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Frequency of common CFTR gene mutations in Venezuelan patients with cystic fibrosis.

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Abstract. Mutations in the CFTR gene in Cystic Fibrosis (CF) patients have geographic differences and there is scant data on their prevalence in Venezuelan patients. This study determined the frequency of common CFTR gene mutations in these patients. We amplified and sequenced exons 7, 10, 11, 19, 20 and 21, which contain the most common CFTR mutations, from 105 Venezuelan patients in the National CF Program. Eleven different mutations were identified, four with frequencies greater than 1%: p.Phe508del (26,17%), p.Gly542X (3,33%), p.Arg334Trp (1,43%) and p.Arg1162X (1.43%). No mutations were found in 63.3% of patients. This report represents the largest group of Venezuelan CF patients ever examined and includes a wider mutation panel than has been previously studied in this population. Southern European CFTR mutations predominate in the Venezuelan population, but a high percentage of the causative alleles remain unidentified.

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Frecuencia de mutaciones comunes en el gen CFTR en pacientes venezolanos con fibrosis quística.

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Palabras clave: fibrosis quística, CFTR: regulador de la conductancia transmembrana de la fibrosis quística, Venezuela, p.Ser1235Arg, p.Glu1308X, p.Leu558Ser.

Resumen. Mutaciones en el gen CFTR en pacientes con Fibrosis Quística tienen diferencias geográficas y hay escasos datos de su prevalencia en pacientes Venezolanos. Este estudio determinó la frecuencia de mutaciones comunes presentes en el gen CFTR en estos pacientes. Nosotros examinamos los exones 7, 10, 11, 19, 20 y 21, que contienen las mutaciones más comunes reportadas, de pacientes Venezolanos del Programa Nacional de FQ, usando la reacción en cadena de la polimerasa y secuenciación automatizada. Once mutaciones diferentes fueron identificadas en 105 pacientes estudiados. Las mutaciones con frecuencias mayores a 1% fueron p.Phe508del (26,17%), p.Gly542X (3,33%), p.Arg334Trp (1,43%) y p.Arg1162X (1.43%). En el 63,35 de los pacientes ninguna mutación fue encontrada. Este reporte representa el grupo más grande de pacientes Venezolanos con FQ que ha sido examinado e incluido en el más amplio panel de mutaciones que ha sido examinado en esta población. Las mutaciones en el gen CFTR predominantes en el sur de Europa resultan ser las más predominantes en la población venezolana, pero un alto número de alelos resulta aún desconocido.

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INTRODUCTION

Cystic fibrosis (CF; MIM # 219700) is caused by mutations in the gene encoding the chloride channel –Cystic Fibrosis transmembrane conductance regulator (CFTR; #602421) (1-3)– the seventh member of the C subfamily of the ATP-binding cassette (ABC) transporter gene super family (ABCC7) (4). CFTR is located at chromosomal region 7q31.2 and is comprised of 27 exons (6) that span approximately 190 kb of genomic DNA (5).

Cystic fibrosis (CF) is a severe autosomal recessive disorder and more than 1400 different CFTR causative gene mutations have been reported (7). The distribution of these mutations varies among different populations according to the geographical and ethnic origin of patients (8, 9). A few mutations, such as p.Phe508del (Exon 10), p.Asn1303Lys (Exon 21) and p.Gly542X (Exon 11) are frequent worldwide. A review of studies from Latin American countries reported that the following mutations were found with a frequency greater than 1%: p.Phe508del (Exon 10), p.Gly542X (Exon 11) p.Asn1303Lys (Exon 21), p.Trp1282X (Exon 20) and p.Arg1162X (Exon 19) (10). In Venezuela, the true incidence of this disease is unknown. Previous studies have focused their analysis on defining the frequency of the Delta F508 mutation, which was reported as 50% (n= 41) and 79% (n=30) (11, 12), but there are no

reports of the CFTR mutations found in the other 21%-50% of Venezuelan CF patients.

