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The Most Common Mutations in the CFTR Gene in the Population of Bosnia and Herzegovina

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Cystic fibrosis is an autosomal recessive multisystem disease caused by a mutation of the CFTR gene. To date, more than 1900 mutations of this gene are known. Studies have shown that the most common mutation is delF508. In Bosnia and Herzegovina, the prevalence of individual mutations in the general population has not been thoroughly studied, so this study aimed to determine the prevalence of the mutation concerning the countries of the region and the rest of the world.
Study Design: Retrospective study.
Place and Duration of Study: Thirty-nine subjects with suspected Cystic fibrosis were referred to the Center for Genetics of the Medical Faculty in Sarajevo between 2018-2020.

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Methodology: 29 common CFTR gene mutations were analysed with the ELUCIGENE CF29 v2 kit (Elucigene Diagnostics, UK) using four multiplex PCR.
Results: The most common mutation in our study was the F508 deletion, present in 14 subjects (73.68%). R347P and G542X mutations were confirmed in two subjects in the heterozygous state in combination with delF508 (M) 5.26% of each of these mutations. 621+1G>T was found in a homozygous state in one subject, while in another, it was in a heterozygous state in combination with delF508(M) mutation, 10.52%. Mutation 2184 delA was found in one subject in the homozygous state with a total frequency of 5.26%.
Conclusion: Subjects with cystic fibrosis in Bosnia and Herzegovina are most often carriers of the delF508 mutation. Considering the existence of many mutations and that it is difficult to test them all, targeting the most common mutations in a clinical environment might help in approving therapy, and increasing patients' quality of life.

Keywords: Cystic fibrosis; mutation F508; heterozygous; homozygous.

1. INTRODUCTION

Cystic fibrosis (CF) is a genetic multisystem disease that predominantly affects the lungs and other organs such as the pancreas, liver, intestines, etc.

CF is caused by a mutation in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) protein, which spans the cell membrane. CFTR mainly functions as a chloride channel. Mutations in the CFTR protein prevent the transport of chloride ions, which leads to the accumulation of chloride ions and associated water molecules in epithelial cells and the lack of hydration of extracellular mucus and secretions [1]. Increased viscosity of mucus secretion affects the respiratory tract, pancreas, liver and intestines, which is associated with high morbidity and reduced life expectancy [2].

Cystic fibrosis is inherited in an autosomal recessive manner. The gene that causes cystic fibrosis was discovered in 1989 and is located on chromosome 7, at position 7q13. More than 1900 mutations of this gene have been defined to date, of which 1500 lead to the manifest clinical presence of cystic fibrosis [3,4]. Mutations that cause CF are usually divided into six classes based on their effect on protein production, namely, class one (defective synthesis), class two (defective processing and maturation), class three (defective channel regulation), class 4 (reduced channel conductance), class 5 (reduced amount of CFTR protein with normal function) and class 6 (decreased CFTR stability) [5]. Some scientists have also proposed a seventh class for separate consideration of large deletions that can abrogate CFTR mRNA production [6]. By far, the most common, and also the first identified mutation, is the deletion of codon 508, which leads to the loss of phenylalanine from the amino acid sequence and belongs to class two [7]. The discovery of the gene responsible for CF and many mutations improved the diagnosis and treatment of subjects with CF. They opened the way to new therapeutic approaches and the emergence of targeted therapies. Thanks to this, the number of adult CF subjects exceeds that of children in developed countries, and the estimated median survival age is nearly 50 years [8].

2. MATERIALS AND METHODS

The research included 39 people suspected of cystic fibrosis, who were referred for molecular analysis to the Center for Genetics of the Faculty of Medicine from 2017 to 2020. All relevant data on the subjects are in the appropriate protocols of the Center.

Taking the sample was preceded by information about the goals and diagnostic potential of the analysis and the signing of the voluntary consent of the subjects that the results, without revealing the identity of the issues, can be used for scientific purposes and published. Along with each biological sample, the respondents also filled out questionnaires with appropriate questions relevant to the analysis.

