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Letter to the Editor

Prevalence of ΔF508 cystic fibrosis carriers in a Romanian population group

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Dear Editor,

Cystic fibrosis (CF) is the most common autosomal recessive disorder in European populations. The overall incidence in Europe is estimated at 1/3500, with wide variations among countries(1). This health condition is caused by mutations in the CF transmembrane conductance regulator (CFTR) gene, which encodes an ATP-binding cassette transporter that functions as a ligand-gated anion channel (2).

CF usually presents as an association of exocrine pancreatic insufficiency, chronic obstructive pulmonary disease, and increased levels of chlorine and sodium in sweat, although some patients can present a milder phenotype. Other clinical features may include sinusitis, diabetes mellitus, bowel obstruction, and male infertility caused by the aplasia of vas deferens. These clinical features lead to a marked decrease in the quality of life, the current median predictive age of survival being estimated at 40 years (3).

Although more than 1600 mutations have been described in the CFTR gene, F508del (also referred to as ΔF508) is the most prevalent, accounting for up to 66% of all alleles in CF patients (4). It consists of a 3-bp deletion in exon 10 and a loss of phenylalanine 508, leading to sequestration of an abnormal protein in the endoplasmic reticulum, followed by degradation in the proteasome (2). The clinical picture of patients carrying this mutation consists of the classical CF phenotype (3).

CF follows an autosomal recessive pattern of inheritance, with heterozygous individuals exhibiting a normal phenotype. The carrier frequency is estimated between 1/20 to 1/80 in different European countries (4). Although carrier testing is usually conducted in relatives of CF patients, no family history of the disease can be found in most of the cases of affected infants. Thus, carrier screening has been recommended for pregnant women, as well as for couples planning a family (5).

In Romania, CF has an estimated incidence of 1/2056 (1), F508del mutation being the most frequently detected (6–8). Our study objective was to assess the F508del carrier frequency in a group of 415 healthy unrelated individuals.

A group of 415 healthy unrelated subjects were randomly selected from a group of medical students from Cluj and Iasi counties in 2008 and 2012. Informed consent was obtained from all the study participants.

For genetic testing, 3 ml of peripheral blood was collected on EDTA (ethylenediaminetetraacetic acid) as anticoagulant. Genomic DNA was extracted using a commercially available extraction kit (Wizzard Genomic DNA Purification Kit, Promega) from blood leucocytes contained in a volume of 300µl.

Genotyping was carried out by real-time PCR allelic discrimination with TaqMan probes. The experiment was conducted using a Quant Studio 3 Real-Time PCR System (Applied Biosystems, USA), and the data were analyzed with Quant Studio Design & Analysis software v1.4.3.

The mean frequency of F508del mutation was calculated and the 95% confidence interval was determined. In order to estimate the overall carrier frequency (of all CF mutations), we divided the F508del carrier frequency by the relative

frequency of F508del in CF chromosomes from Romanian patients. Chi-squared test was applied for comparing the F508del carrier frequency in males and females. The software used to collect the data was Microsoft® Excel 2019 and the statistical package used was IBM® SPSS® Statistics v26.

A total of 415 subjects were tested for F508del carrier status, 233 men (56%), and 182 women (44%). Thirteen F508del carriers were detected (7 men and 6 women). Thus, the carrier frequency in our group was 1 in 32 (95% CI: 1/21-1/69), with no significant differences between men and women. The subjects originated from most of the Romanian counties, as shown in Figure 1. However, most of the subjects originated from Iasi and Cluj counties (17% and 26%), and only a minority from the southern region. Three carriers were from Bistrita-Nasaud county and two carriers were found to be from Iasi and Harghita counties. No significant differences regarding carrier frequency were found between subjects originating from Cluj and Iasi counties.

The relative frequency of F508del in CF chromosomes in 203 Romanian patients was 0.62 (7). Presuming that the relative frequency of the F508del mutation among carriers is similar to the F508del frequency in CF patients (62%), the CF carrier frequency including other mutations can be estimated at 1 in 20 (95% CI: 1/14-1/34).

To date, limited data are available regarding mutation prevalence in Romanian CF patients. The largest Romanian patient cohort shows that F508del is present in 62% of the CF chromosomes, with 80% of patients carrying at least one F508del allele (44,3% homozygotes and 35.9% heterozygotes) (7). However, other studies conducted on Romanian CF patients show different F508del frequency, from 56% (6) to 71% (8) of the tested CF chromosomes. These differences could be explained by small sample sizes as well as different patient origins, as F508del frequencies were previously observed to vary between different regions of the same country (9). Frentescu et al. conducted the most extensive study on Romanian CF patients and although several mutations were discovered in more than 1% of the CF chromosomes, the detection rate of mutations was 72.3% (6). Thus, further research is required in order to improve the detection rate and to determine the mutation frequency in Romanian CF patients.

