EXHIBIT 11

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[54] **CYSTIC FIBROSIS MUTATION CLUSTER**

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[58] **Field of Search** 435/6, 91, 91.2; 436/94; 536/27, 23.2, 24.31; 935/77, 78

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[57] **ABSTRACT**

Four mutations have been found clustered in exon 11 of the CFTR (cystic fibrosis transmembrane conductance regulator) gene. These mutations occur within a set of amino acids highly conserved among ATP-dependent transport proteins. Humans can be tested to determine whether they carry one of these mutations using a number of methods and/or probes taught herein. Specifically the mutations include: Asn_{549}, Asp_{551}, $Stop_{553}$, and Thr_{559}.

33 Claims, 2 Drawing Sheets

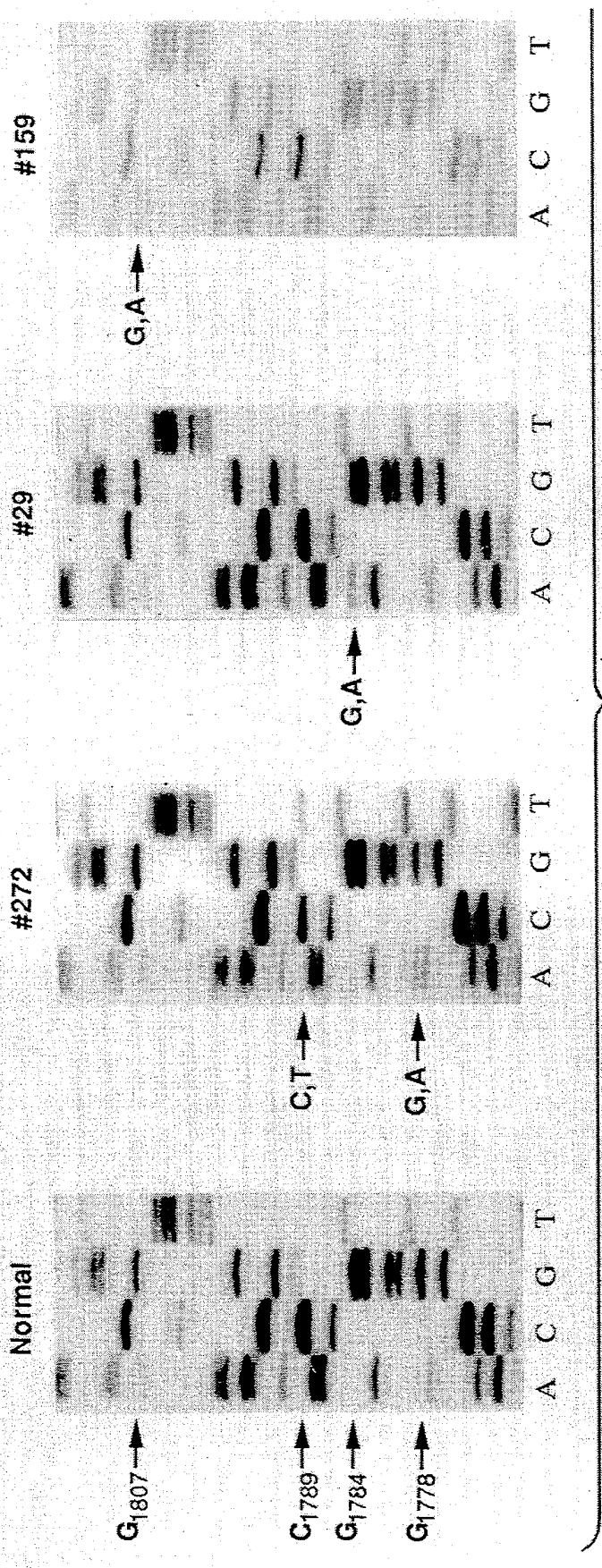
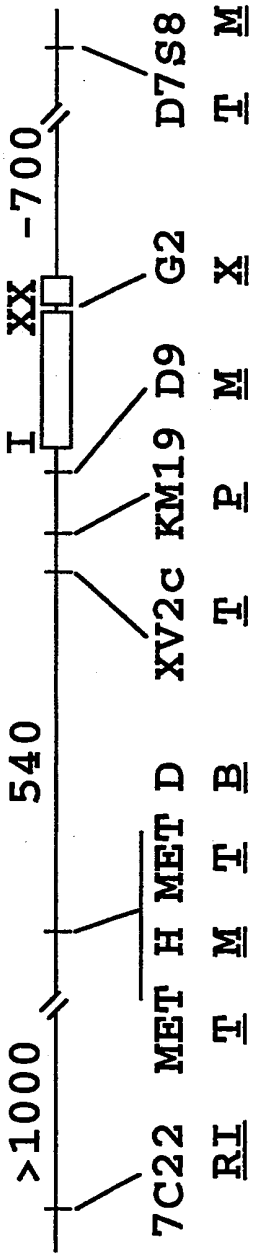


FIG. 1

FIG. 2


D7S8
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CYSTIC FIBROSIS MUTATION CLUSTER

This invention was made using U.S. government funds awarded by the National Institutes of Health as DK 39635 and DK 34944. Therefore the government has certain rights in the invention.

BACKGROUND OF THE INVENTION

Cystic fibrosis (CF), the most common lethal autosomal genetic disorder in the Caucasian population, occurs approximately once in every 2500 live births (Boat, et al., *The Metabolic Basis of Inherited Disease,* eds. Shriver, et al., McGraw-Hill, New York (1989) pp. 2649-2680). A single locus for CF has been mapped to chromosome 7q31 by linkage analysis using DNA marker probes.

Several markers have been shown to have a high degree of linkage disequilibrium with the CF locus in Caucasians suggesting that one mutation of the CF gene predominates in this population (Estivill, et al., Nature, (1987), 326:840; Estivill, et al., Genomics, (1987), 1:257). DNA polymorphism haplotypes from phenotypically and racially diverse patient populations indicated that several additional mutations of the CF gene may exist in these groups (Ober, et al., Am. J. Hum. Genet., vol. 41, p. 1145, 1987; Estivill, et al., ibid., vol. 43, p. 23 (1988); Fujiwara, et al., ibid., vol. 44, p. 327, (1989); Kerem, et al., ibid. p. 827 and Cutting, et al., ibid., p. 307).

The gene responsible for CF has recently been identified (Rommens, et al., Science, vol. 245, p. 1059 (1989); Riordan, et al., ibid., p. 1066); it comprises 20 exons and encodes a protein of 1480 amine acids called the CF Transmembrane Conductance Regulator (CFTR). Several regions are postulated to have functional importance in the CFTR protein, including two areas for ATP binding, termed Nucleotide Binding Folds (NBF), a Regulatory (R) region that has multiple potential sites for phosphorylation by protein kinases A and C, and two hydrophobic regions believed to interact with cell membranes.

One mutation has been identified in the CF gene which leads to the omission of phenylalanine residue 508 within the first putative NBF domain, indicating that this region is functionally important. This mutation, termed ΔF_{508}, accounts for about 70% of the CF chromosomes in Caucasian patients and was highly associated with the predominant haplotype found on chromosomes of Caucasian CF patients (Kerem, et al., Science, vol. 245, p. 1073 (1989); Lemna, et al., New Engl. J. Med., vol. 322, p. 291 (1990)); the haplotypes associated with Caucasian CF chromosomes without ΔF508 are less common, confirming predictions that allelic heterogeneity exists in CF (Ober, et al., Am. J. Hum. Genet., vol. 41, p. 1145, 1987; Estivill, et al., ibid., vol. 43, p. 23 (1988); Fujiwara, et al., ibid., vol. 44, p. 327, (1989); Kerem, et al., ibid. p. 827 and Cutting, et al., ibid., p. 307; Kerem, et al., Science, vol. 245, p. 1073 (19S9)).

