



Short Communication

A high frequency of the Cystic Fibrosis 2184insA mutation in Western Ukraine: Genotype–phenotype correlations, relevance for newborn screening and genetic testing[☆]

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Abstract

We present the first comprehensive report on the distribution and genotype–phenotype correlations of CF-causing mutations in Western Ukraine (former Galicia). The 2184insA mutation was identified in 17 unrelated CF patients, 2 of whom are homozygotes for this allele. This mutation is associated with the classical form of CF. The high frequency of 2184insA mutation (7.20% of all mutated CF chromosomes) suggests that it is likely of Galician origin, from where it has spread throughout Europe and beyond. The achieved 83.71% mutation detection rate fulfills the minimal pre-requisite for introduction of the “two-tier” (IRT/DNA) newborn screening program.

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1. Introduction

The incidence of CF in Ukraine has not been determined at nation-wide or regional levels, thus far. However, our previous results based on testing of 720 random individuals for common

CFTR alleles suggest a carrier frequency of 1 in 29 (data not shown). Therefore, the extrapolated frequency of CF homozygotes is approximately 1 in 3300 live births. Based on these estimates and the annual national birth rate of 509,000 newborns, 143 children with CF are expected to be born every year in the country [1]. Given the average annual birth rate within the last decade (1998–2008) of 119,000 newborns in Western Ukraine (WU), 47 of them with CF should be born in this region.

The core of historic Galicia comprises current regions of Lviv, Ternopil and Ivano-Frankivsk, from where most of the CF patients included in this report originate (Fig. 1). The region's dominant Ukrainian population (approximately 90%) is complemented by Russian (2.7%), Polish (2.1%), Romani (1.9%), Hungarian and Ashkenazi Jewish (1.5% each), Slovak (1.0%), German, Armenian and Czech (together 0.3%) minorities [extrapolated WU 2001 census; 2].

Within the last decade (1998 to 2008), molecular genetic screening of common European CF-causing mutations was

Abbreviations: BMI, body mass index; CF, Cystic Fibrosis; CFF, Cystic Fibrosis Foundation; *CFTR*, gene for the Cystic Fibrosis transmembrane conductance regulator protein; CFLD, Cystic Fibrosis liver disease; DNA, deoxyribonucleic acid; IRT, immunoreactive trypsinogen; FVC, forced vital capacity; FEV₁, forced expiratory volume at one second; M, million; MLPA, multiplex ligation-dependent probe amplification; PS, pancreatic sufficiency; PCR, polymerase chain reaction; WU, Western Ukraine.

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performed in a total of 420 individuals of WU origin where CF was clinically suspected, including the examination of 630 first degree relatives from these families. From the total number of cases studied the diagnosis of the classical form of CF was unambiguously established in 132 patients (132/420; 31.43%) using consensual diagnostic criteria [3]. Since this cohort is representative of the WU population (Fig. 1), we could characterize the distribution of the most common CF-causing alleles by utilizing a panel of 10 mutations comprised within set a “home brew” methods (F508del, CFTRdele2,3(21 kb), I507del, 1677delTA, G542X, N1303K, W1282X, G551D, R553X, 1717-1G>A) [4,5]. Moreover, we continuously improved CF DNA diagnostics by successfully participating in the CF Thematic Network external quality assessment scheme since 2005 (cf. eqascheme.org).

In this study, in 57 patients where one or both *CFTR* mutations remained unidentified, we initially examined 23 additional common European CF alleles [6] (E60X, P67L, G85E, R117H, 621+1G>T, 711+1G>T, 1078delT, R334W, R347P, A455E, S549R T>R, R560T, 1898+1G>A, 2184delA, 2789+5G>A, 3120+1G>A, M1101K, D1152H, R1162X, 3659delC, 3849+10kbC>T, S1251N, 3905insT) comprised within a commercial assay, followed by DNA sequencing of selected *CFTR* exons 7 and 13, since these contain majority of the mutations found in Central and Eastern European CF populations [6,7]. The scope of this project was limited by the short duration of the Eurogentest project (www.eurogentest.org) incoming fellowship and its major aim was to

provide sufficient evidence for public health purposes aimed at the introduction of newborn screening (IRT/DNA) in Ukraine.

To our knowledge, this is the first comprehensive report on the distribution of *CFTR* mutations in WU and their genotype–phenotype correlations, which has general implications for genetic testing and newborn screening in patients of this origin.

2. Methods

Leukocyte DNA was extracted either by the Proteinase K/phenol-based procedure or by our modification of the “salting out” technique [8]. Extended *CFTR* mutation/rearrangement screening was performed in sequential order by a/commercial assay comprising 32 most common European CF-causing mutations — Elucigene™ CF-EU1 (Gen-Probe Life Sciences Ltd.; United Kingdom), b/multiplex ligation-dependent probe amplification (MLPA)-SALSA MLPA KIT P091-B1 *CFTR* (MRC-Holland; The Netherlands) and c/sequencing on the 3130xl Genetic Analyzer (Applied Biosystems; USA).

