Cystic fibrosis carrier frequency and estimated prevalence of the disease in Morocco

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Abstract

Background: The epidemiology of cystic fibrosis (CF) is poorly known in North African populations, in particular in Morocco and the CF carrier frequency in the general Moroccan population has never been evaluated.
Methods: To estimate the prevalence of CF mutations in Morocco, blood samples from 150 healthy Moroccans were tested for frequent CFTR mutations and the intron 8 polyT variant.
Results: Two subjects were heterozygous for F508del and eight others for the (T)\textsuperscript{5} variant.
Conclusion: These findings indicate that the Moroccan population is at risk for CF and CFTR-related disorders. CF prevalence could be in the range of that found in European populations. Wider studies are necessary to identify the clinical pattern and accurately determine the prevalence and molecular basis of CF in Morocco.

Keywords: CF epidemiology; CF prevalence; Cystic fibrosis; Morocco

1. Introduction

Cystic fibrosis transmembrane conductance regulator (CFTR) gene (MIM*602421) mutations are associated with a broad range of phenotypes, from severe classical cystic fibrosis (CF) to CFTR-related disorders, such as isolated male infertility by congenital bilateral absence of the vas deferens (CBAVD), disseminated bronchiectasis and chronic pancreatitis. Over 1500 CFTR sequence changes have been described, F508del being the most frequent mutation, along with geographic and ethnic variations in their distribution and frequency\textsuperscript{[1,2]}. Little is known about the spectrum and frequency of CFTR gene mutations in North African populations: data are available on CF patients living in Algeria and Tunisia\textsuperscript{[3-5]} and on CF Moroccan patients living in Europe\textsuperscript{[1,6]}. To our knowledge, there is no data on the prevalence of CF mutations among the native Moroccan population.

We carried out a preliminary study by screening healthy Moroccan individuals for 32 CFTR gene mutations. From the CF mutation frequency estimates obtained on this sample, we determined the probable range of CF prevalence in Morocco.

2. Patients and methods

2.1. Patients

Blood samples were collected from 150 unrelated healthy Moroccan volunteers, 71 males and 79 females, aged 18 to 55 years, who were recruited during a blood donation programme at the Department of Medical Genetics of the Institut
National d’Higiane in Rabat, Morocco. They originated from different regions of Moroccan and confirmed the Moroccan origin of their parents and grand-parents. They had no discernable symptoms suggestive of CF. The study was approved by the local ethics committee (Institut National d’Higiene, Rabat) and written consent to the genetic study was obtained from all subjects.

2.2. Methods


The presence of F508del was confirmed using denaturing gradient gel electrophoresis of exon 10 [8]. Whenever the (T)5 variant of intron 8 was detected, determination of the (TG)m(T)n haplotype was performed using a validated fluorescent PCR assay (manuscript submitted).

Allele frequencies and 95% Confidence Interval (CI) were obtained by allele counting. The expected frequency of homozygotes for a given mutation (M) in a population (P) was obtained by using the Wright formula [9]:

\[
P(M) = p^2 + Fp(1 - p)
\]

where \( p \) is prevalence of the mutation and \( F \) the mean inbreeding coefficient of population. We considered 25% of marriages between relatives with a large majority between first-cousins in the Moroccan population, based on available regional data [10].

Given that the inbreeding coefficient of a first-cousin offspring is 1/16, a rough estimate of the mean inbreeding coefficient in the Moroccan population will thus be 1/64 ≈ 0.015.

From the observed CF mutation frequency in our sample, an estimate of the expected prevalence of CF in the Moroccan population was obtained by using the following formula:

\[
\text{prev}_\text{CF} = FP_1 + (1 - F)p_1^2 + 2p_1p_2 + p_2^2
\]

where \( F \) is the inbreeding coefficient, \( p_1 \) the prevalence of allele F508del in our sample and \( p_2 \) the estimated prevalence of all non F508del CF alleles in the population. An estimate of \( p_2 \) can be obtained from \( p_1 \) and the proportion \( P_{F508del} \) of F508del alleles among all CF alleles:

\[
p_2 = (1 - P_{F508del})^{-1} \times p_1 / P_{F508del}
\]

\( P_{F508del} \) is unknown in the native Moroccan population but estimates can be found in the literature. Note that in Eq. (2), inbreeding only increases the frequency of F508del homozygotes. It probably also has an impact on other CF mutations but to account for it, individual estimates of the frequencies of all non F508del CF mutations in the population will be needed.

3. Results

Two men aged 32 and 43 years were found to be carriers for F508del. These results were given individually within the framework of a genetic counselling session. The carrier frequency for F508del was 1/75, i.e. 1.3% with a 95% Confidence Interval (CI) ranging from 0 to 3%, and the F508del allelic prevalence was 1/150 (0.7% with a 95% CI ranging from 0 to 1.6%). No other CF mutation was found in our population sample.

