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ORIGINAL RESEARCH

Cystic Fibrosis Polymorphic Variants in a Russian Population

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Purpose: Cystic fibrosis (CF) is one of the most common monogenic diseases with an autosomal recessive inheritance. Carrier screening leads to a reduction in the number of children born with CF disease. The aim of this study was to develop the custom panel for the diagnosis of heterozygous carriage of polymorphic variants in the *CFTR* gene and to establish their allelic frequencies (AF) in one of the Russian regions where ethnic Russians predominate.

Patients and Methods: The diagnostic panel was designed on the basis of data from the register of CF patients in Russia for 2017 and validated on 22 blood samples of patients with previously genetically established CF. The study participants (n=642) for CF variants estimation were randomly selected from the population-based cohort study ESSE-Vologda. Genotypes were determined by real-time PCR on the QuantStudio 12K Flex Real-Time PCR System. Data processing was performed using the TaqMan Genotyper Software.

Results: The proposed diagnostic panel allowed simultaneous analysis of 60 variants of the *CFTR* gene. A total of 23 carriers of the following variants were identified among 642 participants: F508del (rs113993960) with a frequency of 2.02%, L138ins (rs397508686) and 394delTT (rs121908769) – 0.47%, CFTRdele2.3 (c.54–5940_273+10250del21080; p. S18Rfs*16) – 0.31%, R117H (rs78655421), and G542X (rs113993959) – 0.16%. The frequency of heterozygotes in the Russian population was 3.58% or 1:28 (CI95%: 2.28–-5.33% by Clopper–Pearson exact method).

Conclusion: High frequency of heterozygous *CFTR* variants carriers and availability of highly productive diagnostic panel for detection of *CFTR* variants suggest the prospect of carrier screening for some common CF variants among Russian population.

Keywords: cystic fibrosis, *CFTR*, genetic analysis, carrier screening, carrier testing

Introduction

Cystic fibrosis (CF) is one of the most common life-threatening monogenic diseases with an autosomal recessive inheritance that affects different organ systems, mostly the lungs and pancreas. In CF patients viscous secretions accumulate in the airways, causing pathological changes and destruction of lung tissue. In the ducts of the pancreas, an increased viscosity of secrets results in organ damage which leads to nutrient deficiency. CF is the cause of early mortality for most untreated patients.[1]

CF symptoms arise as a result of homozygosity or compound heterozygosity of mutant alleles in the gene of cystic fibrosis transmembrane conductance regulator (*CFTR*), which is located on the long arm of the seventh chromosome, has a size of about 189 Kb and includes 27 exons.[2] It was shown that the carriers of one mutant allele had significantly increased risk for 57 CF-related conditions.[3] According to

the Cystic Fibrosis Mutation Database, more than 2090 mutations of the *CFTR* gene are found, 360 of them are CF-causing.[4] According to their effect on CFTR, these mutations are divided into six classes. The usage of the proposed classification by Marson et al, 2016 helps in determining the CFTR defect: the first class IA (no mRNA), IB (no protein), II (no traffic), III (impaired gating), IV (decreased conductance), V (less protein), and VI (less stable).[5]

The introduction of screening programs and the creation of national registers in many countries improve our knowledge of CF epidemiology, diagnostics, and clinical progression. Thus, thanks to the mandatory neonatal screening program for CF in 2006 among newborns in Russia, millions of newborns were screened to exclude this disease. It was found that the average frequency of this disease among newborns in Russia is 1:10,250 (0.009%).[6] Moreover, CF frequency in various regions of Russia varies from 1:2500 to 1:17,000 (0.04–0.005%).[6]

Neonatal screening promotes early diagnosis and early treatment but does not reduce the number of CF patients in the population. Considering the psychosocial and economic burden of CF, carrier screening seems more promising for resolving the problems associated with CF. Carrier screening for CF resulted in a reduction of 50–75% of live births with CF in some countries.[7] Carrier screening before pregnancy in Russia is not common.

An increase in the number of genetic tests for CF in Russia and the creation of the national registry of CF patients[8] allowed obtaining data on the spectrum of variants in the *CFTR* gene in Russia. Data on the frequency of variants associated with CF based on a population study in Russia are not available.

The aim of our study was to develop the custom panel for CF carrier screening and to estimate allelic frequency (AF) of *CFTR* variants in the Russian population to predict the potential effectiveness of CF carrier screening in Russia.

