June 1, 2015

1	
2	Functional characteristics of L1156F-CFTR associated with alcoholic chronic
3	pancreatitis in Japanese
4	
5	Shiho Kondo <sup>1</sup> , Kotoyo Fujiki <sup>2</sup> , Shigeru B. H. Ko <sup>3</sup> , Akiko Yamamoto <sup>1</sup> , Miyuki Nakakuki <sup>1</sup> ,
6	Yasutomo Ito <sup>4</sup> , Nikolay Shcheynikov <sup>5</sup> , Motoji Kitagawa <sup>2</sup> , Satoru Naruse <sup>6</sup> , Hiroshi Ishiguro <sup>1</sup>
7	
8	<sup>1</sup> Department of Human Nutrition, Nagoya University Graduate School of Medicine, Nagoya,
9	Japan; <sup>2</sup> Department of Nutrition, Nagoya University of Arts and Sciences, Nisshin, Japan;
10	<sup>3</sup> Department of Systems Medicine, Keio University School of Medicine, Tokyo, Japan;
11	<sup>4</sup> Division for Medical Research Engineering, Nagoya University Graduate School of
12	Medicine, Nagoya, Japan; <sup>5</sup> Epithelial Signaling and Transport Section, National Institute of
13	Dental and Craniofacial Research, Bethesda, USA; <sup>6</sup> Miyoshi Municipal Hospital, Miyoshi,
14	Japan
15	
16	Running Title: L1156F-CFTR and alcoholic chronic pancreatitis
17	
18	Address for correspondence:
19	Hiroshi Ishiguro, MD, PhD
20	Human Nutrition, Nagoya University Graduate School of Medicine
21	Research Center of Health, Physical Fitness, and Sports, Nagoya University
22	Furo-cho E5-2 (130), Chikusa-ku, Nagoya 464-8601, Japan; Tel/Fax: +81-52-744-2183
23	Email: <u>ishiguro@htc.nagoya-u.ac.jp</u>
24	

- 25 <u>Author contributions</u>
- 26 Shiho Kondo: Genomic analysis, protein expression, and preparation of manuscript
- 27 Kotoyo Fujiki: Planning the study and preparation of manuscript
- 28 Shigeru B. H. Ko: Pancreatic exocrine function
- 29 Akiko Yamamoto: Planning the study, measurement of Cl<sup>-</sup>HCO<sub>3</sub><sup>-</sup> exchange activity, and
- 30 preparation of manuscript
- 31 Miyuki Nakakuki: Measurement of sweat Cl<sup>-</sup> and genomic analysis
- 32 Yasutomo Ito: molecular modeling
- 33 Nikolay Shcheynikov: Analysis of HCO<sub>3</sub><sup>-</sup> and Cl<sup>-</sup> fluxes
- 34 Motoji Kitagawa: Planning the study
- 35 Satoru Naruse: Planning the study and analysis of the clinical data
- 36 Hiroshi Ishiguro: Planning the study and preparation of manuscript

June 1, 2015

#### 38 ABSTRACT

39 Although cystic fibrosis is rare in Japanese, measurement of sweat Cl<sup>-</sup> has suggested mild 40dysfunction of cystic fibrosis transmembrane conductance regulator (CFTR) in some patients 41with chronic pancreatitis. In the present study, we have investigated the association of CFTR 42variants and chronic pancreatitis in Japanese and the functional characteristics of a Japanese-, 43and pancreatitis-specific CFTR variant, L1156F. Seventy patients with alcoholic chronic 44pancreatitis, 18 patients with idiopathic chronic pancreatitis, and 180 normal subjects 45participated. All exons and their boundaries and promoter region of the CFTR gene were 46sequenced. HEK 293 cells were transfected with 3 CFTR variants (M470V, L1156F, and 47M470V+L1156F) and the protein expression was examined. Xenopus laevis oocytes were 48injected with the CFTR variants and HCO<sub>3</sub><sup>-</sup>/Cl<sup>-</sup> transport activity was examined. CFPAC-1 49 cells were transfected with the CFTR variants and CI-HCO<sub>3</sub><sup>-</sup> exchange activity was examined. 50Six variants (E217G, I556V, M470V, L1156F, Q1352H, and R1453W) were identified in the 51coding region of the CFTR gene. Cystic fibrosis-causing mutations were not found. The allele 52frequencies of L1156F and Q1352H in alcoholic chronic pancreatitis (5.0 and 7.9 %) were significantly (p<0.01) higher than those in normal subjects (0.6 and 1.9 %). L1156F was 5354linked with a world-wide CFTR variant M470V. Combination of M470V and L1156F significantly reduced CFTR expression to  $\sim 60\%$ , impaired CFTR-mediated HCO<sub>3</sub><sup>-</sup>/Cl<sup>-</sup> 5556transport activity to 50-60%, and impaired CFTR-coupled CI-HCO<sub>3</sub><sup>-</sup> exchange activity to 5720-30%. The data suggest that the Japanese-specific CFTR variant L1156F causes mild 58dysfunction of CFTR and increases the risk of alcoholic chronic pancreatitis in Japanese. 59Key words: CFTR gene, L1156F, alcoholic chronic pancreatitis, Japanese

60

June 1, 2015

#### 61 INTRODUCTION

62 Cystic fibrosis transmembrane conductance regulator (CFTR) is a cAMP regulated anion 63 channel that is expressed in the apical membrane of epithelial cells (18). In the pancreas 64 CFTR is found in centroacinar cells, intralobular ducts, and small interlobular ducts and plays 65 a key role in  $HCO_3^-$  and fluid secretion in the pancreatic juice (3, 21, 28). CFTR-mediated 66 HCO<sub>3</sub><sup>-</sup> secretion dilutes and alkalinizes the protein-rich acinar secretion, which is thought to 67 prevent the formation of protein plugs and blockage of the ducts (12). A recent study using 68 Na<sup>+</sup>-H<sup>+</sup> exchanger regulatory factor-1 knock out mice demonstrated that mislocalization of 69 CFTR worsened experimental acute pancreatitis (35) and suggested that CFTR protects from 70 acute pancreatitis. 71Over 1,900 mutations and polymorphic loci have now been identified in the CFTR gene 72(Cystic Fibrosis Mutation Database; www.genet.sickkids.on.ca/) and there are considerable 73 regional and ethnic variations in the spectrum (38). Combination of mutations and 74polymorphisms confer variable phenotypes (11). Loss of CFTR function (< 1%) due to severe 75mutations on both alleles causes cystic fibrosis (CF) with chronic airway disease and 76 pancreatic insufficiency. Decrease of CFTR function to  $\sim$ 5% due to compound heterozygote 77 of 1 severe mutation and 1 mild mutation causes "pancreatic sufficient" CF. Non-classic or 78atypical CF presents single organ disease phenotypes, including disseminated bronchiectasis, 79 congenital bilateral absence of the vas deferens (CBAVD), and chronic pancreatitis, which are 80 classified as CFTR-related disorders (5, 36). 81 Chronic pancreatitis is a progressive inflammatory disease of the pancreas that causes the loss 82 of acinar cells, ductal irregularities often with intraductal stones, and irregular fibrosis. 83 Although alcohol abuse is the major cause of chronic pancreatitis, only a minority of heavy 84 drinkers develop pancreatitis. Thus chronic pancreatitis is thought to result from a

85	combination of environmental (alcohol, cigarettes, etc.) and genetic factors (CFTR, PRSS1,
86	SPINK1, etc.) (6). In European populations, CFTR mutations have been frequently (~ 30%)
87	found in patients with idiopathic chronic pancreatitis (4). Although some of the patients were
88	classified as CF or CFTR-related disorders after search for rare CFTR mutations and sweat Cl
89	measurement, many of the patients with chronic pancreatitis carrying one CFTR mutation
90	were not diagnosed as CF or CFTR-related disorders (5). The risk of chronic pancreatitis
91	increased 4.3 times in CF carriers (8). Subjects carrying one mild/uncommon CFTR mutation
92	were also reported to be at an increased (~ threefold) risk of chronic pancreatitis (45).
93	Although CF is quite rare in Asian populations including Japanese (19, 47), the incidence of
90	
94	chronic pancreatitis in Japan (11.9 per 100,000) (17) is similar to that in the United States (46).
95	Measurement of sweat Cl <sup>-</sup> suggested that about one-third to one-half cases of chronic
96	pancreatitis (both idiopathic and alcoholic) in Japanese are related to the dysfunction of CFTR
97	(16, 34). Our previous study (13) indicated the association of 2 types of CFTR variants:
98	c.4056G>C, p.Arg1352His (Q1352H) and c.4357C>T, p.Arg1453Trp (R1453W) and chronic
99	pancreatitis in Japanese. Defects of protein expression and ion transport of Q1352H-CFTR
100	were confirmed by heterologous expression system, while R1453W-CFTR showed mild
101	reduction of open probability (27). In the present study, we have shown that a
102	Japanese-specific CFTR variant c.3468G>T, p.Leu1156Phe (L1156F) is related to alcoholic
103	chronic pancreatitis in Japanese. The risk of L1156F carrier for developing chronic
104	pancreatitis was 9.0 times higher compared to non-carrier. Since L1156F is linked to
105	c.1408A>G, p.Met470Val (M470V), a world-wide CFTR variant, we have examined the
106	protein expression and functional characteristics of M470V+L1156F-CFTR using
107	heterologous expression systems. We have found that combination of M470V and L1156F
108	partially impaired expression and channel function of CFTR and substantially impaired the
109	CFTR-coupled Cl <sup>-</sup> -HCO <sub>3</sub> <sup>-</sup> exchange activity.

#### 110 MATERIALS AND METHODS

### 111 Subjects

112This study was approved by the ethical committee of Nagoya University Graduate School of 113 Medicine and written informed consent was obtained from each subject prior to the study. 114 Seventy patients with alcoholic chronic pancreatitis (65 males, 5 females; mean age 61.3 115years, range 37–82), 18 patients with idiopathic chronic pancreatitis (13 males, 5 females; 116 mean age 55.6 years, range 24–85), and 180 normal subjects (156 males, 24 females; mean 117age 52.3 years, range 19–87) participated in this study. Diagnosis of chronic pancreatitis was 118 based on the criteria of the Japan Pancreas Society (42). Patients who consumed ethanol over 60 g/day for more than 10 years were considered alcoholic. Patients with no or occasional 119 120 social alcohol intake were classified as idiopathic following the exclusion of known rare 121causes of chronic pancreatitis.

### 122 Analysis of the CFTR, SPINK1, and PRSS1 genes

123 Genomic DNA was extracted from blood leukocytes. PCR was carried out using the primers

shown in Table 1. PCR products were purified using the High Pure PCR Product Purification

125 Kit (Roche Diagnostics, Mannheim, Germany). The sequence reaction was carried out using

126 the GenomeLab Dye Terminator Cycle Sequencing with Quick Start kit (Beckman Coutler,

127 Fullerton, CA). The reaction products were purified using the Centri-Sep spin columns

128 (Applied Biosystems, Foster, CA) and sequenced by CEQ 8000 system (Beckman Coutler).

All 27 exons and their boundaries (100-300 bp including c.1210-12T (5-9), poly T and

130 c.1210-34GT (9-13), TG repeats in intron 9) and promoter region (up to 1,028 bp upstream of

131 the translation initiation codon of exon 1) of the CFTR gene were sequenced for samples from

132 alcoholic and idiopathic chronic pancreatitis. Six CFTR variants: c.650A>G, p.Glu217Gly

133 (E217G); c.1666A>G, p.Ile556Val (I556V); M470V; L1156F; Q1352H; and R1453W were

detected. The presence of these variants in normal subjects was screened by SNP typing with

135 Masscode system (Shimadzu, Kyoto, Japan) and confirmed by direct sequencing in positive

and equivocal cases. In subjects carrying CFTR variants, the presence of c.101A>G,

137 p.Asn34Ser (N34S) and IVS3+2T>C mutations in the SPINK1 gene and c.365G>A,

p.Arg122His (R112H) and c.86A>T, p.Asn29Ile (N29I) mutations in the *PRSS1* gene were

139 analyzed by sequencing.

#### 140 Measurement of sweat Cl<sup>-</sup> concentration

141 Cl<sup>-</sup> concentrations in insensible sweat were estimated by dividing the amount of Cl<sup>-</sup> recovered 142 from one thumb by the amount of sweat measured in the other thumb as we described 143 previously (33, 34). This method is based on the observation that the sweat rates of the right 144 and left fingers are almost identical (44). Sweat rate from one thumb was measured by a 145 perspiration meter (Perspiro 201, Suzuken, Nagoya, Japan) and sweat during the period was 146 collected from the other thumb. The Cl<sup>-</sup> content was measured by capillary electrophoresis 147 (Bio-Rad, Hercules, CA).

#### 148 **Evaluation of Pancreatic Exocrine Function**

149 Pancreatic exocrine function was evaluated by the secretin test as we previously described

150 (25). Duodenal intubation was performed to collect pancreatic juice. After an acclimation

151 period, 80 U/body of human secretin (ChiRhoStim, ChiRhoClin, Burtonsville, MD) was

- administered intravenously and pancreatic juice was collected every 10 minutes for 60
- 153 minutes. Total secreted volume (mL/h) and total amylase output (U/h) were measured. The

154 HCO<sub>3</sub><sup>-</sup> concentration in each sample was measured and the highest value was set as the

155 maximum HCO<sub>3</sub><sup>-</sup> concentration (mEq/L). The lower limits of normal range of volume,

amylase output, and the maximum HCO<sub>3</sub><sup>-</sup> concentration (MBC) are 183 mL/h, 99,000 U/h

157 and, 80 mEq/L (25).

#### 158 Preparation of DNA constructs of CFTR variants

- 159 Wild-type human CFTR cDNA in pCMV vector (pcDNA3-CFTR) was provided by Prof. K.
- 160 Kirk (University of Alabama School of Medicine). CFTR mutations (M470V, L1156F, and
- 161 both of M470V and L1156F) were introduced by site-directed mutagenesis (QuickChange
- 162 mutagenesis kit, Stratagene, La Jolla, CA). The primers used for M470V are: sense
- 163 5'-GGCAAGACTTCACTTCTAATGGTGATTATGGGAGAACTGG-3', antisense
- 164 5'-CCAGTTCTCCCATAATCACCATTAGAAGTGAAGTCTTGCC-3'. The primers used
- 165 for L1156F are: sense
- 166 5'-ATAGATGTGGATAGCTTTATGCGATCTGTGAGCCGAGTCT-3', antisense
- 167 5'-AGACTCGGCTCACAGATCGCATAAAGCTATCCACATCTAT-3'.
- 168 Expression of CFTR variants in HEK293 and CFPAC-1 cells
- 169 Cells were cultured in Dulbecco's modified Eagle's medium with high glucose supplemented
- 170 with 10% fetal calf serum, penicillin (100 U/ ml) and streptomycin (100 mg/ml) in 35 mm
- 171 dishes. Cells were transfected with 2.5 µg of each plasmid using Lipofectamin or
- 172 Lipofectamine LTX & PLUS Reagent (Life Technologies, Carlsbad, CA). Cells were used
- 173 24-72 h post-transfection for experiments.
- 174 Real-time PCR was performed to confirm the successful transfection. Total RNA was
- 175 extracted using the RNeasy Mini Kit (Qiagen GmbH, Hilden, Germany) and reverse
- transcribed using the PrimeScript® RT reagent Kit with gDNA Eraser (Perfect Real Time)
- 177 (Takara Bio, Otsu, Japan). A fluorogenic SYBR Green and Mx3000P QPCR System (Agilent
- 178 Technologies, Santa Clara, CA) were used for real time quantification. The primers used for
- 179 CFTR are: sense 5'- TGCCCTTCGGCGATGTTTTT-3', antisense

#### 180 5'-GTTATCCGGGTCATAGGAAGCTA-3'.

- 181 For western blot analysis, cells were lysed in a Passive Lysis Buffer (Promega, Madison, WI).
- 182 Thirty micrograms of protein were suspended in sodium dodecyl sulfate (SDS) sample buffer
- 183 (Life Technologies) and separated by SDS-polyacrylamide gel electrophoresis. The separated
- 184 proteins were transferred to a polyvinylidene difluoride membrane and probed with a
- 185 monoclonal antibody against the NBD2 domain of CFTR (M3A7, Millipore, Billerica, MA,
- 186 or sc-10747, Santa Cruz, Dallas, TX). After treating with the secondary antibody, protein
- 187 bands were visualized by the enhanced chemiluminescence using ECL Western Blotting
- 188 Starter Kit (GE Healthcare Bio-Sciences, Piscataway, NJ).
- 189 Effects of ethanol (EtOH), acetaldehyde (ALD), palmitoleic acid (POA) (Santa Cruz, Dallas,
- 190 TX), and palmitoleic acid ethyl ester (POAEE) (Santa Cruz, Dallas, TX) on the protein
- 191 expression were examined by treating cells for 24-48 hours. POA and POAEE were
- 192 solubilized according to a previous work (29).

#### 193 Cl<sup>-</sup> channel activity of CFTR variants in HEK293 cells

- 194 Macroscopic Cl<sup>-</sup>-current recordings by whole-cell patch-clamp were performed on HEK293
- 195 cells transfected with CFTR variants. The pipette solution contained 150 mM
- 196 N-methyl-D-glucamine-Cl (NMDG-Cl), 1 mM MgCl<sub>2</sub>, 1 mM EGTA, 0.5 mM ATP, and 10
- 197 mM HEPES at pH 7.3. The bath solution contained 150 mM NMDG-Cl, 1 mM MgCl<sub>2</sub>, 1 mM
- 198 CaCl<sub>2</sub>, and 10 mM HEPES-Tris at pH 7.4. Cells were stimulated with 10 µM forskolin. The
- 199 membrane potential was held at -60 mV. CFTR Cl<sup>-</sup> current was confirmed by the complete
- 200 inhibition of the current with 100 µM glibenclamide. Currents were recorded using the
- 201 Axopatch 200B patch-clamp amplifier (Axon Instruments, Union City, CA). Data were
- 202 collected at 5 kHz and filtered at 1 kHz.

June 1, 2015

#### 203 HCO<sub>3</sub><sup>-</sup> and Cl<sup>-</sup> transport by CFTR variants expressed in *Xenopus laevis* oocytes

204 Oocytes were obtained by partial ovariectomy of anesthetized female *Xenopus*. Healthy

205 oocytes in stages V-VI were injected with 5 ng cRNA of CFTR variants in a final volume of

50 nl. Injected oocytes were incubated at 18°C in a ND96 solution and used 48-120 h after
injection.

208Intracellular pH (pH<sub>i</sub>) and Cl<sup>-</sup> concentration (Cl<sup>-</sup><sub>i</sub>) were measured at room temperature as 209 previously described (41). In brief, the electrodes were prepared from single-barreled 210 borosilicate glass tubes and vapor silanized with bis (dimethylamino) dimethyl silane. The 211tips of the pH electrodes were filled with H<sup>+</sup> exchanger resin (hydrogen ionophore I, cocktail 212B; Sigma-Aldrich, St. Louis, MO). The electrodes were fitted with a holder with an Ag-AgCl 213wire attached to a high-impedance probe of a two-channel electrometer (FD-223; World 214Precision Instruments, Sarasota, FL). A second channel was used for the measurement of 215membrane potential by standard reference microelectrodes. The signal from the voltage 216electrode was subtracted from the voltage of the pH electrode. HCO<sub>3</sub><sup>-</sup> fluxes were calculated 217from the change of pH<sub>i</sub> and the buffering capacity.  $Cl_i$  was measured with a Cl-sensitive liquid ion exchanger (477913; Corning, Corning, NY). The tips of vapor-silanized electrodes 218were filled with the Cl<sup>-</sup>-selective liquid ion exchanger and backfilled with 3 M KCl. Cl<sup>-</sup><sub>i</sub> was 219calculated according to the equation:  $Cl_i = Cl_{cal} \times 10^{(\Delta V/S)}$ , where  $Cl_{cal}$  is the Cl activity of 220221the calibration solutions,  $\Delta V$  is the difference in voltage between the Cl<sup>-</sup> electrode and 222reference electrode, and S is the slope measured in response to a 10-fold change in Cl<sup>-</sup> activity. 223Membrane current was measured with two-electrode methods using an OC-725C Oocyte 224Clamp System (Warner Instrument, Hamden, CT).

