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THE FREQUENCY OF MUTATIONS IN EXON 11 OF THE CF GENE IN POLISH CYSTIC FIBROSIS PATIENTS

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Results of mutation analysis in exon 11 of the CF gene have been presented. Using the SSCP technique 18 mutations (of four different types) were detected in cystic fibrosis patients of Polish origin. Thus, we were able to detect in exon 11 about 10% of all CF mutations occuring in the affected population examined.

Cystic fibrosis (CF) is one of the most common autosomal genetic disorders in the Caucasian population. The CF gene contains 27 exons and encodes a protein of 1480 amino acids [1- 4]. This protein, named "Cystic Fibrosis Transmembrane Regulator" (CFTR), regulates Cl^- transport across the cell membrane [5, 6].

The most frequent CF mutation, ΔF508, was found in exon 10 [2]. The average frequency of this mutation in Caucasian CF patients is about 68%. It differs, however, in different populations examined [7]. A "hot spot" for less frequent mutations was localized in exon 11. In a 34 nucleotides long fragment ten different mutations were found [8].

In this paper the results of mutation analysis in exon 11 in Polish CF patients are presented.

MATERIAL AND METHODS

Material. DNA samples from 62 unrelated CF patients of Polish origin were tested. The diagnosis was based on the clinical features and sweat test.

DNA analysis. Standard methods were applied for DNA isolation. Restriction fragment length polymorphism (RFLP) analysis using KM19 and XV2c [9] probes was performed by Southern blotting and hybridization with radioactive probes.

Mutation analysis. Mutations in exon 11 were detected by the single strand conformation polymorphism (SSCP) analysis [10]. For identification of R553X, G551D and S549N mutations, the digestion of PCR products with appropriate restriction enzymes was used as an alternative method [8]. The PCR conditions and oligonucleotide (primer) sequence were applied according to Zielinski *et al.* [4].

The ΔF508 mutation in exon 10 was examined as described earlier [11]. Identification of the N1303K mutation in exon 21 was performed by allele specific PCR [12].

Characteristic of tested mutations is presented in Table 1.

Table 1
Type of mutations examined

Mutation	Localization		Description	
	Exon	Intron	Nucleotide change	Reference
ΔF508	10		delCTT	[2]
G542X	11		G→T	[14]
R553X	11		C→T	[8]
G551D	11		G→A	[14]
1717-1G-A		10	atagGA→ataaGA	[16]
S549N	11		G→A	[14]
N1303K	21		C→G	[17]

RESULTS AND DISCUSSION

Mutations in exon 11 were examined for 81 non ΔF508 CF alleles of 62 unrelated Polish CF patients. Using the SSCP technique, 4 different types of mutation (G542X, R553X, G551D, 1717-1G-A) were found in 18 chromosomes (Table 2).

Table 2

The frequency of mutations detected in exon 11 in the Polish population

Chromosomes		Mutation								Summary	
		R553X		1717-1G→A		G551D		G542X			
type	no*	no	%	no	%	no	%	no	%	no	%
CF	188	6	3.2	5	2.6	1	0.5	6	3.2	18	9.5
non ΔF508	81		7.4		6.2		1.2		7.4		22.2

*number of cases

In the Polish population examined the frequency of mutations detected (Table 2) did not differ significantly from the results of other workers. However, the frequency of the R553X mutation was higher (7.4%) and identical with the frequency observed for the G542X mutation. Thus, we were able to detect in exon 11 about 22% of the non ΔF508 CF mutations. For comparison, in the German population approximately 36% [10] and in South Europeans about 13% [13] of these mutations could be detected in exon 11.

The XV2c/KM19 haplotype distribution among DNA samples with at least one identified mutation is presented in Table 3. A strong association between haplotype B and G542X and 1717-1G-A mutations is apparent. This confirms previous observations that haplotype B very often accompanies CF mutations [14, 15]. In our experiments, a linkage between haplotype A and R553X could also be observed.

In Polish CF patients, the ΔF508 mutation occurs with a frequency of approximately 56%. So far, besides ΔF508 and mutations in exon 11, the

Table 3

XV2c/KM19 haplotypes among CF mutations in the Polish population

Genotype	No of cases	XV2c/KM19 haplotype*
ΔF508/G542X	5	BB
ΔF508/1717-1G-A	3	BB
	1	BD
ΔF508/R553X	4	AB
R553X/ –	2	AC
G542X/ –	1	AC
G551D/ –	1	BC
1717-G→A/N1303K	1	AB

– Unknown CF mutation

* XV2c/KM19 haplotypes: A (1.1); B (1.2); C (2.1); D (2.2)

frequency of N1303K [12] and R1162X (Bal, J., unpublished) mutations have been examined. This indicates that it is possible to detect only approximately 67% of all CF mutations. The search for other mutations is therefore important for characterization of mutations in the CF gene in the Polish population.

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