrum of *CFTR* mutations, class II mutations, because these contain F508del, are by far the most frequent. According to the data available to the ECFSPR, therapeutic approaches that address F508del may help more than 80% of CF patients in most European countries. By comparison all other *CFTR* mutations are relatively rare. Since the ultimate aim is to develop treatments for all mutation categories, it is important to know where the respective patients are mainly found. The most useful example is the geographic distribution of the G551day and other gating mutations reported in the current paper. The CFTR potentiator ivacaftor has proven efficacy in patients carrying the class III mutation G551day [16]. It has also proven in vitro efficacy to potentiate other gating mutations [23] and clinical benefit in patients with these mutations was also recently shown [17]. Jointly, the 9 non-G551D gating mutations account for only 18.3% of patients with a class III mutation. However, knowing the countries where patients with these mutations reside is needed to provide them access to the drug. Since ivacaftor also potentiates normal CFTR [23] clinical trials are ongoing in subjects carrying class IV or V mutations, other mutation categories that differ considerably between countries.

The current report has limitations. Data representativeness differs between countries and therefore also the correctness of the estimate of occurrence of a mutation class. This is pointed out in Table 2 and in Fig. 1. Whereas in many long established national registries coverage is high, in more recent national registries this might not yet be the case and in some countries reporting is from selected centers only. For further details on the representativeness of data collection to the ECFSPR, we refer to the ECFSPR annual report [25]. The difference in mutation class frequency between countries reflects the known genetic diversity between countries [9,24] but may also to some extent reflect differences in the thoroughness of DNA analysis between countries and between CF centers (Table 2). Furthermore, reliability of genetic analysis may not be optimal in all centers [26]. Lastly, assigning a *CFTR* mutation exclusively to one class is not entirely accurate, since mutations may have properties of several mutation classes. A fraction of F508del mutant CFTR is known to escape the cellular quality control system and appear at the apical cell membrane [27,28]. Such F508del-CFTR protein has however reduced function since it has at most 20% of wild type CFTR activity [29]. F508del thus combines the properties of class II and class III–IV mutations. Non-maturing mutants when rescued by a corrector may thus also behave as a class III or IV mutation. For drug development this points towards the necessity to combine CFTR correctors and potentiators. Patients carrying splice site mutations that generate a new exon harboring a stop codon (e.g.; 3849+10kbC>T) may thus, like patients with nonsense mutations, benefit similarly from treatment with drugs promoting 'read-through' of stop codons. Lastly, for many mutations it is unknown to what mutation class they belong. The *CFTR* 2 project [30] was set up to fill this knowledge gap by investigating the effect of mutations with a frequency above 0.01% on the synthesis and function of the CFTR protein in vitro. This will thus help further grouping of mutations in mutation classes.

In conclusion, the frequency of *CFTR* mutation classes varies considerably between countries. In 18/23 countries, 80% or

more of the patients had at least one class II mutation. Overall 16.4% of European patients had at least one class I mutation but this varied from 3 countries with more than 30% to 4 countries with less than 10% of subjects. Overall only respectively 3.9, 3.3 and 3.0% of European subjects had at least one mutation of classes III, IV and V with again great variability: 14% of Irish patients had at least one class III mutation, 7% of Portuguese patients had at least one class IV mutation, and in 6 countries more than 5% of patients had at least one class V mutation. These differences reflect the known genetic heterogeneity, but they might also to some degree be due to differences in detail of genetic analysis between countries.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jcf.2013.12.003.

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