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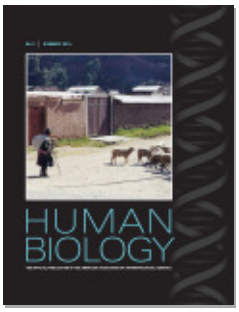

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Cystic Fibrosis Gene Variability in Two Southern Brazilian Amerindian Populations: Analysis of the ΔF508 Mutation and the KM19 and XV2C Haplotypes

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Abstract The frequencies of the ΔF508 deletion, the most common cystic fibrosis mutation in Europeans and European-derived populations, and the XV2C and KM19 restriction fragment length polymorphisms that are tightly linked to the *CFTR* locus vary among populations. To determine the distribution of these extragenic markers and of the ΔF508 mutation, we analyzed 326 chromosomes of individuals from two South American Indian populations, the Guarani and the Kaingang. The allele and haplotype frequencies differed greatly between the two populations as well as among Amerindians and normal European Brazilians and European Brazilian cystic fibrosis patients. The absence of the ΔF508 mutation and the B haplotype are in agreement with the hypothesis that the ΔF508 mutation occurred after the divergence of these two populations. This finding is useful for populations containing a large Amerindian component and helps us to understand the origins of the ΔF508 deletion, the most common cystic fibrosis mutation in Europeans and European-derived populations, as well as the different incidences of cystic fibrosis in continental groups.

Cystic fibrosis (CF) is an autosomal recessive disorder with an incidence of about 1/2,500 live births in European and other European-descended populations; this incidence corresponds to a carrier frequency of about 1/25 (Welsh et al. 1995). In European Brazilians the incidence is estimated as 1/7,500, whereas in African Brazilians this incidence is approximately 1/15,300 (Raskin et al. 2007b). Since the CF gene was mapped to chromosome 7 in 1985, a variety of polymorphic DNA segments tightly linked to the CF gene have been identified (Knowlton et al. 1985; Estivill et al. 1987a, 1987b). These segments have several restriction fragment length polymorphisms (RFLPs) that are useful for the analysis of families with a history of CF and for population studies. In 1989 the gene responsible for CF, referred

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Table 1. Standard XK Haplotypes

Haplotype	*XV2C*[a]	*KM19*[b]
A	1	1
B	1	2
C	2	1
D	2	2

a. 1 = absence and 2 = presence of restriction site for *Taq*I (Beaudet et al. 1989).
b. 1 = absence and 2 = presence of restriction site for *Pst*I (Beaudet et al. 1989).

to as the cystic fibrosis transmembrane conductance regulator (*CFTR*) was cloned. It spans more than 250 kb and contains 27 exons that encode a protein product of 1,480 amino acids. The most common *CFTR* mutation in Europeans and their descendants all over the world is a 3-bp deletion that causes the loss of a phenylalanine residue at position 508 in its tenth exon (ΔF508) (Kerem et al. 1989). The frequency of the ΔF508 mutation in European Brazilian CF patients is about 48%, and in African Brazilian CF patients it is about 12% (Raskin et al. 2003).

Two DNA segments that are tightly linked to the *CFTR* gene, called XV2C and KM19, have RFLPs detected by *Taq*I and *Pst*I restriction enzymes, respectively. These loci are located 260 kb from the upstream (5′) end of the *CFTR* gene and are predicted to have less than a 1% recombination rate with the *CFTR* locus (Estivill et al. 1987a). By convention, the allele lacking the polymorphic restriction site is denominated as the **1* allele, whereas the allele containing the restriction site is the **2* allele. These XV2C and KM19 (or simply XK) RFLPs in combination result in four possible allele combinations per chromosome, or XK haplotypes, named A through D (Table 1) (Beaudet et al. 1989). The distribution of XK haplotypes has been determined in a large number of European-derived CF patients worldwide (Estivill et al. 1987b, 1988; Cutting et al. 1989; Gasparini et al. 1990; Nunes et al. 1991; Raskin et al. 1997a, 1997b) and in some African populations (Martin et al. 1988; Cutting et al. 1989; Denter et al. 1992; Raskin et al. 2007a). Specific haplotypes are nonrandomly associated with specific *CFTR* alleles. In most Caucasian populations haplotype B (X1-K2; see Table 1) is strongly associated with CF-bearing chromosomes, mainly the ones that carry the ΔF508 mutation. In African and African-derived populations this association is also present (Martin et al. 1988; Cutting et al. 1989; Denter et al. 1992; Raskin et al. 2007a).

