Journal of Cystic Fibrosis 6 (2007) 194–208

Journal of Cystic Fibrosis

www.elsevier.com/locate/jcf

CFTR gene analysis in Latin American CF patients: Heterogeneous origin and distribution of mutations across the continent

Martín M. Pérez [a], María Cecilia Luna [a], Omar H. Pivetta [a], Genoveva Keyeux [b,*]

[a] *Centro Nacional de Genética Médica, ANLIS-Dr. Carlos G. Malbrán, Avenue Las Heras 2670, 4° Piso, 1425 Buenos Aires, Argentina*
[b] *Instituto de Genética, Universidad Nacional de Colombia, Calle 53 Carrera 38, Ciudad Universitaria, Bogotá, Colombia*

Received 23 June 2006; received in revised form 28 July 2006; accepted 28 July 2006
Available online 11 September 2006

Abstract

Background: Cystic Fibrosis (CF) is the most prevalent Mendelian disorder in European populations. Despite the fact that many Latin American countries have a predominant population of European-descent, CF has remained an unknown entity until recently. Argentina and Brazil have detected the first patients around three decades ago, but in most countries this disease has remained poorly documented. Recently, other countries started publishing their results.
Methods: We present a compilation and statistical analysis of the data obtained in 10 countries (Argentina, Brazil, Chile, Colombia, Costa Rica, Cuba, Ecuador, Mexico, Uruguay and Venezuela), with a total of 4354 unrelated CF chromosomes studied.
Results: The results show a wide distribution of 89 different mutations, with a maximum coverage of 62.8% of CF chromosomes/alleles in the patient's sample. Most of these mutations are frequent in Spain, Italy, and Portugal, consistent with the origin of the European settlers. A few African mutations are also present in those countries which were part of the slave trade. New mutations were also found, possibly originating in America.
Conclusion: The profile of mutations in the *CFTR* gene, which reflects the heterogeneity of its inhabitants, shows the complexity of the molecular diagnosis of CF mutations in most of the Latin American countries.
© 2006 European Cystic Fibrosis Society. Published by Elsevier B.V. All rights reserved.

Keywords: CFTR mutations; Cystic Fibrosis; Latin America; Diagnosis; CFTR; Ethnic background; ABCC7

1. Introduction

The most frequent recessive genetic disorder in Caucasian populations is Cystic Fibrosis (CF; MIM # 219700). The incidence of the disease as well as the frequency of the most common mutation, p.F508del, reflect ancient migratory and demographic expansion processes, and possibly a selective advantage of heterozygous carriers [1–6]. Extended haplotype studies suggest that the p.F508del and a few other, common, mutations originated between 11,000 and 34,000 years ago in a population genetically different from any present-day European group, and spread to different areas of Europe [3, 7]. Furthermore, some mutations were subject to founder and genetic drift events, thus explaining regional and local elevated frequencies [2,8,9].

Latin America and the Caribbean have become in the last 500 years a melting pot of different ethnicities. Many countries have a strong Amerindian heritage mixed with Europeans (predominantly Spanish or Portuguese, but also Italians, Germans, French, English, near- and middle-Easterners and central-European Jews), and in some of them (e.g. Cuba, Colombia, Brazil), the predominant population in particular regions is African-descended. The belief that CF was typically a disease of northern Europeans and Anglo-Saxon descended populations had misled the diagnosis in patients from Latin America. However, the disease started to be studied in Argentina in the sixties and later in the other countries [10].

* Corresponding author. Tel.: +57 1 3165000x11614 or 11610; fax: +57 1 3165526.
E-mail address: gkeyeuxb@unal.edu.co (G. Keyeux).

1569-1993/$ - see front matter © 2006 European Cystic Fibrosis Society. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.jcf.2006.07.004

In the last decade, some countries began screening for particular mutations, and only recently four Latin American countries, namely Argentina [11, 12], Mexico [13], Colombia [14] and Brazil [15], reported extensive *CFTR* locus (MIM # 602421) analyses. Still, few data are available for some of the rest of the Latin American and Caribbean countries and for others there are no data at all. In spite of this, a comprehensive view of the disease incidence and most frequent mutations is beginning to emerge in this region of the world.

Following the recommendations of a WHO workshop on the worldwide molecular epidemiology of CF held in 2002 [16], the aim of the present investigation was to perform a meta-analysis of the distribution of *CFTR* mutations across the Central and South American sub-continent in order to establish a comprehensive picture of the molecular epidemiology of CF in this region. It should also help to design a common diagnostic panel for the main mutations, as well as regionally adapted additional mutation tests, according to the results so far obtained.

DATABASES: OMIM: 602421 (CFTR); 219700 (CF); http://www.sickkids.on.ca/cftr; GDB: 120584; XM_004980; HGMD: CFTR; GeneCard: CFTR.

2. Materials

Data from patients from 10 Latin American countries were taken from the literature. Since we believe it is important to have as broad a picture as possible, some data were taken from congress presentations, communications to the Cystic Fibrosis Genetic Analysis Consortium (CFGAC; Population variation of 24 common cystic fibrosis mutations. Table 1.5. http://www.genet.sickkids.on.ca/cftr/newfreq/All.html), or personal communications. A total of 4354 unrelated CF chromosomes were studied between 1990 and 2004. The information was gathered from 6 different CF studies from Argentina, 10 from Brazil, 4 from Chile, 2 from Colombia, 2 from Mexico, 3 from Ecuador, 2 from Venezuela, and 1 each from Costa Rica, Cuba, and Uruguay.

All mutations considered in the present survey are CF-causing, as specified in the published articles. The geographical origin of patients is as follows: *Argentina*: Buenos Aires (N=49) [17]; Buenos Aires, La Plata (N=220) [12]; Buenos Aires, Santa Fé, Mendoza, San Juan, Tucumán, Córdoba, Entre Ríos, Corrientes, Formosa, Neuquen (N=155) [18]; Buenos Aires, Formosa (N=111) (Varela and Targovnik, personal communication); Buenos Aires (N=13) [19]; Córdoba (N=75) (Oller-Ramirez, personal communication); *Brazil*: Rio Grande do Sul, Santa Catarina, Paraná, São Paulo, Minas Gerais (N=234) [20]; São Paulo (N=37) [21]; São Paulo (N=160) [22]; Rio Grande do Sul (N=61) [23]; Sao Paulo (N=60) [24]; Rio de Janeiro (N=74) [25, 26]; Sao Paulo (N=24) [27]; Rio Grande do Sul (N=77) [28]; Santa Catarina, Parana, Mina Gerais (N=193) [15]; Sao Paulo (N=9) [29]; *Chile*: Santiago (N=36) [30]; Valparaíso (N=18) [31]; Santiago (N=22) [32]; Valdivia, Santiago (N=50) [33]; *Colombia*: Bogotá, Antioquia, Caldas, Bolívar, Valle (N=92) [14]; Bogotá (N=17) [34]; *Costa Rica*: San José (N=24) [35]; *Cuba*: La Habana (N=72) [36]; *Ecuador*: Quito (N=16) [37]; Guayaquil (N=25) (Cassiman 2004, personal communication); Quito (N=10) [38]; *Mexico*: Mexico DF (N=97) [13]; Monterrey, Guadalajara, Puebla (N=40) [39]; *Uruguay*: Montevideo (N=38) [40, 41]; *Venezuela*: Maracaibo (N=27) [34]; Caracas (N=41) [42].

