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Journal of Cystic Fibrosis 10 (2011) 217 – 220

Journal of Cystic Fibrosis

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Short communication

Distribution of *CFTR* mutations in Eastern Hungarians: Relevance to genetic testing and to the introduction of newborn screening for cystic fibrosis ☆

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29 September 2010; 21 December 2010; 26 December 2010

Abstract

Background: The aim of this study was characterization of an updated distribution of *CFTR* mutations in a representative cohort of 40 CF patients with the classical form of the disease drawn from Eastern Hungary. Due to the homogeneity of the Hungarian population our data are generally applicable to other regions of the country, including the sizeable diaspora.
Methods: We utilized the recommended "cascade" *CFTR* mutation screening approach, initially using a commercial assay, followed by examination of the common "Slavic" deletion CFTRdele2,3(21 kb). Subsequently, the entire *CFTR* coding region of the CFTR gene was sequenced in patients with yet unidentified mutations.
Results: The Elucigene CF29[Tm] v2 assay detected 81.25% of all CF causing mutations. An addition of the CFTRdele2,3(21 kb) increased the mutation detection rate to 86.25%. DNA sequencing enabled us to identify mutations on 79/80 CF alleles. Mutations [CFTRdele2,3(21 kb), p.Gln685ThrfsX4 (2184insA) were found at an unusually high frequency, each comprising 5.00% of all CF alleles.
Conclusion: We have identified common CF causing mutations in the Hungarian population with the most common mutations (p.Phe508del, p.Asn1303Lys, CFTRdele2,3(21 kb), 2184insA, p.Gly542X, and p.Leu101X), comprising over 93.75% of all CF alleles. Obtained data are applicable to the improvement of DNA diagnostics in Hungary and beyond, and are the necessary prerequisite for the introduction of a nationwide "two tier" CF newborn screening program.
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Keywords: Cystic fibrosis; Hungary; Eastern Hungarians; 2184insA; CFTRdele2,3(21 kb); DNA diagnostics; Newborn screening

1. Introduction

More than 1600 sequence alterations have been reported in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene, among which less than 20 mutations display clear ethnic and geographic affiliation. To date, three previous publications regarding the prevalence of a limited number of *CFTR* mutations in Hungary [1–3] are available, however the most recent being from 1996.

The aim of this study is to provide updated data on the distribution of *CFTR* gene mutations in a representative cohort

Abbreviations: CF, cystic fibrosis; IRT, immunoreactive trypsinogen; MLPA, multiplex ligation-dependent probe amplification.

☆ This work has been presented in a poster form in the 33rd European Cystic Fibrosis Conference, Valencia, Spain, 16–19 June 2010. Ivady G, Madar L, Nagy B, Gonczi F, Macek M, Ajzner E, Gombos E, Kappelmayer J and Balogh I. Complete screening of CFTR gene mutations in cystic fibrosis patients from Eastern Hungary. J Cyst Fibros 2010; 9(S2):S5.

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doi:10.1016/j.jcf.2010.12.009

of Eastern Hungarian patients with the classical form of cystic fibrosis (CF). Due to the population homogeneity in Hungary our results can be of utility for the entire Hungarian population, including the sizeable (approximately 2 million) Hungarian diaspora. We hope that our data will enable development of a screening panel optimized for the CF mutation distribution and foster introduction of newborn screening.

2. Methods

Diagnosis of CF was established on the basis of standard consensus clinical and laboratory criteria [4]. Sweat chloride concentrations were measured using a Sweat Chek Conductivity Analyzer (Wescor, USA) and/or using the Sanasol Sweat Analyzer (Sanasol, Hungary). Altogether 40 unrelated CF patients were included (mean age±SD; 14.4±8.7 years). The geographical origin of studied patients and their families is shown in Fig. 1.