#### 225 Cl<sup>-</sup>HCO<sub>3</sub><sup>-</sup> exchange activity in CFPAC-1 cells transfected with CFTR variants

226 CFPAC-1 cells transfected with CFTR variants were loaded with BCECF and first bathed in

June 1, 2015

231	Molecular modeling of CFTR
230	$pH_i$ increase upon removal of Cl <sup>-</sup> .
229	the presence of forskolin. The activity of Cl <sup>-</sup> HCO <sub>3</sub> <sup>-</sup> exchange was estimated from the rate of
228	switched to the Cl <sup>-</sup> -free HCO <sub>3</sub> <sup>-</sup> -CO <sub>2</sub> -buffered solution (Cl <sup>-</sup> was replaced with glucuronate) in
227	the standard $HCO_3^CO_2$ -buffered solution containing 1 $\mu$ M forskolin. The bath solution was

- 232 A molecular model of CFTR protein was constructed by homology modeling using Discovery
- 233 studio (Accelrys, San Diego, CA). The sequence alignment of 2 transmembrane domains
- 234 (TMD1 and TMD2) and 2 nucleotide-binding domains (NBD1 and NBD2) were performed
- using 3G5U (for TMD1 and TMD2), 1XMI (for NBD1), and 3GD7 (for NBD2) as
- template-structures according to a previous work (14). Energy minimization and equilibration
- were performed using the CHARMM22 force field. This atomic model lacks the regulatory
- domain (R domain, residues 656-857), N-terminal (residues 1-44) and C-terminal (residues

239 1461-1480) regions.

### 240 Statistical analysis

- For clinical data,  $\chi^2$  test with Yates correction if appropriate was used for statistical analysis.
- 242 Data of *in vitro* experiments are presented as means  $\pm$  SEM of the indicated number of
- 243 experiments. Statistical analysis was carried out by Student's *t*-test or ANOVA followed by
- 244 Dunnett's test for multiple comparisons.

#### 246 **RESULTS**

#### 247 CFTR variants in Japanese patients with chronic pancreatitis

- Established CF-causing mutations were not found. Six variants (E217G, I556V, M470V,
- L1156F, Q1352H, and R1453W) were identified in coding regions of the CFTR gene (Table
- 250 2). The allele frequencies of L1156F and Q1352H in alcoholic chronic pancreatitis (5.0 and
- 251 7.9 %) were significantly (p<0.01) higher than those in normal subjects (0.6 and 1.9 %). The
- allele frequency of R1453W in idiopathic pancreatitis (11.1%) was significantly (p<0.01)
- higher than that in normal subjects (1.9%). The allele frequencies of E217G, I556V, and
- 254 M470V were not different among groups.

# Genotypes, pancreatic exocrine function, and sweat Cl of chronic pancreatitis patients carrying the L1156F

- 257 Table 3 shows the characteristics of 8 patients with alcoholic (7 males) and idiopathic (1
- female) chronic pancreatitis who carry the L1156F variant. Genotypes of CFTR (presence of
- L1156F and G1352H, M/V470, poly T, and TG repeats), sex, age, etiology of pancreatitis,
- 260 presence or absence of pancreatic stone, pancreatic exocrine function (secretin test), sweat Cl
- concentration, and mutations in SPINK1 and PRSS1 are shown. Age (mean 58.8 years, range
- 262 49-73) was not different from that of total patients.
- 263 Pancreatic stone was found in 7 of 8 patients. Secretin test was performed in 3 patients.
- Volume and amylase output of collected pancreatic juice from 3 patients were all below the
- normal lower limits. The maximum HCO<sub>3</sub><sup>-</sup> concentration (MBC) of pancreatic juice from 2 of
- 266 3 patients was below the normal lower limits.
- 267 There was no homozygote of L1156F. All patients carrying the L1156F had M470V on at
- least one allele suggesting that L1156F is linked to M470V. Two patients had the Q1352H

variant which is also associated with alcoholic chronic pancreatitis. Two patients had a

270 genotype of 7/7 poly T and 11/11 TG repeats, while 6 patients had a genotype of 7/7 poly T

and 11/12 TG repeats. One patient had N34S mutation in SPINK1. Sweat Cl<sup>-</sup> was measured in

5 patients. Sweat Cl<sup>-</sup> of 3 patients was >60 mM and that of 2 patients was in the intermediate

273 range (40-60 mM).

#### 274 Expression of CFTR variants in HEK293 cells

- The protein expression wild-type CFTR and 3 CFTR variants (M470V-CFTR, L1156F-CFTR, L1156F-CFTR,
- 276 M470V+L1156F-CFTR) transfected in HEK293 cells was examined by western blot analysis
- 277 (Figure 1). The approximately 170 kD bands corresponding to glycosylated mature 'band C'

278 CFTR proteins and another smaller-size fragment (~150 kD) corresponding to immature

<sup>279</sup> 'band B' CFTR protein were detected. When the band intensities of mature CFTR were

280 normalized against those of  $\beta$ -actin, the expression of M470V+L1156F-CFTR was reduced to

 $60 \pm 10 \%$  (p<0.01) compared to wild-type CFTR. The expression of M470V-CFTR and

282 L1156F-CFTR was not significantly different from that of wild-type CFTR.

283 Effects of ethanol (EtOH) and acetaldehyde (ALD) on the protein expression of wild-type and

284 M470V+L1156F CFTR were examined (Figure 2). Treatment with combination of EtOH (50

mM) and ALD (200  $\mu$ M) for 24 hours significantly (p<0.05) inhibited the expression of

wild-type CFTR, while EtOH and ALD by themselves did not affect the expression. The

expression of M470V+L1156F CFTR was not affected by EtOH, ALD, and their

288 combination.

289 Free fatty acids and fatty acid ethyl esters, non-oxidative metabolites of ethanol have toxic

290 effects on pancreatic acinar cells (9). Previous studies demonstrated that palmitoleic acid

291 (POA) and palmitoleic acid ethyl ester (POAEE) inhibited the protein expression and function

of CFTR in pancreatic duct cells (23, 29). Treatment with POA (100  $\mu$ M) or POAEE (100

- $\mu$ M) for 48 hours significantly (p<0.05) inhibited the expression of wild-type CFTR (Figure
- 3). The expression of M470V+L1156F CFTR was not affected by POA and POAEE.

#### 295 Cl<sup>-</sup> channel activity of CFTR variants in HEK293 cells

- 296 Cl<sup>-</sup> channel current of wild-type CFTR and 3 CFTR variants (M470V-CFTR, L1156F-CFTR,
- 297 M470V+L1156F-CFTR) was measured by the whole cell configuration (Figure 4).
- 298 Stimulation with 10 µM forskolin generated a large Cl<sup>-</sup> current in cells transfected with
- wild-type CFTR. The Cl<sup>-</sup> current was abolished by glibenclamide (100  $\mu$ M). The magnitude
- of CF current by M470V-CFTR and L1156F-CFTR was similar to that by wild-type CFTR.
- 301 The magnitude of Cl<sup>-</sup> current by M470V+L1156F-CFTR was smaller than that by wild-type
- 302 CFTR, but the difference was not statistically significant.

#### 303 HCO<sub>3</sub><sup>-</sup> and Cl<sup>-</sup> transport by CFTR variants expressed in *Xenopus laevis* oocytes

- 304 HCO<sub>3</sub><sup>-</sup> and Cl<sup>-</sup> fluxes were examined in *Xenopus laevis* oocytes expressing wild-type CFTR
- or 3 CFTR variants (M470V-CFTR, L1156F-CFTR, M470V+L1156F-CFTR) (Figure 5).
- 306 When oocytes expressing wild-type CFTR were perfused with the  $HCO_3^--CO_2$ -buffered
- 307 solution and stimulated with forskolin, removal of extracellular Cl<sup>-</sup> caused a rapid decrease of
- $Cl_i$  (Figure 5B) and elevation of pH<sub>i</sub> (Figure 5A), which are probably largely due to Cl<sup>-</sup> efflux
- and HCO<sub>3</sub><sup>-</sup> influx via activated CFTR, respectively. Thus the activity of HCO<sub>3</sub><sup>-</sup> and Cl<sup>-</sup>
- transport by CFTR can be estimated from the initial rate of  $pH_i/Cl_i$  changes (Figure 5C). In
- 311 oocytes expressing M470V+L1156F-CFTR, removal of extracellular Cl<sup>-</sup> caused a slower Cl<sup>-</sup><sub>i</sub>
- decrease (Figure 5B) and a slower  $pH_i$  elevation (Figure 5A). The activity of HCO<sub>3</sub><sup>-</sup> and Cl<sup>-</sup>
- transport by M470V+L1156F-CFTR was significantly (p<0.01) reduced to 52 and 57 % of
- that by wild-type CFTR (Figure 5C). Unexpectedly, membrane current by
- 315 M470V+L1156F-CFTR was similar to that by wild-type CFTR. M470V and L1156F by
- themselves did not affect CFTR-mediated  $HCO_3^{-1}/Cl^{-1}$  transport.

#### 317 CI<sup>-</sup>HCO<sub>3</sub><sup>-</sup> exchange activity coupled with CFTR variants

- 318 It has been known that CFTR and SLC26 Cl<sup>-</sup>HCO<sub>3</sub><sup>-</sup> exchangers (SLC26A3 and A6) are
- $_{319}$  physically and functionally coupled to work as machinery for HCO<sub>3</sub> secretion and that the
- 320 Cl<sup>-</sup>HCO<sub>3</sub><sup>-</sup> exchange activity of SLC26A3 and A6 is dependent on the presence of functional
- 321 CFTR (26). CFPAC-1, a human pancreatic duct cell line bearing F508del-CFTR
- endogenously expresses SLC26A3 and A6 (15). The regulation of SLC26 Cl<sup>-</sup>HCO<sub>3</sub><sup>-</sup>
- exchangers by CFTR variants were examined by transfecting the variants to CFPAC-1 cells
- (Figure 6). The Cl<sup>-</sup>HCO<sub>3</sub><sup>-</sup> exchange (AE) activity was estimated from the rate of  $pH_i$  increase
- upon removal of Cl<sup>-</sup>. In cells transfected with wild-type CFTR, stimulation with forskolin (1
- 326  $\mu$ M) increased the rate of pH<sub>i</sub> increase by ~4 fold from 0.77 ± 0.10 (n = 18) to 3.28 ± 0.13 (n
- 327 = 25). The stimulated pH<sub>i</sub> response was decreased by only 8.8% to  $2.99 \pm 0.07$  (n = 18) with
- 328 CFTRinh-172 (10 µM) and thus largely mediated by AE, which is consistent with a previous
- report that transfection of wild-type CFTR augmented DIDS-sensitive AE activity in
- 330 CFPAC-1 cells (15).
- 331 The CFTR-dependent AE activity was compared under forskolin stimulation (Figure 6).
- 332 Transfection of wild-type CFTR increased the AE activity by ~5 fold (compared with mock).
- 333 The AE activity in cells transfected with 3 CFTR variants; M470V, L1156F, and
- M470V+L1156F-CFTR was all much smaller (p<0.01) compared to cells transfected with
- wild-type CFTR. The AE activity coupled with M470V, L1156F, and M470V+L1156F-CFTR
- was 33%, 35%, and 26% of that coupled with wild-type CFTR. The AE activity coupled with
- M470V+L1156F-CFTR was significantly (p<0.05) smaller compared to that with
- 338 L1156F-CFTR.

#### 339 Localization of M470 and L1156 in homology model of CFTR

340 To examine the possible effects of M470V and L1156F on CFTR function at the molecular

- 341 level, we constructed a homology model of CFTR in the inward-facing conformation (closed
- state). Figure 7 indicates M470 in NBD1 and L1156 in the junctional residues between TMD2
- and NBD2. Thus M470V may affect ATP binding to NBD1 and L1167F may affect
- 344 conformational transition between closed and open states. However, M470 and L1156F are
- not close to each other, and it is not clear why the double mutation (combination of M470V
- and L1156F) but not single mutations affected the transport Cl<sup>-</sup> and HCO<sub>3</sub><sup>-</sup> (Figure 5).

#### 348 **DISCUSSION**

#### 349 Spectrum of CFTR variants in Japanese

- 350 The spectrum of *CFTR* mutations/polymorphisms shows considerable regional and ethnic
- variations (13, 38) and some of the polymorphisms (such as poly T, TG repeats, and M470V)
- as well as mutations are known to significantly affect CFTR function. Thus the association of
- 353 CFTR variants/dysfunction and CF/CFTR-related diseases including chronic pancreatitis may
- 354 vary among different ethnic groups.
- Common disease-causing mutations in Europeans such as F508del have never been identified
- in Japanese CF patients. Most of the mutations found in alleles inherited from Japanese/Asian
- ancestry are of rare types (22, 30, 48). Our recent data suggest that
- $c.2908+1085_3367+260 del7201$  (*CFTR* dele16-17b) is the major CF-causing mutation in
- 359 Japanese (20, 31).
- 360 Among polymorphisms, longer TG repeats in IVS8 increase the probability of exon 9
- skipping (43) and M470V-CFTR causes mild channel dysfunction to ~60% (10, 27). In a
- 362 previous study, we have performed haplotype analysis of *CFTR* polymorphisms in Japanese
- 363 (13). While (TG)10 and (TG)11 are common in Europeans, Japanese had longer (TG)11 or
- (TG)12. The haplotype frequencies of (TG)n-M/V470 in Japanese were ~51% for
- 365 (TG)11-V470, ~31% for (TG)12-M470, and ~16% for (TG)12-V470. The data predict that
- 366 background CFTR function in Japanese is lower than that in Europeans.
- The association of chronic pancreatitis and 3 Japanese/Asian types of CFTR variants (L1156F,
- 368 Q1352H, and R1453W) were demonstrated in our present and previous (13) studies.
- 369 Twenty-three of 88 patients with chronic pancreatitis (26.1%) carried 1 or 2 of the 3 variants,
- which was 2.9 times higher than normal subjects (Table 2). None of the CF-causing mutations

371	was found and thus the spectrum of pancreatitis-related CFTR variants is different from that
372	of CF-related mutations. Although 2 other studies indicated a higher frequency of 5T in
373	Japanese patients with chronic pancreatitis (2, 24), we have not found similar association

374 (Table 2) (13).

### 375 Epidemiology of L1156F-CFTR

While Q1352H and R1453W are also found in Koreans (27) and thus categorized to

377 Asian-type CFTR variants, L1156F is probably a Japanese-specific CFTR variant and has not

been reported from other countries. Guanine to thymine substitution at nucleotide position

379 3468 in the *CFTR* gene (c.3468G>T) results in the leucine to phenylalanine substitution at

1156 in the polypeptide. L1156F was previously found in an adult, Japanese healthy female

- 381 (Cystic Fibrosis Mutation Database), and a recent study reported the association of L1156F
- and chronic pancreatitis in Japanese (32).
- In the present study, we have found that L1156F is associated with alcoholic chronic
- pancreatitis in Japanese (the odds ratio = 9.0 when compared to normal subjects, Table 2).
- L1156F was found in 10.0% in patients with alcoholic chronic pancreatitis. While Q1352H
- was also found in patients with CBAVD (1) and diffuse panbronchiolitis and R1453W in
- patients with diffuse panbronchiolitis (Cystic Fibrosis Mutation Database), L1156F has not
- been found in other CFTR-related diseases. Thus L1156F is probably a pancreatitis-specific
  CFTR variant.

#### 390 Functional characteristics and molecular modeling of L1156F-CFTR

391 Although some CFTR variants have been reported to increase the risk for developing chronic

- 392 pancreatitis, the functional characteristics were examined only for a few variants (10, 27, 39).
- 393 Since L1156F is most likely linked to M470V (Table 2), we analyzed the protein expression,

394	Cl <sup>-</sup> channel activity, HCC	<sub>3</sub> <sup>-</sup> /Cl <sup>-</sup> transport activity	, and CFTR-coupled	Cl <sup>-</sup> HCO <sub>3</sub> <sup>-</sup> exchange
-----	---------------------------------------	---	--------------------	--

activity of 3 CFTR variants (M470V-CFTR, L1156F-CFTR, M470V+L1156F-CFTR) using

- 396 heterologous expression systems.
- 397 The combination of M470V and L1156F reduced the expression of mature 'band C' form of
- 398 CFTR protein to 60~70% (Figures 1, 2, and 3). The effects of M470V and L1156F by
- themselves on the protein expression were not significant due to a considerable variation
- 400 between experiments. The variable effects of M470V may be related to the different rate of
- 401 maturation between M470 and V470 CFTR proteins (10).
- 402 The M470V+L1156F-CFTR variant expressed in *Xenopus laevis* oocytes showed a reduction
- 403 of HCO<sub>3</sub><sup>-</sup>/Cl<sup>-</sup> transport activity to 50~60% of wild-type CFTR (Figure 5), while L1156F by
- 404 itself caused almost no reduction. However, the M470V+L1156F-CFTR variant expressed in
- 405 HEK293 cells showed a weak (by  $\sim 20\%$ ) and non-significant reduction of whole cell Cl<sup>-</sup>
- 406 channel current compared to wild-type CFTR (Figure 4). The difference may be due to the
- 407 different systems of heterologous expression. To the contrary, CFTR-coupled Cl<sup>-</sup>HCO<sub>3</sub><sup>-</sup>
- 408 exchange activity was substantially impaired by M470V, L1156F, and the double mutation
- 409 (M470V+L1156F, Figure 6).
- 410 M470 and L1156F are not close to each other in the inward-facing conformation (closed state)
- in a predicted model of CFTR (Figure 7), which do not explain why the combination of
- 412 M470V and L1156F impaired Cl<sup>-</sup>/HCO<sub>3</sub><sup>-</sup> transport activity.
- 413 L1156F-CFTR variant and chronic pancreatitis
- 414 Genotype-phenotype correlation in CF is most evident for exocrine pancreatic status (11).
- 415 Particular cases of chronic pancreatitis develop as atypical CF with single organ disease
- 416 phenotypes or CFTR-related disorders (5). CFTR mutations have been frequently found in

June 1, 2015

417	patients with chronic	pancreatitis (4). Thus	pancreas is probably	y more sensitive to mild CFTR
-----	-----------------------	------------------------	----------------------	-------------------------------

418 dysfunction than the other organs. The CFTR variant, L1156F, identified in the present study

is associated with chronic pancreatitis but not CF, which is consistent with the idea.

420 The present 8 cases of adult-onset chronic pancreatitis carrying L1156F are probably not

- 421 categorized to atypical CF. However high level of sweat Cl<sup>-</sup> in 3 patients indicates CFTR
- 422 dysfunction. We may predict total CFTR function *in vivo* from genotypes (Table 3) and the

423 CFTR channel activity of each variant. The activity of M470V+L1156F-CFTR is 50~80% of

424 the wild type CFTR (Figures 4 and 5), that of M470V+Q1352H-CFTR was ~1% of the wild

425 type (27), and that of M470V-CFTR was  $60\sim95\%$  of the wild type (10, 27) (Figures 4 and 5).

426 Thus the predicted total CFTR function of the present 8 cases with chronic pancreatitis

427 carrying L1156F may vary from ~30 to ~90% of the wild type. In addition, CFTR-coupled

428 Cl<sup>-</sup>HCO<sub>3</sub><sup>-</sup> exchange activity was substantially affected by M470V, L1156F, and the double

429 mutation (Figure 6). Thus CFTR-dependent HCO<sub>3</sub><sup>-</sup> transport by pancreatic duct is probably

430 further impaired, which may increase the risk of developing chronic pancreatitis.