The experimental methods used to analyse the *CFTR* gene varied from one group to another. Some have concentrated in the search of specific mutations that are

Table 1
Mutations found in the Latin American CF patients

Exon 1	p.L6V*
Exon 3	p.W57X, p.R75X, p.G85E
Exon 4	p.R117H
Exon 6a	p.H199Y, p.V201M, p.L206W, p.Q220X, p.V232D, c.846delT*
Exon 6b	p.Y275X*, c.935delA
Exon 7	p.R334W, p.R347P, p.Y362X*, c.1078delT, c.1215delG
Exon 8	c.1323_1324insA*
Exon 9	c.1460_1461delAT*, c.1353_1354insT*,#
Exon 10	p.I506T, p.I507del, p.F508del
Exon 11	p.G542X, p.S549N, p.S549R, p.G551D, p.G551S, p.R553X, p.L558S, p.A559T, c.1782delA
Exon 12	p.S589I
Exon 13	p.H609R*, p.P750L, p.V754M, c.1924_1930del, c.2055_2063del, c.2183AA>G;c.2184delA, c.2184delA, c.2185_2186insC, c.2347delG, c.2566_2567insT*, c.2594_2595delGT*
Exon 14a	p.R851L, c.2686_2687insT*
Exon 15	c.2869_2870insG
Exon 16	c.3120+1G>A
Exon 17a	p.I1027T, c.3171delC, c.3199_3204del
Exon 17b	p.G1061R, p.R1066C, p.W1069X#, p.W1089X, p.Y1092X, p.W1098C*
Exon 19	p.R1162X, p.W1204X, p.Q1238X, c.3617_3618delGA*#, c.3659delC
Exon 20	p.W1282X, p.R1283M
Exon 21	p.N1303K, c.4016_4017insT
Exon 22	c.4160_4161insGGGG*
5′ flanking	c.-834G>T
Intron 2	c.297-1G>A*, c.297-2A>G
Intron 3	c.406-1G>A
Intron 4	c.621+1G>T
Intron 5	c.711+1G>T
Intron 8	c.IVS8-5T
Intron 10	c.1716G>A, c.1717-1G>A
Intron 11	c.1811+1.6KbA>G, c.1812-1G>A
Intron 12	c.1898+1G>A, c.1898+3A>G
Intron 14	c.2789+2_2789+3insA, c.2789+5G>A
Intron 17a	c.3272-26A>G
Intron 17b	c.3500-2A>G*
Intron 19	c.3849+1G>A, c.3849+10KbC>T
Intron 20	c.4005+1G>A, c.4005-1G>A#

Mutations are listed according to their position in the gene. Mutation names reported by the original authors have been changed to the new nomenclature, according to the HGVS (2003) recommendations.
*: new mutations; #: mutations in the original author's paper included in the present meta-analysis that have not been reported in the Cystic Fibrosis Mutation Database.

Table 2
Mutation frequencies in Latin American CF patients

Country	No. of chromosomes analysed	p.F508del	p.G542X	p.W1282X	p.N1303K	p.R1162X	p.L6V*	p.W57X	p.R75X	p.G85E	p.R117H	p.H199Y	p.V201M	p.L206W	p.Q220X	p.V232D	p.Y275X*	p.R334W	p.R347P	p.Y362X*	p.I506T
Argentina	98	61																			
	440	258	18	12	12	2	1	1		3					1			5		1	
	310	181	20	7	5	5				7	0							5	0		
	222	135	15	7	5	1															
	26	14	2	1						1											
	150	88	6		6	1				2								3			
Subtotal and	1246	737	61	27	28	9	1	1		13					1			13		1	
frequency (%)	100	59.15	4.90	2.17	2.25	0.72	0.08	0.08		1.04					0.08			1.04		0.08	
Brazil	468	221	26		11																
	74	38	2	1																	
	320	155	28	3	8	8				4			1	2		1	1	8			
	122	62																			
	120	38	10		3																
	148	38	4	0	0																
	48	15																			
	154	75	5	1	0													2	0		
	386	154	24	6	10	17				9	0							10	1		
	18	4	0	0	2	0				0								0	0		
Subtotal and	1858	800	99	11	34	25				13			1	2		1	1	20	1		
frequency (%)	100	43.06	5.33	0.59	1.83	1.35				0.70			0.05	0.11		0.05	0.05	1.07	0.05		
Chile	72	21																			
	36	11	3		0																
	44	22	4	3		1												1			
	100	45	7	5	0	2					0							2	0		
Subtotal and	252	99	14	8		3												3			
frequency (%)	100	41.28	5.55	3.17		1.19												1.19			
Colombia	184	77	7	2	1	2												1			
	34	13	2	1	1																
Subtotal and	218	90	9	3	2	2												1			
frequency (%)	100	41.28	4.13	1.38	0.92	0.92												0.46			
Costa Rica	48	11	12	0	0						0							0	0		
Frequency (%)	100	22.91	25.00																		
Cuba	144	49																			
Frequency (%)	100	34.03																			
Ecuador	32	11			1																
	50	16	2							2											
	20	5	0	0	0																
Subtotal and	102	32	2		1					2											
frequency (%)	100	31.37	1.96		0.98					1.96											
Mexico	194	79	12		4				3	1	1	1									2
	80	36	4		1																
Subtotal and	274	115	16		5				3	1	1	1									2
frequency (%)	100	41.97	5.84		1.82				1.09	0.36	0.36	0.36									0.73
Uruguay	76	43	6		2	3				3								2			
Frequency (%)	100	56.58	7.89		2.63	3.95				3.95								2.63			
Venezuela	54	16	2																		
	82	41																			
Subtotal and	136	57	2																		
frequency (%)	100	41.91	1.47																		
Total	4354	2033	221	49	72	42	1	1	3	32	1	1	1	2	1	1	1	39	1	1	2
Frequency (%)	100	46.69	5.08	1.13	1.65	0.96	0.02	0.02	0.07	0.73	0.02	0.02	0.02	0.05	0.02	0.02	0.02	0.90	0.02	0.02	0.05

The five most frequent mutations are shown on the left-hand side, followed by the rest of the mutations in 5′–3′ and exon–intron order. Where indicated, the mutation was screened and found in 0 chromosomes. The empty spaces indicate that the mutations were not tested. Nomenclature of the mutations is according to the recent guidelines of the Human Genome Variation Society (2003) Nomenclature for the description of sequence variations. http://www.genomic.unimelb.edu.au/mdi/mutnomen/.

p.I507del	p.S549N	p.S549R	p.G551D	p.G551S	p.R553X	p.L558S	p.A559T	p.S589I	p.H609R*	p.P750L	p.V754M	p.R851L	p.I1027T	p.G1061R	p.R1066C	p.W1069X#	p.W1089X	p.Y1092X	p.W1098C*	p.W1204X
3	0	1	0		1								1	1	1					
			1		0															
					4															
					1			2							3		1			
3		1	1		6			2					1	1	4		1			
0.24		0.08	0.08		0.48			0.16					0.08	0.08	0.32		0.08			
			1		4															
					1															
					2							1					1			
			0		0															
0			1		0															
	0		0		1															
1	0	1	0		2		0										1	3		
0	0	0	0		0													0		
1		1	1		10							1					2	3		
0.05		0.05	0.05		0.54							0.05					0.11	0.16		
					3															
	0		0		0															
			1																	
0			1		1															
			2		4															
			0.79		1.58															
		4					1		1						1			1		
		4					1		1						1			1		
		1.83					0.46		0.46						0.46			0.46		
0	0		0		0															
0			0		0															
5	5		1	1	1	1				1	1					1		1	1	1
	1																			
5	6		1	1	1	1				1	1					1		1	1	1
1.82	2.19		0.36	0.36	0.36	0.36				0.36	0.36					0.36		0.36	0.36	0.36
1					1										1					
1.31					1.31										1.31					
10	6	6	6	1	22	1	1	2	1	1	1	1	1	1	6	1	3	5	1	1
0.23	0.14	0.14	0.14	0.02	0.51	0.02	0.02	0.05	0.02	0.02	0.02	0.02	0.02	0.02	0.14	0.02	0.07	0.11	0.02	0.02