DNA isolation from blood leukocytes was performed using a commercial system (QIAgen Blood Mini Kit, Qiagen, Germany). Three different approaches, in accordance with updated European recommendations [5], were used for the identification of the common European CF-causing mutations: a/ initially Elucigene CF29[Tm] v2 Kit, Tepnel Diagnostics, UK was utilized, b/ followed by the examination of the common "Slavic" CFTRdele2,3(21 kb) deletion [6] and finally c/ patients with unidentified CF mutations were subjected to sequencing of the entire coding region of *CFTR* gene [7], except that for exon 6b modified primers were used — 6BF (5′-CTG TAC AGC GTC TGG CAC AT-3′) and 6BR (5′-CAA ACA TCA AAT ATG AGG TGG AA-3′). The Elucigene CF29[Tm] v2 Kit is capable of detecting the following mutations: p.Asp1152His (c.3454 G>C), c.1585-1 G>A, p.Gly542X (c.1624 G>T), p.Trp1282X (c.3846 G>A), p.Asn1303Lys (c.3909 C>G), p.Phe508del (c.1521_1523delCTT), c.3717+12191 C>T, p.Leu88IlefsX22 (c.262_263delTT), c.489+1 G>T, p.Ser1251Asn (c.3752 G>A), p.Gly551Asp (c.1652 G>A), p.Arg117His (c.350 G>A), p.Arg1162X (c.3484 C>T), p.Arg334Trp (c.1000 C>T), p.Ala455Glu (c.1364 C>A), p.Lys684SerfsX38 (c.2051_2052delAAinsG), p.Lys1177SerfsX15 (c. 3528delC), p.Phe316-LeufsX12 (c.948delT), p.Ile507del (c.1519_1521delATC), p.Arg347Pro (c.1040 G>C), p.Arg553X (c.1657 C>T), p.Glu60X (c.178 G>T), c.2988+1 G>A, c.2657+5 G>A, c.1766+1 G>A, c.579+1 G>T, p.Gly85Glu (c.254 G>A), c.p.Lys684AsnfsX38 (c.2052delA), and p.Arg560Thr (c.1679 G>C). For DNA sequencing the PCR products were cleaned using ultra-filtration micro-columns (Microcon YM-100, Millipore, USA). Purified PCR products were sequenced using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA). Removal of unincorporated nucleotides was performed using gel filtration (DyeEx Kit, Qiagen). Capillary electrophoresis was performed on the ABI Prism 310 Genetic Analyzer (Applied Biosystems). Intragenic *CFTR* rearrangements were examined by multiplex ligation-dependent probe amplification (MLPA)-SALSA MLPA KIT P091-B1 CFTR (MRC-Holland, The Netherlands). With regards to mutation nomenclature we used the commonly used "legacy name" followed by the currently recommended description in parentheses (Table 1).

3. Results

The mean sweat chloride concentration was 108 mmol/L, ranging 55–173 mmol/L (in line with the manufacturer's specifications), with all but one patient having concentrations over 60 mmol/L. With the Elucigene CF29[Tm] v2 assay we identified the p.Phe508del mutation on 56/80 CF alleles (70.00%), p.Asn1303Lys (4x; 5.00%), p.Gly542X (3x; 3.75%), 1717-1 G>A and pArg347Pro (1x each; 1.25%). The CFTRdele2,3(21 kb) mutation was present on 4 CF alleles (5.00%). Only one mutant allele could be detected in 11 samples. Therefore these were analyzed further by sequencing: in 4 patients the presence of p.Gln685ThrfsX4 (2184insA) (5.00%) was revealed. Two patients had the p.Leu101X (2.50%) mutation, while the remainder bore p.Gln220X, p.Ser466X, p.Tyr1092X, and p.Glu831X alleles (one each; 1.25%). In one patient, no other CF causing mutation was found.

Fig. 1. Origin of the patients included in this study.

Table 1
Comparison of *CFTR* mutation distribution in selected Central European populations.

