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CFTR allelic heterogeneity in Mexican patients with cystic fibrosis: implications for molecular screening

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ABSTRACT

Introduction. Cystic fibrosis, the most common autosomal recessive disorder, is caused by defects in the CF transmembrane conductance regulator gene (*CFTR*) that encodes a chloride channel. To date, over 1,800 mutations have been described related to the causative gene of CF, showing a variable frequency among populations. In a previous extensive analysis of the *CFTR* locus in 97 Mexican patients, 34 different mutations (75% of CF alleles) were found using several strategies for mutation screening; however, 63% had at least an uncharacterized allele. Despite the combined technologies used, there are still a great number of unknown mutations in the Mexican population. **Objective.** Screening of the *CFTR* gene to provide additional evidence of the mutational wide spectrum responsible for CF in Mexican patients. **Material and Methods.** In this study, the number of unrelated CF patients was increased to 230, 133 new cases and the 97 previously reported to include 63% with at least an uncharacterized allele. Additional tools were used to improve the detection rate of CF mutations, such as a commercial kit for 36 mutations plus a single chain conformational polymorphism method and DNA sequencing. **Results.** By using a combination of these strategies we characterized 77.7% of all the CF alleles, resulting in a total of 46 different mutations detected, including the identification of 12 additional mutations (p.R334W, p.A455E, c.3120+1G > A, c.3272-26A > G, c.711+1G > T, p.Q552X, p.W1282X, c.IVS8-5T, p.R1162X and p.R347P, p.D1152H and p.T1036N). Although these 12 mutations have been reported in other populations, they have not yet been reported in Mexican patients. This report shows that Mexico has one of the widest spectra of *CFTR* mutations worldwide. The knowledge of the ethnic and geogra-

Heterogeneidad alélica de CFTR en pacientes mexicanos con fibrosis quística: implicaciones para el tamizaje molecular

RESUMEN

Introducción. *La fibrosis quística, la enfermedad autosómica recesiva más común, es ocasionada por defectos en el gen regulador de la conductancia transmembranal de la fibrosis quística (CFTR), que codifica para un canal de cloro. Hasta la fecha se han descrito más de 1,800 mutaciones relacionadas con este gen, que muestran una frecuencia variable entre las distintas poblaciones. En un análisis previo del locus CFTR en 97 pacientes mexicanos, fueron detectadas 34 diferentes mutaciones (75% de los alelos FQ) mediante diversas estrategias; sin embargo, 63% tenían al menos un alelo sin caracterizar. A pesar de la combinación de diferentes tecnologías utilizadas, aún hay un gran número de mutaciones desconocidas en la población mexicana.* ***Objetivo.*** *Tamizar el gen CFTR como una evidencia adicional del amplio espectro mutacional responsable de la FQ en pacientes mexicanos.* ***Material y Métodos.*** *En este estudio se incluyeron 230 pacientes no relacionados con FQ, 133 casos nuevos y 97 previamente reportados, de estos últimos 63.9% tenían al menos un alelo no caracterizado. Para mejorar la tasa de detección de las mutaciones FQ se utilizó un kit comercial para 36 mutaciones y el método de polimorfismo conformacional de cadena sencilla, seguido de secuenciación.* ***Resultados.*** *Al utilizar una combinación de estrategias, en este trabajo se logró caracterizar 77.7% de todos los alelos FQ, detectando un total de 46 mutaciones diferentes, incluyendo la identificación de 12 mutaciones adicionales (p.R334W, p.A455E, c.3120+1G > A, c.3272-26A > G, c.711+1G > T, p.Q552X, p.W1282X, c.IVS8-5T, p.R1162X,*

phic distribution of *CFTR* mutations in this population will allow the development of more effective methods for diagnosis and treatment.

Key words. *CFTR* gene. Mutations. Cystic fibrosis. Mexican patients.

p.R347P, p.D1152H, p.T1036N). Aunque estas 12 mutaciones han sido reportadas en otras poblaciones, ellas no habían sido reportadas en pacientes mexicanos. Este reporte muestra que México presenta uno de los espectros más amplios de mutaciones de CFTR a nivel mundial. El conocimiento de la distribución étnica y geográfica de las mutaciones de CFTR en esta población permitirá el desarrollo de métodos más eficaces para el diagnóstico y tratamiento de la enfermedad.