(continued on next page)

Table 2 (*continued*)
Mutation frequencies in Latin American CF patients

Country	p.Q1238X	p.R1283M	c.-834G>T	c.297-1G>A*	c.297-2A>G	c.406-1G>A	c.621+1G>T	c.711+1G>T	c.846delT*	c.935delA	c.1078delT	c.1215delG	c.1323_1324insA*	c.1353_1354insT*#	c.1460_1461delAT*
Argentina					1		3							1	1
		1					1	1							
Subtotal and frequency (%)		1 0.08			1 0.08		4 0.32	1 0.08						1 0.08	1 0.08
Brazil							1								
	1						1 0	1			0				
Subtotal and frequency (%)	1 0.05						2 0.11	1 0.05							
Chile							0				0				
Subtotal and frequency (%)															
Colombia												1	1		
Subtotal and frequency (%)												1 0.46	1 0.46		
Costa Rica							0								
Frequency (%)															
Cuba															
Frequency (%)															
Ecuador															
Subtotal and frequency (%)															
Mexico				1		3	1		1	2	1				
Subtotal and frequency (%)				1 0.36		3 1.09	1 0.36		1 0.36	2 0.73	1 0.36				
Uruguay			1												
Frequency (%)			1.31												
Venezuela															
Subtotal and frequency (%)															
Total	1	1	1	1	1	3	7	2	1	2	1	1	1	1	1
Frequency (%)	0.02	0.02	0.02	0.02	0.02	0.07	0.16	0.05	0.02	0.05	0.02	0.02	0.02	0.02	0.02

c.1716G>A	c.1717-1G>A	c.1782delA	c.1811+1,6KbA>G	c.1812-1G>A	c.1898+1G>A	c.1898+3A>G	c.1924_1930del	c.2055_2063del	c.2183AA>G;c.2184delA	c.2184delA	c.2185_2186insC
	5 0 1	1	4			1			1	1	
			2		2						
	6 0.48	1 0.08	6 0.48		2 0.16	1 0.08			1 0.08	1 0.08	
	1										
	0										
	6 0 7 0.37			5 5 0.27	1 0 1 0.05				3 3 0.16	0 0	
	0								0		
			12								1
	0		12 5.50								1 0.46
	0										
1							1	2	2		
1 0.36	1 1.31						1 0.36	2 0.73	2 0.73		
1 0.02	14 0.32	1 0.02	18 0.41	5 0.11	3 0.07	1 0.02	1 0.02	2 0.05	6 0.14	1 0.02	1 0.02

(continued on next page)

Table 2 (*continued*)
Mutation frequencies in Latin American CF patients

Country	c.2347delG	c.2566_2567insT*	c.2594_2595delGT*	c.2686_2687insT*	c.2789+2_2789+3insA	c.2789+5G>A	c.2869_2870insG	c.3120+1G>A	c.3171delC	c.3199_3204del	c.3272-26A>G	c.3500-2A>G*
Argentina												
		2	1	2	2	3						
						3	1	1			2	
Subtotal and		2	1	2	2	6	1	1			2	
frequency (%)		0.16	0.08	0.16	0.16	0.48	0.08	0.08			0.16	
Brazil												
	2			1		1			1			
								6				
						0	0	4				
						0						
Subtotal and	2			1		1		10	1			
frequency (%)	0.11			0.05		0.05		0.54	0.05			
Chile												
Subtotal and												
frequency (%)												
Colombia						1		1				1
Subtotal and						1		1				1
frequency (%)						0.46		0.46				0.46
Costa Rica												
Frequency (%)												
Cuba												
Frequency (%)												
Ecuador												
Subtotal and												
frequency (%)												
Mexico										2		
Subtotal and										2		
frequency (%)										0.73		
Uruguay						1						
Frequency (%)						1.31						
Venezuela												
Subtotal and												
frequency (%)												
Total	2	2	1	3	2	9	1	12	1	2	2	1
Frequency (%)	0.05	0.05	0.02	0.07	0.05	0.21	0.02	0.28	0.02	0.05	0.05	0.02

c.3617_3618delGA*,#	c.3659delC	c.3849+1G>A	c.3849+10kbC>T	c.4005+1G>A	c.4005-1G>A#	c.4016_4017insT	c.4160_4161insGGGG*	c.IVS8-5T	Unknown	Authors
									37	Aulehla-Scholz [17]
	2		4		1	2		4	76	Visich [12]
			1						78	Ibañez [18]
									54	Varela 2004
									8	Prieto [19]
	2		1	1				1	18	Oller-Ramirez 2004
	4		6	1	1	2		5	271	
	0.32		0.48	0.08	0.08	0.16		0.40	21.75	
									205	Raskin [20]
									32	Chiba [21]
1									89	Bernardino [22]
									60	Marostica [23]
									69	Parizotto [24]
									99	Cabello [25,26]
									33	Martins [27]
									70	Streit [28]
	0		5						120	Raskin [15]
	0		0						12	Goloni-Bertollo [29]
1			5						789	
0.05			0.27						42.46	
									48	Rios [30]
									22	Molina [31]
			1						11	Navarro [32]
	0		3						34	Repetto [33]
			4						115	
			1.58						45.63	
		1							67	Keyeux [14]
									17	Restrepo [34]
		1							84	
		0.46							38.53	
			0						25	Venegas [35]
									52.08	
									95	Collazo [36]
									65.97	
									20	Merino [37]
									30	Cassiman 2004
									15	Paz-y-Mino [38]
									65	
									63.72	
			1				1		53	Orozco [13]
			2						35	Villalobos [39]
			3				1		88	
			1.09				0.36		32.11	
									11	Luzardo [40,41]
									14.47	
									36	Restrepo [34]
									41	Alvarado [42]
									77	
									56.62	
1	4	1	18	1	1	2	1	5	1620	
0.02	0.09	0.02	0.41	0.02	0.02	0.05	0.02	0.11	37.21	

most frequently found in Caucasians, by allele specific polymerase chain reaction (AS-PCR), enzymatic digestion, allele specific oligonucleotide hybridization (ASO), or using mainly commercial kits, whereas other studies used a systematic approach to analyse the promoter, coding and exon/intron boundaries of the *CFTR* region in the search for any possible mutation. The methods used in the latter include Denaturing Gradient Gel Electrophoresis (DGGE), Single Strand Conformational Polymorphism (SSCP) and Heteroduplex Analysis methods, followed by sequencing of the observed electrophoretical variants [11–15].

2.1. Statistical analyses

The genetic structure of the groups of patients was analyzed by AMOVA (*A*nalysis of *Mo*lecular *V*ariance), using the Arlequin Ver 2.000 software [43], in order to test the genetic variance in the distribution of CF-causing mutations by country.

Dendrograms were constructed using the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) and NJ (Neighbour Joining) methods with Reynolds' distance to visualize the differences in the distribution of the CFTR mutations in the Latin American countries so far studied. These were constructed using Arlequin to calculate the pairwise Fst, and the Populations [44] and TreeView (Win32) [45] programs to build the dendrograms.

3. Results

Out of 4354 CF chromosomes, 89 mutations were found (Table 1) with a detection rate of 62.79% of the total CF chromosomes studied. The frequency data obtained from this compilation are summarized in Table 2.