CFTR mutation	Germany 1994	Romania 2008	Austria 1997	Slovakia 2008	Hungary 1992	This study
deltaF508 (c.1521_1523 delCTT)	72.0%	56.3%	74.6%	38.2%	64.3%	70.0%
G551D (c.1652 G>A)	1.0%	N/F	1.6%	N/F	N/F	N/F
R553X (c.1657 C>T)	2.3%	N/F	N/F	1.2%	2.4%	N/F
G542X (c.1624 G>T)	1.4%	3.9%	2.4%	2.4%	1.2%	3.75%
621+1 G>T (c.489+1 G>T)	0.1%	0.8%	N/F	N/F	N/F	N/F
1717-1 G>A (c.1585-1 G>A)	0.9%	N/F	0.8%	0.6%	1.2%	1.25%
W1282X (c.3846 G>A)	0.7%	2.3%	N/F	N/F	1.2%	N/F
N1303K (c.3909 C>G)	2.3%	0.8%	N/F	1.2%	1.2%	5.0%
R347P (c.1040 G>C)	1.6%	N/F	1.6%	1.2%	N/A	1.25%
CFTRdele2,3(21 kb)	1.5%[a]	1.6%	2.6%[a]	1.1%[a]	N/A	5.0%
2184insA (c.2052_2053 insA)	0.6%	N/F	N/F	2.4%	N/A	5.0%
L101X (c.302 T>G)	N/F	N/F	N/F	N/F	N/A	2.5%
Q220X (c.658 C>T)	N/F	N/F	N/F	N/F	N/A	1.25%
S466X (c.1397 C>G)	N/F	N/F	N/F	N/F	N/A	1.25%
E831X (c.2491 G>T)	N/F	N/F	N/F	0.6%	N/A	1.25%
Y1092X (c.3276 C>A)	0.3%	N/F	N/F	N/F	N/A	1.25%

Legend: data for Germany [8], Romania [9], Austria [10], Slovakia [11] and Hungary [3]; N/A: not analyzed; N/F: not found, [a]frequencies reported by Dork et al. in 2000 [6], mutations included in the Elucigene CF29 v2 assay are formatted in italics; the original "legacy name" is followed by the recommended mutation nomenclature [17].

In 12 out of the 19 compound heterozygote patients testing of the mutation phase was performed in their respective families, with all detected mutations present *in trans*. Only one tested case remained with p.Phe508del together with an unidentified allele *in trans,* with *CFTR* rearrangement analysis by MLPA being negative. The population distribution of mutations detected in this study was compared to German [8], Romanian [9], Austrian [10], Slovakian [11] and previous Hungarian [3] studies as shown in Table 1.

4. Discussion

Hereby, we present the first comprehensive study of *CFTR* mutation distribution in the Eastern Hungarian population which complements decade old partial studies of this subject [1–3]. Given the homogeneity of the general Hungarian population, based on previous population genomic studies showing relatively "small spread" within principle component-based analyses [12–15], data drawn from Eastern Hungary are very likely relevant for the remainder of the country. At present, there are approximately 10 million citizens living in Hungary. According to the Official Gazette of the Ministry of Health (2008) the prevalence for cystic fibrosis is 1:4000 in Hungary. The Eastern part of Hungary is neighboring Slovakia, Ukraine and Romania, with approximately 2 million inhabitants. Population admixture increased when subsequently Romanian shepherds, Flemmish and Slovakian settlers colonized this region. According to a local survey from 1910 the population reported to be of 54.5% Hungarian, 16.1% Romanian, 10.7% Slovakian, and 10.2% German origin, including several other minorities. However, Romani origin was not reported at that time. We use this survey for illustration, since self-reporting of ethnicity substantially changed during the last century, when over 94% of the inhabitants had declared to be of Hungarian origin in 2001.

In general the observed degree of mutation heterogeneity is between the reported Northern and Southern European mutation spectra [16], whereby all mutations were previously detected in South German, Ashkenazi Jewish and other Balkans populations [17] and filed in the Cystic Fibrosis Mutation Database [17]. Altogether six mutations reached a higher prevalence than 1.30%: p.Phe508del, p.Asn1303Lys, CFTRdele2,3(21 kb), 2184insA, p.Gly542X and p.Leu101X, in decreasing order of their frequencies. As the population under study is not primarily of Slavic origin, it is interesting, that the Slavic mutation CFTRdele2,3(21 kb) was found on 5.00% of CF alleles, which is the third highest prevalence after Czech Republic (6.37%) and Russia (5.69%) [6]. In this respect Eastern Hungary was formerly inhabited by Slavic tribes who later gradually assimilated with Hungarians (from 895 AD), which likely explains the high frequency of this allele.

