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Original Research Article

Specifics of cystic fibrosis genetic spectrum in Georgia

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ABSTRACT

Background: Cystic fibrosis (CF) is a life-threatening autosomal recessive disease caused by mutations in the cystic fibrosis transmembrane conductance regulator gene (CFTR). F508del is the most common mutation in the world. Other mutations are rare and population specific.
Aim: The study aimed to comprehensively analyze the distribution of CFTR mutations in Georgian CF patients. Knowing the prevalence and characteristics of specific mutations can be reflected in genetic counseling and management strategies.
Materials and Methods: We reviewed the data of 129 CF patients, aged < 18 years, from all parts of Georgia. 91 patients with a clinically confirmed CF diagnosis were tested for CF-causing mutations (constituting 70.5% of all currently registered CF patients in the country). These patients have been analyzed for rare CFTR variants by massively parallel sequencing of the entire CFTR coding region and adjacent introns combined with the analysis of intra-CFTR rearrangements.
Results: CFTR gene analysis revealed 29 mutations in Georgian CF patients. The most common mutation was c.1545_1546delTA (1677delTA) with a frequency of 42.7%, while the second most common mutation, W1282X, was detected in 11.2% of all CF alleles. Another 27 CFTR mutations have low frequency, including F508del (6.7% of alleles). 3 novel mutations were found (c.708dupT; CFTRdele16_17; c.3170C>G) and reported to CFTR2 database.
Conclusions: According to the data, the distribution of CFTR mutations in the Georgian CF population differs regarding the high frequency of mutation c.1545_1546delTA (1677delTA) and the low frequency of the predominant F508del mutation. Compared to patients with F508 del and W1282X mutations, patients with 1677delTA have typical manifestations and complications; however, the frequency of growth retardation and liver damage is 3 times, and the frequency of chronic respiratory manifestations and chronic malnutrition is 2 times lower, though pancreatic insufficiency is more severe in patients with 1677delTa. CF-associated diabetes, distal intestinal obstruction syndrome, and hemoptysis were only observed in patients with the 1677delTA mutation. The mortality rate is lower compared to patients with F508del and W1282X mutations.

1. Background

Cystic fibrosis is one of the most common life-threatening autosomal recessive diseases caused by a mutation in the

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cystic fibrosis trans membrane conductance regulator gene (CFTR). The estimated incidence among Caucasians is 1 in 2,000-4,000 births. More than 2000 mutations are identified. The most common mutation in the world is F508del. [1–3]

The frequency and spectrum of mutations can differ among countries, populations, and ethnic groups. Knowing the distribution of the CF-causing mutations among each population, especially with mixed ethnic groups, can be beneficial in developing diagnostic tools, disease management, and prenatal diagnosis, also developing a system to screen a panel of the most frequent mutations without sequencing the entire CFTR gene. [4–6]

The total population of Georgia in 2012 -2020 was about 3 716 000. There are three major ethnic groups: Georgians, Azerbaijanis, and Armenians. There is only 1 CF center, located in the capital city, Tbilisi. All diagnosed cases of CF from birth to 18 years are followed up at this center. The neonatal screening program was implemented in 2012 in Tbilisi only and has become nationwide since 2018.

This study aims to report the distribution of CF-causing mutations in a representative group of Georgian CF patients and assess the clinical course of the disease with different CFTR genotypes.

According to our information, no such study was ever performed.

2. Materials and Methods

The study cohort comprised 129 patients aged between 25 days and -17,38 years, originating from all parts of Georgia (119 Georgians, 4 Georgian Azerbaijanis, 6 Georgian Armenians) and regularly following up at the Georgian CF center. The diagnosis of CF was based on clinical and laboratory consensus diagnostic criteria. [7,8] Informed consent was obtained from every patient, their parents, or legal representatives. Blood samples of 91 patients were sent to the Department of Biology and Medical Genetics, University Hospital Motol Prague, Czech Republic. Complete analysis of the CFTR gene, coding region, including analysis in intra-CFTR rearrangements and of adjacent intronic sequences, was performed according to an established methodology. [9–13]

To assess the genotype-phenotype correlation from 91 consented Georgian CF patients, we collected the following parameters: age at diagnosis and at the last examination visit, sweat chloride test results, weight, height, Body mass index, and clinical information, including the pancreatic sufficiency, lung function, thyroid function, chronic colonization by P. aureginosa and S. Aureus, complications (liver disease, meconium ileus, CF–related diabetes).

