RESEARCH PAPER

Mutational Spectrum of the *CFTR* Gene in the Kazakhstan Population

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Objective: To study the frequency and spectrum of *CFTR* gene variants in different ethnic groups of Kazakhstan. **Methods:** We reviewed the records of 58 patients with cystic fibrosis. All the patients underwent molecular genetic analysis to reveal genotype-phenotype correlations. **Results:** The median (IQR) age of the patients was 5.4 year (7 months, 18 year); 40% were diagnosed at the age of 5-10 year. The study identified 28 specific variants: p.Phe508del, the variant most common in the European population, was detected in 30 patients (51.7%). Variants other than p.Phe508del were revealed in 31% (21 patients). **Conclusions:** We found a number of specific variants characteristic of the Kazakhstani population. A pronounced regression of disease symptoms was detected in patients with mild mutations; whereas in patients with severe mutations, therapy produced very little effect.

Keywords: *p.Phe508del, Pancreatic elastase, Pseudomonas aeruginosa, Sweat test*.

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Cystic fibrosis (CF) is a monogenically inherited autosomal recessive disorder characterized by the presence of mutations in *Cystic fibrosis transmembrane conductance regulator (CFTR)* gene [1-3]. There are more than 2000 known variants of the CF gene- the following variants are most common in the European population: p.Phe 508del (52.8%), p.Ser18ArgfsX16 (6.3%), p.Glu92Lys (2.6%), p.Gln685Thrfs (2%), 3849+10kbC>T (1.6%), p.Ser670_Leu671insTer (1.6%), p.Gly542Ter (1.3%), p.Asn1303Lys (1.3%), p.Trp1282Ter (1.1%), p.Leu138dup (1.1%) [4]. Incidence of cystic fibrosis varies in different populations of the world; varying from 1:1800 newborns in Ireland, to 1:9000 in Africa, and 1:3,50,000 in Japan. These data indicate differences in the population gene pool of Europe, Asia and Africa [5]. There is minimal data on the frequency and spectrum of *CFTR* gene variants in different ethnic groups of Kazakhstan.

A study of the CF variants spectrum most specific to a population helps to identify groups at increased risk of cystic fibrosis, conduct timely measures to prevent secondary complications, optimize the therapeutic algorithms and, consequently, ensure a more favorable prognosis of the disease. Thus, the purpose of this study was to analyze the CF variants most specific to the Kazakhstani population.

METHODS

We reviewed the records of patients with cystic fibrosis, based on clinical symptoms, undergoing treatment at the Scientific Center of Pediatrics and Pediatric Surgery at Almaty, Kazakhstan between 2016-2019. The diagnosis was confirmed by sweat test (sweat chloride concentration >80 mmol/mL) and decreased levels of stool pancreatic elastase. To study genotype-phenotype correlations and the possible effect of identified variants on the aspects of the disease, all the patients underwent molecular genetic analysis at the Molecular Laboratory of the Department of Biology and Human Genetics of Motol University Hospital, Prague, Czech Republic. The patients went through a so-called 'cascading' approach in molecular genetics diagnostics, which involves identifying gene variants in several stages of testing – starting from the most common variants and gradually moving on to rarer ones. First, the most common variants of the *CFTR* gene were examined using the commercial Elucigene CF-EU2 kit (Longwood Diagnostics), which permits simultaneous detection of 50 mutations (supple-mentary material). Then, additional multiplex ligation-dependent probe amplification (MLPA) was performed for identifying extensive intragenic rearrangements i.e., deletions or deletions by duplication of one or more exons within the entire *CFTR* gene, which are not detectable by conventional PCR-based methods. Finally, MPS-based analysis of the entire *CFTR* coding region, adjacent splice site junctions and several introns was done using a locus-specific library preparation assay (CFTR NGS assay; Devyser) and MPS sequencing was performed on

MiSeqSystem (Illumina). Bioinformatic analysis was carried out using the SOPHiA platform for hereditary disorders (*www.sophiagenetics.com*). Positive cases were confirmed by targeted Sanger DNA sequencing on ABI 3130xl DNA Analyser (ThermoFisher). MLPA analysis of intra-*CFTR* rearrangements and copy number variation was performed using the SALSA MLPA P091 CFTR Assay followed by the analysis of raw data on the proprietary software Coffalyser.Net (MRC-Holland).