Published molecular studies of *CFTR* in Amerindians are limited. Highsmith et al. (1991, 1994) described a Zuni patient with one copy of the R1162X mutation and one copy of the 3849 + 10 kb C → T mutation. Later, Grebe et al. (1992) tested 11 CF patients from a Native American Pueblo population (6 of them from the Zuni Pueblo, a geographically isolated community) for the six most common *CFTR* mutations in Caucasians (ΔF508, G542X, G551D, R553X, N1303K, and W1282X). They reported that the ΔF508 mutation was not detected among the affected individuals and that only one G542X mutation was found. Ten patients had the A/A (3), A/C (2), or C/C (5) haplotype combinations, but the one carrying the

G542X mutation had the A/B haplotype combination. Five of six Zunis presented the C/C and one the A/C haplotype combination. The same investigators also analyzed 15 non-CF chromosomes from the parents of 22 patients and found 9 chromosomes with haplotype A, 5 chromosomes with haplotype C, and 1 chromosome with haplotype B. These 15 parents had haplotype combinations A/A (5), A/B (1), A/C (4), C/C (4), and B/C (1). Twenty chromosomes from 10 randomly surveyed normal individuals from Zuni Pueblo were also analyzed; 12 showed haplotype C and 8 showed haplotype A. They had haplotype combinations A/A (1), A/C (6), and C/C (3).

Mercier et al. (1994) decided to analyze the entire coding sequence of the *CFTR* gene in 8 of the 11 Pueblo CF patients described by Grebe et al. (1992), including the 6 CF patients from Zuni Pueblo. They found the R1162X mutation in 11 out of 16 chromosomes (all in the 6 Zuni Pueblo samples) and identified three other mutations, including G542X (1/16) and 3849 + 10 kb C → T (3/16), which had been previously described in other non-Amerindian populations, and a new mutation (D648V) in one chromosome. Using microsatellites, they showed that all the detected R1162X mutations shared a common *CFTR* haplotype that had previously been found in R1162X chromosomes of Italian and Spanish origin, where the mutation was initially reported (Estivill et al. 1987b; Gasparini et al. 1991; Nunes et al. 1991). They therefore concluded that these chromosomes could be a result of admixture of Spanish or Italian settlers.

Kessler et al. (1996) screened 588 normal Zuni individuals for the R1162X mutation and found 39 carriers, leading to a carrier frequency of 6.7% for this specific mutation. They also retested for the R1162X mutation in four CF Pueblo patients [the same patients previously tested by Grebe et al. (1992) for six non-R1162X mutations] and found that all of them were negative. Paz-y-Mino et al. (1999), on the other hand, studied 10 Ecuadoran "Mestizo" (Spaniard-Amerindian) CF patients and found the frequency of the ΔF508 mutation to be 25%. None of the other seven prevalent European *CFTR* mutations were found, although Yee et al. (2000) hypothesized that a novel mutation they detected (L1093P), should have native American origin.

CF is the most common severe autosomal recessive disorder, at least in the southern states of Brazil, but it is still underdiagnosed in Latin America (Raskin et al. 2007b). Nevertheless, in the last 15 years, with the development of medical centers that specialize in the diagnosis and treatment of CF patients, the number of recognized CF cases has increased dramatically. Although more than 2,000 cases are currently included in the Brazilian CF registers, there is no published CF case in a nonadmixed Brazilian Amerindian group.