3. Results

Over the period from 2012 to 2020, a total of 129 patients were registered at the Georgian Cystic Fibrosis Center. Among these, CF was confirmed in 34 children through positive neonatal screening. The age at diagnosis ranged from a minimum of 28 days to a maximum of 143 days, with the mean age for diagnosis being 51 days. The prevalence of CF in Georgia, estimated as 1 in 4000 births, underscores the significance of this genetic disorder within the country. (Data is taken from the National Statistics Office of Georgia).

For the first time in Georgia, our study analyzed the genetic mutations in 129 CF patients, <18 yerasold from all parts of Georgia. 91 clinically confirmed CF patients, revealing a total of 29 CF-causing mutations. (see the table) The most prevalent mutations in Georgian CF patients were 1677delTA (42.7%), W1282X (11.2%), F508del (6.7%), 3120+1G>A (4.5%), D11OH (3.4%), 3199del6/I148T (2.8%), E92K (2.2%), and G542X (1.7%). Notably, the 1677delTA mutation accounted for 42.7% of detected mutations.

Among the patients, 22 (34.8%) were found to be homozygous for the 1677delTA mutation, while 32 were heterozygous.

Our study identified three novel CF-causing mutations (c708dupT, CFTRdele16_17, and c3170C>G) not previously documented in CF mutation databases.Following mutations were added to the CFTR2 database. The cFTRdele16-17 mutation was found in two patients of Armenian origin from the same family. The patient's clinical course with a combination of CFTRdele16_17 and 2183delAA>G mutations was very severe. A lethal outcome was detected in one case.

Our research provides a foundation for the development of a national mutation panel for Georgia. We propose a basic mutation panel consisting of 14 mutations with a prevalence exceeding 1% in our analyzed data, and an extended panel comprising all 29 mutations identified in Georgian CF patients. These panels will support population-based CF screening and more targeted treatment strategies.

3.1. Basic Mutation panel (14 mutations)

1667delTA; W1282X; F508del; 3120+1G>A; D11OH; 3199del6/I148T; E92K; G542X; 2183delAA>G; c.3185T>A ; G1069R; I1234V; cFTRdele16-17; 1248+1G>A.

3.2. Additional mutations for extended panel (15 mutations)

N1303K; 3170C>G; 2789+5G->A; 3821delT; CFTRdel2.3(21kb); E94K; H1054D; L997F; R1158; 1716+1G->A; c.708dupT; I148T; M470V; N1303K; E831X.

Table 1: Allelic frequencies of the 21 most common mutations in Gergian CF patients

cDNA	Protein Name	Legacy Name	Alleles-Frequency, no(%)
c. 1545_1546delTA	p.Tyr515X	1677delTA	76 (42.7)
c.3846G>A	p. Trp1282X	W1282X	20 (11.2)
c.1521_1523delCTT	p.Phe508del	[delta]F508	12 (6.7)
c.2988+1G>A	-	3120+ 1G- >A	8 (4.5)
c.328G>C	p.Asp110His	D110H	6 (3.4)
c.3067_3072delATAGTG	p.Ile1023_Val1024del	3199del6	5 (2.8)
c.274G>A	p.Glu92Lys	E92K	4 (2.2)
c.1624G>T	p.Gly542X	G542X	3 (1.7)
-	-	2183delAA>G	2 (1.1)
c.3185T>A	p.Leu1062Gln	-	2 (1.1)
c.3205G>A	p.Gly1069Arg	G1069R	2 (1.1)
c.3700A>G	p.Ile1234Val	I1234V	2 (1.1)
-	-	CFTRdele16- 17b	2 (1.1)
c.1116+1G>A	-	1248+ 1G- >A	2 (1.1)
c.3909C>G	p.Asn1303Lys	N1303K	1 (0.55)
c.54-5940_273+10250del21kb	-	CFTRdele2,3	1 (0.55)
c.1408A>G	p.Met470Val	M470V	1(0.55)
c.2657+5G>A	-	2789+5G->A	1 (0.55)
c.3691delT	p.Ser1231ProfsX4	3821delT	1 (0.55)
c.274G>T	p.Glu92X	E92X	1 (0.55)
c.3160C>G	p.His1054Asp	H1054D	1 (0.55)
c.2991G>C	p.Leu997Phe	L997F	1 (0.55)
c.3472C>T	p.Arg1158X	R1158X	1 (0.55)
c.1584+1G>A	-	1716+1G->A	1 (0.55)
c.3170C >G	-	-	1 (0.55)
c.708dupT	p.Gln237SerfsX21	-	1 (0.55)
c.2491G>T	p.Glu831X	E831X	1 (0.55)
c.443T>C	p.Ile148Thr,	I148T	1 (0.55)
Unidentified			2 (1.1)