Genomic DNA was extracted from 200 µL of whole blood using QIAamp DNA Mini Kit (*QIAGEN GmbH*, Germany). We employed the protocol for purifying total DNA from whole blood using a microcentrifuge [9]. The concentration of the obtained DNA was determined by Qubit 3.0 Fluorometer (*ThermoScientific*, USA). Twenty-nine common CFTR gene mutations (D1152H, 1717-1G>A, G542X, W1282X, N1303K, ΔF508, 3849+10kbC>T, 394delTT, 621+1G>T, S1251N,

G551D, R117H, R1162X, R334W, A455E, 2183AA>G, 3659delC, 1078delT, ΔI507, R347P, R553X, E60X, 3120+1G>A, 2789+5G>A, 1898+1G>A, 711+1G>T, G85E, 2184delA, and R560T) were analysed with the ELUCIGENE CF29 v2 kit (Elucigene Diagnostics, UK) using four multiplex PCR. The thermal condition of PCR were 35 cycles of a Chain reaction of denaturation step at 94^0C for 30 s, annealing at 58^0C for 120 sec and elongation at 72^0C for 60 s, after a single initial denaturation step at 95^0C for 5 min and followed by a single step at 72^0C for 5 min. Electrophoresis of the PCR product was performed in a 3% agarose gel with ethidium bromide (0.1 mg/ml) at 80 V and 2 A. Visualisation was performed in a UV transilluminator (*Uvitec*, Cambridge).

3. RESULTS

From analysed 39 samples for CFTR gene mutation we confirmed it in 16 subjects. Out of 16 subjects with a mutation, 12 (75%) were younger than 18, while four (25%) were older than 18. Important information is that this study included a four-member family (husband, wife, and two children), where the model of autosomal recessive inheritance of cystic fibrosis was shown as an example.

Analysis of DNA material from 16 subjects revealed the presence of five different mutations in the CFTR gene and their haplotypes. Table 1 shows the percentage of detected mutations, with the most prevalent mutation being delF508, found in 14 subjects (*73.68*%). Six of the subjects presented in the homozygous state (delF508 (M)), and eight of them in the heterozygous state (delF508 (M)/ delF508 (N)) (Fig. 1). R347P and G542X mutations were confirmed in two subjects in the heterozygous state in combination with delF508 (M). Two subjects had 621+1G>T, one in the homozygous while the other in heterozygous state in combination with the delF508(M) mutation (5.26% respectively). The fifth mutation, 2184 delA, was found in one subject in the homozygous state.

Table 1. Prevalence of CFTR gene mutations in 16 subject

Mutation	Homozygous	Heterozygous	Prevalence % (n=19)
delF508	6	8	73.68
R347P / delF508	/	1	5.26
G542X / delF508	/	1	5.26
621+1G>T	1		5.26
621+1G>T / delF508		1	5.26
2184 delA	1	/	5.26
			Total: 100

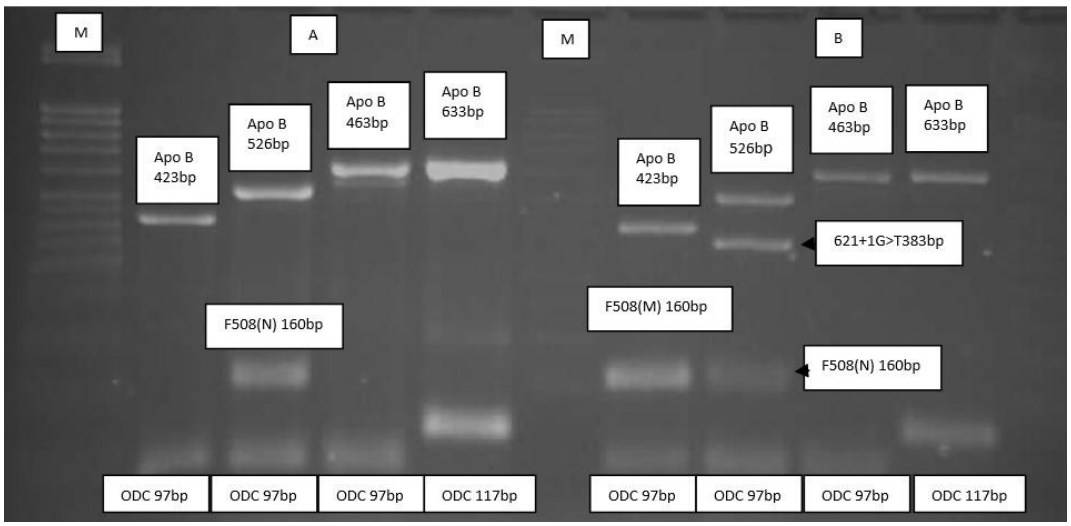

Fig. 1. M- molecular weight marker (50 bp ladder); A- patient with normal ΔF508(N) allele; B- patient with heterozygous for the mutation ΔF508(M) allele and the 621+1G>T383 mutation; ApoB-Upper control; ODC-lower control

4. DISCUSSION

Cystic fibrosis is a complex disease; therefore, the various symptoms and severity can vary from person to person. Many factors (genetics, age at CF diagnosis, environment, diet) can also affect a person's health and disease course.

