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(54) Title: Q4N2NEG2 ENHANCES CFTR ACTIVITY

(57) Abstract: Phosphorylation of the cystic fibrosis transmembrane conductance regulator (CFTR) by cyclic AMP- dependent protein kinase (PAK) is essential for opening the CFTR chloride channel. A short segment containing many negatively charged amino acids (817-838, NEG2) within the regulatory (R) domain of CFTR is a critical regulator of the chloride channel activity. An isolated NEG2 polypeptide may be expressed as a separate sequence that stimulates CFTR channel openings at lowe concentrations, but that inhibits CFTR channel openings at higher concentrations. Residues in the NEG2 sequence were substituted to produce a polypeptide that exerts only an activating effect on CFTR. One such polypeptide is the Q4N2NEG2 polypeptide. Exogenous Q4N2NEG2 exerts stimulatory effects on both wild-type and mutant G551D CFTR function, without exhibiting inhibitory activity at any concentration.

Q4N2NEG2 ENHANCES CFTR ACTIVITY

[01] This invention was made with government support under RO1 HL/DK 49003, P30 DK27651 and RO1 DK51770 awarded by the National Institute of Health. The government has certain rights in the invention

TECHNICAL FIELD OF THE INVENTION

[02] This invention is related to the field of cystic fibrosis. More particularly, it is related to the area of therapeutic treatments and drug discovery for treating cystic fibrosis.

BACKGROUND OF THE INVENTION

[03] Defects in CFTR, a chloride channel located in the apical membrane of epithelial cells, are associated with the common genetic disease, cystic fibrosis (Quinton, 1986, Welsh and Smith, 1993, Zielenski and Tsui, 1995). CFTR is a 1480 amino acid protein that is a member of the ATP binding cassette (ABC) transporter family (Riordan et al., 1989, Higgins, 1992). Each half of CFTR contains a transmembrane domain and a nucleotide binding fold (NBF), and the two halves are connected by a regulatory, or R domain. The R domain is unique to CFTR and contains several consensus PKA phosphorylation sites (Cheng et al., 1991, Picciotto et al., 1992).

Opening of the CFTR channel is controlled by PKA phosphorylation of serine residues in the R domain (Tabcharani et al., 1991, Bear et al., 1992) and ATP binding and hydrolysis at the NBFs (Anderson et al., 1991, Gunderson and Kopito, 1995). Phosphorylation adds negative charges to the R domain, and introduces global conformational changes reflected by the reduction in the α-helical content of the R domain protein (Dulhanty and Riordan, 1994). Thus, electrostatic and/or allosteric changes mediated by phosphorylation are likely to be responsible for interactions between the R domain and other CFTR domains that regulate channel function (Rich et al., 1993, Gadsby and Nairn, 1994).

[04] Rich et al., 1991 showed that deletion of amino acids 708-835 from the R domain (ΔR-CFTR), which removes most of the PKA consensus sites, renders the CFTR channel PKA independent, but the open probability of ΔR-CFTR is one-third that of

the wild type channel and does not increase upon PKA phosphorylation (Ma et al., 1997, Winter and Welsh, 1997). Thus, it is possible that deletion of the R domain removes both inhibitory and stimulatory effects conferred by the R domain on CFTR chloride channel function. This conclusion is supported by studies that show that addition of exogenous unphosphorylated R domain protein (amino acids 588-858) to wt-CFTR blocks the chloride channel (Ma et al., 1996), suggesting that the unphosphorylated R domain is inhibitory. Conversely, exogenous phosphorylated R domain protein (amino acids 588-855 or 645-834) stimulated the ΔR-CFTR channel, suggesting that the phosphorylated R domain is stimulatory (Ma et al., 1997, Winter and Welsh, 1997). Therefore, it appears that the manifest activity (stimulatory or inhibitory) depends on the phosphorylation state of the R domain.

[05] About 25% of the known 700 mutations in CFTR produce a mutant CFTR protein which is properly transported to the apical membrane of epithelial cells but have only low level, residual channel activity. There is a need in the art for agents which can boost the level of channel activity in those mutants having low level activity.

SUMMARY OF THE INVENTION

[06] These and other objects of the invention are achieved by providing one or more of the embodiments described below. In one embodiment of the invention an isolated polypeptide is provided. The polypeptide comprises an amino acid sequence of SEQ ID NO: 6 wherein the polypeptide retains a net negative charge of 1-8. More preferably the variant of said CFTR protein has the sequence of SEQ ID NO: 1.

[07] In another embodiment of the invention a method is provided for activating a CFTR protein. An effective amount of the polypeptide is administered to a cell comprising a CFTR protein that forms a cAMP regulated chloride channel. The polypeptide comprises the sequence of SEQ ID NO: 6. The CFTR protein is consequently activated. More preferably, the polypeptide has the sequence of SEQ ID NO: 1.

[08] According to another embodiment of the invention a method is provided for activating a CFTR protein. An effective amount of a polypeptide is contacted with a CFTR protein in a lipid bilayer wherein the polypeptide comprises the amino acid sequence

of SEQ ID NO: 6. The CFTR protein is thereby activated. More preferably, the polypeptide comprises the amino acid sequence of SEQ ID NO: 1.

[09] In another embodiment of the invention a method is provided for synthesizing a CFTR-related polypeptide. Units of one or more amino acid residues are linked to form a polypeptide comprising the amino acid sequence of SEQ ID NO: 6. More preferably, the polypeptide has the sequence of SEQ ID NO: 1.

[10] In another embodiment of the invention an isolated polypeptide is provided. The polypeptide comprises the amino acid sequence of SEQ ID NO: 2.

[11] In yet another embodiment of the invention a nucleic acid molecule is provided. The nucleic acid comprises a nucleotide sequence encoding a polypeptide according to SEQ ID NO: 2.

[12] In another embodiment of the invention a method of activating a CFTR protein is provided. A nucleic acid comprising a sequence encoding a polypeptide according to SEQ ID NO: 2 is administered to a cell comprising the CFTR protein, whereby the polypeptide is expressed and the CFTR protein is activated.

[13] These and other embodiments of the invention, which will be apparent to those of skill in the art, provide the art with reagents and tools for enhancing function of cAMP regulated chloride channels that are defective in cystic fibrosis patients.

BRIEF DESCRIPTION OF THE DRAWINGS

[14] Figure. 1A and 1B and 1C: Demonstration of increase in open probability of CFTR channel with addition of the Q4 N2 NEG2 peptide.

[15] (Figure 1A) Single channel trace of the CFTR channel before addition of peptide.

[16] (Figure 1B) Single channel trace after addition of Q4 N2 NEG2 peptide (4 μM).

[17] (Figure 1C) Summary of five separate experiments. Addition of Q4N2 NEG2 peptide increases the Po by about two-fold.

DETAILED DESCRIPTION OF THE INVENTION

[18] It is a discovery of the present inventors that the channel inhibitory properties of the R domain of CFTR protein can be separated from the channel activating properties. Thus activating polypeptides can be used to treat CFTR defective cells, without concern for inhibition at certain concentrations. Activating polypeptides may also be used to enhance the activity of normal CFTR, including that delivered by gene transfer.

[19] A polypeptide for use in treating CFTR-defective cells contains a 22 amino acid sequence, GLXISXXINXXXLKXXFFXXXX, as shown in SEQ ID NO: 6. The amino terminal residue is acetylated and the carboxy terminal residue is amidated. The residue X, at positions 3, 6, 7, 10, and 11 is either glutamic acid or glutamine; at position 12 is aspartic acid or asparagine; at position 15 is glutamic acid or glutamine; at position 16 is cysteine or serine; at positions 19 or 20 is aspartic acid or asparagine; at position 21 is methionine or norleucine; at position 22 is either glutamic acid or glutamine. The amino acid residue at position 16 is more preferably serine. The amino residue at position 21 is more preferable norleucine. The polypeptide of SEQ ID NO: 6 has a net negative charge. The net negative charge is preferably within the ranges of 1-8, 2-8, 3-8, 4-8, 5-8, 6-8, or 7-8.

[20] The polypeptide more preferably has the sequence of SEQ ID NO: 1, GLEISEQINQQNLKQSFFNDLE, wherein L at position 21 is norleucine. The amino terminal residue of the polypeptide is preferably acetylated and the carboxy terminal residue is preferably amidated.

[21] The polypeptide may also be present in a composition with a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers are well known to those in the art. Pharmaceutically acceptable carriers include, but are not limited to, large, slowly metabolized macromolecules, such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and inactive virus particles. The composition can also contain liquids, such as water, saline, glycerol, and ethanol, as well as substances such as wetting agents,

emulsifying agents, or pH buffering agents. Buffering agents include Hanks' solution, Ringer's solution, or physiologically buffered saline.

[22] It may be desirable that the polypeptide be fused to another polypeptide to provide additional functional properties. For example, fusion to another protein such as keyhole limpet hemocyanin can be used to increase immunogenicity. Another desirable fusion partner is a membrane-penetrating peptide. Such peptides include VP-22 (SEQ ID NO: 3), as well as the peptides shown in SEQ ID NO: 4 and SEQ ID NO: 5. Such peptides can be used to facilitate the uptake of the polypeptide by target cells. The polypeptides of the invention may also be fused to proteins that cause specific targeting to lung epithelial cells. For instance, the peptide THALWHT directs DNA to human airway epithelial cells. Single chain antibody variable domains may be used to do the same.

[23] A CFTR protein can be activated by the polypeptide. The CFTR protein can be in a cell, preferably in the cell membrane and the CFTR protein forms a cAMP-regulated chloride channel. An effective amount of a polypeptide that comprises the sequence of SEQ ID NO: 6 can be administered to the cell, and administration of the polypeptide activates the CFTR protein. The polypeptide administered more preferably comprises the sequence of SEQ ID NO: 1.

[24] The cells may be any cells that contain or express a CFTR protein. The cells may naturally express the CFTR protein, such as lung epithelial cells, or the cells may express the CFTR protein after transient or stable transformation. The cells may be primary cells isolated from individuals that express a wild-type CFTR protein, or may be primary cells isolated from individuals that express a mutant CFTR protein. The cells may also be of a stable cell line. The cells may also exist in the body.

[25] The CFTR protein is a wild type or a mutant CFTR protein. The mutant CFTR protein is a CFTR protein that is expressed by the cells and that is transported to the cell surface. The mutant CFTR protein also forms a cAMP-regulated chloride channel. The mutant CFTR protein may contain alterations that are known and characterized, or may contain alterations that have not yet been discovered. A mutant CFTR protein that fails to undergo full activation is a CFTR protein that does not

conduct ions to the same degree as wild-type CFTR. The mutant CFTR protein may not conduct ions at all. The mutant protein may also conduct ions to a similar extent as wild type CFTR but be present in the membrane in substantially lower amounts than is true for normal individuals.

[26] Activated is defined as any increase in conductance by the CFTR protein. An increase in conductance may result when the opening of the CFTR channel occurs with greater frequency than previously observed. An increase in CFTR conductance may result when the duration of opening is increased each time the CFTR channel opens. An increase in conductance may also result due to greater ability to conduct ions each time the CFTR protein channel is open. The increase in open probability of the CFTR protein is preferably at least 25%, at least 50%, at least 75%, at least 100%, at least 125%, at least 150%, at least 175%, at least 200%, or at least 300%.

[27] An effective amount is any amount of polypeptide that is sufficient to activate the CFTR protein, as activate is defined above. Preferably, the polypeptide is administered to achieve a concentration of 0.5 to 14 μM. More preferably, the polypeptide is administered to achieve a concentration of 4-6 μM.

[28] The polypeptide may be administered by any means acceptable in the art. For instance, the polypeptide may be administered *in vitro*, or to cells in culture, by addition to the medium. The polypeptide may be administered *in vivo*, to a patient, by any route including intravenous, intrathecal, oral, intranasal, transdermal, subcutaneous, intraperitoneal, parenteral, topical, sublingual, or rectal. Most preferably, the polypeptide is administered to a patient in an aerosol.

[29] The aerosolized polypeptide can be co-administered with an expression vector that encodes wild type CFTR protein. An expression vector may be linear DNA that encodes wild type CFTR protein, or a plasmid or human artificial chromosome that expresses wild type CFTR protein. The vector may be administered as naked DNA or may be administered complexed to lipid molecules such as with liposomes, short polypeptides such as the THALWHT polypeptide, or polycations such as polylysine, with or without stabilizing agents and/or receptor ligands. The DNA may also be administered in a viral vector. Viral vectors are known in the art. Several nonlimiting

examples include retroviruses, adenoviruses, adeno-associated viruses, lentiviruses, and herpes simplex virus. The gene encoding the wild type CFTR protein may additionally comprise a promoter sequence to drive expression of the CFTR gene. Any promoter known in the art may be used. Promoters include strong promoters such as the promoters of cytomegalovirus, SV40, or Rous sarcoma virus. The promoter may also be a tissue specific promoter. Preferably the tissue specific promoter is a lung specific promoter. Lung specific promoters include the promoters of surfactant protein A, keratin 18, Du Clara cell secretory protein, and the promoter of CFTR.

