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Cystic Fibrosis in the Brazilian Population: DF508 Mutation and KM-19/XV-2C Haplotype Distribution

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Abstract We have used PCR amplification of DNA obtained from Guthrie cards to identify the DF508 mutation and correlate it with the allele frequencies at two polymorphic loci (*XV-2C* and *KM-19*) closely linked to the cystic fibrosis gene. The DNA came from 193 white Brazilian families affected by cystic fibrosis and living in five different states of Brazil. The distribution of the haplotypes derived from the DF508 and non-DF508 *XV-2C/KM-19* genotypes indicates that 88% of the DF508 alleles are linked to haplotype *B* and suggests that high heterogeneity exists among the non-DF508 cystic fibrosis alleles occurring in different states. Our data can be used to compare linkage disequilibrium between Brazilians and other heterogeneous populations where the DF508 mutation frequency is low and where many different rare mutations account for the remaining recessive cystic fibrosis alleles.

Since 1989 when the cystic fibrosis gene was cloned and its major mutation identified, more than 650 different cystic fibrosis mutations have been detected and correlated with the *XV-2C* and *KM-19* (*XK*) restriction fragment length polymorphisms (RFLPs), which are tightly linked to the cystic fibrosis gene (Estivill, Farral et al. 1987; Estivill, Scambler et al. 1987; Cystic Fibrosis Genetics Analysis Consortium, Newsletter 67, 1996). The most frequent mutation, DF508, consists of a three-nucleotide deletion in exon 10, located in the first putative ATP binding domain of the predicted protein (Rommens et al. 1989; Riordan et al. 1989; Kerem et al. 1989).

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Linkage and linkage disequilibrium analyses of *XK* haplotypes of non-DF508 cystic fibrosis alleles proved to be useful in detecting carriers when DF508 and other common mutations were not detected (Lemna et al. 1990). Worldwide data on DF508 frequency (Tsui 1992; Cystic Fibrosis Genetics Analysis Consortium, Newsletter 67, 1996) and the distribution of the *XK* haplotypes (European Working Group on CF Genetics 1990) showed that great variations of mutation frequencies and their respective *XK* haplotypes can be found between and within populations of different countries. For example, southern European populations show lower DF508 frequencies (≈50%) and more heterogeneous haplotype distributions of DF508- and non-DF508-bearing cystic fibrosis chromosomes than northern European and North American populations (Estivill, Farral et al. 1987; Estivill et al. 1988; Duarte et al. 1990; Novelli et al. 1990; Ferrari et al. 1990; Morral et al. 1993).

In previous studies we found that the *XK* haplotype distribution in Brazilian cystic fibrosis patients is as heterogeneous as that seen in southern European countries (Raskin et al. 1997b). Although the overall frequency of the DF508 mutation in Brazil is 47%, similar to that of southern Europe, variations have been seen between Brazilian states (Raskin et al. 1993, 1997a). These results are not surprising because Brazil was conquered and colonized by Portugal in 1500 and a major European migration flow started in 1802, mainly from Italy, Spain, and Germany (Salzano and Freire-Maia 1967; Salzano and Pena 1987).

In this study we correlate the DF508 mutation status with the *XK* haplotypes of 193 Brazilian cystic fibrosis patients (266 alleles with a known haplotype from a total of 386 cystic fibrosis alleles) from 5 different states of Brazil. Our data can be used to improve prenatal diagnosis and carrier detection and to infer the haplotypes of the 53% non-DF508 alleles that occur in the Brazilian population affected by cystic fibrosis.

Materials and Methods

Subject Population. Analyses were done on samples from 193 unrelated white subjects diagnosed with cystic fibrosis and their parents. These subjects were born in 5 different states of southeastern and southern Brazil (58 patients were born in the state of Rio Grande do Sul, 23 in the state of Santa Catarina, 22 in the state of Paraná, 56 in the state of São Paulo, and 34 in the state of Minas Gerais). Fifty-five percent of the cystic fibrosis patients were male; the patients ranged in age from 2 months to 32 years, with a mean age of 6.7 years. The age at diagnosis ranged from 1 month to 26 years, with a mean age of 3.03 years. All patients and 90% (345/384) of their parents were born in Brazil.