Table 2: Total number of deaths.genetic characteristics.

N	Age at diagnosis	Age at death	Gender	Sweat Chloride	Mutation 1	Mutation 2
1	3 yr	13yr	M	86mmol/l	W1282X	W1282X
2	7mo	3 yr	M	97mmol/l	-	-
3	3 yr	10yr	M	116mmol/l	F508	W1282X
4	7 mo	13yr	F	108mmol/l	W1282X	W1282X
5	11 yr	15yr	M	115mmol/l	CFTRdele2,3	F508
6	2 mo	15yr	F	124 mmol/l	3199del6/I148T	1677delTA
7	3 yr	18 yr	F	90mmol/l	2183delAA>G	CFTRdel16-17
8	6 mo	17 yr	F	-	1677delTA	unidentified

We attempted to correlate genetic and clinical characteristics in Georgian CF patients, with a focus on pancreatic failure, liver damage, meconium ileus, and distal intestinal obstruction syndrome. Genotype-phenotype correlations were established in the context of exocrine pancreatic function. Notably, 70% of individuals with pancreatic insufficiency(56 patients) had mutations in classes I, II, and III, with 57% having the 1677delTA mutation.[14–16]

The majority of CF patients in our study presented with CF-related pulmonary symptoms. Pseudomonas aeruginosa was isolated in 18.9% of patients, while sputum cultures were positive for Staphylococcus aureus in 35.7%.

The 1677delTA mutation emerged as a leading cause of liver damage in Georgian CF patients. Additionally, this mutation was associated with meconium ileus (12 patients) and distal intestinal obstruction (4 patients) in most cases, with four individuals being homozygous for this mutation.

A total of 8 children died from complications of CF from 2012-2020. Most of the patients had the W1282X mutation. Nearly all of them died at the age of 13-18 years.

4. Discussion

Our study presents a significant contribution to the understanding of cystic fibrosis (CF) in Georgia, as it is the first to report on the CFTR mutation distribution in a representative cohort of 91 Georgian CF patients. Our findings reveal several important insights into the genetic and clinical aspects of CF in this population.

Our study highlights that eight mutations with a prevalence greater than 1.5% play a crucial role in Georgian CF patients, among which 1677delTA is the most prevalent. The cDNA name for this mutation is c1545_c1546delTA, protein p.Tyr515X, it is located on the 11th exon. The mutation causes cystic fibrosis in the homozygous state and in combination with other mutations.[7] This mutation is unique in its distribution, being practically absent in the American continent and rare in Northern and Central European countries. In 2011, only 32 patients with such a mutation were recorded in mutation database.

Our research confirms that 1677delTA is the leading mutation in Georgia and neighboring countries, including Turkey, Cyprus, Greece, Bulgaria, the Russian Federation (particularly the northern Caucasian region), Armenia, and Iran.[17–21] The concentration of this mutation is found the most in the southern regions of the Russian Federation, especially Chechnya.[22–25]

Our research confirmed that 1677delTa is the leading mutation for the population of Georgia. A high prevalence of the 1677delta in Georgia and neighboring countries could be associated with the origin of mutation from the Caucasus region.[26]

Our study not only identified the high prevalence of the 1677delTA mutation but also highlighted its clinical significance. Patients with this mutation exhibited a rather severe disease course, resembling the typical manifestations and complications of CF. However, some distinct characteristics were observed, including a higher frequency of growth retardation and liver damage, as well as a lower frequency of chronic respiratory manifestations and chronic malnutrition. Notably, pancreatic insufficiency was more severe in individuals with the 1677delTA mutation.[27,28] Moreover, CF-associated diabetes, distal intestinal obstruction syndrome, and hemoptysis were exclusively observed in individuals with this mutation. Despite these severe clinical manifestations, the mortality rate was lower compared to patients with the F508del and W1282X mutations.[29,30]

We identified three novel mutations (c708dupT, CFTRdele16_17, and c3170C>G) not previously documented in CF mutation databases. These mutations have been added to the CFTR2 database, contributing to the global understanding of CF mutations and their implications.