[30] A CFTR protein can also be activated by applying an effective amount of a polypeptide to a CFTR protein in a lipid bilayer. The polypeptide comprises the amino acid sequence of SEQ ID NO: 6. The polypeptide more preferably comprises the amino acid sequence of SEQ ID NO: 1. Activating a CFTR protein in a lipid bilayer is useful to the art for screening agents for the treatment of cystic fibrosis.

[31] A CFTR protein in a lipid bilayer may be a CFTR protein that is expressed in cells in culture. The cells may express the CFTR protein without manipulation, or may be stably or transiently transfected to express the CFTR protein. The lipid bilayer may also be such artificial preparations as, without limitation, a microsome preparation, a lipid-bilayer vesicle preparation, or liposomes. The polypeptide may be applied to the protein by its addition to cell culture media, or solution in which the lipid bilayers are maintained. A change in conductance may be measured by any means known in the art, such as patch clamping.

[32] A CFTR activating polypeptide can be synthesized by sequentially linking units of one or more amino acid residues to form a polypeptide comprising the amino acid sequence of SEQ ID NO: 6. Preferably the polypeptide has the amino acid sequence of SEQ ID NO: 1. Synthesis of the CFTR polypeptide can be performed using solid-phase synthesis, liquid-phase synthesis, semisynthesis, or enzymatic synthesis techniques. Preferably the polypeptides are synthesized by solid-phase synthesis. More preferably the peptides are synthesized by F-moc synthesis.

[33] The polypeptide of the invention may alternatively comprise the sequence of SEQ ID NO: 2, GLEISEQINQQNLKQSFFNDME. The polypeptide of SEQ ID NO: 2 is not modified. It is similar to the sequence of SEQ ID NO: 1, but for a methionine at position 21, rather than a norleucine. Like SEQ ID NO: 1 and SEQ ID NO: 6, it may be fused to a membrane penetrating polypeptide.

[34] Nucleic acid molecules comprise a nucleotide sequence that encodes the polynucleotide sequence of SEQ ID NO: 2. One of skill in the art will recognize that many sequences will encode the polypeptide, as more than one codon can specify a given amino acid. The nucleic acid may further comprise regulatory sequences that enhance the expression of the polypeptide. Promoters may be strong constitutive promoters, as discussed above, or may be tissue-specific promoters. Preferably the tissue-specific promoter is a lung-specific promoter. The nucleic acid molecules may further comprise a vector. The vector can be any suitable vector for the delivery of the polynucleotide sequence into the lungs of a patient, resulting in expression of the polypeptide in the lungs of the patient.

[35] A CFTR protein can be activated by expression of a polynucleotide. A nucleic acid comprising a sequence encoding a polypeptide according to SEQ ID NO: 2 is administered to a cell comprising the CFTR protein. The polypeptide is expressed and the CFTR protein is thereby activated. The polynucleotide may be administered by any acceptable means in the art. Preferably the polynucleotide is administered as an aerosol.

[36] The administration of the polypeptides of the present invention are most useful in treatment of a class of mutations that encode CFTR proteins that are properly delivered to the plasma membrane but that are residually or minimally active. Minimally or residually active CFTR proteins have the ability to mediate or modulate channel conductance. However, channel conductance is insufficient to sustain the healthy, not cystic fibrotic phenotype. Residually or minimally active includes proteins for which the activity of the CFTR can be recorded but may be at a level that is barely detectable. This invention will also be useful for CFTR mutants that are, to a large extent, misprocessed and thus reach the plasma membrane in much lower quantities than normally processed CFTR, and for CFTR mutants that are, to a large

extent, improperly spliced, but retain production of some properly spliced CFTR. Known mutants of CFTR are listed in Table 1. In addition to its utility in the activation of mutant forms of CFTR, this invention will be a useful adjunct to gene therapy for cystic fibrosis. By enhancing the per-CFTR molecule chloride transport activity, this peptide will increase the chloride transport activity obtained at any level of expression of CFTR, thereby increasing its effective efficacy.

Table 1

Name	Nucleotide_change	Exon	Consequence	Reference
-816C->T	C to T at -816	5' flanking	promoter mutation?	Bienvenu et al. (NL#60)
-741T->G	T to G at -741	5' flanking	promoter mutation?	Bienvenu et al. (NL#59)
-471delAGG	deletion of AGG from -471	5' flanking	promoter mutation?	Grade et al. 1994
-363C/T	C to T at -363	5' flanking	promoter mutation	Zielenski et al. 1999*
-102T->A	T to A at -102	5' flanking	regulatory mutation?	Claustres et al. (NL#69)
-94G->T	G to T at -94	5' flanking	promoter mutation?	Claustres et al. (NL#70)
-33G->A	G to A at -33	5' flanking	promoter mutation?	Claustres et al (NL#67)
132C->G	C to G at 132	1	altered translation initiation?	Claustres et al (NL#67)
P5L	C to T at 146	1	Pro to Leu at codon 5	Chillón et al. (NL#59)
S10R	C to A at 160	1	Ser to Arg at codon 10	Hughes et al. (NL # 65)
S13F	C to T at 170	1	Ser to Phe at 13	Cao et al. (NL#69)
185+1G->T	G to T at 185+1	intron 1	mRNA splicing defect	Férec 1998*
185+4A->T	A to T at 185+4	intron 1	mRNA splicing defect? (CBAVD)	Culard et al. 1994
186-13C->G	C to G at 186-13	intron 1	mRNA splicing defect?	Férec et al. (NL#50)
W19C	G to T at 189	2	Trp to Cys at 19	Macek et al. (NL#62)
G27E	G to A at 212	2	Gly to Glu at 27	Bienvenu et al. 1994a
R31C	C to T at 223	2	Arg to Cys at 31	Costes et al. (NL #56)
R31L	G to T at 224	2	Arg to Leu at 31	Zielenski et al. 1995
232del18	Deletion of 18 bp from 232	2	Deletion of 6 aa from Leu34 to Gln39	Faucz et al. (NL#69)
S42F	C to T at 257	2	Ser to Phe at 42	Férec et al. 1995
D44G	A to G at 263	2	Asp to Gly at 44	Fanen et al. 1992

Name	Nucleotide_change	Exon	Consequence	Reference
A46D	C to A at 269	2	Ala to Asp at 46	Andoniadi et al. (NL#64)
279A/G	A to G at 279	2	No change (Leu at 49)	Bienvenu et al. (NL#69)
I50T	T to C at 280	2	Ile to Thr at codon 50	Casals et al. (NL #65)
S50P	T to C at 280	2	Ser to Pro at 50	Casals et al. (NL#65)
S50Y	C to A at 281	2	Ser to Tyr at 50 (CBAVD)	Zielenski et al. (NL#63)
296+3insT	insertion of T after 296+3	intron 2	mRNA splicing defect?	Casals et al. 1998*
296+1G->T	G to T at 296+1	intron 2	missense; mRNA splicing defect?	Walker et al. 2000*
296+1G->C	G to C at 296+1	intron 2	mRNA splicing defect	Tzetis et al. (NL#64)
296+2T->C	T to C at 296+2	intron 2	mRNA splicing defect	Férec et al. (NL#63)
296+9A->T	A to T at 296+9	intron 2	mRNA splicing defect?	Zielenski et al. (NL#68)
296+12T->C	T to C at 296+12	intron 2	mRNA splicing defect?	Cuppens et al. (NL#53)
297-28insA	insertion of A after 297-28	intron 2	mRNA splicing defect?	Scheffer & Dijkstra (NL#60)
297-3C->A	C to A at 297-3	intron 2	mRNA splicing defect?	Zielenski et al. (NL#70)
297-3C->T	C to T at 297-3	intron 2	mRNA splicing defect?	Bienvenu et al. (NL#55)
297-2A->G	A to G at 297-2	intron 2	mRNA splicing defect	Schwarz et al (NL#67)
297-10T->G	C to G at 297-10	intron 2	splice mutation?	Zielenski et al. 1999*
297-12insA	insertion of A at 297-12	intron 3	splice mutation?	Girodon et al. 1999*
E56K	G to A at 298	3	Glu to Lys at 56	Dörk et al. (NL#69)
W57G	T to G at 301	3	Trp to Gly at 57	Ferrari et al. (NL#47)
W57R	T to C at 301	3	Trp to Arg at 57	Malone et al. (NL#69)
D58N	G to A at 304	3	Asp to Asn at 58	Dörk et al. (NL#69)
D58G	A to G at 305	3	Asp to Gly at 58	Claustres et al. 2000*
E60K	G to A at 310	3	Glu to Lys at 60	Claustres et al. 2000*
E60L	G to A at 310	3	Glu to Leu at 60	Casals et al. 2000*
N66S	A to G at 328	3	Asn to Ser at 66	Cashman et al. (NL#55)
P67L	C to T at 332	3	Pro to Leu at 67	Hamosh et al. (NL#54)

Name	Nucleotide_change	Exon	Consequence	Reference
K68E	A to G at 334	3	Lys to Glu at 68	Kilinc et al. (NL#70)
K68N	A to T at 336	3	Lys to Asn at 68	Dörk & Tümmler (NL#48)
A72T	G to A at 346	3	Ala to Thr at 72	Pacheco et al. 1999*
A72D	C to A at 347	3	Ala to Asp at 72	Le Gall et al. (NL#68)
R74W	C to T at 352	3	Arg to Trp at 74	Claustres et al. 1993
R74Q	G to A at 353	3	Arg to Gln at 74	Malone et al. 2000*
R75L	G to T at 356	3	Arg to Leu at 75	Costes et al. (NL#55)
W79R	T to C at 367	3	Trp to Arg at 79	Macek et al. (NL#56)
G85E	G to A at 386	3	Gly to Glu at 85	Zielenski et al. 1991b
G85V	G to T at 386	3	Gly to Val at 85	Casals et al. (NL#67)
F87L	T to C at 391	3	Phe to Leu at 87	Bienvenu et al. 1994c
L88S	T to C at 395	3	Leu to Ser at 88	Malone et al. (NL#51)
Y89C	A to G at 398	3	Tyr to Cys at 89	Seia et al. 1999*
L90S	T to C at 401	3	Leu to Ser at 90	Férec 1998*
G91R	G to A at 403	3	Gly to Arg at 91	Guillermit et al. 1993
405+1G->A	G to A at 405+1	intron 3	mRNA splicing defect	Dörk et al. 1993e
405+3A->C	A to C at 405+3	intron 3	mRNA splicing defect?	Hamosh et al. (NL#54)
405+4A->G	A to G at 405+4	intron 3	mRNA splicing defect?	Ghanem et al. 1994
406-10C->G	C to G at 406-10	intron 3	mRNA splicing defect?	Greil et al. (NL#55)
406-6T->C	T to C at 406-6	intron 3	mRNA splicing defect?	Claustres et al. 1993
406-3T->C	T to C at 406-3	intron 3	mRNA splicing defect?	Kilinc et al. (NL#70)
406-2A->G	A to G at 406-2	intron 3	mRNA splicing defect	Dörk et al. (NL#69)
406-2A->C	A to C at 406-2	intron 3	mRNA splicing defect	Costes et al. (NL#60)
406-1G->C	G to C at 406-1	intron 3	mRNA splicing defect	Bonizzato et al. 1992
406-1G->A	G to A at 406-1	intron 3	mRNA splicing defect	Wang et al. 1998*
406-1G->T	G to T at 406-1	intron 3	mRNA splicing defect	Bienvenu et al. (NL #55)
E92K	G to A at 406	4	Glu to Lys at 92	Nunes et al. 1993
A96E	C to A at 419	4	Ala to Glu at 96	Férec 1998*