Apart from the two F508del carriers, eight other subjects (5.3%) were heterozygous for the (T)5 variant and the estimated allelic prevalence of this variant was thus 2.7% with a 95% CI ranging from 0.8 to 4.5%. Four of these eight subjects carried the (TG)12 allele in association with the (T)5 variant and four carried the (TG)11 allele.

4. Discussion

4.1. Estimate of CF prevalence in the native Moroccan population

The epidemiology of CF in Morocco is poorly documented compared to its neighbours Algeria and Tunisia, probably because of misdiagnosis of the disease and lack of genetic studies. No data is available on CF prevalence in the country and the CFTR molecular pathology has not been studied in the native Moroccan population. The populations of these countries are quite closed: the main ethnic groups are the Berbers and Arabs, but there have also been currents of Phoenicians, Romans, Vandals, Byzantines, Turks, Moriscos, sub-Saharan Africans, and European remnants of the colonial period [3]. Unfortunately, we had no information about the precise ethnic origin of the 150 studied Moroccan individuals but, as they originated from different regions of Morocco, we have assumed that the cohort was fairly representative of the overall Moroccan population. Despite the limited size of the study population, our results document the presence of CF alleles in this population. Drawing the pedigrees with the names of relatives of the two F508del carriers clearly indicated that they were unrelated. It could also be argued that they might not be representative of the native Moroccan population and could be of recent European origin. This cannot be definitely ruled out. However, as with the other individuals included in this study, the Moroccan origin of their parents and grand-parents was clearly established.

Based on the F508del allelic prevalence of 1/150 and a coefficient of inbreeding of 0.015, the expected frequency of F508del homozygosity would thus be:

\[
1/22,500 + 0.015 \times 1/150 \times 149/150 = \frac{1}{7000}
\]

To estimate the prevalence of CF, one needs to take into account the proportion of F508del alleles among all CF alleles, which is unknown in the native Moroccan population. Calculations were thus made based on published data from studies in CF patients (Table 1). In the first Tunisian and Algerian studies of 10 and 39 CF patients, respectively, the proportion of F508del was found quite similar: 17.9% and 20.0% of CFTR alleles, respectively [3,5]. In a larger Tunisian cohort, Messaoud
et al. reported a proportion of 50.7% of F508del among 540 CF alleles [4]. This proportion is closer to those reported in Southern European populations such as Portuguese (44.5%) [1], Spanish (51.7%) [11], Greek (53.4%) [12] and South Italian (55.6%) [13] populations. Two large studies reported on CF molecular epidemiology in North African patients living in Europe. In the collaborative study of Estivill et al., F508del was found in 32% of 147 alleles and, more specifically, in 40% of CF alleles in Moroccan patients [1]. However, the number of Moroccan CF alleles was not mentioned. In the French collaborative study, F508del accounted for 31% of 158 CF alleles in French patients originating from North Africa (Morocco, Algeria, Tunisia) [6].

Beside F508del, other frequent mutations were found among North African populations, in particular 711+1G originating from North Africa (Morocco, Algeria, Tunisia) [6]. F508del accounted for 31% of 158 CF alleles in French patients (51.7%) [11], Greek (53.4%) [12] and South Italian (55.6%) [13].

The (T)5 variant of intron 8 is considered as a mild splicing CFTR mutation with incomplete penetrance. Although it is not considered a CF-causing mutation and has no impact on genetic counselling when found in isolation, it is frequently found in trans with severe mutations such as F508del in CBAVD patients [6,14-17]. Its overall prevalence in our population (2.7%) was similar to that previously observed in series of healthy individuals from European countries [14,15,18,19]. As widely documented by functional and epidemiological studies, the disease penetrance of the (T)5 variant increases with the adjacent (TG) length [20,21]. The (TG)12(T)5 allele, whose penetrance was assessed 78% [21], was found in four of eight (T)5 heterozygous individuals, a result which indicates that the Moroccan population may also be at risk for CFTR-related disorders. Given the small size of the sample, it is not possible to state that the proportion of (TG)12(T)5 among (T)5 alleles is indeed more frequent in the Moroccan population than in European or North American populations [21].

In conclusion, our preliminary study shows that the Moroccan population is at risk for cystic fibrosis and related disorders. We need to sensitise clinicians to the disease, and urge them to carry out large-scale studies to define the clinical pattern and determine more accurately the prevalence and the molecular basis of CF in Morocco. This could help to define diagnosis strategies and patients care, and would also have implications for genetic counselling.

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References