The CFTR mutations that cause CF vary in different ethnic groups, and the Venezuelan population contains a mix of different ethnicities (13, 14). The aim of this study was therefore to look for the CFTR mutations reported as the most common in other populations –those located in exons 10, 11, 19, 20 y 21– in Venezuelan patients with a clinical diagnosis of CF, who are in the National CF Program. This information can be used for implementing a diagnostic method that could rapidly identify a large percentage of CF patients in the Venezuelan population.

PATIENTS AND METHODS

Patients and sampling

From 2011 to 2013 we recruited 105 patients from an equal number of unrelated families in the National CF Program of the Ministry of Health of Venezuela. The National CF Program incorporates patients who meet the program's diagnostic criteria of classic signs and symptoms of the disease and have corroborative laboratory results (15). Most of these patients, however, lack molecular confirmation of the disease and the implementation of routine molecular diagnosis is one of the long-term goals of this work.

Detailed questionnaires, including clinical and family history, were obtained from each participating family. Local Ethics Committees approved the study and informed consent was obtained from all participating families.

For data analysis, this study divided the patients into two groups based on family origin: 1) those whose families had been in Venezuela for at least three generations and 2) the rest of the population. The patients in the two groups were of similar for age distribution and sex ratio.

Molecular analysis

A peripheral blood sample was collected from each patient and genomic DNA was extracted using the QIAamp DNA Mini Kit (QIAGEN). Amplification of six complete CFTR exons (7, 10, 11, 19, 20 and 21) was performed using CFTR gene specific primers previously described for CFTR analysis (16, 17) and the amplicons were sequenced in both directions on an ABI-3130 XL Genetic Analyzer (Applied Biosystems). The sequences were analyzed with the Sequencing Analysis 5.3.1 and SeqScape softwareV2.5 programs (Applied Biosystems).

Data analysis

Statistical analysis was carried out using program R version 3.0.1 and Microsoft Excel 2007-based data sheets.

RESULTS

All of our patients were in the National Program for Cystic Fibrosis. Seven families had more than one sibling with CF: six cases had 2 affected siblings and one case had 3 affected siblings. We included only one case per family for the statistical analysis, which resulted in 210 alleles derived from 105 patients. In our cohort there were 36 females (34.28%) and 69 males (65.72%), with ages ranging from 2 months-old to 30 years-old. Sixty of these patients were at least third-generation Venezuelan (57%) and 45 patients (43%) were either foreign born or children of parents who were not born Venezuelan.

Mutation analysis.

Sixteen mutant genotypes were found in 105 patients, which are shown in Table I. In 28/105 patients (26.67%) two mutations were identified, while in 21/105 patients (20%) only one mutation was detected. Figs. 1 and 2 show partial electro-

TABLE I
GENOTYPE FREQUENCY OF MUTATIONS FOUND FOR THE SIX EXONS STUDIED

Alelle 1	Alelle 2	N	%
WT	WT	56	53.33
p.Phe508del	p.Phe508del	17	16.19
p.Phe508del	WT	14	13.33
p.Gly542X	WT	2	1.90
p.Arg334Trp	WT	2	1.90
p.Arg1162X	WT	2	1.90
p.Asn1303Lys	p.Phe508del	2	1.90
p.Arg553X	p.Phe508del	1	0.95
p.Leu558Ser	p.Phe508del	1	0.95
p.Glu1308X	p.Gly542X	1	0.95
p.Trp1282X	WT	1	0.95
p.Gly542X	p.Phe508del	1	0.95
p.Arg334Trp	p.Phe508del	1	0.95
p.Ser549Arg	p.Phe508del	1	0.95
p.Gly542X	p.Gly542X	1	0.95
p.Arg1162X	p.Gly542X	1	0.95
p.Ser1235Arg	p.Ser1235Arg	1	0.95

Variants are described using the designation of amino acid changes at the protein level (p.) as recommended by the Human Genome Variation Society (18).Wt: (Wild Type) indicates no mutation was found.

