

## **Analysis of CFTR gene mutations and Cystic Fibrosis incidence in the Ecuadorian population.**

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**Key words:** Cystic fibrosis, CF, CFTR, Ecuadorian incidence, F508del, G85E.

**Abstract.** Ecuador is one of the Latin American countries where cystic fibrosis has not been thoroughly studied. The goal of this study was to establish the incidence of this specific pathology and the incidence of the 29 most common European CF mutations in Ecuador's population. We performed a prospective-descriptive study with the intention of including patients registered at the Ecuadorian Cystic Fibrosis Foundation as well as the main pediatric hospitals in Ecuador. The inclusion criteria were clinical manifestations of cystic fibrosis plus two positive pilocarpine iontophoresis sweat tests (CI > 60 mEq/L). We tested F508del mutation by heteroduplex method and then, we confirmed these results and searched for other 28 frequent European mutations aside from F508del by a reverse dot blot technique (INNO-LiPA CFTR 29 + Tn). Sixty two unrelated patients were included. Both heteroduplex and reverse dot blot methods identified 53.22% of all mutations. The estimated Ecuadorian cystic fibrosis incidence was 1:11,252. The mutations found and their incidence were F508del (37.1%), G85E (8.9%), G542X (2.4%), N1303K (2.4%), G551D (1.6%) and R334W (0.8%). The incidence of cystic fibrosis in Ecuador is closely similar to other Latin American countries where there is a large "mestizo" population. We are reporting one of the highest incidences of G85E in the world.

## **Análisis de las mutaciones del gen CFTR y de la incidencia de Fibrosis Quística en la población ecuatoriana.**

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**Palabras clave:** Fibrosis quística, CF, CFTR, incidencia ecuatoriana, F508del, G85E.

**Resumen.** Ecuador es uno de los países latinoamericanos donde la fibrosis quística no ha sido profundamente estudiada. El objetivo de este estudio fue establecer la incidencia de esta patología identificar las 29 mutaciones europeas mas frecuentes que afectan a los pacientes fibroquísticos ecuatorianos. Se realizó un estudio descriptivo-prospectivo que incluyó pacientes provenientes de las Fundación Fibrosis Quística y de los principales hospitales pediátricos del Ecuador. Los criterios de inclusión fueron la presencia de manifestaciones clínicas clásicas de fibrosis quística más dos pruebas del sudor por iontoforesis de pilocarpina positivas  $CI > 60$  mEq/L. Se utilizó heteroduplex para identificar la mutación F508del y posteriormente se confirmó dichos resultados e identificó otras 28 mutaciones frecuentes de la población europea utilizando un test de hibridación reversa *in situ* (INNO-LiPA CFTR 29 + Tn). Sesenta y dos pacientes fueron incluidos y se logró identificar el 53,22% de todos los alelos mutados. Las mutaciones encontradas fueron F508del (37,1%), G85E (8,9%), G542X (2,4%), N1303K (2,4%), G551D (1,6%) y R334W (0,8%). La incidencia estimada de la fibrosis quística en el Ecuador (1:11.252) es muy similar a la de otros países latinoamericanos que poseen un gran porcentaje de población mestiza. Reportamos una de las frecuencias más altas de la mutación G85E en el mundo.

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### **INTRODUCTION**

Cystic fibrosis (CF; MIM# 219000) is an autosomal recessive disease caused by a mutant protein called Cystic Fibrosis Transmembrane Regulator (CFTR) which functions as a chloride channel that has regulatory functions over other ion channels (1, 2). The defective protein leads to increased viscosity of glandular secretions and is responsible for all clinical manifestations in CF patients (3). There are more than one thousand reported mutations at the cystic fibrosis gene (4).

Cystic fibrosis is the most common severe autosomal recessive genetic disorder,

and its incidence ranges from 1:2,500 to 1:90,000 affected newborns among different ethnicities (5, 6).