The exact incidence of cystic fibrosis in Bosnia and Herzegovina is unknown since no national registry of subjects exists. Accordingly, numerous epidemiological data regarding this disease in our country are unknown. To expand the data on the frequency and distribution of mutations of the CFTR gene in our country, we analysed 39 subjects, 16 of whom had a proven mutation of the CFTR gene. We have not found a similar study in the PubMed database that refers to the population of Bosnia and Herzegovina at the time of this paper's publication. Five mutations were found, with different percentages. The most common mutation in our study was the F508 deletion, presented in 14 subjects (73,68%).

These results are in coordination with the studies done in the region. The prevalence of F508 mutation in the CFTR gene is about 70% which is proven in further studies: 72.28% in Serbia and Montenegro [10], 58.33% in Croatia [11], and 62.7-68.6 in Slovenia [12]. Globally, some countries, such as Denmark, show a higher rate of this mutation presence (87.2 %). In contrast, some countries have a significantly lower percentage of this mutation (e.g. Algeria, with the world's lower presentation of 26.3%) [13]. R347P and G542X mutations were confirmed in two subjects in the heterozygous state (together with delF508), a percentage of 5.26% for each mentioned mutation. R347P is a missense with an overall worldwide frequency of about 0.2%. The subjects described initially with this mutation were compound heterozygotes with the delta F508 mutation and had a very mild course of CF, suggesting that R347P, like other missense mutations, causes a mild phenotype. However, severe cases of carriers of this mutation have also been described [14]. G542X, one of the most common mutations in European populations (2.6%), was detected in 6.1% of CF alleles in Mediterranean countries and is found in the region's countries [10]. 621+1G>T was found in a single subject in the homozygous state and another subject in the heterozygous state with the delF508 mutation, a percentage of 10.52%. The 621+1G>T mutation has an average frequency of 0.54% in the European population, being the most common in central Greece (5.72%) [15].

2184 delA mutations were found in one subject in the homozygous state, with a percentage of 5.26%. Since the 2184 insA mutation is not included in standard commercial tests, routine DNA diagnostics have not identified this allele. However, some targeted studies have shown a high frequency of this mutation in certain countries, such as Ukraine [16]. In the results tabular presentation of our research, one family was included, for which the analysis determined that the mother and father were heterozygous for delF508, and two male children, one of whom was homozygous and possessed only F508(M), and the other was heterozygous and had F508 (N). This data shows the autosomal recessive mode of inheritance of cystic fibrosis. The sample of 39 subjects included in this study is relatively small. It would be preferable to use a larger number of subjects to obtain more accurate statistical results. This small sample may limit the generalizability of our findings to the entire cystic fibrosis subject population. According to research by Moonesinghe et al. [17] and Alice et al. [18], small samples can lead to unreliable results and wrong conclusions. They emphasise expanding the study to more subjects to provide more accurate statistics.

More accurate and better data on different mutations of the CFTR gene in the population lead to a better understanding of phenotypic variability and, thus, significant progress in the diagnosis and treatment of subjects with CF. Today's CFTR modulator therapies have shifted from medicines that treat symptoms to therapies that also restore the function of the CFTR protein [19]. By doing so, they enabled a better and better quality of life for people with CF. Some of these therapies, which use monoclonal antibodies [20], and the CRISP method [21,22], are clinical, and some are in preclinical development [23].

5. CONCLUSION

According to our results, subjects with cystic fibrosis in Bosnia and Herzegovina are most often carriers of the delF508 mutation, in line with neighbouring countries. Including all subjects suspected of CF in a study is necessary to obtain accurate results on prevalence in Bosnia and Herzegovina.

More reliable results and a better understanding of the disease's cellular and molecular basis will

