Online journal ISSN - 1676-5680
GMR Genetics and Molecular Research
EVOLUTION AND TECHNOLOGY

A low prevalence of cystic fibrosis in Uruguayans of mainly European descent

Horacio Cardoso[1], Beatriz Crispino[1], Adriana Mimbacas[1,2] and Manuel Enrique Cardoso[3]

[1]Departamento de Citogenética, Instituto de Investigaciones Biológicas Clemente Estable (IIBCE), Montevideo, Uruguay
[2]Departamento de Citogenética, IIBCE, Unidad Asociada al Instituto de Biología, Facultad de Ciencias, Montevideo, Uruguay
[3]Facultad de Ciencias Sociales y Comunicación,
Universidad Católica del Uruguay, Montevideo, Uruguay
Corresponding author: H. Cardoso
E-mail: hca@iibce.edu.uy

Genet. Mol. Res. 3 (2): 258-263 (2004)
Received September 12, 2003
Accepted March 1, 2004
Published May 25, 2004

ABSTRACT. Cystic fibrosis is the most common hereditary disease in populations of European descent, with its prevalence depending on the populations and ethnic groups studied. In contrast to Europe and North America, there is little information about this disease in Latin America. Uruguay currently has a human population of 3,000,000, with a low rate of miscegenation and no remaining isolated Amerindian groups. In the present study, we estimated the prevalence of cystic fibrosis in this country based on the detection of ΔF508 mutation carriers in 500 unrelated individuals and on the frequency of individuals homozygous for this mutation within the affected population. The latter was calculated from the frequency of the different mutations and genotypes observed in a sample of 52 previously described patients with confirmed cystic fibrosis. A theoretical estimate of the prevalence of cystic fibrosis based on anthropological data suggested a frequency of 25 affected individuals/100,000 inhabitants. However, our data indicated that the true prevalence in the population was considerably lower (6.9 cases/100,000 inhabitants).

Key words: Cystic fibrosis, Hereditary disease, European descent

INTRODUCTION

Cystic fibrosis (CF) is the most common hereditary disease in populations of European descent. To date, more than 1000 mutations have been described in the CF transmembrane regulator gene. The prevalence observed depends on the populations and ethnic groups studied (Zielenski and Tsui, 1995). Variations in the prevalence between 1:1,700 and 1:7,700 have been described in northern European populations (Tsui, 1992), with lower values being reported for an Asian population in Hawaii (1:90,0000) (Estivill, 1991) and an African population (1:100,000) (Tsui, 1992). In contrast, there is little information on this disease in Latin America (Raskin et al., 1993; Chertkoff et al., 1997). The few studies that have been reported have yielded contradictory results (Ríos et al., 1994) or have estimated the prevalence of CF using indirect (Pivetta and Olek, 1991) or theoretical (Valenzuela, 1988) approaches.

In contrast to other Latin American countries, Uruguay has a population with peculiar characteristics that include: a) a total population of 3,000,000 inhabitants, half of whom live in the capital Montevideo, b) no remaining isolated Amerindian groups living in the country since the end of the 19th century, c) a low rate of miscegenation (Sans et al., 1993,1997), and d) one of the highest health care standards in South America, with 92.5% of the population being attended by the health care system (PNUD, 1997).

We have previously estimated the frequency of various mutations and genotypes in a sample of 52 patients with confirmed CF (Luzardo et al., 2002). Considering the peculiar characteristics of the Uruguayan population and the lack of molecular data on CF in this population, we have now estimated the prevalence of CF in this country based on the detection of ΔF508 mutation carriers in a representative sample of 500 unrelated individuals, and on the frequency of individuals homozygous for this mutation in the affected population.

