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CYSTIC FIBROSIS: FREQUENCY OF ΔF508 AND G542X MUTATIONS IN CORDOBA, ARGENTINA

MARIA C. SALEH[1], ALFREDO BOTELLI[2], MYRNA MELANO de BOTELLI[2], CARLOS A. REZZONICO[2], CARLOS E. ARGARAÑA[1]

[1] *Centro de Investigaciones en Química Biológica de Córdoba, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba;* [2] *Hospital de Niños, Córdoba*

Summary Using Polymerase Chain Reaction (PCR) based techniques we analyzed the frequency and genotypic distribution of two mutations (ΔF508 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 ΔF508/ΔF508, 2 individuals with non-determined mutations (X/X) and 9 compound heterozygotes (ΔF508/X). The mutation G542X was not found. The mutation ΔF508 was detected in 25 chromosomes resulting in an incidence of 66%.

Key words: cystic fibrosis, ΔF508 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 infections[1].

Since 1959 the diagnosis of the disease is confirmed by the quantitative determination of chloride and/or sodium concentration in sweat[3]. 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 gene[8]. However, only a few mutations are found in high proportion, specially the mutation ΔF508, a deletion of a phenylalanine in the position 508 of the protein, which represents approximately 70% of the worldwide CF mutations[9].

The incidence of different mutations varies according to the ethnic group analyzed; for example the mutation ΔF508 varies from 30% in Turkey to 80% in Denmark[9]. 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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Postal address: Dr. Carlos E. Argaraña, Centro de Investigaciones en Química Biológica de Córdoba, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Ciudad Universitaria CC 61, 5016 Córdoba, Argentina

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 ΔF508 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]. ΔF508 was assayed according to Campbell et al[11] using the polyacrylamide gel electrophoresis system described by Makowaski 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 ΔF508 is shown in Table 1. From 38 mutated non related chromosomes 66% carried the ΔF508 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 coincidence with the worldwide frequency of 3.2%[7]. A number of chromosomes 13 carries a non determined mutation different from ΔF508 and G542X.

Table 1.— *Genotypic Distribution of the Mutation ΔF508 in CF Patients*

Genotype[1]	Patients[2]	Allele ΔF508		Others (X)[3]	
		N°	(%)	N°	(%)
ΔF508/ΔF508	8	16	42.1		
ΔF508/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 ΔF508 y G542X. The presence of mutations in the CFTR gene in these individuals is assumed due to the clinical phenotype.

The frequency of ΔF508 is similar to that reported in 1990 for Argentina (63%) by the Cystic Fibrosis Genetic Analysis Consortium[14] and coincident to the global European frequency[15]. 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 Cooke[3].

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 population[18].

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 ΔF508 is present in almost 90% of the patients (homozygotes plus compound hetero-zygotes; see Table 1), the determination of only this mutation is important as a first approach to decide further molecular analysis.

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Resumen

Fibrosis quística: frecuencia de las mutaciones Δ508 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 (ΔF508 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 ΔF508/ΔF508, 2 individuos con mutaciones no determinadas (X/X) y 9 heterocitogas compuestos ΔF508/X. La mutación G542X no fue encontrada. La mutación ΔF508 se detectó en 25 cromosomas representando una incidencia del 66%.

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The sense of existence is the greatest happiness.

La mayor felicidad está en la percepción de la existencia.

Benjamin Disraeli (1804-1881)