Palabras clave. *Gen CFTR. Mutaciones. Fibrosis quística. Pacientes mexicanos.*

INTRODUCTION

Cystic fibrosis (CF, MIM# 219700), the most common of autosomal recessive diseases, has an incidence in Caucasians of 1 in 2,000–4,000 live newborns, and shows a variable frequency among populations.[1,2] The incidence of this disorder in Latin America is unknown; however, in Mexico, the Mexican Cystic Fibrosis Association (AMFQ) has estimated that it is approximately 1 in 8,000 live newborns (personal communication).

CF is caused by defects in the CF transmembrane conductance regulator gene (*CFTR*, MIM# 602421), which encodes a chloride channel. Defects in the *CFTR* gene result in chronic obstructive lung problems, pancreatic failure, elevated sweat electrolytes and male infertility.[3-5] Clinically, the disease has a variable expressivity that ranges from mild to severe.[6]

To date, over 1,800 different mutations have been reported for the *CFTR* gene.[7,8] The distribution and frequency of the mutations in the Mexican population is different from those reported for populations that are genetically homogeneous[9-13] or admixtures, such as those from Brazil, Colombia, Uruguay, Argentina and Ecuador.[14-18] In an extensive *CFTR* locus analysis of 97 Mexican CF patients, Orozco, *et al.*[9] found that this population displayed a wide spectrum of mutations in the *CFTR* gene, with 34 different mutations having been characterized; however, only 75% of the *CFTR* alleles were identified. In populations such as Mexican, with high heterogeneity derived from admixture, it is possible that several techniques must be combined in order to detect and characterize a greater number of mutations in patients with CF.[19] In the present study, we provide additional evidence of the mutational wide spectrum responsible for CF in Mexican patients increasing the number of mutations of the previously characterized 34 to 46.

MATERIALS AND METHODS

Patients

A total of 230 CF patients were studied, including 133 new cases and 97 patients previously reported (35 full-characterized patients and 62 with at least one uncharacterized allele).[9] The previously full-characterized patients were included in order to avoid a possible bias to estimate frequencies of the *CFTR* mutations.

In this study, 123 were males (53.5%) and 107 females (46.5%), with a mean (± SD) age at onset of 4.6 ± 5.8 years. The patients included in this study were recruited from the AMFQ, Hospital Infantil de México and Instituto Nacional de Pediatría in Mexico City; all of them were of Mexican Mestizo origin. Diagnosis was based on abnormally elevated sweat chloride concentrations (> 60 mEq/l) and on clinical symptoms typical of CF.[20,21] Patients had chloride sweat values ranging from 62 to 129 mEq/1. Infections by *Pseudomonas aeruginosa* (*Pae*) were identified in 121 patients (52.6%) and pancreatic failure was found in 131 patients (56.9%). The study was conducted with the approval of the ethics and research committees. All participating individuals or their parents or legal guardians signed an informed consent form.

Samples

DNA was isolated from the peripheral blood lymphocytes from all new CF patients following conventional methods.[22] Mutation detection was performed in 195 patients with at least one uncharacterized allele (133 new cases and 62 uncharacterized or partially characterized in Orozco, *et al.*).[9]

Detection of known mutations

To identify *CFTR* known mutations, we used a kit containing 36 *CFTR* mutations and the three Tn

alleles (5T, 7T and 9T) of intron 8. The screening was performed through a reverse dot blot according to the manufacturer's instructions (Kit Inno-LiPa *CFTR*/36, INNOGENETICS, NV, Brussels, Belgium).[23] Analysis was carried out in duplicate.