Our study presents several limitations. We had a small sample size and the study group may not be representative for the entire Romanian population because most of our participants originated from the northern region of the country. Nevertheless, this is the first study to assess CF carrier frequency in Romanian population, which is an important step in determining whether a

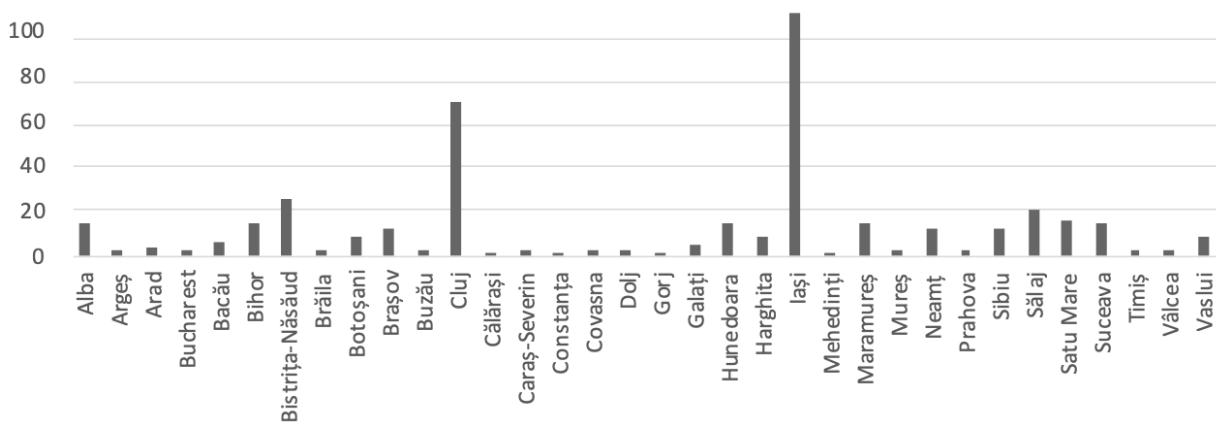

Fig. 1. Subject distribution on counties

CF carrier screening program in this population could be beneficial.

Knowing how mutations are distributed in the targeted population is also important for choosing the right mutation panel for screening. This choice affects not only the screening effectiveness, but also the costs of the program. Because of the different F508del prevalence among countries, with a higher prevalence in northern and western Europe, and also due to the "founder" effect, the commercial assays available which achieve a high detection rate in the North- and West-European countries could be less effective in South-Eastern European populations (4).

Several other studies are also required in order to determine if a screening program could be beneficial in Romania. Psychological studies and social studies are necessary in order to determine the general interest of the population in such a program and the psychological impact the screening could have. A cost-effectiveness study is also important for choosing the most suitable screening strategy (4).

In recenct decades, the carrier detection for CF has become more accessible, and several carrier screening programs have been conducted in different countries like Italy, the Netherlands, Australia, or the USA(5). A CF carrier screening program aims to detect carriers without any family history of CF (4). Knowledge of CF carrier status enables informed procreative choices like prenatal diagnosis. The implementation of screening programs of carriers in couples with no familial history of CF was associated with a decreased CF incidence (5).

Several studies showed an increased interest in the general population for CF carrier screening. Moreover, more than 80% of the carrier couples detected by population-based screening programs utilized prenatal diagnostic, most of them choosing to terminate if the fetus was affected (5). A potential negative outcome of a CF carrier screening program could be the psychological harm which might occur in some cases. However, there is little evidence for long-term negative psychological effects (5).

Preimplantation screening for couples was found to be a cost-effective program in the Australian population in whom the CF incidence (1/2940) (10) is significantly lower than in the Romanian population (1/2056) (2).

Our results show that CF carrier frequency in the Romanian population is comparable with the average carrier frequency of 1 in 25 in persons with European descent(5). Although further studies regarding mutation prevalence are required, our findings suggest that population-based carrier screening for CF should be taken into consideration.

Sergiu Nicolae Osan[1], Iona Hrapsa[1], Constantin Ionut Coroama[1], Diana Laura Miclea[1,2], Camelia Al-Khzouz[2,3], Calin Lazar[3,4], Marius Florin Farcas[1*]

1. Department of Medical genetics, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania

2. Medical genetics Department, Clinical Emergency Hospital for Children, Cluj-Napoca, Romania

3. Mother and child Department, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania

4. First Pediatrics Clinic, Clinical Emergency Hospital for Children, Cluj-Napoca, Romania

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Corresponding author

Marius Florin Farcas
E-mail: mf.farcas@yahoo.com

Abbreviations

CF- cystic fibrosis
CFTR- cystic fibrosis transmembrane conductance regulator

Authors' contribution

SNO (Investigation, writing- original draft preparation)
IH (Formal analysis, Investigation, Writing- original draft preparation)
ICC (Investigation)
DLM (Writing- review and editing)
CA (Writing- review and editing)
CL (Writing- review and editing, Methodology, Supervision)
MFF (Conceptualization, Supervision, Resources)

Conflicts of interests

The authors declare no conflict of interests.

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