There is a need in the art of genetic screening for knowledge of other mutant alleles of CFTR which are present on the other 30% of CF chromosomes in Caucasian CF patients, as well as other alleles found in other racial groups. Knowledge of such alleles can be used to design probes for screening, as well as to devise other screening methods. The more complete the set of probes available for CF mutant alleles, the more accurate diagnoses can be made.

SUMMARY OF THE INVENTION

It is an object of the invention to provide nucleic acid probes for detecting mutant CFTR alleles other than ΔF_{508}.

It is another object of the invention to provide methods of testing a DNA sample of a human for the presence of mutant alleles of the CFTR gene other than ΔF_{508}.

These and other objects of the invention are provided by one or more of the embodiments described below. In one embodiment a nucleic acid probe is provided which is complementary to a mutant allele of the CFTR gene selected from the group consisting of: Asn_{549}, Asp_{551}, $Stop_{553}$, and Thr_{559}.

In another embodiment of the invention a method is provided for testing a DNA sample of a human to determine if the human is a carrier of Cystic Fibrosis or if the human is affected with Cystic Fibrosis, comprising:

providing a DNA sample from a human;

testing the sample for the presence of a mutation in exon 11 of the CFTR gene of the human, the presence of the mutation indicating that the human is a carrier of Cystic Fibrosis or is affected with Cystic Fibrosis.

These and other embodiments are described with more particularity below. They provide the art with the knowledge of four hitherto unknown mutant alleles which are present in human populations and which can lead to cystic fibrosis if they are not present in a heterozygous configuration with a wild-type allele. In the case of a heterozygote, the individual is a "carrier", but will not be affected himself. If the mutant alleles are present with other mutant alleles, then the individual will be affected with the cystic fibrosis disease. These newly discovered alleles allow for genetic screening to provide more accurate diagnoses. Previously, without knowledge of these alleles, individuals carrying these alleles would have been "false negatives", i.e., they would have appeared to carry a wild-type allele because they did not carry any of the known mutant alleles.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows direct sequence analysis of PCR amplified genomic DNA from exon 11 of CFTR using the primer 11i-5'. The order of nucleotides in each gel panel is A,C,G,T. Patient #272 has exon 11 mutations on each chromosome, A_{1778} and T_{1789}. Patient #29 has the A_{1784}. Patient #159 has the A_{1807} mutation.

FIG. 2 shows a map of the relative positions and approximate distances in kb between the markers 7C22, MET, XV2c, KM19, D9, G2 and D7S8 and the CF gene which 4s shown as a box (Kerem, et al., *Science,* (1989) vol. 245, p. 1073; Estivill, et al., *Am. J. Hum. Genet.* (1989), vol. 44, p. 70 and Ramsay, et al., *Genomics* (1990), vol. 6, p. 39). Roman numerals denote exons 1 and 20 respectively. Enzyme abbreviations are as indicated in legend to Table 3.

DETAILED DESCRIPTION

It is a finding of the present invention that four mutations which cause cystic fibrosis (if present in an individual who lacks a wild-type allele) are clustered in a region of exon 11 of the CFTR gene consisting of nucleotides 1778–1807. (The numbering of nucleotides used herein follows the numbering of Riordan et al., Science vol. 245, p.1066, 1989.) See SEQ ID NO: 1. Thus exon

11 comprises a "hotspot" for CF mutations. The corresponding region of the protein is contained within the postulated first (N-terminal) nucleotide binding fold domain, a region which is highly conserved among a large number of homologous proteins. Each of the four mutations is a transversion, three causing amino acid substitutions and one producing a termination codon.

One mutation. G_{1784} to A, was found in 4% of the Caucasian CF chromosomes studied. (The allele which carries this mutation is termed the Asp551 allele herein.) The stop codon mutation (caused by a thymidine at nucleotide number 1789 leading to a translational stop after 552 amino acid residues,) was found in 5% of the American Black CF chromosomes studied. The other two mutations are rare in the American Black patients. These are both G to A mutations located at nucleotides 1778 and 1807, and lead to an asparagine and threonine residue, respectively. See Table 2.

The possibility that the three missense mutations are normal variants of the CFTR gene was ruled out by sequencing or restriction digestion of non-CF chromosomes with the same haplotype as that associated with each particular mutation. As shown in Table 3, none of the non-CF chromosomes of the same haplotype carried the mutations described herein. Therefore the missense mutations are not normal variants of the gene.

The Asp_{551} allele taught herein is to date the second most common CF mutation in Caucasian chromosomes. The mutation on the allele causes the substitution of glycine, a neutral amino acid, with aspartic acid, a polar amino acid. This charge change makes it unlikely that the allele codes for a normal polymorphic variant of the CFTR protein. In addition, even though the mutation occurs on 4% of Caucasian CF chromosomes, it has not been found on three normal chromosomes with the same ten site haplotype or twenty-four other normal Caucasian chromosomes. In six out of seven Caucasian patients who were found to have this mutation, it was paired with the ΔF_{508} mutation.

The CF gene was identified solely by its location in the human genome (Rommens, et al., Science, vol. 245, p. 1059 (1989)). Little is known of the function of its protein product, CFTR, except by analogy to well-characterized proteins that have similar amino acid sequences (Riordan, et al., Science, vol. 245, p. 1066 (1989)). The four mutations described here occur within a 13 amino acid segment (codons 548 to 560) (see SEQ ID NO: 2) of the putative first NBF region in the CFTR protein which is highly conserved among similar regions of other membrane-associated transport proteins (Riordan, et al., Science, vol. 245, p. 1066 (1989)). Five amino acids in this region are completely conserved in comparable regions from the multiple drug resistance proteins indicating that these positions are probably crucial to protein function (FIG. 2). It appears to be significant that the amino acid substitutions described in this study occur at three of the five completely conserved residues. Moreover, the substitutions occur at the three most conserved residues in that region between CFTR and 14 other membrane associated proteins which bind ATP (Riordan, et al. *Science* (1989), vol. 245, p. 1066). Therefore, the location of these mutations supports the theory that the CFTR protein is a member of the ATP-dependent transport protein superfamily (Riordan, et al., Science, vol. 245, p. 1066 (1989); Higgins, Nature, vol. 341, p. 103 (1989)).

Nucleic acid probes are provided according to the present invention which comprise either ribonucleic or deoxyribonucleic acids. Typically, the size of the probes varies from approximately 18 to 22 nucleotides. Functionally, the probe is long enough to bind specifically to the homologous region of the CFTR gene, but short enough such that a difference of one nucleotide between the probe and the D N A being tested disrupts hybridization. Thus the nucleic acid probes of the present invention are capable of detecting single nucleotide changes in the CFTR gene. The probes of the present invention are complementary to the mutant alleles described here: Asn_{549}, Asp_{551}, $Stop_{553}$, Thr_{559}. The homology of the probes to the mutant alleles is 100%. Probes corresponding to the wild-type sequences in this, region of the CFTR gene can also be used. These probes will bind to wild-type sequences but not mutant alleles in the region of nucleotides 1776–1807. Thus, for example, one could determine whether an individual was homozygous or heterozygous for a particular allele using both a wild-type and an allele-specific probe. If no wild-type allele is present in an individual carrying one of these mutant alleles, the individual will be affected by cystic fibrosis.