Complete Genetic Screening of *CFTR* Gene

After screening by the method mentioned above, we analyzed the DNA from those cases having still at least one unknown allele. We used single strand conformation polymorphism (SSCP) analysis[24] on MDE™ gel matrix and silver staining of DNA (Silver Stain Kit, Bio-Rad Laboratories Richmond, CA). We screened first priority mutations in *CFTR* exons 4, 7, 10, 11, 13, 17b, 20, 21,[25] followed by exons 1, 2, 6a, 6b, 16, 17a and 18, including introns 8 and 19 as previously described. Abnormal band shift electrophoretic patterns were direct DNA sequenced using the ABI PRISM Big Dye terminator cycle sequencing reaction kit (v.3) (Applied Biosystems Foster City, CA, USA).

Nomenclature

For mutation nomenclature the recommendations provided by the Human Genome Variation Society (HGVS)[26] were followed.

RESULTS

Molecular Screening

One hundred and ninety-five patients with at least one uncharacterized allele (133 new cases and 62 patients from those previously reported) were screened for CF mutations using a two-tier approach: *CFTR*/36 kit analysis combined with SSCP analysis. Both the wild type and mutant bands for every CF mutation were searched, using the *CFTR*/36 kit (Figure 1). Nineteen mutations were detected using the *CFTR*/36 kit (Table 1). It is worth noting that of the patients for whom Tn polymorphism were analyzed, the vast majority carried the 7T and 9T alleles (92%) (Table1). In this first step, both alleles were fully characterized in 110 patients, including those 35 previously reported. Of the remaining 120 analyzed with SSCP, eight of them were informative for exons 7, 11, 13a, 17a, 18, and 20 and for intron 19 and the presence of c.3272-26A > G, p.R334W, p.R347P, p.S549N, c.2055_2063del, p.T1036N, p.D1152H and p.W1282X mutations was documented by automated direct DNA sequencing (Table 1). Although the mutations p.R334W, p.A455E, c.3120+1G > A, c.3272-26A > G, c.711+1G > T, p.Q552X, p.W1282X, c.IVS8-5T, p.R1162X and p.R347P, p.D1152H and p.T1036N, have been reported in other populations, they had not yet been reported in Mexican patients. Thus, the results from the present screening include 12 distinct mutations, resulting in a total of 46 different mutations detected in the Mexican population sampled. As follows, by using a combination of research strategies over 77% of CF alleles were characterized (Table1).

Seven mutations showed a frequency greater than 1%: p.F508del was the most common mutation (44.6%), followed by p.G542X (7.4%), p.N1303K (2.4%), p.I507del (1.5%), p.R334W (1.5%), p.S549N (1.5%) and c.3849 + 10kbC > T (1.3%) (Table1). These seven mutations represented 60.2% (277/460) of all Mexican CF alleles studied in this work. Except for p.F508del, these mutations are more frequent in Mexican patients than in the worldwide population or in other Hispanic populations (Table 2). The remaining 39 mutations accounted only for 17.5% of identified CF alleles. Mutations c.621 + 1G > T, c.394delTT, c.2789 + 5G > A, c.3659delC, c.2143delT, p.E60X, c.2184delA, c.711 + 5G > A, c.1717-1G>A, c.*CFTR*del2,3 (21kb), c.3905insT, p.R560T, c.1898 + 1G > A and p.S1251N, which were contained in the kit, were not detected in our patients.

Forty-one patients (17.8%) were homozygous for p.F508del and 123 (53.5%) were classified as being compound heterozygous. Regarding the p.G542X mutation, 5 patients (2.2%) were homozygous and 24 (10.4%) heterozygous.

DISCUSSION

Mexicans are an admixed population with a very complex genetic structure, where Native American genes account for 51%, European genes for 45.4% and African genes for 3.7%[27]. Perhaps because of this complexity, Mexico has a very broad spectrum of *CFTR* mutations, including some found only in Mexicans.[9,28] Therefore detection of *CFTR* mutations in the Mexican patients is complicated. In this study, the analysis of the most common mutations (p.F508del, p.G542X, p.N1303K, p.R553X and p.G551D), allowed the characterization of only ~50% of the affected chromosomes, whereas in Caucasians, the search for the same mutations could achieve a 98% detection rate of affected alleles.[8,13,29] In popula-

Table 1. Frequency of *CFTR* gene mutations in 230 Mexican patients.