The five states chosen for study represent the Brazilian white population well because demographic data show that the states make up almost 50% of

Table 1. Standard Haplotype Nomenclature

Haplotype	*KM-19*	*XV-2C*
A	1[a]	1
B	2[a]	1
C	1	2
D	2	2

a. (1) Absence of restriction site for *Pst*I or *Taq*I; (2) presence of restriction site for *Pst*I or *Taq*I.

the Brazilian population, the states' white population includes about 65% of all whites in Brazil (Brazilian Census 1989, 1991), and four of the states (except Minas Gerais) have a low admixture rate (Salzano and Freire Maia 1970). Each of these five states is served by well-established cystic fibrosis regional reference centers, and the 193 patients make up about 80% of living patients with cystic fibrosis referred to regional reference centers in the entire country (Macri et al. 1992). All white patients followed by these centers between 1990 and 1992 were included in the study. Criteria for diagnosis included clinical findings of cystic fibrosis and positive sweat tests.

Blood Samples. Blood samples were collected by fingerstick on Guthrie cards. Care was taken to avoid contamination of samples by using gloves and plastic bags to separate samples during collection, mailing, and analysis.

DNA Analysis. DNA analysis for the DF508 mutation and *XK* RFLPs was done using pieces of filter paper cut from a single Guthrie card blood spot, using internal markers as controls, as described by Raskin et al. (1992a,b). The polymerase chain reaction (PCR) products were then visualized by ethidium bromide staining and exposure to ultraviolet light.

Haplotype Analysis. All parents of affected children were assumed to be carriers, having one normal and one cystic fibrosis allele; the chance of uniparental disomy and nonpaternity were ignored. Recombination between the *XV-2C* and *KM-19* loci was ignored because of the tight linkage that has been established. Cystic fibrosis chromosomes of parents were defined as those inherited by affected children, and parental non–cystic fibrosis chromosomes were not inherited by affected children. The *XK* haplotypes for parental chromosomes were inferred by comparing the affected child's and parents' genotypes, using standard nomenclature (Table 1), and the RFLP frequencies were calculated by simple gene counting (Beaudet et al. 1989). We were not able to obtain samples from 63 of 386 (16%) parents, but the cystic fibrosis haplotypes could be established for 266 parents.

Table 2. Haplotype Distribution on DF508 and Non-DF508 Brazilian Cystic Fibrosis Alleles

	Rio Grande do Sul			*Santa Catarina*			*Paraná*		
Haplotype	*Number of Alleles*	*DF508*	*non-DF508*	*Number of Alleles*	*DF508*	*non-DF508*	*Number of Alleles*	*DF508*	*non-DF508*
A	8	0	8	5	0	5	3	1	2
B	60	44	16	11	8	3	21	17	4
C	11	0	11	4	0	4	7	1	6
D	7	3	4	2	0	2	3	0	3

Haplotypes could not be established in 57 cystic fibrosis chromosomes because of a lack of DNA samples or noninformative markers. Therefore the results of 144 DF508 and 122 non-DF508 alleles were used in this analysis.

Statistical Methods. Statistical significance in 2×2 tables was calculated using Fisher's exact test (two-tailed), and a standard chi-square was calculated for larger tables.

Linkage disequilibrium was calculated from the formula

$$k(1 - k)(q - p)/k(1 - k)[(1 - k)p + kq][1 - (1 - k)p - kq]^{1/2} \quad (1)$$

(Krawczak et al. 1988), where p is the prevalence of haplotype B in non-DF508 cystic fibrosis genes, q is the prevalence of haplotype B in DF508 cystic fibrosis genes, and k is the proportion of cystic fibrosis alleles with the DF508 deletion.