RESULTS

We studied the data of 58 children (52% boys) with CF with a median (IQR) age of 5.4 year (7 month, 18 year) (range 3 month – 18 year). Majority of the patients (46.6%) were between 1 year and 5 year of age. Of the remaining, 16 (27.6%) were infants and 13 (18.9%) were between 5-10 years of age. Majority (55%) of patients were ethnic Kazakhs or Russians (35%). Pulmonary form of the disease was seen in 15 patients, 10 patients had intestinal manifestations, and the rest of the children were diagnosed with a mixed form of the disease.

In 88% of cases (51 patients), variations associated with CF were identified, whereas in the remaining children (*n*=7, 12%) no variations were detected, and the diagnosis was based on high sweat chloride levels, pancreatic insufficiency, low levels of stool pancreatic elastase, and presence of *Pseudomonas aeruginosa* in culture.

We identified 28 variants specific to cystic fibrosis. p.Phe508del, the variant most common in the European population, was detected in 30 patients (51.7%) (9, homozygous state and 21, heterozygous state). Variants other than this were found in 21 (41.2%) patients (3 in homozygous state).

In patients with p.Phe508del variant (homozygous or compound) *P. aeruginosa* was found in culture more commonly than in patients with other variants (46.5% vs 21.6%; *P*=0.04). Moreover, 66.7% of patients homozygous for p.Phe508del and positive for *P. aeruginosa* were infants. Secondary complications in the form of bronchiectasis in patients with p.Phe508del (homozygous or composite) were detected more commonly (21,70%) than in patients with other mutation (14, 50%) (*P*=0.12). However, there was no difference in frequency of fibrotic changes in lung tissue between the two variants.

Of the identified variants, 21 (75%) were classified as severe mutations, whereas the remaining 25% (*n*=7) were ranked as mild (supplementary material). The following variants, not common to the European population, were identified in the study: p.Arg553X, c.2818_2819delAC, c.185G> T, p.Tyr515_Arg516delinsTer, p.Val392Gly, c.488A>C, p.Tyr1092His, p.Gln290Ter, c.4111_4113dupGAA (severe mutations) and, mild mutations (c.2491G>T, p.Ser1235Arg).

p.Tyr515_Arg516delinsTer variant was detected in four patients (1 homozygous). This variant had originated from the Caucasus geographic region [6], but despite its occurrence in many other regions of Russia, it is mainly found in Georgians, Megrels and Chechens [4,7,8]. Some of the patients in the study were ethnic Kazakhs, but since Kazakhstan is a mult-ethnic country with a wide prevalence of inter-ethnic marriages, the probability of transfer of this mutation to the Kazakh population is high. In patients with the heterozygous variant, the disease develops in a more severe form, most likely due to it often being a compound with one of the other severe variants. P.Trp1282Arg variant was identified in one case in the study – in an ethnically Russian child; although, this variant is reported to occur in Ashkenazi Jews [9].

Patients with mild variants, either in the homozygous or compound/heterozygous state, had complete absence of steatorrhea or moderate steatorrhea; moderate pancreatic elastase concentrations accompanying mild pancreatic dysfunction; no *P. aeruginosa* in culture, and significantly fewer gastrointestinal complications.

We identified Å92Ê (p. Glu92Lys, c.274G>A) and p.Gln290Ter variants, each of them in homozygous state, for the first time in the Kazakhstani population; of which, p.Glu92Lys is reported to be prevalent among the Turkish people.

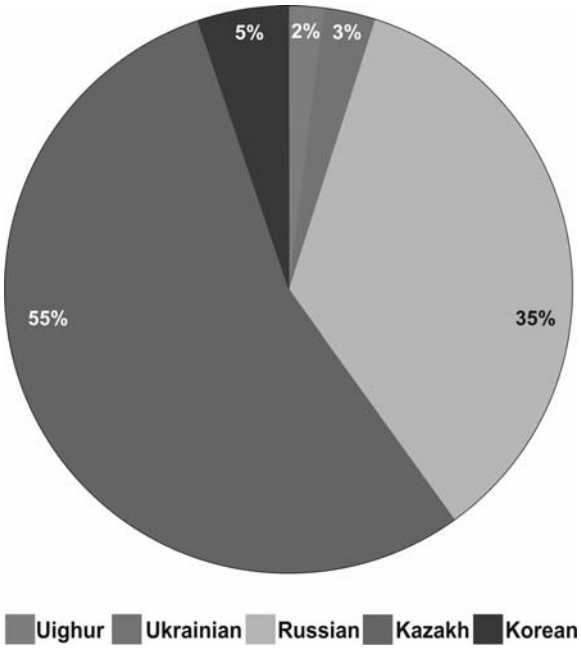

Fig.1 Distribution by ethnicity.