Mutations	Location in *CFTR* gen	Affected alleles/number of chromosomes. In Orozco, *et al*., 2000 (n = 194)	Affected alleles/ number of chromosomes in this work (n = 350)	Affected alleles/total of chromosomes (n)	Total frequency (%)	Detection Systems
p.F508del	Exon 10	79/194	126/350	205/460	44.6	KIT-CFTR/36
p.G542X	Exon 11	12/194	22/350	34/460	7.4	KIT-CFTR/36
p.N1303K	Exon 21	4/194	7/350	11/460	2.40	KIT-CFTR/36
p.S549N	Exon 11	5/194	2/350	7/460	1.52	SSCP
p.I507del	Exon 10	5/194	2/350	7/460	1.52	mHET, KIT-CFTR/36
p.R334W*	Exon 7	-	7/350	7/460	1.52	SSCP, KIT-CFTR/36
c.3849 + 10kbC > T	Intron 19	1/194	5/350	6/460	1.3	KIT-CFTR/36, mHET
c.3199_3204del	Exon 17a	2/194	2/350	4/460	0.87	KIT-CFTR/36
p.A455E*	Exon 9	-	4/350	4/460	0.87	KIT-CFTR/36
p.I148T	Exon 4	3/194	1/350	4/460	0.87	mHET, KIT-CFTR/36
p.R75X	Exon 3	3/194	-	3/194**	1.54**	mHET
c.406 - 1G > A	Intron 3	3/194	-	3/194**	1.54**	mHET
c.2055_2063del	Exon 13	2/194	1/350	3/460	0.65	SSCP
c.1078delT	Exon 7	1/194	1/350	2/460	0.43	mHET, KIT-CFTR/36
c.2183AA > G	Exon 13	2/194	-	2/460	0.43	mHET, KIT-CFTR/36
c.3120 + 1G > A*	Intron 16	-	2/350	2/460	0.43	KIT-CFTR/36
c.3272 - 26A > G*	Intron 17a	-	2/350	2/460	0.43	KIT-CFTR/36, SSCP
c.711 + 1G > T*	Intron 5	-	2/350	2/460	0.43	KIT-CFTR/36
c.935delA	Exon 6b	2/194	-	2/460	0.43	mHET, SSCP
p.G85E	Exon 3	1/194	1/350	2/460	0.43	mHET, KIT-CFTR/36
p.I506T	Exon 10	2/194	-	2/460	0.43	SSCP
p.Q552X*	Exon 11	-	2/350	2/460	0.43	KIT-CFTR/36
p.W1282X*	Exon 20	-	2/350	2/460	0.43	SSCP, KIT-CFTR/36
c.1716G > A	Exon 10	1/194	-	1/194**	0.51**	mHET
c.1924_1930del	Exon 13	1/194	-	1/460	0.21	SSCP
c.297 - 1G > A	Intron 2	1/194	-	1/194**	0.51**	mHET
c.4160_4161insGGGG	Exon 22	1/194	-	1/194**	0.51**	mHET
c.846delT	Exon 6a	1/194	-	1/460	0.21	mHET, SSCP
p.D1152H*	Exon 18	-	1/350	1/460	0.21	SSCP
p.G551D	Exon 11	1/194	-	1/460	0.21	SSCP
p.G551S	Exon 11	1/194	-	1/460	0.21	SSCP
p.H199Y	Exon 6a	1/194	-	1/460	0.21	mHET, SSCP
c.IVS8-5T*	Intron 8	-	1/350	1/460	0.21	SSCP
p.L558S	Exon 11	1/194	-	1/460	0.21	mHET, SSCP
p.P750L	Exon 13	1/194	-	1/460	0.21	mHET, SSCP
p.R1162X*	Exon 19	-	1/350	1/460	0.21	KIT-CFTR/36
p.R117H	Exon 4	1/194	-	1/460	0.21	mHET, SSCP
p.R347P*	Exon 7	-	1/350	1/460	0.21	SSCP, KIT-CFTR/36
p.R553X	Exon 11	1/194	-	1/460	0.21	SSCP
p.R75Q	Exon 3	1/194	-	1/194**	0.51**	mHET
p.T1036N*	Exon 17a	-	1/350	1/460	0.21	SSCP
p.V754 M	Exon 13	1/194	-	1/460	0.21	mHET, SSCP
p.W1069X	Exon 17b	1/194	-	1/460	0.21	mHET, SSCP
p.W1098C	Exon 17b	1/194	-	1/460	0.21	mHET, SSCP
p.W1204X	Exon 19	1/194	-	1/194**	0.51**	mHET
p.Y1092X	Exon 17b	1/194	-	1/460	0.21	mHET, SSCP