The immigration history and heterogeneity of the Brazilian population makes the analysis of the geographic distribution of *CFTR* mutations and of their tightly linked RFLPs of particular interest to trace the origin and spread of the CF gene mutations; this is especially true for the study of Amerindians, because of the scarcity of data. In this study we intend to present information on the prevalence of the ΔF508 mutation and of the KM19 and XV2C alleles and haplotypes in 163

Amerindians from southern Brazil and to compare them to those of North American Amerindians and to normal and diseased individuals of other ethnic groups living in Brazil or elsewhere.

Materials and Methods

Subject Population. Brazil is a huge country, larger than all of Western Europe and larger than the United States exclusive of Alaska. It has a population of approximately 170 million people (IBGE 2007), making it the fifth most populous country in the world. The Portuguese arrived in Brazil about 500 years ago, and since then, various other European, Middle Eastern, and Asian immigrant groups have settled there. From the 16th to the 19th centuries Africans were brought to Brazil as slaves, whereas indigenous people of diverse cultures and languages immigrated to the region much earlier. When the Portuguese arrived, there were 2.5 million autochthonous Indians (Salzano and Freire-Maia 1970; Bethel 1997). Although the major European contribution was composed of Portuguese, subsequent waves of Europeans have contributed to the diverse biological and cultural heritage of Brazil. Intermarriage between the Portuguese and Indians was common and started soon after the arrival of the first colonizers. Actually, mating between European men and indigenous women became commonplace and later was even encouraged as a strategy for population growth and colonial occupation of the country (Morner 1967).

Because the admixture rate varied from place to place, the five main Brazilian regions (north, northeast, southeast, south, and center-west) differ in ethnic background and Amerindian contribution. The Amerindian populations underwent a drastic demographic decline as a result of diseases and conflicts with European colonizers (Salzano and Freire-Maia 1967; Monteiro 1994; Ribeiro 1995). Today there are about 326,000 Amerindians in Brazil, located mainly in the northern and western border regions as well as in the upper Amazon basin. Although Amerindians constitute less than 1% of the present-day Brazilian population (Salzano and Callegari-Jacques 1988; IBGE 2007), they still carry the genetic features from the early colonization phase, as shown by recent mitochondrial DNA data. These data suggest that the Indian matrilineal contribution to the total present-day Brazilian European-derived pool may be as high as 33%, varying from 22% in the south to 54% in the northern region (Alves-Silva et al. 2000). Studies of nuclear markers have shown that the overall (matrilineal plus patrilineal) Amerindian contribution is lower, varying from 7–11% in the southern regions (Dornelles et al. 1999; Probst et al. 2000) to 41% in the northern regions (Santos and Guerreiro 1995).

According to archeologists, the Amerindians arrived in southern Brazil about 9,000 years ago. The Guarani and Kaingang are currently the two most populous Amerindian groups in Brazil (41,000 and 22,000, respectively), and they are also the major southern Brazilian Indian populations. The Guarani speak a Tupi language, whereas the Kaingang belong to the Ge linguistic group. Besides language,

the two groups differ in many aspects of their cultures and are genetically distinct (Petzl-Erler et al. 1993; Petzl-Erler and McDevitt 1994; Salzano et al. 1997; Parham et al. 1997; Sotomaior et al. 1998; Faucz et al. 2000; Tsuneto et al. 2003). The Guarani were further subdivided into several groups, which also differ with regard to allele frequencies (Tsuneto et al. 2003). The Guarani population sampled in this study belongs to the Guarani-M'byá subgroup.

Tsuneto et al. (2003) estimated that the Kaingang's rate of admixture with non-Amerindians is about 7% and that the Guarani-M'byá rate is approximately 4%. Differences in the *HLA* alleles and haplotypes between the Kaingang and Guarani have also allowed the calculation of intertribal gene flow. Approximately 1.4% of the present Guarani-M'byá gene pool could be of Kaingang origin, and 0.5% of the Kaingang gene pool could be derived from the Guarani (Petzl-Erler et al. 1993). Taken together, these estimates indicate that both populations still retain more than 90% of their ancient genetic constitution.