5. Conclusion

According to the results, the distribution of CFTR mutations in Georgia CF patients differs from neigbouring counties, regarding the high frequency of mutation c. 1545-1546delTa (1677delTA). Our study documented a higher frequency of pancreatic insufficiency, liver damage, meconial ileus, and intestinal obstruction in patients with this mutation, than the general CF patient population. . The identification of common mutations and the discovery of novel mutations lay the foundation for improved diagnosis and management of CF in the country.

We hope this study can play a role in the future and provide a basis for recommendations on testing, will contribute to the development of a more targeted and comprehensive mutation panel and our own national diagnostic algorithm, and implementing variant-specific CF therapies in the future.

6. Conflict of Interest

The authors declare no conflict of interest.

7. Source of Funding

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References

1. Boyle MP. Nonclassic cystic fibrosis and CFTR-related diseases. *Curr Opin Pulm Med*. 2003;9(6):498–503.
2. Mehta G, Macek M, Mehta A. Cystic fibrosis across Europe: EuroCareCF analysis of demographic data from 35 countries. *J Cyst Fibros*. 2001;9(Suppl 2):5–21.
3. Farrell PM, White TB, Ren CL. Diagnosis of Cystic Fibrosis: Consensus Guidelines from the Cystic Fibrosis Foundation. *J Pediatr*. 2017;181:4–15. doi:10.1016/j.jpeds.2016.09.064.
4. European Cystic fibrosis Society Patient Registry Annual data report 2020 . [September 23, 2021]. Available from: https://www.ecff.ou/projects/ecfs-patient-registry/project.
5. Levy H, Farrel PM. New challenges in diagnosis and management of cystic fibrosis. *J Pediatr*. 2015;166(6):1337–41.
6. Smyth AR, Bell SC, Bojcin S, Bryon M, Duff A, Flume P, et al. Europe Cystic Fibrosis Society Standards of Care: best Practice Guidelines. *J Cyst Fibros*. 2014;13(Suppl 1):23–42.
7. CFTR2 project (Clinical and Functional Translation of CFTR) 2020 . Available from: http://www.cftr2.org/index.phpCFTR2project.
8. Farrell PM, Farrel TB, Ren CL, Hempstead SE, Accurso F, Derichs N, et al. Diagnosis of Cystic Fibrosis: Consensus Guidelines from the Cystic Fibrosis Foundation. *J Pediatrics*. 2017;181:4–15. doi:10.1016/j.jpeds.2016.09.064.
9. 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_updatedv European recommendations. *Eur J Hum Genet*. 2009;17(1):51–65.
10. Vallieres E, Elborn L. Cystic fibrosis gene mutations: evaluation and assessment of disease severity. *JCyst Fibr*. 2014;4:161–72. doi:10.2147/AGG.S53768.

