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A Cluster of Highly Polymorphic Dinucleotide Repeats in Intron 17b of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Gene

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Summary

A cluster of highly polymorphic dinucleotide repeats has been detected in intron 17b of the CFTR gene, 200 bp downstream from the preceding exon. At least 24 alleles, with sizes ranging from 7 to 56 units of a TA repeat, have been identified in a panel of 92 unrelated carriers of cystic fibrosis (CF). The common ones are those with 7, 30, and 31 dinucleotide units, with frequencies of .22, .19, and .12, respectively, among the non-CF chromosomes. Mendelian, codominant segregation of the alleles has been demonstrated in family studies, as expected. A less polymorphic dinucleotide (CA repeat) cluster has also been detected in a region 167 bp downstream from the TA repeat. The length of the CA repeat cluster varies from 11 to 17 dinucleotide units, and it appears to have an inverse relationship to that of the TA repeats. These dinucleotide repeats should be useful in genetic linkage studies, in counseling for CF families with unknown mutations, and in tracing the origins of the various mutant CF alleles.

Introduction

The gene responsible for cystic fibrosis (CF), a common autosomal recessive disorder, has been identified (Kerem et al. 1989; Riordan et al. 1989; Rommens et al. 1989), and the structure and organization of the gene have been characterized in detail (Zielenski et al. 1991b). The gene is predicted to encode a transmembrane protein of 1,480 amino acids and is named cystic fibrosis transmembrane conductance regulator (CFTR). In addition to the major mutation, which is a deletion of 3 bp in exon 10 of the gene ($\Delta F508$), accounting for approximately 70% of the CF mutant genes (Kerem et al. 1989, 1990; Cystic Fibrosis Genetic Analysis Consortium 1990), many other mutations in CFTR have been identified (Cutting

et al. 1990a, 1990b; Dean et al. 1990; Guillermit et al. 1990; Kerem et al. 1990; Vidaud et al. 1990; White et al. 1990; Zielenski et al. 1991a). The majority of these mutations are rare, however, or confined to particular geographic locations (Zielenski et al. 1991a). Except for a few regions in the world, the fraction of known mutations among most CF populations is around 80%–85% of the total CF chromosomes screened (Cystic Fibrosis Genetic Analysis Consortium, unpublished data).

Direct DNA analysis is one of the immediate applications of the knowledge about the CF mutations. With an average detection rate of 85%, however, only 72% of the couples who seek genetic counseling will receive fully informative answers; the remaining cases will have to rely on closely linked DNA markers to obtain a more complete diagnosis. The presence of many long dinucleotide repeats in the CFTR gene (Zielenski et al. 1991b) offers an alternative strategy to RFLPs in tracking the parental chromosomes in a family, as simple dinucleotide repeat sequences are often found to be excellent markers for family studies (Tautz 1989; Weber and May 1989).

Chehab et al. (1991) reported a polymorphic GATT

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tetranucleotide repeat in the 5' flanking region of exon 6b (intron 6a), and Morral et al. (1991) noted a polymorphic GT dinucleotide repeat in intron 8. While these DNA markers showed a limited number of alleles useful for family analysis, we turned our attention to two potentially more informative clusters of dinucleotide repeats in the intron 17b region of the CFTR gene. As shown in figure 1, sequence analysis of the genomic DNA clones revealed a TA repeat, (TA)₇TG(TA)₁₄CA(TA)₆, and a CA repeat, (CA)₁₃, separated by 167 bp of apparently unique sequence (Zielenski et al. 1991b). In the present communication, we report the characterization of this region and its application in CF analysis.

Material and Methods

Analysis of Intron 17b Sequences

The DNA sequence containing the dinucleotide repeats of interest in intron 17b has been reported elsewhere (Zielenski et al. 1991b). The genomic DNA fragment (4.5-kb *EcoRI* fragment) (Rommens et al.