Cystic fibrosis has been studied in few Latin American countries. Its incidence varies throughout Hispanic countries, 1:9,000 in México (7), 1:9,600 in Uruguay (8), 1:6,573 in Argentina (9, 10) which probably reflects ethnic variation in these countries. This fact is supported by the varying incidence that mutations have among countries. F508del is the most common mutation worldwide, but its incidence in Colombia is 41.8% (11), in Chile is 45% (12), in Argentina is 58.64% (10), in Uruguay is 40.4% (8), in Belém (North Brazil) is 22.7%

(13), in Costa Rica is 23% (14), in México is 40.72% (7) and in Venezuela 26.79% (15).

In a previous study conducted in Ecuador the F508del incidence was found in a small sample of patients (16). We tried to extend the knowledge about CF in Ecuador by screening the 29 most frequent European mutations in the CF Ecuadorian population and by reporting the estimative incidence of this pathology throughout the country.

## MATERIALS AND METHODS

### Sample collection

We contacted the Ecuadorian CF Foundation to collect patients. This Foundation registers the majority of the CF patients throughout the country. There are practically no CF patients outside of this entity in Ecuador. The Foundation approved the project's ethical fields, set up the hospital appointments, and we planned several visits to obtain consent and draw blood samples from the patients.

The inclusion criteria for each patient were CF clinical manifestations plus two positive pilocarpine iontophoresis sweat tests (chloride >60 mEq/L) (17). The exclusion criteria were a negative sweat test or not having it undergone.

Sixty two patients were collected from June 1996 until January 2003. They came from 7 different provinces (Pichincha: n = 28, Guayas: n = 26; El Oro n = 2, Azuay n = 2, Los Ríos n = 2, Manabí n = 1 and Tungurahua n = 1).

### Pathology incidence

To calculate the Ecuadorian CF incidence, we obtained data from the most recent population census (year 2001) and used the city Guayaquil, part of Guayas province. This city represents 16.7% of the Ecuadorian population, groups the largest number of migrants from the country's in-

ner provinces (18) and was the most representative source of patients in our study. Finally, we projected Guayaquil's incidence rate to the country's newborn population reported in the last census to calculate the estimative CF incidence in Ecuador during 2001.

### Detection of CFTR mutations

DNA was isolated from white blood cells using a modified salting out technique (19). DNAs were stocked with a final concentration of 3 ng/ $\mu$ L.

Using polymerase chain reaction (PCR), exon 10 was amplified in all patients (20) to look for F508del mutation using CF1 5'-GTTGGCATGCTTTGATGACGCTTC-3' and CF2 5'-GTTTTCCTGGATTATGCCTGGCAC-3' primers by heteroduplex method (21). Once F508del results were obtained, we employed INNO-LiPA CFTR 29 + Tn, a reverse dot blot technique, to confirm the previous results and to look for the 29 most frequent European CF mutations: E60X; G85E; 394delTT; 621 + 1G  $\rightarrow$  T; R117H; 711 + 5G  $\rightarrow$  A; 1078delT; R347P; R334W; A455E; Tn; F508del; I507del; G542X; 1717-1G  $\rightarrow$  A; G551D; R553X; R560T; Q552X; 2183AA  $\rightarrow$  G; 2184delA; 2143delT; 2789 + 5G  $\rightarrow$  A; R1162X; 3659delC; 3849 + 10kb  $\rightarrow$  T; 3905insT; W1282X; S1251N y N1303K (22).

The statistical analysis of mutation incidences was performed using Pearson Chi square test.

## RESULTS

Blood was collected from 62 non-familial patients aging 0 to 25. There was a small number of patients older than 15 years (14.51% of the sample), with no patients older than 25 years.

Our study included patients from 7 of the 22 provinces of Ecuador (Table I). The origin of the majority of the patients was

