Frequency of ΔF508 in a Mexican sample of cystic fibrosis patients

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Abstract
This paper reports the frequency of the ΔF508 mutation in a cohort of 50 Mexican patients with cystic fibrosis (CF). The mutation was detected by PCR mediated site directed mutagenesis. ΔF508 was found in 39% of CF chromosomes, a frequency lower than that reported in Argentina and Spain. The high rate of CF cases who die undiagnosed, the ethnic origin of Mexican populations, and the limited number of cases studied could account for the low frequency of the ΔF508 mutation found in this preliminary report.

(Cystic fibrosis (CF) is the most common recessive disease among Caucasians.

Results
Fifty unrelated CF index cases and three normal controls were studied using the PSM technique. The resulting amplified product is a 219 bp fragment which is digested by MboI on a normal allele giving rise to a 202 bp band and a 17 bp fragment which is not visible on electrophoresis.

In this study, all normal non-carriers (controls) exhibited the 202 bp band. Affected subjects homozygous for the ΔF508 mutation showed the 219 bp fragment while compound heterozygotes carrying ΔF508 and a different mutation showed 202 and 219 bp bands plus a heteroduplex fragment which is only observed with deletion mutations (figure).

Methods
Blood samples were obtained from CF patients diagnosed on the basis of typical clinical manifestations or positive sweat test or both.

Genomic DNA was extracted from peripheral blood leukocytes according to standard protocols and the mutation was detected by PCR mediated site directed mutagenesis (PSM). The oligonucleotide primer sequences used were: 5′GCACCATTAAAGAAAATATGATAT... T3′ (forward), 5′CATTCACAGTAGCTT-ACCCA3′ (reverse). The 3′ end of the forward primer overlaps the deletion locus and includes a single base mismatch designed to alter the sequence of the amplified products. The PSM gives rise to products carrying a new MboI restriction site associated with the wild type allele but not the mutant allele.

A thermal cycler (Perkin Elmer) was used for DNA amplification. We added 1 μg of DNA to 100 μl amplification mixture containing 300 ng of the appropriate primers and 2.5 U of Taq DNA polymerase (Gibco), all in a solution containing 200 mmol/l of each deoxyribonucleotide triphosphate, 1.5 mmol/l MgCl₂, 67 mmol/l Tris (pH 8.8), 10 mmol/l 2-mercaptoethanol, 16.6 mmol/l ammonium sulphate, and 6.5 mmol/l EDTA. Amplification was initiated with 10 minutes of incubation at 94°C followed by 35 cycles consisting of two minutes of annealing at 50°C, two minutes of extension at 72°C, and two minutes of denaturation at 94°C. A 10 minute incubation at 72°C completed the amplification.

After PCR, 25 μl of the reaction mixture were incubated overnight with MboI restriction endonuclease (New England, Biolabs). Electrophoresis was carried out at 100 V for two hours on a 10% polyacrylamide gel.
Detection of CF \(\Delta F508\) mutation using PCR mediated site directed mutagenesis. The upper part shows the pedigree of a family. Lane 1, HaeIII digested Phi X174 DNA size marker. Lane 2, \(AF508/AF508\) homozygote (proband). Lanes 3 and 4, \(AF508/wt\) heterozygous (obligate carriers). Lane 5, wild type homozygous (normal).

Frequency of \(AF508/UK\) and \(UK/UK\) genotypes among Mexican CF patients.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No cases</th>
<th>% of AF508</th>
<th>% of UK</th>
</tr>
</thead>
<tbody>
<tr>
<td>(AF508/AF508)</td>
<td>10*</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>(AF508/UK)</td>
<td>19</td>
<td>38</td>
<td>19</td>
</tr>
<tr>
<td>(UK/UK)</td>
<td>21</td>
<td>42</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100</td>
<td>39</td>
</tr>
</tbody>
</table>

* One case was diagnosed at necropsy. UK = unknown mutation.

Discussion

The results obtained from this preliminary report show that the frequency of \(AF508\) in the sample studied (39%) is lower than that reported in several other countries, including two Hispanic populations such as Argentina (63%) and Spain (54%).

It is known that in developing countries a great number of children affected with CF die without diagnosis or appropriate treatment. This was observed at our Institute where only one \(AF508\) case was found in a sample of 2945 admissions during 1976, 1978, and 1980, while 32 were diagnosed in 3260 consecutive necropsies in the same hospital. The age of necropsy cases ranged from newborn to 4 years and only nine were diagnosed as having CF before death. This situation has probably changed because awareness of the disease has improved during the past decade. However, one patient in the present study was a 6 month old child who died at the Institute undiagnosed during his life but diagnosed at necropsy. The molecular study of a tissue sample confirmed homozygosity for \(AF508\).

We therefore have to consider that the patients in the present study may only represent the survivors and that patients homozygous for \(AF508\) with severe pulmonary and pancreatic disease might be more frequent among dead children than among living ones.

The other important consideration is the ethnic origin of the Mexican population which is mainly Amerindian–Hispanic, where Indian genes account for more than 50% and white (Hispanic) genes for 40%. The remainder being of black origin. Indian ancestry is also very different in Mexico from other Latin American populations such as Argentina. This difference could account for variations in the frequency of \(AF508\) or other CF mutations that must be analysed.

It is important to study a larger cohort taking into account the ethnic origin of the CF patients and to search for other common mutations in order to identify variations and to characterise the CF population attending clinics. It would also be interesting to carry out molecular studies on CF cases diagnosed post mortem to compare mutation frequencies in both dead and living patients.

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