As expected, the p.F508del is the most frequent mutation, with an average frequency of 46.69% (2033/4354 chromosomes). Nevertheless, the range is broad, from 22.92% in Costa Rica to 59.15% in Argentina (Table 3). The p.G542X mutation, with a total frequency of 5.07% (25% in Costa Rica to 1.47% in Venezuela), is the second most frequent mutation in Latin America, with the exception of Colombia and Costa Rica. p.N1303K, p.W1282X and p.R1162X are the next most frequent mutations, with variations from 0.59% to 3.95% (Table 3). Costa Rica is a unique case, where p.G542X (12/48 chromosomes) and p.F508del (11/48 chromosomes) have almost the same frequencies. In Colombia, the second mutation is c.1811+1.6KbA>G, with a frequency of 5.5% (12/218 chromosomes) (Table 2). The p.G85E mutation was found in several countries such as Argentina [[12,18,19], Oller Ramirez, personal communication], Brazil [15,22], Ecuador (Cassiman, 2004, personal communication), Mexico [13] and Uruguay [41] with frequencies ranging from 0.36% in Mexico up to 3.95% in Uruguay. The frequency of the rest of the mutations varies from one country to another, and many are restricted to one or two countries only (Tables 2 4 and 5).

From the 89 mutations found, 41 are present in more than 1 patient (Table 2). As shown in Table 4, 19 of them have overall frequencies of 0.1% to 1% in Latin America and could be considered as rare, but some of them have local elevated frequencies, like c.1811+1.6KbA>G in Colombia (from 4.2% to 10.5% in the different geographical regions studied) [14, 46], p.G85E in Ecuador (8.9%) (Ruiz et al., personal communication), Uruguay (3.95%) [41], Brazil (2.33%) [15] and Argentina (2.26%) [18], p.R334W in Brazil (2.6%) [15, 22] and p.I507del (2.58%) and p.S549N (2.5%) in Mexico [13] (Table 2). p.R1162X showed an overall frequency of 0.96% (Table 3), but with some regional elevated frequencies, like in Santa Catarina, Brazil, with a presence of 10.4% in the sample studied [15]. c.3120+1G>A, normally present in African and Afrodescendant populations, was found in Rio de Janeiro, Brazil, with a frequency of 4.05% [26].

So far, 48 mutations are present only once in the whole set of chromosomes (4354), and 16 of them have not been described in other populations (mutations c.1460_ 1461delAT*, c.2566_2567insT*, p.L6V*, p.Y362X*,*c.1353_1354insT*, c.2594_2595delGT*, p.W1098C*, c.846delT*, c.4160_4161insGGGG*, c.297-1G>A*, c.3617delGA*, c.2686_2687insT*, p.Y275X*, c.3500-2A>G*, p.H609R* and c.1323_1324insA*) (Table 5B).

The p.G551D mutation, which shows an elevated frequency in the world, was found only in one Argentinean [18], two Brazilian [20,25], two Chilean [33, 32] and one Mexican [13] patient. p.R117H, a frequent mutation in the British patients, was investigated in the patients from Argentina [[18], Varela and Targovnik, personal communication], Brazil [15,28,29], Colombia [14], Costa Rica [35] and Mexico [13], and was found only in one CF patient from Mexico. Nevertheless, it was found in 2 patients from a sample of 35 Argentinean males with congenital bilateral absence of the vas deference [47]. Finally, the percentage of unidentified mutations in the whole sample of Latin American patients is 37.21% (Tables 2 and 3). However, four countries are far away from this mean value (Costa Rica, Cuba, Ecuador and Venezuela), and only two, Argentina (21.75%) and Uruguay (14.47%), have lower percentages of unknown mutations.

At least another 38 mutations have been searched for, but none of them were found in the CF patients from Latin America: p.E60X, p.Y122X, p.G178R, p.G330X, p.R347H, p.R352Q, p.S364P, p.A455E, p.Q493X, p.V520F, p.C524X, p.R560T, p.Y563D, p.P574H, p.K710X, p.Q890X, p.R1158X, p.S1196X, p.S1255X, p.D1270N, p.W1310X, p.W1316X, c.405+1G-A, c.444delA, c.556delA, c.574delA, c.1677delTA, c.2043delG, c.2307insA, c.2909delT, c.3120G-A, c.3358delAC, c.3662delA, c.3750delAG, c.3791delC, c.3821delT, c.3849+4A-G, c.3905insT.

For the calculation of the distance matrix used in the AMOVA analyses and the construction of dendrograms, we included the frequency data of all mutations greater or equal to 0.1% (Tables 3 and 4), of the very rare mutations listed in Table 5, grouped as a single set of "other" mutations, different in each country, and of the unknown mutations.

Table 3
Most frequent mutations (>1%) in Latin American patients

Country	Chromosomes analysed	p.F508del		p.G542X		p.N1303K		p.W1282X		p.R1162X		Unknown	
		n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Argentina	1246	737	59.15	61	4.90	28	2.25	27	2.17	9	0.72	271	21.75
Brazil	1858	800	43.06	99	5.33	34	1.83	11	0.59	25	1.35	789	42.46
Chile	252	99	39.28	14	5.55	0	0.00	8	3.17	3	1.19	115	45.63
Colombia	218	90	41.28	9	4.13	2	0.92	3	1.38	2	0.92	84	38.53
Costa Rica	48	11	22.92	12	25.00	–	–	–	–	–	–	25	52.08
Cuba	144	49	34.03	–	–	–	–	–	–	–	–	95	65.97
Ecuador	102	32	31.37	2	1.96	1	0.98	–	–	–	–	65	63.72
Mexico	274	115	41.97	16	5.84	5	1.82	–	–	–	–	88	32.11
Uruguay	76	43	56.58	6	7.89	2	2.63	–	–	3	3.95	11	14.47
Venezuela	136	57	41.91	2	1.47	–	–	–	–	–	–	77	56.62
Total	4354	2033	46.69	221	5.08	72	1.65	49	1.13	42	0.96	1620	37.21

A – sign indicates that these mutations were not tested in the sample of patients, therefore their real frequency remains unknown.

Very low FST values, indicating a low genetic differentiation of the CF-causing mutations [48] were found between the Argentinean and Uruguayan (FST=0.00187, $p>0.18$), the Brazilian and Chilean (FST=0.00088, $p>0.20$), the Brazilian and Colombian (FST=0.0028, $p>0.19$) and the Cuban and Ecuadorian (FST=− 0.0062, $p>0.69$) patient groups. On the contrary, some of the other group comparisons show moderate to elevated genetic differences between each other (FST=0.05–0.23, $p>0.000$), such as in the case of Uruguay or Argentina versus Costa Rica, Cuba, Ecuador and Venezuela, indicating that the mutations contributing to the disease are significantly different between any of the latter four countries and Argentina or Uruguay.

The dendrograms maintain the same clustering pattern with the different methods used, indicating the strength and good resolutions of the methods employed. Fig. 1 shows the results obtained with the UPGMA method using Reynolds' distance. Reflecting the FST data, Argentina and Uruguay, Chile, Brazil and Colombia, and Cuba, Ecuador and Venezuela form separate clusters and show very short branching distances within each cluster, due to a large similarity in the type and frequency of the CF-causing mutations. Costa Rica sits on an isolated branch, apparently due to the weight given by the exceptionally high frequency of the p.G542X mutation in these patients (25%). Although some of the clustering could be artificial, due to the lack of mutation data in some countries (e.g. Cuba, Costa Rica and Venezuela), it is clear that the general pattern will be maintained for those countries where a great number of mutations as well as a significant number of patients have been studied.