1989) containing these repeats was isolated from the phage clone TE33II, cloned in the Bluescript (KS+) phagemide vector (Stratagene), and named pTE33IIE4.5.

The PCR (Mullis et al. 1986; Saiki et al. 1988) was used to amplify the genomic DNA segment containing the TA repeats in intron 17b (fig. 1). The standard reaction (50 μl) contained 0.5 μg of genomic DNA, 0.2 mM dNTP, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.001% (w/v) gelatin, 100 ng each of the two oligonucleotide primers 17B-R1 (5'-GCTGCATTCTATAGGTTATC-3') and 17B-RE3 (5'-AAACTTACCGACAAGAGGAA-3'), and 2 units of *Taq* polymerase (Cetus). A typical reaction consisted of 30 cycles of denaturation (30 s, 94°C), annealing (30 s, 55°C), and extension (60 s, 72°C) in a thermal cycler (Perkin-Elmer), as described elsewhere (Zielenski et al. 1991b). The size of the amplified products from the control plasmid (pTE33IIE4.5) was 414 bp, as expected.

PCR-amplified DNA fragments (from 17B-R1 and 17B-RE3 PCR primers) corresponding to selected TA and CA repeat alleles were subcloned into Bluescript

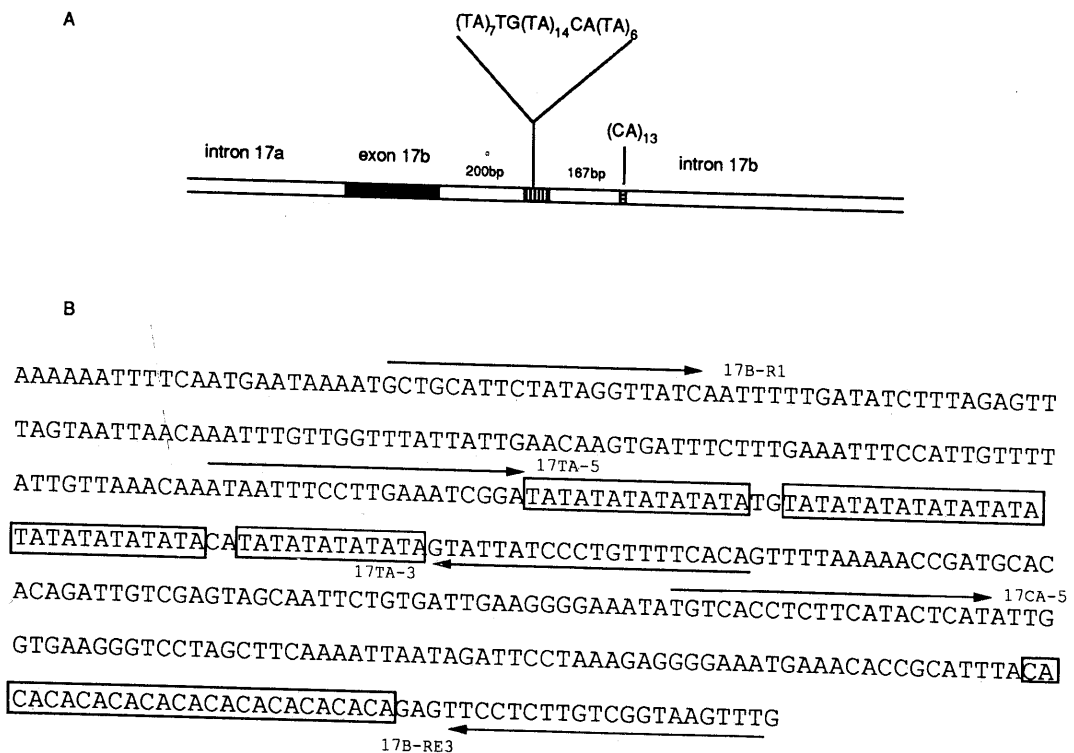


Figure 1 Dinucleotide repeats in intron 17b of CFTR gene. A, Location of repeats with respect to exon 17b. B, Nucleotide sequence of repeat region, as determined in genomic clone pTE33IIE4.5.