3. Results

The “starting” distribution of CF mutations in the WU cohort based on our previous analyses [4,5] is listed in Table 1, whereby 76.14% (201/264 of all CF alleles) were identified (Table 1).



Fig. 1. Origin of patients with the 2184insA mutation included in this study.

Table 1
Distribution of *CFTR* mutations in Western Ukrainian CF patients.

<i>CFTR</i> mutations	N. of alleles	%
F508del (c.1520_1522delTCT)	143	54.17
<i>2184insA</i> (c.2052_2053insA)	19	7.20
N1303K (c.3909C>G)	13	4.92
CFTRdele2,3(21 kb) (c.54_273)	11	4.17
G542X (c.1624G>T)	7	2.65
W1282X (c.3846G>A)	6	2.27
1898+1G>A (c.1766+1G>A)#	3	1.14
2143delT (c.2012delT)#	3	1.14
621+1G>T (c.489+1G>T)#	2	0.76
R334W (c.1000C>T)#	2	0.76
3272-11 A>G (c.3140-11A>G) #	2	0.76
3849+10kbC>T (c.3717+12191C>T)#	2	0.76
185+1G>T (c.53+1G>T)#	1	0.38
E92K (c.274G>A)#	1	0.38
R347H (c.1040G>A)#	1	0.38
<i>Y362X*</i> (c.1086 T>A)	1	0.38
1717-1G>A (c.1585-1G>A)	1	0.38
R553X (c.1657C>T)	1	0.38
2183AA>G (c.2051_2052delAA)#	1	0.38
2721del11 (c.2589_2599delAATTTGGTGCT)#	1	0.38
Identified total	221	83.71
Unidentified	43	16.29

CFTR nomenclature is listed where applicable as by its more frequently used “legacy name” followed by the cDNA name in parenthesis [8]; mutations in italics were identified in this study, while the one in bold is novel; # these mutations were detected through previous collaborations (see Acknowledgment); * this mutation is novel.

We did not detect any intragenic *CFTR* rearrangements using MLPA in 63 *CFTR* genes (23.86%) with unknown CF mutations [9]. Sequencing of exon 7 revealed a novel allele Y362X [using the “legacy nomenclature”; 7]. The patient with this mutation bears the F508del allele in *trans* and suffers from the classical form CF associated with high sweat chloride concentrations. In exon 13 we found a high prevalence of the 2184insA mutation since this allele was detected in a total of 17 unrelated cases. Two patients are homozygotes for this mutation, while the analysis of parental origin revealed that 13 had F508del, 1 had the “Slavic” deletion CFTRdele2,3(21 kb) and one case had the R334W mutation in *trans*. Sixteen CF patients with 2184insA mutation originated were of WU origin, while only 1 case came from the eastern (Lugansk) part of the country. This mutation was not identified in 25 CF patients from the collaborating southeastern Zaporizhzhya CF centre (Fig. 1; data not shown). Identification of additional 20 CF mutations in the WU cohort increased the mutation detection rate to 83.71% (221/264; Table 1).

Genotype/phenotype correlations in 17 (8 females/9 males) bearing the 2184insA mutation are presented in Table 2. These data strongly suggest its “CF-causing” nature.

4. Discussion

Since the 2184insA mutation is not included within mutation panels of commonly used commercial assays [10], this allele is not identified by routine DNA diagnostics [11], thus far. As a consequence, there were only sporadic reports of this mutation in

patients of Slavic origin [6,7,12–17] and of its associated phenotype, e.g. in a Polish patient [12]. The 2184insA mutation is located within the poly A tract in which deletions of one or two nucleotides are commonly found [9]. Consequently, the “mirror deletion” at this site, i.e. 2184delA, is included in the most frequently used commercial assays [10,11]. Similarly, the 2183AA>G belongs to the most common CF-causing mutations [6,17].

Of all populations studied to date, the prevalence of the 2184insA mutation is highest in WU (7.20% of all mutated CF chromosomes). Based on the observed population gradient, it is likely that the 2184insA mutation has its origin in Slavic populations of WU-Galicia from where it has disseminated throughout Europe and beyond.

More than half of the cases with this mutation were diagnosed within their first year of life, which attests the presence of typical CF symptoms in these children, and 1 patient presented with meconium ileus at birth (Table 2).

Fecal elastase-1, measured in 15 of the 17 cases with 2184insA, was low (Table 2), thereby substantiating the clinically ascertained pancreatic insufficiency [18]. Fecal elastase-1 levels in all patients treated at the Lviv CF centre are within the range of 1.6–566.0 µg/g of stool (average 6.5 µg/g; data not shown). Approximately 10% of them are pancreatic sufficient (PS, fecal elastase-1 > 200 µg/g). The percentage of PS cases and the high proportion of patients diagnosed within the first year of life are comparable to e.g. the CFF Registry [19]. This general comparison confirms that the clinical ascertainment of CF in WU for this study is not biased.