4. Discussion

In the present meta-analysis we have examined the results obtained in the research of 4354 CF chromosomes from Latin American patients, where 89 mutations were found.

Table 4
Mutations with frequencies between 0.1% and 1%

Mutation	Frequency		Country
	Number of chromosomes	%	
p.R334W	39	0.90	Argentina, Brazil, Chile, Colombia, Uruguay
p.G85E	32	0.73	Argentina, Brazil, Ecuador, Mexico, Uruguay
p.R553X	22	0.51	Argentina, Brazil, Chile, Mexico, Uruguay
c.1811+1.6KbA>G	18	0.41	Argentina, Colombia
c.3849+10KbC>T	18	0.41	Argentina, Mexico
c.1717-1G>A	14	0.32	Argentina, Brazil, Uruguay
c.3120+1G>A	12	0.28	Argentina, Brazil Colombia
p.I507del	10	0.23	Brazil, Mexico, Uruguay
c.2789+5G>A	9	0.21	Argentina, Brazil, Colombia, Uruguay
c.621+1G>T	7	0.16	Argentina, Brazil, Mexico
p.S549N	6	0.14	Mexico
p.S549R	6	0.14	Argentina, Brazil, Colombia
p.G551D	6	0.14	Argentina, Mexico
p.R1066C	6	0.14	Argentina, Colombia, Mexico
c.2183A>G;c.2184delA	6	0.14	Argentina, Mexico
p.Y1092X	5	0.11	Colombia, Mexico
c.1812-1G>A	5	0.11	Brazil
c.IVS8-5T	5	0.11	Argentina
c.3659delC	4	0.09	Argentina

Table 5
Mutations with frequencies less than 0.1%

Panel A

Mutation	Number of chromosomes	%	Country
p.R75X	3	0.07	Mexico
c.W1089X	3	0.07	Argentina, Brazil
c.406-1G>A	3	0.07	Mexico
c.1898+1G>A	3	0.07	Argentina, Brazil
c.2686_2687insT*	3	0.07	Argentina, Brazil
p.L206W	2	0.05	Brazil
p.I506T	2	0.05	Mexico
p.S589I	2	0.05	Argentina
c.711+1G>T	2	0.05	Argentina
c.935delA	2	0.05	Mexico
c.2055_2063del	2	0.05	Mexico
c.2347delG	2	0.05	Brazil
c.2566_2567insT*	2	0.05	Argentina
c.2789+2_2789+3insA	2	0.05	Argentina
c.3199_3204del	2	0.05	Mexico
c.3272-26A>G	2	0.05	Argentina
c.4016_4017insT	2	0.05	Argentina

Panel B

Mutation	*N*	% each	Country
p.L6V*, p.W57X, p.Q220X, p.Y362X*, p.I1027T, p.G1061R, p.R1283M, c.297-2A>G, c.1353_1354insT*, c.1460_1461delAT*, c.1782delA, c.1898+3A>G, c.2184delA, c.2594_2595delGT*, c.2869_2870insG, c.4005*1G>A, c.4005-1G>A#	17	0.02	Argentina
p.R117H, p.H199Y, p.G551S, p.L558S, p.P750L, p.V754M, p.W1069X#, p.W1098C*, p.W1204X, c.297-1G>A*, c.846delT*, c.1078delT, c.1716G>A, c.1924_1930del, c.4160_4161insGGGG*	15	0.02	Mexico
p.V201M, p.V232D, p.Y275X*, p.R347P, p.R851L, p.Q1238X, c.3171delC, c.3617_3618delGA*#	8	0.02	Brazil
p.A559T, p.H609R*, c.1215delG, c.1323_1324insA*, c.2185_2186insC, c.3500-2A>G*, c.3849+1G>A,	7	0.02	Colombia
c.-834G>T	1	0.02	Uruguay

The upper part (Panel A) shows the mutations found in more than one patient, whereas the lower part (Panel B) of the table shows all the mutations that are present only once in each country. *N*=number of different mutations.

Unfortunately, at present not all Latin-American countries have started molecular studies in their patients with a probable Cystic Fibrosis diagnosis. Also, the method used to investigate the *CFTR* mutations varied from one group to the other: some authors have concentrated in the search of specific mutations that are most frequently found in Caucasians (using mainly commercial kits), whereas other studies made a systematic analysis of part or all the coding region of the *CFTR* gene (DGGE, SSCP or mHET methods, followed by sequencing). Therefore, not all studies can be directly compared one to another, and most important, at present it is not possible to know if the absence of particular mutations is real, or results from a screening bias. However, this is the most complete compilation of the Latin American CF-causing mutations, and the general trend deduced in the present study is consistent with the data presented in the first survey of 2174 CF chromosomes prepared by the authors for a meeting convened by the World Health Organization in 2002 [16].

The profile of mutations in the *CFTR* gene in the different countries and areas reflects the heterogeneity of its inhabitants, since Latin America is a region that shows a very strong diversity in certain features such as population origin, cultural aspects and environment. In Mexico, Colombia, Ecuador or Chile, for instance, between 57% and 85% of the population is Mestizo, which means that it is a highly admixed population between Europeans (mainly Spaniards), Amerindians and, in some regions, Afrodescendants. This is clearly reflected in the mitochondrial DNA haplogroups found in the Mestizo population from Mexico and Colombia, for example, where up to 78% of the maternally inherited lineages are Amerindian (A-D) [49, 50]. As a consequence, the frequencies of the CF mutations differ significantly between the South-eastern countries (Argentina, Uruguay and south eastern of Brazil), which have had the major European immigration, and the others.

As an example, in the case of Argentina and Uruguay, the p.F508del mutation shows the highest frequencies (59% and

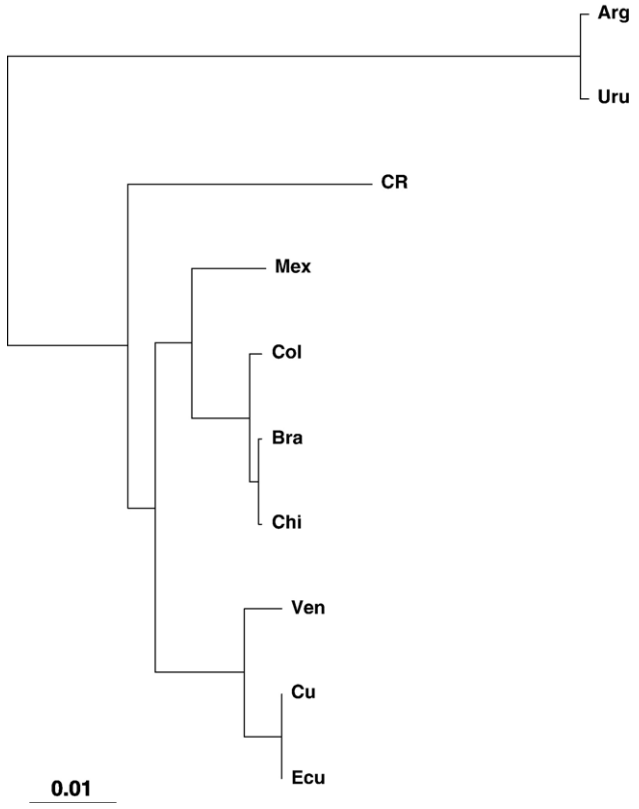

Fig. 1. UPGMA dendrogram using Reynolds' distance. The tree shows the clustering of CF patients according to the CF-causing mutations found in each country (Arg=Argentina, Bra=Brazil, Chi=Chile, Col=Colombia, CR=Costa Rica, Cu=Cuba, Ecu=Ecuador, Mex=Mexico, Uru=Uruguay, Ven=Venezuela).

