Frequency of $\Delta F508$ in a Mexican sample of cystic fibrosis patients

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Abstract

This paper reports the frequency of the $\Delta F508$ mutation in a cohort of 50 Mexican patients with cystic fibrosis (CF). The mutation was detected by PCR mediated site directed mutagenesis. $\Delta F508$ 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 $\Delta F508$ mutation found in this preliminary report.

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Cystic fibrosis (CF) is the most common recessive disease among Caucasians.¹ The CF gene has recently been identified and characterised² and a major mutation consisting of a 3 bp deletion ($\Delta F508$) has been described.³ Over 200 mutations in the CF gene have now been identified; however, $\Delta F508$ is the most common and its frequency has been estimated in different populations showing wide variation.⁴ It occurs in about 70% of northern European and North American CF chromosomes, though it is found at a much lower rate in southern Europe.⁴⁵ The first study in a Latin American population came from Argentina, where $\Delta F508$ was found in 58 out of 92 (63%) CF chromosomes.⁴

In Mexico the population is heterogeneous but mainly Mestee (Amerindian-Hispanic) and the incidence of CF is unknown. Knowledge of the frequency of $\Delta F508$ as well as that of other mutations in a population is required for accurate genetic counselling. Therefore, we have analysed a cohort of CF patients attending the Cystic Fibrosis Clinic of the Mexican Association of Cystic Fibrosis-Instituto Nacional de Pediatría.

In this communication we report the data obtained on the $\Delta F508$ mutation in a sample of Mexican CF patients.

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 leucocytes according to standard protocols and the mutation was detected by PCR mediated site directed mutagenesis (PSM).^{6,7} The oligonucleotide primer sequences used were: 5'GCACCATTAAAGAAAATATGA-

T3' (forward), 5'CATTACAGTAGCTT-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.⁸ 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 μ g of DNA to 100 μ l amplification mixture containing 300 ng of the appropriate primers and 2.5 U of *Taq* DNA polymerase (Gibco), all in a solution containing 200 nmol/l of each deoxynucleotide 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 μ mol/l EDTA.⁷ 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 μ l 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.

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 $\Delta F508$ mutation showed the 219 bp fragment while compound heterozygotes carrying $\Delta F508$ and a different mutation showed 202 and 219 bp bands plus a heteroduplex fragment which is only observed with deletion mutations (figure).

The table shows the distribution of patients homozygous for $\Delta F508$, $\Delta F508$ compound heterozygotes, and cases with unknown mutations; the frequency of the $\Delta F508$ mutation on CF chromosomes was 39%.

Among the $\Delta F508$ homozygotes one was diagnosed after death at 6 months of age and the others had a mean age of 6 years when the molecular study was performed. The age of $\Delta F508$ /unknown compound heterozygotes was

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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, $\Delta F508/\Delta F508$ homozygote (proband). Lanes 3 and 4, $\Delta F508/wt$ heterozygous (obligate carriers). Lane 5, wild type homozygous (normal).

Frequency of the $\Delta F508$ mutation in 50 Mexican CF patients.

Genotype	CF cases		CF chromosomes	
	No	%	% $\Delta F508$	% UK
$\Delta F508/\Delta F508$	10*	20	20	0
$\Delta F508/UK$	19	38	19	19
UK/UK	21	42	0	42
Total	50	100	39	61

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

variable between 2 months and 34 years as it was for patients carrying unknown mutations.

Discussion

The results obtained from this preliminary report show that the frequency of $\Delta F508$ 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%).⁴⁹

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 CF case was found in a sample of 2945 admissions during 1976, 1978, and 1980,¹⁰ while 32 were diagnosed in 3260 consecutive necropsies in the same hospital.¹¹ 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 $\Delta F508$.

We therefore have to consider that the patients in the present study may only represent the survivors and that patients homozygous for $\Delta F508$ 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.^{12,13} 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 $\Delta F508$ 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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