*Mutations detected in this study. **Frequency from 194 alleles reported by Orozco, *et al*., 2000 (these mutations were not screened in this study).
mHET: Multiplex Heteroduplex Analysis. **SSCP:** Single Strand Conformation Polymorphism.

Table 2. Comparison of the frequencies of the most common *CFTR* gene mutations in 230 (460 chromosomes) Mexican patients with those in other populations.

Mutations	Mexicans This study	Worldwide[7] (%)	Frequency (%) in Latin Americans[31]	Frequency (%) in patients of Spanish Ancestry[32]
p.F508del	44.60	66.00	46.69	51.74
p.G542X	7.40	2.40	5.08	7.70
p.I507del	1.52	0.20	0.23	1.07
p.N1303K	2.40	1.30	1.65	2.92
p.R334W*	1.52	0.10	0.90	1.79
p.S549N	1.52	0.10	0.14	0.00
c.3849 + 10kbC > T	1.30	0.20	0.41	0.00
TOTAL	60.30	70.30	55.10	65.22

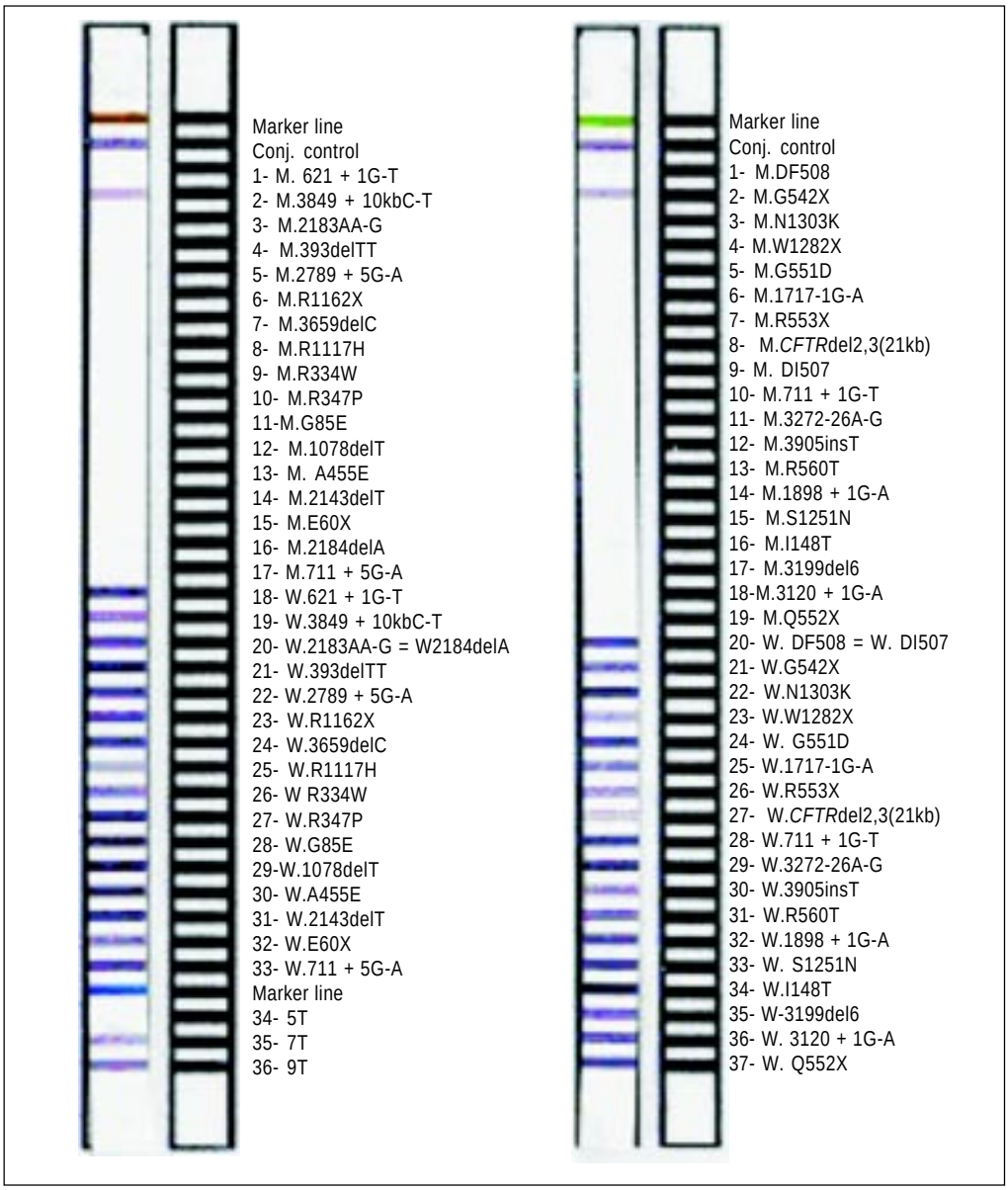

Figure 1. *Detection of known mutations using the assay's strips (Inno-Lipa* CFTR*36 assay). An example of a heterozygous compound patient p.G542X/c.3949 + 10kbC > T. The patient shows the polymorphisms 7T and 9T.*

tions with a high heterogeneity, such as in Mexico, a variety of methodologies should be combined to improve the detection rate of the *CFTR* mutations.

In fact, in this study different strategies were used in order to improve the detection rate of *CFTR* mutations in the 230 Mexican patients, and we found 46 mutations, 12 of them (p.R334W, p.A455E, c.3120 + G > A, c.3272-26A > G, c.711 + 1G > T, p.Q552X, p.W1282X, c.IVS8-5T, p.R1162X and p.R347P, p.D1152H and p.T1036N) had not yet been reported previously for Mexican people. Overall, our strategy led to the identification of 77.7% of all affected alleles, which document that Mexico has one of the widest spectrums of *CFTR* mutations worldwide due in