431 In European populations, not only CF carriers (8) but also subjects carrying one

432 mild/uncommon CFTR mutation (45) are at an increased risk of chronic pancreatitis. A study

433 from United States reported that a risk of chronic pancreatitis was increased by the presence

434 of p.R75Q, a non CF-causing, HCO<sub>3</sub><sup>-</sup>-conductance impairing CFTR variant (40). Thus non

435 CF-causing CFTR variants increase the susceptibility to chronic pancreatitis in both

436 Europeans and Japanese. Chronic pancreatitis is now thought to be a complex disease caused

437 by complicated interactions between various genes and environmental factors (7). Mild CFTR

- 438 dysfunction due to non CF-causing variants may be one genetic factor. A recent study from
- 439 Germany reported that compound and trans-heterozygosity of CFTR, SPINK1, CTRC, and
- 440 *PRSS1* variants is an overt risk factor of idiopathic chronic pancreatitis (39).

June 1, 2015

441	Although alcohol abuse is the major cause of chronic pancreatitis, the mechanisms by which
442	ethanol induces pancreatic injury have not well been understood. Recent studies demonstrated
443	that ethanol and its non-oxidative metabolites, fatty acid ethyl esters inhibited the protein
444	expression and function of CFTR in pancreatic duct cells (23, 29). In the present study,
445	combination of EtOH and ALD (Figure 2) and POAEE (Figure 3) decreased the protein
446	expression of wild-type CFTR in HEK293 cells. Only a minority of heavy drinkers develop
447	pancreatitis, which suggests the existence of predisposing and protective genetic factors.
448	N34S-SPINK1 allele and CTRC variants were reported to be more frequent in patients with
449	alcoholic chronic pancreatitis than in controls, while loss-of-function variants of PRSS1 and
450	PRSS2 were overexpressed in controls when compared to patients with alcoholic pancreatitis
451	(7). On the other hand, the association of CFTR variants and alcoholic chronic pancreatitis
452	has not yet been clarified (37).
453	Although one of 18 patients (5.6%) with idiopathic chronic pancreatitis had L1156F, L1156F
454	was more prevalent (10.0%) in the patients with alcoholic chronic pancreatitis in the present
455	study (Table 2). One patient with alcoholic chronic pancreatitis had M470V+L1156F-CFTR
456	and N34S-SPINK1 (Table 3). In the present study, we have for the first time identified the
457	alcoholic chronic pancreatitis-susceptibility genotype, M470V+L1156F-CFTR. The protein
458	expression of M470V+L1156F-CFTR was not affected by EtOH, ALD, and POAEE (Figures
459	2 and 3). The association of L1156F and ethanol needs to be further investigated.
460	In summary, our present data suggest that the Japanese-specific CFTR variant, L1156F, is
461	associated with alcoholic chronic pancreatitis. L1156F is linked with the world-wide CFTR
462	variant M470V. Combination of L1156F and M470V impairs protein expression and
463	HCO <sub>3</sub> <sup>-</sup> /Cl <sup>-</sup> transport activity of CFTR and CFTR-coupled Cl <sup>-</sup> -HCO <sub>3</sub> <sup>-</sup> exchange activity. The
464	molecular mechanisms underlying the synergistic effects of L1156F and M470V need to be

465 investigated.

# 466 **ACKNOWLEDGEMENTS**

467 We thank Dr. Shmuel Muallem for suggestions.

## 468 **GRANTS**

- 469 This work was supported by Pancreas Research Foundation of Japan and grants from the
- 470 Japan Society for the Promotion of Science and the Research Committee of Intractable
- 471 Pancreatic Diseases (principal investigators: Tooru Shimosegawa, Yoshifumi Takeyama)
- 472 provided by the Ministry of Health, Labour, and Welfare of Japan.

473

# 475 **REFERENCES**

476	1.	Anzai C, Morokawa N, Okada H, Kamidono S, Eto Y, Yoshimura K. CFTR gene
477		mutations in Japanese individuals with congenital bilateral absence of the vas deferens. $J$
478		<i>Cyst Fibros</i> 2: 14-8, 2003.
479	2.	Aoyagi H, Okada T, Hasatani K, Mibayashi H, Hayashi Y, Tsuji S, Kaneko Y,
480		Yamagishi M. Impact of cystic fibrosis transmembrane conductance regulator gene
481		mutation on the occurrence of chronic pancreatitis in Japanese patients. J Int Med Res 37:
482		378-84, 2009.
483	3.	Argent B, Gray M, Steward M, Case M. Cell Physiology of Pancreatic Ducts. In:
484		Physiology of the Gastrointestinal Tract, edited by Johnson LR, Barrett KE, Ghishan FK,
485		Merchant JL, Said HM, Wood JD. pp. 1371-1396. Elsevier Academic Press, 2006.
486	4.	Audrézet MP, Chen JM, Le Maréchal C, Ruszniewski P, Robaszkiewicz M, Raguénès O,
487		Quéré I, Scotet V, Férec C. Determination of the relative contribution of three genes-the
488		cystic fibrosis transmembrane conductance regulator gene, the cationic trypsinogen gene,
489		and the pancreatic secretory trypsin inhibitor gene-to the etiology of idiopathic chronic
490		pancreatitis. Eur J Hum Genet 10: 100-6, 2002.
491	5.	Bombieri C, Claustres M, De Boeck K, Derichs N, Dodge J, Girodon E, Sermet I,
492		Schwarz M, Tzetis M, Wilschanski M, Bareil C, Bilton D, Castellani C, Cuppens H,
493		Cutting GR, Drevínek P, Farrell P, Elborn JS, Jarvi K, Kerem B, Kerem E, Knowles M,
494		Macek M Jr, Munck A, Radojkovic D, Seia M, Sheppard DN, Southern KW, Stuhrmann
495		M, Tullis E, Zielenski J, Pignatti PF, Ferec C. Recommendations for the classification of
496		diseases as CFTR-related disorders. J Cyst Fibros 10 Suppl 2: S86-102, 2011.

497 6. Braganza JM, Lee SH, McCloy RF, McMahon MJ. Chronic pancreatitis. *Lancet* 377

- 498 (9772): 1184-97. 2011.
- 499 7. Chen JM, Férec C. Chronic pancreatitis: genetics and pathogenesis. *Annu Rev Genomics*500 *Hum Genet* 10: 63-87, 2009.
- 8. Cohn JA, Neoptolemos JP, Feng J, Yan J, Jiang Z, Greenhalf W, McFaul C, Mountford R,
   Sommer SS. Increased risk of idiopathic chronic pancreatitis in cystic fibrosis carriers.
   *Hum Mutat* 26: 303-7, 2005.
- 504 9. Criddle DN, Murphy J, Fistetto G, Barrow S, Tepikin AV, Neoptolemos JP, Sutton R,
- 505 Petersen OH. Fatty acid ethyl esters cause pancreatic calcium toxicity via inositol
- trisphosphate receptors and loss of ATP synthesis. *Gastroenterology* 130: 781-93, 2006.
- 507 10. Cuppens H, Lin W, Jaspers M, Costes B, Teng H, Vankeerberghen A, Jorissen M,
- 508 Droogmans G, Reynaert I, Goossens M, Nilius B, Cassiman JJ. Polyvariant mutant cystic
- 509 fibrosis transmembrane conductance regulator genes. The polymorphic (Tg)m locus
- 510 explains the partial penetrance of the T5 polymorphism as a disease mutation. *J Clin*
- 511 *Invest* 101: 487-96, 1998.
- 512 11. Ferec C, Cutting GR. Assessing the Disease-Liability of Mutations in CFTR. *Cold Spring*513 *Harb Perspect Med* 2: a009480, 2012.
- 12. Freedman SD. New concepts in understanding the pathophysiology of chronic
- 515 pancreatitis. *Int J Pancreatol* 24: 1-8, 1998.
- 516 13. Fujiki K, Ishiguro H, Ko SB, Mizuno N, Suzuki Y, Takemura T, Yamamoto A,
- 517 Yoshikawa T, Kitagawa M, Hayakawa T, Sakai Y, Takayama T, Saito M, Kondo T,
- 518 Naruse S. Genetic evidence for CFTR dysfunction in Japanese: background for chronic
- 519 pancreatitis. J Med Genet 41: e55, 2004.

520	14. Furukawa-Hagiya T, Furuta T, Chiba S, Sohma Y, Sakurai M. The power stroke driven
521	by ATP binding in CFTR as studied by molecular dynamics simulations. J Phys Chem B
522	117: 83-93, 2013.

- 523 15. Greeley T, Shumaker H, Wang Z, Schweinfest CW, Soleimani M. Downregulated in
- adenoma and putative anion transporter are regulated by CFTR in cultured pancreatic
  duct cells. *Am J Physiol Gastrointest Liver Physiol* 281: G1301-8, 2001.
- 16. Hanawa M, Takebe T, Takahashi S, Koizumi M, Endo K. The significance of the sweat
  test in chronic pancreatitis. *Tohoku J Exp Med* 125: 59-69, 1978.
- 528 17. Hirota M, Shimosegawa T, Masamune A, Kikuta K, Kume K, Hamada S, Kihara Y,
- 529 Satoh A, Kimura K, Tsuji I, Kuriyama S; Research Committee of Intractable Pancreatic
- Diseases. The sixth nationwide epidemiological survey of chronic pancreatitis in Japan.
   *Pancreatology* 12: 79-84, 2012.
- 18. Hwang TC, Kirk KL. The CFTR ion channel: gating, regulation, and anion permeation. *Cold Spring Harb Perspect* Med 3 (1): a009498, 2013.
- Imaizumi Y. Incidence and mortality rates of cystic fibrosis in Japan, 1969-1992. *Am J Med Genet* 58: 161-168, 1995.
- 536 20. Ishiguro H, Nakakuki M, Yamamoto A, Fujiki K, Naruse S, Yoshimura K, Shimosegawa
- 537 T, and the Research Committee of Intractable Pancreatic Diseases, the Ministry of Health,
- Labor, and Welfare of Japan. Incidence, prognosis, and *CFTR* mutations of cystic fibrosis
- in Japan. *Pediatr Pulmonol* Suppl 36: 375-376, 2013 (abstract).
- 540 21. Ishiguro H, Steward MC, Naruse S, Ko SB, Goto H, Case RM, Kondo T, Yamamoto A.
- 541 CFTR functions as a bicarbonate channel in pancreatic duct cells. *J Gen Physiol* 133:

543	22.	Izumikawa K, Tomiyama Y, Ishimoto H, Sakamoto N, Imamura Y, Seki M, Sawai T,
544		Kakeya H, Yamamoto Y, Yanagihara K, Mukae H, Yoshimura K, Kohno S. Unique
545		mutations of the cystic fibrosis transmembrane conductance regulator gene of three cases
546		of cystic fibrosis in Nagasaki, Japan. Intern Med 48: 1327-31, 2009.
547	23.	Judák L, Hegyi P, Rakonczay Z Jr, Maléth J, Gray MA, Venglovecz V. Ethanol and its
548		non-oxidative metabolites profoundly inhibit CFTR function in pancreatic epithelial cells
549		which is prevented by ATP supplementation. <i>Pflugers Arch</i> 466: 549-62, 2014.
550	24.	Kimura S, Okabayashi Y, Inushima K, Yutsudo Y, Kasuga M. Polymorphism of cystic
551		fibrosis gene in Japanese patients with chronic pancreatitis. Dig Dis Sci 45: 2007-12,
552		2000.
553	25.	Kitagawa M, Naruse S, Ishiguro H, Nakae Y, Kondo T, Hayakawa T. Evaluating
554		exocrine function tests for diagnosing chronic pancreatitis. Pancreas 15: 402-8, 1997.
555	26.	Ko SB, Shcheynikov N, Choi JY, Luo X, Ishibashi K, Thomas PJ, Kim JY, Kim KH, Lee
556		MG, Naruse S, Muallem S. A molecular mechanism for aberrant CFTR-dependent HCO <sub>3</sub> <sup>-</sup>
557		transport in cystic fibrosis. EMBO J 21: 5662-72, 2002.
558	27.	Lee JH, Choi JH, Namkung W, Hanrahan JW, Chang J, Song SY, Park SW, Kim DS,
559		Yoon JH, Suh Y, Jang IJ, Nam JH, Kim SJ, Cho MO, Lee JE, Kim KH, Lee MG. A
560		haplotype-based molecular analysis of CFTR mutations associated with respiratory and
561		pancreatic diseases. Hum Mol Genet 12: 2321-32, 2003.
562	28.	Lee MG, Ohana E, Park HW, Yang D, Muallem S. Molecular mechanism of pancreatic
563		and salivary gland fluid and HCO <sub>3</sub> <sup>-</sup> secretion. <i>Physiol Rev</i> 92: 39-74, 2012.

- 29. Maléth J, Balázs A, Pallagi P, Balla Z, Kui B, Katona M, Judák L, Németh I, Kemény LV,
- 565 Rakonczay Z Jr, Venglovecz V, Földesi I, Pető Z, Somorácz Á, Borka K, Perdomo D,
- Lukacs GL, Gray MA, Monterisi S, Zaccolo M, Sendler M, Mayerle J, Kühn JP, Lerch
- 567 MM, Sahin-Tóth M, Hegyi P. Alcohol disrupts levels and function of the cystic fibrosis
- transmembrane conductance regulator to promote development of pancreatitis.
- 569 *Gastroenterology* 148: 427-39, 2015.
- 570 30. Morokawa N, Iizuka S, Tanano A, Katsube A, Muraji T, Eto Y, Yoshimura K. Severe
- 571 cystic fibrosis in a Japanese girl caused by two novel CFTR (ABCC7) gene mutations:
- 572 M152R and 1540del10. *Hum Mutat* 15: 485, 2000.
- 573 31. Nakakuki M, Fujiki K, Yamamoto A, Ko SB, Yi L, Ishiguro M, Yamaguchi M, Kondo S,
- 574 Maruyama S, Yanagimoto K, Naruse S, Ishiguro H. Detection of a large heterozygous
- deletion and a splicing defect in the CFTR transcripts from nasal swab of a Japanese case
- 576 of cystic fibrosis. *J Hum Genet* 57: 427-33, 2012.
- 577 32. Nakano E, Masamune A, Niihori T, Kume K, Hamada S, Aoki Y, Matsubara Y,
- 578 Shimosegawa T. Targeted next-generation sequencing effectively analyzed the cystic
- fibrosis transmembrane conductance regulator gene in pancreatitis. *Dig Dis Sci* 60:
- 580 1297-307, 2015.
- 33. Naruse S, Ishiguro H, Shirota K, Nakakuki M, Yamamoto A, Kondo T. Sweat chloride
  measurement with a highly sensitive electrode. *Pancreas* 33: 100, 2006.
- 583 34. Naruse S, Ishiguro H, Suzuki Y, Fujiki K, Ko SB, Mizuno N, Takemura T, Yamamoto A,
- 584Yoshikawa T, Jin C, Suzuki R, Kitagawa M, Tsuda T, Kondo T, Hayakawa T. A finger
- sweat chloride test for the detection of a high-risk group of chronic pancreatitis. *Pancreas*28: e80-85, 2004.
- 587 35. Pallagi P, Balla Z, Singh AK, Dósa S, Iványi B, Kukor Z, Tóth A, Riederer B, Liu Y,

588		Engelhardt R, Jármay K, Szabó A, Janovszky A, Perides G, Venglovecz V, Maléth J,
589		Wittmann T, Takács T, Gray MA, Gácser A, Hegyi P, Seidler U, Rakonczay Z Jr. The
590		role of pancreatic ductal secretion in protection against acute pancreatitis in mice. Crit
591		<i>Care Med</i> 42: e177-88, 2014.
592	36.	Paranjape SM, Zeitlin PL. Atypical cystic fibrosis and CFTR-related diseases. Clin Rev
593		Allergy Immunol 35: 116-23, 2008.
594	37.	Perri F, Piepoli A, Stanziale P, Merla A, Zelante L, Andriulli A. Mutation analysis of the
595		cystic fibrosis transmembrane conductance regulator (CFTR) gene, the cationic
596		trypsinogen (PRSS1) gene, and the serine protease inhibitor, Kazal type 1 (SPINK1) gene
597		in patients with alcoholic chronic pancreatitis. Eur J Hum Genet 11: 687-92, 2003.
598	38.	Rohlfs EM, Zhou Z, Heim RA, Nagan N, Rosenblum LS, Flynn K, Scholl T, Akmaev
599		VR, Sirko-Osadsa DA, Allitto BA, Sugarman EA. Cystic fibrosis carrier testing in an
600		ethnically diverse US population. Clin Chem 57: 841-8, 2011.
601	39.	Rosendahl J, Landt O, Bernadova J, Kovacs P, Teich N, Bödeker H, Keim V, Ruffert C,
602		Mössner J, Kage A, Stumvoll M, Groneberg D, Krüger R, Luck W, Treiber M, Becker M,
603		Witt H. CFTR, SPINK1, CTRC and PRSS1 variants in chronic pancreatitis: is the role of
604		mutated CFTR overestimated? Gut 62: 582-92, 2013.
605	40.	Schneider A, Larusch J, Sun X, Aloe A, Lamb J, Hawes R, Cotton P, Brand RE,
606		Anderson MA, Money ME, Banks PA, Lewis MD, Baillie J, Sherman S, Disario J,
607		Burton FR, Gardner TB, Amann ST, Gelrud A, George R, Rockacy MJ, Kassabian S,
608		Martinson J, Slivka A, Yadav D, Oruc N, Barmada MM, Frizzell R, Whitcomb DC.

- 609 Combined bicarbonate conductance-impairing variants in CFTR and SPINK1 variants are
- 610 associated with chronic pancreatitis in patients without cystic fibrosis. *Gastroenterology*

June 1, 2015

- 611 140: 162-71, 2011.
- 41. Shcheynikov N, Wang Y, Park M, Ko SB, Dorwart M, Naruse S, Thomas PJ, Muallem S.
  Coupling modes and stoichiometry of Cl<sup>-</sup>/HCO<sub>3</sub><sup>-</sup> exchange by slc26a3 and slc26a6. *J Gen Physiol* 127: 511-24, 2006.
- 42. Shimosegawa T, Kataoka K, Kamisawa T, Miyakawa H, Ohara H, Ito T, Naruse S, Sata
- 616 N, Suda K, Hirota M, Takeyama Y, Shiratori K, Hatori T, Otsuki M, Atomi Y, Sugano K,
- 617 Tanaka M. The revised Japanese clinical diagnostic criteria for chronic pancreatitis. J
  618 *Gastroenterol* 45: 584-91, 2010.
- 43. Strong TV, Wilkinson DJ, Mansoura MK, Devor DC, Henze K, Yang Y, Wilson JM,
- Cohn JA, Dawson DC, Frizzell RA, Collins FS. Expression of an abundant alternatively
  spliced form of the cystic fibrosis transmembrane conductance regulator (CFTR) gene is
  not associated with a cAMP-activated chloride conductance. *Hum Mol Genet* 2: 225-30,
  1993.
- 44. Tsuda T, Noda S, Kitagawa S, Morishita T. Proposal of sampling process for collecting
  human sweat and determination of caffeine concentration in it by using GC/MS. *Biomed Chromatogr* 14: 505-10, 2000.
- 45. Weiss FU, Simon P, Bogdanova N, Mayerle J, Dworniczak B, Horst J, Lerch MM.
- 628 Complete cystic fibrosis transmembrane conductance regulator gene sequencing in
- patients with idiopathic chronic pancreatitis and controls. *Gut* 54: 1456-60, 2005.
- 46. Yadav D, Lowenfels AB. The epidemiology of pancreatitis and pancreatic cancer. *Gastroenterology*144: 1252-61, 2013.
- 47. Yamashiro Y, Shimizu T, Oguchi S, Shioya T, Nagata S, Ohtsuka Y. The estimated

- 633 incidence of cystic fibrosis in Japan. J Pediatr Gastroenterol Nutr 24: 544-547, 1997.
- 48. Yoshimura K, Wakazono Y, Iizuka S, Morokawa N, Tada H, Eto Y. A Japanese patient
- homozygous for the H1085R mutation in the CFTR gene presents with a severe form of
- 636 cystic fibrosis. *Clin Genet* 56: 173-5, 1999.