Name	Nucleotide_change	Exon	Consequence	Reference
Q98R	A to G at 425	4	Gln to Arg at 98	Romey et al. 1995
P99L	C to T at 428	4	Pro to Leu at 99	Schwartz & Holmberg(NL#50)
I105N	T to A at 446	4	Ile to Asn at 105	Claustres et al. 2000*
S108F	C to T at 455	4	Ser to Phe at 108	Seydewitz et al. 1995
Y109N	T to A at 457	4	Tyr to Asn at 109	Schaedel et al. 1998*
Y109C	A to G at 458	4	Tyr to Cys at 109	Schaedel et al. 1994
D110H	G to C at 460	4	Asp to His at 110	Dean et al. 1990
D110Y	G to T at 460	4	Asp to Tyr at 110	Casals et al. 2000*
D110E	C to A at 462	4	Asp to Glu at 110	Seia et al. 1999*
P111A	C to G at 463	4	Pro to Ala at 111	Férec et al. (NL#69)
P111L	C to T at 464	4	Pro to Leu at 111	Claustres et al. (NL#62)
delta E115	3 bp deletion of 475-477	4	deletion of Glu at 115	Chillón et al. 1995 (NL#61)
E116Q	G to C at 478	4	Glu to Gln at 116	Walker et al. 2000*
E116K	G to A at 478	4	Glu to Lys at 116	Costes et al. (NL#60)
R117C	C to T at 481	4	Arg to Cys at 117	Dörk et al. 1994b
R117H	G to A at 482	4	Arg to His at 117	Dean et al. 1990
R117P	G to C at 482	4	Arg to Pro at 117	Feldmann et a. (NL#64)
R117L	G to T at 482	4	Arg to Leu at 117	Férec et al. 1995
A120T	G to A at 490	4	Ala to Thr at 120	Chillón et al. 1994
I125T	T to C at 506	4	Ile to Thr at 125	Mittre (NL#70)
G126D	G to A at 509	4	Gly to Asp at 126	Wagner et al. 1994
L137R	T to G at 542	4	Leu to Arg at 137	Chevalier-Porst & Bozon (NL#70)
L137H	T to A at 542	4	Leu to His at 137	Wallace (NL#69)
L138ins	insertion of CTA, TAC or ACT at nucleotide 544, 545 or 546	4	insertion of leucine at 138	Dörk et al. (NL#69)
H139R	A to G at 548	4	His to Arg at 139	Férec et al. 1995
P140S	C to T at 550	4	Pro to Ser at 140	Férec et al. (NL#61)

Name	Nucleotide_change	Exon	Consequence	Reference
P140L	C to T at 551	4	Pro to Leu at 140	Tzetis et al. (NL#70)
A141D	C to A at 554	4	Ala to Asp at 141	Gouya et al. (NL#65)
H146R	A to G at 569	4	His to Arg at 146 (CBAVD)	Bienvenu et al. (NL#68)
I148T	T to C at 575	4	Ile to Thr at 148	Bozon et al. 1994
I148N	T to A at 575	4	Ile to Asn at 148	Casals et al. (NL#69)
G149R	G to A at 577	4	Gly to Arg at 149	Mercier et al. 1995
M152V	A to G at 586	4	Met to Val at 152 (mutation?)	Edkins & Creegan (NL#54)
M152R	T to G at 587	4	Met to Arg at 152	Yoshimura 1998*
591del18	deletion of 18 bp from 591	4	deletion of 6 a.a. from	Varon & Reis (NL#64)
A155P	G to C at 595	4	Ala to Pro at 155	Zielenski et al. (NL#70)
S158R	A to C at 604	4	Ser to Arg at 158	Girodon et al. 1999*
Y161N	T to A at 613	4	Tyr to Asn at 161	Claustres et al. 2000*
Y161D	T to G at 613	4	Tyr to Asp at 161	Zielenski et al. 1999*
Y161S	A to C at 614 (together with 612T/A)	4	Tyr to Ser at 161	Andrew et al. 1999*
K162E	A to G at 616	4	Lys to Glu at 162	Tzetis et al. (NL#70)
621G->A	G to A at 621	4	mRNA splicing defect	Mackova et al. (NL#64)
621+1G->T	G to T at 621+1	intron 4	mRNA splicing defect	Zielenski et al. 1991b
621+2T->C	T to C at 621+2	intron 4	mRNA splicing defect	Schwarz et al. (NL#66)
621+2T->G	T to G at 621+2	intron 4	mRNA splicing defect	Claustres et al. 1993
621+3A->G	A to G at 621+3	intron 4	mRNA splicing defect	Tzetis et al. (NL#70)
622-2A->C	A to C at 622-2	intron 4	mRNA splicing defect	Cuppens et al. 1993
622-1G->A	G to A at 622-1	intron 4	mRNA splicing defect	Zielenski et al. (NL#66)
L165S	T to C at 626	5	Leu to Ser at 165	Férec et al. (NL#51)
K166Q	A to G at 628	5	Lys to Gln at 166	Macek et al. (NL#62;#66)
R170C	C to T at 640	5	Arg to Cys at 170	Férec et al. (NL#62)
R170G	C to G at 640	5	Arg to Gly at 170	Claustres et al. (NL# 49)

Name	Nucleotide_change	Exon	Consequence	Reference
R170H	G to A at 641	5	Arg to His at 170	Brownsell et al. 2001*
I175V	A to G at 655	5	Ile to Val at 175	Romey et al. 1994a
I177T	T to C at 662	5	Ile to Thr at 177	Bienvenu et al. (NL#68)
G178R	G to A at 664	5	Gly to Arg at 178	Zielenski et al. 1991b
Q179K	C to A at 667	5	Gln to Lys at 179	Zhang & Wong 2000*
N186K	C to A at 690	5	Asn to Lys at 186	Claustres & Carles (NL#70)
N187K	C to A at 693	5	Asn to Lys at 187	Arduino et al. 1998*
D192N	G to A at 706	5	Asp to Asn at 192	Costes et al. (NL#62)
delta D192	deletion of TGA or GAT from 706 or 707	5	deletion of Asp at 192	Feldmann et al. (NL#66)
D192G	A to G at 707	5	Asp to Gly at 192	Audrézet et al. 1994
E193K	G to A at 709	5	Glu to Lys at 193	Ferrari et al. (NL#62); et al. Mercier et al. 1995
711+1G->T	G to T at 711+1	intron 5	mRNA splicing defect	Zielenski et al. 1991b
711+3A->C	A to C at 711+3	intron 5	mRNA splicing defect	Macek MJr et al. (NL#61)
711+3A->G	A to G at 711+3	intron 5	mRNA splicing defect	Petreska et al. 1994
711+3A->T	A to T at 711+3	intron 5	mRNA splicing defect?	Casasl et al. (NL#67)
711+5G->A	G to A at 711+5	intron 5	mRNA splicing defect	Bisceglia et al. 1994
711+34A->G	A to G at 711+34	intron 5	mRNA splicing defect?	Tzetis et al. (NL#68)
712-1G->T	G to T at 712-1	intron 5	mRNA splicing defect	Chillón et al. (NL#59)
G194V	G to T at 713	6a	Gly to Val at 194	Férec 1998*
A198P	G to C at 724	6a	Ala to Pro at 198	Walker et al. 1999*
H199Y	C to T at 727	6a	His to Tyr at 199	Dörk & Tümmler (NL#45)
H199Q	T to G at 729	6a	His to Gln at 199	Dean et al. (NL#28)
V201M	G to A at 733	6a	Val to Met at 201	Férec 1998*
P205S	C to T at 745	6a	Pro to Ser at 205	Chillón et al. 1993b
L206W	T to G at 749	6a	Leu to Trp at 206	Claustres et al. 1993
L206F	G to T at 750	6a	Leu to Phe at 206	Férec et al. (NL#69)

Name	Nucleotide_change	Exon	Consequence	Reference
A209S	G to T at 757	6a	Ala to Ser at 209	Férec 1998*
E217G	A to G at 782	6a	Glu to Gly at 217	Zielenski et al. (NL#70)
Q220R	A to G at 791	6a	Gln to Arg at 220	Férec 1998*
C225R	T to C at 805	6a	Cys to Arg at 225	Fanen et al. 1992
L227R	T to G at 812	6a	Leu to Arg at 227	Ghanem et al. (NL#59)
V232D	T to A at 827	6a	Val to Asp at 232 (CBAVD)	Costes et al. (NL#60)
Q237E	C to G at 841	6a	Gln to Glu at 237	Costes et al. (NL#62)
G239R	G to A at 847	6a	Gly to Arg at 239	Zielenski et al. (NL#60)
G241R	G to A at 852	6a	Gly to Arg at 241	Férec et al. (NL#69)
M243L	A to C at 859	6a	Met to Leu at 243 (ATG to CTG)	Yoshimura 1999*
M244K	T to A at 863	6a	Met to Lys at 244	Claustres et al. (NL#64)
R248T	G to C at 875	6a	Arg to Thr at 248 (CBAVD)	Scheffer et al. (NL#70)
875+1G->C	G to C at 875+1	intron 6a	mRNA splicing defect	Zielenski et al. (NL#58)
875+1G->A	G to A at 875+1	intron 6a	mRNA splicing defect	Duarte et al. (NL#63)
876-14del12	deletion of 12 bp from 876-14	intron 6a	mRNA splicing defect?	Audrézet et al. 1993a
876-10del8	deletion of 8 bp from 876-10	intron 6a	mRNA splicing defect?	Costes et al. (NL#46,47)
876-3C->T	C to T at 876-3	intron 6a	splicing mutation?	Chevalier-Porst & Bozon 1999*
R258G	G to A at 904	6b	Arg to Gly at 258	Mercier et al. 1995
V920L	G to T at 289	15	Val to Leu at 920	Girodon et al. 1999*
M265R	T to G at 926	6b	Met to Arg at 265	Schwarz et al. (NL#65)
E278del	deletion of AAG from 965	6b	deletion of Glu at 278	Casals et al. (NL#70)
N287Y	A to T at 991	6b	Asn to Tyr at 287	Shrimpton & Borowitz (NL#69)

Name	Nucleotide_change	Exon	Consequence	Reference
994del9	deletion of TTAAGACAG from 994	6b	mRNA splicing defect	Zielenski et al. (NL#70)
1002-3T->G	T to G at 1002-3	intron 6b	mRNA splicing defect	Mackova et al. (NL#64)
E292K	G to A at 1006	7	Glu to Lys at 292	Bienvenu et al. (NL#68)
R297W	C to T at 1021	7	Arg to Trp at 297	Dörk et al. (NL#69)
R297Q	G to A at 1022	7	Arg to Gln at 297	Graham et al. 1991
A299T	G to A at 1027	7	Ala to Thr at 299	Férec 1999*
Y301C	A to G at 1034	7	Tyr to Cys at 301	Constantinou-Deltas (NL#58)
S307N	G to A at 1052	7	Ser to Asn at 307	Onay & Kirdar (NL#70)
A309D	C to A at 1058	7	Ala to Asp at 309	Ferrari et al. (NL#64)
A309G	C to G at 1058	7	Ala to Gly at 309	Bienvenu et al. (NL#68)
delta F311	deletion of 3 bp between 1059 and 1069	7	deletion of Phe310, 311 or 312	Meitinger et al. 1993
F311L	C to G at 1065	7	Phe to Leu at 311	Férec et al. 1992
G314R	G to C at 1072	7	Gly to Arg at 314	Nasr et al. (NL#56)
G314V	G to T at 1073	7	Gly to Val at 324	Chevalier-Porst & Bozon (NL#70)
G314E	G to A at 1073	7	Gly to Glu at 314	Golla et al. 1994
F316L	T to G at 1077	7	Phe to Leu at 316	Férec 2000*
V317A	T to C at 1082	7	Val to Ala at 317	Férec et al. (NL#55)
L320V	T to G at 1090	7	Leu to Val at 320 CAVD	Bienvenu et al (NL#67)
L320F	A to T at 1092	7	Leu to Phe at 320	Macek et al. (NL#64)
V322A	T to C at 1097	7	Val to Ala at 322 (mutation?)	Férec et al. (NL#63)
L327R	T to G at 1112	7	Leu to Arg at 327	Ravnik-Glavac et al. (NL#53)
R334W	C to T at 1132	7	Arg to Trp at 334	Estivill et al. 1991
R334L	G to T at 1133	7	Arg to Leu at 334	Dörk et al. (NL#69)