The 2184insA frameshift mutation was found at a particularly high frequency (5.00%). In this regard a recent paper of Makukh et al. (2010) [18] reported that this allele is the second most common mutation in Western Ukraine, comprising 7.20% of all mutated CF alleles. Since Western Ukraine is bordering the area from which our cohort was drawn (Fig. 1.), this result shows population relatedness of both regions given their close long-term historical ties. Therefore, our data confirm the "Galician origin" of this mutation [18] given its decreasing gradient towards the region from which our patients were drawn. It will be of interest to study similar cohorts of CF patients in neighboring Eastern Slovakia, Southeastern Poland, Belarus and Northwestern Romania in order to further substantiate this likely regional founder effect.

For the analysis of mutations in the cohort under study we used the recommended "cascade approach" [5,19] analyzing common mutations first followed by sequencing and rearrangement analysis as specified in the Methods section. Thus, when using the Tepnel CF29[Tm] v2 assay 81.25% of CF-causing mutations

Table 2
Increasing detection rate using the cascade screening strategy in the Eastern Hungarian CF population.

	Detected CF alleles	
	Number	%
Elucigene CF 29^{Tm} v2 assay	65	81.25%
Elucigene CF 29 ^{Tm} v2 assay+CFTRdele2,3(21 kb)[a]	69	86.25%
Elucigene CF 29 ^{Tm} v2 assay+CFTRdele2, 3(21 kb)[a]+2184insA[b]+L101X[b]	75	93.75%
DNA sequencing of the remaining CFTR exons	79	98.75%

Legend:
[a]"Junction PCR" method [6].
[b]Identified by targeted sequencing of *CFTR* exons 13 and 4.

were identified. Incorporation of the CFTRdele2,3(21 kb) mutation increased the detection rate to 86.25%. Sequencing was essential for the detection of the unexpectedly highly prevalent mutation 2184insA (Table 2).

In total, we identified 98.75% of the CF causing mutations in Eastern Hungarians in line with other CF populations which were thoroughly investigated by the set of techniques utilized in this study [20] and where only patients with the classical form of the disease where included. In the case of the only remaining patient, the most likely explanation is that an intronic or promoter mutation which was not analyzed by our methods might be producing the null allele.

In summary, we present a *CFTR* mutation panel which could be used in DNA diagnostics in the entire Hungarian population. Moreover, since there is a substantial Hungarian diaspora of 2 millions [21] living in neighboring countries and North America, our data are pertinent to these populations as well. The observed mutation spectrum is in line with previous Central European studies [16]. Finally, our study will serve as the necessary prerequisite for the introduction of nation-wide "two tier" (IRT/DNA) newborn screening programs [22].

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgements

This work was supported by a MZOFNM2005, IGA NS9986-3 from the Czech Ministry of Health to MM Jr., by the TAMOP 4.2.1./B-091/1/KONV-2010-0007 project to IB.

References

[1] Nemeth K, Fekete G, Kiss E, Varadi A, Holics K, Ujhelyi R. Analysis of five CFTR mutations in Hungarian cystic fibrosis patients. J Inherit Metab Dis 1996;19(3):378.

[2] Nemeti M, Louie E, Papp Z, Johnson JP. Molecular analysis of cystic fibrosis in the Hungarian population. Hum Genet 1991;87(4):511–2.

[3] Nemeti M, Johnson JP, Papp Z, Louie E. The occurrence of various non-delta F508 CFTR gene mutations among Hungarian cystic fibrosis patients. Hum Genet 1992;89(2):245–6.

[4] Rosenstein BJ, Cutting GR. The diagnosis of cystic fibrosis: a consensus statement. Cystic Fibrosis Foundation Consensus Panel. J Pediatr 1998;132(4):589–95.

[5] Dequeker E, Stuhrmann M, Morris MA, Casals T, Castellani C, Claustres M, et al. Best practice guidelines for molecular genetic diagnosis of cystic fibrosis and CFTR-related disorders—updated European recommendations. Eur J Hum Genet 2009;17(1):51–65.