11. Villanueva C, Marceniuk G, St, Murphy. Cystic Fibrosis: diagnosis and management. *BMJ*. 2017;359:1–7.
12. Moskowitz SM, Chmiel JF, Sternen DL, Cheng E, Gibson RL, Marshall SG, et al. Clinical practice and genetic counseling for cystic fibrosis and CFTR-related disorders. *Genet Med*. 2008;10(12):851–68.
13. Levy H, Farrell PM. New challenges in the diagnosis and management of cystic fibrosis. *J Pediatr*. 2015;166(6):1337–41.
14. Castellani C, Cuppens H, Macek 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.
15. Salvatore D, Buzetti R, Baldo E, Forneris MP, Lucidi V, Manunza D, et al. An overview of international literature from cystic fibrosis registries. Part 3. Disease incidence, genotype/phenotype correlation, microbiology, pregnancy, clinical complications, lung transplantation, and miscellanea. *J Cyst Fibros*. 2011;10(2):71–85.
16. Boeck KD, Weren M, Proesmans M, Kerem E. Pancreatitis among patients with cystic fibrosis: correlation with pancreatic status and genotype. *Pediatrics*. 2005;115(4):463–9.
17. Onay T, Zielenski J, Topaloglu O, Gokgoz N, Kayserili H, Apak MY, et al. Cystic fibrosis mutations and associated haplotypes in Turkish cystic fibrosis patients. *Hum Biol*. 2001;73(2):191–203.
18. Hangul M, Pekcan S, Kose M, Acıcan D, Şahlar T, Erdoğan M, et al. The incidence of Cystic Fibrosis in the Central Region of Anatolia in Turkey Between. *Balkan Med J*. 2015;36(3):179–83.
19. Alibakhshi R, Kianishirazi R, Cassiman JJ, Zamani M, Cuppens H. Analysis of the CFTR gene in Iranian cystic fibrosis patients: identification of eight novel mutations. *J Cyst Fibros*. 2008;7(2):102–9.
20. Bonyadi M, Omrani O, Rafee M, Bilan N. Spectrum of CFTR gene mutations in Iranian Azeri Turkish patients with Cystic Fibrosis. Geneting testing and Molecular. *Genet Test Mol Biomarkers*. 2010;15(1-2):89–92.
21. Bonyadi M, Rafeey M, Vahedi A, Vahed L. Genetic pattern of cystic fibrosis patients in Azeri Turkish population. *Russian Open Med J*. 2017;1:e0101. doi:10.15275/rusomj.2017.0101.
22. Petrova N, Bainova N, Marakhonov A. Ethnic Differences in the Frequency of CFTR Gene Mutations in Populations of the European and North Caucasian Part of the Russian Federation. *Front Genet*. 2021;p. 678374. doi:10.3389/fgene.2021.678374.
23. Petrova N, Kashirskaya N, Saydaeva D. Spectrum of CFTR mutations in Chechen cystic fibrosis patients: high frequency of c1545-1546 delTA (1677delTA) and c274G>A (E92K)mutations in North Caucasus. *BMC Med Genet*. 2019;20(1). doi:10.1186/s12881-019-0785-z.
24. Petrova NV, Kashirskaya NY, Vasilyeva TA, Kondratyeva E, Zhekaite EK, Voronkova AY, et al. Analysis of CFTR Mutation Spectrum in Etnic Russion Cystic Fibrosis Patients. *Genes (Basel)*. 2020;11(5):554. doi:10.3390/genes11050554.
25. Stepanova A, Krakovsky S, Polyakov A. Reliability of the Search for 19 Common Mutations in the CFTR Gene in Russian Cystic Fibrosis Patients and the Calculated Frequency of the Disease in Russian Federation. *Russion J Gnetics* . 2016;52(2):204–13.
26. Gorinova Y, Savostyanov K, Pushkov A, Nikitin A, Pen'kov EL, Krasovskiy SA, et al. Genotype-Phenotype Correlations of the Course of Cystic Fibrosis in Russian Children. *Current Pediatrics*. 2018;17(1):61–9.
27. Rueda-Nieto S, Mondejar-Lipez P, Mira-Escolano M, Cutillas-Tolín A, Maceda-Roldán L, Arense-Gonzalo JJ, et al. Analysis of the genotype profile and its relationship with the clinical manifestations in people with cystic fibrosis: study from a rare disease registry. *Orphanet J Rare Dis*. 2022;17(1):222. doi:10.1186/s13023-022-02373-y.
28. Salvatore D, Buzetti R, Baldo E, Forneris MP, Lucidi V, Manunza D, et al. An overview of international literature from cystic fibrosis registries. Part 3. Disease incidence, genotype/phenotype correlation, microbiology, pregnancy, clinical complications, lung transplantation, and miscellanea. *J Cyst Fibros*. 2011;10(2):71–85.
29. Vallieres E, Elborn L. Cystic fibrosis gene mutations: evaluation and assessment of disease severity. *JCyst Fibr*. 2014;4:161–172.
30. Cutting GR. Cystic fibrosis genetics: from molecular understanding to clinical application. *Nat Rev Genet*. 2015;16(1):45–56.

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