Studied Populations. We investigated 163 Amerindians from three populations: 83 individuals designated as Kaingang Total [19 of them are designated here as KIV (Kaingang who live in Ivai, located in Manoel Ribas county) and 64 are designated as KRC (Kaingang who live in Rio das Cobras, in Laranjeiras do Sul county)] and 80 individuals designated as GRC (Guarani, living in Rio das Cobras). These Amerindians, living in the Indian areas of Rio das Cobras and Ivai (Paraná, southern Brazil), were contacted in 1988 by M. L. Petzl-Erler. A more detailed description of the populations can be found in Petzl-Erler et al. (1993). These populations have been studied for several MHC and non-MHC genes (Petzl-Erler et al. 1993; Petzl-Erler and McDevitt 1994; Salzano et al. 1997; Parham et al. 1997; Sotomaior et al. 1998; Faucz et al. 2000; Tsuneto et al. 2003). Samples were coded for anonymity before being sent to us. The study was approved by the institutional review board of the Federal University of Paraná, CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), FUNAI (Fundação Nacional do Índio), and the Kaingang and Guarani leaders.

Typing Procedure. Genomic DNA was obtained from peripheral blood leukocytes using the phenol-chloroform extraction method with some modifications (Sambrook and Russell 2001). Methods to determine the ΔF508 and XK alleles were essentially those described by Saiki et al. (1988), Feldman et al. (1988), Rosenbloom et al. (1989), and Raskin et al. (1992a, 1992b).

The DNA segment of chromosome 7 that contains the XK loci is tightly linked to the *CFTR* gene locus with a recombination rate of less than 1% (Estivill et al. 1987a, 1987b). When PCR products containing the XK segments are digested with *Taq*I or *Pst*I enzymes, characteristic patterns are seen after electrophoresis (Raskin et al. 1992b). Internal controls were used to certify the quality of digestion (Raskin et al. 1992a).

Statistical Analysis. Estimated haplotype frequencies were obtained using the computer program package Arlequin (Schneider et al. 1997). Arlequin was also

used to calculate allele frequencies and to verify the fit to Hardy-Weinberg equilibrium expectations. To verify the statistical significance of the differences between populations, we used the RXC computer program (Miller 1997).

Results

Allele Frequencies. It was possible to directly screen all 163 samples for the ΔF508 mutation, but no mutation was found.

For KM19 it was possible to type all 160 Guarani alleles, but 4 KM19 Kaingang alleles could not be typed because of technical problems, so 2.41% of the KM19 alleles remained unidentified.

As shown in Table 2, there are no statistically significant differences in the XK allele and haplotype prevalence between Kaingang from Rio das Cobras and Kaingang from Ivai ($p = 0.573$) or between the Kaingang and Guarani populations ($p = 0.260$), not even when we compared the pooled Brazilian Amerindian data with North American healthy individuals ($p = 1.0$) or with Zuni Pueblo CF patients ($p = 0.59$). The *KM19*1* frequencies varied in these groups between 94% and 100%. These prevalences are significantly lower (35–75%) in samples from other ethnic groups. KM19 allele frequencies fit Hardy-Weinberg equilibrium expectations.

For XV2C it was possible to type all 160 Guarani alleles, but 4 XV2C Kaingang alleles could not be typed because of technical problems, so 2.41% of the XV2C alleles remained unidentified.

We did not observe any statistically significant differences between the two Kaingang communities (*XV2C*1* varying from 74% to 76%, $p = 0.833$), but we did observe a marked dissimilarity between the two Amerindian populations ($p < 1 \times 10^{-6}$), with the *XV2C*1* and *XV2C*2* alleles showing a reverse distribution between them (much higher frequency of *XV2C*1*, 74% in the Kaingang). There were no significant differences between the Guarani data (*XV2C*1* = 39%) and the Zuni Pueblo Indians of North America (*XV2C*1* = 45–55%, $p = 0.186$ and $p = 0.641$, respectively). However, the pooled Kaingang data differed significantly from the Zuni Pueblo Indians ($p = 0.014$ and $p = 0.010$). The *XV2C*1* allele frequency in the other ethnic groups varied from 55% to 66% (see Table 2). XV2C allele frequencies fit Hardy-Weinberg equilibrium expectations.