Materials and Methods

Population-Based Cohort Sampling

The study included subjects from the Epidemiology of Cardiovascular Risk Factors and Diseases in Regions of the Russian Federation Study (ESSE-RF).[9] The ESSE-RF is a multicenter population-based study, conducted in 2012–2013, covering 13 regions of Russia. The multistage clustered samples of about 2000 people, aged 25–64, from every region, were obtained using Kish methods.[10] Blood samples of all individuals were stored at −70°C in the biobank of the National Medical Research Center for Therapy and Preventive Medicine. The study was approved by the Independent Ethic Committee of the National Medical Research Center for Therapy and Preventive Medicine and was conducted according to the principles expressed in the Declaration of Helsinki. Informed written consent was obtained from all participants.

Our study included participants from ESSE-RF, conducted in the Vologda region of North-West Federal District of Russia (ESSE-Vologda). A total of 642 out of 1642 participants from ESSE-Vologda were randomly selected for the study (44% were men), and the average age was 44±11 years old. The Vologda region was chosen as a typical region dominated by people of Russian nationality.[11]

DNA extraction was performed from blood samples using QIAamp® DNA Blood Mini Kit (Qiagen, Hilden, Germany). DNA concentration was measured on NanoDrop OneC Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

CF Sampling

Twenty-two DNA samples of CF patients, used for the panel validation, were obtained in the framework of cooperation between biobanks of the National Medical Research Center for Therapy and Preventive Medicine and the Research Centre for Medical Genetics (Moscow, Russia).

Real-Time PCR

The genetic diagnostic panel was developed on the basis of QuantStudio 12K Flex Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA). The reaction mixture consisted of a DNA sample with 2 × TaqMan OpenArray Real-Time PCR Master Mix (Thermo Fisher Scientific, Waltham, MA, USA) and was loaded onto the OpenArray plates using QuantStudio 12K Flex AccuFill system (Thermo Fisher Scientific, Waltham, MA, USA). The plates were coated with immersion liquid and loaded into QuantStudio 12K Flex Real-Time PCR System for amplification according to the manufacturer's standard protocol. Data analysis was performed using the TaqMan Genotyper Software package, version 1.4.0 (Thermo Fisher Scientific, Waltham, MA, USA).

Sanger Verification

The validation of Real-time PCR data was done in the selected samples by Sanger sequencing of the PCR products. The PCR products were sequenced using ABI PRISM BigDye Terminator v3.1 reagent kit (Thermo Fisher Scientific, Waltham, MA, USA) and then analysed on DNA sequencer Applied Biosystem 3500 DNA Analyzer (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's protocol.

Statistical Analysis

The frequency of heterozygotes (HF) and the AF were worked out as a percentage for all participants and all alleles, accordingly. The confidence interval was calculated using the Clopper–Pearson exact method.

Results

Variants included in our custom panel were selected according to the data published in the CF register of Russia,[8] as well as the data on the frequencies of heterozygous carriage among the Russian samples.[12,13] Thus, 60 variants with the highest frequencies were selected (Supplementary Table 1).

The average accuracy of genotyping - the call rate using QuantStudio 12K Flex Real-Time PCR system was 93.5%. The reproducibility of the genotyping results was evaluated on two OpenArray plates on different days by different researchers. As a result, the call rate of one plate was 93%, of the second – 99%. The reproducibility of the results by parallels was 90%. These data can be explained by three samples with a low call rate on one of the plates. After filtering samples with a call rate of less than 90%, the call rate was 97.92% and 99.29%, correspondingly. The reproducibility of the results by parallels after quality filtering was 98%.