MATERIAL AND METHODS

Population sample

The sample size (500 individuals) for the detection of ΔF508 carriers in a Montevidean population was determined based on a probable frequency of 2% (estimated with 95% confidence) which is similar to that observed in Europe (The Cystic Fibrosis Genetic Analysis Consortium, 1994) for this mutation. The subjects were selected at random from among unrelated individuals attended at 15 (5 public and 10 private) medical health institutions. Data from a previous unpublished study (Cardoso, M.E., personal communication) allowed us to stratify the population at each health center into six arbitrary socio-economic categories and to determine their corresponding frequencies. The number of subjects enlisted at each institution was established based on the socio-economic categories identified above. The individuals were selected while attending one of the health centers for blood analyses unrelated to CF and were informed that they would be enrolled in the study anonymously and that they would not receive the test results. After providing informed consent, 5 ml of peripheral blood was collected from each subject. This study (Ref. 1081/96) was approved by the Ministerio de Salud Pública (Uruguay).

DNA extraction and PCR

DNA was extracted using a standard protocol modified from Valenzuela and Méndez (1982). The primers, cycles and temperatures for the PCR amplification of CFTR exon 10 were as described by Kerem et al. (1989) and Riordan et al. (1989). Samples from all of the individuals studied were amplified in groups of four DNAs each. The PCR amplification products were separated on 8% polyacrylamide gels. Normal homozygous and known heterozygous (normal/ΔF508) PCR products were used as controls in each run. When more than one band was observed in any of these groups, the corresponding four DNAs were amplified separately. As a control for the multiple DNA procedure, 25% of the samples (143 individuals) were randomly selected and amplified individually. Complete agreement between the multiple and individual methods was observed in all cases. No DNA or PCR amplifications were obtained in 31 of 531 individuals (21 for DNA, 3.95%, and 10 for PCR, 1.88%) because of technical problems (broken tubes, coagulated blood, improperly identified samples).

Affected population

The mutation and genotype frequencies were previously established using a sample of 52 patients with clinically confirmed CF selected from a group of 160 individuals (Luzardo et al., 2002).

RESULTS

Cystic fibrosis population

Twenty-one already-known mutations were detected in the 52 unrelated patients considered to be an affected population based on their clinical features of CF. According to these data (Luzardo et al., 2002), 40.4% of the observed mutations corresponded to ΔF508. The other mutations were detected in 38 cases (38.2%), and no mutation was identified in 21.2% of the cases (Table 1). The mutation ΔF508 was present in heterozygous (ΔF508/unknown 17.3%, ΔF508/other 32.7%) or homozygous (15.4%, 8/52) form in a total of 65.4% (34/52) of the cases (Table 2).

Table 1. Mutations observed in the population studied.

Mutation	No. of chromosomes	Frequency (%)
ΔF508	42	40.4
Others*	38	36.5
Unknown	24	23.1

Total number of chromosomes analyzed = 104.
*G542X, R1162X, G85E, N1303K, R334W, R75Q, R74W, D1270N, W1282X, ΔI507, 2789+5G->A, R1066C, -816C/T, and R553X.

Non-cystic fibrosis population

Four heterozygous subjects were detected in the sample of 500 individuals. Thus, the

Table 2. Genotypes observed in the population studied.

Genotypes	No. of individuals	Frequency (%)
ΔF508/ΔF508	8	15.4
ΔF508/Others*	17	32.7
ΔF508/Unknown	9	17.3
Other/Other	6	17.5
Other/Unknown	11	21.2
Unknown/Unknown	1	1.9

Total number of individuals = 52.
*G542X, R1162X, G85E, N1303K, R334W, R75Q, R74W, D1270N, W1282X, ΔI507, 2789+5G->A, R1066C, -816C/T, R553X.

corresponding ΔF508 carrier status was observed in 1/125 individuals in the population. Based on these data, one would expect to find a homozygous individual (ΔF508/ΔF508) in a proportion of 1:62,500 (1.6×10^{-5}).

