

CYSTIC FIBROSIS: FREQUENCY OF $\Delta F508$ AND G542X MUTATIONS IN CORDOBA, ARGENTINA

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Summary Using Polymerase Chain Reaction (PCR) based techniques we analyzed the frequency and genotypic distribution of two mutations ($\Delta F508$ and G542X) that produce Cystic Fibrosis. The study was carried out in 19 non-related patients (38 chromosomes) born in the Province of Córdoba. The distribution of genotypes showed the presence of 8 homozygote patients $\Delta F508/\Delta F508$, 2 individuals with non-determined mutations (X/X) and 9 compound heterozygotes ($\Delta F508/X$). The mutation G542X was not found. The mutation $\Delta F508$ was detected in 25 chromosomes resulting in an incidence of 66%.

Key words: cystic fibrosis, $\Delta F508$ and G542X mutations, CF in Argentina

Cystic Fibrosis (CF) is the most common lethal autosomal recessive disease in whites. The disease is produced by an alteration of a chloride ion channel in the apical membrane in cells of exocrine glands that produces a decrease in chloride permeability and increase in sodium absorption^{1,2}. This dysfunction leads to impaired fluid secretion and the production of an abnormally thick exocrine secretion that results in pancreatic insufficiency and susceptibility to chronic obstructive pulmonary infections¹.

Since 1959 the diagnosis of the disease is confirmed by the quantitative determination of chloride and/or sodium concentration in sweat³. Although most patients show elevated sweat electrolyte concentrations (>60 mmol/L), exceptionally, some patients (~1%) show intermediate concentration values (45-59 mmol/L) between those found in normal and affected individuals that make a conclusive diagnosis difficult^{4,5}. Another pitfall

of the methodology is the failure to detect carriers of the disease who do not have an increased chloride concentration although they have only one functional allele.

The cloning and identification of the gene responsible for CF^{6,7}, the «Cystic Fibrosis Transmembrane Conductance Regulator» (CFTR) gene, allowed the finding of mutations that produce CF and the implementation of techniques to detect them. This new methodology can be used as an alternative for patients not detected by the biochemical test and for the identification of carriers of the disease.

In patients with CF, more than 400 different mutations have been described in the CFTR gene⁸. However, only a few mutations are found in high proportion, specially the mutation $\Delta F508$, a deletion of a phenylalanine in the position 508 of the protein, which represents approximately 70% of the worldwide CF mutations⁹.

The incidence of different mutations varies according to the ethnic group analyzed; for example the mutation $\Delta F508$ varies from 30% in Turkey to 80% in Denmark⁹. This variation makes the determination of the frequency of each mutation in a given region very important, before using this methodology as an alternative diagnostic test.

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Due to the lack of information in Córdoba about the frequency of mutations leading to CF, we started a screening program for the determination of $\Delta F508$ and G542X mutations in patients clinically diagnosed and in obligated heterozygotes.

Materials and methods

Analyses of the mutations were performed with amplified DNA fragments by the Polymerase Chain Reaction (PCR). Genomic DNA from leukocytes was prepared according to Nordvag et al.¹⁰. $\Delta F508$ was assayed according to Campbell et al.¹¹ using the polyacrylamide gel electrophoresis system described by Makowski et al.¹². G542X was detected according to Friedman et al.¹³. Patients of CF clinically diagnosed as well as the parents and some relatives were tested for the analysis of the mutations.

Results and Discussion

The frequency and genotypic distribution of the mutation $\Delta F508$ is shown in Table 1. From 38 mutated non related chromosomes 66% carried the $\Delta F508$ mutation. This mutation is present, at least in one chromosome, in 17 of the 19 patients analyzed.

The mutation G542X was not detected. Even though the number of chromosomes analyzed is not high, this result indicates that this mutation probably accounts for no more than 2-3% of the total mutations present in the region in coinci-

dence with the worldwide frequency of 3.2%⁷. A number of chromosomes 13 carries a non determined mutation different from $\Delta F508$ and G542X.

The frequency of $\Delta F508$ is similar to that reported in 1990 for Argentina (63%) by the Cystic Fibrosis Genetic Analysis Consortium¹⁴ and coincident to the global European frequency¹⁵. These percentages are in agreement with the relatively high proportion of Argentineans with European immigrant ancestors.

Since 1974, 225 cases of children with CF born in the Province of Córdoba were diagnosed by one of us (M. M. de Botelli) by the method of Gibson and Cooke³.

According to the annual growth rate of the Argentinean population (2.11%) it is possible to calculate for the Province of Córdoba (population: 2.8 million) 59,000 newborn per year. If the same frequency of CF is assumed for this population as in Caucasian populations (1 carrier/25 and 1 patient/2,500 individuals) approximately 2,400 carriers would be expected for Córdoba and 24 CF newborn per year. Since in the last three years in average, 11 CF newborn/year, were detected we would be detecting around half of the total cases. Anyway, if we were detecting all the cases, the incidence of the disease in Córdoba would be approximately one affected newborn every 5,400.

The convenience of mass population screening of CF mutations for detection of heterozygotes is controversial^{16, 17}. Besides ethical and psychological considerations, one of the main problems is the low sensitivity of the test for identification of mutations. In our case, 34% of the chromosomes remained without diagnosis. In some countries, the inclusion of some low frequency mutation increases the probability of detection to about 80-90% of the chromosomes but it may be very difficult to reach 100% due to the existence of many extremely rare mutations, some of them detected only once in a given population¹⁸.