Results

The overall distributions of the DF508 and non-DF508 mutations seen in the five Brazilian states are shown in Table 2.

Strong linkage disequilibrium was found between the *XK* and cystic fibrosis alleles ($p < 0.001$) with the DF508 mutation and with non-DF508 mutations (see Table 2). Linkage disequilibrium between *XK* haplotype *B* and cystic fibrosis alleles was stronger on chromosomes with the DF508 deletion. Most of the DF508 alleles (88%) and 41% of the non-DF508 cystic fibrosis alleles were associated with haplotype *B*, although this is the second less frequent haplotype among non–cystic fibrosis Brazilian chromosomes (Raskin et al. 1997b).

We found a relatively high number (12%) of DF508 alleles with the *A* (5%) and *D* (7%) *XK* haplotypes. This frequency is larger than that reported in other studies [4.1% for the English population ($p = 0.01$) and 2.3% for the Danish population ($p < 0.01$)] but similar to the findings for German

São Paulo			*Minas Gerais*			*Total*		
Number of Alleles	*DF508*	*non-DF508*	*Number of Alleles*	*DF508*	*non-DF508*	*Number of Alleles*	*DF508*	*non-DF508*
8	6	2	7	0	7	31	7	24
56	35	21	30	25	5	178	129	49
1	0	1	11	0	11	34	1	33
7	4	3	4	3	1	23	10	13

(9.2%, $p > 0.5$), Spanish (12.2%, $p > 4$), Italian (9.6%, $p > 0.5$), and Portuguese (9.1%, $p > 0.5$) populations (European Working Group on CF Genetics 1990).

Significant differences are found in the haplotype distributions of non-DF508 cystic fibrosis mutations across state of birth ($\chi^2 = 14.6$, d.f. $= 4$, $p < 0.01$).

Discussion

The finding that 88% of DF508 alleles and 41% of non-DF508 cystic fibrosis alleles are associated with haplotype *B* supports previous data suggesting a single origin for the DF508 mutation and a selective advantage of mutations arising in *XK* haplotype *B* (European Working Group on CF Genetics 1990). The relatively high number (12%) of DF508 alleles with the *A* or *D XK* haplotype could be derived from DF508 mutations on the common cystic fibrosis haplotype *B* by a single recombination event in heterozygotes, point mutation, or gene conversion. A single recombination event is possible because the region around *XV-2C* is reported to be a hot spot for recombination and because at least 200 kb separate the *XK* loci from the cystic fibrosis locus (Kerem et al. 1989; Dork et al. 1992). The observation of the DF508 mutation in association with non-*B* haplotypes is not unexpected. The relatively high frequency of DF508 mutations bearing non-*B* haplotypes may be due to (1) greater ethnic heterogeneity of European populations that migrated to Brazil (because the degree of association is lower in southern European than in northern European populations); (2) Brazilian internal migration, allowing intensive intrapopulation exchanges; or (3) admixture with nonwhite populations of Brazil (mainly blacks) who have a different distribution of cystic fibrosis linked haplotypes.

Significant differences are found in the haplotype distributions of non-DF508 cystic fibrosis mutations across state of birth. Haplotype *B* is the most frequent haplotype found in non-DF508 cystic fibrosis alleles from the states

of Rio Grande do Sul and São Paulo, indicating that other mutations in these two states occurred on the same relatively uncommon haplotype on non–cystic fibrosis chromosomes in which the DF508 mutation arose, representing between 41% and 78% of the non-DF508 cystic fibrosis alleles in the population of these two states. However, in the states of Paraná and Minas Gerais haplotype *C* is the most frequent haplotype on non-DF508 alleles, and haplotype *A* is the most frequent haplotype on non-DF508 alleles in Santa Catarina state. Because the proportion of the nonwhite population is low in Santa Catarina and Paraná (Brazilian Census 1991), admixture with nonwhite populations does not seem to play an important role in changing haplotypes frequencies in these two states; one explanation for the increased frequency of mutations bearing haplotypes *A* and *C* in Paraná and Santa Catarina could be a founder effect brought mainly by the Italian immigration and/or Portuguese colonization. This hypothesis is supported by the following data:

1. Among the southern European countries populations that emigrated to Santa Catarina and Paraná, haplotypes *A* and *C* are overrepresented in non–cystic fibrosis chromosomes from Italy (70%) (Novelli et al. 1990; Ferrari et al. 1990) and in non-DF508 cystic fibrosis chromosomes from Portugal (87%) (Duarte et al. 1990).
2. Santa Catarina and Paraná are geographically close.
3. The DF508 mutation frequency previously found in subjects from Paraná and Santa Catarina is disproportionately low (Raskin et al. 1993, 1997a).
4. Recent data indicate that 35% of Santa Catarina cystic fibrosis patients are Italian descendants through both sets of grandparents, and at least 40% are Italian descendants through either the maternal or paternal side of the family (Raskin et al. 1997a).
5. Demographic data show that most whites living in the capital of Santa Catarina state (Florianopolis), where most Santa Catarina subjects and respective parents were born, are Portuguese descendants, and as many as 83% of individuals born in Florianopolis have both sets of grandparents of Portuguese origin (Salzano and Freire Maia 1967; Salzano and Pena, 1987).

In the state of Minas Gerais 46% of the non-DF508 mutations are linked to haplotype *C*. Interestingly, among the five states included in this study Minas Gerais received the least flow of immigrants between 1802 and 1950, and its white population is composed mainly of descendants of Portuguese families that arrived in Brazil after 1500, when Brazil was conquered by Portugal (Salzano and Freire-Maia 1967; Salzano and Pena 1987). Once again a founder effect may explain the high frequency of haplotype *C* among non-DF508 cystic fibrosis alleles in Minas Gerais state. Another nonexclusive explanation would be admixture with the nonwhite population. This is plausible because of the five states studied Minas Gerais has the highest rate of admixture between whites and blacks (Salzano and Freire Maia 1967); 8.3% of the state population is composed of blacks and 34% is composed of mulattoes (Brazilian Census 1991). Interestingly, haplotype *C*, which reaches its highest frequency in non-DF508 cystic fibrosis alleles from the state of Minas Gerais, is frequent in black Americans: It is the most frequent haplotype in non–cystic fibrosis chromosomes (39%) and is overrepresented on cystic fibrosis chromosomes (19%) (Cutting et al. 1989).

Further studies will be needed to obtain information about the ethnic background of the families affected by cystic fibrosis and the degree of admixture within different ethnic groups in these populations. The incidence of cystic fibrosis and the frequency of its mutations in different states and ethnic groups of Brazil are just now being clarified (Raskin et al. 1997a; Chakraborty et al. 1993) and may explain the heterogeneity seen in the haplotypes of Brazilian cystic fibrosis patients.

Use of linkage disequilibrium data with linkage analysis can provide accurate estimates of carrier status and prenatal diagnosis for many families affected by cystic fibrosis and information on the origins and the degree of homogeneity of the cystic fibrosis mutations. Haplotype distributions can be used in population studies to infer the presence of known mutations, to detect carriers, and to diagnose family members at risk by linkage analysis when sweat tests give equivocal results and it is not possible to directly detect specific cystic fibrosis mutations. When linkage analysis cannot be done (e.g., when certain family members are uninformative or the genotype of an essential member is unknown), the departure from random association or linkage disequilibrium can be used to calculate the relative risk that a chromosome carries a cystic fibrosis mutation without analysis for specific mutations (Beaudet et al. 1989).