The DNA samples of humans to be tested according to the present invention include DNA of fetuses, juveniles, and adults. The DNA can be directly analyzed upon removal from the human source, or the DNA may be amplified by the PCR technique (Saiki, et al., Science, vol. 230, p. 1350 (1985)). The PCR technique amplifies certain regions of the DNA selectively according to the primers which are used. Alternatively, cells may be isolated from the human source and grown in culture prior to isolation of DNA. Growth in culture may be required where the number of cells available for DNA isolation is limited. Amplification according the present invention is of exon 11 sequences. All or part of exon 11 may be amplified prior to testing in one of the methods of the present invention. Primers which may be used include the oligonucleotide primers 11i-5' (SEQ ID NO: 3) (5'-CAACTGTGGTTAAAGCAATAGTGT-3') and 11i-3' (SEQ ID NO: 4) (5'-GCACAGATTCTGAGTAACCATAAT-3'). These primer sequences are selected from intron sequences flanking 5' and 3' of exon 11 of the CFTR gene. Other primers may be selected from known CFTR sequences which flank nucleotides 1778 to 1807.

According to one method of the present invention, mutations are detected by sequencing a region of exon 11 of the CFTR gene. The region includes nucleotides 1778 to 1807, which encompasses all four mutational sites taught herein. The sequences can be inspected by eye or by machine to determine if one of the mutations taught herein is present. These include an adenine at nucleotides nos. 1778, 1784, or 1807, or a thymidine residue at nucleotide no. 1789. Sequencing can be accomplished according to any means known in the art. Most simply this region of the genome can be amplified and then the sequence of the amplified region can be determined.

According to other methods of the present invention, the presence of the mutant alleles taught herein can be detected indirectly by testing for the loss or acquisition of specific restriction endonuclease sites. In particular, in the case of the Asn_{549} allele, the mutation leads to a loss of a DdeI site (CTNAG) which can be detected using methods known in the art. Similarly, the Asp_{551} allele carries a mutation which creates an MboI site (GATC) not present on the wild-type allele. Isoschizomers of DdeI and MboI can also be used. Both the

Asp551 and the Stop553 alleles carry mutations which destroy a Hinc II site (GTYRN). In addition, any other restriction enzyme having a recognition sequence including one of the nucleotides 1778, 1784, 1807 or 1789 may be used, provided that the mutation either creates or destroys the recognition site. Detection of the new or missing restrictions enzyme sites can be accomplished according to any means known in the art. For example, Southern gels of genomic DNA can be used. The genomic DNA is digested with the appropriate restriction endonuelease and separated on an electrophoretic gel matrix such as agarose or acrylamide, as is known in the art. DNA separated on the gel matrix can be then transferred to another solid support on which hybridization can occur. The transfer can be accomplished according to any means known in the art such as wicking or electroblotting. Transferred DNA can be detected by hybridization with a nucleic acid probe which spans nucleotides 1778, 1784, or 1789. The probe should extend far enough beyond nucleotides 1778, 1784, or 1789 such that it is able to hybridize to a piece of DNA which has one end at nucleotide 1778, 1784, or 1789. Further the probe preferably does not span additional sites for the restriction endonuelease being used; this simplifies the analysis but is not necessary. Alternatively, the genomic DNA can be amplified as described above and then tested for the size and number of fragments generated with DdeI, MboI, HincII or other restriction endonuclease which recognize a sequence which includes nucleotides 1778, 1784, or 1789. If enzymes are found which specifically recognize the sequences at nucleotide 1807, they may also be used to detect the Thr559 mutant allele.

According to still other methods of the present invention rapid screening techniques are used to determine whether exon 11 of the CFTR gene carries any mutations. Such techniques can be followed by one of the techniques already described above which are specific for a particular allele or mutation. One such rapid screening technique involves the determination of the conformation of single strands of DNA which have been amplified from exon 11 sequences. The single strands are run in non-denaturing electrophoretic gels, such as are typically used for sequencing DNA. The mobility of single stranded DNA on such gels is sensitive to the conformation of the DNA fragments. The conformation of the single stranded DNA is dependent on its base sequence, alterations in even one base affecting the conformation. Thus the presence of one of the CF alleles described herein can be detected by amplifying exon 11 sequences, denaturing the duplex molecules, and separating them on the basis of their conformation on non-denaturing polyacrylamide gels. If mutant alleles are present, they will have a different mobility than wild-type sequences amplified with the same primers. Most conveniently, the amplified sequences will be radiolabeled to facilitate visualization on gels. This can be readily accomplished using labeled primers or a labeled nucleotide. For a general reference on this technique see Orira, et al., Genomics vol. 5, pp. 874–879 (1989).

According to another rapid screening technique of the present invention amplified fragments containing mutations are detected using denaturing gradient gel electrophoresis (DGGE). For a general reference on this technique see Sheffield, et al., Proc. Natl. Acad. Sci. vol. 86, pp. 232–236 (1989). Briefly, double stranded fragments which are generated by amplification (PCR) can be subjected to DGGE. "DGGE is a gel system that separates DNA fragments according to their melting properties. When a DNA fragment is electrophoresed through a linearly increasing gradient of denaturants, the fragment remains double stranded until it reaches the concentration of denaturants equivalent to a melting temperature (t_m) that causes the lower-temperature melting domains of the fragment to melt. At this point, the branching of the molecule caused by partial melting sharply decreases the mobility of the fragment in the gel. The lower-temperature melting domains of DNA fragments differing by as little as a single-base substitution will melt at slightly different denaturant concentrations because of differences in stacking interactions between adjacent bases in each DNA strand. These differences in melting cause two DNA fragments to begin slowing down at different levels in the gel, resulting in their separation from each other." Sheffield, et al., ibid. Use of a GC clamp as taught in Myers et al., Nucleic Acids Res. vol. 13, pp. 3111–3146 (1985) increases the sensitivity of detection of this method from about 40% to about 100%. If mismatches are present, which would be the case if the DNA sample amplified was heterozygous for an exon 11 CFTR allele, they will be visible on these DGGE gels. Double stranded fragments containing one wild-type strand and one mutant strand will have a different mobility on these gels than will double stranded fragments which contain two wild-type or two mutant strands, due to the different melting temperatures of these species. Thus, the melting temperature of fragments amplified from exon 11 can be determined by DGGE and can indicate whether a mutant allele is present.

The following examples are not intended to limit the scope of the invention, but to illustrate various aspects of the invention.

EXAMPLE 1

This example shows the association of certain haplotypes with the ΔF_{508} and unknown CFTR mutations.

Haplotypes for four DNA markers were determined on 155 Caucasian and 43 Black CF chromosomes using three markers 5′ of the CF gene (XV2c, KMI9 and Mp69.9) and one within the gene (G2).