Based on the observed frequency of homozygous ΔF508 individuals, we estimated that the prevalence of CF (considering all types of mutations) would be 1:9,613 (1×10^{-4}). Considering the marginality of the analyzed proportions, the variability of this prevalence (1:9,613) was calculated from the statistical distribution of the random variable:

$$X = \frac{1}{4}\,\frac{B(500, 0.008)}{500}\,\frac{B(500, 0.008)}{500}\,\frac{52}{B(52, 0.225)}$$

where B(n, p) are independent binomial distributions. In all, 9999 simulations were run on a computer program. The 0.025 and 0.975 percentiles of the resulting distribution (0 and 25×10^{-5}, respectively) were taken as the boundaries for the 95% confidence interval. The 0.5 percentile (6.93×10^{-5}) coincided with the expected value.

DISCUSSION

The prevalence of CF is closely related to ethnic factors (Tsui, 1992). Unlike other parts of the world including North America, South America has undergone an important miscegenation of its original inhabitants (Amerindians) with people of European and African origin who arrived during and following the Portuguese-Hispanic colonization of the 16th century. The resulting admixture of races has probably modified the prevalence of CF in Uruguay and other South American countries, although there are still no consistent data to support this conclusion (Orozco et al., 2000; Venegas et al., 2003). Since it is impossible to obtain reliable information about ethnic admixture, we selected our subjects based on socio-economic considerations. This strategy allowed us to maintain the ethnic composition of the sample as similar as possible to that of the actual population.

Genetic studies of 21 nuclear gene polymorphisms (blood groups, proteins, enzymes, and HLA loci) have shown that the Montevidean population is composed mainly of people of European descent (≥86%) with African and Amerindian admixtures of 6-11 and 1-2%, respectively (Sans et al., 1993, 1997). An estimate of the prevalence of CF based on this information

indicated a theoretical value of 25 affected persons/100,000 inhabitants.

In contrast, the results obtained here demonstrated that the prevalence of CF was moderately low in our population, with 1.6 individuals/100,000 inhabitants probably being homozygous for ΔF508. If all of the mutations were considered, this figure would increase to 6.9/100,000. This frequency is much lower than those reported for Spain (28.6/100,000), Italy (33.3/100,000) and France (50/100,000), countries where the European ancestors of most Uruguayans originated. This estimated value was only about 53% of the lowest prevalence observed among European countries (Sweden, 13/100,000) (Tsui, 1992), but was seven-fold higher than that found in populations of Asian origin (Hawaii, 1.1/100,000) (Estivill, 1991) and approximately the same as that observed in "Hispanic" individuals of the USA population (Schidlow, D., personal communication). Thus, as with the frequency of ΔF508 in the CF population, these variations in prevalence cannot be explained solely by ethnic admixture.

ACKNOWLEDGMENTS

The authors thank the following institutions for providing blood samples: Hospital Maciel (Ministerio de Salud Pública, MSP), Hospital Pereira-Rossell (MSP), Hospital Pasteur (MSP), Hospital Central de las Fuerzas Armadas (SSFFAA), Hospital Policial (SP), Hospital Evangélico, Centro de Asistencia del Sindicato Médico del Uruguay, Asociación Española Primera de Socorros Mutuos, Casa de Galicia, Centro Integral de Medicina Asistencial, Círculo Católico, Gremial Médica Centro Asistencial, Instituto Médico de Prevensión y Asistencia SA, Mutualista Israelita Del Uruguay, and Universal Sociedad de Producción Sanitaria, and also Dr. Fernando Nieto (deceased) for his contribution to the statistical analysis of the data. Research partially supported by a grant from Roche International Ltd. (Uruguay).

REFERENCES

Chertkoff, L., Visich, A., Bienvenu, T., Grenoville, M., Segal, E., Carniglia, L., Kaplan, J.C. and **Barreiro, C.** (1997). Spectrum of CFTR mutations in Argentine cystic fibrosis patients. *Clin. Genet. 51*: 43-47.

Estivill, X. (1991). Molecular genetics of cystic fibrosis. *Ann. Nestlé 49*: 23-37.

