Frequency of F508del Variant in Patients with Cystic Fibrosis from Paraguay

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Abstract

Cystic fibrosis (CF) is an autosomal recessive disorder and is caused by variants in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene. We aimed to study the frequency of the F508del variant, the most common variant worldwide, in patients with CF from Paraguay. The frequency of the F508del variant in Paraguayan patients with a clinical diagnosis of CF was assessed using a polymerase chain reaction followed by the sequencing of the PCR products. 43 of the 86 patients (50%) were homozygous for the F508del variant, 28 were heterozygous (32.56%), and the remaining 15 (17.44%) were non-carriers. In terms of alleles, there were 114 mutated (114/172 or 66.28%) and 58 did not correspond to this variant (58/172 or 33.72%). This is the first study of the frequency of the F508del variant in patients with CF in Paraguay. This information is of utmost relevance when planning and offering treatments from health services.

Keywords

CTFR, variant, PCR, ΔF508.

Introduction

Cystic fibrosis (CF) is one of the most common autosomal recessive disorders and it is caused by variants in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene. These variants cause a multisystemic alteration, which involves lung damage, exocrine pancreatic insufficiency, liver disease, intestinal motility disorders, and high concentrations of chloride in sweat [1].

The gene that encodes the CFTR protein is located on the long arm of chromosome 7, at position q31.2. To date, more than 2110 gene variants in the sequence of this protein have been reported, only 15 % off them not associated with clinic manifestations or desiase [2–4].

Despite the diversity of variants in this gene, the frequency of one of them, the F508del variant, is remarkably high worldwide. This variant results from a specific 3 base pair deletion of the CTT triplet in the coding region (exon 10) of the CFTR gene, which results in the loss of a phenylalanine residue at position 508 of the amino acid [3–5].

Due to the ethnic diversity and heterogeneity of genetic backgrounds in Latin-American population, the allelic prevalence of the p.(Phe508del) variant very according to geographical region with a rage from 22.92% in Costa Rica to 59.1% in Argentina. Even in sames country the prevalence reported is different by regions, as we can see in northeastern Mexico whith 61.5% meanwhile in the southeastern is 35.7% [6–8].

In 2017, the average incidence of CF in Paraguay was 1 in 5,112 newborns [7], but there is no work on the frequency of variants in the country. This work has been carried out to determine the frequency of the F508del variant, in the CTFR gene, in the group of people who carry out their treatment in the National Neonatal Detection Program.

Methodology

Eighty-six patients with a clinical diagnosis of CF born in the different departments of Paraguay and followed by the "National

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Neonatal Detection Program" were analyzed. The age range of the patients was between 1 and 25 years. All patients included in this study were diagnosed on the basis and standard clinical and laboratory criteria agreed upon Rosenstein & Cutting (1998) [9]. Chloride concentrations in sweat were measured using a Sweat Check conductivity analyzer (Wescor, USA). All participants or their legal representatives previously signed an informed consent.

Between January 2017 and July 2018, peripheral blood samples (5ml) were collected by venipuncture in tubes with EDTA. Of the 86 patients analyzed, 4 patients already had results of a previous molecular study and submitted them so that those data could be part of the study.

The extraction and purification of genomic DNA from the blood samples were carried out using the commercial GeneJET Genomic DNA Purification Kit (# K0722 Thermo Scientific®), following the manufacturer's instructions. The purity of the genetic material was evaluated using a spectrophotometer (DS-11FX + DeNovix®).

In order to analyze the presence of the F508del variant, exon 10 of the CFTR gene was amplified by PCR using the primers described by Kerem *et al.* (1989) [4]· C16B 5'-GTTTTCCTGGAT TATGCCTGGGCAC-32' and C16D 5'-GTTGGCATGCTTT GATGACGCTTC-3'. Amplification was carried out with the Rotor-Gene Q thermocycler (Qiagen®) in a final volume of 25 µl. Subsequently, the amplification products were analyzed by means of vertical electrophoresis in 6% polyacrylamide gels (PAGE). The gels were then silver stained, washed and fixed. Samples from previously diagnosed and genotyped patients were used as positive controls. To confirm the results, all PCR products were sequenced at Macrogen (Geumcheon-gu, Seoul, Korea). Comparison of the sequences obtained in GenBank allowed us to identify the F508del variant.

Results

All of the 86 samples from the Cystic Fibrosis Prevention Program were molecularly analyzed. In order to identify the presence of the F508del variant, a conventional PCR and subsequent sequencing of the products were performed. Figure 1 shows the detection of PCR products in a 6% polyacrylamide gel, where patients homozygous for the F508del variant present a single 95bp band, which represents both alleles with the variant; heterozygotes have a 95 bp and a 98 bp product, indicating an allele with the F508del variant and a non-carrier allele (wild type); and finally, in unaffected or non-carrier individuals, a band of 98 bp is observed, which represents the two non-mutated alleles.