XK Haplotype Frequencies. Four Kaingang haplotypes could not be assigned, so 2.41% of the haplotypes remained unidentified.

Based on Amerindian values, the four haplotypes occur in the healthy European Brazilian and CF patient samples but with marked differences between them ($p = 0.0005$ in the Guarani; $p < 10^{-6}$ in the Kaingang comparison). Haplotypes A and C are seen in 96% of the chromosomes of these two Amerindian populations, whereas haplotype B is absent in all of them (see Table 2). Haplotype C is most frequent (56%) in the Guarani, whereas haplotype A is most frequent in the Kaingang (74%). The haplotype distribution in healthy and CF Pueblo Indians does not differ from that seen in the Guarani, but it does differ from the distribution observed in the Kaingang ($p = 0.005$ and $p = 0.002$, respectively).

Table 2. Distribution of XK Alleles and Haplotypes in Different Populations

XK Alleles and Haplotypes	*Guarani, N (%)*	*Kaingang from Rio das Cobras, N (%)*	*Kaingang from Ivai, N (%)*	*Kaingang, Total, N (%)*	*European Brazilians, N (%)*[a]	*African Brazilians, N (%)*[b]	*Zuni Pueblo Amerindians, N (%)*[c]	*European Brazilian CF Patients, N (%)*[a]	*African Brazilian CF Patients, N (%)*[b]	*Amerindian Pueblo CF Patients, N (%)*[c]
XV2C allele	160	124	38	162	463	146	35	521	74	22
**1*	61 (38.7)	91 (73.4)	29 (76.3)	120 (74.1)	254 (54.9)	66 (45.8)	18 (51.4)	398 (66.4)	41 (55.4)	10 (45.4)
**2*	99 (61.2)	33 (26.5)	9 (23.7)	42 (25.9)	209 (45.1)	78 (54.2)	17 (48.6)	123 (23.6)	33 (44.6)	12 (54.5)
KM19 allele	160	124	38	162	463	146	35	521	74	22
**1*	152 (93.7)	120 (96.8)	38 (100)	158 (97.6)	340 (73.4)	78 (53.4)	34 (97.1)	182 (34.9)	44 (59.5)	21 (95.4)
**2*	8 (6.3)	4 (3.2)	–	4 (2.4)	123 (26.6)	68 (46.6)	1 (2.8)	339 (65.1)	30 (40.5)	1 (4.5)
XK haplotype	160	124	38	162	463	146	35	517	74	22
A	62 (38.7)	91 (73.4)	29 (76.3)	120 (74.1)	193 (41.7)	25 (23.1)	17 (48.6)	94 (18.2)	18 (28.1)	9 (40.9)
B	–	–	–	–	61 (13.2)	**21 (19.4)**	1 (2,8)	300 (58.3)	18 (28.1)	1 (4.5)
C	90 (56.2)	29 (23.4)	9 (23.7)	38 (23.4)	146 (31.5)	**35 (32.4)**	17 (48.6)	87 (16.8)	21 (32.8)	12 (54.5)
D	8 (5.0)	4 (3.3)	–	4 (2.5)	63 (13.6)	**27 (25.0)**	–	36 (6.9)	7 (10.9)	–

a. Raskin et al. (1997a, 1997b).
b. Raskin et al. (2007a).
c. Grebe et al. (1992).

The four haplotypes occur in the healthy European Brazilian and CF patient samples but with marked differences between them, which also depart from the Amerindian values ($p = 0.0005$ in the Guarani; $p < 10^{-6}$ in the Kaingang comparison). Haplotype B, not seen in Brazilian Amerindians, is the most prevalent (58%) among European Brazilian CF patients; it is also frequent in African Brazilian CF patients (28%) and less prevalent in control African Brazilians (19%) and European Brazilians (17%).