#### 638 FIGURE CAPTIONS

#### 639 Figure 1: Protein expression of CFTR variants transfected in HEK293 cells

- (A) HEK293 cells transfected with wild-type CFTR and 3 CFTR variants (M470V-CFTR, 640
- 641 L1156F-CFTR, M470V+L1156F-CFTR) were lysed and the separated proteins were blotted
- 642 with antibodies against the NBD2 domain of CFTR (M3A7, Millipore, Billerica, MA) and
- 643 β-actin. Negative control: non-transfected cells. (B) The staining intensities of CFTR and
- $\beta$ -actin bands were analyzed and the CFTR/ $\beta$ -actin ratios were compared. Data are shown as 644
- 645 means  $\pm$  SEM of 8 independent transfection experiments. \* p <0.01, compared to wild-type
- 646 CFTR.

657

#### 647 Figure 2: Effects of ethanol and acetaldehyde on the protein expression of wild-type and 648 M470V+L1156F CFTR in HEK293 cells

- 649 (A) HEK293 cells were transfected with wild-type or M470V+L1156F CFTR and treated
- 650 with ethanol (EtOH, 50 mM) and/or acetaldehyde (ALD, 200 µM) for 24 hours. The separated
- 651proteins were blotted with antibodies against the NBD2 domain of CFTR (sc-10747, Santa
- 652 Cruz, Dallas, TX) and  $\beta$ -actin. C: non-transfected cells. (B) The CFTR/ $\beta$ -actin ratios were
- compared. Data are shown as means  $\pm$  SEM of 5 independent transfection experiments. \* 653
- p < 0.05, compared to non-treated control. # p < 0.05, compared to wild-type CFTR. 654

#### 655Figure 3: Effects of fatty acid and fatty acid ethyl ester on the protein expression of wild-type and M470V+L1156F CFTR in HEK293 cells 656

- (A) HEK293 cells were transfected with wild-type or M470V+L1156F CFTR and treated
- 658 with palmitoleic acid (POA, 100  $\mu$ M) or palmitoleic acid ethyl ester (POAEE, 100  $\mu$ M) for 48
- 659 hours. The separated proteins were blotted with antibodies against the NBD2 domain of
- 660 CFTR (sc-10747, Santa Cruz, Dallas, TX) and  $\beta$ -actin. (B) The CFTR/ $\beta$ -actin ratios were

- 661 compared. Data are shown as means  $\pm$  SEM of 6 independent transfection experiments. \*
- p < 0.05, compared to non-treated control. # p < 0.05, compared to wild-type CFTR.

#### 663 Figure 4: Cl<sup>-</sup> channel activity of CFTR variants expressed in HEK293 cells

- 664 Cl<sup>-</sup> channel activity of wild-type CFTR (black trace) and 3 CFTR variants (M470V-CFTR:
- blue; L1156F-CFTR: green; M470V+L1156F-CFTR: red) were measured in the whole cell
- 666 configuration. After establishing the whole cell configuration with NMDG-Cl rich solutions,
- 667 CFTR was activated with 10 μM forskolin. Currents were recorded at a holding potential of
- -60 mV and peak currents were normalized as current densities (pA/pF). After the peak
- 669 current was observed, glibenclamide (100  $\mu$ M) was added to the bath solution. Data are
- shown as representative traces (A) and means  $\pm$  SEM of 7-9 experiments (B).

# Figure 5: HCO<sub>3</sub><sup>-</sup> and Cl<sup>-</sup> transport by CFTR variants expressed in *Xenopus laevis*oocvtes

- 673 Xenopus oocytes expressing wild-type CFTR or 3 CFTR variants (M470V-CFTR,
- L1156F-CFTR, M470V+L1156F-CFTR) were first bathed in HCO<sub>3</sub><sup>-</sup>-free media. The bath
- solution was switched to HCO<sub>3</sub><sup>-</sup>-CO<sub>2</sub>-buffered solution as indicated. After the stabilization of
- $pH_i$ , the bath solution was switched to Cl<sup>-</sup>-free HCO<sub>3</sub><sup>-</sup>-CO<sub>2</sub>-buffered solution in the presence
- of forskolin (1  $\mu$ M). The initial rates of pH<sub>i</sub> (A) and Cl<sup>-</sup><sub>i</sub> (B) changes were used to calculate
- the HCO<sub>3</sub><sup>-</sup> and Cl<sup>-</sup> fluxes. (C) Means  $\pm$  SEM of HCO<sub>3</sub><sup>-</sup>/Cl<sup>-</sup> fluxes and membrane current of
- 3-6 experiments. \* p < 0.01, compared to wild-type CFTR.

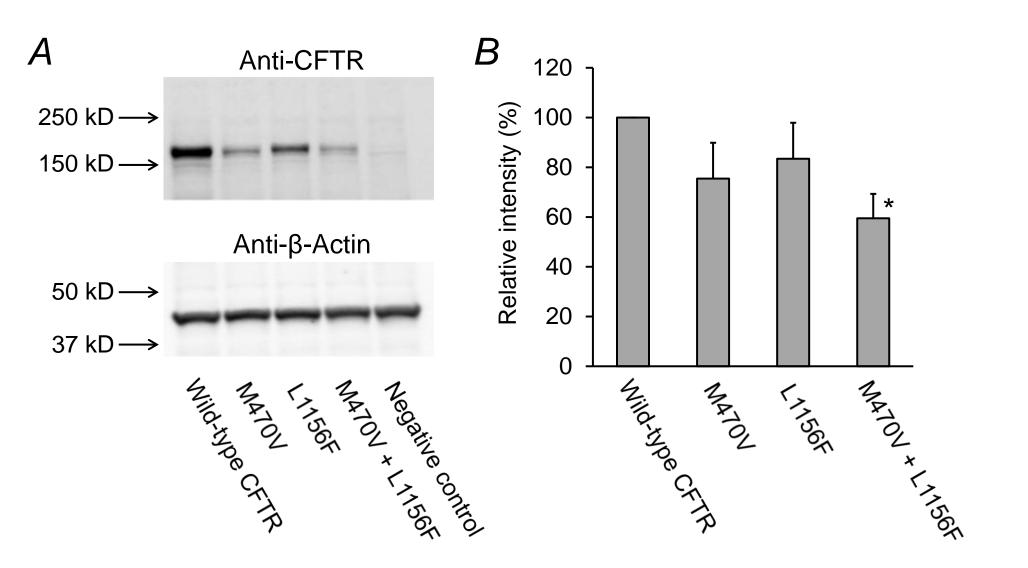
### 680 Figure 6: Cl<sup>-</sup>HCO<sub>3</sub><sup>-</sup> exchange activity in CFPAC-1 cells transfected with CFTR variants

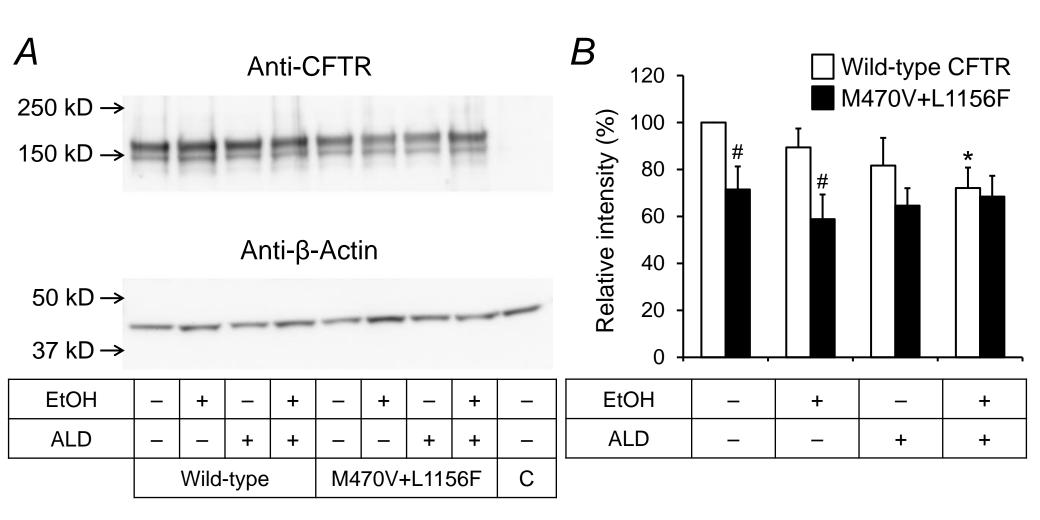
- 681 CFPAC-1 cells transfected with wild-type CFTR (black trace), 3 CFTR variants
- 682 (M470V-CFTR: blue; L1156F-CFTR: green; M470V+L1156F-CFTR: red), and vector alone
- (gray) were first bathed in the standard HCO<sub>3</sub><sup>-</sup>-CO<sub>2</sub>-buffered solution containing 1  $\mu$ M

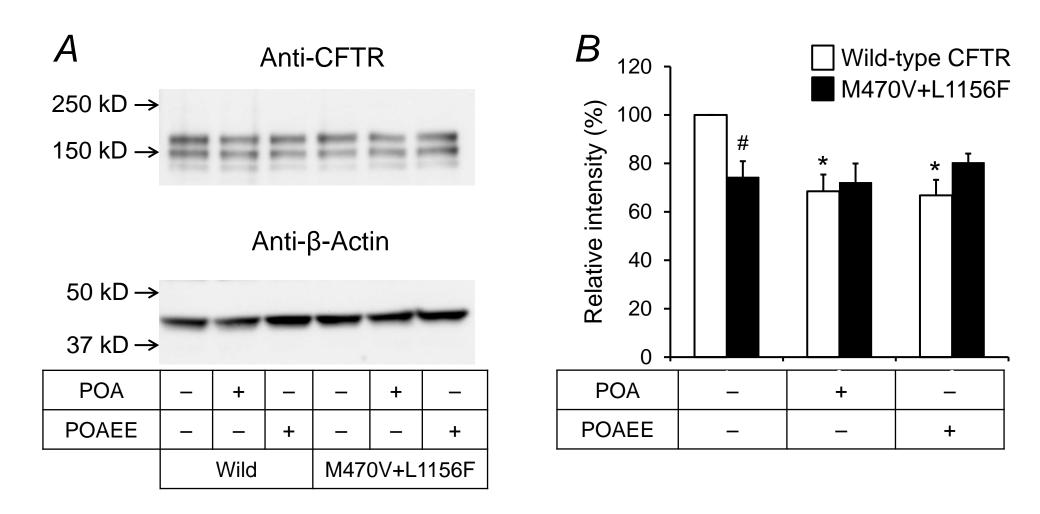
- 684 forskolin. The bath solution was switched to Cl<sup>-</sup>-free HCO<sub>3</sub><sup>-</sup>-CO<sub>2</sub>-buffered solution in the
- 685 presence of forskolin as indicated. (A) Representative traces are shown. (B) The activity of
- $CI^{-}HCO_{3}^{-}$  exchange was estimated from the rate of pH<sub>i</sub> increase upon removal of Cl<sup>-</sup>. Data
- are shown as means  $\pm$  SEM of 20-25 experiments. \* p<0.01, compared to wild-type CFTR. #
- 688 p<0.05, compared to L1156F-CFTR.

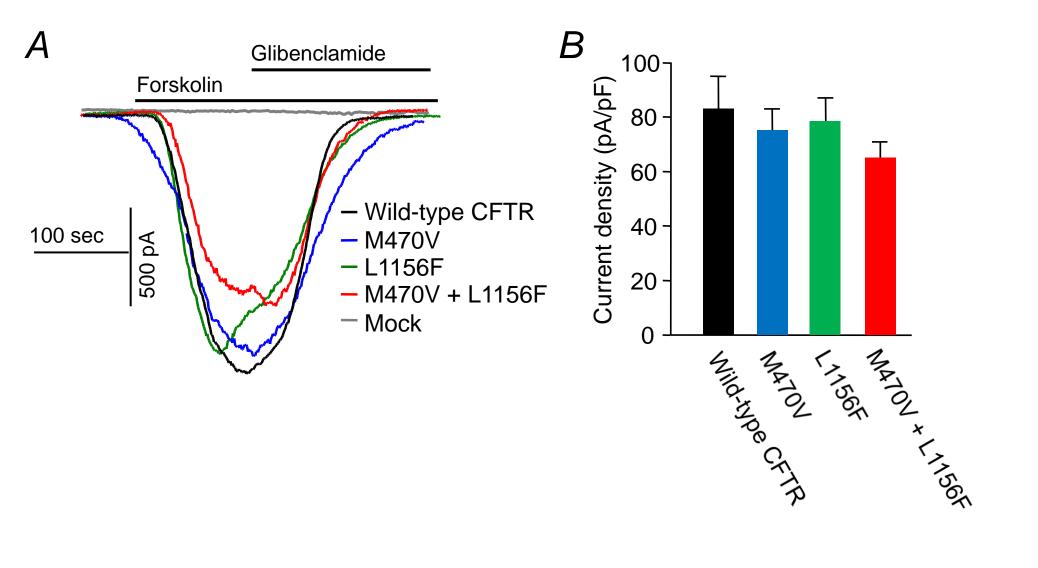
## 689 Figure 7: Localization of M470 and L1156 in homology model of CFTR

- An molecular model of CFTR in the inward-facing conformation (closed state). The TMD1,
- NBD1, TMD2, and NBD2 are colored in green, lime, blue, and purple, respectively, with
- ribbon representation. M470 and L1156F are indicated.









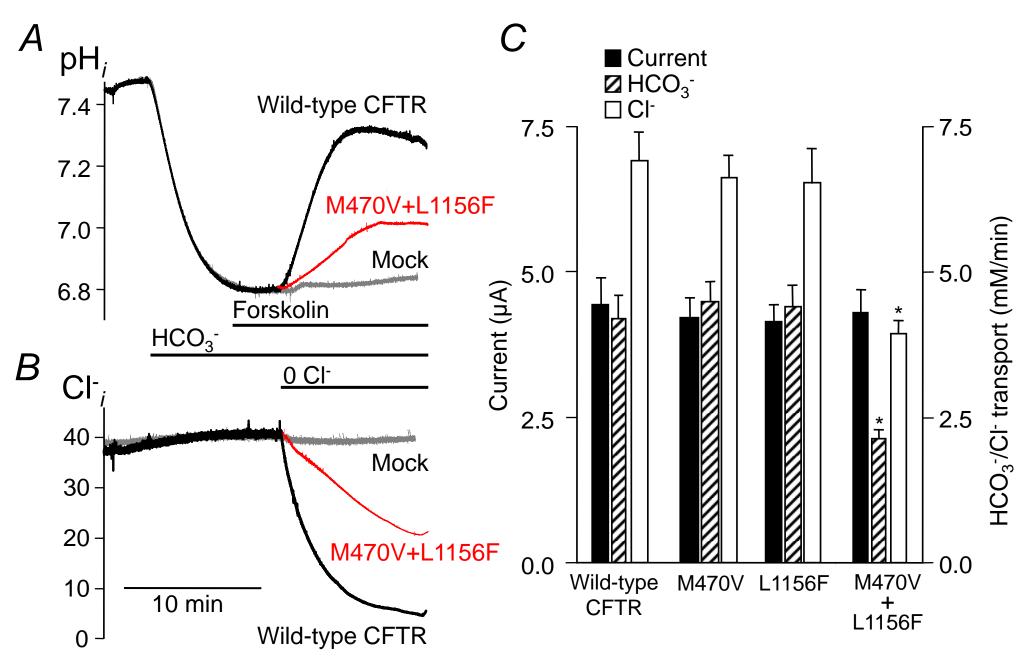
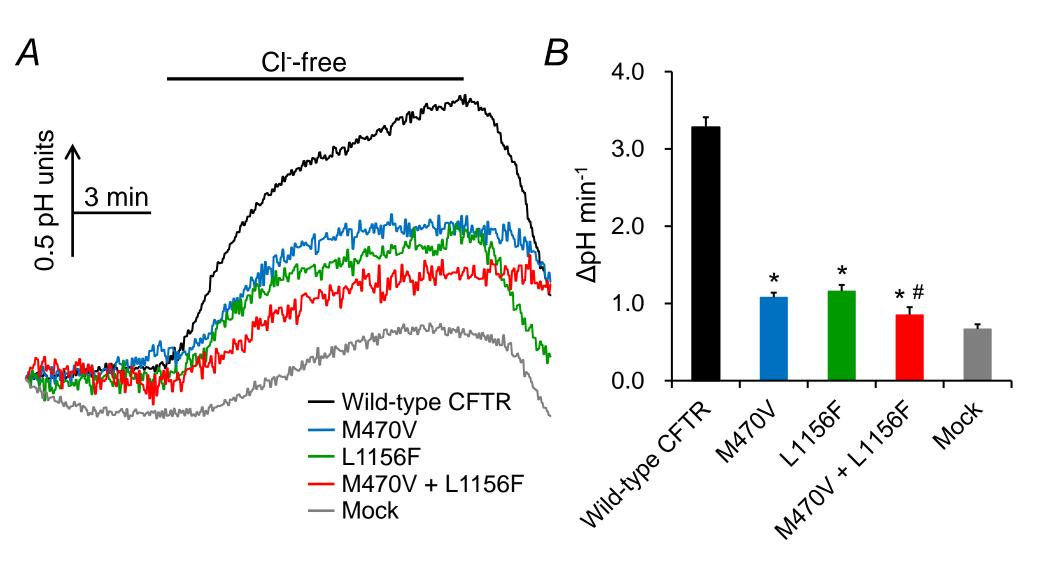
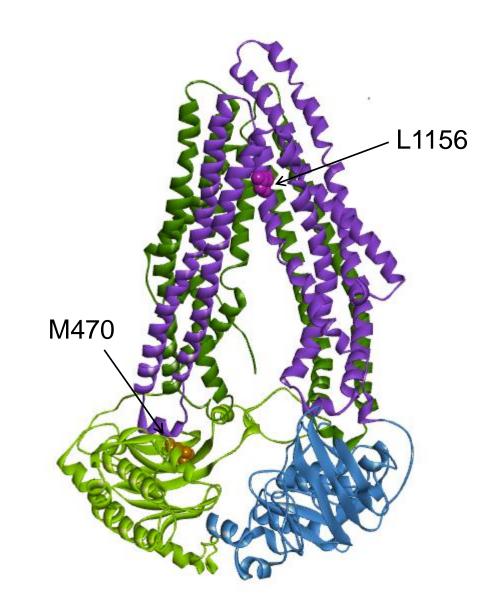