Name	Nucleotide_change	Exon	Consequence	Reference
R334Q	G to A at 1133	7	Arg to Gln at 334	Férec et al. (NL#65)
I336K	T to A at 1139	7	Ile to Lys at 336	Cuppens et al. 1993
T338I	C to T at 1145	7	Thr to Ile at 338	Saba et al. 1993
E474K	G to A at 1152	10	Glu to Lys at 474	Girodon et al. 1999*
L346P	T to C at 1169	7	Leu to Pro at 346	Constantinou (NL #58)
R347C	C to T at 1171	7	Arg to Cys at 347	Férec et al. (NL#56)
R347H	G to A at 1172	7	Arg to His at 347	Cremonesi et al., 1992
R347P	G to C at 1172	7	Arg to Pro at 347	Dean et al. (NL #6)
R347L	G to T at 1172	7	Arg to Leu at 347	Audrézet et al. 1993a
M348K	T to A at 1175	7	Met to Lys at 348	Audrézet et al. 1993b
A349V	C to T at 1178	7	Ala to Val at 349	Audrézet et al. 1993a
R352W	C to T at 1186	7	Arg to Trp at 352	Byrne et al. (NL#69)
R352Q	G to A at 1187	7	Arg to Gln at 352	Cremonesi et al. 1992
Q353H	A to C at 1191	7	Gln to His at 353	Ferec et al. (NL #65)
Q359K/T360K	C to A at 1207 and C to A at 1211	7	Glu to Lys at 359 and Thr to Lys at 360	Shoshani et al. 1992
Q359R	A to G at 1208	7	Gln to Arg at 359	Férec 1999*
W361R(T->C)	T to C at 1213	7	Trp to Arg at 361	Bienvenu et al. (NL#56)
W361R(T->A)	T to A at 1213	7	Trp to Arg at 361	Telleria & Alonso 1998*
S364P	T to C at 1222	7	Ser to Pro at 364	Hamosh et al. (NL#54)
L365P	T to C at 1226	7	Leu to Pro at 365	Casals et al. 2000*
1243ins6	insertion of ACAAAA after 1243	7	insertion of Asp and Lys after Lys370	Shackleton et al (NL#67)
1248+1G->A	G to A at 1248+1	intron 7	mRNA splicing defect	Schwarz et al. (NL#58)
1249-29delAT	deletion of AT from 1249-29	intron 7	mRNA splicing defect?	Zielenski et al. (NL#69)
1249-27delTA	deletion of TA at 1249-27	intron 7	mRNA splicing defect?	Egan et al. (NL#70)
1249-5A->G	A to G at 1249	intron 7	mRNA splicing defect?	Bienvenu et al. (NL#62)
L375F	A to C at 1257	8	Leu to Phe at 375 (CUAVD)	Jézéquel (NL#65)

Name	Nucleotide_change	Exon	Consequence	Reference
E379X	G to T at 1267	8	Glu to Stp at 379	Glaeser & Mehnert 2000*
L383S	T to C at 1280	8	Leu to Ser at 383	Casals et al. (NL#69)
T360R	C to G at ?	7	Thr to Arg at 360	Férec 1998*
V392A	T to C at 1307	8	Val to Ala at 392 CAVD	Bienvenu et al (NL#67, NL#68)
V392G	T to G at 1307	8	Val to Gly at 392	Zielenski et al. Larder et al. (NL#70)
M394R	T to G at 1313	8	Met to Arg at 394	Férec 1998*
A399V	C to T at 1328	8	Ala to Val at 399	Yoshimura & Azuma 2000*
E403D	G to C at 1341	8	Glu to Asp at 403	Férec 1999*
1341G->A	G to A at 1341	8	?	Telleria & Alonso 1998*
1341G->A	G to A at 1341	8		Tellería 1999*
1341+1G->A	G to A at 1341+1	intron 8	mRNA splicing defect	Dörk et al. (NL#69)
1341+18A->C	A to C at 1341+18	intron 8	mRNA splicing defect?	Claustres et al. (NL#60)
1342-11TTT->G	TTT to G at 1342-11	intron 8	mRNA splicing defect?	Dörk & Tümmler (NL#59)
1342-2A->C	A to C at 1342-2	intron 8	mRNA splicing defect	Dörk et al. 1993b
1342-1G->C	G to C at 1342-1	intron 8	mRNA splicing defect	Cutting & Curristin (NL #30)
E407V	A to T at 1352	9	Glu to Val at 407	Zielenski et al. 1999*
N418S	A to G at 1385	9	Asn to Ser at 418	Sava et al. (NL#64)
G424S	G to A at 1402	9	Gly to Ser at 424	Bienvenu et al. 2000*
D443Y	G to T at 1459	9	Asp to Tyr at 443	Bienvenu et al. (NL#63)
I444S	T to G at 1463	9	Ile to Ser at 444	Zielenski et al. 1999*
Q452P	A to C at 1487	9	Gln to Pro at 452	Claustres et al. (NL#70)
delta L453	deletion of 3 bp between 1488 and 1494	9	deletion of Leu at 452 or 454	Dörk et al (NL#67)
A455E	C to A at 1496	9	Ala to Glu at 455	Kerem et al. 1990

Name	Nucleotide_change	Exon	Consequence	Reference
V456F	G to T at 1498	9	Val to Phe at 456	Dörk et al. 1994a
G458V	G to T at 1505	9	Gly to Val at 458	Cuppens et al. 1990
1524+6insC	insertion of C after 1524+6, with G to A at 1524+12	intron 9	mRNA splicing defect?	Bienvenu et al. (NL#61)
1525-1G->A	G to A at 1525-1	intron 9	mRNA splicing defect	Dörk et al. 1993a
S466L	C to T at 1529	10	Ser to Leu at 466 (CBAVD)	Costes et al. (NL#66)
G480S	G to A at 1570	10	Gly to Ser at 480	Kawasoe et al. 2001*
G480C	G to T at 1570	10	Gly to Cys at 480	Smit et al. 1991
G480D	G to A at 1570	10	Gly to Asp at 480	Hawworth et al. (NL#66)
H484Y	C to T at 1582	10	His to Tyr at 484 (CBAVD?)	Casals et al. (NL#69)
H484R	A to G at 1583	10	His to Arg at 484	Férec 1998*
S485C	A to T at 1585	10	Ser to Cys at 485	Andrew et al. 1999*
C491R	T to C at 1603	10	Cys to Arg at 491	Chevalier-Porst & Bozon (NL#70)
S492F	C to T at 1607	10	Ser to Phe at 492	Férec et al. 1992
Q493R	A to G at 1610	10	Gln to Arg at 493	Savov et al. 1994a
P499A	C to G at 1627	10	Pro to Ala at 499 (CBAVD)	Arduino et al. (NL#68)
T501A	A to G at 1633	10	Thr to Ala at 501	Claustres et al. 1999*
I502T	T to C at 1637	10	Ile to Thr at 502	Chevalier-Porst & Bozon (NL#70)
E504Q	G to C at 1642	10	Glu to Gln at 504	Baranov (NL#34,#35)
I506L	A to C at 1648	10	Ile to Leu at 506	Zielenski et al. (NL#70)
delta I507	deletion of 3 bp between 1648 and 1653	10	deletion of Ile506 or Ile507	Kerem et al. 1990; Schwarz et al. 1991
I506S	T to G at 1649	10	Ile to Ser at 506	Deufel et al. 1994
I506T	T to C at 1649	10	Ile to Thr at 506	Desgeorges et al. 1995
delta F508	deletion of 3 bp between 1652	10	deletion of Phe at 508	Rommens et al., Riordan

Name	Nucleotide_change	Exon	Consequence	Reference
	and 1655			et al., Kerem et al. 1989
F508S	T to C at 1655	10	Phe to Ser at 508	Férec 1998*
D513G	A to G at 1670	10	Asp to Gly at 513 (CBAVD)	Bienvenu et al. (NL#70)
Y517C	A to G at 1682	10	Tyr to Cys at 517	Arduino et al. (NL#70)
V520F	G to T at 1690	10	Val to Phe at 520	Jones et al. 1992
V520I	G to A at 1690	10	Val to Ile at 520	Malone et al. (NL#60)
1706del16	16 bp deletion from 1706	10	deletion of spice site	
1706del17	deletion of 17 bp from 1706	10	deletion of splice site	Leoni et al. 1993
E527Q	G to C at 1711	10	Glu to Gln at 527	Byrne et al. (NL#70)
E527G	A to G at 1712	10	Glu to Gly at 527	Benetazzo et al. (NL#70)
1716-1G->A	G to A at 1716-1	intron 10	mRNA splicing defect	Jordanova et al. (NL#69)
E528D	G to T at 1716	10	Glu to Asp at 528 (splice mutation?)	Girodon et al. 1999*
1716+2T->C	T to C at 1716+2	intron 10	mRNA splicing defect	Claustres et al. (NL#68)
1717-8G->A	G to A at 1717-8	intron 10	mRNA splicing defect?	Savov et al. 1994a
1717-3T->G	T to G at 1717-3	intron 10	mRNA splicing defect?	Férec et al. (NL#68)
1717-2A->G	A to G at 1717-2	intron 10	mRNA splicing defect	Hawworth et al (NL#67)
1717-1G->A	G to A at 1717-1	intron 10	mRNA splicing defect	Kerem et al. 1990
1717-9T->A	T to A at 1717-9	intron 10	mRNA splicing mutation?	Vouk & Komel 1999*
D529H	G to C at 1717	11	Asp to His at 529	Férec 1998*
A534E	C to A at 1733	11	Ala to Glu at 534	Audrézet et al. 1993a
I539T	T to C at 1748	11	Ile to Thr at 539	Chomel & Kitzis (NL#66)

Name	Nucleotide_change	Exon	Consequence	Reference
G544S	G to A at 1762	11	Gly to Ser at 544	Férec et al. (NL#61)
G544V	G to T at 1763	11	Gly to Val at 544 (CBAVD)	Claustres et al. (NL#69)
S549R(A->C)	A to C at 1777	11	Ser to Arg at 549	Sangiuolo et al. 1990
S549N	G to A at 1778	11	Ser to Asn at 549	Cutting et al. 1990a
S549I	G to T at 1778	11	Ser to Ile at 549	Kerem et al. 1990
S549R(T->G)	T to G at 1779	11	Ser to Arg at 549	Kerem et al. 1990
G550R	G to A at 1780	11	Gly to Arg at 550	Férec et al. (NL#66)
G551S	G to A at 1783	11	Gly to Ser at 551	Strong et al. 1991
G551D	G to A at 1784	11	Gly to Asp at 551	Cutting et al. 1990a
Q552K	C to A at 1786	11	Gln to Lys	Faucz et al. (NL#69)
R553G	C to G at 1789	11	Arg to Gly at 553	Férec et al. (NL#59)
R553Q	G to A at 1790	11	Arg to Gln at 553 (associated with delta F508;	Dörk et al. 1991b
R555G	A to G at 1795	11	Arg to Gly at 555	Zielenski et al 1999*
I556V	A to G at 1798	11	Ile to Val at 556 (mutation?)	Ghanem et al. (NL#50)
L558S	T to C at 1805	11	Leu to Ser at 558	Maggio et al. (NL#31)
A559T	G to A at 1807	11	Ala to Thr at 559	Cutting et al. 1990a
A559E	C to A at 1808	11	Ala to Glu at 559	Girodon et al. 1999*
R560K	G to A at 1811	11	Arg to Lys at 560	Férec et al. 1992
R560T	G to C at 1811	11	Arg to Thr at 560; mRNA splicing defect?	Kerem et al. 1990
1811+1G->C	G to C at 1811+1	intron 11	mRNA splicing defect	Petreska et al. (NL#50)
1811+1.6kbA->G	A to G at 1811+1.2kb	intron 11	creation of splice donor site	Chillón et al. 1995
1811+18G->A	G to A at 1811+18	intron 11	mRNA splicing defect?	Teng et al. (NL#65)
1812-1G->A	G to A at 1812-1	intron	mRNA splicing defect	Chillón et al. 1994