57%, respectively) (Table 3) and around 90% European ancestry. Furthermore, if we take three of the most frequent mutations in Europeans (p.F508del, p.G542X and p.N1303K), along with a very frequent mutation in Italians, p.R1162X [9], the percentage of these in Argentinean and Uruguayan CF patients adds up 67% and 71%, respectively, of the mutations (Table 3). This is also manifest in Brazil, where the States that have a predominant European ancestry with little admixture with Africans or Amerindians (Santa Catarina, Parana) show a frequency of 63% for these mutations, compared to 14% in the Afro-Brazilian patients [15].

Another feature of Latin American countries with higher European descent, is the lower frequency of unknown mutations, as seen in Argentina (22%) and Uruguay (14%) (Table 3), and in the more European States from Brazil (19%) [15], which is comparable to the global value given for Europe (18%), particularly for Spain, France, Germany and the United Kingdom [9]. Even if this overall observation could partly be due to a better CF diagnosis, particularly in Argentina where the clinical expertise has been developed over decades, this does not seem to explain the significant differences observed in some other countries, like Colombia (39% unknown mutations), for instance, where patients with borderline or normal sweat test values and mild clinical signs were indeed found to have rare mutations in the CFTR locus [14]. Therefore, we presume that among the true unknown CF genotypes, a high number of mutations are probably contained in the unexplored regions of the gene (deep intronic sequences and 5′ and 3′ regulatory regions).

The ethnic background of the different Latin American CF patients and the diversity of origin of the European ancestors is also apparent in the rest of the profile of *CFTR* mutations so far found: the rare mutations (<1%) are very different in each country, and many of them are present in only one country, as shown in Tables 4 and 5. Furthermore, each of the four countries where an exhaustive search for mutation has been achieved (Argentina, Brazil, Colombia and Mexico), shows a set with a high number of private mutations, present in only one patient (Table 5, panel B).

In particular regions from Colombia, Venezuela, Brazil, Uruguay and Ecuador, the presence of Afrodescendant populations is important, although its global percentage does not exceed 10%. Anthropological and genetic studies, however, show that in some countries like Colombia, the African component is present in up to 25% of the population [51], it being in the Mulattos or in the Mestizo. Actually, two African mutations (p.A559T and c.3120+1G>A) have been found in two regions with high African admixture in Colombia (Bolivar and Antioquia-Chocó) [14], and the c.3120+1G->A mutation has also been found in Brazil [15,26], with a frequency of 4% in Rio de Janeiro, and Argentina (Oller Ramirez, personal communication). As discussed, another way to disclose similarities or differences in the distribution of mutations in the CF patients from Latin

Table 6
Screening panel of CFTR mutations

Country	Total number of mutations	Minimum panel	Detection power
Uruguay	12	6 mutations: p.F508del, p.G542X, p.R1162X, p.N1303K (p.R334W, p.G85E)	78%
Argentina	52	7 mutations: p.F508del, p.G542X, p.R1162X, p.W1282X, p.N1303K (p.R334W, p.G85E)	71%
México	35	8 mutations: p.F508del, p.G542X, p.N1303K (p.R75X, p.I507del, p.S549N,c.406-1G>A, c.3849+10kbG>A)	58%
Colombia	19	7 mutations: p.F508del, p.G542X, p.R1162X, p.W1282X, p.N1303K (p.S549R, c.1811+1.6kbA>G)	56%
Brazil	41	6 mutations: p.F508del, p.G542X, p.R1162X, p.W1282X, p.N1303K (p.R334W)	53%

The total number of mutations found in each country is indicated in the second column from left. The number of mutations useful in a first routine screening is indicated in the third column: this minimum panel of CF-causing mutations includes all those mutations shown in Table 3 and other mutations having a frequency close or above 1% in the population considered, indicated in parenthesis. The last column shows the detection power reached, when using this minimum panel.

America is to construct dendrograms based on the genetic diversity found in the series of patients grouped by country. This statistical analysis gives a numerical value (Fst) to the differences in the mutations observed in the groups of patients. The separate branch formed by Ecuador, Cuba and Venezuela (Fig. 1) probably reflects this contribution of African genes, since the patients from Ecuador (Guayaquil) [[37], Cassiman, personal communication] and Venezuela (Maracaibo) [34] come from regions with high admixture with Africans. Descendants of people from the Middle East are also numerous in these same regions, and thus could partly explain the distinct clustering. In other words, although the specific mutations found in Afrodescendant CF patients have not been screened for in the Ecuadorian, Cuban and Venezuelan CF patients, the present analysis shows that, even with the scarce data available to date, their genetic makeup, as regards the CF-related mutations, is distinct from that of the rest of Latin American countries.

Contrary to the argument sometimes found in the literature, we do not believe that the discrete clustering of Mexico, Brazil, Chile and Colombia, but also Ecuador, Cuba and Venezuela (Fig. 1), reflects a more indigenous contribution to the CF mutation panel, which could have been overlooked by the actual methods used to screen the patients. Asia, which is the major source of the Native Americans, is not a region with a particularly high incidence of CF, nor have the frequent "Asian" mutations [52–54] been found in Latin American patients, as has been the case with the African and Afro-American mutations [54–57]. New mutations found in the Latin American patients could be regarded as genuine "American" private mutations (unless they are found in patients of the source populations), and the actual number (16 in 4356 chromosomes) could be suggestive of other private mutations not detected so far.

The case of Costa Rica is unusual, since although being one of the most "European" countries in Latin America (sometimes called the Latin-American Switzerland), only two common European mutations, from a panel of 16 frequent mutations, were found [35], and a high proportion of mutations (52%) remain unknown. In this country, p.G542X (25%) is the first mutation, followed by p.F508del (22.92%) (Table 3). As argued by the authors, this unusual high frequency of p.G542X, which is almost four times that observed overall in Spain (7.66%) and Andalusia (8.6%) [9], could be a consequence of a strong founding effect due to the small number of settling families from this region established in Costa Rica in the XVIth–XVIIth century. Due to this, a part of this elevated frequency can also be attributed to endogamy for many generations, and thus non-randomness of the patients studied. Another example of a possible founder effect has been observed in Colombia, where two mutations show unusually high frequencies, c.1811+1,6kbA>G (10.8%) in Bogotá [14], and c.621+1G>A (17.65%) in Boyacá [Mateus et al., unpublished results].

We constructed dendrograms using the pairwise genetic distances obtained using both, a reduced (consisting of 2174 CF chromosomes) [16] and an enlarged set of patients (present data). The only relevant difference observed when using a higher number of patients per country is a shift in the clustering of the Chilean CF cohort, which in the first study [30] had a similar mutation profile as Cuba, and clustered together with these patients on a separate branch (data not shown). We therefore believe that the actual grouping shown in the dendrograms (Fig. 1) not only is a reliable view of the origin of the mutations in the different Latin American countries, but also, and more important, points to the need of a diversity of mutation panels advisable to reach a better diagnostic efficacy. Argentina and Uruguay should screen a common panel of 7 mutations which would reach a detection power of 71% and 78%, respectively, of the CF mutations. This panel, besides the common mutations shown in Table 3, should include the p.R334W and p.G85E mutations. Brazil, using the same panel, would reach only 53% of detection (Table 6), the rest of the mutations being present at frequencies of <0.6% in the whole sample. The situation of Colombia and Mexico is quite different, since the minimum panel includes the common mutations (Table 3) plus a set of other mutations (5 in Mexico and 2 in Colombia), which are different in each of the two countries (Table 6).