However, variations in linkage disequilibrium observed in different populations are difficult to interpret, and linkage disequilibrium data of one ethnic group can differ significantly from those of another. Thus, although the haplotype frequencies and linkage disequilibrium can be established for a given population, either or both can differ dramatically between populations, and therefore it is necessary to have linkage disequilibrium data for each group being compared. One approach is to geographically select a population and then to draw a random sample from it. Allele frequencies found in affected individuals from the sample can be compared with frequencies found in normal individuals drawn from the same sample. However, the implicit assumption that the population is at equilibrium is confounded by ethnic and racial differences. Thus for studies to be accurate, they should be carried out in the geographic region where the patients are born so that risks can be calculated with more precision.

The immigration history and heterogeneity of the Brazilian population make analysis of the geographic distribution of the cystic fibrosis tightly

linked RFLPs of particular interest. The results of this study support the hypothesis that high molecular heterogeneity will be found among the non-DF508 alleles that constitute 53% of the Brazilian cystic fibrosis gene pool. The appearance of the DF508 mutation on non-*B* haplotype chromosomes in this population is likely the result of recombination between the linked markers and the mutations, as happens in populations where the mutation is more ancient. Thus the Brazilian group resembles the populations found geographically close to where the DF508 mutation seems to have originated.

Our haplotype data improve the efficiency of cystic fibrosis DNA analysis in this population. Although RFLP studies of *XK* markers were informative in 48% of the families (Raskin et al. 1997b), direct detection of the DF508 mutation alone would make only 25% of the families fully informative for prenatal diagnosis (Raskin et al. 1993). Therefore diagnosis based on analysis of the DF508 deletion alone would be of limited value for prenatal diagnosis of and carrier screening for cystic fibrosis in Brazilian families. Our results suggest that the standard two-step strategy of screening for the DF508 mutation followed by linkage and linkage disequilibrium analyses when DF508 is not detected can be applied to the Brazilian population. Identification of other mutations in white and nonwhite Brazilian cystic fibrosis patients and correlations of these mutations to haplotypes of *XK* loci and loci closer to the mutation sites (Dork et al. 1992; Morral et al. 1993, 1996) could provide further insights into linkage disequilibrium and possibly facilitate screening for the less common mutations. Comparison of this database with southern European databases should also provide relevant information on the historical origin and diffusion of cystic fibrosis in Brazil and will increase the efficiency of cystic fibrosis DNA analysis for this population.

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Low-Molecular-Weight Acid Phosphatase (ACP1), Obesity, and Blood Lipid Levels in Subjects with Non-Insulin-Dependent Diabetes Mellitus

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Abstract Low-molecular-weight acid phosphatase (*ACP1*) is a polymorphic protein-tyrosine phosphatase present in all human tissues, including adipocytes. A positive association between the low-activity *ACP1*A/*A* genotype and extreme body mass index has previously been shown by us in obese subjects from the population of Rome. We have now studied a sample of 265 subjects with non-insulin-dependent diabetes mellitus (NIDDM) from another Italian population. There is a significant interaction between *ACP1*, body mass index, and blood lipid level. A highly significant positive association between *ACP1*A/*A* and high body mass index similar to that previously observed in obese subjects from the population of Rome is present only in those NIDDM subjects with blood lipid levels within the normal range. In NIDDM subjects the low-activity *ACP1*A/*A* variant seems to favor the increase of body mass or blood lipid levels or both. The pattern of association is independent of sex, age at survey, age at onset of diabetes, duration of disease, and therapy.

We have previously shown in different classes of obese subjects from the population of Rome, Italy, a relationship between low-molecular-weight acid phosphatase (*ACP1*) genetic polymorphism and body mass index (BMI) (Bottini et al. 1990; Paggi et al. 1991). The proportion of *ACP1*A/*A* genotype, which is associated with the lowest enzymatic activity, is much higher among subjects with high BMI than among subjects who are moderately obese, suggesting a strong tendency of the *ACP1*A/*A* genotype to influence extreme body mass deviation.

ACP1 is a protein-tyrosine phosphatase (PTPase) (Boivin and Galand 1986) controlled by a locus on chromosome 2. The locus shows three common

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