Figure 5





CFTR       SUTR       GCCCCTCAGAGAGTTGAAGA       TGATCCTAGTCGGGTTCCTG         S' UTR       AAACGTAACAGGAACCCGACTA       CTCAACCCTTTTTCTCTGACCT         Svon 1       GGAGAAAGCCGCTAGAGCAAA       GTGGCTCTCTATTCAATCAGCC         Exon 2       TGTAAGAGATGAAGCCTGGTA       GCTCCTATTTCAATCAGCC         Exon 3       CCATGAGATTTTGTCTCTATA       GAGTTGGATTCACTCTTTATA         Exon 4       AAGAGTTCACATATGGTATG       TGCCATTTATTTAATAGGCAT         Exon 5       GAAGATAGTAAGCAGATGAA       AATTGACCTTTCTTAATA         Exon 6a       TGCTCAGAACCACGAAGTGT       ATTAGCTGGGTGGTGGTGCAT (1st)         CTGAGGCAGGAAAGACTCTGAA       TCCTAGTATTAGCTGGCAACT       Exon 6b         Exon 7       TATAGCAGAAAGACTCTAGA       TCCTAGTATTAGCTGGCAACT         Exon 8       TGGGTATTCTGACAGG       TCCTAGTATAGCAGAAGATCCT         Exon 9       GGCCATGTGCTTTCAAACT       TCGCCATGGCAAAAGACT         Exon 10       TTGTGCATAGCAGAGTACCTGAAA       ATTGATCCATTCACAGGAACACA         Exon 12       CTTCTGCACCACTTTTGAAGA       GCTACATTCCATGCAAAGACT         Exon 13       GGTACCATTTAGTTGCACA       GGAAGATATTCACAGTACTTTGC         Exon 14       ACACTTAGATTCAAGTAATCCTATGAGAACACACACACAC			
5' UTRGCCCCTCAGAGAGTTGAAGATGATCCTAGTCGGGTTCCTG5' UTRAAACGTAACAGGAACCCGACTACTCAACCCTTTTTTCTCTGACCTExon 1GGAGAAAGCCGCTAGAGCAAAGTGGCTCTCATTCAATCAGCExon 2TATTCCCTCCCCAATCCCTTTTGGGATTACAGGCATTAGCCExon 3CCATGAGATTTGTCTCTATAGACTTGGATTCATCCTTTAAATAGExon 4AAGAGTTCACATATGGTATGTGCCATTTATTAATAGGCATExon 5GAAGATAGTAAGCAGAGTAGAAAATTGACCTTTCTTAGTTTCCExon 6aTGCTCAGAACCACGAAGGATCTAGGTGGTGGTGCAT (1st)Exon 7TATAGGCAGGAAGCACCACGAAGGATCCTAGAATTGACAGAACTExon 8TGGGTAATTCAGGGTGCTTCAAACTCGCCATTAGATTAGCAGAACTExon 9GGCCATGTCCTTTCAAAACTTCGCCATGGCATGAAATCCAExon 10TTGTGCATAGCAGGAGTACCTGAAAATTGATCCATTCACAGTAGCAExon 11TCAGCAATGTGTTTTTGACCCCAAGATACGGGCACAGATTExon 12CTTCTGACAACTTTTGACAGATTGATCCATTCACAGTAGCAExon 13GGTACCAATTTAATACTACAGATTGAAGGGAGTCTTTTGCExon 13TGGGATGGGATGCTCCTGTCGGAAGAGATATGTCCATTGCExon 14ACACTTAGATTCAGGTAGCTCTTCAGAAGCAAGAACTATATGCExon 15GGGTCAAGGGCCTGCTGTCAGAAGCAAGAACTATATGCExon 16TCTGAAGGGTGCATGCTCTTTAGACCAAGGACTGCACTGExon 17aAGAAATAACCAGACACAAAGCAAATTCCACTACCATAAGCTGGCACTGGExon 18GAGCCAGGAACCACAAGCAAATTCCACAAGGACTCCATGGTCAExon 17aAGAAATAACCAGACGACTGTGTCAAAAGGAGCACCACGTGTExon 17aAGAAATAACCAGGCACTGGAATTCCACAACGCACTGGGAACTAAGAExon 17aAGAAATAACCAAGGACCACGTGTCCAAACGCACGGACTCCAAA </td <td>Exon/Intron</td> <td>Forward primers (5'-3')</td> <td>Reverse primers (5'-3')</td>	Exon/Intron	Forward primers (5'-3')	Reverse primers (5'-3')
5'UTRAAACGTAACAGGAACCCGACTACTCAACCCTTTTTCCATCAGCExon 1GGAGAAAGCCGCTAGAGCAAAGTGGCTCTCTATTCCAGCExon 2TATTCCCTCCCATCCCTTTTGGGATTACAGGCATTAGCCExon 3CCATGAGATTTGTCTCTATAGAGTTGGATTCATCCTTTATAExon 4AAGAGTTTCACATATGGTATGTGCCATTTTTTAAATAGCATExon 5GAAGATAGTAAGCAGCTAGATGAAATTGACCTTCTTATTTAATAGGCATExon 6TGCTCAGAACCACGAAGTGATATTAGCTGGGTGGTGCAT (1st) CTGAGGCAGGAAGATGCTGATGACAGCGTGCAGGACAGGAAATTCGCACExon 6aTGCTCAGAACCACGAAGTGATCCTAGGATAGTAGCTGGCAACExon 7TATAGGCAGAAAGACTCTAGATCCTAGTATTAGCTGGCAACTExon 8TGGGTAATTCAGGGTTGCTTCGCCATTAGGATGAAATCCAExon 9GGCCATGTGCTTTTCAAACTTCGCCATGGCAACAGAACACExon 10TTGTGCATAGCAGAGTACCTGAAATTGATCCATCACAGTACGCExon 11TCAGCAATGTTGTTTTTGACCCCAAGATACTGGCATACCAAExon 12CTTCTGCACCACTTTGAGAAATTGTCAGCAAGATCTCACAAExon 13TGGGTAGTGTTTCTTCTCACCGGAAGAGATATTGCCATTGCExon 14GACCCAGGAACCAAAAGCAAAATTCCACTATGATTTAGTExon 15GGTACAAGTTAGTCCATTCCAGAAGCAAGAACAATGTCCATTGExon 16GCTCAAGGAACCAAAGCAAATTCCACTACCATAATGCTGGExon 17CACCAGCATGGCATCCTGTTAGACTCAAGGTCAACCExon 18GGGTAAGGAACCAAAGCAAATTCCACTACGCATAAGGTCAACCExon 19GCCCAGAAAGAACACAAGCAACCCAATGACACAGGACTTCAACExon 17CACACAGCATGGCACTGTGAGCTACAGGTACACAGGACTTCAACExon 17CACACAGCAAGGACCACAGGATTCCAATGACACAGGACTTGGACACCExon 17 </td <td></td> <td></td> <td></td>			
Exon 1GGAGAAAGCCGCTAGAGCAAAGTGGCTCTCTATTCAATCAGCExon 2TGTAAGAGATGAAGCCTGGTAGCTCCTATTTTAACAGGCATTAGCCExon 3CCATGAGATTTGCTCTATAGAGTTGGATTCACTCTTTTAAExon 4AAGAGTTTCACATATGGTATGTGCCATTATTTAATAGGCATExon 5GAAGATAGTAAGCTAGATGAAAATTGACCTTTCTTAGTTTCCExon 6aTGCTCAGAACCACGAGAGAAAATTGACCTTTGTAGTGGTGGCAT (1st)Exon 7TATAGGCAGAAACACGGGTGCATGAAATTGACAGAACTExon 8TGGGTAATTCAGGTTGTACAGCGTCCAGGACGGAAATTGCTT (2nd)Exon 9GGCCAGTGCCTTTCAAACTCGCCATGAGATACAGExon 9GGCCATGTGCTTTCCAAACTCGCCATGGGCAGAAAGCCExon 9GGCCATGGCTTTTCAAACTTCGCCATGGCAGAGAACACAExon 9GGCCATGTGCTTTCAAACTCGCCATGGCAGAGACACAExon 10TTGTGCATAGCAGAGAGACCTGAAAATTGATCACAGCAGAGACACAExon 11TCAGCAATGTGTTTTGACCCCAAGATACGGGCACAGATExon 12CTTCTGCACCACTTTTGAGAAACTTAAAGGAGATGCTCTGTExon 13GGTACCAATTAAATACTACAGATTGATAGGGAGATCTTTGCExon 14aAACCTTAGATCAAGTAAAACATCCCATCACATAAGAACTAATGACExon 15GGTAAGGGTGCATGCTCTGTTCGAAAGCAAAGAACAATAGGAExon 16TCTGAAGGCGCACAGTGTACCAAAGAAGACACAAGACAAAGAACAATATGGAExon 17CAACCAGGAAGTCCTGGAGCAAAGCACACAAGGACGCACTGCACATAExon 18AGAACCAAGGACACACAACACAACACCCATGCTGCACATTAGGTCCACTExon 19GCCCAGCAAAGTAATCACGACACACCCAAGGTTGGACCATTGGTGAACACAExon 17CAACAGAGGACGCACAGTGTGGACACACACCAACGGTGGCACCTGGAAAExon 17AGAAATAATCACTGGCACACAGAACACAACACAACACAC			
Exon 2TATTCCCTCCCAATCCCTTTTGGGATTACAGGCATTAGCCExon 3CCATGAGATTTGTCTCTATAGAGTTGGATTCTTTAATAAGExon 4AAGAGTTTCACATATGGTATGTGCCATTATTTAATAGGCATExon 5GAAGATAGTAAGCTAGATGAAAATTGACCTTTCTTAGTTTCCExon 6aTGCTCAGAACCACGAAGTGTATTAGCTGGGTGGGTGCAT (Jst)CTGAGGCAGAGATTGACAGGATGCATGATTGACAGAACTExon 7TATAGGCAGAAAGACTCTAGATCCTAGTATTGACAGAAACTExon 8TGGGTAATTCAGGGTTGTTCGCCATGATTAGCTGGCAACACTExon 9GGCCATGTGCTTTCAAACTTCGCCATGGAGAAAATCCAExon 10TTGTGCATAGCAGAGTACTGAAAATTGATCACTGCAAGAATCCAExon 11TCAGCAATGTGTTTTCAAACTTCGCCATGGGCACAGATACAGExon 12CTTCTGCACACCTTTTGAGAAATTGATCCATTCACAGTGACTExon 13GGCACAGTGTGATTCTTTCGACAGGGAAGGAGTCTTTTGCExon 14ACACTTAGATTCATGACTCTGTTCGTAATCCTATGATTATGTExon 15GGTTAAGGGTGCATGCTCTTCAGAAGCTAAGAACTATATGAExon 14ACACTTAGATTCAAGTACAGCAAATTCCACTACCATATGGGExon 15GGTTAAGGGTGCATGCTCTTCAAGCCAAGGTACATTGTCCATTExon 16TCTGAAGCGTCACTGTATCCAAATGACAGGACTTCAATExon 17CACCAGCATGGCACCAGTGTACCAAAATGAAGAAGAAATAAExon 17CACAGGAAGGACCACAGTGTCCAAAATGAAGAAATAAGGExon 17CACCAGCATGGCACCAGTGTACCAAAATGAAGAAATAAGGExon 17CACAGAATAAATCACTGACACACCATGTGTGCACCAGGAATTAExon 17CACCAGCATGGCACCAGTGTACCAAAATGAAGAAATAGGCCACAGGAAExon 17CACCAGCATGGCACCAGTGTACCAAAATGAAAGTCACATGGGCACTGAAExon 17CACA	5' UTR	AAACGTAACAGGAACCCGACTA	CTCAACCCTTTTTCTCTGACCT
Exon 2TGTAAGAGATGAAGCCTGGTAGCTCCTATTTTTAAATATAAGExon 3CCATGAGATTTTGTCTCTATAGAGTTGGATTCATCCTTTAAExon 4AAGAGTTTCACATATGGTATGTGCCATTTATTAATAGGCATExon 5GAAGATAGTAAGCAGAGAGAAAATTGACCTTTCTTAGTTTCCExon 6aTGCTCAGAACCACGAAGTGTATTAGCTGGGTGGGTGGAT (1st)CTGAGGCAGGAGAATGAGTCTGTACAGGGTGCATGAATTTGACAGGAACTExon 7TATAGGCAGAAAGACTCTAGATCCTAGGATATTGACAGAACTExon 8TGGGTAATTCAAGGGTTGCTTCGCCATTAGGTGGAAACAGExon 9GGCCATGTGCTTTTCAAACTTCGCCATGTGCAAGATACAGExon 10TTGTGCATAGCAGAGTACCTGAAAATTGATCCATTCACAGGTGCTExon 11TCAGCAATGTGTTTTGACCCCAAGATACGGGCACAGAGTExon 12CTTCTGCACCACTTTTGAGAAGCTACATTCTGCCCATACCAAExon 13GGTACCAATTTAATTACTACAGATTTGTAAGGGAGTCTTTGCExon 14ACACTTAGATTCAAGAGAATACTCAGAAGGTAAGTCCATTGCExon 13CGTAAGGAGATGCTCCTGTTCGTAATCCATGAGATTTAGTExon 14ACACTTAGATTCAAGAAATACTCAGAAGCTAAGAGCATTAGAExon 15TGGAATGCAAGCACAAAGCAAATTCCACTACCATAAGCTTGGExon 16TCTGAATGCGTCATGCTTCAAGCACAGGACTTCAACExon 17TAGACCAGGACACAAGACACACTATGAATGCATTGCTATGGExon 17AGAAATAACACTGGCACAGTGTCCAAAATGAAGTCACATGGTGCAExon 17AGAAATAGCCAGTGTGATTCCACTACGAGTACTGGGAATExon 17CACCAGCATGGCACAGTGTACCAAAATGAAGTCACATGGTGCATCGExon 17CACCAGGAAGGAAGTGCACAGGGCCATGTGTGCATTGCAACGGACTTCAACExon 17CAGAAGAGGGACCCAGTGGTCCAAAATGGGCACCTCAAATExon 1	Exon 1	GGAGAAAGCCGCTAGAGCAAA	GTGGCTCTCTATTCAATCAGC
Exon 3CCATGAGATTITGTCTCTATAGAGTTGGATTCATCCTTTATAExon 4AAGAGTTTCACATATGGTATGTGCCATTTATTTAATAGGCATExon 5GAAGATAGTAAGCTAGATGAAAATTGACCTTTCTTAGTTTCCExon 6aTGCTCAGAACCACGAAGTGTATTAGCTGGGTGGTGGTGCAT (1st)CTGAGGCAGGAGAATTGCTTCAGAGTCATCAGCGTGCATGAGATATTGACAGAAACCExon 7TATAGGCAGAAAGACTCTAGATCCTAGTATAGCTGGCAACTExon 8TGGGTAATTCAGGGTTGCTTCGCCATGTGCCAAGATCCAExon 9GGCCATGTGCTTTCCAAAACTTCGCCATGTGCCAAGATCAGExon 10TTGTGCATAGCAGAGAGACCTGAAAATTGATCCATTCACAGTGAGCTExon 11TCAGCAATGTTGTTTTGAGCACCCAAGATACGGGCACAGATTExon 12CTTCTGCACACCTTTTGAGAAGCTACATTCTGCCATACCAAExon 13GGTACCAATTTAATTACTACAGATTTGTAAGGAGATCTTTTGCExon 14ACACTTAGATTCAAGTAATACTCAGAAGCAAGATATGTCCATTGCExon 13CGTAAGGAGACACAAAGCAAATTCCACTACCATATATGAExon 14GACCCAGGAACACAAAGCAAATTCCACTACCAATATGTCCATTExon 15TAGACTCAAGTTTAGTTCCATTTAGACCAAGCATAGTGGCACTTAExon 16TCTGAATGCGTCAGATATATCAAAGAATGGCACCACTGCCATTAExon 17aCACAGCATGGCACATGTGCCCAAATGAACAGGACTTCAACExon 17aAGAAATAACCAGTGGCACATTGTGCGAAAATGACAGGACTTCAACExon 17aAGAAATAACCAGTGGACATTGTGGGATCACACGTGCACATTGTGGCACATTExon 17aAGAAATAACCAAGTGGAACACCCTATGGAGAAACCACAGTGGCACTCAAAAExon 17aAGAAATAACCAGGACACACTTGTGCGACAAACGCAATTAAGGExon 17aAGAAATAACCAAGTGTGAAAACCCAAAATGCACAGTGTGCACCGCAATTExon 17aAGAAATAACCAGTGGACATTGT	Exon 2	TATTCCCTCCCAATCCCTTT	TGGGATTACAGGCATTAGCC
Exon 4AAGAGTTTCACATATGGTATGTGCCATTATTTAATAGGCATExon 5GAAGATAGTAAGCTAGATGAAAATTGACCTTTCTTAGTTTCCExon 6aTGCTCAGAACCACGAAGTGTATTAGCTGGGTGGTGGTGCAT (1st)Exon 6bTGGAATGAGTCTGTACAGCGTGCATGAATATTGACAGAACTExon 7TATAGGCAGAAAAGACTCTAGATCCTAGTATTAGCTGGCAACTExon 8TGGGTAATTCAAGGTTGCTTCGCCATGTGAAAATCCAExon 9GGCCATGTGCTTTTCAAACTTCGCCATGGCAAGAACACGExon 10TTGTGCATAGCAGAGTACCTGAAAATTGATCCATTCACAGTACAGExon 11TCAGCAATGTTGTTTTGACCCCAAGATACGGGCACAGAGTAExon 12CTTCTGCACCACTTTGAGAAGCTACATTCTGCCATACCAAExon 13GGGATGCGATTCTTCGACCGGGAAAGGATATGTCCATTGCExon 14ACACTTAGATTCAAGTAATACTCAGAAGGTAAGTCTTTGGExon 15GGTACCAATTTAATTAGTCCATTTAGACTCAAGTTAGATExon 16TCTGAAAGGGAGGTCTCTGTCAGAAGCAAAGCAAAExon 17GAGCCAGGAACCAAAAGCAAATTCCACTACCATAAGGTTGCATTExon 18GAGCCAGGACTCAATGTCTTCAAGCCAGGACCTGCATTAExon 17CACCAGCATGGCACATGATACCAAAATGAAGTACCAGGACTTCAACExon 17CACCAGCATGGCACATGATACCAAAGAAGCAGCACTGCATTAExon 17CACCAGCATGGCACATGATCCAATGAACAGAACTATAGCAExon 17CAGAAAATAAATCACTGGACACACCCATGGTGTACTGGTGCATCGExon 17CACAAGAATGGCACCAGTGTCCAATGAAATTGACATGGACTCGExon 17CAAAAAAAGGGGCACCAGTGTGCGAAAATCGAACTGGACTGGAATTAExon 17CAAAAAAAGCAGGAACCAAAAAGATACACAGGAACTCAAAGCAGGATTAExon 17CAAAAAAAGGGGACCCAGTGTCCATGGGGAAATAGCCACTGGAATA	Exon 2	TGTAAGAGATGAAGCCTGGTA	GCTCCTATTTTTAAATATAAG
Exon 5GAAGATAGTAAGCTAGATGAAAATTGACCTTTCTTAGTTTCCExon 6aTGCTCAGAACCACGAAGTGTATTAGCTGGGTGGTGGTGCAT (1st) CTGAGGCAGGGAAATTGCTT (2nd)Exon 6bTGGAATGAGTCTGTACAGGGTGCATGAATATTGACAGCAGCAAATGCTExon 7TATAGGCAGAAAGACTCTAGATCCTAGTATAGCTGGCAACTExon 8TGGGTAATCAGGGTTGCTTCGCCATTAGGATGAAATCCAExon 9GGCCATGTGCTTTTCAAACTTCGCCATGTGCAAGATACAGExon 10TTGTGCATAGCAGAGTACTGAAAATTGATCCATTCACAGTAGCTExon 11TCAGCAATGTTGTTTTGACACCCAAGATACGGGCACAGATExon 12CTTCTGCACCACTTTTGAGAAGCTACATTCTGCCATACCAAExon 13GGGATGTGATTCTTTCGACCGGAAGAAGTATGTCCATTGCExon 13TGGGATGTGATTCTTTCGACCGGAAGCAAGAATATGTCExon 14ACACTTAGATCAAGTACAAGCAAATTCCACTAACTAATGAExon 15GGTTAAGGGTGCATGCTCTTCAGAAGCAAGAACTTAAGGExon 16GACCAGGAACCAAAGCAAATTCCACTACCATAATGGACTCAACExon 17CACAGCATGGCACTGTGTGCAAAAGAACAGGACTCAACExon 17CACAGCATGGCACATGTATCAAAATAATCACTGAGCTCATTExon 16TCTGAATGCGCTCATGTGAGCAAATGACAGGACACTCAACExon 17CAAAGAATGGCACCAGTGTGCGACAATCGTGTGCATCGExon 17TTAACCAAATAACAAGGAGTGTGAACACCCATGTGTGCACTGGACTCGExon 18TAGGAGAAGTGTGAAAACAGATCACAGGTGACCCTCAAAAExon 20CAGGATGAAATGGCACCAGTGTTCTAGCGTAAGAGAGCACTTGAAAExon 21TGTTCACAAGGGACCCAGTTTCCACGCGGAAAACTGCACTGGACTExon 22AGAGCCATGTCACAGTATTCACGCGGGAAAGCACTTCAAAAExon 23CCAAGGATGGCACCAGTTTCTGCGTGCAAC	Exon 3	CCATGAGATTTTGTCTCTATA	GAGTTGGATTCATCCTTTATA
Exon 6aTGCTCAGAACCACGAAGTGTATTAGCTGGGTGTGGTGCAT (1st) CTGAGGCAGGAAATTGCTT (2nd)Exon 6bTGGAATGAGTCTGTACAGCGTGCATGAATATGACAGAACTExon 7TATAGGCAGAAAGACTCTAGATCCTAGTATAGCTGGCAACTExon 8TGGGTAATCCAGGGTTGCTTCGCCATTGGAATGAAATCCAExon 9GGCCATGTGCTTTTCAAACTTCGCCATGTGCAAGAACAGExon 10TTGTGCATAGCAGAGGTACCTGAAAATTGATCCATTCACAGTAGCTExon 11TCAGCAATGTTGTTTTGACAGGCTACATTCTGCCATACCAAExon 12CTTCTGCACCACTTTTGAGAAGCTACATTCTGCCATACCAAExon 13GGTACCAATTAATTACTACAGATTGATAAGGAAGTCTTTTGCExon 13TGGGATGTGATTCTTCGACCGGGAAGAGATATGTCCATTGCExon 13CGTACAGATTAGTCCATTCCAGAAGCTAAGAACTATATGAExon 14ACACTTAGATCAAGAAGCAACAAATTCCACTACACATAATGGAExon 15GGTTAAGGGTGCATGCTCTCAAGCCAAGCAACAATGTGGExon 16CTGGAATGCGTCTACTGTGAGCAAACACAAGCAGCACTCAACExon 17aCAGAACTAAGGCACAGTGTACCCAAAATGAACTCACTGGExon 17aCACAAGCATGGCACAGTGTACCAAAATGAACTCACATGGTCAExon 17aGAAATAAATCACTGACACACCCATGTGTGCATCGExon 17aGAAAATAACTGACTGACACACCCATGTGTGCATCGExon 17bTTTAACCAATGACATTGTGAATACCGATTCAAGGAAATTAAExon 17bTTAACCAATGACATTGTGTGACACAAGCAGTGCCACAGTGTExon 17bTTAACCAATGACATTGTGCAACACATGTGGCAACTCGAATAExon 17bTTAACCAATGACATTGTGTGACACAGCAGTTCCAAATExon 17bTTAACCAATGACATGTGCACACCATGTGTACCATGGGAATCGAExon 17bTTAACCAATGACATTGTGCAACACTATGGGAAAACTGCACTCGAA	Exon 4	AAGAGTTTCACATATGGTATG	TGCCATTTATTTAATAGGCAT