KS + vector (Stratagene). The sequence of clones of PCR-amplified DNA was determined by the dideoxy-chain termination method with the Sequenase kit (U.S. Biochemicals) essentially according to a method described elsewhere (Sanger et al. 1977; Kerem et al. 1990).

Detection of TA and CA Length Polymorphisms

To detect the length of the TA and CA repeats in intron 17b, two end-labeled primers, internal to the amplified genomic DNA, were used in an additional round of extension. The primer 17TA-3 (5'-TGTGA-AAACAGGGATAATAC-3') was used to identify the TA repeats, and the primer 17CA-5 (5'-TGTCACC-TCTTCATACTCAT-3') was used for the CA repeats (fig. 1). The primers (60 pmol each) were labeled in a 20- μ l reaction with 40 μ Ci of [γ - 32 P]ATP and 5 units of polynucleotide kinase (Pharmacia) in a buffer containing 50 mM Tris-HCl (pH 7.6), 10 mM MgCl₂, 5 mM DTT, and 0.1 mM EDTA, at 37°C for 10 min. Approximately 0.2 pmol of the end-labeled primers were then mixed with 5 μ l of the crude PCR product and the addition of fresh PCR buffer and *Taq* polymerase (1 unit), to a total volume of 25 μ l. The PCR was conducted as described above. The products were displayed on a 6% polyacrylamide sequencing-type gel as described elsewhere (Zielenski et al. 1991a, 1990b). The sizes of fragments extended from primers 17TA-3 and 17CA-5 were, as predicted from the sequence of pTE33IIE4.5, 217 and 137 bp, respec-

tively. Attempts to amplify the TA repeat separately with the oligonucleotide pair described above (17B-R1/17TA-3) or with combinations of other oligonucleotides were unsuccessful.

Families and Haplotype Analysis

Forty-seven Canadian CF families were used in the present study; 43 of them have been described elsewhere (Tsui et al. 1986), and the others were subsequently recruited for linkage and mutation analyses (Kerem et al. 1990; Zielenski et al. 1991a). DNA samples were not available for two of the parents. The mutations identified in these CF families have been reported elsewhere (Kerem et al. 1989, 1990; Zielenski et al. 1991a). Extensive DNA marker haplotype information was available for 43 families (Kerem et al. 1989).

Results

The TA and CA repeats noted in the intron 17b region of CFTR were analyzed in the 47 families previously used for CF linkage and mutation analyses. Except for two missing normal chromosomes and one CF chromosome, unequivocal data were obtained from 185 chromosomes (92 normal and 93 CF). Among them, 24 different length variations (alleles) were detected for the TA repeat sequence named CF17B200 (fig. 2). The variable number of repeating units appeared to differ from each other by two-

<u>allele1</u>	<u>7</u>	<u>31</u>	<u>31</u>	<u>32</u>	<u>26</u>	<u>32</u>	<u>29</u>	<u>31</u>	<u>31</u>	<u>32</u>	<u>33</u>	<u>34</u>	<u>35</u>	<u>36</u>	<u>39</u>	<u>44</u>	<u>45</u>	<u>45</u>	<u>46</u>	<u>47</u>	<u>48</u>	<u>51</u>	<u>53</u>	<u>56</u>
allele2	7	8	22	24	7	28	7	7	30	7	22	31	7	31	32	31	31	31	32	35	32	32	7	31