DNA markers XV2e and KM19 and their associated polymorphisms are described for these populations elsewhere (Cutting, et al., Am. J. Hum. Genet., vol. 44, p. 307 (1989)). Probes D9 (Mp6d.9) and G2, which detect MspI and XbaI polymorphism sites respectively, were obtained from Professor Robert Williamson (Estivill, et al., Am. J. Hum. Genet., vol. 44, p. 70 (1989); Ramsay, et al., Genomies, vol. 6, p. 39 (1990)). Direct detection of the ΔF_{508} mutation was performed by PCR amplification of genomic DNA using primers C16B and C16D followed by vacuum blotting of amplified DNA to nitrocellulose filters and hybridization with either oligo N (Normal sequence) or oligo F (deletion Phe_{508} sequence) as previously described (Kerem, et al., Science, vol. 245, p. 1073 (1989)). The results are shown in Table I below. Parentheses indicate frequency, — indicates that a polymorphism was uninformative or unknown, 1 is the absence and 2 the presence of a restriction site.

TABLE 1

	XV2c	KM19	D9	G2	Caucasian		American Black	
Haplotype	TaqI	PstI	MspI	XbaI	Unk	ΔF_{508}	Unk	ΔF_{508}
A	1	1	1	2	6	0	1	0
B	1	1	2	2	2	0	2	0
C	1	2	1	2	0	0	1	0
D	1	2	2	1	12	5	1	0
E	1	2	2	2	6	92	2	7
F	2	1	1	2	1	0	4	0
G	2	2	1	1	0	0	1	0
H	2	2	2	1	2	1	1	0
I	2	2	2	2	0	6	2	2
	—	—	—	—	6	16	12	7
					35(.23)	120(.77)	27(.63)	16(.37)

The ΔF_{508} mutation was almost exclusively associated with one haplotype (E in Table 1) and accounted for 77% of the mutations on CF chromosomes from our Caucasian patients, similar to other studies of North American Caucasians (Kerem, et al., Science, vol. 245, p. 1073 (1989); Lemna, et al., New. Engl. J. Med., vol. 322, p. 291 (1990)). In contrast, only 16 of 43 (37%) CF chromosomes from American Black patients had the ΔF_{508} mutation, confirming that racial admixture alone does not account for the incidence of CF in this group. Caucasian CF chromosomes without the ΔF_{508} mutation. i.e., unknown, occur on 6 haplotypes with 24 of 29 chromosomes having either an A, D or E haplotype (Table 1). Unknown CF mutations in the American Black patients are associated with a wider distribution of haplotypes than in Caucasians, two of which may be unique to American Black patients.

EXAMPLE 2

This example demonstrates how the four new CFTR mutations were found.

An initial panel of ten Caucasian CF patients having fourteen of the 35 unknown mutations shown in Table 1 representing each haplotype group was selected for nucleotide sequencing (Orkin, et al., Nature, vol. 296, p. 627 (1982).) All of eighteen American Black patients with twenty-seven unknown mutations were examined. Exon 11 was sequenced in these patients as part of a systematic study of regions believed to be functionally important in the CFTR protein.

Four mutations were detected in exon 11 of these patients, three cause amino acid substitutions, while the fourth produces a termination codon (FIG. 1). One mutation (A_{1784}) was found in 4% of our Caucasian CF chromosomes; the stop codon mutation (T_{1789}) occurred in 5% of our American Black CF chromosomes, while the remaining mutations (A_{1807} and A_{1778}) are rare mutations in American Black patients (Table 2). The presence of each mutation has been confirmed in at least one relative of each patient.

TABLE 2

		CF Chromosome	
Nucleotide	Amino Acid	Racial origin	Haplotypes (# of Chromosomes)*
$G_{1778}\rightarrow A$	$Ser_{549}\rightarrow Asn$	American Black	A15(1)
$G_{1784}\rightarrow A$	$Gly_{551}\rightarrow Asp$	Caucasian	D16/18(5) D03(1)
$C_{1789}\rightarrow T$	$Arg_{553}\rightarrow Stop$	American Black	I12(1) Ii06(1)
$G_{1807}\rightarrow A$	$Ala_{559}\rightarrow Thr$	American Black	F(1)

TABLE 2-continued

		CF Chromosome	
Nucleotide	Amino Acid	Racial origin	Haplotypes (# of Chromosomes)*

*Haplotype codes are created as follows: the first capitalized letter indicates the 4 site (XV2c, KM19, D9 and G2) haplotype shown in Table 1; numbers following the letter indicate the extended haplotype (7C22, MET, D7S8); i indicates an incomplete haplotype that is informative for at least three of the sites in the four site haplotype.

EXAMPLE 3

This example demonstrates how mutations A_{1778} and A_{1784} can be detected without sequencing.

PCR amplification of genomic DNA was performed as previously described (Saiki et al. Science, vol. 280, p. 1880 (1985)) using oligonucleotide primers 11i-5' (5'-CAACTGTGGTTAAAGCAATAGTGT-3') and 11i-3' (5'-GCACAGATTCTGAGTAACCATAAT-3') selected from intron sequences flanking 5' and 3' of exon 11 of the CFTR gene. Approximately 500 ng of genomic DNA extracted from peripheral lymphocytes of each subject was amplified using 2 microliters of a 10 micromolar solution of each primer described above in a total volume of 100 microliters containing 1X Taq Polymerase Buffer (50 mM KCl, mM Tris (pH 8.3), 1.5 mm $MgCl_2$, 0.01% (w/v) gelatin); 0.02 micromoles of each 2' deoxynueleotide 5' triphosphate (Pharmacia) and 2.5 units of Taq Polymerase (Cetus). Amplification was performed by 30 cycles of annealing at 58° for 30 seconds extension at 72° for 1 minute and denaturing at 94° for 30 seconds.

Amplification produced a 425 basepair fragment. Dde I digestion of DNA amplified from normal exon 11 sequence created two fragments of 174 and 251 bp whereas DNA amplified from exon 11 sequence containing the A_{1778} mutation was not cut with Dde I. DNA amplified from normal exon 11 sequence (425 bp) cannot be cut with MboI whereas digestion of DNA amplified from exon 11 sequence containing the A_{1784} mutation creates two fragments of 182 and 243 bp.

EXAMPLE 4

This example demonstrates that the three missense mutations are nor normal polymorphic variants of the CFTR gene.

In order to eliminate the possibility that the missense mutations are normal variants, non-CF chromosomes with the same haplotype as that associated with each particular mutation were analyzed by nucleotide sequencing or restriction digestion (Table 3).