part to the great ethnic diversity and the heterogeneity in the genetic background. With the combination of these technologies it was possible to identify seven mutations that showed a frequency greater than 1% in the Mexican population: p.F508del (44.6%), p.G542X (7.4%), p.N1303K (2.4%), p.I507del (1.5%), p.S549N (1.5%), p.R334W (1.5%) and c.3849+10kbC > T(1.3%) which is relevant to optimize future screening of the *CFTR* mutations in populations like ours.

It should be noted that despite the combination of techniques used, ~23% of the alleles remained still uncharacterized, showing that there are still a great number of unknown alleles, perhaps rare mutations, however we cannot rule out the possibility that in the Mexican population there could exist an undetermined endemic mutation with a high frequency. On the other hand, in this study only 19 of the 36 mutations included in the kit *CFTR*36 were detected, may be due to the fact that the kit was fashioned specifically for Caucasian population. Therefore it is necessary to identify all *CFTR* mutations affecting Mexican population to design a kit targeted for our population, including the most frequent mutations, and the remaining described in this study (Table 1). Improvements in the mutational screening analysis and strategies employed, as shown by the use of the two technologies in this study, will aid in elucidating the mutations involved in CF in admixed populations. Finally, more detailed knowledge of the ethnic and geographical distribution of *CFTR* mutations will improve the CF detection programs in a given population[30-32] and allow the development of more effective methods of diagnosis and treatment.

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