Name	Nucleotide_change	Exon	Consequence	Reference
		11		
R560S	A to C at 1812	12	Arg to Ser at 560	Costes et al. (NL#54)
A561E	C to A at 1814	12	Ala to Glu at 561	Duarte et al. (NL#55)
V562L	G to C at 1816	12	Val to Leu at 562	Hughes et al. (NL#65)
V562I	G to A at 1816	12	Val to Ile at 562	Feldmann et al (NL#67)
Y563D	T to G at 1819	12	Tyr to Asp at 563	Hamosh et al. (NL#54)
Y563N	T to A at 1819	12	Tyr to Asn at 563	Kerem et al. (NL #13)
Y563C	A to G at 1821	12	Tyr to Cys at 563	Delhaize C (NL#67)
L568F	G to T at 1836	12	Leu to Phe at 568 (CBAVD?)	Dörk et al. (NL#69)
Y569D	T to G at 1837	12	Tyr to Asp at 569	Malone et al. (NL#65)
Y569H	T to C at 1837	12	Tyr to His at 569	Costes et al. (NL#52)
Y569C	A to G at 1838	12	Tyr to Cys at 569	Plaseska et al. (NL#45)
L571S	T to C at 1844	12	Leu to Ser at 571	Savov et al. (NL#60)
D572N	G to A at 1846	12	Asp to Asn at 572	Férec et al. (NL#59)
P574H	C to A at 1853	12	Pro to His at 574	Kerem et al. 1990
G576A	G to C at 1859	12	Gly to Ala at 576 (CAVD)	Sarginson et al. (NL#69)
Y577F	A to T at 1862	12	Tyr to Phe at 577	Dörk et al (NL#67)
D579Y	G to T at 1867	12	Asp to Tyr at 579	Harris et al. (NL#63)
D579G	A to G at 1868	12	Asp to Gly at 579	Ferrari et al. (NL#53)
D579A	A to C at 1868	12	Asp to Ala at 579	Pacheco et al. (NL#70)
T582I	C to T at 1877	12	Thr to Ile at 582	Claustres et al (NL#67)
T582R	C to G at 1877	12	Thr to Arg at 582	Casals et al. (NL#55)
S589N	G to A at 1898	12	Ser to Asn at 589 (mRNA splicing defect?)	Scheffer et al. (NL#68)
S589I	G to T at 1898	12	Ser to Ile at 589 (splicing?)	Schwarz et al. 1999*
1898+1G->T	G to T at 1898+1	intron 12	mRNA splicing defect	Morris (NL #62)

Name	Nucleotide_change	Exon	Consequence	Reference
1898+1G->C	G to C at 1898+1	intron 12	mRNA splicing defect	Cuppens et al. 1993
1898+1G->A	G to A at 1898+1	intron 12	mRNA splicing defect	Strong et al. 1992
1898+3A->C	A to C at 1898+3	intron 12	mRNA splicing defect?	Mercier et al. 1995
1898+3A->G	A to G at 1898+3	intron 12	mRNA splicing defect?	Ferrari et al. (NL#35)
1898+5G->T	G to T at 1898+5	intron 12	mRNA splicing defect	Zielenski et al.1995
1898+5G->A	G to A at 1898+5	intron 12	mRNA splicing defect	Férec et al. (NL#69)
1898+73T->G	T to G at 1898+73	intron 12	mRNA splicing defect?	Smit et al. (NL#37)
R600G	A to G at 1930	13	Arg to Gly at 600	Bienvenu et al. (NL#69)
I601F	A to T at 1933	13	Ile to Phe at 601	Schwarz et al. (NL#68)
V603F	G to T at 1939	13	Val to Phe at 603	Zielenski et al. (NL#70)
T604I	C to T at 1943	13	Thr to Ile at 604	Girodon et al. 1999*
1949del84	deletion of 84 bp from 1949	13	deletion of 28 a.a. (Met607 to Gln634)	Granell et al. 1992
H609R	A to G at 1958	13	His to Arg at 609	Bienvenu et al. (NL#69)
L610S	T to C at 1961	13	Leu to Ser at 610	Férec et al. (NL#52)
A613T	G to A at 1969	13	Ala to Thr at 613	Liechti-Gallati (NL#68)
D614Y	G to T at 1972	13	Asp to Tyr 614	Girodon et al. 1999*
D614G	A to G at 1973	13	Asp to Gly at 614	Audrézet et al. 1993b
I618T	T to C at 1985	13	Ile to Thr at 618	Macek et al. (NL#62)
L619S	T to C at 1988	13	Leu to Ser at 619	Dörk et al. 1991
H620P	A to C at 1991	13	His to Pro at 620	Haworth et al. (NL#66)
H620Q	T to G at 1992	13	His to Gln at 620	Dörk and Sturhmann (NL#68)
G622D	G to A at 1997	13	Gly to Asp at 622 (oligospermia)	Zielenski et al. (NL#68)

Name	Nucleotide_change	Exon	Consequence	Reference
G628R(G->A)	G to A at 2014	13	Gly to Arg at 628	Fanen et al. 1992
G628R(G->C)	G to C at 2014	13	Gly to Arg at 628	Cuppens et al. 1993
L633P	T to C at 2030	13	Leu to Pro at 633	Haworth et al. (NL#62)
L636P	T to C at 2039	13	Leu to Pro at 636	Bombieri et al. (NL#70)
D648V	A to T at 2075	13	Asp to Val at 648	Férec et al. (NL#44)
D651N	G to A at 2083	13	Asp to Asn at 651	Bombieri et al. (NL#70)
T665S	A to T at 2125	13	Thr to Ser at 665	Férec et al. (NL#63)
E672del	deletion of 3 bp between 2145-2148	13	deletion of Glu at 672	Claustres et al. (NL#69)
K683R	A to G at 2180	13	Lys to Arg at 683	Chevalier-Porst & Bozon 2000*
F693L(CTT)	T to C at 2209	13	Phe to Leu at 693	Audrézet et al. 1993b
F693L(TTG)	T to G at 2211	13	Phe to Leu at 693	Meyer et al. 2001*
K698R	A to G 2225	13	Lys to Arg at 698	Férec et al. (NL#69)
E725K	G to A at 2305	13	Glu to Lys at 725	Tzetis et al. (NL#70)
P750L	C to T at 2381	13	Pro to Leu at 750	Chevalier-Porst & Bozon 2000*
V754M	G to A at 2392	13	Val to Met at 754	Wallace (NL#69)
T760M	C to T at 2411	13	Thr to Met at 760	Zielenski et al. 1999*
R766M	G to T at 2429	13	Arg to Met at 766	Glavac et al. (NL#66)
N782K	C to A at 2478	13	Asn to Lys at 782	Girodon et al. 1999*
R792G	C to G at 2506	13	Arg to Gly at 792	Glavac et al. (NL#66)
A800G	C to G at 2531	13	Ala to Gly at 800	Mercier et al. 1995
E822K	G to A at 2596	13	Glu to Lys at 822	Mercier et al. 1993a
E826K	G to A at 2608	13	Glu to Lys at 826	Bombieri et al (NL#67)
2622+1G->T	G to T at 2622+1	intron 13	splice mutation	Girodon et al. 1999*
2622+1G->A	G to A at 2622+1	intron 13	mRNA splicing defect	Audrézet et al. 1993a
2622+2del6	deletion of TAGGTA from 2622+2	intron 13	mRNA splicing defect	Zielenski et al. (NL#70)

Name	Nucleotide_change	Exon	Consequence	Reference
D836Y	G to T at 2638	14a	Asp to Tyr at 836	Ghanem & Goossens (NL#47)
R851L	G to T at 2684	14a	Arg to Leu at 851	Casals et al. (NL#68)
C866Y	G to A at 2729	14a	Cys to Tyr at 866	Audrézet et al. (NL#41)
L867X	T to A at 2732	14a	Leu to Stop at 867	Haworth et al. (NL#69)
2751G->A	G to A at 2751	14a	mRNA splicing defect?	Wagner et al. (NL#65)
2751+2T->A	T to A at 2751+2	intron 14a	mRNA splicing defect	Antoniadi et al. (NL#68)
2751+3A->G	A to G at 2751+3	intron 14a	mRNA splicing defect? (CBAVD)	Casals et al. (NL#65)
2752-26A->G	A to G at 2752-26	intron 14a	mRNA splicing defect?	Tzetis et al. (NL#66)
2752-1G->T	G to T at 2752-1	intron 14a	mRNA splicing defect	Férec et al. (NL#65)
2752-1G->C	G to C at 2752-1	intron 14a	splice mutation	Dubourg & Blayau 1999*
T908N	C to A at 2788	14b	Thr to Asn at 908	Férec et al. (NL#69)
2789+2insA	insertion of A after 2789+2	intron 14b	mRNA splicing defect? (CAVD)	Dubourg et al. (NL#70)
2789+3delG	deletion of G at 2789+3	intron 14b	mRNA splicing defect	Macek et al. (NL#63)
2789+5G->A	G to A at 2789+5	intron 14b	mRNA splicing defect	Highsmith et al. 1990
2790-2A->G	A to G at 2790-2	intron 14b	mRNA splicing defect	Marigo et al. (NL#61)
2790-1G->C	G to C at 2790-1	intron 14b	mRNA splicing defect	Schwartz et al. (NL#54)
2790-1G->T	G to T at 2790-1	intron 14b	mRNA splicing defect	Bienvenu et al. (NL#63)
Q890R	A to G at 2801	15	Gln to Arg at 890	Casals et al. 1998*
D891G	A to G at 2804	15	Asp to Gly at 891	Kilinc et al. (NL#70)
S895T	G to T at 2816	15	Ser to Thr at 895	Férec 1999*

Name	Nucleotide_change	Exon	Consequence	Reference
T896I	C to T at 2819	15	Thr to Ile at 896	Lázaro et al. 2000*
N900T	G to A at 2831	15	Asn to Thr at 900	Férec 1999*
2851A/G	A or G at 2851	15	Ile or Val at 907	Claustres et al. 2000*
S912L	C to T at 2867	15	Ser to Leu at 912	Ghanem et al. 1994
Y913C	A to G at 2870	15	Tyr to Cys at 913	Vidaud et al. 1990
Y917D	T to G at 2881	15	Tyr to Asp at 917	Schwarz et al. (NL#69)
Y917C	A to G at 2882	15	Tyr to Cys at 917	Edkins & Creegan (NL#60)
I918M	T to G at 2886	15	Ile to Met at 918	Girodon et al. 1999*
Y919C	A to G at 2888	15	Tyr to Cys at 919	Savov et al. 1994a
V920M	G to A at 2890	15	Val to Met at 920	Bienvenu et al. (NL#63)
D924N	G to A at 2902	15	Asp to Asn at 924	Girodon et al. 1999*
L927P	T to C at 2912	15	Leu to Pro at 927	Hermans et al. 1994
F932S	T to C at 2927	15	Phe to Ser at 932	Férec 1999*
R933S	A to T at 2931	15	Arg to Ser at 933 (CBAVD)	Dörk et al. (NL#69)
V938G	T to G at 2945	15	Val to Gly at 938 (CAVD)	Dörk et al. (NL#69)
H939D	C to G at 2947	15	His to Asp at 939	Férec et al. (NL#54)
H939R	A to G at 2948	15	His to Arg at 939	Férec et al. (NL#69)
S945L	C to T at 2966	15	Ser to Leu at 945	Claustres et al. 1993
K946X	A to T at 2968	15	Lys to Stop at 946	Haworth et al. (NL#69)
H949Y	C to T at 2977	15	His to Tyr at 949	Ghanem et al. 1994
H949R	A to G at 2978	15	His to Arg at 949	Férec et al. (NL#65)
M952T	T to C at 2987	15	Met to Thr at 952	Zielenski et al. 1999*
M952I	G to C at 2988	15	Met to Ile at 952 CBAVD mutation?	Girodon et al (NL#67)
M961I	G to T at 3015	15	Met to Ile at 961	Malone et al. 2000*
L967S	T to C at 3032	15	Leu to Ser at 967 (oligospermia?)	Zielenski et al. (NL#70)
G970R	G to C at 3040	15	Gly to Arg at 970	Cuppens et al. 1993

Name	Nucleotide_change	Exon	Consequence	Reference
3040+2T->C	T to C at 3040+2	intron 15	mRNA splicing defect	Poncin (NL#69)
3041-1G->A	G to A at 3041-1	intron 15	mRNA splicing defect	Malone et al (NL#67)
G970D	G to A at 3041	16	Gly to Asp at 970	Vassilakis et al. (NL#69)
L973F	TC to AT at 3048 and 3049	16	Leu to Phe at 973 (CBAVD)	Dörk and Sturhmann (NL#68)
L973P	T to C at 3050	16	Leu to Pro at 973	Férec 1998*
S977P	T to C at 3061	16	Ser to Pro at 977	Dörk et al. (NL#51)
S977F	C to T at 3062	16	Ser to Phe at 977	Férec et al. (NL#69)
D979V	A to T at 3068	16	Asp to Val at 979	Feldmann et al. (NL#68)
D979A	A to C at 3068	16	Asp to Ala at 979 (CBAVD?)	Dörk and Sturhmann (NL#68)
I980K	T to A at 3071	16	Ile to Lys at 980	Bienvenu et al. (NL#62)
D985H	G to C at 3085	16	Asp to His at 985	Claustres & Guittard (NL#70)
D985Y	G to T at 3085	16	Asp to Tyr at 985	Bienvenu et al. (NL#63)
I991V	A to G at 3103	16	Ile to Val at 991	Bombieri et al. 2000*
D993Y	G to T at 3109	16	Asp to Tyr at 993	Claustres et al (NL#67)
F994C	T to G at 3113	16	Phe to Cys at 994	Claustres et al. (NL#70)
3120G->A	G to A at 3120	16	mRNA splicing defect	Zielenski et al. 1994
3120+1G->A	G to A at 3120+1	intron 16	mRNA splicing defect	Macek et al. (1997)
3121-2A->T	A to T at 3121-2	intron 16	mRNA splicing defect	Férec et al. 1995
3121-2A->G	A to G at 3121-2	intron 16	mRNA splicing defect	Macek et al. (NL#60)
3121-1G->A	G to A at 3121-1	intron 16	mRNA splicing defect	Feldmann et al (NL#67)
L997F	G to C at 3123	17a	Leu to Phe at 997	Kabra et al. (NL#69)
3131del15	deletion of 15 bp from 3130,	17a	deletion of Val at 1001	Wallace & Tassabehji