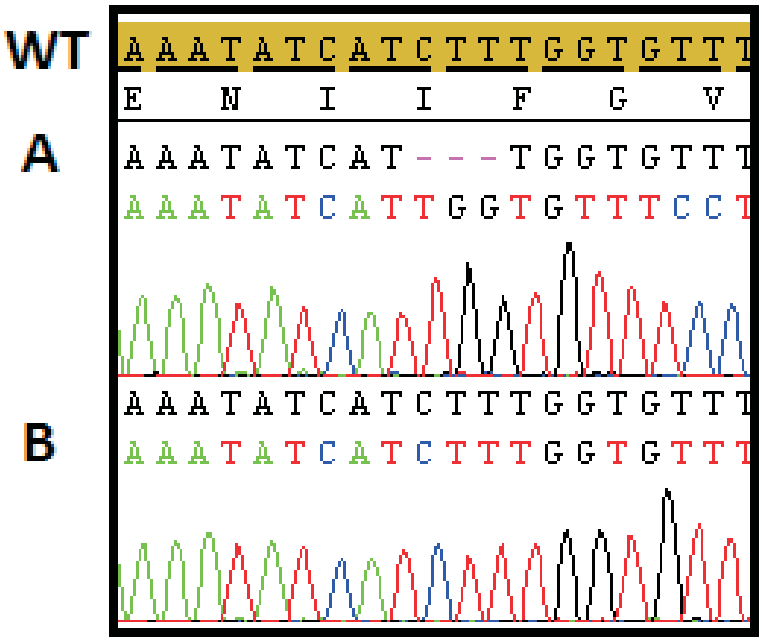

Fig. 1. Electropherograms showing part of a DNA sequence of the region of exon 10 of the CFTR gene, in which is observed the presence of the deletion of the triplet CTT, coding for a phenylalanine at position 508 (c.1521_1523delCTT). (WT) reference sequence, (A) Top: presence of the homocygote deletion, (B) Bottom: wild type sequence.

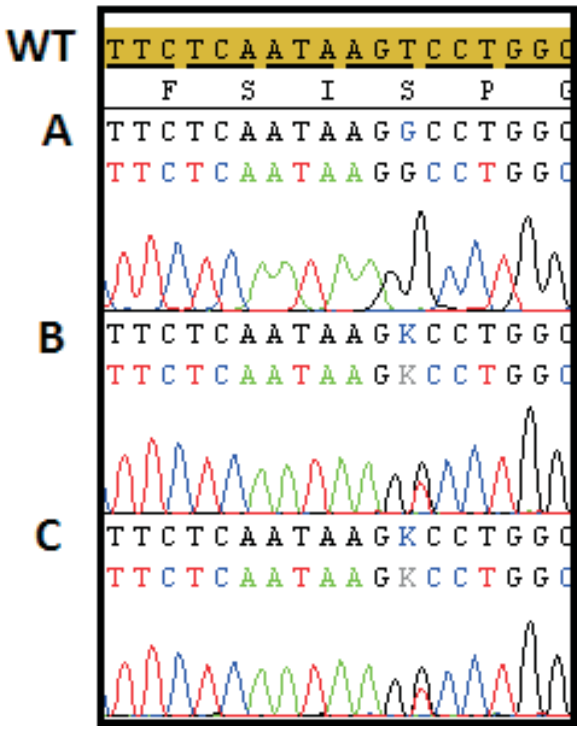

Fig. 2. Electropherograms showing part of a DNA sequence of the region of exon 19 of the CFTR gene, corresponding to the p.Ser1235Arg (c.3705T>G) mutation in which is observed the presence of mutation, (WT) reference sequence, (A) Top: presence in Homocygotecondition (patient), (B and C) Bottom: mutation Heterocygote condition (both parents).

pherograms of some of the mutations detected.

Seventeen patients (16.2%) were homozygotes for p.Phe508del, one patient (0.95%) was a homozygote for p.Ser1235Arg and one patient (0.95%) was a homozygote for p.Gly542X. Thirty patients were compound heterozygotes: twenty one patients (20%) had a WT allele, seven patients (5.7%) had one p.Phe508delallele and another mutated allele, and two patients (2.8%) had two alleles different than p.Phe508del. In the remaining 56 patients (53.33%), we failed to detect any CFTR mutation in the exons studied.