**TABLE I**  
Patients' distribution according to origin and CFTR mutation incidences

Mutation	Guayas	Pichincha	El Oro	Manabí	Tungurahua	Azuay	Los Ríos	Total country
	n = 26 N (%)	n = 28 N (%)	n = 2 N (%)	n = 1 N (%)	n = 1 N (%)	n = 2 N (%)	n = 2 N (%)	n = 62 N (%)
F508del	19 (41.3)	25 (54.3)	2 (4.3)	0	0	0	0	46 (37.1)
G85E	6 (54.5)	1 (9.09)	0	0	2 (18.18)	2 (18.18)	0	11 (8.9)
G542X	0	2 (66.6)	0	0	0	0	1 (33.3)	3 (2.4)
N1303K	2 (66.6)	1 (33.3)	0	0	0	0	0	3 (2.4)
G551D	1 (50)	1 (50)	0	0	0	0	0	2 (1.6)
R334W	1 (100)	0	0	0	0	0	0	1 (0.8)
Mutated alleles identified	29	30	2	0	2	2	1	66 alleles (53.2)
WT alleles	23	26	2	2	0	2	3	58 alleles (46.8)
Total alleles	52	56	4	2	2	4	4	124

n = number of patients. N = number of alleles.

Guayas (n = 26) and Pichincha (n = 28), probably because these are the provinces where there are first level pediatric hospitals and where the CF Foundation works as a non-profit organization.

Considering that during the year 2001 there were 45,011 newborns (18) and 4 new patients reported in Guayaquil, the estimated CF incidence in Guayaquil was 1:11,252 newborns. When this incidence rate was projected to the entire Ecuadorian newborn population (278,170 newborn) we inferred a total of 25 affected newborns during year 2001 in the whole country, which would be the estimative incidence of cystic fibrosis in Ecuador.

Of 124 alleles studied by heteroduplex analysis, forty six (37.1%) had F508del mutation (Table I). Of these 46 alleles, 24 (57.2%) were identified as homozygotes for

the F508del genotype (12 patients) (Table II). Afterwards, these results were confirmed using the INNO-LiPA CFTR 29 + Tn method, which found 28 additional mutated alleles. We resolved 17 complete genotypes (27.4 %), detected one mutated allele in 32 patients (51.6 %) and didn't find the mutated allele (WT: Wild Type) in 13 patients (21.0 %) (Table II). The CFTR mutation incidences and the patients' origin distribution among Ecuadorian provinces are shown in Table I.

## DISCUSSION

Considering the estimative incidence we calculated (1:11,252 newborns), the fact that there are practically no CF patients outside the Cystic Fibrosis Foundation in Ecuador and that our study included all its

**TABLE II**  
Patient's genotypes

Genotype	Patients	Percentage
WT/WT	13	21.0
G85E/WT	5	8.1
G542/WT	3	4.8
G551D/WT	2	3.2
DF508/WT	18	29.0
R334W/WT	1	1.6
DF508/DF508	12	19.4
DF508/G85E	4	6.5
N1303K/WT	3	4.8
G85E/G85E	1	1.6
Total	62	100.00

WT Wild Type –non identified mutated alleles.

members, it can be assumed that although these 62 cases represent the highest number of CF patients studied in Ecuador so far, there is probably a large number of non-reported CF patients. This phenomenon probably can be explained by the lack of good health systems in Ecuador coupled with the medical belief that it is an uncommon pathology in non-Caucasian populations and the lack of information on this particular disease and its clinical manifestations among health workers.

The fact that there were no patients older than 25 years old in our sample probably reflects that although the mean survival age in First World countries is 32 year (23), this would not be applicable to Third World patients as our sample, where only a small group of patients survive past 15 years.

Ecuador's most frequently found mutation was F508del (37.1%). Its incidence is not statistically higher ( $p = 0.66$ ) than the one reported in a previous Ecuadorian publication where it was identified in 25% of all alleles (16), supporting the theory that the Ecuadorian F508del incidence is much

lower than the 66.8 reported worldwide (24).

Despite the varying incidences of F508del among Hispanic Countries, the Ecuadorian F508del incidence was only statistically different than the one reported in Spain and Argentina (Table III). This F508del data may reflect the varying ethnic distributions among Hispanic Countries. Argentina and Spain have a large Caucasian population, while Ecuador is a country where mestizos make up two thirds of the population (18). This Caucasian distribution might be the reason why the cystic fibrosis incidence in Ecuador is lower than the ones described in Argentina and Spain (9, 10, 25).