Name	Nucleotide_change	Exon	Consequence	Reference
	3131, or 3132		to Ile at 1005	(NL#61)
I1005R	T to G at 3146	17a	Ile to Arg at 1005	Dörk et al. 1994b
A1006E	C to A at 3149	17a	Ala to Glu at 1006	Férec et al. 1995
V1008D	T to A at 3155	17a	Val to Asp at 1008	Casals et al. (NL#70)
A1009T	G to A at 3157	17a	Ala to Thr at 1009	Bombieri et al. 2000*
P1013L	C to T at 3169	17a	Pro to Leu at 1013	Onay et al. (NL#69)
Y1014C	A to G at 3173	17a	Tyr to Cys at 1014	Bozon (NL#70)
P1021S	C to T at 3193	17a	Pro to Ser at 1021 (CBAVD)	Casals et al. (NL#69)
3195del6	deletion of AGTGAT from 3195 to 3200	17a	deletion of Val1022 and Ile1023	Claustres et al. 1994
3196del54	deletion of 54 bp from 3196	17a	deletion of 18 aa from codon 1022	Desgeorges et al. (NL#65)
3199del6	deletion of ATAGTG from 3199	17a	deletion of Ile at 1023 and Val at 1024	Bozon (NL#70)
I1027T	T to C at 3212	17a	Ile to Thr at 1027	Andrew et al. 2001 *
M1028R	T to G at 3215	17a	Met to Arg at 1028	Lázaro et al. 2000*
M1028I	G to T at 3216	17a	Met to Ile at 1028	Onay et al. (NL#69)
Y1032C	A to G at 3227	17a	Tyr to Cys at 1032 (CBAVD)	Dörk et al. (NL#69)
I1366T	T to C at 4229	22	Iso to Thr at 1366	Férec 1999*
3271delGG	deletion of GG at 3271	17a	framshift for exon 17b, loss of splice site	Wang 1998*
3271+1G->A	G to A at 3271+1	intron 17a	mRNA splicing defect	Mercier et al. 1994
3271+1delGG	deletion of GG at 3271+1	intron 17b	mRNA splicing defect	Wang et al. 1998*
3272-26A->G	A to G at 3272-26	intron 17a	mRNA splicing defect?	Fanen et al. 1992
3272-9A->T	A to T at 3272-9	intron 17a	mRNA splicing defect?	Chomel et al (NL#67)
3272-4A->G	A to G at 3272-4	intron	mRNA splicing defect?	Kanvakis (NL#63)

Name	Nucleotide_change	Exon	Consequence	Reference
		17a		
3272-1G->A	G to A at 3272-1	intron 17a	mRNA splicing defect	Mercier et al. 1993b
G1047D	G to A at 3272	17b	Gly to Asp at 1047 and mRNA splicing defect? (CBAVD?)	Teng et al. (NL#68)
F1052V	T to G at 3286	17b	Phe to Val at 1052	Mercier et al. 1993b
T1053I	C to T at 3290	17b	missense mutation	Bienvenu et al. 1998*
T1053I	C to T at 3290	17b	Thr to Ile at 1053 (CBAVD?)	Bienvenu et al. (1998)
H1054D	C to G at 3292	17b	His to Asp at 1054	Férec et al. 1993
T1057A	A to G at 3301	17b	Thr to Ala at 1057	Ghanem et al. (NL#68)
K1060T	A to C at 3311	17b	Lys to Thr at 1060	Casals et al. 1995 (NL#61)
G1061R	G to C at 3313	17b	Gly to Arg at 1061	Mercier et al. 1993b
L1065F	C to T at 3325	17b	Leu to Phe at 1065	Tzetis et al. (NL#70)
L1065R	T to G at 3326	17b	Leu to Arg at 1065	Casals et al (NL#67)
L1065P	T to C at 3326	17b	Leu to Pro at 1065	Ghanem et al. 1994
R1066S	C to A at 3328	17b	Arg to Ser at 1066	Férec et al. (NL#65)
R1066C	C to T at 3328	17b	Arg to Cys at 1066	Fanen et al. 1992
R1066H	G to A at 3329	17b	Arg to His at 1066	Férec et al. 1992
R1066L	G to T at 3329	17b	Arg to Leu at 1066	Mercier et al. 1993b
A1067T	G to A at 3331	17b	Ala to Thr at 1067	Férec et al. 1992
A1067D	C to A at 3332	17b	Ala to Asp at 1067	Girodon et al. 1999*
G1069R	G to A at 3337	17b	Gly to Arg at 1069	Savov et al. 1994a
R1070W	C to T at 3340	17b	Arg to Trp at 1070	Macek et al. (NL#58)
R1070Q	G to A at 3341	17b	Arg to Gln at 1070	Mercier et al. 1993b
R1070P	3341 G to C	17b	Arg to Pro at 1070	Shrimpton & Borowitz
Q1071P	A to C at 3344	17b	Gln to Pro at 1071	Ghanem et al. 1994
Q1071H	G to T at 3345	17b	Glu to His at 1071	Clasutres et al. 2000*
P1072L	C to T at 3347	17b	Pro to Leu at 1072	Bombieri et al. (NL#70)

Name	Nucleotide_change	Exon	Consequence	Reference
F1074L	T to A at 3354	17b	Phe to Leu at 1074	Casals et al. (NL#65)
L1077P	T to C at 3362	17b	Leu to Pro at 1077	Bozon et al. 1994
H1085R	A to G at 3386	17b	His to Arg at 1085	Mercier et al. 1993b
T1086I	C to T at 3389	17b	Thr to Ile at 1086	Bienvenu et al (NL#67)
N1088D	A to G at 3394	17b	Asn to Asp at 1088	Zielenski et al. (NL#70)
Y1082H	T to C at 3406	17b	Tyr to His at 1082	Egan et al. (NL#69)
L1093P	T to C at 3410	17b	Leu to Pro at 1093	Wine et al. (NL#69)
L1096R	T to G at 3419	17b	Leu to Arg at 1096	Claustres & Guittard 1998*
W1098R	T to C at 3424	17b	Trp to Arg at 1098	Zielenski et al. 1995
Q1100P	A to C at 3431	17b	Gln to Pro at 1100	Nunes et al. (NL#55)
M1101R	T to G at 3434	17b	Met to Arg at 1101	Mercier et al. 1993b
M1101K	T to A at 3434	17b	Met to Lys at 1101	Zielenski et al. 1993
S1118F	C to T at 3485	17b	Ser to Phe at 1118	Férec 1998*
S1118C	C to G at 2485	17b	Ser to Cys at 1118	Zielenski et al. 1999*
G1123R	G to C at 3499	17b	Gly to Arg at 1123 mRNA splicing defect?	Wallace & Tassabehji (NL#60)
3499+2T->C	T to C at 3499+2	intron 17b	mRNA splicing defect	Creegan & Edkins (NL#64)
3499+3A->G	A to G at 3499+3	intron 17b	mRNA splicing defect?	Haworth et al. (NL#68)
3499+6A->G	A to G at 3499+6	intron 17b	mRNA splicing defect?	Férec et al. (NL#65)
3500-2A->G	A to G at 3500-2	intron 17b	mRNA splicing defect	Vidaud et al. (NL#70)
E1123del	Deletion of AAG at 3504 - 3506	18	deletion of Glu at 1123	Ellis (NL#70)
G1127E	G to A at 3512	18	Gly to Glu at 1127	Bienvenu et al. (NL#63)
3523A->G	A to G at 3523	18	Ile to Val at 1131	Giorgi et al. 1999*
A1136T	G to A at 3538	18	Ala to Thr at 1136	Férec 2000*
M1137V	A to G at 3541	18	Met to Val at 1137	Zielenski et al. (NL#59)

Name	Nucleotide_change	Exon	Consequence	Reference
M1137R	T to G at 3542	18	Met to Arg at 1137	Duarte et al. (NL#65)
I1139V	A to G at 3547	18	Ile to Val at 1139	Teng et al. 1994
delta M1140	deletion of 3 bp between 3550 and 3553	18	deletion of Met at 1140	Férec et al. (NL#64)
M1140K	T to A at 3551	18	Met to Lys at 1140	Férec 1998*
T1142I	C to T at 3557	18	Thr to Ile at 421	Lázaro et al. 2000*
V1147I	G to A at 3571	18	Val to Ile at 1147	Kilinc et al. (NL#70)
N1148K	C to A at 3576	18	Asn to Lys at 1148	Casals et al. 2000*
D1152H	G to C at 3586	18	Asp to His at 1152	Highsmith et al. (NL#49)
V1153E	T to A at 3590	18	Val to Glu at 1153 (CBAVD)	Dörk et al. (NL#68)
D1154G	A to G at 3593	18	Asp to Gly at 1154 (CBAVD)	Costes et al. (NL#64)
3600G->A	G to A at 3600	18	mRNA splicing defect	Zielenski et al. 1994
3600+2insT	insertion of T after 3600+2	intron 18	mRNA splicing defect?	Zielenski et al. (NL#70)
3600+5G->A	G to A at 3600+5	intron 18	mRNA splicing defect?	Bienvenu et al. (NL#66)
3601-20T->C	T to C at 3601-20	intron 18	mRNA splicing mutant?	Kabra et al. (NL#69)
3601-17T->C	T to C at 3601-17	intron 18	mRNA splicing defect?	Audrézet et al. 1993a
3601-2A->G	A to G at 3601-2	intron 18	mRNA splicing defect	Dörk et al. 1993a
S1159P	T to C at 3607	19	Ser to Pro at 115p	Macek et al. (NL#55)
S1159F	C to T at 3608	19	Ser to Phe at 1159	Férec 1999*
D1168G	A to G at 3635	19	Asp to Gly at 1168	Macek et al. (NL#58)
K1177R	A to G at 3662	19	Lys to Arg at 1177	Baralle et al. (NL#61)
3696G/A	G to A at 3696	18	No change to Ser at 1188	Malone et al. 1999*
V1190P	T to A at 3701	19	Val to Pro at 1190	Glavac et al. (NL#64)

Name	Nucleotide_change	Exon	Consequence	Reference
3750delAG	deletion of AG from 3750	19	frameshift	Mercier et al. 1993a
3755delG	deletion of G between 3751 and 3755	19	frameshift	Claustres et al. (NL#70)
M1210I	G to A at 3762	19	Met to Ile at 1210	Nukiwa & Seyama (NL#55)
V1212I	G to A at 3766	19	Val to Ile at 1212	Macek et al. (NL#55)
L1227S	T to C at 3812	19	Leu to Ser at 1227	Dubourg & David (NL#70)
E1228G	A to G at 3815	19	Glu to Gly at 1228	Kilinc et al. 2000*
I1230T	T to C at 3821	19	Ile to Thr at 1230	Claustres & Maugard (NL#69)
I1234V	A to G at 3832	19	Ile to Val at 1234	Claustres et al. 1992b
S1235R	T to G at 3837	19	Ser to Arg at 1235	Cuppens et al. 1993
G1237S	G to A at 3841	19	Gly to Ser at 1237	Casals et al. 2000*
Q1238R	A to G at 3845	19	Gln to Arg at 1238	Férec C et al. (NL#58)
3849G->A	G to A at 3849	19	mRNA splicing defect?	Cutting et al. 1992
3849+1G->A	G to A at 3849+1	intron 19	mRNA splicing defect	Greil et al. 1993
3849+4A->G	A to G at 3849+4	intron 19	mRNA splicing defect?	Ronchetto et al. 1992
3849+10kbC->T	C to T in a 6.2 kb EcoRI fragment 10 kb from 19	intron 19	creation of splice acceptor site	Highsmith et al. 1994
3849+5G->A	G to A at 3849+5	intron 19	mRNA splicing defect?	Kilinc et al. (NL#70)
3850-3T->G	T to G at 3850-3	intron 19	mRNA splicing defect	Dörk et al. 1993a
3850-1G->A	G to A at 3850-1	intron 19	mRNA splicing defect	Audrézet et al. 1993a
V1240G	T to G at 3851	20	Val to Gly at 1240	Zielenski et al. 1999*
G1244V	G to T at 3863	20	Gly to Val at 1244	Savov et al. 1994b
G1244E	G to A at 3863	20	Gly to Glu at 1244	Devoto et al. 1991
T1246I	C to T at 3869	20	Thr to Ile at 1246	Férec et al. (NL#64)