The panel validation on 22 CF patients revealed that 13 samples were compound heterozygotes, one sample had a homozygous variant F508del (rs113993960), and one – L138ins (rs397508686). Only one heterozygous *CFTR* variant was identified for five samples. The presence of *CFTR* variants was not detected for two samples. A total of 19 mutant alleles were detected, among them the most frequent were the following variants: F508del (rs113993960) was found in one sample in homozygous state and in 11 samples in heterozygous state; L138ins (rs397508686) – in one sample in homozygous state and in one sample in heterozygous state; 2143delT (rs121908812) and E92K (rs121908751) – in heterozygous state in two samples each; 3944delG (rs397508612), S1196X (rs121908763), 621 + 1G> T (rs78756941), 712–1G> T (rs121908793), 1248 + 1G (rs397508158), S1159F (rs397508573), 3667ins4 (rs387906378), G542X (rs113993959), N1303K (rs80034486), S466X (c.1397C> G) (rs121908805), 2789 + 5G190280 (rs121908783), 3849 + 10kbC> T (rs75039782), and 2183AA> G (rs121908799) – in heterozygous state in one sample each (Table 1). Thus, the custom panel validation on CF patients detected that mutant alleles were identified in 79.5% of cases (with fully established CF-mutant genotypes – 68.2%), at least one mutant allele was identified in 90.9% of the cases. The most frequent were: F508del (rs113993960) – 29.5%, L138ins (rs397508686) – 6.8%, 2143delT (rs121908812) and E92K (rs121908751) – 4.5%.

Twenty-three heterozygous carriers of *CFTR* variants were identified among 642 participants. The HF was 3.58% (CI95%: 2.28–5.33%) or 1:28. In total, six mutant alleles were found: F508del (rs113993960) with a frequency of 2.02%, L138ins (rs397508686) – 0.47%, 394delTT (rs121908769) – 0.47%, CFTRdele2.3 (c.54–5940_273 +10250del21080; p.S18Rfs*16) – 0.31%, R117H

Table 1 Results of the Custom Panel Validation on Russian CF Patients (N=22)

Patient ID	Genotypes
1	rs113993960/rs397508612
2	rs113993960/NA
3	rs121908751/rs78756941
4	NA/NA
5	rs121908793/NA
6	rs113993960/rs397508158
7	rs397508573/NA
8	rs121908812/rs75961395
9	rs113993960/rs387906378
10	rs113993959/NA
11	rs113993960/rs113993960
12	rs113993960/rs80034486
13	rs113993960/rs121908751
14	rs397508686/rs397508686
15	rs113993960/rs121908805
16	rs121908812/NA
17	rs113993960/rs80224560
18	rs113993960/rs121909011
19	NA/NA
20	rs113993960/rs121908783
21	rs113993960/rs75039782
22	rs397508686/rs121908799

(rs78655421)– 0.16% and G542X (rs113993959) – 0.16% (Table 2).

DNA sequencing by Sanger was used for validation of the results. Sanger sequencing was performed on 1–3 of heterozygous samples identified using the custom panel, as well as the wild-type homozygous samples as controls. The genotypes for six *CFTR* variants were confirmed (Figure 1). The proportion of confirmed results was 70%.

Although the genotype analysis with two assays C__64676246_10 for genotyping rs74767530 and C___656878C_30 for rs77932196 on QuantStudio 12K Flex Real-Time PCR System using the TaqMan Genotyper Software (Thermo Fisher Scientific, Waltham, MA, USA) detected some heterozygous samples and one mutant homozygous sample, they were not verified by Sanger sequencing (Figure 2). In this case, we decided not to include these assays in the future redesign of our custom panel. The proportion of confirmed results by Sanger sequencing without them was 91%.

Discussion

In our study using the custom panel for detecting 60 *CFTR* variants among 642 participants from the population-based cohort study, ESSE-Vologda was identified 23 CF carriers, among them 13 carriers had F508del, 3 – L138ins, 3 – 394delTT, 2 – CFTRdele2.3, 1 – R117H, and 1 – G542X. In total, 6 mutant alleles were found, 5 of them are among the 15 most common variants found in Russian CF patients.[8] The detection efficiency of carriers using this custom panel was 80.94%, which was calculated as a sum of disease allele frequencies (DAF) for variants included in the custom panel among CF patients according to the Russian CF register.[8] The HF was 3.58% (1:28), expected disease frequency was 0.032%.

Our results can be compared to the results of other studies in Russia. In the study based on the results of whole-exome sequencing of 372 individuals selected from different research and clinical projects the HF was 2.96% for people living in the North-West region of Russia, the disease frequency was 0.022.[15] In the study based on 1000 Russian blood donors genotyped for the 24 most common *CFTR* variants the HF was 2.9%.[12] In the study of 922 samples from various regions of Russia tested for 19 variants it was 2.82%.[13] It is important to note that our study is the only population-based study evaluating the frequency of variants associated with CF in Russia, and therefore indicates a greater accuracy of the AF assessment.