TABLE 3

	DNA polymorphism haplotypes associated with each exon 11 mutation															
Haplotype*	RI	T	M	T	B	T	P	M	X	T	M	CF Chromosomes**	Caucasian	Black	Normal Chromosomes***	Caucasian	Black
Mutation $G_{1778}\rightarrow A$												$G_{1778}\rightarrow A$	Normal at 1778		$G_{1778}\rightarrow A$	Normal at 1778	
A15	1	1	1	1	2	1	1	1	2	2	1	1	0	0	0	0	0
A	—	—	—	—	—	1	1	1	2	—	—	0	5****	0	0	32	6
1c	—	—	—	—	—	—	1	1	2	—	—	0	1	4	0	0	29
c	—	—	—	—	—	—	—	1	2	—	—	0	0	3	0	8	18
Other	—	—	—	—	—	—	—	—	—	—	—	0	47	28	0	40	40
												1	53	35	0	80	93
Mutation $G_{1784}\rightarrow A$												$G_{1784}\rightarrow A$	Normal at 1784		$G_{1784}\rightarrow A$	Normal at 1784	
D16/18	1	2	2	1	2	1	2	2	1	1	—	5	0	0	0	3	0
D03	1	1	1	1	1	1	2	2	1	1	—	1	5	0	0	1	0
D	—	—	—	—	—	1	2	2	1	—	—	0	1	1	0	7	1
Other	—	—	—	—	—	—	—	—	—	—	—	0	42	35	0	16	35
												6	48	36	0	27	36
Mutation $C_{1789}\rightarrow A$												$C_{1789}\rightarrow A$	Normal at 1789		$C_{1784}\rightarrow A$	Normal at 1789	
I12	1	1	1	1	2	2	2	2	2	2	1	1	0	0	0	0	0
Ii06	1	1	1	1	2	2	2	—	2	—	—	1	0	0	0	0	0
d	—	—	—	—	—	—	—	2	2	—	—	0	6	7	0	0	0
Other	—	—	—	—	—	—	—	—	—	—	—	0	14	27	0	0	36
												2	20	34	0	0	36
Mutation $G_{1807}\rightarrow A$												$G_{1807}\rightarrow A$	Normal at 1807		$G_{1807}\rightarrow A$	Normal at 1807	
F	—	—	—	—	—	2	1	1	2	—	—	1	1	3	0	0	8
Ic	—	—	—	—	—	—	1	1	2	—	—	0	7	4	0	0	17
c	—	—	—	—	—	—	—	1	2	—	—	0	0	3	0	0	2
Other	—	—	—	—	—	—	—	—	—	—	—	0	12	25	0	0	9
												1	20	35	0	0	36

Table 3 Legends

*Sites 7C22, MET and D7S8 have been previously described (Cutting, et al., Am. J. Hum. Genet. (1989), vol. 44, p. 307); 1 indicates the absence and 2 the presence of a particular site, — indicates that the site is different or uninformative. Enzyme abbreviations — RI(EcoRI), T(TaqI) M(MspI), B(BamI), P(PstI) and X(XbaI). The relative positions of the markers are indicated in FIG. 2.

**CF chromosomes include 21 Caucasian and 9 Black chromosomes with the ΔF_{508} mutation in the $G_{1778}\rightarrow A$ and $G_{1784}\rightarrow A$ group and 6 Caucasian and 9 Black chromosomes with the ΔF_{508} mutation in the $C_{1789}\rightarrow T$ and $G_{1807}\rightarrow A$ group. Twenty Caucasian CF and 36 Black CF chromosomes were directly sequenced in each case.

***Normal Caucasian chromosomes are from parents and/or siblings of CF patients and are therefore non-CF bearing. Normal Black chromosomes are either non-CF bearing chromosomes from healthy family members or chromosomes from Black patients heterozygous for sickle cell anemia or β-thalassemia (CF carrier frequency in American Blacks is 1 in 65 persons (Cutting, et al., Am. J. Hum. Genet. (1989), vol. 44, p. 307). Normal chromosomes with 4 site haplotypes (XV2c, KM19, Mp6d.9 and G2) identical to the mutation-bearing chromosomes were examined whenever possible. However, two or three site haplotypes, which included the intragenic marker G2 and the closest 5' markers (Mp6d.9± KM19), were also employed. Screening of normal chromosomes and additional Caucasian CF chromosomes for each mutation was as follows: PCR amplification of exon 11 followed by DdeI digestion to detect the $G_{1778}\rightarrow A$ mutation or MboI digestion to detect the $G_{1784}\rightarrow A$ mutation or direct sequencing to detect either the $C_{1789}\rightarrow T$ or $G_{1807}\rightarrow A$ mutations (Table 2).

****DNA from only five of the six Caucasian patients with an unknown mutation associated with haplotype A was available.

The $G_{1778}\rightarrow A$ ($Ser_{549}\rightarrow Asn$) mutation was identified on one chromosome from a Black CF patient and was inherited from the patient's mother. This mutation causes a conservative substitution between uncharged polar amino acids. To help confirm that this mutation is deleterious, normal chromosomes with the same haplotypes were analyzed. This mutation is associated with an eleven site haplotype (A15) seen only once in 198 (43 American Black, 155 Caucasian) chromosomes. However, the four site haplotype, composed of XV2C, KM19, D9 (Mp6d.9) and G2, designated A, is not rare in either race. This mutation could not be detected on 40 Caucasian or 53 American Black normal chromosomes with at least two sites in common with the haplotype (Table 3).

The $G_{1784}\rightarrow A$ ($Gly_{551}\rightarrow Asp$) mutation was discovered on six Caucasian chromosomes, five of which have the same ten site haplotype D16/18. The sixth occurred on a Chromosome which was identical at four sites closest to the gene (DO3 haplotype in Table 3) but which differed at the more distant sites. To date, this is the second most common CF mutation in Caucasians. It is unlikely that this mutation is a protein polymorphism since it replaces a neutral with a charged amino acid. Furthermore, the mutation occurs on 4% of Caucasian CF chromosomes in our sample and has not been found on 3 normal chromosomes with the same 10 site haplotype or 24 other normal Caucasian chromosomes. In 6 or 7 Caucasian patients (including two siblings) who were found to have this mutation, it was paired with the ΔF_{508} mutation. Three of these patients, ages 11 to 13 years, have mild lung disease with normal pulmonary function test results, while the other three patients, ages 15–17 years, have moderate to severe pulmonary disease. The seventh patient with the $Gly_{551}\rightarrow Asp$ mutation, age 31 years, has an unknown mutation on his other CF chromosome and manifests mild lung disease. All of the patients except one from the sibling pair have exocrine pancreatic insufficiency requiring pancreatic enzyme supplements. The range of illness severity and small number of patients precluded a meaningful assessment of the effect of this mutation on phenotype. All patients are of Northern European ancestry representing different ethnic groups.

The nucleotide substitution C_{1789} to T ($Arg_{553}\rightarrow$ Stop) is the first nonsense mutation observed in the CFTR gene. It occurs at a CG dinucleotide, a "hotspot" for mutations, and it conforms to the CG→TG rule (Youssoufian, et al., Nature, vol. 324, p. 380 (1986); Soria, et al., Proc. Natl. Acad. Sci. USA, vol. 86, p. 587 (1989)). This mutation was found on two Black chromosomes having haplotypes identical at 8 informative sites (Table 3) suggesting a common origin of this mutation. It is unknown whether a stable truncated CFTR protein is present in vivo; however, in other disorders, nonsense mutations have been associated with unstable protein products (Adams, et al., Sem. Hematol., in press). Interestingly, one of the two patients with this nonsense mutation is a genetic compound with the $G_{1778}\rightarrow A$

(Ser$_{549}$→Asn) mutation (Patient #272 in FIG. 1). This 13 year old patient has mild disease compared to patients homozygous for the ΔF_{508} mutation.

The fourth mutation, a G_{1807} to A substitution was found on one chromosome from an American Black patient. This mutation causes a conservative change (Ala$_{559}$→Thr) and since the remainder of the CF gene has not yet been sequenced, we were not convinced that this mutation is associated with disease. Fortunately, this mutation is associated with a relatively common four site haplotype (F) in the Black population. Direct sequencing of twenty-seven chromosomes with at least two sites in common with haplotype F from American Black sickle cell or β thalassemia carriers did not reveal this mutation.

EXAMPLE 5

This example compares the sequence of CFTR in the region of the four disclosed mutations to other known proteins.