Name	Nucleotide_change	Exon	Consequence	Reference
			(mutation?)	
G1247R	G to A at 3871	20	Gly to Arg at 1247	Casals et al. (NL#69)
G1249R	G to A at 3877	20	Gly to Arg at 1249	Dijkstra et al. 1994
G1249E	G to A at 3878	20	Gly to Glu at 1249	Greil et al. 1994
S1251N	G to A 3884	20	Ser to Asn at 1251	Kälin et al. 1992a; Mercier et al. 1993a
T1252P	A to C at 3886	20	Thr to Pro at 1252	Wallace (NL#69)
S1255P	T to C at 3895	20	Ser to Pro at 1255	Lissens et al. 1992
S1255L	C to T at 3896	20	Ser to Leu at 1255	Bienvenu et al. (NL#69)
F1257L	T to G at 3903	20	Phe to Leu at 1257	Férec 1998*
delta L1260	deletion of ACT from either 3909 or 3912	20	deletion of Leu at 1260 or 1261	Hermans et al. 1994
3922del10->C	deletion of 10 bp from 3922 and replacement with 3921	20	deletion of Glu1264 to Glu1266	Schwarz et al. (NL#69)
I1269N	T to A at 3938	20	Ile to Asn at 1269	McDowell et al. (NL#66)
D1270N	G to A at 3940	20	Asp to Asn at 1270	Dean et al. 1991
W1282G	T to G at 3976	20	Trp to Gly at 1282	Faucz et al. (NL#69)
W1282R	T to C at 3976	20	Trp to Arg at 1282	Ivaschenko et al. 1993
W1282C	G to T at 3978	20	Trp to Cys at 1282	Férec et al. (NL#69)
R1283M	G to T at 3980	20	Arg to Met at 1283	Cheadle et al. 1992
R1283K	G to A at 3980	20	Arg to Lys at 1283	Chevalier & Bozon (NL#54)
F1286S	T to C at 3989	20	Phe to Ser at 1286	Dorval et al. 1993
Q1291R	A to G at 4004	20	Gln to Arg at 1291	Dörk et al. 1994b
Q1291H	G to C at 4005	20	Gln to His at 1291; mRNA splicing defect (?)	Jones et al. 1992
4005+1G->A	G to A at 4005+1	intron 20	mRNA splicing defect	Férec et al. 1992
4005+2T->C	T to C at 4005+2	intron	mRNA splicing defect	Boman (NL#69)

Name	Nucleotide_change	Exon	Consequence	Reference
		20		
4006-61del14	deletion of 14 bp from 4006-61 to 4006-47	intron 20	mRNA splicing defect?	Friedman et al. (NL#59)
4006-19del3	deletion of 3 bp from 4006-19	intron 20	mRNA splicing defect?	Naseem et al. (NL#36)
4006-14C->G	C to G at 4006-14	intron 20	mRNA splicing defect?	Poncin (NL#69)
4006-8T->A	T to A at 4006-8	intron 20	mRNA splicing defect?	Chevalier-Porst & Bozon (NL#70)
4006-4A->G	A to G at 4006-4	intron 20	mRNA splicing defect?	Chomel et al. (NL#68)
V1293I	G to A at 4009	21	Val to Ile at 1293	Férec et al. (NL#69)
T1299I	C to T at 4028	21	Thr to Ile at 1299	Liechti-Gallati (NL#68)
F1300L	T to C at 4030	21	Phe to Leu at 1300	Poncin (NL#69)
N1303H	A to C at 4039	21	Asn to His at 1303	Claustres et al. 1992b
N1303I	A to T at 4040	21	Asn to Ile at 1303	Lissens et al. (NL#66); Férec et al. (NL#66)
N1303K	C to G at 4041	21	Asn to Lys at 1303	Osborne et al. 1991
D1305E	T to A at 4047	21	Asp to Glu at 1305	Claustres et al. (NL#69)
Q1313K	C to A at 4069	21	Gln to Lys at 1313	Malone et al. (NL#68)
V1318A	T to C at 4085	21	Val to Ala at 1318	Férec 1998*
E1321Q	G to C at 4093	21	Glu to Gln at 1321	Férec et al. (NL#64)
4096-28G->A	G to A at 4096-28	intron 21	mRNA splicing defect?	Claustres et al. (NL#68)
4096-3C->G	C to G at 4096-3	intron 21	mRNA splicing defect?	Claustres et al. (NL#69)
L1335P	T to C at 4136	22	Leu to Pro at 1335	Zielenski et al. (NL#70)
F1337V	T to G at 4138	22	Phe to Val at 1337 (CBAVD)	Scheffer et al. (NL#70)
L1339F	C to T at 4147	22	Leu to Phe at 1339	Girodon et al. 1999*
G1349S	G to A at 4177	22	Gly to Ser at 1349	Yoshimura 1999*

Name	Nucleotide_change	Exon	Consequence	Reference
G1349D	G to A at 4178	22	Gly to Asp at 1349	Beaudet et al. 1991
K1351E	A to G at 4183	22	Lys to Glu at 1351 (CBAVD)	Dörk et al. (NL#69)
Q1352H*	G to C at 4188	22	Gln to His at 1352	Nukiwa & Seyama (NL#55)
R1358S	A to T at 4206	22	Arg to Ser at 1358	Férec 1999*
A1364V	C to T at 4223	22	Ala to Val at 1364 CBAVD	Claustres et al (NL#67)
D1377H	G to C at 4261	22	Asp to His at 1377	Costes et al. (NL#56)
L1388Q	T to A at 4295	23	Leu to Gln at 1388 (CBAVD)	Dörk et al. (NL#68)
V1397E	T to A at 4322	23	Val to Glu at 1397	Petreska et al. 1994
E1409V	A to T at 4358	23	Glu to Val at 1409	Claustres et al. (NL#55)
Q1412X	C to T at 4366	23	Gln to Stop at 1412	Wallace & Tassabehji (NL#60)
4374+10T->C	T to C at 4374+10	intron 23	splicing?	Férec 1998*
4374+1G->A	G to A at 4374+1	intron 23	mRNA splicing defect	Fanen et al. 1992
4374+1G->T	G to T at 4374+1	intron 23	mRNA splicing defect	Dörk et al. (NL #38)
4375-1G->C	G to C at 4375-1	intron 23	splicing mutation	Chevalier-Porst & Bozon 1999*
R1422W	C to T at 4396	24	Arg to Trp at 1422	Claustres et al. (NL#70)
S1426P	T to C at 4408	24	Ser to Pro at 1426	Férec 1999*
D1445N	G to A at 4465	24	Asp to Asn at 1445	Antoniadi et al. (NL#69)
R1453W	C to T at 4489	24	Arg to Trp at 1453	Yoshimura 1999*
CFTRdele14a	deletion of >=1.2 kb including exon 14a	14a	aberrant mRNA splicing	Egan et al. (NL#68)
CFTRdele19	deletion of 5.3kb, removing exon 19	19	?	Girodon et al. 1999
2104insA+2109-	insertion of A at 2104,	13	?	Girodon et al. 1999*

Name	Nucleotide_change	Exon	Consequence	Reference
2118del10	deletion of 10bp at 2109			
CF25kbdel	Complex deletion/rearrangement	intron 3	?	Shackleton et al. (NL# 70)

[37] The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples which are provided herein for purposes of illustration only, and are not intended to limit the scope of the invention.

EXAMPLES

Development of a polypeptide that exerts only an activating effect on CFTR

[38] The activating peptide of Q4N2NEG2 was created by substituting glutamine residues for glutamic acid residues at four sites and asparagines for aspartic acid residues at two sites of the authentic NEG2 peptide sequence GLEISFEINEEDLKECFFDDME (SEQ ID NO: 7). In addition, a serine residue was substituted for cysteine, to prevent peptide dimerization, and norleucine was substituted for methionine, to prevent oxidation. These changes create a peptide with reduced chemical reactivity and high predicted helical structure, confirmed by circular dichroism, as well as reduced net negative charge (from –9 to –3). Attempts to eliminate negative charge completely resulted in an insoluble peptide. When this peptide was added to the cis (intracellular) side of CFTR channels captured in the planar lipid bilayer, at concentration ranging 0.5 to 14 μM, marked dose-related stimulation of channel activity was observed. At concentrations of 4-6 μM Po of CFTR doubles. No inhibitory activity was seen in any experiment at any concentration of peptide.

Q4N2NEG2 polypeptide stimulates wild-type CFTR protein.

[39] To test whether the Q4N2NEG2 polypeptide is responsible for increasing the open probability of the CFTR channel, synthetic Q4N2NEG2, a 22 amino acid peptide, was added to the cis-intracellular side of single CFTR channels captured in the planar lipid

bilayer (Figure 1). The diary plot of open probability as a function of time shows the activity of a single wt-CFTR channel during the course of the experiment (Figure 1A). During stimulation, the open probability doubles and more transitions are observed between the open and closed states (Figure 1B). The open probability observed in 5 experiments at 4 μM concentration Q4N2NEG2 is shown to be increased by about two-fold in the graph (Figure 1C).

Q4N2NEG2 polypeptide stimulates mutant G551D CFTR protein.

[40] The Q4 N2 NEG2 peptide sequence has been tested on one mutant form of CFTR, G551D, which reaches the plasma membrane. In the planar lipid bilayer, Q4N2NEG2 increased the open probability of G551 by about threefold. Thus, this peptide is useful to stimulate channel activity in mutant forms of CFTR that reach the plasma membrane.

The NEG2 polypeptide can be rendered inhibitory to CFTR

[41] The NEG2 sequence can also be rendered inhibitory, with no stimulatory activity, by scrambling the sequence such that the resulting peptide is predicted to not have helical tendencies, as confirmed by circular dichroism measurements, but retains the full net negative charge of –9. This peptide, called scrambled NEG2, inhibits channel activity by about 90% at 6 μM concentration, with no stimulation observed at any concentration. In addition, insertion of a proline residue into the middle of the NEG2 sequence also results in a peptide which inhibits channel activity by about 60%, but does not stimulate. Proline residues are known to disrupt helical structures.

METHODS USED IN EXAMPLES

Subcloning of CFTR gene

[42] The wt CFTR cDNA was subcloned into an Epstein-Barr virus-based episomal eukaryotic expression vector, pCEP4 (Invitrogen, San Diego, CA), between the Nhe1 and Xho1 restriction sites.

Expression of CFTR in HEK 293 cells

[43] A human embryonic kidney cell line (293-EBNA HEK; Invitrogen) was used for transfection and expression of the CFTR proteins (Ma et al., 1997, Ma et al., 1996, Xie et al., 1995). The HEK-293 cell line contains a pCMV-EBNA vector, which constitutively expresses the Epstein-Barr virus nuclear antigen-1 (EBNA-1) gene product and increases the transfection efficiency of Epstein-Barr virus-based vectors. The cells were maintained in Dulbecco's Modified Eagle Medium with 10% FBS and 1% L-glutamine. Geneticin (G418, 250 (g/ml) was added to the cell culture medium to maintain selection of the cells containing the pCMV-EBNA vector. Lipofectamine reagent (Life Technologies, Inc) in Optimem media (serum-free) was used to transfect the HEK-293 cells with pCEP4(wt). After 5 hours, serum was added to the media (10% final serum concentration). Twenty-four hours after transfection, the transfection media was replaced with fresh media. The cells were harvested two days after transfection and microsomal membrane vesicles were prepared for single channel measurements in the lipid bilayer reconstitution system.

Vesicle preparation from transfected HEK 293 cells

[44] HEK-293 cells transfected with pCEP4(CFTR) were harvested and homogenized using a combination of hypotonic lysis and Dounce homogenization in the presence of protease inhibitors (Ma et al., 1997, Ma et al., 1996, Xie et al., 1995). Microsomes were collected by centrifugation of postnuclear supernatant (4500 x g, 15 min) at 100,000 x g for 20 min and resuspended in a buffer containing 250 mM sucrose, 10 mM HEPES, pH 7.2. The membrane vesicles were stored at -75°C until use.