For all 6 *CFTR* variants, the AF calculated in our study was higher than the AF for European (Non-Finnish) population according to EXAC, except for R117H that can be due to its low penetrance. The higher AF could possibly be explained by the northern location of the Vologda region. The AF of F508del, 394delTT, and R117H variants are likely higher in northern Europe.[16–18]

Among other cohorts in the world, the following results were obtained. In the Italian population during screening for 47 variants, overall HF in the general population (57,999 subjects) was 3.23% (1:31).[19] In the United States, a panel containing 23 variants was recommended for carriers screening by American College of Medical Genetics and Genomics and American College of Obstetricians and Gynecologists. The detection efficiency of carriers screening using this panel ranged from 43% to 88% in different ethnic groups.[20] Among Caucasian individuals (757,198 participants) the HF was 1:29.[20] To increase the level of detection based on this panel, two panels were created containing 32 and 69 variants. The HF among Caucasians was 1:28 (438,026 participants) and 1:27 (16,242 participants), respectively.[21] CF carrier screening in Australia using a panel with 38 variants identified 342 CF carriers among 12,000 participants, the HF was 2.91%.[22] Studies conducted earlier in Russia aimed at identifying carriers of mutant alleles among a healthy population included either the determination of one variant (F508del)[23,24] or from 7 to 24 variants.[12,13,25] Percentage of variants included in our custom panel that are present in above-mentioned studies is 71.7%.[12,13,19–22]

The F508del (rs113993960) variant is the most common among CF-causing in the European population.[21,26] According to the data of the Russian CF patients register, the DAF of this variant is 52.81%.[8] In our study 13 participants were identified as heterozygous for this variant, the HF was 2% (56.52% of all identified variants). The value obtained for the variant proportion among all identified variants (56.52%) corresponds to the DAF from the Russian CF patients register (52.81%),[8] which confirms the data on the high penetrance of this variant, that tends toward 100% depending on which variant is combined.[27,28] A slightly higher value in our study can be explained by the fact that not all rare variants from the Russian CF patients register[8] were included in our custom panel. In the study by Gurina in a representative

Table 2 Identified *CFTR* Variants Among 642 Participants of the ESSE-Vologda Study

Variant	HGVS	dbSNP	Number of identified alleles	Variant proportion among all identified variants, %	HF, %	At 95% CI, %	AF (ESSE-Vologda), %	AF, European (Non-Finnish), EXAC, GNOMAD Exome, GNOMAD Genome, %[14]	Proposed Classification[5]	DAF among Russian CF patients, %[8]	HF among 1000 Russian samples, %[12]	HF among 922 Russian samples, %[13]
F508del	p.F508del	rs113993960	13	56.52	2.02	1.08-3.44	1.01	1.06	II	52.81	1.5	1.4
CFTRdele2,3	p.S18Rfs*16	hg19::chr7:117138367-117159446	2	8.69	0.31	0.04-1.12	0.16	0.01312[b]	IA	6.21	0.1	0.43
G542X	p.G542*	rs113993959	1	4.34	0.16	0-0.86	0.08	0.03	IB	1.35	0	0.22
L138ins	p.L138dup	rs397508686	3	13.04	0.47	0.1-1.36	0.23	0[a]	IV	1.24	0.1	0.33
394delTT	p.L88Ifs*22	rs121908769	3	13.04	0.47	0.1-1.36	0.23	0.04	IB	0.94	0	0
R117H	p.R117H	rs78655421	1	4.34	0.16	0-0.86	0.08	0.26	IV	0.04	0.4	NA
Total			23		3.58	2.28-5.33						

Notes: [a]Allele frequency in GNOMAD Exome. [b]Allele frequency in GNOMAD Genome.