The four mutations described here occur within a thirteen amino acid segment (codons 548 to 560) of the first NBF region in the CFTR protein that is highly conserved with similar regions of other membrane-associated transport proteins (Riordan et al. Science vol. 245, pp. 1066–1073 (1989)). Five amino acids in this region are completely conserved in comparable regions from the multiple drug resistance proteins, indicating that these positions are probably crucial to protein function. It appears significant that the amino acid substitutions described in this study occur at three of the five completely conserved residues. Moreover, the substitutions occur at the three most conserved residues in that region between CFTR and fourteen other membrane associated proteins which bind A TP (shown in Riordan). The location of these mutations suggests that the CFTR protein is a member of the ATP-dependent transport protein superfamily (Riordan, supra; and Higgins, Nature, 341:103 (1989)).

We claim:

1. A nucleic acid probe which is complementary to a mutant allele of the CFTR gene said allele being selected from the group consisting of:

Asn$_{549}$, Asp$_{551}$, Stop$_{553}$, and Thr$_{559}$.

2. The probe of claim **1** which is complementary to the Asn$_{549}$ allele.

3. The probe of claim **1** which is complementary to the Thr$_{559}$ allele.

4. The probe of claim **1** which is complementary to the ASP$_{551}$ allele.

5. The probe of claim **1** which is complementary to the Stop$_{553}$ allele.

6. A method of testing a DNA sample of a human to determine if the human is a carder of Cystic Fibrosis or if the human is affected with Cystic Fibrosis, comprising:

providing a sample of DNA from a human;

testing the sample for the presence of a mutation in exon 11 of the CFTR gene, said mutation comprising a nucleotide selected from the group consisting of: an adenine at nucleotide number 1778, 1784, or 1807, and a thymidine at nucleotide 1789, the presence of the mutation indicating that the human is a carder of Cystic Fibrosis or is affected with Cystic Fibrosis.

7. The method of claim **6** wherein the step of testing comprises amplifying exon 11 of said gene in a sample of DNA from the human to form a population of amplified DNA.

8. The method of claim **7** further comprising the step determining the conformation of single strands of the amplified DNA, a conformation different from that of single strands of amplified exon 11 of wild-type CFTR allele indicating an exon 11 mutation.

9. The method of claim **7** wherein the step of amplifying is performed in the presence of radiolabeled deoxynucleotide triphosphates or radiolabeled primers to form labeled amplified DNA.

10. The method of claim **8** wherein the conformation is determined by electrophoresis on non-denaturing gels.

11. The method of claim **7** further comprising the step of;

determining the melting temperature of double strands of the amplified DNA, the presence of species of amplified DNA in the population of amplified DNA having different melting temperatures from DNA amplified from exon 11 of wild-type CFTR allele indicating a mutation in exon 11 of at least one allele of the CFTR gene in the human.

12. The method of claim **11** wherein the melting temperatures are determined by means of denaturing gradient gel electrophoresis.

13. The method of claim **6** further comprising:

contacting the human DNA sample with a nucleic acid probe complementary to a mutant allele of the CFTR gene, said allele being selected from the group consisting of: Asn$_{549}$, Asp$_{551}$, Stop$_{553}$, and Thr$_{559}$, under conditions where totally homologous sequences anneal but sequences differing in one nucleotide do not;

detecting whether the human DNA sample anneals to one of said probes, annealing to one of said probes indicating the presence of a mutant CFTR allele which can cause cystic fibrosis if no wild type allele is present in the human.

14. The method of claim **13** wherein the human DNA sample has been amplified to increase the number of copies of exon 11 of the CFTR gene.

15. The method of claim **6** further comprising:

determining the nucleotide sequence of a region of exon 11 of a CFTR allele of a human, said region comprising nucleotides 1778–1807;

inspecting the sequence to determine if there is an adenine at nucleotides number 1778, 1784, or 1807, or a thymidine at nucleotide number 1789, the presence of at least one of said nucleotides indicating a mutation in a CFTR allele which can cause cystic fibrosis if no wild-type CFTR allele is present in the human.

16. The method of claim **7** further comprising:

digesting an aliquot of the amplified exon 11 DNA with a restriction endonuclease which recognizes a sequence CTNAG which occurs at nucleotide 1778 of the wild-type CFTR allele, to form DNA fragments;

measuring the size of the amplified exon 11 DNA and the DNA fragments, DNA fragments which are the same size as the amplified exon 11 DNA indicating a mutation in a CFTR allele which can cause cystic fibrosis if no wild-type CFTR allele is present in the human.

17. The method of claim **16** wherein the endonuclease is DdeI.

18. The method of claim 7 further comprising:
digesting an aliquot of the amplified exon 11 DNA with a restriction endonuclease which recognizes a sequence GTYRAC which occurs at nucleotide 1784 of the wild-type CFTR allele, to form DNA fragments;
measuring the size of the amplified exon 11 DNA and the DNA fragments, DNA fragments which are the same size as the amplified exon 11 DNA indicating a mutation in a CFTR allele which can cause cystic fibrosis if no wild-type CFTR allele is present in the human.

19. The method of claim 18 wherein the endonuclease is HincII.

20. The method of claim 7 further comprising:
digesting an aliquot of the amplified exon 11 DNA with a restriction endonuclease which recognizes a sequence GTYRAC which occurs at nucleotide 1789 of the wild-type CFTR allele to form DNA fragments;
measuring the size of the amplified exon 11 DNA and the DNA fragments, DNA fragments which are the same size as the amplified exon 11 DNA indicating a mutation in a CFTR allele which can cause cystic fibrosis if no wild-type CFTR allele is present in the human.

21. The method of claim 20 wherein the endonuelease is HineII.

22. The method of claim 7 further comprising:
digesting an aliquot of the amplified exon 11 DNA with a restriction endonuclease which recognizes a sequence GATC which occurs at nucleotide 1784 of a mutant CFTR allele but does nor recognize the sequence in a wild-type CFTR allele, to form DNA fragments;
measuring the size of the amplified exon 11 DNA and the DNA fragments, DNA fragments which are not the same size as the amplified exon 11 DNA indicating a mutation in a CFTR allele which can cause cystic fibrosis if no wild-type CFTR allele is present in the human.

23. The method of claim 22 wherein the endonuclease is MboI.

24. The method of claim 6 further comprising:
digesting DNA of the human with a restriction endonuclease which recognizes a sequence GATC which occurs at nucleotide 1784 of a mutant CFTR allele but not of a wild-type CFTR allele:
separating the digested DNA on a gel matrix;
hybridizing the separated, digested DNA with an exon 11 probe which spans nucleotides 1784 but does not span any other sequence which the endonuclease recognizes;
detecting the DNA which hybridizes to the probe, two hybridizing fragments indicating a mutation in a CFTR allele of the human which can cause cystic fibrosis if no wild-type CFTR allele is present in the human.

25. The method of claim 24 wherein the endonuclease is MboI.

26. The method of claim 6 further comprising:
digesting DNA of the human with a restriction endonuclease which recognizes a sequence CTNAG which occurs at nucleotide 1778 of the wild-type CFTR allele;
separating the digested DNA on a gel matrix;
hybridizing the separated, digested DNA with an exon 11 probe which spans nucleotide 1778 but does not span any other sequence which the endonuclease recognizes;
detecting the DNA which hybridizes to the probe, only one hybridizing fragment indicating a mutation in a CFTR gene of the human which can cause cystic fibrosis if no wild-type CFTR allele is present in the human.

