Author’s response to reviews

Title: Spectrum of CFTR mutations in Chechen cystic fibrosis patients: high frequency of c.1545_1546delTA (p.Tyr515X; 1677delTA) and c.274G>A (p.Glu92Lys, E92K) mutations in North Caucasus.

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Author’s response to reviews:

Dear Isabella Zanella,
We are very thankful for all the remarks and advices that were given by the reviewers. We took into account all the comments and changed the text according to them. Table 2 was completely changed.

Vito Terlizzi (Reviewer 1)

- It is very useful to know the common mutation spectrum in a given population but the paper provides too little information to be accepted as the article article. I recommend presenting the work as a short communication or brief report.

Answer: As we know BMC Medical Genetics Journal does not accept short communications. So we cannot change the format of the article.

- Quality of written English - Needs some language corrections before being published

Answer: English editing of the manuscript was handled by Secrest Editing s.r.o. (Prague, Czech Republic).

Maria Tzetis, Ph.D (Reviewer 2)

Quality of written English - Needs some language corrections before being published

- The manuscript does not add any essential knowledge on CFTR epidemiology and is instead more of an ethnological study on the Chechen population. The methodology is vague. How was the specific mutation identified from RFLP studies???

Answer: An in-house molecular genetic methods including amplified fragment length (AFLP) and restriction fragment length (RFLP) polymorphism techniques were utilized to detect insertion/deletion variants and nucleotide substitutions, respectively. This information is included in the text on page 7, line 4.

- There are no conclusions to the manuscript. Do the authors conclude that the Chechen population should only be tested for the 2 described mutations?

Answer. We changed our conclusion, page 12, line 6. Testing of the two CF-causing mutations is thus recommended in Chechen CF patients since it allows to identify one or both mutant CFTR alleles in more than 99% patients suspected of being affected by CF. Furthermore, we have confirmed the genetic delineation of the Chechen population from other ethnic groups of the Northern Caucasus (e.g. by low prevalence of the c.3846G>A mutation which is dominant in adjacent Karachay-Cherkessia; Figure 1.), as well as the role of historic migrations of Turkic speaking peoples from Central Asia to the Northern Caucasus with the c.274G>A mutation very...
likely being their “marker”. Analysis of genotype-phenotype correlations in two groups of Chechen CF patients (i.e. c.1545_1546delTA homozygotes versus c.1545_1546delTA / c.274G>A compound heterozygotes - demonstrated that presence of the c.274G>A mutation is associated with generally less severe course of the disease. Our data will improve genetic counselling and provide a basis for the introduction of mutation-specific therapies in the future. Also this information is added into the abstract.

Ana E. Fernández-Lorenzo (Reviewer 3)

Quality of written English – Acceptable

- I think this is an interesting paper because shows the variety of CF mutations in a group of population.

- The number of patients is low, I would that were indicated prevalence of CF in this region.

Answer: In the Chechen Republic, the 33 Chechen CF patients are officially registered. The prevalence of CF is 2,455 per 100,000 Chechens in 2017. Nearly all of the known CF patients residing in Chechnya (32/33) were analysed. We added this information on the page 9, line 1, of the text.

- I think it could be interesting describing common manifestations of Cystic Fibrosis, that there are different presentations of the disease and the clinical status of patients can be very different. I would like to know how is clinical status of studied patients, describing how is health of this patients when they are diagnosed and in the evolution of the disease.

Answer: We added the information about the clinical features in the Material and Methods – page 7 To assess the course of the disease in Chechen CF patients with different CFTR genotypes, the following key parameters were taken into account: the patient’s age at the last examination, the age at diagnosis, sweat test (chlorides, mM/L), body mass index (BMI) (kg/m2), spirometry parameters: FEV1 (% predicted) and FVC (% predicted), pancreatic insufficiency (fecal elastase 1 (<200 mg/g)), complications (meconium ileus, liver cirrhosis (with/without portal hypertension), CF-related diabetes mellitus, allergic bronchopulmonary aspergillosis (ABPA), chronic sino-bronchial colonization by S. aureus, P. aeruginosa, B. cepacia complex, Achromobacter spp, S. maltophilia, nontuberculous mycobacteria (NTM), including Gram-negative microflora).

The statistical analysis was performed using the program STATISTICA8.0. To compare observed categorical variables, the Fisher test was used, while for quantitative tests the Mann-Whitney test was utilised. Results were considered as significant when p≤0,05.

In the Results – page 9-10 and Table 2 with summation of the results was added on page 16.
In order to compare the clinical course of CF in studied cohort, patients were divided in two the most prevalent groups: 17 homozygous for c.1545_1546delTA (Group 1) and 8 compound heterozygotes for c.1545_1546delTA and c.274G>A variants (Group 2). We did not find significant differences in the patients age at last clinical examination, their age at diagnosis, sweat Cl concentrations or BMI values. None of the most common before mentioned complications (meconium ileus, liver cirrhosis, diabetes, polyposis) were revealed in both groups. Significant differences were observed only in terms of pancreatic insufficiency in that all patients from Group 1 had fecal elastase 1 concentrations below 50 µg/g, while all patients from Group 2 concentrations were over 200 µg/g (p<0.0001) indicating lower degree of pancreatic exocrine dysfunction associated with the presence of c.274G>A. Similarly the proportion of patients with chronic P. aeruginosa lung colonization was significantly higher in Group 1 compared to Group 2 (69.0% vs. 14.0%, accordingly; p=0.024). The differences in other examined microorganisms under investigation were not significant. Overall, presence of the c.274G>A mutation is associated with less severe course of the disease than in c.1545_1546delTA homozygotes (Table 2).

And in Discussion section on pages 11-12.

The comparison of key clinical parameters in two groups of Chechen patients with different genotypes demonstrated that the allele c.274G>A is associated with higher residual pancreatic function and lower chronic lung colonization with pathognomonic microorganisms in accordance with CFTR2 database data [1].