The most common mutations we identified (Table I) do not match with the most common mutations worldwide (F508del 66.8%, G542X 2.6%, N1303K 1.6%, G551D 1.5%, y W1282X 1%) (24). More interestingly, according to our literature search, the incidence of our second most common mutation (G85E 8.9 %) is by far one of the highest among Hispanic Countries (Table III) and worldwide (24, 25), even

TABLE III  
Main CF mutations found in Hispanic countries

Mutation	Ecuador <sup>¥</sup> n = 62	Spain <sup>1§</sup>	Argentina <sup>2</sup> n = 220	Uruguay <sup>3</sup> n = 52	Chile <sup>4</sup> n = 57	Costa Rica <sup>5</sup> n = 24	México <sup>6</sup> n = 97	Brazil <sup>*7</sup> n = 33	Colombia <sup>8</sup> n = 92	Venezuela <sup>9</sup> n = 30
	% p	% p	% p	% p	% p	% p	% p	% p	% p	% p
F508del	37.1	52.7 0.016	58.6 0.002	40.4 N.S.	39.5 N.S.	23.0 N.S.	40.7 N.S.	22.7 N.S.	41.8 N.S.	26.79 N.S.
G85E	8.9	0.8 <0.05	0.7 <0.05	2.9 <0.05	N.T.	N.T.	0.5 <0.05	N.T.	0.0 <0.05	N.T.
G542X	2.4	8.0 <0.05	4.1 0.016	5.7 <0.05	6.1 <0.05	25.0 N.S.	6.2 <0.05	0.0 0.002	3.8 N.S.	N.T.
G551D	1.6	N.R.	0.0 <0.05	0.0 0.002	0.9 N.S.	0.0 0.036	0.5 0.02	3.0 N.S.	0.0 <0.05	N.T.
R334W	0.8	1.2 N.S.	1.1 N.S.	1.9 N.S.	1.7 N.S.	N.T.	0.5 N.S.	N.T.	0.5 N.S.	N.T.
N1303K	2.4	2.5 N.S.	2.7 N.S.	2.9 N.S.	0.0 <0.05	0.0 0.008	2.1 N.S.	N.T.	0.5 <0.05	N.T.

n = number of patients. ¥: Present Study. §: Review article. \*Belém, North of Brazil. p: Pearson Chi square in relation to Ecuadorian mutation incidences. N.R.: Not reported. N.T.: Not tested. N.S.: Not statistically significant p > 0.05.  
References are: (1) Bobadilla *et al.* 2002; (2) Visich *et al.*, 2002; (3) Luzardo *et al.*, 2002; (4) Repetto *et al.*, 2001; (5) Venegas *et al.*, 2003; (6) Orozco *et al.*, 2000; (7) Araújo *et al.*, 2005; (8) Keyeux *et al.*, 2003; (9) Morales-Machin *et al.*, 2004.



higher than the one reported in the Mediterranean region and the south of Greece (3.4%) where it is said to have originated (24, 26). G85E although with lower incidence, was more widespread than F508del in our study (Table I), which reflects that in the case of performing a larger study including all the Ecuadorian provinces, its incidence is more likely to be even higher than the one reported in this work. G85E is a severe class II missense mutation in exon 3 at nucleotide position 386 associated with severe lung disease. Guanine is replaced by adenosine, resulting in substitution of glutamic acid for glycine-85 in the first membrane-spanning domain (26).

Although Ecuador was colonized by the Spaniards, its population has a genetic makeup notably different from that of the Spanish population and instead reflects the numerous indigenous ethnicities to its region. The genetic makeup of the Ecuadorian population is even quite different from that of neighbouring Colombia (11). The differing genetics can be seen in the incidence of F508del and G542X mutations in CF patients in Ecuador, Spain, and Colombia (Table II).

According to Table I, Ecuador's Spanish history would only influence the population of Pichincha where F508del and G542X are the most commonly found mutations.

Although 53.22% is an acceptable sensitivity if we compare it with other studies (25), it is clear that other methods, including sequencing and deletion testing, will be needed to identify the mutations in about half of Ecuador's CF patients as well as to describe some novel mutations characteristic of this particular population as other Hispanic studies have done (7, 11).

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