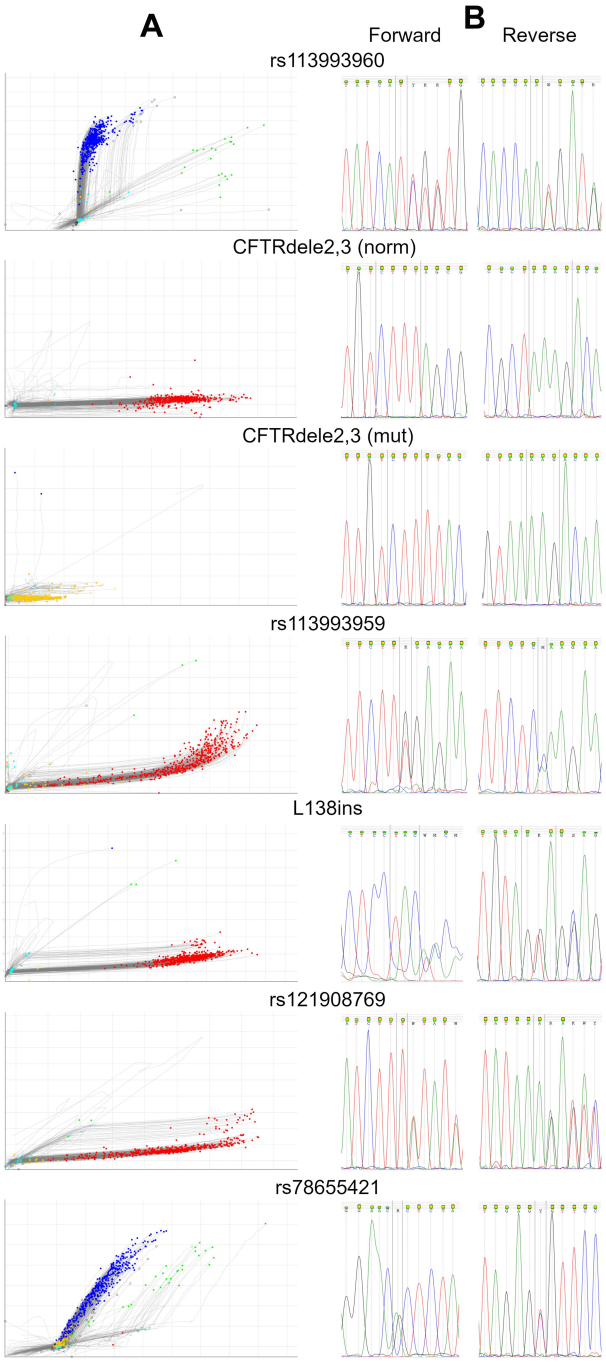

Figure 1 Genotyping results and verification by Sanger sequencing of heterozygous carriers. (**A**) Genotyping results. (**B**) Verification by Sanger sequencing.

sample of residents from Novosibirsk, one of the Russian cities, consisting of 9397 participants, 109 carriers of the F508del variant were identified, the HF was 1.15%.[24] Earlier it was shown that the AF of F508del in Russians from the European part of Russia was 0.532%.[25] In other studies on Russian individuals, the HF was 1.5%,[12] 2.25%,[23] and 1.4%.[13] Among different studies the similar results can be found: in the Italian population 42.6% of all detected CF carriers had F508del,[19] in the United States among Caucasians using panels with 23 variants – 75%,[20] using panels with 32 and 69 variants – 68.69% and 60.49%, respectively,[21] and in Australia – 80.06%.[22]

The CFTRdele2.3 variant is the second most common among Russian patients (6.21%).[8] Its frequency is high in the countries of Central and Eastern Europe. It is suggested that this variant originated from the common Slavic ancestral population.[29] In our study, the HF of CFTRdele2.3 variant was 0.31%, in other studies – 0.1%,[12] 0.01%,[25] and 0.43%.[13]

The DAF of the G542X variant in Russian CF patients is 1.35%, in the Northwestern Federal District – 1.54%.[8] In our study, the HF was 0.15%, in another study – 0.22%.[13] This variant has high penetrance,[28] but in our study, it was found only in one sample, so it does not seem possible to compare the data from our study with the data from the Russian CF patients register.[8] The frequency of this variant is the third among carriers in the studies conducted in the United States using panels of 23 variants (the frequency was 1:2190),[20] 2.56% of all identified heterozygotes among Caucasians using a panel of 32 variants and 3.17% using a panel of 62 variants[21] and the fifth in the Italian population (4.2% of carriers).[19]

The DAF of the L138ins variant among Russian CF patients is 1.24%.[8] In our study three carriers were identified (HF was 0.47%), 13.04% of all detected variants. In other studies, the HF was 0.33%[13] and 0.1%.[12]

In our study, three carriers of the 394delTT variant were found (HF was 0.47%). This variant is one of the most prevalent in the Northern Europe populations.[16] Its DAF among Russian patients is 0.94%.[8] In the studies by Abramov et al and Archibald et al no carriers of this variant were identified.[12,22]