XK Genotype Frequencies. Ten possible haplotype combinations occur in healthy European Brazilians from Paraná (Raskin et al. 1997a, 1997b) and all but the C/D haplotype combination occur in CF patients born in Paraná, differing from the Amerindian values (Table 3). The Guarani displayed only five haplotype combinations, the Kaingang from Rio das Cobras displayed four, and the Kaingang from Ivai displayed two. The A/C haplotype combination has the highest frequency in the Guarani, whereas in the Kaingang the A/A haplotype combination has the highest frequency. The B/B haplotype combination is the most frequent (39%) in European Brazilian CF patients. Although this is not true for the general European Brazilian population from Paraná, in which the A/C haplotype combination is the most frequent; haplotype combinations containing the B haplotype are present in 24.42% (65%) of this group (Raskin et al. 1997a, 1997b). The genotype frequencies of the Guarani but not the Kaingang are similar to those of the general North American Pueblo Indian population. We found statistically significant differences in genotype frequencies between the pooled Kaingang data on the one hand and the general population and CF patients from North American Pueblo Indians on the other ($p < 10^{-6}$).

Discussion

Our data provide the first information on the distribution of the ΔF508 *CFTR* mutation and XK *CFTR* alleles and haplotypes in Brazilian Amerindians. No ΔF508 mutation or XK haplotype B were found in any of the Amerindian chromosomes studied. This finding contrasts with the frequency of these markers in other Brazilian ethnic groups (Raskin et al. 1993, 1999, 2003, 2006a) and agrees with the strong association between the ΔF508 mutation and the XK haplotype B first demonstrated in CF patients by Kerem et al. (1989). This association is likely to reflect a single origin of the ΔF508 mutation, which was probably first introduced about 52,000 years ago during the Paleolithic Age in the "Caucasoid" population, after the divergence of the major continental groups (Kerem et al. 1989; Morral et al. 1994).

Although we did not detect the ΔF508 allele or XK haplotype B in 326 Brazilian Amerindian chromosomes, we do not believe that we have enough data to make broad conclusions about the incidence of CF in Amerindians. Because there is no comprehensive study published on the incidence of CF in Amerindians, it has been assumed that the incidence may be similar to or less than those of other non-European groups, that is, 1/15,300 live births for blacks (Hamosh et al. 1998)

Table 3. Distribution of XK Genotypes in Different Populations

XK Alleles and Haplotypes	*Guarani, N (%)*	*Kaingang from Rio das Cobras, N (%)*	*Kaingang from Ivai, N (%)*	*Kaingang, Total, N (%)*	*European Brazilians, N (%)*[a]	*African Brazilians, N (%)*[b]	*Amerindian Population from Zuni Pueblo, N (%)*[c]	*European Brazilian CF Patients, N (%)*[a]	*African Brazilian CF Patients, N (%)*[b]	*Amerindian Pueblo CF Patients, N (%)*[c]
Total	80	62	19	81	463	64	25	231	32	11
A/A	13 (16.25)	31 (50.00)	10 (52.63)	41 (50.62)	81 (17.5)	5 (7.81)	6 (24.00)	10 (4.33)	3 (9.37)	3 (27.27)
A/B	–	–	–	–	51 (11.02)	7 (10.94)	1 (4.00)	59 (25.54)	6 (18.75)	1 (9.10)
A/C	34 (42.5)	25 (40.32)	9 (47.37)	34 (41.97)	122 (26.35)	8 (12.5)	10 (40.00)	9 (3.9)	6 (18.75)	2 (18.18)
A/D	2 (2.5)	4 (6.45)	–	4 (4.94)	52 (11.23)	–	–	6 (2.6)	–	–
B/B	–	–	–	–	8 (1.73)	–	–	90 (38.96)	4 (12.5)	–
B/C	–	–	–	–	38 (8.21)	–	1 (4.00)	17 (7.36)	–	–
B/D	–	–	–	–	16 (3.46)	14 (21.88)	–	19 (8.23)	4 (12.5)	–
C/C	25 (31.25)	2 (3.23)	–	2 (2.47)	46 (9.94)	9 (14.06)	7 (28.00)	13 (5.63)	7 (21.87)	5 (45.45)
C/D	6 (7.50)	–	–	–	40 (8.64)	16 (25)	–	4 (1.73)	1 (3.13)	–
D/D	–	–	–	–	9 (1.94)	5 (7.81)	–	4 (1.73)	1 (3.13)	–