Of the 86 patients included in the study (Table 1), 41 were female and 45 were male. The F508del variant was detected in 71 people (82.56%) and 15 people did not present this variant (17.44%). Of the positive patients, 43 were homozygous (60.56%) (27 men and 16 women) and 28 were heterozygous (39.44%) (13 men and 15 women). In terms of alleles, 114 were detected with the F508del variant (66.28%) and 58 did not correspond to this variant (33.72%).

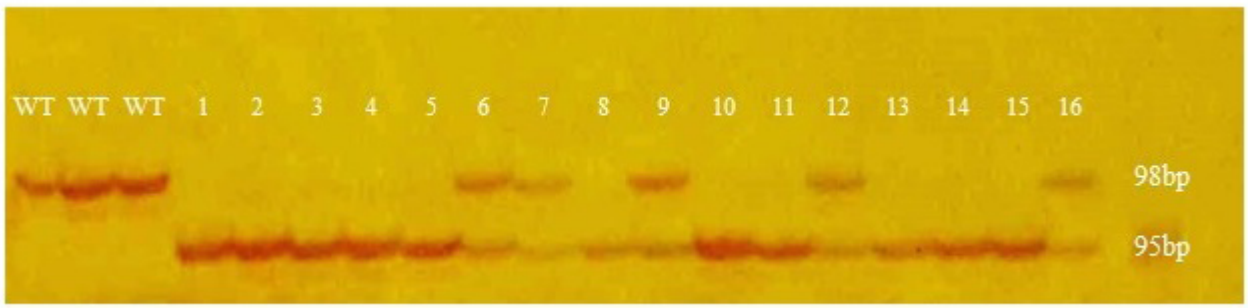

Figure 1. Detection of the F508del mutation by PCR on a 6% polyacrylamide gel. WT: positive non-carrier controls (two 98bp fragments). Lanes 1, 2, 3, 4, 5, 8, 10, 11, 13,14 and 15 are homozygous patients for the variant (two 95bp fragments). Lanes 6, 7, 9, 12 and 16 are heterozygous patients for the variant (one 95bp and one 98bp fragment). MWM: 25 bp molecular weight marker.

Table 1. Age, sex and genotype of CF patients included in the study.

Age range	F508del/ F508del		F508del/X		X/X	
	Men	Women	Men	Women	Men	Women
0-5	12	6	8	6	3	4
6-10	11	5	2	4	0	4
11-15	2	5	2	4	1	2
16-20	1	0	1	0	1	0
21-25	1	0	0	1	0	0
Variant %	43 (60.56%)		-28 (39.44%)		–	
	71 (82.56%)				15 (17.44%)	

X: wild-type alleles or alleles with other variants

Discussion

CF is a common and serious autosomal recessive disease that affects many populations throughout the world. In 2017, the average incidence of CF in Paraguay was 1 in 5,112 newborns [10], however, the frequency of CTFR gene variants associated with CF is unknown. This study constitutes the first molecular report of the frequency of the F508del variant in patients with cystic fibrosis in Paraguay.

The F508del variant is the most common variant of the CTFR gene worldwide, but its incidence varies in different regions. In Europe, this variant presents a descending gradient from northwest to southeast, with a maximum (87.2% of all CF chromosomes) in Denmark and a minimum (31.0%) in Lithuania, the average for the European continent being 61.4% [5]. In the United States, the average frequency of the F508del variant is 68.6%, but this value varies in each ethnic group present in the country [5]. In Latin America the average frequency of this variant is 45%, with Costa Rica being the country with the lowest frequency reported, with a frequency of 22.92%, and the highest frequency recorded so far is Argentina with 59.15% of incidence [6].

In this study, a frequency of 66.28% of alleles with the F508del variant is reported in Paraguay, being the highest frequency recorded for the continent. 82.56% of the people included in this study had at least one allele with this variant. This information is of utmost relevance when planning and offering treatments from health services, considering that CF molecular research has resulted in the development of new specific therapies according to the different classes of CTFR gene variants. The use of specific treatments for the F508del variant would result in the improvement of conditions and life expectancy for a large proportion of CF patients in the country.

It is essential to expand this type of study to be able to identify the true frequency of the allele in the population to recognize possible carriers, and complement it by analyzing the frequency of other variants of the CFTR gene. Thus, there will be more information on the manifestation and prevalence of this disease in the Paraguayan population.

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Declaration of Conflicting Interests

The authors declare that there are no conflicts of interest.

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