The R117H variant has low penetrance,[27,28,30] so the DAF among patients from the Russian CF Register (0.04%)[8] is lower than AF obtained in our population-based study (0.08%). In our study, the HF was 0.16%, in the cohort study by Abramov et al – 0.4%.[12] One of the factors influencing low penetrance is the intron 8 splice acceptor.[30]

Our study has some limitations. Unfortunately, due to the technical issues, one variant (rs121908776, 1677delTA) with DAF of more than 1% among Russian CF patients[8] was not included in our study. In our future studies, we are planning to include this variant in the redesign of this custom panel.

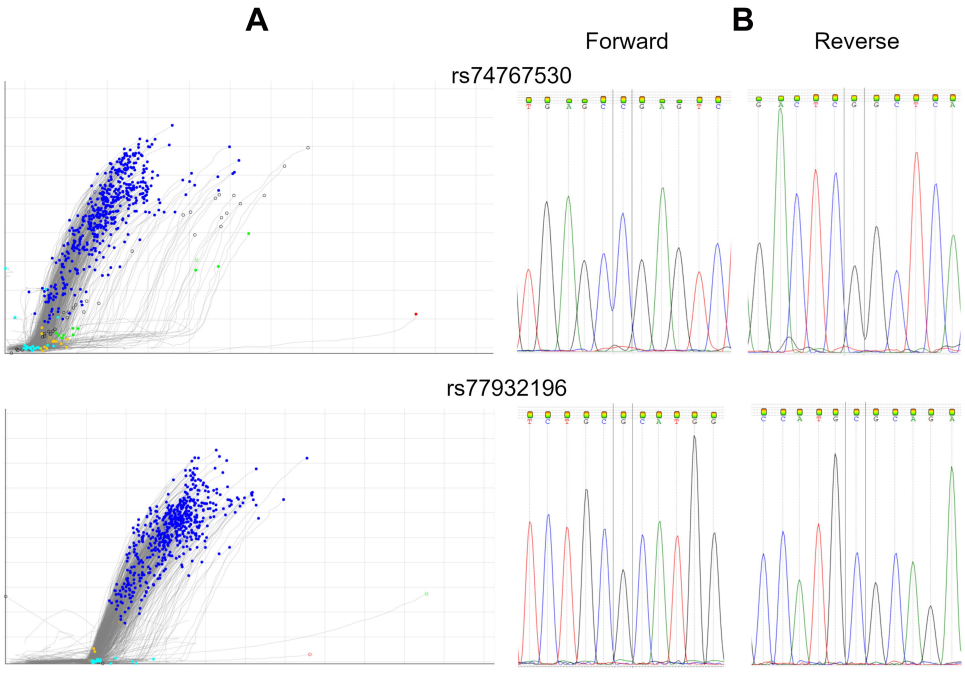

Figure 2 Genotyping results and verification by Sanger sequencing of samples using rs74767530 (assay C__64676246_10) and rs77932196 (assay C___656878C_30). (**A**) Genotyping results. (**B**) Verification by Sanger sequencing.

Conclusion

A custom panel was developed to identify heterozygous carriage of *CFTR* gene variants. The method of genotyping using QuantStudio 12K Flex Real-Time PCR system is characterized by high reproducibility, speed, and has a relatively low cost of analysis. The proposed panel allows a simultaneous analysis of 60 variants of the *CFTR* gene and can be used for CF carriage screening. The data obtained indicate a high frequency of heterozygous carriage of *CFTR* variants in the Russian population. High frequency of heterozygous *CFTR* variants carriers and availability of high efficient diagnostic panel for detection of 60 *CFTR* gene variants may contribute to improving CF carrier screening efficiency in Russia.

Data Sharing Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Approval and Informed Consent

The study was approved by the Independent Ethics Committee of the National Medical Research Center for Therapy and Preventive Medicine and was conducted according to the principles expressed in the Declaration of Helsinki. A statement on ethics approval №07-03/12 from 03.07.2012 of meetings of the Independent Ethics Committee of Federal State Institution «National Medical Research Center for Therapy and Preventive Medicine» of the Ministry of Healthcare of the Russian Federation.

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Disclosure

The authors declare that they have no competing interests.

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