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Haplotypes of microsatellite markers of the CFTR gene in Polish and German CF chromosomes suggest an ancient origin of the most frequent cystic fibrosis mutations

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Abstract. In this study we have analysed haplotypes of microsatellite markers of the CFTR gene: IVS8CA, IVS17BTA, IVS17BCA in 17 CF chromosomes of Polish origin and in 19 chromosomes of German origin bearing CF mutations other than ΔF508. In the Polish population, the G542X mutation is connected with haplotypes 16/17-28/32/38-13; in the German population, a more diverse haplotype association has been detected (23-33-13 and 16-32-13). The 1717-1G->A mutation is associated with the 15/16-7-13 haplotype in the Polish population, like the G551D mutation in Germany. The only analysed case of N1303K of Polish origin is connected with the 23-30-13 haplotype, like in the German population. One N1303K chromosome of an entirely different haplotype (16-29-17) turned out to be of Greek origin. These data suggest an ancient, Palaeolithic or Neolithic origin of these mutations in the territory of current Northern Europe.

Key words: cystic fibrosis, microsatellites, mutations.

Introduction

Microsatellites are a universal resource for the study and diagnosis of genetic disorders (TODD 1992). The variable nature of these repetitive blocks gives high heterozygosities which allow to trace certain alleles in the population and/or families. Three highly informative microsatellite dinucleotide markers (also known as variable number of tandem repeats – VNTR or short tandem

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repeat sequences – STR) have been described in intronic regions of the CFTR gene, mutations of which cause cystic fibrosis (CF): IVS8CA, IVS17BTA, IVS17BCA (MORRAL et al. 1991, ZIELENSKI et al. 1991). They have revealed to be extremely useful for indirect diagnosis of CF and for characterization of mutant haplotypes. Especially in the case of unidentified mutations, the indirect analysis with intragenic markers is a method of choice for prenatal diagnosis and carrier detection (MORNET et al. 1992, MAGNANI et al. 1994).

An analysis of microsatellite marker haplotypes of the CFTR gene bearing the ΔF508 mutation in different European populations (MORRAL et al. 1994) demonstrated that this most frequent CF mutation occurred more than 52,000 years ago and spread throughout Europe in two chronologically distinct expansions. The first expansion onto the current European territory was associated mainly with the 23-31-13 haplotype and took place about 50,000 years ago; further expansions targeted mainly on Central and Northern Europe were associated with the 17-31-13 and 17-32-13 haplotypes and were caused by migrations about 10,000 years ago (MORRAL et al. 1994, SCHWARTZ et al. 1995). Ancestral haplotypes 23-31-13, 17-31-13 and 17-32-13, through slippage mechanism, generated a whole series of derivative haplotypes (WEBER 1990, MORRAL et al. 1994). Two genetically distinct geographical groups were revealed: (1) the Mediterranean/British group (predominant haplotype 23-31-13); and (2) Central/North European group (predominant haplotype 17-31-13). A broad CFTR gene microsatellite study (WITT et al. 1996) showed that the Polish population remains in an intermediate position between North- and South-European countries, which correlates with gradients established so far: a gradient of frequency of haplotypes of extragenic linked markers XV2c/KM19 (MACIEJKO et al. 1989) and a gradient of frequency of the ΔF508 mutation (BAL et al. 1991).

We have analysed haplotypes of CFTR gene microsatellite markers in a sample of CF chromosomes containing several mutations causing CF in Polish and German populations. Preliminary conclusions with regard to the history of these mutations in Poland have been drawn.

Patients and methods

A total of 17 CF chromosomes of Polish origin and 19 CF chromosomes of German origin bearing mutations of the CFTR gene other than ΔF508 have been used in this study. A clinical diagnosis of CF patients was based on usual criteria (WELSH et al. 1995). In all families, detection of a standard set of five

most frequent mutations of the CFTR gene has been performed (ΔF508; 1717-1G->A; G542X; G551D; N1303K). In all assays standard PCR protocols were used. Microsatellites within the CFTR gene were analysed by amplification in the presence of radioactive precursors of each marker locus as was described previously (MORRAL, ESTIVILL 1992). The PCR products were analysed on standard 6% polyacrylamide sequencing gels and visualized through autoradiography. The PCR products of DNA samples carrying alleles of known size were used as size markers.

Results and discussion

Relative frequencies of mutations of the CFTR gene of Polish CF chromosomes investigated in our laboratory were as follows: ΔF508 52.82%; 1717-1G->A 3.23%; G542X 3.23%; N1303K 2.82%; G551D 0.40%. Previous investigations showed that only four non-ΔF508 mutations (G542X, G551D, W1282X, N1303K) are present in most geographical and ethnic subgroups worldwide with frequencies higher than 1% each, but with considerable variation (CYSTIC FIBROSIS GENETIC ANALYSIS CONSORTIUM 1994).