27. The method of claim 26 wherein the endonuclease is DdeI.

28. The method of claim 6 further comprising:
digesting DNA of the human with a restriction endonuclease which recognizes a sequence GTYRAC which occurs at nucleotide 1784 of the wild-type CFTR allele;
separating the digested DNA on a gel matrix;
hybridizing the separated, digested DNA with an exon 11 probe which spans nucleotide 1784 but does not span any other sequence which the endonuclease recognizes;
detecting the DNA which hybridizes to the probe, only one hybridizing fragment indicating a mutation in a CFTR gene of the human which can cause cystic fibrosis if no wild-type CFTR allele is present in the human.

29. The method of claim 28 wherein the endonuclease is HincII.

30. The method of claim 6 further comprising:
digesting DNA of the human with a restriction endonuclease which recognizes a sequence GTYRAC which occurs at nucleotide 1789 of the wild-type CFTR allele;
separating the digested DNA on a gel matrix;
hybridizing the separated, digested DNA with an exon 11 probe which spans nucleotide 1789 but does not span any other sequence which the endonuclease recognizes;
detecting the DNA which hybridizes to the probe, only one hybridizing fragment indicating a mutation in a CFTR gene of the human which can cause cystic fibrosis if no wild-type CFTR allele is present in the human.

31. The method of claim 30 wherein the endonuclease is HincII.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i i i) NUMBER OF SEQUENCES: 4

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6129 base pairs
(B) TYPE: nucleic acid

-continued

(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

(v i) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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CATTTGTGT                                                           6129
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1480 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(v i) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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            20                  25                  30

Glu Leu Ser Asp Ile Tyr Gln Ile Pro Ser Val Asp Ser Ala Asp Asn
        35                  40                  45

Leu Ser Glu Lys Leu Glu Arg Glu Trp Asp Arg Glu Leu Ala Ser Lys
    50                  55                  60
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                85                  90                  95

Val Gln Pro Leu Leu Leu Gly Arg Ile Ile Ala Ser Tyr Asp Pro Asp
            100                 105                 110

Asn Lys Glu Glu Arg Ser Ile Ala Ile Tyr Leu Gly Ile Gly Leu Cys
        115                 120                 125

Leu Leu Phe Ile Val Arg Thr Leu Leu Leu His Pro Ala Ile Phe Gly
    130                 135                 140

Leu His His Ile Gly Met Gln Met Arg Ile Ala Met Phe Ser Leu Ile
145                 150                 155                 160

Tyr Lys Lys Thr Leu Lys Leu Ser Ser Arg Val Leu Asp Lys Ile Ser
                165                 170                 175

Ile Gly Gln Leu Val Ser Leu Leu Ser Asn Asn Leu Asn Lys Phe Asp
            180                 185                 190

Glu Gly Leu Ala Leu Ala His Phe Val Trp Ile Ala Pro Leu Gln Val
        195                 200                 205

Ala Leu Leu Met Gly Leu Ile Trp Glu Leu Leu Gln Ala Ser Ala Phe
    210                 215                 220

Cys Gly Leu Gly Phe Leu Ile Val Leu Ala Leu Phe Gln Ala Gly Leu
225                 230                 235                 240

Gly Arg Met Met Met Lys Tyr Arg Asp Gln Arg Ala Gly Lys Ile Ser
                245                 250                 255

Glu Arg Leu Val Ile Thr Ser Glu Met Ile Glu Asn Ile Gln Ser Val
            260                 265                 270

Lys Ala Tyr Cys Trp Glu Glu Ala Met Glu Lys Met Ile Glu Asn Leu
        275                 280                 285

Arg Gln Thr Glu Leu Lys Leu Thr Arg Lys Ala Ala Tyr Val Arg Tyr
    290                 295                 300

Phe Asn Ser Ser Ala Phe Phe Phe Ser Gly Phe Phe Val Val Phe Leu
305                 310                 315                 320

Ser Val Leu Pro Tyr Ala Leu Ile Lys Gly Ile Ile Leu Arg Lys Ile
                325                 330                 335

Phe Thr Thr Ile Ser Phe Cys Ile Val Leu Arg Met Ala Val Thr Arg
            340                 345                 350

Gln Phe Pro Trp Ala Val Gln Thr Trp Tyr Asp Ser Leu Gly Ala Ile
        355                 360                 365

Asn Lys Ile Gln Asp Phe Leu Gln Lys Gln Glu Tyr Lys Thr Leu Glu
    370                 375                 380

Tyr Asn Leu Thr Thr Thr Glu Val Val Met Glu Asn Val Thr Ala Phe
385                 390                 395                 400

Trp Glu Glu Gly Phe Gly Glu Leu Phe Glu Lys Ala Lys Gln Asn Asn
                405                 410                 415

Asn Asn Arg Lys Thr Ser Asn Gly Asp Asp Ser Leu Phe Phe Ser Asn
            420                 425                 430

Phe Ser Leu Leu Gly Thr Pro Val Leu Lys Asp Ile Asn Phe Lys Ile
        435                 440                 445

Glu Arg Gly Gln Leu Leu Ala Val Ala Gly Ser Thr Gly Ala Gly Lys
    450                 455                 460

Thr Ser Leu Leu Met Met Ile Met Gly Glu Leu Glu Pro Ser Glu Gly
465                 470                 475                 480

Lys Ile Lys His Ser Gly Arg Ile Ser Phe Cys Ser Gln Phe Ser Trp
                485                 490                 495
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Ile Met Pro Gly Thr Ile Lys Glu Asn Ile Ile Phe Gly Val Ser Tyr
500 505 510