enable the development of more effective therapy adapted to specific mutations and the quality of life of CF subjects in this population.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Chen Q, Shen Y, Zheng J. A review of cystic fibrosis: Basic and clinical aspects. Animal Model Exp Med. 2021;4(3): 220–32.
2. Modaresi MR, Faghinia J, Reisi M, Keivanfar M, Navaie S, Seyyedi J, et al. Cystic fibrosis prevalence among a group of high-risk children in the main referral children hospital in Iran. J Educ Health Promot. 2017;6:54.
3. Al-Abadi B, Al-Hiary M, Khasawneh R, Al-Momani A, Bani-Salameh A, Al-Saeidat S, et al. Cystic fibrosis gene mutation frequency among a group of suspected children in king hussein medical center. Med Arch. 2019;73(2):118–20.
4. Bepari KK, Malakar AK, Paul P, Halder B, Chakraborty S. Allele frequency for cystic fibrosis in Indians vis-a/-vis global populations. Bioinformation. 2015;11(7): 348–52.
5. Deletang K, Taulan-Cadars M. Splicing mutations in the CFTR gene as therapeutic targets. Gene Ther. 2022;29:399– 406.
6. Santos L, Nascimento R, Duarte A. et al. Mutation-class dependent signatures outweigh disease- associated processes in cystic fibrosis cells. Cell Biosci. 2023; 13:26.
7. De Boeck K, Zolin A, Cuppens H, Olesen HV, Viviani L. The relative frequency of CFTR mutation classes in European subjects with cystic fibrosis. Journal of Cystic Fibrosis. 2014 ;13(4):403–409.
8. Scotet V, L'Hostis C, Férec C. The changing epidemiology of cystic fibrosis: Incidence, survival and impact of the CFTR gene discovery. Genes (Basel). 2020;11(6):589.
9. QIAamp DNA Mini Kit: For DNA Purification from Lymphocytes;2016.
10. Radivojevic D, Djurisic M, Lalic T, Guc-Scekic M, Savic J, Minic P, et al. Spectrum of cystic fibrosis mutations in Serbia and Montenegro and strategy for prenatal diagnosis. Genet Test. 2004;8(3):276–80.
11. Knezević J, Tanacković G, Matijević T, Barisić I, Pavelić J. Analysis of cystic fibrosis gene mutations and associated haplotypes in the Croatian population. Genet Test. 2007;11(2):133–8.
12. Vouk K, Strmecki L, Liovic M, Kopriva S, Micetic-Turk D, Komel R. Mutational analysis of 30 Slovenian cystic fibrosis subjects compared to known Slovenian and European CF mutation spectra. Pflugers Arch. 2000;439(3 Suppl):R63-65.
13. Estivill X, Bancells C, Ramos C. Geographic distribution and regional origin of 272 cystic fibrosis mutations in European populations. The Biomed CF Mutation Analysis Consortium. Hum Mutat. 1997;10(2):135–54.
14. Varon R, Stuhrmann M, Macek M, Kufardjieva A, Angelicheva D, Magdorf K, et al. Pancreatic insufficiency and pulmonary disease in German and Slavic cystic fibrosis subjects with the R347P mutation. Hum Mutat. 1995;6(3):219–25.
15. Kanavakis E, Efthymiadou A, Strofalis S, Doudounakis S, Traeger-Synodinos J, Tzetis M. Cystic fibrosis in Greece: molecular diagnosis, haplotypes, prenatal diagnosis and carrier identification amongst high-risk individuals. Clin Genet. 2003;63(5):400–9.
16. Makukh H, Křenková P, Tyrkus M, Bober L, Hančárová 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. Journal of Cystic Fibrosis. 2010;9(5): 371–5.
17. Moonesinghe R, Yang Q, Khoury MJ. Sample size requirements to detect the effect of a group of genetic variants in case-control studies. Emerg Themes Epidemiol. 2008;(3)5:24.
18. Alice S. Whittemore , Lorene M. Nelson. Study Design in Genetic Epidemiology: Theoretical and Practical Considerations. JNCI Monographs. 1999;1999(26): 61–69.

19. Lopes-Pacheco M. CFTR Modulators: The Changing Face of Cystic Fibrosis in the Era of Precision Medicine. Front. Pharmacol. 2020;10:1662.
20. Moni SS, Al Basheer A. Molecular targets for cystic fibrosis and therapeutic potential of monoclonal antibodies. Saudi Pharm J. 2022;30(12):1736-1747. DOI:10.1016
21. Lee JA, Cho A, Huang EN. et al. Gene therapy for cystic fibrosis: new tools for precision medicine. J Transl Med. 2021; 19: 452 Available: https://doi.org/10.1186/s12967-021-03099-4
22. Sanchez SA, Paunovska K, Cristian A, Dahlman EJ.Treating Cystic fibrosis with mRNA and CRISPR. Human Gene Therapy. 2020;31(17):940-955.
23. Pranke I, Golec A, Hinzpeter A, Edelman A, Sermet-Gaudelus I. Emerging Therapeutic Approaches for Cystic Fibrosis. From Gene Editing to Personalised Medicine. Front Pharmacol. 2019;27;10:121. DOI: 10.3389/fphar.2019.00121

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