The allelic frequency obtained in this study is shown in Table II, which also shows the frequencies reported for all of the mutations in the exons examined both in Latin America and the rest of the world. The p.Phe508del mutation had the highest prevalence and another four mutations had frequencies greater than 1% (p.Gly542X, p.Arg334Trp, p.Asn1303Lys and p.Arg1162X). Three additional mutations had not been previously reported in the Venezuelan population: p.Ser1235Arg (exon19), p.Glu1308X (exon 21) and p.Leu558Ser (exon 11) (Table II).

The allelic frequencies reported for p.Phe508del are lower than have been previously reported. In Latin America a total of 26 mutations have been described in the six exons we studied and of these we found 11 in the Venezuelan population. We also found three mutations that had not been previously reported in Latin America (p.Ser1235Arg in exon19, p.Glu1308X in exon 21 and p.Leu558Ser in exon 11), but have been reported as non-common mutations in the Cystic Fibrosis Mutation Database (7) (Table II).

Analysis of the Venezuelan population, individuals of third generation

In order to determine whether there are any endogenous Venezuelan mutations, we compared the mutations in those patients who were at least third generation Venezuelans to those whose families who arrived in Venezuela more recently. For this purpose, we divided the population studied into two groups, as shown in Table III.

There were no significant differences ($p = 0.34$) between the allelic frequencies of the two groups shown in Table III, when analyzed by an independency test based on the Chi-square, nor when the same data was analyzed using 2×2 contingency tables. We then performed an additional analysis

TABLE II
ALLELIC FREQUENCY OF FOUND MUTATIONS FOR THE SIX EXONS STUDIED
(Data of exons 10, 11, 19,20 and 21)

Mutation	This study	Perez et al. (10)	CFGAC (19)
		(n = 4102) (%)	(n = 43,849) (%)
		Latin-America	Common mutation
Unknown	63.33	37.21	22.7
p.Phe508del	26.19	46.7	66
p.Gly542X	3.33	5	2.4
p.Arg334Trp	1.43	0.9	0.1
p.Arg1162X	1.43	1	0.3
p.Ser1235Arg	0.95	-	-
p.Asn1303Lys	0.95	1.7	1.3
P.Trp1282X	0.48	1.1	1.2
p.Ser549Arg	0.48	0.1	0
p.Arg553X	0.48	0.5	0.7
p.Leu558Ser	0.48	-	-
p.Glu1308X	0.48	-	-
p.Arg347Pro	0	0	0.2
p.Tyr362X	0	0.02	-
p.Phe316LeufsX12	0	0.02	0.1
p.Ile506Thr	0	0.05	-
p.Ile507Del	0	0.2	0.2
p.Ser549Asn	0	0.1	0.1
p. Gly551Asp	0	0.1	1.6
p. Gly551Ser	0	0.1	-
p.Ala559Thr	0	0.02	-
p.Gly551ValfsX8	0	0.02	-
p.Trp1204X	0	0.02	-
p.Gln1238X	0	0.02	-
p.Lys1177SerfsX15	0	0.1	0.1
p.Arg1283Met	0	0.02	-
p.Ser1297PhefsX5	0	0.05	-

Variants are described using the designation of amino acid changes at the protein level (p.) as recommended by the Human Genome Variation Society(18).

TABLE III
ALLELIC FREQUENCY OF MUTATIONS FOUND FOR THE SIX EXONS STUDIED FOR EACH GROUP

Allele	Venezuelans		Foreigners	
WT	81	67.5	52	57.78
p.Phe508del	29	24.2	26	28.89
p.Gly542X	3	2.5	4	4.44
p.Asn1303Lys	2	1.67	0	0
p.Trp1282X	1	0.83	0	0
p.Ser549Arg	1	0.83	0	0
p.Arg553X	1	0.83	0	0
p.Arg334Trp	1	0.83	2	2.22
p.Arg1162X	1	0.83	2	2.22
p.Ser1235Arg	0	0	2	2.22
p.Leu558Ser	0	0	1	1.11
p.Glu1308X	0	0	1	1.11
Total	120	100	90	100

Variants are described using the protein designation, Wt: (Wild Type) no mutation was found (18).