Asp Glu Tyr Arg Tyr Arg Ser Val Ile Lys Ala Cys Gln Leu Glu Glu
515 520 525

Asp Ile Ser Lys Phe Ala Glu Lys Asp Asn Ile Val Leu Gly Glu Gly
530 535 540

Gly Ile Thr Leu Ser Gly Gly Gln Arg Ala Arg Ile Ser Leu Ala Arg
545 550 555 560

Ala Val Tyr Lys Asp Ala Asp Leu Tyr Leu Leu Asp Ser Pro Phe Gly
565 570 575

Tyr Leu Asp Val Leu Thr Glu Lys Glu Ile Phe Glu Ser Cys Val Cys
580 585 590

Lys Leu Met Ala Asn Lys Thr Arg Ile Leu Val Thr Ser Lys Met Glu
595 600 605

His Leu Lys Lys Ala Asp Lys Ile Leu Ile Leu Asn Glu Gly Ser Ser
610 615 620

Tyr Phe Tyr Gly Thr Phe Ser Glu Leu Gln Asn Leu Gln Pro Asp Phe
625 630 635 640

Ser Ser Lys Leu Met Gly Cys Asp Ser Phe Asp Gln Phe Ser Ala Glu
645 650 655

Arg Arg Asn Ser Ile Leu Thr Glu Thr Leu His Arg Phe Ser Leu Glu
660 665 670

Gly Asp Ala Pro Val Ser Trp Thr Glu Thr Lys Lys Gln Ser Phe Lys
675 680 685

Gln Thr Gly Glu Phe Gly Glu Lys Arg Lys Asn Ser Ile Leu Asn Pro
690 695 700

Ile Asn Ser Ile Arg Lys Phe Ser Ile Val Gln Lys Thr Pro Leu Gln
705 710 715 720

Met Asn Gly Ile Glu Glu Asp Ser Asp Glu Pro Leu Glu Arg Arg Leu
725 730 735

Ser Leu Val Pro Asp Ser Glu Gln Gly Glu Ala Ile Leu Pro Arg Ile
740 745 750

Ser Val Ile Ser Thr Gly Pro Thr Leu Gln Ala Arg Arg Arg Gln Ser
755 760 765

Val Leu Asn Leu Met Thr His Ser Val Asn Gln Gly Gln Asn Ile His
770 775 780

Arg Lys Thr Thr Ala Ser Thr Arg Lys Val Ser Leu Ala Pro Gln Ala
785 790 795 800

Asn Leu Thr Glu Leu Asp Ile Tyr Ser Arg Arg Leu Ser Gln Glu Thr
805 810 815

Gly Leu Glu Ile Ser Glu Glu Ile Asn Glu Glu Asp Leu Lys Glu Cys
820 825 830

Leu Phe Asp Asp Met Glu Ser Ile Pro Ala Val Thr Thr Trp Asn Thr
835 840 845

Tyr Leu Arg Tyr Ile Thr Val His Lys Ser Leu Ile Phe Val Leu Ile
850 855 860

Trp Cys Leu Val Ile Phe Leu Ala Glu Val Ala Ala Ser Leu Val Val
865 870 875 880

Leu Trp Leu Leu Gly Asn Thr Pro Leu Gln Asp Lys Gly Asn Ser Thr
885 890 895

His Ser Arg Asn Asn Ser Tyr Ala Val Ile Ile Thr Ser Thr Ser Ser
900 905 910

Tyr Tyr Val Phe Tyr Ile Tyr Val Gly Val Ala Asp Thr Leu Leu Ala
915 920 925

Met Gly Phe Phe Arg Gly Leu Pro Leu Val His Thr Leu Ile Thr Val

-continued

930 935 940

Ser Lys Ile Leu His His Lys Met Leu His Ser Val Leu Gln Ala Pro
945 950 955 960

Met Ser Thr Leu Asn Thr Leu Lys Ala Gly Gly Ile Leu Asn Arg Phe
965 970 975

Ser Lys Asp Ile Ala Ile Leu Asp Asp Leu Leu Pro Leu Thr Ile Phe
980 985 990

Asp Phe Ile Gln Leu Leu Leu Ile Val Ile Gly Ala Ile Ala Val Val
995 1000 1005

Ala Val Leu Gln Pro Tyr Ile Phe Val Ala Thr Val Pro Val Ile Val
1010 1015 1020

Ala Phe Ile Met Leu Arg Ala Tyr Phe Leu Gln Thr Ser Gln Gln Leu
1025 1030 1035 1040

Lys Gln Leu Glu Ser Glu Gly Arg Ser Pro Ile Phe Thr His Leu Val
1045 1050 1055

Thr Ser Leu Lys Gly Leu Trp Thr Leu Arg Ala Phe Gly Arg Gln Pro
1060 1065 1070

Tyr Phe Glu Thr Leu Phe His Lys Ala Leu Asn Leu His Thr Ala Asn
1075 1080 1085

Trp Phe Leu Tyr Leu Ser Thr Leu Arg Trp Phe Gln Met Arg Ile Glu
1090 1095 1100

Met Ile Phe Val Ile Phe Phe Ile Ala Val Thr Phe Ile Ser Ile Leu
1105 1110 1115 1120

Thr Thr Gly Glu Gly Glu Gly Arg Val Gly Ile Ile Leu Thr Leu Ala
1125 1130 1135

Met Asn Ile Met Ser Thr Leu Gln Trp Ala Val Asn Ser Ser Ile Asp
1140 1145 1150

Val Asp Ser Leu Met Arg Ser Val Ser Arg Val Phe Lys Phe Ile Asp
1155 1160 1165

Met Pro Thr Glu Gly Lys Pro Thr Lys Ser Thr Lys Pro Tyr Lys Asn
1170 1175 1180

Gly Gln Leu Ser Lys Val Met Ile Ile Glu Asn Ser His Val Lys Lys
1185 1190 1195 1200

Asp Asp Ile Trp Pro Ser Gly Gly Gln Met Thr Val Lys Asp Leu Thr
1205 1210 1215

Ala Lys Tyr Thr Glu Gly Gly Asn Ala Ile Leu Glu Asn Ile Ser Phe
1220 1225 1230

Ser Ile Ser Pro Gly Gln Arg Val Gly Leu Leu Gly Arg Thr Gly Ser
1235 1240 1245

Gly Lys Ser Thr Leu Leu Ser Ala Phe Leu Arg Leu Leu Asn Thr Glu
1250 1255 1260

Gly Glu Ile Gln Ile Asp Gly Val Ser Trp Asp Ser Ile Thr Leu Gln
1265 1270 1275 1280

Gln Trp Arg Lys Ala Phe Gly Val Ile Pro Gln Lys Val Phe Ile Phe
1285 1290 1295

Ser Gly Thr Phe Arg Lys Asn Leu Asp Pro Tyr Glu Gln Trp Ser Asp
1300 1305 1310

Gln Glu Ile Trp Lys Val Ala Asp Glu Val Gly Leu Arg Ser Val Ile
1315 1320 1325

Glu Gln Phe Pro Gly Lys Leu Asp Phe Val Leu Val Asp Gly Gly Cys
1330 1335 1340

Val Leu Ser His Gly His Lys Gln Leu Met Cys Leu Ala Arg Ser Val
1345 1350 1355 1360

Leu Ser Lys Ala Lys Ile Leu Leu Leu Asp Glu Pro Ser Ala His Leu
1365 1370 1375

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Asp Pro Val Thr Tyr Gln Ile Ile Arg Arg Thr Leu Lys Gln Ala Phe
                1380                1385                1390

Ala Asp Cys Thr Val Ile Leu Cys Glu His Arg Ile Glu Ala Met Leu
            1395                1400                1405

Glu Cys Gln Gln Phe Leu Val Ile Glu Glu Asn Lys Val Arg Gln Tyr
        1410                1415                1420

Asp Ser Ile Gln Lys Leu Leu Asn Glu Arg Ser Leu Phe Arg Gln Ala
1425                1430                1435                1440

Ile Ser Pro Ser Asp Arg Val Lys Leu Phe Pro His Arg Asn Ser Ser
                1445                1450                1455

Lys Cys Lys Ser Lys Pro Gln Ile Ala Ala Leu Lys Glu Glu Thr Glu
                1460                1465                1470

Glu Glu Val Gln Asp Thr Arg Leu
            1475                1480
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: DNA (genomic)

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

(v i) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CAACTGTGGT TAAAGCAATA GTGT 24

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: DNA (genomic)

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

(v i) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GCACAGATTC TGAGTAACCA TAAT 24

32. The probe of claim **1** which comprises 18 to 22 nucleotides.

33. The method of claim **13** wherein said nucleic acid probe comprises 18 to 22 nucleotides.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,407,796
DATED : April 18, 1995
INVENTOR(S) : Garry R. Cutting, et. al.

It is certified that error appears in the above-indentified patent and that said Letters Patent is hereby corrected as shown below:

In claim 6, column 11, lines 55 and 65 please delete each occurrence of the word "carder" and insert therefor --carrier--.

Please move claims 1-31 which appear at column 11, line 41 to column 14, line 55 and insert them after the sequence listing at column 27.

Signed and Sealed this

Twelfth Day of September, 1995

Attest:

BRUCE LEHMAN

Attesting Officer

Commissioner of Patents and Trademarks