CTGAGGCAGGAGAATTGCTT (2nd)Exon 6bTGGAATGAGTCTGTACAGCGTGCATGAATATTGACAGAAACTExon 7TATAGGCAGAAAGACTCTAGATCCTAGTATTAGCTGGCAACTExon 8TGGGTAATTCAGGGTGCTTCGCCATGGCAGAGTACCAExon 9GGCCATGTGCTTTTCAAACTTCGCCATGTGCAAGATACAGExon 10TTGTGCATAGCAGAGTACCTGAAAATTGATCCATTCACAGTAGCTExon 11TCAGCAATGTTGTTTTTGACAATTGATCCATTCACCAGTAGCTExon 12CTTCTGCACCACTTTGCAAGAGCTACCATTCTGCCATACCAAExon 13GGTACCAATTTAATTACTACAGATTTGTAAGGGAGTCTTTGCExon 13CATTAGAAGGAGATCCTCTGTTCGTAATCCTATGACATTGCExon 14ACACTTAGATTCAAGTAATACTCAGAAGCTAAGAACTATATGAExon 15GGTACCAAGTATAGTCCATTCAGAAGCTAAGAACTATATGAExon 16CTTGAAGGTGCATGCTCTTCAAGCACACACAAAGCAAAExon 17TAGACCAGGACTCAAGTTAGTTCCATTTAGACCAGGACTTCAACExon 16TCTGAATGCGTCTACTGTGAGCAATAGACAGGACTTCAACExon 17CACCAGCATGGCACATGATCCAAAATGAAGTCACATGGTCAExon 16TCTGAATGCGCCCAGTGTGGCAAAATGAAGTCACATGGTCAExon 17CAACAGAATGGCACCAGTGTGCAAAATAATCACTGGCACTGAExon 17CAAAGAATGGCACCAGTGTGCAACACAGTGGCACTCAAATExon 17TTTAACCAATGGCACAGTGTGCAAAACACAGTGGCACTGAACExon 17CAAGGAATGGTGAATAAAGCTATGAGGAAACTGCACTGGAATExon 17CAAGGAATGGTGAAAAGTGGAACATCCACTGGGCTACTGGAAATExon 17CAAGGAATGGCACCAGTGTGCAAAATAATCACAGGACTCCAAATExon 17TTTAACCAATGGCACCAGTGTGCAAAGAAACTGCACTGGAAACExon 17CAAGGAAAGGGAACGAAAGGA <td>Exon 5</td> <td>GAAGATAGTAAGCTAGATGAA</td> <td>AATTGACCTTTCTTAGTTTCC</td>	Exon 5	GAAGATAGTAAGCTAGATGAA	AATTGACCTTTCTTAGTTTCC
Exon 6bTGGAATGAGTCTGTACAGCGTGCATGAATATTGACAGAACTExon 7TATAGGCAGAAAGACTCTAGATCCTAGTATTAGCTGGCAACTExon 8TGGGTAATTCAGGGTGCTTCGCCATGTGCATGAAATCCAExon 9GGCCATGTGCTTTTCAAACTTCGCCATGTGCAAGATACAGExon 10TTGTGCATAGCAGAGATACTGAAAATTGATCCATTCACAGTAGCAExon 11TCAGCAATGTTGTTTTGACCCCAAGATACGGGCACAGATTExon 12CTTCTGCACCACTTTTGAGAAGCTACATTCTGCCATACCAAExon 13GGGACGATGTGTTTTTGACCGGGAAGAGATATGTCCATGCExon 13TGGGATGTGATTCTTCGACCGGGAAGAGAATGTCCATGGCExon 14ACACTTAGAAGGAGATGCTCCTGTTCGTAATCCATGAGATTATGACExon 15GGTTAAGGGTGCATGCTCTCAAGACTAAGAACTATATGACExon 14GACCCAGGAACACAAAGCAAATTCCACTACCATAAGACTATATGACExon 15GGTTAAGGGTGCATGCTCTCAAGCCAGCACTGCCATTAExon 16TCTGAATGCGTCTACTGTGAGCAATGACAGAGACTATAGACExon 17CACCAGCATGGCACACTGTCTCAAAAGAATGACGCACACACACExon 17CACCAGCATGGCACACTGTACCCATGTGTACTGGACTCGExon 17CAACAGAATGGCACACAGTGTCGAAAATAATAAExon 17CAAGAATGGCACACAGTGGGGCCTCAAGGTACTGGExon 17CAAGAATGGCACACAGTGGCGCTCAGGCTACTGGGATTExon 18TAGGAGAGTGTGAATAAAGCAATGCGACTCGAAAAExon 19GCCCGACAAATAACCAAGTCATGGGTAGGTCCAGTCAAAAExon 20CAGGATGGCACGGTTTCCACCAGGGTAGGCCACTGCAAAAExon 21TGTTCACAAGGGACTCGAAAAGGGGTAGGTCCAGTGAAAAAExon 22AGGACACTGTGCCACGTATTTCCACGGCTGCAAGTATTCATExon 23CCATGGTTGAAAAGCTGATTG	Exon 6a	TGCTCAGAACCACGAAGTGT	ATTAGCTGGGTGTGGTGCAT (1st)
Exon 7TATAGGCAGAAAGACTCTAGATCCTAGTATTAGCTGGCAACTExon 8TGGGTAATTCAGGGTTGCTTCGCCATTAGGATGAAATCCAExon 9GGCCATGTGCTTTTCAAACTTCGCCATGTGCAAGATACAGExon 10TTGTGCATAGCAGAGTACCTGAAAATTGATCCATTCACAGTAGCTExon 12CTTCTGCACCACTTTTGACACCAAGATCGGGCACAGATTExon 13GGTACCAATTTAATTACTACAGACTTGTAAGGGAGTCTTTGCExon 13TGGGATGTGATTCTTTCGACCGGGAAGAGATATGTCCATTGCExon 13CATTAGAAGGAGATGCTCCTGTTCGTAATCCTATGATTTTAGTExon 13CATTAGAAGGAGATGCTCCTGTTCGTAATCCTATGATTTTAGTExon 14ACACTTAGATTCAAGTAATACTCAGAAGCAAAGCAATGCCATTAExon 15GGTAAGGGTGCATGCTCTTCAAGCCCAGCACTGCCATTAExon 15GGTAAGGGTGCATGCTCTTCAAGCCAAGGACTTCAACExon 16TCTGAATGCGTCATGTGTGAGCAATAGACAGGACTTCAACExon 17CACCAGGATGGCACACTGTACTCAAAAGAAGTAATATAGExon 17AGAAATAAATCACTGACAACACCCATGTGTACTGTGTGCATCGExon 17CACCAGGATGGCACACAGTGGCGACAATCTGTGTGCATCGExon 17TTAACCAATGACTGTGAATAAGGATACACAGTGACCCCAATTExon 17TAAGGAGAAGTGTGAATAAAGGATACACAGTGACCCTCAATTExon 17TTAACCAATGGCACAGTGGCGACATCTGTGGGATTExon 18TAGGAGAAGTGTGAATAAAGGATACACAGTGACCCTCAATTExon 20CAGGATTGAAAGTGGCACACACTATGAGAAAACCAAAGExon 21TGTTCACAAGGGACTCAAAAAGGGGTAGGTCAGTCAAAAAExon 22AGAGCATGTGCCACTGTTGTTCCACACGGTAGAGCACACAAGA(Ist)Exon 24TTTCTGTCCCTGCTCTGGTTCTGGCTTGCAAAACACAAAG(Ist)FXNI <td< td=""><td></td><td></td><td>CTGAGGCAGGAGAATTGCTT (2nd)</td></td<>			CTGAGGCAGGAGAATTGCTT (2nd)
Exon 8TGGGTAATTCAGGGTTGCTTCGCCATTAGGATGAAATCCAExon 9GGCCATGTGCTTTTCAAACTTCGCCATGGCAAGATACAGExon 10TTGTGCATAGCAGAGATACCTGAAAATTGATCCATTCACAGTAGCTExon 11TCAGCAATGTTGTTTTGACCCCAAGATACGGGCACAGATTExon 12CTTCTGCACCACTTTTGAGAAGCTACATTCTGCCATACCAAExon 13GGTACCAATTAATACTACAGATTTGTAAGGGAGTCTTTTGCExon 13TGGGATGTGATTCTTCGACCGGGAAGAGATGTCCATTGCExon 13CATTAGAAGGAGAGTGCTCCTGTTCGTAATCCTATGATTTTAGTExon 14ACACTTAGATTCAAGTAATACTCAGAAGCTAAGAACTATATGAExon 15GGTTAAGGGTGCATGCTCTTCAAGCCAGCACTGCCATTGExon 15GGTAAGGGTGCATGCTCTTCAAGCCAGCACTGCCATTAExon 16TCTGAATGCGTCTACTGTGAGCAATAGACAGGACTCCAACExon 17aAGAATAAATCACTGACACACCCATGTGTACTTTGTAATATAGExon 17aAGAAATAAATCACTGACACACCCATGTGTACTTTGTAATATAGExon 17aAGAAATAAATGCACTGACACACCCATGTGTACCTGGGACACTGGExon 17aAGAAATAAATGACAGGACACTGTGGCGACAATCTGTGGCACCGGATTExon 17aAGAAATAAATGACAAGTGGCTCCAGCAACTGGACACTGGAExon 17bTTAACCAATGACATTGTGAATACCGATTCAAGGAAATTAExon 18TAGGAGAAGTGTGAATAAAGGATACACACTGGCACCTCGAATExon 20CAGGATTGAAAGGTGACTGCAACACTATGGGCTACTGGGATCAAGGACTExon 21TGTTCACAAGGGACTCCAAAAGGGCTAGGCTGCAGTCAAAAExon 22AGACCATGTGCCACGTATTTCCACTGGCTGCAAAACACAAGG (1st)Exon 23CCATGGTTGAAAAGCTGATGGTGTGGCTTGCCTGCAGAAACACAAGG (1st) <i>PINKI</i> Intron 3-Exon 3CCAACCACGCAACAGAATA	Exon 6b	TGGAATGAGTCTGTACAGCG	TGCATGAATATTGACAGAACT
Exon 9GGCCATGTGCTTTTCAAACTTCGCCATGTGCAAGATACAGExon 10TTGTGCATAGCAGAGTACCTGAAAATTGATCCATTCACAGTAGCTExon 11TCAGCAATGTTGTTTTGACCCCAAGATACGGGCACAGATTExon 12CTTCTGCACCACTTTGAGAAGCTACATTCTGCCATACCAAExon 13GGTACCAATTTAATACTACAGATTTGTAAGGAGATCTTTGCExon 13TGGGATGGATTCTTCGACCGGGAAGAATAGTCCATGCExon 13CATTAGAAGGAGATGCTCCTGTTCGTAATCCTATGATTTTAGTExon 14ACACTTAGATTCAAGTAATACTCAGAAGCAAGAACATATGCExon 15GGTTAAGGGTGCATGCTCTTCAAGCCAGCACTGCCATTAExon 15TAGACTCAAGTTAGTTCATTTAGACTCAAGTTTAGTTCCATTExon 16TCTGAATGCGTCACTGTGAGCAATAGAACAGAAGTAATAGExon 17aAGAAATAAATCACTGACACACCCATGTGTACTTGTGGACATGGTCAExon 17aAGAAATAAATGCACTGACACACCCATGTGTACTTTGTAATATAGExon 17aAGAAATAAATGCACTGACACACCCATGTGTACTTTGTAATATAGExon 17aAGAAATAAATGCACTGACACACCCATGTGTACTTGTGGAATGExon 17aAGAAATAAATGCACTGACACACCCATGTGTCACTGGGACACGExon 17aAGAAATAAGCACAGTGTGAATAAAGGATACACAGTGACCCTCAATTExon 17aTTAACCAATGGCACCAGTGTCCACGACAATTATCACAGGAACTGGAACExon 17bTTTAACCAATGGCACCAGTGTCCACAGCATGGCACTGGAAExon 17bTTTAACCAATGGCACCAGTGTCCACAGCATGGCACTGGAACExon 17bTTTAACCAATGGCACCAGTGTCTTCGGCTAGGACAGCATTGGAAExon 17bTTTAACCAATGGCACCAGATTCTCACCACAGTACTGGGAACAGGAAGTGTGAAAAExon 17bTTTAACCAATGGCACCAGTATTCCACCACAGGAAACCAAAGExon 12CCAGGATGAGCACTGGAACACTTG	Exon 7	TATAGGCAGAAAGACTCTAGA	TCCTAGTATTAGCTGGCAACT
Exon 10TTGTGCATAGCAGAGTACCTGAAAATTGATCCATTCACAGTAGCTExon 11TCAGCAATGTTGTTTTGACCCCAAGATACGGGCACAGATTExon 12CTTCTGCACCACTTTTGAGAAGCTACATTCTGCCATACCAAExon 13GGTACCAATTTAATTACTACAGATTTGTAAGGGAGTCTTTTGCExon 13TGGGATGGATTCTTTCGACCGGGAAGAGATATGTCCATTGCExon 13CATTAGAAGGAGATGCTCCTGTTCGTAATCCATAGATTTTAGTExon 14ACACTTAGATTCAAGTAATACTCAGAAGCAAAGCAAATGGCACTACAGAExon 15GGTTAAGGGTGCATGCTCTCAAGCCAGCACGCATTAExon 15TAGACTCAAGTTAGTTCCATTTAGACTCAAGTTTAGTTCCATTExon 15TAGACTCAAGTTAGTCCATTTAGACTCAAGTTCAACExon 16TCTGAATGCGTCTACTGTGAGCAATAGACAGGACACAAGCAExon 17aCACCAGCATGGCACATGTATCCAAAATGAAGTCACATGGTCAExon 17aAGAAATAAATCACTGACACACCCATGTGTGCACTGExon 17aAGAAATAAATCACTGACACACCCATGTGTGCACTGGExon 17aAGAAATAAATCACTGACACACCCATGGTGTACTTGTGAATATAGExon 17bTTTAACCAATGACATTGTGAATACCGATTCAAGGAATTAExon 17bTTTAACCAATGACATTGTGAATACCGATTCAAGGAATTAExon 17bTTTAACCAATGACATTGTGATCCTACGGTACTGGGATTExon 17bCACGACAATGGCACCAGTGTTCCTACGGGTACGCACTGAAAExon 18TAGGAGAGTGCACAGTGTTCCACTGGGCTACTGGAAAAExon 21TGTTCACAAGGACCCACGATTTCCACTGGCTGCAGAAAACTGCACTGGAAExon 22AGAGCCATGTGCCCACGATTTCCACTGGCTGCAAAAACCAAGAG(1st)Exon 24TTTCTGTCCCTGCTCTGGCCTCTGGCTTGCAAAAACACAAAG(1st)Fxon 24TTTCTGTCCCAGGTAATGCCACGAGTTTGTGTCTTTCTGGGGGAGACCATTG	Exon 8	TGGGTAATTCAGGGTTGCTT	CGCCATTAGGATGAAATCCA
Exon 11TCAGCAATGTTGTTTTTGACCCCAAGATACGGGCACAGATTExon 12CTTCTGCACCACTTTTGAGAAGCTACATTCTGCCATACCAAExon 13GGTACCAATTTAATTACTACAGATTTGTAAGGGAGTCTTTTGCExon 13TGGGATGTGATTCTTTCGACCGGGAAGAGATATGTCCATTGCExon 13CATTAGAAGGAGATGCTCCTGTTCGTAATCCTATGATTTAGTExon 14aACACTTAGATTCAAGTAATACTCAGAAGCATAGCCATAGCATATATGAExon 15GGTTAAGGGTGCATGCTCTTCAAGCCAGCACTGCCATTAExon 15GGTTAAGGGTGCATGCTCTTCAAGCCAGCACTGCCATTAExon 16TCTGAATGCGTCTACTGTGAGCAATAGACAGGACTTCAACExon 17aCACCAGCATGGCACATGATATCCAAAATGAAGTCACAGGTCAExon 17aAGAAATAAATCACTGACACACCCATGTGTACTGTGGAAGTCACAGGTCAExon 17aAGAAATAAATCACTGACAACACCCATGTGTACTGTGGCATCGExon 17aAGAAATAAATCACTGACACACCCATGTGTACTGTGGCATCGExon 17bTTTAACCAATGACATTGTGAAATACCGATTTCAAGGAAATTAExon 17bTTTAACCAATGACATTGTGGAATACCAAGTGACCCTCAATTExon 17bTTTAACCAATGACATTGTGCAACACTATGAGAAAAACTGCACTGGAExon 17bTTTAACCAATGACAAGTGTGCAACACTATGAGAAAACTGCACTGGAATTAExon 17bTTTAACCAAAGGGACTCCAAAAGGGGTAGGTCCAGTGCAACAExon 12TGTTCACAAGGGACTCCAAAAGGGGTAGGTCCAGTGGAAGACExon 23CCATGGTTGAAAAGCTGATTGTCACACTGGCAAAAACCAAGG (1st)Exon 24TTTCTGTCCCTGCTCTGGTCTCTGGCTTTCTCTGGGGGAGAGCACTGCAAAFSPINKIIntro 3-Exon 3CCAATCACAGTAATAGCATGTGTAATGGGCACTCGAAA (1st)Exon 22AGACCAGCAACAGAAAAGCAAGAATGTGTAATGGGCACTGGAAACCAAGGAFS	Exon 9	GGCCATGTGCTTTTCAAACT	TCGCCATGTGCAAGATACAG
Exon 12CTTCTGCACCACTTTTGAGAAGCTACATTCTGCCATACCAAExon 13GGTACCAATTTAATTACTACAGATTTGTAAGGGAGTCTTTTGCExon 13TGGGATGTGATTCTTTCGACCGGGAAGAGATATGTCCATTGCExon 13CATTAGAAGGAGATGCTCCTGTTCGTAATCCTATGATTTTAGTExon 14aACACTTAGATTCAAGTAATACTCAGAAGCTAAGAACTATATGAExon 15GGTTAAGGGTGCATGCTCTCAAGCCAGGACCCATAGCATTAExon 15GGTAAGGGTGCATGCTCTCAAGCCAAGCATGCCCATTAExon 15TAGACTCAAGTTTAGTTCCATTTAGACTCAAGTTTAGTTCCATTExon 16TCTGAATGCGTCTACTGTGAGCAATAGACAGGACTCAACAExon 17aCACCAGCATGGCACAGTGTCCAAAATGAAGTCACAGGTCAExon 17aCACCAGCATGGCACCAGTGTGCGACAATCGTGTGCATCGExon 17aAGAAATAAATCACTGACACACCCATGTGTACTTGTGAATATAGExon 17bCAAAGAAGGGACACCAGTGTGCGACAATCGTGTGCACTCGExon 17bTTTAACCAATGACATTTGTGAATACCGATTTCAAGGAAATTAExon 17bTTAACCAATGACATTGTGATGCTTCAGGCTACTGGGATTExon 17bTTAACCAATGACATTGTGATGCTTCAGGCTACTGGGATTExon 17bTTAACCAATGACAGTGTGCAACACTATGAGAAAACTGCACTGGAExon 17bTGTCACAAGGGACTCCAAAAGGGGTAGGTCCAGTCAAAAExon 17bTGTTCACAAGGGACTCCAAAAGGGGTAGGTCCAGTCGAGTGGAExon 17bTGTTCACAAGGGACTCCAAAAGGGGTAGGTCCAGTCGAGTGGAGExon 17bTGTTCACAAGGGACTCCAAAAGGGGTAGGTCCAGTCAAAAExon 18TAGGAGCATGTGCCACGTATTTCCACTGGGCAATTATTTCATExon 21TGTTCACAAGGGACTCCAAAAGGGGTAGGTCCAGTCAAAACACAAG (1st)Exon 22AGAGCCATGTACTCCTGCTGGCCAGTATTTCCACTGGGCAAA	Exon 10	TTGTGCATAGCAGAGTACCTGAAA	ATTGATCCATTCACAGTAGCT
Exon 13GGTACCAATTTAATTACTACAGATTTGTAAGGGAGTCTTTTGCExon 13TGGGATGTGATTCTTTCGACCGGGAAGAGATATGTCCATTGCExon 13CATTAGAAGGAGATGCTCCTGTTCGTAATCCTATGATTTTAGTExon 14aACACTTAGATTCAAGTAATACTCAGAAGCTAAGAACTAATGAExon 14bGACCCAGGAACACAAAGCAAATTCCACTACCATACGCATGCCATTAExon 15GGTTAAGGGTGCATGCTCTTCAAGCCAGCACTGCCATTAExon 15TAGACTCAAGTTTAGTTCCATTTAGACTCAAGTTAGTTCCATTExon 16TCTGAATGCGTCTACTGTGAGCAATAGACAGGACTTCAACExon 17aCACCAGCATGGCACATGTATCCAAAATGAAGTCACATGGTCAExon 17aAGAAATAAATCACTGACACACCCATGTGTACTTTGTAATATAGExon 17bCAAAGAATGGCACCAGTGTGCGACAATTCAGGACATCGExon 17bTTTAACCAATGACATTGTGAATACCGATTTCAAGGAAATTAExon 17bGCCGACAAATAACCAAGTGTGCTTCAAGGCTACTGGGATTExon 17bTTAACCAATGACATTGTGAAAGATACACAGTGACCCTCAATTExon 17bGCCGACAAATAACCAAGTGTGCTTCAGGCTACTGGGATTExon 17bGCCGACAAATAACCAAGTGTGCTTCAGGCTACTGGGATTExon 17bGCCGACAAATAACCAAGTGTATGGGGAACCCTCAAAAExon 17bGCCGACAAATAACCAAGTGTATGGGTAGGCTGCTExon 17bGCCGACAAATAACCAAGTGGCTCCAAAAGGGGTAAGGTCCAGTCAAAAExon 17bGCCGACAAATAACCAAGTGGTGCACACACTATGAGGAAACTGACTGGAGExon 17bTTTACCAAGGGACTCCAAAAGGGGTAAGGTCCAGTCAAAACCAAGExon 17bGCCGACAAAGTGGCCCCGAAATGCACACTGGGCAGGACGCGTExon 21TGTTCACAAGGGCACCGACTTTTCCACTGGCAAGACAGCACTTExon 22AGAGCATGACAGTGATTGTGAGTAAAGCTGGATGGCTGT<	Exon 11	TCAGCAATGTTGTTTTTGACC	CCAAGATACGGGCACAGATT
Exon 13TGGGATGTGATTCTTTCGACCGGGAAGAGATATGTCCATTGCExon 13CATTAGAAGGAGATGCTCCTGTTCGTAATCCTATGATTTTAGTExon 14aACACTTAGATTCAAGTAATACTCAGAAGCTAAGAACTATATGAExon 14bGACCCAGGAACACAAAGCAAATTCCACTACCATAAGACTATATGAExon 15GGTTAAGGGTGCATGCTCTTCAAGCCAGGACTGCCATTAExon 15TAGACTCAAGTTTAGTTCCATTTAGACTCAAGTTTAGTTCCATTExon 16TCTGAATGCGTCTACTGTGAGCAATAGACAGGACTTCAACExon 17aCACCAGCATGGCACATGTATCCAAAATGAAGTCACATGGTCAExon 17aAGAAATAAATCACTGACACACCCATGTGTACTTTGTAATATAGExon 17bCAAAGAATGGCACCAGTGTGCGACAAATCAGTGACATCGExon 17bTTTAACCAATGACATTTGTGAATACCGATTTCAAGGAAATTAExon 18TAGGAGAAGTGTGAATAAAGGATACACAGTGACCCTCAATTExon 20CAGGATTGAAAGTGTGCAACACTATGAGAAAACTGCACTGGAExon 21TGTTCACAAGGGACTCCAAAAGGGGTAGGCCAGTCAAAAExon 23CCATGGTTGAAAAGCTGATTGTGAGAAACTGGACGGCTGTExon 24TTTCTGTCCCTGCTCTGGTCTCTGGCTTGCAAAACACAAG (1st) ACTATTGCCAGGAAGCACTCGAAA (1st)Fxon 2TCCCAACTCCTATCCCACGGTGTGTAATGGGCACTCGAAA (1st) TACCACCCACTGTTCGTTGCAACGACAGAAA(2nd)	Exon 12	CTTCTGCACCACTTTTGAGAA	GCTACATTCTGCCATACCAA
Exon 13CATTAGAAGGAGATGCTCCTGTTCGTAATCCTATGATTTAGTExon 14aACACTTAGATTCAAGTAATACTCAGAAGCTAAGAACTATATGAExon 14bGACCCAGGAACACAAAGCAAATTCCACTACCATAAGACTATATGAExon 15GGTTAAGGGTGCATGCTCTTCAAGCCAGCACTGCCATTAExon 15TAGACTCAAGTTTAGTTCCATTTAGACTCAAGTTTAGTTCCATTExon 16TCTGAATGCGTCTACTGTGAGCAATAGACAGGACTTCAACExon 17aCACCAGCATGGCACATGTATCCAAAATGAAGTCACATGGTCAExon 17aAGAAATAAATCACTGACACACCCATGTGTACTTTGTAATATAGExon 17bCAAAGAATGGCACCAGTGTGCGACAATCGTGTGCATCGExon 17bTTTAACCAATGACATTTGTGAATACCGATTTCAAGGAAATTAExon 17bTAGGAGAAGTGTGAATAAAGGATACACAGTGACCCTCAATTExon 17bTAGGAGAAGTGTGCAACAGTGTGCTTCAGGCTACTGGGATTExon 17bTAGGAGAAGTGTGCAACAACTATGAGAAAACTGCACTGGAExon 17bTAGGAGAAGTGTGCAACAACTATGAGAAAACTGCACTGGAAExon 17bTAGGAGAAGTGTGCAACAACTATGAGAAAACTGCACTGGAATTAExon 17bTAGGAGAAGTGTGCAACAACTATGAGAAAACTGCACTGGAAExon 17bTTTAACCAAAGGGACTCCAAAAGGGGTAGGTCCAGTCAAAACACAGAGExon 20CAGGATTGAAAAGCTGATTGTCCACTGGCTAGGACACTGGAExon 21TGTTCACAAGGGACTCCAAAAGGGGTAGGTCCAGTAAAACACAAG (1st)Exon 24TTTCTGTCCCTGCTCTGGCCTCTGGCTTTTCTCGGGGTGAGPRNKIIntron 3-Exon 3CCGACAGCAACAGAATAGCATGTGATATGGGCACTCGAAA (1st)Fxon 2TCCCAACTCCTATCCCACTGTACCACCCACTGTTCGTTGA (2nd)	Exon 13	GGTACCAATTTAATTACTACAG	ATTTGTAAGGGAGTCTTTTGC