In conclusion, the present meta-analysis shows the complexity of the molecular diagnosis of CF mutations in most of the Latin American countries. Therefore, the knowledge of the molecular genetic epidemiology of CF in the different countries is important to schedule more straightforward molecular diagnostic of the patients in order to improve the cost-effectiveness of the studies, especially when economical resources are scarce. From a clinical point of view, a first screen should include the main mutation panel described in Table 6, after which a locally (geographically) adapted choice of other relevant regions of the CFTR gene to be screened (particular mutations, or exons and exon/intron boundaries) seems advisable in those patients where the genotype remains unknown after the basic panel screening, like for instance the p.R553X mutation in Sao Paulo [20] and Formosa (Varela and Targovnik, personal communication), since otherwise an important proportion of mutations in these patients could be overlooked.

Acknowledgments

We are indebted to Drs. João Lavinha, John Dodge and Jean-Jacques Cassiman for critical review of the present manuscript. We would like to acknowledge Prof. William Usaquén for helping in the statistical analyses.

References

[1] Romeo G, Galietta JL, Devoto M. Why is the CF gene frequent? Hum Genet 1989;84:1–5.
[2] EWGCFG European Working Group on Cystic Fibrosis Genetics. Gradient of distribution in Europe of the major CF mutation and of its associated haplotype. Hum Genet 1990;85:436–45.

[3] Morral N, Nunes V, Casals T, Chillón M, Giménez J, Bertranpetit J, et al. Microsatellite haplotypes for cystic fibrosis: mutation frameworks and evolutionary tracers. Hum Mol Genet 1993;2:1015–22.
[4] Gabriel SE, Bringman KN, Koller BH, Boucher RC, Stutts MJ. Cystic fibrosis heterozygote resistance to cholera toxin in the cystic fibrosis mouse model. Science 1994;266:107–9.
[5] Schroeder SA, Gaughan DM, Swift M. Protection against bronchial asthma by CFTR delta F508 mutation: a heterozygote advantage in cystic fibrosis. Nat Med 1995;1:1100–2.
[6] Pier GB, Grout M, Zaidi T, Meluleni G, Mueschenborn S, Banting G, et al. *Salmonella typhi* uses CFTR to enter intestinal epithelial cells. Nature 1998;393:79–82.
[7] Mateu E, Calafell F, Ramos MD, Casals T, Bertranpetit J. Can a place of origin of the main cystic fibrosis mutation be identified? Am J Hum Genet 2002;70:257–64.
[8] Lucotte G, Hazout S. Geographic and ethnic distribution of the more frequent cystic fibrosis mutations in Europe show that a founder effect is apparent for several mutant alleles. Hum Biol 1995;67:561–76.
[9] Estivill X, Bancells C, Ramos C. Biomed CF mutation analysis consortium. Geographic distribution and regional origin of 272 cystic fibrosis mutations in European populations. Hum Mutat 1997;10:135–54.
[10] Pivetta OH, Luna MC, Granados P, Cafferata EG. Enfermedad Fibroquística del páncreas. In: Emiliani Roque A, editor. Gastroenterología en clínica pediátrica. CP 67 SA. Buenos Aires: Libreria Técnica; 1997. p. 190–221.
[11] Chertkoff L, Visich A, Bienvenu T, Grenoville M, Segal E, Carniglia L, et al. Spectrum of CFTR mutations in Argentine cystic fibrosis patients. Clin Genet 1997;51:43–7.
[12] Visich A, Zielenski J, Castanos C, Diez G, Grenoville M, Segal E, et al. Complete screening of the CFTR gene in Argentine cystic fibrosis patients. Clin Genet 2002;61:207–13.
[13] Orozco L, Velasquez R, Zielenski J, Tsui LC, Chávez M, Lezana JL, et al. Spectrum of CFTR mutations in Mexican cystic fibrosis patients: identification of five novel mutations (W1098C, 846delT, P750L, 4160insGGGG and 297-1G→A). Hum Genet 2000;106:360–5.
[14] Keyeux G, Rodas MC, Bienvenu T, Garavito P, Vidaud D, Sanchez D, et al. Main cystic fibrosis mutations in patients from Colombia: implications of the mutational spectrum for local and regional molecular diagnosis programs. Hum Mutat 2003:1–7 Mutation in Brief # 644 Online.
[15] Raskin S, Pereira L, Reis F, Rosario NA, Ludwig N, Valentim L. High allelic heterogeneity between Afro-Brazilians and Euro-Brazilians impacts cystic fibrosis genetic testing. Gen Test 2003;7:213–8.
[16] WHO (2004) The Molecular Genetic Epidemiology of Cystic Fibrosis. Report of a Joint Meeting of WHO/ECFTN/ICF(M)A/ECFS. http://www.who.org/genomics.
[17] Aulehla-Scholz C, Kaiser R, Weber J, Pivetta OH, Eigel A, Dworniczak B, et al. The frequency of the CF Delta F508 deletion in CF chromosomes of different ethnic origin. Hum Genet 1990;85:392–3.
[18] Ibañez C, Pérez M, Pastorale C, Granados P, Keyeux G, Pivetta OH, et al. Correlación entre las mutaciones del gen CFTR y la expectativa de vida de los pacientes fibroquísticos de América Latina. Actas XXXIII Congreso Argentino de Genética. Basic Appl Genet 2004;S-70.
[19] Prieto L, Chiesa A, Macri C, Gruñeiro-Papendieck L. 1999. Pesquisa neonatal de Fibrosis Quística (FQ): Programa piloto con IRT y DNA. II Congreso Argentino de Neumonología Pediátrica, IX Congreso Latinoamericano de Fibrosis Quística, Buenos Aires, Argentina.
[20] Raskin S, Phillips JA, Kaplan G, McLure M, Vnencak-Jones C, Rozov T. Geographic heterogeneity of four common worldwide cystic fibrosis non F508 mutations in Brazil. Hum Biol 1999;71:103–13.
[21] Chiba MS, Gomes CET, Silva GP, Bernardino A, Lima C, Zatz M. 2001. Caracteristicas fenotipicas dos pacientes portadores de fibrose cistica. Anais do IX Congresso Brasileiro de Pneumunología Pediatrica, V Congresso da Sociedade Latino Americana de Pneumologia Pediatrica; X Congresso Latino Americano de Fibrose Cistica; IX Jornada Brasileira de Fibrose Cistica; II Jornada Brasileira de Fisioterapia Respiratoria em Pediatria. 28 de septiembre al 3 de octubre 2001 Florianopolis-SC-Brasil.
[22] Bernardino AL, Ferri A, Passos-Bueno MR, Kim CE, Nakaie CM, Gomes CE, et al. Molecular analysis in Brazilian cystic fibrosis patients reveals five novel mutations. Gen Test 2000;4:69–74.
[23] Marostica PJ, Raskin S, Abreu-e-Silva FA. Analysis of the delta F508 mutation in a Brazilian cystic fibrosis population: comparison of pulmonary status of homozygotes with other patients. Braz J Med Biol Res 1998;31:529–32.
[24] Parizotto EA, Ribeiro AF, Bertuzzo CS. Molecular characterization of cystic fibrosis patients in the state of Sao Paulo (Brazil). J Med Genet 1997;34:877.
[25] Cabello GM, Moreira AF, Horovitz D, Correia P, Santa Rosa A, Llerena Jr J, et al. Cystic fibrosis: low frequency of DF508 mutation in 2 population samples from Rio de Janeiro, Brazil. Hum Biol 1999;71:189–96.
[26] Cabello GM, Cabello PH, Llerena Jr J, Fernandez O, Harris A. The 3120+1G-A splicing mutation in CFTR is common in Brazilian cystic fibrosis patients. Hum Biol 2001;3:403–9.
[27] Martins CS, Ribeiro F, Costa FE. Frequency of the cystic fibrosis delta F508 mutation in a population from Sao Paulo State, Brazil. Braz J Med Biol Res 1993;26:1037–40.
[28] Streit C, Burlamaque-Neto AC, de Abreu e Silva F, Giugliani R, Saraiva Pereira ML. CFTR gene: molecular analysis in patients from South Brazil. Mol Genet Metab 2003;78:259–64.
[29] Goloni-Bertollo EM, Rossit ARB, Batista J, Junior S, Fett-Conte AC, Raskin S. CFTR molecular analysis reveals infrequent allele frequencies in nine cystic fibrosis patients from Sao Paulo State, Brazil. Hum Biol 2003;3:393–8.
[30] Rios T, Orellana O, Aspillaga M, Avendaño Y, Largo Y, Riveros N. CFTR mutations in Chilean cystic fibrosis patients. Hum Genet 1994;94:291–4.
[31] Molina G, Gonzalez FJ, Cave R, Cornejo de M, Navarro S, Deglin M, et al. Estudio clínico–genético molecular de la fibrosis quística en la V Región, Chile (Clinical and molecular genetic study of cystic fibrosis in the 5th Region of Chile). Rev Méd Chile 2002;130:850–8.
[32] Navarro H, Kolbach M, Repetto G, Guiraldes E, Harris P, Foradori A. Correlation between phenotype and genotype in a group of patients with cystic fibrosis. Rev Méd Chile 2002;130:475–81.
[33] Repetto G, Poggi H, Harris P, Navarro H, Sanchez I, Guiraldes E, et al. Identification of mutation in the gene cystic fibrosis transmembrane regulator (CFTR) in Chilean patients with cystic fibrosis. Rev Med Chile 2001;129:841–7.
[34] Restrepo CM, Pineda L, Rojas-Martínez A, Gutiérrez CA, Gómez Y, Borjas L, et al. Cystic Fibrosis gene mutation in three Latin American countries. Am J Med Genet 2000;91:277–9.
[35] Venegas PB, Novak JM, Oscar CA, Sanchez FL, Gutierrez IG, Rivera JM, et al. Cystic fibrosis mutations in Costa Rica. Hum Biol 2003;75:179–88.
[36] Collazo T, Magarino C, Chávez R, Suardez B, Gispert S, Gómez M, et al. Frequency of delta-F508 mutation and XV2C/KM19 haplotypes in Cuban cystic fibrosis families. Hum Hered 1995;45:55–7.
[37] Merino M, Schollen E, Matthijs G, Dequeker G, Martinez E, Tapia M, et al. 1999. Estudios moleculares de fibrosis quística en pacientes de Ecuador. Actas del II Congreso Argentino de Neumonología Pediátrica. IX Congreso Latinoamericano de Fibrosis Quística. 24 al 27 de noviembre de 1999. Buenos Aires, Argentina.
[38] Paz-y-Mino C, Perez JC, Burgos R, Davalos MV, Leone PE. The DeltaF508 mutation in Ecuador, South America. Hum Mutat 1999;14:348–50.
[39] Villalobos-Torres C, Rojas-Martínez A, Villarreal-Castellanos E, Cantu JM, Sanchez-Anzaldo FJ, Saiki RK, et al. Analysis of 16 cystic fibrosis mutations in Mexican patients. Am J Med Genet 1997;69:380–2.
[40] Luzardo G, Crispino B, Mimbacas A, Martinez L, Cardoso H. 1999. Análisis de las frecuencias de las mutaciones de fibrosis quística según

