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Microsatellite Haplotypes Associations with 5 CFTR Mutations in »Grande Brière«, an Isolate Located in Southern Brittany

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ABSTRACT

The variability at three microsatellites in the Cystic Fibrosis Transmembrane Conductance Regulator Gene (CFTR) locus has been studied for frequent mutations encountered in an isolated population of »Grande Brière«, a small region located in Southern Brittany. Fluorescent multiplex PCR of these microsatellites were assayed in 16 Cystic Fibrosis (CF) families carrying 5 different mutations. The four most frequent haplotypes on df508 chromosomes were the same as those found in Northern France and Europe but the distribution of these haplotypes provides new enlightenment on the population origin of this insular community.

Introduction

Cystic Fibrosis (CF) is one of the most common severe autosomal recessive disorders in Caucasoid populations and was described for the first time by Fanconi in 1936[1]. Since the cloning of the CFTR[2–4], more than 850 mutations causing CF have been detected (Cystic Fibrosis Mutation Database, www.genet.sickkids.on. ca /cftr/), and a geographical distribution has been proposed[5]. The most common mutation, df508, accounts for approximately 70% of CF chromosomes in the population of north Europe and North America[6], and 73% in northwest France[7]. The aim of this study is to analyse the genetic variability observed at three microsate-

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llite loci associated with mutations encountered in a small French region of »Grande Brière«. This region is a marsh and is mostly formed by different islands connected by dike roads. This marsh was formed from two separate entities:

Several islands around a central village in the middle, encircled by marsh.

On the periphery of the marsh, other villages separated from the central village by some kilometers (Figure 1). Inhabitants, in spite of the industrial revolution have constitued an isolat.

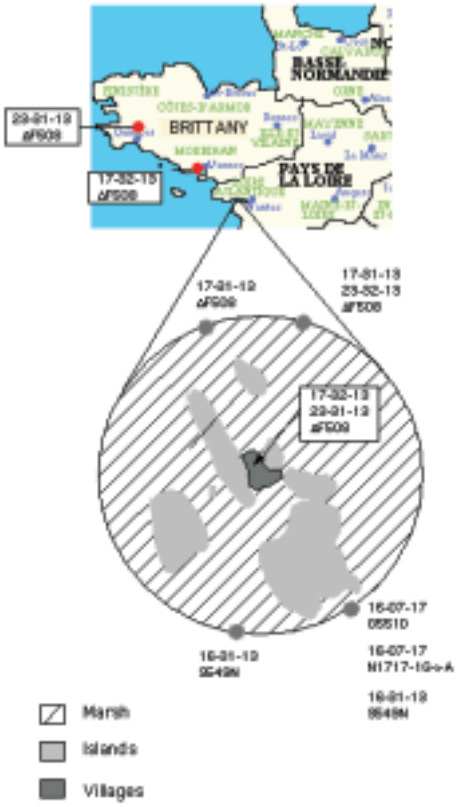

Fig. 1. Representation of two different areas located in "Grande Brière". First islands and central village, and peripheric villages located around, with specific haplotypes and mutations distribution

The aim of this study was to see if founder effects exist in this population for the different mutations and haplotypes encountered.

Materials and Methods

Genealogical reconstruction

This reconstruction has been very difficult, specially in »central Grande Brière« formed from different islands (Fedrun, Pendille, Mazin,...) because inbreeding rate and endogamy in these islands are quite high as illustrated by the numerous administrative authorizations given for consanguinous mariages in the last centuries, or by the fact that 6 family names cover 80% of the population (8). On the other hand, the study proved to be relatively easy in the »peripheric Brière«. We have looked at the remarkably well preserved registers of births, marriages, and death as well as contemporary documents produced by local scholars (mostly unpublished). The genealogies of 16 CF patients were reconstructed over 11 to 16 generations representing a period of 350 to 400 years. All the data have been processed using Heresis 4 Pro sofware after having rendered anonymous all those concerned. All individuals in the study came from families that did not have recent common ancestors in the pedigree. A very low migration rate characterizes the population (mostly immigration), and contribute to their relative isolation.