Exon 14aACACTTAGATTCAAGTAATACTCAGAAGCTAAGAACTATATGAExon 14bGACCCAGGAACACAAAGCAAATTCCACTACCATAATGCTTGGExon 15GGTTAAGGGTGCATGCTCTTCAAGCCAGCACTGCCATTAExon 15TAGACTCAAGTTTAGTTCCATTTAGACTCAAGTTTAGTTCCATTExon 16TCTGAATGCGTCTACTGTGAGCAATAGAAGGACTTCAACExon 17aCACCAGCATGGCACATGTATCCAAAATGAAGTCACATGGTCAExon 17aAGAAATAAATCACTGACACACCCATGTGTACTTTGTAATATAGExon 17bCAAAGAATGGCACCAGTGTGCGACAATCGGTGCACCGExon 17bCAAAGAATGGCACCAGTGTGCGACAATCGGTGCACCGExon 17bTTTAACCAATGACATTGTGAATACCGATTTCAAGGAAATTAExon 17bTAGGAGAAGTGTGAATAAAGGATACACAGTGACCCTCAATTExon 17bCAGGATTGAAAGTGTGCAACATGCTTCAGGCTACTGGGATTExon 17bTTTAACCAATGACATTGTGAATACCGATTTCAAGGAACTGCACTGGAExon 17bCAGGATTGAAAGTGTGCAACACTATGAGAAAACTGCACTGGAATExon 17bGCCCGACAAATAACCAAGTGTGCTTCAGGCTACTGGGATTExon 20CAGGATTGAAAGTGTGCAACAAAGGGGTAGGTCCAGTCAAAAExon 21TGTTCACAAGGGACTCCAAAAGGGGTAGGCTGGATGGCTGTExon 22AGAGCCATGTGCCACGTATTTCCACTGGCTGCAAAACACAAAG (1st)Exon 24TTTCTGTCCCTGCTCTGGTCTCTGGCTTGCAAAAACACAAAG (1st)PRNSIIntron 3-Exon 3CCGACAGCAACAGAATAGCATGTGTAATGGGCACTCGAAAA (1st)Exon 2-Exon 3CCGACAGCAACAGAATAGCATGTGTAATGGGCACTCGAAAA (1st)Exon 2-Exon 3CCGACAGCAACAGAATAGCATGTGTAATGGGCACTGGTTGA (2nd)	Exon 13	TGGGATGTGATTCTTTCGACC	GGGAAGAGATATGTCCATTGC
Exon 14bGACCCAGGAACACAAAGCAAATTCCACTACCATAATGCTTGGExon 15GGTTAAGGGTGCATGCTCTCAAGCCAGCACTGCCATTAExon 15TAGACTCAAGTTAGTTCCATTTAGACTCAAGGTTCAACTExon 16TCTGAATGCGTCTACTGTGAGCAATAGACAGGACTTCAACExon 17aCACCAGCATGGCACATGTATCCAAAATGAAGTCACATGGTCAExon 17aAGAAATAAATCACTGACACACCATGTGTACTTGTGAATAAGExon 17bCAAAGAATGGCACCAGTGTGCGACAATCTGTGTGCATCGExon 17bTTTAACCAATGACATTTGTGAATACCGATTTCAAGGAAATTAExon 17bTAGGAGAAGTGTGAATAAAGGATACACAGTGACCCTCAATTExon 17bTAGGAGAAGTGTGAATAAGCCGACAACGTGACCCTCAATTExon 18TAGGAGAAGTGTGAATAACCAAGTGTGCTTCAGGCTACTGGGATTExon 20CAGGATTGAAAGTGTGCAACACTATGAGAAAACTGCACTGGAExon 21TGTTCACAAGGGACTCCAAAAGGGGTAGGTCCAGTCAAAAExon 22AGAGCCATGTGCCACGTATTTCCACTGGGCAATTATTTCATExon 23CCATGGTTGAAAAGCTGATTGTGAGTAAAGCTGGATGGCTGTExon 24TTTCTGTCCCTGCTCTGGTCTCTGGCTTGCAAAAACACAAAG (1st) <i>PINKI</i> Intron 3-Exon 3CCAATCACAGTATATTCCCCAGAGGTTTGCTTTCTCGGGGGTGAG <i>PRSSI</i> FCCAACTCCTATCCCACTGTGTGTAATGGGCACTCGAAA (1st)Exon 2-Exon 3CCGACAGCAACAGAATAGCATGTGTAATGGGCACTCGAAA (1st)Exon 2-Exon 3CCGACACTCCTATCCCACTGTGTGTAATGGGCACTGGAAC (2nd)	Exon 13	CATTAGAAGGAGATGCTCCTGT	TCGTAATCCTATGATTTTAGT
Exon 15GGTTAAGGGTGCATGCTCTTCAAGCCAGCACTGCCATTAExon 15TAGACTCAAGTTTAGTTCCATTTAGACTCAAGTTTAGTTCCATTExon 16TCTGAATGCGTCTACTGTGAGCAATAGACAGGACTTCAACExon 17aCACCAGCATGGCACATGTATCCAAAATGAAGTCACATGGTCAExon 17aAGAAATAAATCACTGACACACCCATGTGTACTTTGTAATATAGExon 17bCAAAGAATGGCACCAGTGTGCGACAATCGGACCTCCAATTExon 17bTTTAACCAATGACATTGTGAATACCGATTTCAAGGAAATTAExon 17bTTTAACCAATGACATTGTGAATACCGATTTCAAGGAAATTAExon 17bTTTAACCAATGACATGTGCAACGCAACAGTGACCCTCAATTExon 18TAGGAGAAGTGTGAATAAGGATACACAGTGACCCTCAATTExon 19GCCCGACAAATAACCAAGTGTGCTTCAGGCTACTGGGATTExon 20CAGGATTGAAAGTGTGCAACACTATGAGAAAACTGCACTGGAExon 21TGTTCACAAGGGACTCCAAAAGGGGTAGGTCCAGTCAAAAExon 22AGAGCCATGTGCCACGTATTTCCACTGGGCAATTATTTCATExon 23CCATGGTTGAAAAGCTGATTGTGAGTAAAGCTGGATGGCTGTExon 24TTTCTGTCCCTGCTCTGGTCTCTGGCTTGCAAAACACAAAG (1st) <i>PINKI</i> Intron 3-Exon 3CCAATCACAGTTATTCCCCAGAGFxon 2-Exon 3CCGACAGCAACAGAATAGCATGTGTAATGGGCACTCGAAA (1st)Exon 2-Exon 3CCGACAGCAACAGAATAGCATGTGTAATGGGCACTCGAAAA (1st)Fxon 2TCCCAACTCCTATCCCACTGTACCACCCACTGTTCGTTGA (2nd)	Exon 14a	ACACTTAGATTCAAGTAATACT	CAGAAGCTAAGAACTATATGA
Exon 15TAGACTCAAGTTTAGTTCCATTTAGACTCAAGTTTAGTTCCATTExon 16TCTGAATGCGTCTACTGTGAGCAATAGACAGGACTTCAACExon 17aCACCAGCATGGCACATGTATCCAAAATGAAGTCACATGGTCAExon 17aAGAAATAAATCACTGACACACCCATGTGTACTTTGTAATATAGExon 17bCAAAGAATGGCACCAGTGTGCGACAATCTGTGGCATCGExon 17bTTTAACCAATGACATTGTGAATACCGATTTCAAGGAAATTAExon 17bTAGGAGAAGTGTGAATAAAGGATACACAGTGACCCTCAATTExon 18TAGGAGAAGTGTGAATAAAGGATACACAGTGACCCTCAATTExon 19GCCCGACAAATAACCAAGTGTGCTTCAGGCTACTGGGATTExon 20CAGGATTGAAAGTGTGCAACACTATGAGAAAACTGCACTGGAExon 21TGTTCACAAGGGACTCCAAAAGGGGTAGGTCCAGTCAAAAExon 22AGAGCCATGTGCCACGTATTTCCACTGGGCAATTATTTCATExon 23CCATGGTTGAAAAGCTGATTGTGAGTAAAGCTGGATGGCTGTSPINKIIntron 3-Exon 3CCAATCACAGTTATTCCCCAGAGAGTTTGCTTTTCTCGGGGTGAAGFxon 2-Exon 3CCGACAGCAACAGAATAGCATGTGTAATGGGCACTCGAAA (1st)Exon 2-Exon 3CCGACAGCAACAGAATAGCATGTGTAATGGGCACTCGAAAA (1st)Exon 2-Exon 3CCGAACTCCTATCCCACTGTACCACCCACTGTTCGTTGA (2nd)	Exon 14b	GACCCAGGAACACAAAGCAAA	TTCCACTACCATAATGCTTGG
Exon 16TCTGAATGCGTCTACTGTGAGCAATAGACAGGACTTCAACExon 17aCACCAGCATGGCACATGTATCCAAAATGAAGTCACATGGTCAExon 17aAGAAATAAATCACTGACACACCCATGTGTACTTTGTAATATAGExon 17bCAAAGAATGGCACCAGTGTGCGACAATCTGTGTGCATCGExon 17bTTTAACCAATGACATTTGTGAATACCGATTTCAAGGAAATTAExon 18TAGGAGAAGTGTGAATAAAGGATACACAGTGACCCTCAATTExon 19GCCCGACAAATAACCAAGTGTGCTTCAGGCTACTGGGATTExon 20CAGGATTGAAAGTGTGCAACACTATGAGAAAACTGCACTGGAExon 21TGTTCACAAGGGACTCCAAAAGGGGTAGGTCCAGTCAAAAAExon 22AGAGCCATGTGCCACGTATTTCCACTGGGCAATTATTCATExon 23CCATGGTTGAAAAGCTGATTGTGTGGCTACGGAAGCCGTGTExon 24TTTCTGTCCCTGCTCTGGTCTCTGGCTTGCAAAACACAAG (1st) ACTATTGCCAGGAAGCCATT (2nd)SPINKIIntron 3-Exon 3CCAATCACAGTATTCCCCAGAGAGTTTGCTTTCTCGGGGTGAAGExon 2-Exon 3CCGACAGCAACAGAATAGCATGTGTAATGGGCACTCGAAA (1st) TACCACCCACTGTTGGA (2nd)	Exon 15	GGTTAAGGGTGCATGCTCTTC	AAGCCAGCACTGCCATTA
Exon 17aCACCAGCATGGCACATGTATCCAAAATGAAGTCACATGGTCAExon 17aAGAAATAAATCACTGACACACCCATGTGTACTTTGTAATATAGExon 17bCAAAGAATGGCACCAGTGTGCGACAATCTGTGCACTCGExon 17bTTTAACCAATGACATTGTGAATACCGATTTCAAGGAAATTAExon 18TAGGAGAAGTGTGAATAAAGGATACACAGTGACCCTCAATTExon 19GCCCGACAAATAACCAAGTGTGCTTCAGGCTACTGGGATTExon 20CAGGATTGAAAGTGTGCAACACTATGAGAAAACTGCACTGGAExon 21TGTTCACAAGGGACTCCAAAAGGGGTAGGTCCAGTCAAAAExon 22AGAGCCATGTGCCACGTATTTCCACTGGGCAATTATTTCATExon 23CCATGGTTGAAAAGCTGATTGTGAGTAAAGCTGGATGGCTGTExon 24TTTCTGTCCCTGCTCTGGTCTCTGGCTTGCAAAACACAAG (1st) ACTATTGCCAGGAAGCCATTT (2nd)SPINKIIntron 3-Exon 3CCAAATCACAGATATTCCCCAGAGGTTTGCTTTCTCGGGGAGAGA(1st) TGTGTAATGGGCACTCGAAA (1st) TACCACCCACTGTTGA (2nd)Exon 2-Exon 3CCGACAGCAACAGAATAGCATGTGTAATGGGCACTCGAAA (1st) TACCACCCACTGTTGA (2nd)	Exon 15	TAGACTCAAGTTTAGTTCCATT	TAGACTCAAGTTTAGTTCCATT
Exon 17aCACCAGCATGGCACATGTATCCAAAATGAAGTCACATGGTCAExon 17aAGAAATAAATCACTGACACACCCATGTGTACTTTGTAATATAGExon 17bCAAAGAATGGCACCAGTGTGCGACAATCTGTGTGCATCGExon 17bTTTAACCAATGACATTGTGAATACCGATTTCAAGGAAATTAExon 17bTAGGAGAAGTGTGAATAAAGGATACACAGTGACCCTCAATTExon 18TAGGAGAAGTGTGAATAAAGGATACACAGTGACCCTCAATTExon 19GCCCGACAAATAACCAAGTGTGCTTCAGGCTACTGGGATTExon 20CAGGATTGAAAGTGTGCAACACTATGAGAAAACTGCACTGGAExon 21TGTTCACAAGGGACTCCAAAAGGGGTAGGTCCAGTCAAAAExon 22AGAGCCATGTGCCACGTATTTCCACTGGGCAATTATTTCATExon 23CCATGGTTGAAAAGCTGATTGTGAGTAAAGCTGGATGGCTGTExon 24TTTCTGTCCCTGCTCTGGTCTCTGGCTTGCAAAACACAAG (1st) ACTATTGCCAGGAAGCCATTT (2nd)SPINKIIntron 3-Exon 3CCAAACACAGAATAGCAGTTTGCTTTCTCGGGGTGAAG (1st) TACCAACTCCAACAGAATAGCAExon 2-Exon 3CCGACAGCAACAGAATAGCATGTGTAATGGGCACTCGAAA (1st) TACCACCCACTGTTCGTTGA (2nd)	Exon 16	TCTGAATGCGTCTACTGTGA	GCAATAGACAGGACTTCAAC
Exon 17bCAAAGAATGGCACCAGTGTGCGACAATCTGTGTGCATCGExon 17bTTTAACCAATGACATTGTGAATACCGATTTCAAGGAAATTAExon 18TAGGAGAAGTGTGAATAAAGGATACACAGTGACCCTCAATTExon 19GCCCGACAAATAACCAAGTGTGCTTCAGGCTACTGGGATTExon 20CAGGATTGAAAGTGTGCAACACTATGAGAAAACTGCACTGGAExon 21TGTTCACAAGGGACTCCAAAAGGGGTAGGTCCAGTCAAAAExon 22AGAGCCATGTGCCACGTATTTCCACTGGGCAATTATTTCATExon 23CCATGGTTGAAAAGCTGATTGTGAGTAAAGCTGGATGGCTGTExon 24TTTCTGTCCCTGCTCTGGTCTCTGGCTTGCAAAACACAAG (1st) ACTATTGCCAGGAAGCCATTT (2nd)SPINKIIntron 3-Exon 3CCAATCACAGTTATTCCCCAGAGGTTTGCTTTCTCGGGGTGAAG (1st) TGCCAACTCCTATCCCACTGExon 2-Exon 3CCGACAGCAACAGAATAGCATGTGTAATGGGCACTCGAAAA (1st) TACCACCACTGTTCGTCGA (2nd)	Exon 17a	CACCAGCATGGCACATGTAT	CCAAAATGAAGTCACATGGTCA
Exon 17bTTTAACCAATGACATTGTGAATACCGATTTCAAGGAAATTAExon 18TAGGAGAAGTGTGAATAAAGGATACACAGTGACCCTCAATTExon 19GCCCGACAAATAACCAAGTGTGCTTCAGGCTACTGGGATTExon 20CAGGATTGAAAGTGTGCAACACTATGAGAAAACTGCACTGGAExon 21TGTTCACAAGGGACTCCAAAAGGGGTAGGTCCAGTCAAAAExon 22AGAGCCATGTGCCACGTATTTCCACTGGGCAATTATTTCATExon 23CCATGGTTGAAAAGCTGATTGTGAGTAAAGCTGGATGGCTGTExon 24TTTCTGTCCCTGCTCTGGTCTCTGGCTTGCAAAACACAAAG (1st)ACTATTGCCAGGAAGCAATTATTCCCCAGAGACTATTGCCAGGAAGCCATTT (2nd)SPINKIIntron 3-Exon 3CCGACAGCAACAGAATAGCATGTGTAATGGGCACTCGAAA (1st)Exon 2CCGACAGCAACAGAATAGCATGTGTAATGGGCACTCGAAAA (1st)Exon 2TCCCAACTCCTATCCCACTGTACCACCCACTGTTGCACACTGA (2nd)	Exon 17a	AGAAATAAATCACTGACACAC	CCATGTGTACTTTGTAATATAG
Exon 18TAGGAGAAGTGTGAATAAAGGATACACAGTGACCCTCAATTExon 19GCCCGACAAATAACCAAGTGTGCTTCAGGCTACTGGGATTExon 20CAGGATTGAAAGTGTGCAACACTATGAGAAAACTGCACTGGAExon 21TGTTCACAAGGGACTCCAAAAGGGGTAGGTCCAGTCAAAAExon 22AGAGCCATGTGCCACGTATTTCCACTGGGCAATTATTTCATExon 23CCATGGTTGAAAAGCTGATTGTGAGTAAAGCTGGATGGCTGTExon 24TTTCTGTCCCTGCTCTGGTCTCTGGCTTGCAAAAACACAAG (1st)ACTATTGCCAGGAAGCCATTT (2nd)ACTATTGCCAGGAGGGTGAGFPINKIIntron 3-Exon 3CCAATCACAGTTATTCCCCAGAGAExon 2-Exon 3CCGACAGCAACAGAATAGCATGTGTAATGGGCACTCGAAA (1st)Exon 2TCCCAACTCCTATCCCACTGTACCACCCACTGTTCGTTGA (2nd)	Exon 17b	CAAAGAATGGCACCAGTGTG	CGACAATCTGTGTGCATCG
Exon 19GCCCGACAAATAACCAAGTGTGCTTCAGGCTACTGGGATTExon 20CAGGATTGAAAGTGTGCAACACTATGAGAAAACTGCACTGGAExon 21TGTTCACAAGGGACTCCAAAAGGGGTAGGTCCAGTCAAAAExon 22AGAGCCATGTGCCACGTATTTCCACTGGGCAATTATTCATExon 23CCATGGTTGAAAAGCTGATTGTGAGTAAAGCTGGATGGCTGTExon 24TTTCTGTCCCTGCTCTGGTCTCTGGCTTGCAAAAACACAAG (1st)ACTATTGCCAGGAAGCCATTT (2nd)ACTATTGCCAGGAAGCCATTT (2nd)SPINKIIntron 3-Exon 3CCAATCACAGTTATTCCCCAGAGGTTTGCTTTCTCGGGGTGAAGFxon 2-Exon 3CCGACAGCAACAGAATAGCATGTGTAATGGGCACTCGAAA (1st)Exon 2TCCCAACTCCTATCCCACTGTACCACCCACTGTTCGTTGA (2nd)	Exon 17b	TTTAACCAATGACATTTGTGA	ATACCGATTTCAAGGAAATTA
Exon 20CAGGATTGAAAGTGTGCAACA TGTTCACAAGGGACTCCAAACTATGAGAAAACTGCACTGGA AGGGGTAGGTCCAGTCAAAAExon 21TGTTCACAAGGGACTCCAAA AGGGCATGTGCCAGTGAAAATCCACTGGGCAGTCAAAAAExon 22AGAGCCATGTGCACGTATT CCATGGTTGAAAAGCTGATTG TTTCTGTCCCTGCTCTGGTCTGAGTAAAGCTGGATGGCTGTExon 24TTTCTGTCCCTGCTCTGGTC TCTGGCTTGCAAAACACAAAG(1st) ACTATTGCCAGGAAGCCATTT (2nd)SPINKI Intron 3-Exon 3CCAATCACAGTTATTCCCCAGAG PRSSIGTTTGCTTTCTCGGGGTGAAGExon 2-Exon 3CCGACAGCAACAGAATAGCA TCCCAACTCCTATCCCACTGTGTGTAATGGGCACTCGAAA(1st) TACCACCCACTGTTGA (2nd)	Exon 18	TAGGAGAAGTGTGAATAAAG	GATACACAGTGACCCTCAATT
Exon 20CAGGATTGAAAGTGTGCAACACTATGAGAAAACTGCACTGGAExon 21TGTTCACAAGGGACTCCAAAAGGGGTAGGTCCAGTCAAAAExon 22AGAGCCATGTGCCACGTATTTCCACTGGGCAATTATTTCATExon 23CCATGGTTGAAAAGCTGATTGTGAGTAAAGCTGGATGGCTGTExon 24TTTCTGTCCCTGCTCTGGTCTCTGGCTTGCAAAACACAAG (1st)ACTATTGCCAGGAAGCCATTT (2nd)ACTATTGCCAGGAAGCCATTT (2nd)SPINKIIntron 3-Exon 3CCAATCACAGTTATTCCCCAGAGGTTTGCTTTCTCGGGGTGAGFxon 2-Exon 3CCGACAGCAACAGAATAGCATGTGTAATGGGCACTCGAAA (1st)Exon 2TCCCAACTCCTATCCCACTGTACCACCCACTGTTGA (2nd)	Exon 19	GCCCGACAAATAACCAAGTG	TGCTTCAGGCTACTGGGATT
Exon 21TGTTCACAAGGGACTCCAAAAGGGGTAGGTCCAGTCAAAAExon 22AGAGCCATGTGCCACGTATTTCCACTGGGCAATTATTTCATExon 23CCATGGTTGAAAAGCTGATTGTGAGTAAAGCTGGATGGCTGTExon 24TTTCTGTCCCTGCTCTGGTCTCTGGCTTGCAAAACACAAG (1st)ACTATTGCCAGGAAGCCATTT (2nd)ACTATTGCCAGGAAGCCATTT (2nd)SPINKIIntron 3-Exon 3CCAATCACAGTTATTCCCCAGAGGTTTGCTTTTCTCGGGGGTGAGPRSS1Exon 2-Exon 3CCGACAGCAACAGAATAGCATGTGTAATGGGCACTCGAAA (1st)Exon 2TCCCAACTCCTATCCCACTGTACCACCCACTGTTGA (2nd)	Exon 20	CAGGATTGAAAGTGTGCAACA	
Exon 22AGAGCCATGTGCCACGTATT CCATGGTTGAAAAGCTGATTG TTTCTGTCCCTGCTCAGATTG TTTCTGTCCCTGCTCGGTCTCCACTGGGCAATTATTTCAT TGAGTAAAGCTGGATGGCTGT TCTGGCTTGCAAAACACAAG (1st) ACTATTGCCAGGAAGCCATTT (2nd)SPINKI Intron 3-Exon 3CCAATCACAGTTATTCCCCAGAG PRSS1GTTTGCTTTCTCGGGGGTGAG TCCCAACTCCTATCCCACTGExon 2-Exon 3CCGACAGCAACAGAATAGCA TCCCAACTCCTATCCCACTGTGTGTAATGGGCACTCGAAA (1st) TACCACCCACTGTTCGTTGA (2nd)	Exon 21		
Exon 24TTTCTGTCCCTGCTCGGTCTCTGGCTTGCAAAACACAAG(1st) ACTATTGCCAGGAAGCCATTT (2nd)SPINKIKCCAATCACAGTTATTCCCCAGAGGTTTGCTTTTCTCGGGGGTGAGIntron 3-Exon 3CCGACAGCAACAGAATAGCATGTGTAATGGGCACTCGAAA(1st) TACCACCACTGTTGA (2nd)		AGAGCCATGTGCCACGTATT	TCCACTGGGCAATTATTTCAT
Exon 24TTTCTGTCCCTGCTCGGTCTCTGGCTTGCAAAACACAAG(1st) ACTATTGCCAGGAAGCCATTT (2nd)SPINKIKCCAATCACAGTTATTCCCCAGAGGTTTGCTTTTCTCGGGGGTGAGIntron 3-Exon 3CCGACAGCAACAGAATAGCATGTGTAATGGGCACTCGAAA(1st) TACCACCACTGTTGA (2nd)	Exon 23	CCATGGTTGAAAAGCTGATTG	TGAGTAAAGCTGGATGGCTGT
ACTATTGCCAGGAAGCCATTT (2nd)SPINK1Intron 3-Exon 3CCAATCACAGTTATTCCCCAGAGGTTTGCTTTTCTCGGGGGTGAGPRSS1Exon 2-Exon 3CCGACAGCAACAGAATAGCATGTGTAATGGGCACTCGAAA (1st)Exon 2TCCCAACTCCTATCCCACTGTACCACCCACTGTTCGTTGA (2nd)			
SPINK1Intron 3-Exon 3CCAATCACAGTTATTCCCCAGAGGTTTGCTTTTCTCGGGGGTGAGPRSS1Exon 2-Exon 3CCGACAGCAACAGAATAGCATGTGTAATGGGCACTCGAAA (1st)Exon 2TCCCAACTCCTATCCCACTGTACCACCCACTGTTCGTTGA (2nd)			
Intron 3-Exon 3CCAATCACAGTTATTCCCCAGAGGTTTGCTTTTCTCGGGGTGAGPRSS1Exon 2-Exon 3CCGACAGCAACAGAATAGCATGTGTAATGGGCACTCGAAA(1st)Exon 2TCCCAACTCCTATCCCACTGTACCACCCACTGTTCGTTGA(2nd)	SPINKI		······································
PRSS1Exon 2-Exon 3CCGACAGCAACAGAATAGCATGTGTAATGGGCACTCGAAA(1st)Exon 2TCCCAACTCCTATCCCACTGTACCACCCACTGTTCGTTGA(2nd)		CCAATCACAGTTATTCCCCAGAG	GTTTGCTTTTCTCGGGGTGAG
Exon 2 TCCCAACTCCTATCCCACTG TACCACCCACTGTTCGTTGA (2nd)			
Exon 2 TCCCAACTCCTATCCCACTG TACCACCCACTGTTCGTTGA (2nd)	Exon 2-Exon 3	CCGACAGCAACAGAATAGCA	TGTGTAATGGGCACTCGAAA (1st)
	Exon 2	TCCCAACTCCTATCCCACTG	
	Exon 3	CCTCCAGAGCTGTCCATGAG	ATGGGCACTCGAAATGTGTC (2nd)