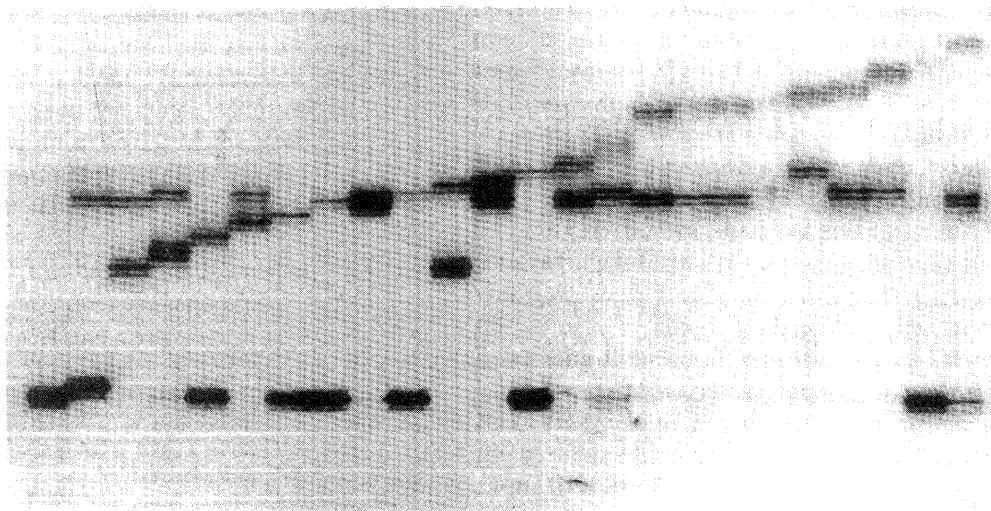


Figure 2 Detection of length variations of TA repeats in intron 17b of CFTR gene: a display of 23 different alleles (length variations) on a sequencing-type gel.

nucleotide increments and to cluster in three size groups; 7–8, 22–48, and 51–56 units.

To confirm that this polymorphism was due to differences in number of dinucleotide repeats, the genomic DNA segments were amplified by PCR, cloned in plasmid vector, and sequenced. The results showed that the alleles (TA)₃₁ and (TA)₇ contained the sequences (TA)₇TG(TA)₁₆CA(TA)₆N₁₆₇(CA)₁₃ and (TA)₇N₁₆₇(CA)₁₃, respectively (fig. 3). Sequencing analysis was not performed for the other alleles. It seemed probable that there would be other sequence variations outside or within the TA repeat in the CF17B200 locus. Without further sequence information, however, it was only possible to distinguish the alleles from each other by the variable number of apparent dinucleotide repeats within the TA region. Three CA repeat variations were detected; their apparent lengths were 11, 13, and 17 dinucleotide units (not shown).

Mendelian inheritance of the TA and CA repeats was confirmed in family studies, and the results

showed that the alleles were segregated in a codominant fashion (not shown). The most frequent allele for the TA repeat polymorphism was (TA)₇, with a frequency of .22 among the normal chromosomes of the CF carrier parents, followed by (TA)₃₀ and (TA)₃₁, with frequencies of .19 and .12 respectively (table 1). The observed heterozygosity for the TA repeat polymorphism in the 92 unrelated CF carriers is 95%; the PIC calculated from the normal chromosomes only is .89. Adding the CA repeat polymorphism in the analysis did not increase the heterozygosity, as complete disequilibrium was detected between the two repeats (not shown). It was, however, of interest to note that the shortest group of TA repeats, (TA)₇ and (TA)₈, was exclusively associated with the longest CA repeat, (CA)₁₇; the medium group of TA repeats, (TA)_{22–48}, with the medium-size CA repeat, (CA)₁₃; and the long TA repeats, (TA)_{51–56} with the shortest CA repeat, (CA)₁₁. The significance of this inverse correlation between the lengths of the two repeat regions was not further investigated, although length compen-