a. Raskin et al. (1997a, 1997b).
b. Raskin et al. (2006a).
c. Grebe et al. (1992).

and 1:90,000 for Hawaiians (Wright and Morton 1968). On the other hand, high frequencies of CF have been detected in a few North American Amerindian populations (Grebe et al. 1992; Mercier et al. 1994; Kessler et al. 1996). Grebe et al. (1992) showed that CF occurs among southwestern Native American Pueblo populations and especially in the geographically isolated community of Zuni, in New Mexico, with an incidence of 1/3,970 in the Pueblo and 1/1,347 in the Zuni, although in the Navajo the incidence may be as low as 1/186,000. Based on the direct analysis of the R1162X mutation, Kessler et al. (1996) estimated the CF incidence in the Zuni to be 1 in 333, with a carrier frequency of the R1162X mutation of 6.7%. They suggested that these high frequencies could be secondary to founder effects. However, Mercier et al. (1994), using both intra- and extragenic *CFTR* markers, showed that all these 11 *CFTR* R1162X mutations occurred in a single haplotype, the same one found in southern European CF patients with that mutation, suggesting that the high frequencies were most probably a result of admixture with Spanish settlers (around 1890), followed by genetic drift. Mercier et al. (1994) stated that if one assumes a frequency of 0.02 for mutant *CFTR* alleles in the general population and a European population average frequency of 0.7 for the ΔF508 mutation among the mutant alleles (CFGAC 2007), then the total population frequency for all the remaining *CFTR* mutations would be 0.006. Therefore, in the case of the absence of the ΔF508 mutation (as we found in this sample of Brazilian Amerindians), if no other mutation reaches its unusual high frequency, CF would be expected to occur at a frequency of only about 1 in 30,000 live-born. The previous estimate suggests that CF may be relatively rare in Amerindians, and according to Mercier et al. (1994), it supports the theory of random drift of the major mutation (Gerdes and Murphy 1985; CFGAC 2007). We believe that this hypothesis should be viewed with caution and tested in future studies.

According to the XK haplotype and genotype distributions, the Kaingang are the most divergent of the three Amerindian groups (Pueblo, Guarani, Kaingang) thus far analyzed. Although the Pueblo sample size is small (Grebe et al. 1992), they are closer to the Guarani than to the Kaingang.

The two Brazilian Amerindian populations were found to differ significantly at the XV2C locus (see Table 2). Also, according to the XK haplotype distributions, the Kaingang are the most divergent of the three Amerindian groups (Pueblo, Guarani, Kaingang) thus far analyzed. In general, differences are less pronounced between populations belonging to the same linguistic group and/or not separated geographically. The significant difference between the Kaingang and Guarani, however, is not unexpected, despite their close geographic proximity, in view of the results of previous studies of the same two populations, which agrees with their cultural and linguistic distinctiveness. Previous comparisons among several South Amerindian ethnic groups have found a high divergence of *HLA* and other genetic polymorphisms between the Guarani and the Kaingang (Petzl-Erler et al. 1993; Petzl-Erler and McDevitt 1994; Salzano et al. 1997; Parham et al. 1997; Sotomaior et al. 1998; Faucz et al. 2000; Tsuneto et al. 2003).

It will be interesting to extend this study, comparing these and other extragenic and intragenic *CFTR* polymorphisms as well as rare mutations in the *CFTR* gene among indigenous populations all over the world, including other Amerindian tribes. This should bring new insights into their origins as well as help to clarify the time of emergence of the many different *CFTR* mutations.

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