Mutation detection

The CF families have been selected by diagnosis on the basis of medical records, biochemical criteria and Polymerase Chain Reaction (PCR) detection. This last investigation was performed as described[9] using Amplification Refractory Mutation System (ARMS) for df508, G551D, and N1717G A. Mutations S549N and W57X have been performed using Single Strand Conformation Polymorphism (SSCP)[10].

Analysis and mutations were confirmed by sequencing.

Microsatellite markers

IVS8CA (intron 8), IVS17BTA, and IVS17BCA (intron 17)[11] were analysed by multiplex PCR. Genomic DNA (100 ng) was amplified using 200 M of each dNTP, 5 pmol of each primer AC8R4, AC8D3, AC17R2, AC17D22, AT17R12, and AT17D12 previously described by Morrall et al.[12], 50 mM KCl, 10mM Tris (pH 8.3), 1.5 mM MgCl2, and 2 U of Taq polymerase in a total volume of 25 l. Unlabelled and 6-FAM (fluorescent component) labelled oligonucleotides were synthetised using an Applied Biosystems 381A. The DNA thermal Cycler 480 (Perkin Elmer Cetus) was programmed to carry out 30 cycles of: 94 °C for 1 min, 50 °C for 2 min, and 74 °C for 3 min. A 5 min incubation step at 94 °C was carried out initially as well as a final step of 10 min at 74 °C.

Amplified products were diluted (1/5 in deionised water). 1 l from this dilution was mixed with 2 l deionised formamide, 0,5 l loading buffer, and 0,5 l labelled standards (Genescan 500 TAMRA). Samples were denatured at 94 °C for 4 min, then loaded on 6% denaturing polyacrylamide gel and electrophoresed at 1000 V for 3h30 on Applied Biosystems 373A DNA sequencer. Allele sizes were determined automatically by Genescan 672.1 software (Applied Biosystems, Foster City, CA 94404) using the second order last square method.

Results

The distribution of the Cystic Fibrosis mutations are summarized in Table 1. Out of 32 CF chromosomes, 27, (i.e. 84%), share the dfF508 mutation.

The haplotypes, established with microsatellites markers IVS8CA, IVS17BTA, and IVS17BCA are shown in Table 2. Fifteen different haplotypes have been described, four of them are linked to the df508 mutation. Two haplotypes account for most of the df508 chromosomes : haplotype 23–31–13 for 55%, and haplotype 17–32–13 for 29.6%. On the 18 normal chromosomes, 11 haplotypes have been found, 8 of them were only found on normal chromosomes and not in mutated CF gene chromosomes.

TABLE 1
DISTRIBUTION OF CF MUTATIONS IN EACH FAMILY ANALYSED IN »GRANDE BRIÈRE«

Genotypes	Number of probands	Frequency
df508/df508	11	68.75%
df508/ W57X	2	12.5%
df508/ G551D	1	6.25%
df508/ S549N	1	6.25%
df508/1717–1G A	1	6.25%
TOTAL	16	100%

Discussion

In our population, df508 mutation account for 84% of all mutations versus 73% for the Breton population of Celtic origin (6). This represents highest value ever observed except for the Basque population (87%) (13). Four other mutations (W57X, S549N, 1717–1G A, and G551D) are also found showing an heterogeneity of CF chromosomes in this population.

Mutation G551D is associated with 16–07–17 haplotype as it is in Spain, England, Scotland, Brittany, Czech, and Slovak Republics and Ireland (12, 14, 15, 16, 17) and may be explained by the Celtic settlement of Brittany.

Mutation N1717–1G A shows the same haplotype 16–07–17. 16–07–17 was found on two normal chromosomes in our study. This was not very surprising because it was the most common haplotype associated with normal chromosomes and mutated non df508 chromosomes (18).