Reconstitution of CFTR channels in lipid bilayer membranes

[45] Lipid bilayer membranes were formed across an aperture of ~200 (m diameter with a mixture of phosphatidylethanolamine:phosphatidylserine:cholesterol in a ratio of 5:5:1. The lipids were dissolved in decane at a concentration of 33 mg/ml. The recording solutions contained: cis (intracellular), 200 mM CsCl, 1 mM $MgCl_2$, 2 mM ATP, and 10 mM HEPES-Tris (pH 7.4); trans (extracellular), 50 mM CsCl, 10 mM

HEPES-Tris (pH 7.4). Vesicles (1-4 (l) containing wild-type CFTR were added to the cis solution. The PKA catalytic subunit was present at a concentration of 50 units/ml in the cis solution unless noted otherwise. Single channel currents were recorded with an Axopatch 200A patch clamp unit (Axon Instruments). The currents were sampled at 1-2.5 ms/point. Single channel data analyses were performed with pClamp and TIPS softwares.

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CLAIMS:

1. A polypeptide comprising an amino acid sequence of SEQ ID NO: 6, wherein the polypeptide retains a net negative charge of 1-8.

2. The polypeptide of claim 1 wherein the polypeptide retains a net negative charge of 2-8.

3. The polypeptide of claim 1 wherein the polypeptide retains a net negative charge of 3-8.

4. The polypeptide of claim 1 wherein the polypeptide retains a net negative charge of 4-8.

5. The polypeptide of claim 1 wherein the polypeptide retains a net negative charge of 5-8.

6. The polypeptide of claim 1 wherein the polypeptide retains a net negative charge of 6-8.

7. The polypeptide of claim 1 wherein the polypeptide retains a net negative charge of 7-8.

8. The polypeptide of claim 1 wherein amino acid residue sixteen is serine.

9. The polypeptide of claim 1 wherein amino acid residue twenty-one is norleucine.

10. The polypeptide of claim 1 which comprises the amino acid sequence of SEQ ID NO: 1.

11. A composition comprising the polypeptide of claim 1 and a pharmaceutically acceptable carrier.

12. The polypeptide of claim 1 consisting of the sequence of SEQ ID NO: 1.

13. The polypeptide of claim 1 wherein the polypeptide is fused to a membrane-penetrating peptide.

14. The polypeptide of claim 13 wherein the membrane-penetrating peptide is selected from the group consisting of: VP-22 (SEQ ID NO: 3), (SEQ ID NO: 4), and (SEQ ID NO: 5).

15. A method of activating a CFTR protein comprising:

administering an effective amount of a polypeptide to a cell comprising a CFTR protein which forms a cAMP-regulated chloride channel, said polypeptide comprising the sequence of SEQ ID NO: 6, whereby the CFTR protein is activated.

16 The method of claim 15 wherein the polypeptide comprises the sequence of SEQ ID NO: 1.

17. The method of claim 15 wherein the effective amount of the polypeptide increases open probability of the channel formed by the CFTR by at least 25%.

18. The method of claim 15 wherein open probability of the channel formed by the CFTR increases by at least 50%.

19. The method of claim 15 wherein open probability of the channel formed by the CFTR increases by at least 75%.

20. The method of claim 15 wherein open probability of the channel formed by the CFTR increases by at least 100%.

21. The method of claim 15 wherein open probability of the channel formed by the CFTR increases by at least 125%.

22. The method of claim 15 wherein open probability of the channel formed by the CFTR increases by at least 150%.

23. The method of claim 15 wherein open probability of the channel formed by the CFTR increases by at least 175%.

24. The method of claim 15 wherein open probability of the channel formed by the CFTR increases by at least 200%.

25. The method of claim 15 wherein open probability of the channel formed by the CFTR increases by at least 300%.

26. The method of claim 15 wherein said polypeptide is administered to achieve a concentration of 0.5 to 14 μM.

27. The method of claim 15 wherein said polypeptide is administered to achieve a concentration of 4-6 μM.

28. The method of claim 15 wherein the CFTR protein is a mutant which reaches the cell's plasma membrane but fails to undergo full activation in the absence of said polypeptide.

29. The method of claim 28 wherein the mutant CFTR protein is selected from the group consisting of -816C->T, -741T->G, -471delAGG, -363C/T, -102T->A, -94G->T, -33G->A, 132C->G, P5L, S10R, S13F, 185+1G->T, 185+4A->T, 186-13C->G, W19C, G27E, R31C, R31L, 232del18, S42F, D44G, A46D, 279A/G, I50T, S50P, S50Y, 296+3insT, 296+1G->T, 296+1G->C, 296+2T->C, 296+9A->T, 296+12T->C, 297-28insA, 297-3C->A, 297-3C->T, 297-2A->G, 297-10T->G, 297-12insA, E56K, W57G, W57R, D58N, D58G, E60K, E60L, N66S, P67L, K68E, K68N, A72T, A72D, R74W, R74Q, R75L, W79R, G85E, G85V, F87L,

L88S, Y89C, L90S, G91R, 405+1G->A, 405+3A->C, 405+4A->G, 406-10C->G, 406-6T->C, 406-3T->C, 406-2A->G, 406-2A->C, 406-1G->C, 406-1G->A, 406-1G->T, E92K, A96E, Q98R, P99L, I105N, S108F, Y109N, Y109C, D110H, D110Y, D110E, P111A, P111L, delta E115, E116Q, E116K, R117C, R117H, R117P, R117L, A120T, I125T, G126D, L137R, L137H, L138ins, H139R, P140S, P140L, A141D, H146R, I148T, I148N, G149R, M152V, M152R, 591del18, A155P, S158R, Y161N, Y161D, Y161S, K162E, 621G->A, 621+1G->T, 621+2T->C, 621+2T->G, 621+3A->G, 622-2A->C, 622-1G->A, L165S, K166Q, R170C, R170G, R170H, I175V, I177T, G178R, Q179K, N186K, N187K, D192N, delta D192, D192G, E193K, 711+1G->T, 711+3A->C, 711+3A->G, 711+3A->T, 711+5G->A, 711+34A->G, 712-1G->T, G194V, A198P, H199Y, H199Q, V201M, P205S, L206W, L206F, A209S, E217G, Q220R, C225R, L227R, V232D, Q237E, G239R, G241R, M243L, M244K, R248T, 875+1G->C, 875+1G->A, 876-14del12, 876-10del8, 876-3C->T, R258G, V920L, M265R, E278del, N287Y, 994del9, 1002-3T->G, E292K, R297W, R297Q, A299T, Y301C, S307N, A309D, A309G, delta F311, F311L, G314R, G314V, G314E, F316L, V317A, L320V, L320F, V322A, L327R, R334W, R334L, R334Q, I336K, T338I, E474K, L346P, R347C, R347H, R347P, R347L, M348K, A349V, R352W, R352Q, Q353H, Q359K/T360K, Q359R, W361R(T->C), W361R(T->A), S364P, L365P, 1243ins6, 1248+1G->A, 1249-29delAT, 1249-27delTA, 1249-5A->G, L375F, E379X, L383S, T360R, V392A, V392G, M394R, A399V, E403D, 1341G->A, 1341G->A, 1341+1G->A, 1341+18A->C, 1342-11TTT->G, 1342-2A->C, 1342-1G->C, E407V, N418S, G424S, D443Y, I444S, Q452P, delta L453, A455E, V456F, G458V, 1524+6insC, 1525-1G->A, S466L, G480S, G480C, G480D, H484Y, H484R, S485C, C491R, S492F, Q493R, P499A, T501A, I502T, E504Q, I506L, delta I507, I506S, I506T, delta F508, F508S, D513G, Y517C, V520F, V520I, 1706del16, 1706del17, E527Q, E527G, 1716-1G->A, E528D, 1716+2T->C, 1717-8G->A, 1717-3T->G, 1717-2A->G, 1717-1G->A, 1717-9T->A, D529H, A534E, I539T, G544S, G544V, S549R(A->C), S549N, S549I, S549R(T->G), G550R, G551S, G551D, Q552K, R553G, R553Q, R555G, I556V, L558S, A559T, A559E, R560K, R560T, 1811+1G->C, 1811+1.6kbA->G, 1811+18G->A, 1812-1G->A, R560S, A561E, V562L, V562I, Y563D, Y563N, Y563C, L568F, Y569D, Y569H, Y569C, L571S, D572N, P574H, G576A, Y577F, D579Y, D579G, D579A, T582I, T582R, S589N, S589I, 1898+1G->T, 1898+1G->C, 1898+1G->A, 1898+3A->C, 1898+3A->G, 1898+5G->T, 1898+5G->A, 1898+73T->G, R600G, I601F, V603F, T604I, 1949del84, H609R, L610S, A613T, D614Y, D614G, I618T, L619S, H620P, H620Q, G622D, G628R(G->A), G628R(G->C), L633P, L636P, D648V,

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30. The method of claim 15 wherein the polypeptide is administered in an aerosol to a patient with a mutant CFTR protein.

31. The method of claim 15 wherein the polypeptide is administered in an aerosol to a patient with insufficient amounts of wild-type CFTR to maintain chloride transport.

32. The method of claim 30 wherein the aerosolized polypeptide is co-administered with an expression vector wherein said expression vector encodes wild-type CFTR protein.

33. The method of claim 31 wherein the aerosolized polypeptide is co-administered with an expression vector wherein said expression vector encodes wild-type CFTR protein.

34. A method of activating a CFTR protein comprising:

applying an effective amount of a polypeptide to a CFTR protein in a lipid bilayer wherein the polypeptide comprises the amino acid sequence of SEQ ID NO: 6, whereby the CFTR protein is activated.

35. The method of claim 34 wherein the polypeptide comprises the amino acid sequence of SEQ ID NO: 1.

36. The method of claim 34 further comprising measuring a change in conductance upon applying the polypeptide.

37. A method of synthesizing a CFTR activating polypeptide comprising:

sequentially linking units of one or more amino acid residues to form a polypeptide comprising the amino acid sequence of SEQ ID NO: 6.

38. The method of claim 37 wherein F-moc synthesis is used.

39. The method of claim 37 wherein the polypeptide has the sequence of SEQ ID NO: 1.

40. A polypeptide comprising the amino acid sequence as shown in SEQ ID NO: 2.

41. The polypeptide of claim 40 wherein the polypeptide is fused to a membrane-penetrating peptide.

42. The polypeptide of claim 41 wherein the membrane-penetrating peptide is selected from the group consisting of: VP-22 (SEQ ID NO: 3), (SEQ ID NO: 4) and (SEQ ID NO: 5).

43. A nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide according to SEQ ID NO: 2.

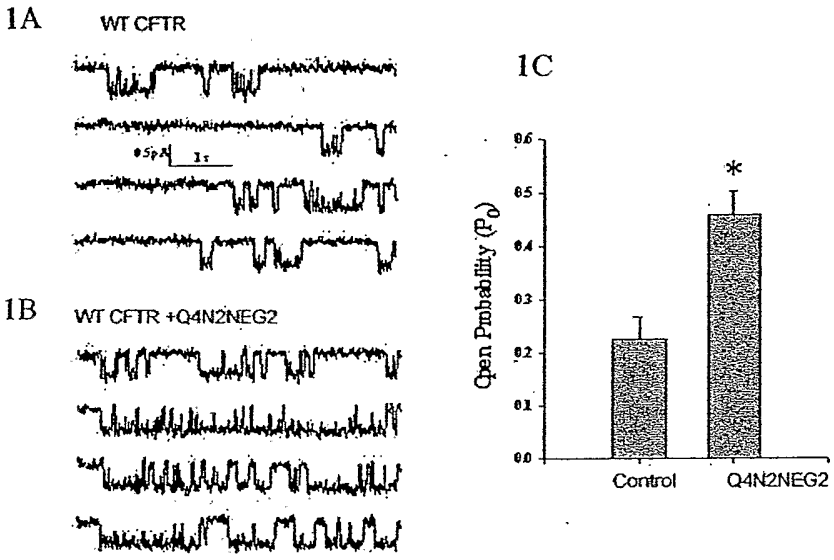
44. A method of activating a CFTR protein, comprising:

administering a nucleic acid comprising a sequence encoding a polypeptide according to SEQ ID NO: 2 to a cell comprising the CFTR protein, whereby the polypeptide is expressed and the CFTR protein is activated.

45. The method of claim 44 wherein the cell is in a patient and the nucleic acid is administered as an aerosol to the patient's airways.

46. The method of claim 45 wherein the nucleic acid molecule is co-administered with an expression vector encoding a wild-type CFTR protein.

Figure 1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