criterios diagnósticos en una muestra de la población Uruguaya. II Congreso Argentino de Neumonología Pediátrica. IX Congreso Latinoamericano de Fibrosis Quística. Buenos Aires, Argentina.
[41] Luzardo G, Aznarez I, Crispino B, Mimbacas A, Martínez L, Poggio R, et al. Cystic fibrosis in Uruguay. Genet Mol Res 2002;1:32–8.
[42] Alvarado J., Acosta L., Carrasquel B., Lugo Z. 2000. Fibrosis Quística. Estudio de 41 Pacientes—Hospital de Niños "J.M. de los Ríos" Acta Otorrinolaringológica 12, Venezuela.
[43] Schneider S, Roessli D, Excoffier L. 2000. ARLEQUIN ver 2.000. A software for population genetics data analysis. http://anthro.unige.ch/arlequin.
[44] Langella O. Populations 2002; 1.2.28, CNRS UPR9034. http://www.cnrs-gif.fr/pge.
[45] Page R 2001; Tree View (Win32) 1.6.5.http://www.taxonomy.zoology.gla.ac.uk/rod/rod.html.
[46] Keyeux G, Bienvenu T, Rodas C, Garavito P, Sanchez D, Stand I, et al. Particular aspects of the molecular basis of cystic fibrosis in Colombia. Am J Hum Genet 1997;4:A202.
[47] Levy EM, Granados P, Rawe V, Brugo Olmedo S, Luna MC, Cafferata E, et al. Mutations in CFTR gene and clinical correlation in argentine patients with congenital bilateral absence of the *vas deferens*. Medicina (Buenos Aires) 2004;64:213–8.
[48] Hartl D. Principles of population genetics. Massachusetts: Sinauer associates, Inc. Sunderland; 1997.
[49] Green LD, Derr JN, Knight A. mtDNA affinities of the peoples of North-Central Mexico. Am J Hum Genet 2000;66:989–98.
[50] Rodas C, Gelvez G, Keyeux. Mitochondrial DNA studies show asymmetrical Amerindian admixture in Afro-Colombian and Mestizo populations. Hum Biol 2003;75:13–30.
[51] Friedemann N, Arocha J. Colombia. In: Livinoff Miles, editor. No longer invisible: Afro-Latin-Americans today. London: Minority Rights Group; 1995. p. 47–76.
[52] Suwanjutha S, Huang NN, Wattanasirichaigoon D, Sura T, Harris A, Macek Jr M. Case report of a Thai male cystic fibrosis patient with the 1898+1G>T splicing mutation in the CFTR gene: a review of East Asian cases. Hum Mutat 1998:1–4 Mutation in Brief # 196 Online.
[53] Wu CL, Shu SG, Zielinski J, Chiang CD, Tsui LC. Novel cystic fibrosis mutation (2215insG) in two adolescent Taiwanese siblings. J Formos Med Assoc 2000;99:564–7.
[54] Bobadilla JL, Macek M, Fine JP, Farell PM. Cystic fibrosis: a worldwide analysis of CFTR mutations—correlation with incidence data and application to screening. Hum Mutat 2002;19:575–606.
[55] Carles S, Desgeorges M, Goldman A, Thiart R, Guittard C, Kitazos CA, et al. First report of CFTR mutations in black cystic fibrosis patients of southern African origin. J Med Genet 1996;33:802–4.
[56] Macek M, Mackova A, Hamosh A, Hilman BC, Selden RF, Lucotte G, et al. Identification of common cystic fibrosis mutations in African-Americans with cystic fibrosis increases the detection rate to 75%. Am J Hum Genet 1997;60:1122–7.
[57] Padoa C, Goldman A, Jenkins T, Ramsay M. Cystic fibrosis carrier frequencies in populations of African origin. J Med Genet 1999;36:41–4.