Table 1. Primer sequences for conventional PCR analysis of CFTR, SPINK1, and PRSS1 genes

	ACP	ICP	NS		
	N=140	n=36	n=360		
E217G (Exo	n 6a)				
Glu	137 (97.9%)	36 (100%)	354 (98.3%)		
Gly	3 (2.1%)	0 (0%)	6 (1.7%)		
M470V (Exc	on 10)				
Met	60 (42.9%)	14 (38.9%)	143 (39.7%)		
Val	80 (57.1%)	22 (61.1%)	217 (60.3%)		
I556V (Exor	n 11)				
Ile	138 (98.6%)	36 (100%)	348 (96.7%)		
Val	2 (1.4%)	0 (0%)	12 (3.3%)		
L1156F (Exc	on 18)				
Leu	133 (95.0%)	35 (97.2%)	358 (99.4%)		
Phe	7 (5.0%) *	1 (2.8%)	2 (0.6%)		
Q1352H (Ex	(xon 22)				
Gln	129 (92.1%)	35 (97.2%)	353 (98.1%)		
His	11 (7.9%) *	1 (2.8%)	7 (1.9%)		
R1453W (Ex	kon 24)				
Arg	138 (98.6%)	32 (88.9%)	353 (98.1%)		
Trp	2 (1.4%)	4 (11.1%) *	7 (1.9%)		

Table 2. The allele frequencies of polymorphisms in the coding regions of *CFTR* gene

\*: p<0.01 vs. NS by Yates Chi square test ACP: alcoholic chronic pancreatitis ICP: idiopathic chronic pancreatitis NS: normal subjects

# Table 3. Characteristics of 8 patients who carry L1156F

CFTR		Sex	Age	Etiology	Pancreatic	Secretin test*		Sweat	SPINK1	PRSS1		
genotypes					Stone	Volume	MBC	Amylase	[Cl <sup>-</sup> ]	mutation	mutation	
1	L1156F+Q1352H	+ V/V470 + 7/7T + 11/11(TG)	М	59	alcoholic	+					-	-
2	L1156F	+ V/V470 + 7/7T + 11/11(TG)	М	65	alcoholic	+	174.5	96.9	21524	42.0	-	-
3	L1156F	+ V/V470 + 7/7T + 11/12(TG)	М	49	alcoholic	-					-	-
4	L1156F+Q1352H	+ M/V470 + 7/7T + 11/12(TG)	М	57	alcoholic	+				88.9	-	-
5	L1156F	+ M/V470 + 7/7T + 11/12(TG)	М	60	alcoholic	+	53.0	50.9	1627	67.0	-	-
6	L1156F	+ M/V470 + 7/7T + 11/12(TG)	М	56	alcoholic	+	149.0	71.3	6891	98.9	-	-
7	L1156F	+ M/V470 + 7/7T + 11/12(TG)	М	51	alcoholic	+					N34S	-
8	L1156F	+ M/V470 + 7/7T + 11/12(TG)	F	73	idiopathic	+				58.9	-	-

Genotypes of CFTR indicate presence of L1156F and G1352H, M/V470, poly T, and TG repeats.

\* The lower limits of normal range of volume, the maximum HCO<sub>3</sub><sup>-</sup> concentration (MBC), and amylase output are 183 mL/h, 80 mEq/L, and 99,000 U/h.