We have analysed microsatellites of the CFTR gene of 17 CF chromosomes of Polish origin and 19 of German origin with CF mutations other than ΔF508, identified in a studied sample of CF chromosomes (Table 1). Germans represent a typical example of the North-European population. In the Polish population, mutation G542X is connected consistently with haplotypes 16/17-28/32/38-13 but in the German population a more diverse haplotype association has been

Table 1. Microsatellite haplotypes of various CF (non-ΔF508) chromosomes in the Polish and German populations

Mutation of the CFTR gene	Polish population		German population	
	haplotype[1]	number	haplotype[1]	number
G542X	16-28-13	2	23-33-13	3
	17-32-13	5	16-32-13	1
	17-38-13	1		
1717-1G->A	15-7-13	2	no data	
	16-7-13	6		
N1303K	23-30-13	1	24-31-13	2
			23-29-13	1
			16-29-17	1
G551D	no data		16-7-13	11

[1] haplotypes of microsatellite markers are presented in the following order: IVS8CA/IVS17BTA/IVS17BCA

detected (23-33-13 and 16-32-13). The 1717->1G-A mutation is associated with the 15/16-7-13 haplotype in the Polish population, the same as the G551D mutation in Germany. The only analysed case of N1303K of Polish origin is connected with the 23-30-13 haplotype, like in the German population. One N1303K chromosome of entirely different haplotype (16-29-17) turned out to be of Greek origin.

In the normal population the microsatellite locus IVS8CA contains 13 alleles ranging from 15 to 34 dinucleotide units. The most variable in size marker IVS17BTA is represented by 36 alleles ranging from 7 to 56 units of a TA repeat. For the less polymorphic IVS17BCA marker 8 alleles were found, varying from 11 to 17 dinucleotide units (ZIELENSKI et al. 1991, MORRAL et al. 1993). No new mutations generating new alleles for these three microsatellites were found suggesting that the loci are stable and reliable for molecular diagnosis (MORRAL, ESTIVILL 1992). Analysis of these intragenic microsatellites has revealed that they are highly polymorphic and useful for analysis of uninformative families: heterozygosities for IVS8CA, IVS17BTA and IVS17BCA were 48%, 87% and 39%, respectively; when the three loci were amplified simultaneously, heterozygosity increased to 95%. When combining microsatellites with mutation analysis of the six most frequent mutations, informativity can be obtained in almost 100% of families (MORRAL, ESTIVILL 1992).

Results presented in this paper, although based on a relatively low number of studied CF chromosomes not allowing for a proper statistical analysis, show the trend in the genotype data supporting the hypothesis that the relatively common mutation N1303K of ancient origin was introduced to Central/Northern Europe on the 23-29/31-13 haplotype background (MORRAL et al. 1993). Identification of a CF chromosome of Greek origin with an entirely different haplotype suggests a possibility of recurrent mutation events enriching a total pool of CF chromosomes in various geographical regions. An ancient origin and association with the ancestral haplotype 23-31/33-13 has been also shown for the G542X mutation in the Spanish population (MORRAL et al. 1993). According to our data, this mutation has been introduced to the Polish population most probably with the 17-32-13 haplotype and chromosomes derived from this haplotype, which might suggest that this mutation in the area of current Poland appeared later than in Spain or Germany. Since in Spain the three most common mutations (ΔF508, G542X, N1303K) originated on the same haplotype background, MORRAL et al. (1993) put forward the hypothesis that either there was a selective advantage for haplotype 23-32-13 and it's derivatives, or that these mutations originated in a population where this

haplotype was very common, or at a time when this haplotype was numerously represented. The estimated age of mutations N1303K and G542X is 34,000-35,000 years (MORRAL et al. 1993). The 1717-1G->A mutation in the Polish population seems to be exclusively connected with chromosomes originating from an ancestral CF chromosome characterized by the 17-32-13 microsatellite haplotype, like the G551D mutation in the German population – this is consistent with the Neolithic appearance of this mutation in the Northern part of the territory of current Europe. Similar haplotype background was shown for the G551D mutation in the Spanish population (MORRAL et al. 1993). Since in the Polish and German populations the 1717-1G->A and G551D mutations were found to be associated with the same respective haplotypes (15/16-7-13), a relatively recent appearance of these mutations in this part of Europe can be postulated.

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