Kerem, B., Rommens, J.M., Butchanan, J.A., Markiewicz, D., Cox, T.K., Chakravarti, A., Buchwald, M. and **Tsui, L.C.** (1989). Identification of the cystic fibrosis gene: genetic analysis. *Science 245*: 1073-1080.

Luzardo, G., Aznarez, I., Crispino, B., Mimbacas, A., Martínez, L., Poggio, R., Zielenski, J., Tsui, L.C. and **Cardoso, H.** (2002). Cystic fibrosis in Uruguay. *Genet. Mol. Res. 1*: 32-38.

Orozco, L., Velásquez, R., Zielenski, J., Tsui, L.C., Chavez, M., Lezana, J.L., Saldana, Y., Hernández, E. and **Carnevale, A.** (2000). Spectrum of CFTR mutations in Mexican cystic fibrosis patients: identification of five novel mutations (W1098C, 846delT, P750L, 4160insGGGG and 297-1G/A). *Hum. Genet. 106*: 360-365.

Pivetta, O.H. and **Olek, K.** (1991). Relación entre la delección ΔF508 y la expresión clínica en pacientes fibroquísticos. *Publicación Oficial del IV Congreso Latinoamericano de Fibrosis Quística*, November, 1991, Montevideo, Uruguay, pp. 205.

PNUD (Programa de las Naciones Unidas para el Desarrollo) (1997). *Human Development: Report 1997*. Oxford University Press, Oxford, UK.

Raskin, S., Phillips III, J.A., Krishnamani, M.R.S., Vnencak-Jones, C., Parker, R.A., Rozov, T., Cardieri, J.M., Marostica, P., Abreu, F., Guigliani, R., Reis, F., Rosario, N.A., Ludwing, N. and **Pilotto, R.F.** (1993). DNA analysis of cystic fibrosis in Brazil by direct PCR amplification from Guthrie cards. *Am. J. Med. Genet. 46*: 665-669.

Riordan, J.R., Rommens, J.M., Kerem, B., Alon, N., Rozmahel, R., Grzelczak, Z., Zielenski, J., Lok, S.,

Plavsic, N., Chou, J.L., Drumm, M.L., Iannuzzi, M.C., Collins, F.S. and **Tsui, L.P.** (1989). Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science 245*: 1066-1073.

Ríos, J., Orellana, O., Aspillaga, M., Avedano, I., Largo, I. and **Riveros, N.** (1994). CFTR mutations in Chilean cystic fibrosis patients. *Hum. Genet. 94*: 291-294.

Sans, M., Salzano, F.M. and **Chakraborty, R.** (1993). Genetic structure and admixture components in Uruguay. *XIII International Congress of Anthropological and Ethnological Science*, August, 1993, Mexico, pp. 98.

Sans, M., Salzano, F.M. and **Chakraborty, R.** (1997). Historical genetics in Uruguay: estimates of biological origins and their problems. *Hum. Biol. 69*: 161-170.

The Cystic Fibrosis Genetic Analysis Consortium (1994). Population variation of common cystic fibrosis mutations. *Hum. Mutat. 4*: 167-177.

Tsui, L.P. (1992). The spectrum of cystic fibrosis mutations. *Trends Genet. 8*: 392-398.

Valenzuela, C. (1988). Genética de la fibrosis quística. *Rev. Chil. Pediatr. 31*: 218-219.

Valenzuela, P. and **Méndez, V.** (1982). *Techniques in Molecular Genetics.* Univ. Cat. Chile, Santiago, Chile, pp. 5.

Venegas, P.B., Novak, J.M., Oscar, C.A., Sanchez, F.L., Gutierrez, I.G., Rivera, J.M., Salas, J.P., Montero, J.F. and **Grody, W.W.** (2003). Cystic fibrosis mutations in Costa Rica. *Hum. Biol. 75*: 179-188.

Zielenski, J. and **Tsui, L.P.** (1995). Cystic fibrosis: genotypic and phenotypic variations. *Annu. Rev. Genet. 29*: 777-807.