When comparing our CF patients with and without the 2184insA mutation, we found no significant differences in their age distribution within specific age brackets (0–1, 1–3, 3–7, 7–15, 15–18 and > 18 years of age), thereby demonstrating similar survival (data not shown). Although there were no differences in the presence of impaired glucose tolerance in patients older than 15 years of age (Table 2; data not shown for non-2184insA cases), the occurrence of CF liver disease (CFLD) was higher in patients with the 2184insA mutation (64.70% vs. 51.51%). We have also observed trends in the distribution of CF-specific infections: patients with the 2184insA mutation had a higher prevalence of *P. aeruginosa* (76.47% vs. 64.53%) and *S. aureus* (70.59% vs. 44.92%), but a lower rate of *H. influenzae* (11.76% vs. 44.94%) (Table 2). Although statistical calculations could not be performed due to the small sample size, based on the evidence presented this mutation can be regarded as “CF-causing”.

In conclusion, the 2184insA is the second most common mutation in WU. As large Ukrainian populations are also found outside of the country with approximate estimates of 4.4 million (M) in Russia, 2 M in USA, 1 M in Canada, 0.25 M in Argentina and 0.3 M in Romania, this mutation should be included in the CF testing panels in patients of Ukrainian origin. Finally, the achieved mutation detection rate of 83.71% fulfills the minimal pre-requisite [20] for the introduction of the “two-tier” (IRT/DNA) newborn screening programs which could alleviate current imbalances in the level of CF clinical diagnosis in other regions of Ukraine with substantial WU populace [1,2].

Table 2
Phenotype parameters of Western Ukrainian CF patients with the 2184insA mutation.

No of patients	2184insA/allele in trans	Current age, full years	Age at diagnosis, month	Sweat test (Cl, mmol/l)	Meconium ileus†	CFLD	Weight for height z-score	Fecal elastase-1 ug/g of stool	Impaired glucose tolerance	Infection				FVC		FEV ₁		Bronchiectases	Blood oxygenation (%)	Finger clubbing	Compliance with therapy
										S. aureus	P. aeruginosa	A. fumigatus	H. influenzae	Liter	% predicted	Liter	% predicted				
1.	2184insA	9	39	87	No	No	-2.21	3.3	No	1	1	0	1	1.8	109	1.6	115	Yes	98	Yes	Good
2.	2184insA	*	42	105	No	Fibrosis	-4.63	NA*		0	1	0	0	NA		NA		Yes	NA	Yes	Unsatisfactory*
3.	F508del	9	3	98	No	No	-1.38	23.1	No	1	1	0	0	NA		NA		Yes	97	Yes	Good
4.	F508del	12	11	104	No	Fibrosis	-2.16	11.3	Yes	1	1	0	0	1.3	100	1.2	106	Yes	99	Yes	Good
5.	F508del	13	60	94	No	fibrosis	-1.11	15.6	Yes	1	1	0	0	1.5	60	0.9	43	Yes	99	Yes	Good
6.	F508del	12	48	105	No	Fibrosis, hypoplasia of biliary ducts	-1.76	2.7	No	0	1	0	0	NA	40	NA	43	Yes	NA	Yes	Good
7.	F508del	6	8	100	No	Fibrosis, hypoplasia of biliary ducts	-1.83	1.9	No	1	1	0	0	y.a		y.a		x	94	Yes	Satisfactory
8.	CFTRdele 2,3 (21kb)	5	4	86	No	Fibrosis	-0.56	NA	NA	1	0	0	0	y.a		y.a		No	NA	No	Unsatisfactory
9.	F508del	7	5	106	No	Fibrosis	0.95	3.2	No	0	1	0	0	1.2	103	1.1	109	No	99	No	Satisfactory
10.	F508del	7	44	105	No	No	-1.48	17.4	No	1	1	0	0	1.0	100	1.0	114	Yes	95	Yes	Good
11.	F508del	14	21	103	No	Fibrosis		44.3	Yes	1	1	1	0	2.1	87	1.7	82	Yes	98	Yes	Unsatisfactory
12.	F508del	3	1	105	Yes	Hypoplasia of biliary ducts	-2.22	8.7	No	0	0	0	1	y.a		y.a		No	NA	No	Unknown
13.	F508del	2	6	105	No	No	-0.70	NA	No	1	1	0	0	y.a		y.a		No	92	No	Good
14.	F508del	6	66	97	No	Fibrosis	NA	NA	NA	1	1	0	0	y.a		NA		Yes	94	Yes	Satisfactory
15.	F508del	5	2	73	No	No	NA	NA	NA	0	0	0	0	y.a.		NA		No	NA	NA	Unknown **
16.	F508del	7	60	100	No	Cirrhosis	-1.96	432.1	No	1	0	0	0	1.0	90	1.0	103	Yes	99	Yes	Satisfactory
17.	R334W	27	5	95	No	No	-0.52	514.2	No	1	1	0	0	3.8	84	1.2	54	Yes	97	Yes	Good

Legend: * — died at age of 7 years; ** — lost from Lviv CF centre care; NA—not available or not performed due to young age (y.a); 1 — presence; 0 — absence; CFLD — Cystic Fibrosis liver disease; FVC — forced vital capacity, FEV₁ — forced expiratory volume at one second, including glucose tolerance test reflect 2009 measurements, bronchiectases were diagnosed by computed tomography.

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