–NMDS (Non-metrics multidimensional scaling)– based on the genetic distance between individuals according to their origin (20) (Fig. 1). The results of this analysis show a group of individuals separated towards the inferior left corner of the plot, which corresponds to the third generation Venezuelans (indicated with an"X" symbol in Fig. 1). In the center of the plot there is a mix of individuals that are difficult to discriminate. The upper right corner of the plot shows a group of individuals that corresponds to non-Venezuelans (indicated with a "Δ" symbol in Fig. 3). The test's stress level indicates that there is a good correlation between the dissimilarity of the individuals measured from the genetic distances and the graphic representation. This analysis shows that while there is a tendency for separation of the two groups, there is also considerable overlap, which confirms the results obtained by the aforementioned tests.

DISCUSSION

This study examined a larger group of Venezuelan CF patients with a more comprehensive mutation panel than has been previously reported, including six complete CFTR gene exons containing mutations p.Phe508del (exon 10), p.Gly542X (exon 11), p.Asn1303Lys (exon 21), p.Trp1282X (exon 20) and p.Arg1162X (exon 19), all of which have frequencies greater than 1% in Latin American countries (10). Similar to other studies from this region, we found relatively low detection rates and high allelic heterogeneity, compared to studies in Europe and the US population of European descent, as reported in the Cystic Fibrosis Mutation Database (7).

As in most countries, the allele p.Phe508del was the most common mutation detected in our patients. Consistent with the findings previously reported from Venezuela, the p.Phe508del allele had a fre-

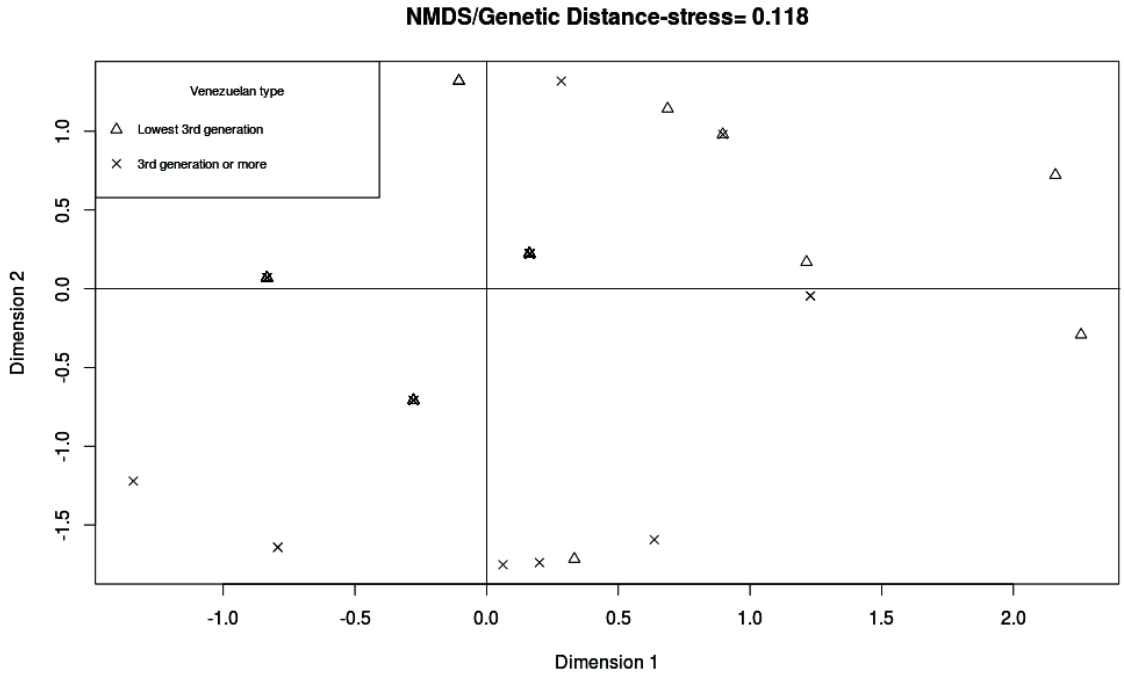

Fig. 3. NMDS ordination (2-Dimensional) of genetic distance matrix representative of the frequency allele distribution on study group. The symbol "X" corresponds to the third generation Venezuelans. The symbol "Δ" corresponds to foreign populations.

quency of 26.79% (12). Similar results have been reported in other Latin-American countries, indicating a higher frequency of heterogeneous mutations in this region (6) than in European countries, where the frequency of the p.Phe508del allele is greater than 45% (7).