[6] Dork T, Macek Jr M, Mekus F, Tummler B, Tzountzouris J, Casals T, et al. Characterization of a novel 21-kb deletion, CFTRdele2, 3(21 kb), in the CFTR gene: a cystic fibrosis mutation of Slavic origin common in Central and East Europe. Hum Genet 2000;106(3):259–68.

[7] Zielenski J, Rozmahel R, Bozon D, Kerem B, Grzelczak Z, Riordan JR, et al. Genomic DNA sequence of the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Genomics 1991;10(1):214–28.

[8] Dork T, Mekus F, Schmidt K, Bosshammer J, Fislage R, Heuer T, et al. Detection of more than 50 different CFTR mutations in a large group of German cystic fibrosis patients. Hum Genet 1994;94(5):533–42.

[9] Frentescu L, Brownsell E, Hinks J, Malone G, Shaw H, Budisan L, et al. The study of cystic fibrosis transmembrane conductance regulator gene mutations in a group of patients from Romania. J Cyst Fibros 2008;7(5): 423–8.

[10] Stuhrmann M, Dork T, Fruhwirth M, Golla A, Skawran B, Antonin W, et al. Detection of 100% of the CFTR mutations in 63 CF families from Tyrol. Clin Genet 1997;52(4):240–6.

[11] Kolesar P, Minarik G, Baldovic M, Ficek A, Kovacs L, Kadasi L. Mutation analysis of the CFTR gene in Slovak cystic fibrosis patients by DHPLC and subsequent sequencing: identification of four novel mutations. Gen Physiol Biophys 2008;27:299–305.

[12] Heath SC, Gut IG, Brennan P, McKay JD, Bencko V, Fabianova E, et al. Investigation of the fine structure of European populations with applications to disease association studies. Eur J Hum Genet 2008;16 (12):1413–29.

[13] Lao O, Lu TT, Nothnagel M, Junge O, Freitag-Wolf S, Caliebe A, et al. Correlation between genetic and geographic structure in Europe. Curr Biol 2008;18(16):1241–8.

[14] Li W, Sun L, Corey M, Zou F, Lee S, Cojocaru A, et al. Understanding the population structure of North American patients with cystic fibrosis. Clin Genet. 2011;79(2):136–46.

[15] Nelis M, Esko T, Magi R, Zimprich F, Zimprich A, Toncheva D, et al. Genetic structure of Europeans: a view from the North-East. PLoS ONE 2009;4(5):e5472.

[16] Bobadilla JL, Macek Jr M, Fine JP, Farrell PM. Cystic fibrosis: a worldwide analysis of CFTR mutations—correlation with incidence data and application to screening. Hum Mutat 2002;19(6):575–606.

[17] Cystic fibrosis mutation database. http://www.genet.sickkids.on.ca/cftr/Home.

[18] Makukh H, Krenkova P, Tyrkus M, Bober L, Hancarova M, Hnateyko O, et al. A high frequency of the Cystic Fibrosis 2184insA mutation in Western Ukraine: genotype–phenotype correlations, relevance for newborn screening and genetic testing. J Cyst Fibros 2010;9(5):371–5.

[19] Castellani C, Cuppens H, Macek Jr M, Cassiman JJ, Kerem E, Durie P, et al. Consensus on the use and interpretation of cystic fibrosis mutation analysis in clinical practice. J Cyst Fibros 2008;7(3):179–96.

[20] Verlingue C, Kapranov NI, Mercier B, Ginter EK, Petrova NV, Audrezet MP, et al. Complete screening of mutations in the coding sequence of the CFTR gene in a sample of CF patients from Russia: identification of three novel alleles. Hum Mutat 1995;5(3):205–9.

[21] Csanyi B, Bogacsi-Szabo E, Tomory G, Czibula A, Priskin K, Csosz A, et al. Y-chromosome analysis of ancient Hungarian and two modern Hungarian-speaking populations from the Carpathian Basin. Ann Hum Genet 2008;72(4):519–34.

[22] Castellani C, Southern KW, Brownlee K, Dankert Roelse J, Duff A, Farrell M, et al. European best practice guidelines for cystic fibrosis neonatal screening. J Cyst Fibros 2009;8(3):153–73.