WHAT THIS STUDY ADDS?

- Mutational spectrum of *CFTR* gene in Kazakhstan population revealed a member of specific variants characteristic to this population and not common in the European population.

Analysis of different gastrointestinal complications showed that milder genetic variants were mostly associated with fewer complications like liver cirrhosis or diabetes. In seven (12%) patients with unidentified variants of the *CFTR* gene, the disease manifested itself mainly through pulmonary symptoms with only slow detrimental effect on other organs.

Data on bacterial infection showed that in infants, *Staphylococcus aureus* was seen in 51% and *H. influenzae* in 33%, while pneumococcus was less prevalent (16%). Thus, the pulmonary form prevailed among clinical manifestations despite the diagnosed mixed form. However, as the disease progressed, the frequency of *P. aeruginosa* colonization steadily increased in patients. Twenty five patients (43%) were carriers of *P. aeruginosa*, whereas three other patients (12%) with bronchiectasis had an intermittent form, which is more favorable due to its milder symptoms and less accumulation of sputum. The remaining 22 patients were found to be chronic carriers who did not respond to treatment (*P. aeruginosa* was constantly present in culture).

Residual wheezing in the lungs was mainly observed in patients with severe variants and with p.Phe508del (homo/heterozygous states), while in children with identified mild variants, residual wheezing was not observed. In children with unspecified variants, residual wheezing persisted in 43% of cases. Following therapy, steatorrhea was still present in most children with severe variants and with p.Phe508del in heterozygous state; whereas in patients with the homozygous variant, steatorrhea was found only in 55%. No steatorrhea was observed in children with mild variants of the gene. In the group of children with unidentified variants, steatorrhea persisted only in one patient.

DISCUSSION

In Kazakhs (32 patients), the p.Phe508del variant was identified in 40.6% (13 patients), while among the representatives of the Russian ethnic group (20 patients) it was seen in 70% of cases (14 patients). According to the available literature, this deletion in codon 508 is most common among Europeans and is much less frequent among Asians [10]. Alibakhshi, et al. [11] analyzed a cohort of Iranian patients, and detected p.Phe508del in 18/1% cases [11], while in the Turkish population, it was detected in 23% [12]. The frequency of p.Phe508del reaches about 60% in Pakistani CF patients, but is much lower in Indian (about 20%) and Japanese patients (about 10%) [13]. In 36 Asian CF patients in the United Kingdom (26 Southern Asians and 10 Central Asians), 26% were homozygous for p.Phe508del [5]. Unfortunately, there is limited information available from most Asian countries. Genetic analysis revealed no p.Phe508del variants in patients of Chinese nationality [6]. It may also be assumed that a fairly high percentage of p.Phe508del variants detected in the Kazakhstani population may be due to a large number of inter-ethnic marriages.

It may be inferred that the severe mutation causes early secondary complications of the respiratory system in children under the age of 1 year due to the poor immune system and poor resistance to external infectious agents. In contrast to the data from other countries [14,15], wherein the first manifestation of the disease in patients homozygous for p.Phe508del is in the second year of life and in the third year in heterozygotes, the present study did not reveal any significant age differences in the first manifestation of symptoms of cystic fibrosis.

We feel that this study of the CF-related variant spectrum most specific to the Kazakhstani population will help to identify groups at increased risk of cystic fibrosis and take timely measures to prevent secondary complications, optimize the therapeutic algorithms and, consequently, ensure a more favorable prognosis of the disease and improve the life expectancy of the patients.

Ethics clearance: Scientific center of Pediatrics and Pediatric Surgery; Protocol No. 2, dated May 05, 2021.
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