The method is very useful for the detection of heterozygotes in families with disease antecedents whenever the obligate heterozygotes bear an identifiable mutation. However, since the mutation $\Delta F508$ is present in almost 90% of the patients (homozygotes plus compound heterozygotes; see Table 1), the determination of only this mutation is important as a first approach to decide further molecular analysis.

Table 1.— Genotypic Distribution of the Mutation $\Delta F508$ in CF Patients

| Genotype ¹ | Patients ² | Allele $\Delta F508$ | | Others (X) ³ | |
|---------------------------|-----------------------|----------------------|------|-------------------------|------|
| | | N ^o | (%) | N ^o | (%) |
| $\Delta F508/\Delta F508$ | 8 | 16 | 42.1 | | |
| $\Delta F508/X$ | 9 | 9 | 23.7 | 9 | 23.7 |
| X/X | 2 | 0 | 0 | 4 | 10.5 |
| TOTAL | 19 | 25 | 65.8 | 13 | 34.2 |

¹ In all cases the parents of the patients were analyzed to confirm the genotype.

² Patients were not related.

³ Mutation not determined, different from $\Delta F508$ y G542X. The presence of mutations in the CFTR gene in these individuals is assumed due to the clinical phenotype.

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Resumen

Fibrosis quística: frecuencia de las mutaciones $\Delta 508$ y G542X en Córdoba, Argentina

Utilizando técnicas basadas en la Reacción en Cadena de la Polimerasa (PCR) hemos analizado la frecuencia y distribución genotípica de dos mutaciones del gen CFTR ($\Delta F508$ y G542X) que ocasionan Fibrosis Quística. El estudio se realizó en 19 pacientes (38 cromosomas) fibroquísticos familiarmente no relacionados, nacidos en la Provincia de Córdoba. La distribución de genotipos indicó la presencia de 8 pacientes homocigotas $\Delta F508/\Delta F508$, 2 individuos con mutaciones no determinadas (X/X) y 9 heterocigotas compuestas $\Delta F508/X$. La mutación G542X no fue encontrada. La mutación $\Delta F508$ se detectó en 25 cromosomas representando una incidencia del 66%.

References

1. Boat TF, Weish MJ, Beaudet AL. Cystic Fibrosis. In: Scriver CR, et al. (eds). The metabolic basis of inherited disease, 6th ed. New York: McGraw Hill, 1989; 2649-80.
2. Quinton PM. Cystic fibrosis: a disease in electrolyte transport. *FASEB J* 1990; 4: 2709-17.
3. Gibson LE, Cooke RE. A test for concentration of electrolytes in sweat in cystic fibrosis of the pancreas utilizing pilocarpine by iontophoresis. *Pediatrics* 1959; 23: 545-9.
4. Davis PB, Hubbard VS, di Sant' Agnese PA. Low sweat electrolytes in a patient with cystic fibrosis. *Am J Med* 1980; 69: 643-6.
5. Stern RC, Boat TF, Abramowsky CR, et al. Intermediate-range sweat chloride concentration and Pseudomonas bronchitis. *JAMA* 1978; 239: 2676-80.
6. Riordan J, Rommens J, Kerem B, et al. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* 1989; 245: 1066-73.
7. Rommens J, Iannuzzi M, Kerem B, et al. Identification of the cystic fibrosis gene: chromosome walking and jumping. *Science* 1989; 245: 1059-65.
8. Claustres M, Laussel M, Desgeorges M, Demaille J. Identification of a 6 bp deletion (3195del6) in exon 17a of the cystic fibrosis (CFTR) gene. *Hum Mol Genet* 1994; 3: 371-2.
9. Tsui LC. The spectrum of cystic fibrosis mutations. *Trends in Genetics* 1992; 8: 392-8.
10. Nordvag B, Husby G, El-Gewely M. Direct PCR of washed blood cells. *BioTechniques* 1992; 12: 490-1.
11. Campbell III PW, Phillips III JA, Krishnamani MR, et al. Cystic Fibrosis: Relationship between clinical status and $\Delta F508$ deletion. *J Pediatr* 1991; 118: 238-41.
12. Makowaski GS, Aslanzadeh J, Hopffer S. Mini-gel PAGE for enhanced resolution of polymerase chain reaction detection of $\Delta F508$ deletion in cystic fibrosis. *Clin Chem* 1993; 39: 2204-5.
13. Friedman KJ, Highsmith WE, Silverman LM. Detecting multiple cystic fibrosis mutations by Polymerase Chain Reaction-mediated Site-Directed mutagenesis. *Clin Chem* 1991; 37: 753-5.
14. Cystic Fibrosis Genetic Analysis Consortium. Worldwide survey of the $\Delta F508$ mutation. Report from the Cystic Fibrosis Genetic Analysis Consortium. *Am J Hum Genet* 1990; 47: 354-9.
15. European Working Group on CF Genetics (EWGCFG). Gradient distribution of the major CF mutation and its associated haplotype. *Hum Genet* 1990; 85: 436-41.
16. Williamson R. Universal Community carrier screening for cystic fibrosis? *Nature Genet* 1993; 3: 195-201.
17. Bekker H, Dennis G, Modell M, et al. The impact of population based screening for carriers of cystic fibrosis. *J Med Genet* 1994; 31: 364-8.
18. Férec C, Audrezet MP, Mercier B et al. Detection over 98% cystic fibrosis mutations in a Celtic population. *Nature Genet* 1992; 1: 188-91.

The sense of existence is the greatest happiness.

La mayor felicidad está en la percepción de la existencia.

Benjamin Disraeli (1804-1881)