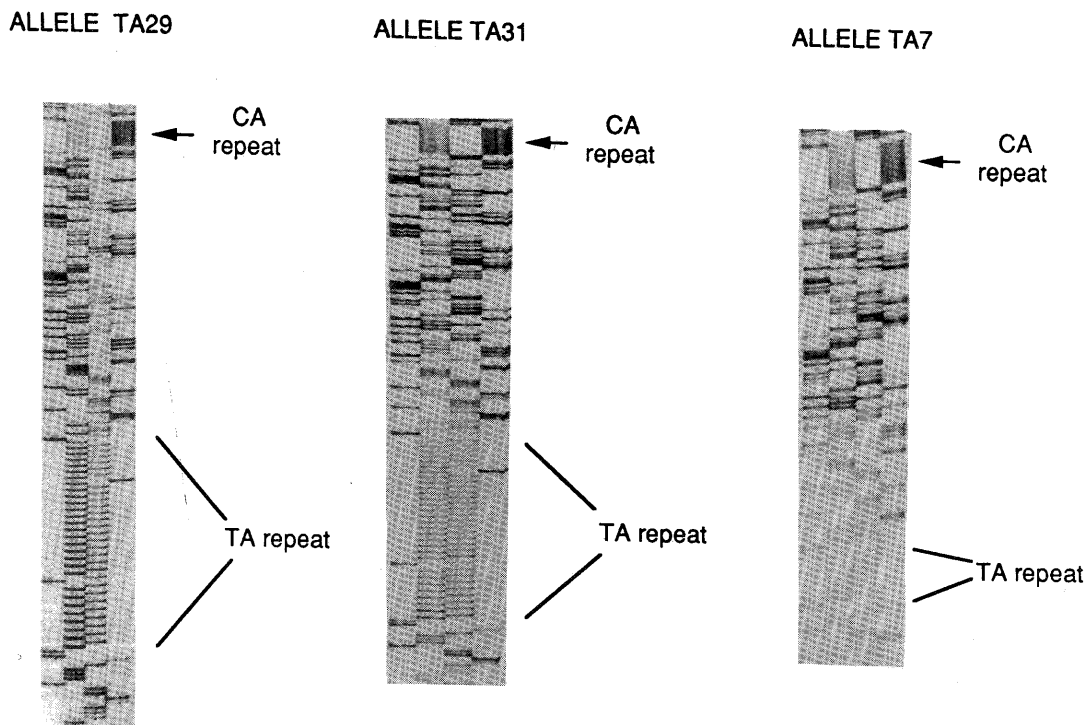


Figure 3 Example of TA repeat alleles in intron 17b of CFTR gene. The DNA sequencing ladders (from left to right, G, A, T, and C) for three TA repeats are shown. The TA dinucleotide repeats are interrupted by an A-to-G and a T-to-C substitution; the length variations in these examples appear to be due to the number of repeats in the second and third block from the sequencing primer 17B-R1 (bottom of the autoradiographs). Allele TA29 represents the sequence of the genomic clone pTE33IIE4.5; the sequences for TA31 and TA7 are both derived from PCR-amplified products from individuals carrying these alleles.

Table 1

Distribution of TA Alleles among $\Delta F508$, Non- $\Delta F508$, and Normal Chromosomes

ALLELE (number of dinucleotide repeats)	FREQUENCY OF CHROMOSOME					
	CF- $\Delta F508$		CF-non- $\Delta F508$		Normal	
	No.	%	No.	%	No.	%
7.....			10	31.3	20	21.7
8.....					1	1.1
22.....					2	2.2
24.....			1	3.1	2	2.2
26.....			1	3.1		
28.....					1	1.1
29.....					3	3.3
30.....	4	6.6	6	18.8	17	18.5
31.....	34	55.7	2	6.3	11	12
32.....	22	36.1	2	6.3	7	7.6
33.....			2	6.3	2	2.2
34.....					3	3.3
35.....			3	9.4	3	3.3
36.....					3	3.3
39.....			1	3.1	1	1.1
43.....			1	3.1	1	1.1
44.....					2	2.2
45.....			2	6.3	4	4.3
46.....	1	1.6			3	3.3
47.....			1	3.1		
48.....					1	1.1
51.....					2	2.2
53.....					2	2.2
56.....					1	1.1
Total.....	61		32		92	

sation was observed for large subunit of rRNA genes of different species and was thought to be functionally important (Hancock and Dover 1990).