TABLE 2
DISTRIBUTION OF THE MICROSATELLITE HAPLOTYPES AMONG NORMAL AND CF CHROMOSOMES FROM THE »GRANDE BRIÈRE« POPULATION. EACH HAPLOTYPE IS NAMED ACCORDING TO THE NUMBER OF REPEATS AT LOCI IVS8CA, IVS17bTA, AND IVS17bCA, RESPECTIVELY

Microsatellites haplotype	df508 mutation	G551D mutation	W57X mutation	S549N mutation	1717–1G A mutation	Normal chromosome
23–31–13	15 (55.6%)					
17–32–13	8 (29.6%)					
17–31–13	3 (11.1%)					
23–32–13	1 (3.7%)					1 (5.5%)
16–07–17		1 (100%)			1 (100%)	2 (11.1%)
16–29–13			2 (100%)			
16–31–13				1 (100%)		2 (11.1%)
16–32–13						4 (22%)
16–30–13						2 (11.1%)
16–35–13						1 (5.5%)
16–33–14						1 (5.5%)
18–35–13						1 (5.5%)
18–34–13						1 (5.5%)
17–35–13						2 (11.1%)
23–34–13						1 (5.5%)
TOTAL	27	1	2	1	1	18

The two W57X chromosomes are associated with 16–29–13 haplotypes and S549N mutation with 16–31–13. They are found in Montoir, a peripheric village known to be a place of population flow.

These results show an unexpected apparent heterogeneity since five different mutations are found, and for the major one, i.e. df508, four different haplotypes. As is the case for Breton (6), and European populations (11), the Grande Brière population share the same genetic features for microsatellites associated with df508 chromosomes. This observation is not in agreement with the known high inbreeding rate and endogamy of this region.

The »Grande Brière« can be divided in two different areas (Figure 1) : the islands located in the middle marsh and the peripheric villages. In the islands, only the df508 mutation is found. Furthermore, this mutation is only linked with two haplotypes 23–31–13, and 17–32–13. These mutations come from two couples who settled in the central islands in the eighteenth, and nineteenth centuries. Haplotypes 17–32–13 and the other one, 23–31–13, came from South Brittany.

Populations from peripheric villages sharing mutations non df508 and df508 associated with less frequent haplotypes 17–31–13, and 23–32–13 do not have any common features with the characteristics of the central islands. This observation shows that the two subpopulations did not mix in the past, and therefore provides another argument showing the strong isolation of the populations of the islands.

The morphology of the ascendants distribution among the central Brière population for the last four centuries is very different from that of periphery, and more generally from panmictic populations. This observation is the logical consequence of the very limited availability of marriageable men. The classical distribution

for the individual ancestors was an inverted pyramid (Figure 2a). For the central »Grande Brière«, this distribution became cylindrical (Figure 2b). The reason for this was, with a few exceptions, the women of the Brière did not choose their husband outside from the islands. Men who were from elsewhere were excluded from the matrimonial market more for economics rather than socials. These observations explain the fact that we easily found the origin for the two microsatellites. This conclusion was supported by the fact that mutations were found only among their own descendants, and on the other hand because of the high level of consanguinity in this population. An older presence of the two microsatellites concerned would have given rise to an homogenous and elevated augmentation of their frequency.

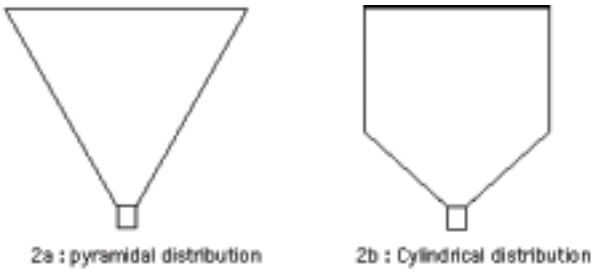

Fig. 2. Comparison of the distribution between individual ancestors in panmictic population (2a) and in the population from central "Grande Brière" (2b)

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POVEZANOST HAPLOTIPOVA MIKROSATELITSKIH S 5 MUTACIJA CFTR U »GRANDE BRIERE«, IZOLATU SMJEŠTENOM U JUŽNOJ BRETANJI

S A Ž E T A K

Varijabilnost tri mikrosatelita regulatornog gena CFTR lokusa cistične fibroze proučavana je zbog učestalih mutacija u izoliranoj populaciji »Grand Brière«, maloj regiji smještenoj u Južnoj Bretanji. Nalaz je proučen (FM PCR) u 16 obitelji s cističnom fibrozom (CF) nosiocima 5 različitih mutacija. Četiri najučestalija haplotipa (df508) isti su onim pronađenim u Sjevernoj Francuskoj i Europi, ali njihova razdioba u populacijama pruža nove spoznaje o porijeklu ove izolirane zajednice.