The Venezuelan population evolved from an unequal mix of Caucasian-Spanish (58.8%), Amerindian (28.5%) and African (12.6%) origins (13).We wished to compare the patients from "Venezuelan" families with this mixed ancestry to those from families recently immigrated to Venezuela. We found a difference in the frequency of mutations in the Venezuelan patients compared to those in the recent immigrants, but when analyzed by statistical tests, (chi-square and NMSD analysis), the differences were not statistically significant. It is possible, however, that the differences might prove to be significant if a larger group of patients and a broader panel of mutations were analyzed.

Four of the mutations commonly found in Southern European CF patients were the most common mutations found in Venezuelan patients: p.Phe508del (26.19%), p.Gly542X (3.33%), p.Arg334Trp (1.43%) and p.Arg1162X (1.43%) (7). However, in over half (53.3%) of the Venezuelan patients the mutations remain uncharacterized (Table 1) –no mutations were found in the exons we examined. These patients may have a European origin, may stem from Amerindians ancestry and thus an Asian origin, or have novel private mutations. Another possibility is that the high percentage of patients for whom no mutations have been found could be an indication that some of the patients do not really have CF and have been misdiagnosed. It is also likely that some mutation(s) may be shared with other countries in South America whose populations have similar ethnic mixes.

Three mutations –p.Ser1235Arg exon19, p.Glu1308X exon21 and p.Leu558Ser exon11– designated as non-

common in the Cystic Fibrosis Mutation Database (7), were found in three patients in the immigrant group who had respiratory and pancreatic insufficiency compatible with a diagnosis of CF. The specific characterization of these cases is as follows: 1. The p.Ser1235Arg mutation was homozygous in a patient whose parents immigrated from the neighboring country of Colombia and were heterozygotes for the mutation (Fig. 2). This mutation has been previously reported in Caucasian CF patients and patients with idiopathic pancreatitis, but phenotypic manifestations appear to be variable because the mutation has only has been reported in the heterozygous state (7, 21-23). 2. The p.Glu1308X mutation was found to be heterozygotic, p.Glu1308X/p.Gly542X, in a patient with German paternal ancestry and Spanish maternal ancestry. This mutation has been previously reported in the same heterozygotic condition, p.Glu1308X/p.Gly542X, in French CF patients (7, 24). 3. The p.Leu558Ser mutation was found to be heterozygotic, p.Phe508del/p.Leu558Ser, in a patient with maternal and paternal Colombian ancestry. This mutation was reported with the same heterozygocity in one Italian CF patient (7, 25). In the last two cases it wasn't possible to obtain samples from both parents, so the exon in question was sequenced three times in order to confirm the presence of each mutation.

This study includes the largest group of CF patients ever studied in Venezuela. Since all patients included in this study come from the national FQ program, the clinical diagnostic criteria for all patients were the same. We used an automatic sequencing method which allowed higher sensitivity and resolution of the mutations detected in this study, in comparison with methods used in previous studies in Venezuela. For the six exons studied here, we sequenced at least 1823 base pairs from each patient. Even though only three new mutations were observed, we also documented the absence of other mutations in these exons, which allows for a better characterization of our population at the molecular level. These results are important for genetic counseling of patients and their family groups.

Sequencing of additional exons and intron junctions (17, 19) and a search for CFTR genomic rearrangements (20, 21), are needed to detect possible regionally prevalent mutations and rare (private) mutations in the 53.3% of Venezuelan patients in whom no mutation was found in the six exons examined. Identification of other mutations common in our population would allow us to improve clinic diagnostic confirmation, carrier analysis and genetic counseling, and would assist in the future development of a cost-effective newborn screening program.

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