Allelic association of the TA repeat was next examined with the most frequent mutation ($\Delta F508$) in the CFTR gene. As shown in table 1, four different TA repeat alleles were found with chromosomes carrying $\Delta F508$; the $(TA)_{31}$ and $(TA)_{32}$ alleles were represented with frequencies of .36 and .52, respectively. This distribution was significantly different from that in the normal chromosome population ($Z = 8.53$; $P < .001$; Smith 1986). In contrast, as could be expected from the large variety of non- $\Delta F508$ mutant alleles, there was no association between the non- $\Delta F508$ chromosome and any TA repeat polymorphisms. This distribution of the TA repeat alleles was also examined with other closely linked DNA markers. No obvious allelic association could be detected, but, when DNA marker haplotypes were constructed, some of the haplotypes

were found associated with only a subset of the TA repeat alleles (data not shown).

Discussion

Among different individuals, length variations are frequently found with DNA sequences containing short tandem repeats; these VNTRs are generally useful for family studies and genetic analysis (Litt and Luty 1989; Tautz et al. 1989; Weber and May 1989; Boylan et al. 1990; Economou et al. 1990; Luty et al. 1990). The most commonly described dinucleotide length polymorphisms are those associated with CA (or GT) repeats, with approximately 50,000 copies scattered throughout the human as well as the mouse genomes (Hamada et al. 1982; Litt and Luty 1989; Weber and May 1989). Permutations of dinucleotide repeats other than CA (or GT), such as TA repeats, are considered to be less common (Semenza et al. 1984;

Stoker et al. 1985; Bowcock et al. 1989; Williamson et al. 1990).

Unequal crossover in meiosis is a plausible mechanism whereby length variation can be generated in the dinucleotide repeat sequences. The multiple TA repeat alleles found associated with $\Delta F508$ are, however, strong evidence arguing against this explanation. From the haplotype data consisting of 25 DNA markers spanning the CFTR gene, it has been shown that all CFTR alleles carrying $\Delta F508$ are descendants of a single mutational event, mainly because the haplotype associated with this mutation is rare among the normal chromosomes. Further, it has been suggested that regions containing TA nucleotides are particularly susceptible to slippage during replication or repair by DNA polymerase and therefore can be highly polymorphic sequences (Kornberg 1980). Our results on the TA dinucleotide repeat region in intron 17b of the CFTR gene are thus in good agreement with this hypothesis. TA repeats may also assume cruciform structures which are susceptible to small sequence insertions and deletions (Freund et al. 1989).

Besides the dinucleotide repeats described in the present study (the CA repeat has also been described by Morral et al., submitted), other polymorphic, simple repeat sequences have also been described in other introns of the CFTR gene—a CA repeat located just upstream of exon 9 (Morral et al. 1991) and a GATT repeat immediately upstream of exon 6b (Chehab et al. 1991). These polymorphic loci thus constitute a group of excellent markers within the CFTR gene. On the basis of the size of the gene, there may be many additional simple repeat polymorphisms elsewhere in the introns. Since 15%–20% of the CF mutations are expected to be rare in the population and therefore escape detection by systems designed to screen for common mutations, these highly polymorphic simple repeats would be particularly useful for genetic diagnosis in CF families where the mutations have not been characterized.

Further, if it is assumed that alteration of the length of the dinucleotide repeats does not occur frequently and that alleles are identical by descent, it may be possible to follow this TA repeat variation among the $\Delta F508$ alleles in different populations, to trace the origin of this mutation. Similarly, the origins of the other CF mutations may also be studied through this and other dinucleotide repeats. In our preliminary analysis, three TA repeat alleles have been found associated with $\Delta F508$ in the French-Canadian families. Since the genealogy of this population can be traced,

it will be possible to evaluate the validity of this approach.

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