Clin Genet 2007: 71: 451–457
Printed in Singapore. All rights reserved

CLINICAL GENETICS
doi: 10.1111/j.1399-0004.2007.00788.x

Short Report

Contribution of the *CFTR* gene, the pancreatic secretory trypsin inhibitor gene (*SPINK1*) and the cationic trypsinogen gene (*PRSS1*) to the etiology of recurrent pancreatitis

Tzetis M, Kaliakatsos M, Fotoulaki M, Papatheodorou A, Doudounakis S, Tsezou A, Makrythanasis P, Kanavakis E, Nousia-Arvanitakis S. Contribution of the *CFTR* gene, the pancreatic secretory trypsin inhibitor gene (*SPINK1*) and the cationic trypsinogen gene (*PRSS1*) to the etiology of recurrent pancreatitis.
Clin Genet 2007: 71: 451–457. © Blackwell Munksgaard, 2007

Acute recurrent/chronic pancreatitis (CP) is a complex multigenic disease. This is a case–control study consisting of 25 Greek patients with CP and a control population of 236 healthy Greek subjects. The whole coding area and neighboring intronic regions of the three genes were screened. Seventeen of 25 patients (68%) had mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene: nine compound heterozygotes with either mild or severe mutations and eight heterozygotes. Four patients (16%) carried CFTR-modulating haplotypes V470-TG11-T5 and V470-TG12-T7. All were negative for *PRSS1* gene mutations, while variants c.486C/T and c.738C/T were found in nine patients each, three homozygotes for the minor alleles. Two carried *SPINK1* gene mutation p.N34S, one being transheterozygote with *CFTR* mutation p.F1052V. The promoter variant −253T>C was found in four individuals (one homozygous for the minor allele), all four being transheterozygotes with mutations in the *CFTR* gene as well. Finally two carried c.272C/T in the 3′ untranslated region, one being a p.N34S carrier as well. In total, 80% (20/25) of patients had a molecular defect in one or both of the *CFTR* and *SPINK1* genes, suggesting that mutations/variants in the *CFTR* plus or minus mutations in the *SPINK1*, but not the *PRSS1* gene, may confer a high risk for recurrent pancreatitis.

M Tzetis[a,*], M Kaliakatsos[a,*], M Fotoulaki[b,*], A Papatheodorou[a], S Doudounakis[c], A Tsezou[d], P Makrythanasis[a], E Kanavakis[a] and S Nousia-Arvanitakis[b]

[a]Department of Medical Genetics, Medical School, Athens University, Athens, [b]Fourth Department of Pediatrics, Medical School, Aristotle University, Thessaloniki, [c]Cystic Fibrosis Center, Aghia Sophia Children's Hospital, Athens, and [d]Laboratory of Cytogenetics and Molecular Genetics, Medical School, University of Thessaly, Larissa, Greece

*These authors contributed equally to the study.

Key words: CFTR – chronic pancreatitis – PRSS1 – recurrent pancreatitis – SPINK1

Corresponding author: Maria Tzetis, PhD, Medical Genetics, Athens University, St Sophia's Children's Hospital, Athens 11527, Greece.
Tel.: +30 210 746 7461;
fax: +30 210 779 5553;
e-mail: mtzeti@cc.uoa.gr

Received 31 October 2006, revised and accepted for publication 26 January 2007

Acute recurrent pancreatitis (ARP) is an inflammatory disease affecting the acinar cells, leading to structural and functional damage to the pancreas (1, 2). Recurrent episodes of pancreatitis may evolve into chronic pancreatitis (CP) and progressive loss of exocrine and endocrine pancreatic function (3–7). The etiological factor of one-third of patients with ARP/CP is unknown. This group of patients is classified as suffering from idiopathic chronic pancreatitis (ICP). Following the description of an autosomal dominant mutation in the cationic trypsinogen gene (*PRSS1*) in families with hereditary pancreatitis (3), investigations began in other genes

that inhibit pancreatic tryptic activity or regulate pancreatic secretory function.

Cystic fibrosis (CF) [cystic fibrosis transmembrane conductance regulator (CFTR); OMIM 602421] the most common (~4% carriers) severe autosomal recessive genetic disease is caused by >1300 mutations (http://www.genet.sickkids.on.ca/cftr) in the gene encoding the *CFTR*. The gene functions as a cAMP-activated chloride channel and promotes cAMP-regulated bicarbonate and fluid secretion thus diluting and alkalinizing the pancreatic juice to prevent obstruction of the smaller pancreatic ducts with proteinaceous plugs (8). Some CFTR mutations may selectively disrupt bicarbonate conductance and, therefore, selectively target the pancreas for CFTR-associated injury (9–11).

CFTR gene mutations are subdivided into six classes (12), with rarer class IV–V mutations retaining residual function (1–10%), therefore associated with pancreatic sufficiency (PS) or monosymptomatic forms of the disease, including ICP and ARP (13–16). For monosymptomatic CFTR, specific variant haplotypes were shown to affect the penetrance of the mutation. The IVS8−5T variant combined with increased number of TG repeats found immediately adjacent to the T's result in the presence of only 5–10% of the normal CFTR messenger RNA, additionally the allele present at the polymorphic locus p. M470V (c.1640A>G) affects the biogenesis of the CFTR protein and the gating of the CFTR channel, with M470 CFTR proteins having a 1.7-fold increased intrinsic chloride activity compared with V470 CFTR proteins (17–19).

In patients with CF, PS diagnosis of pancreatitis may precede the diagnosis for CF. A large study of 10,071 patients (5) found that 10.3% of patients with CF–PS suffered from pancreatitis with the majority carrying at least one mild class IV or V mutation. Other studies have also shown increased susceptibility of CFTR heterozygotes to developing CP (1, 15, 20).

Cationic trypsinogen, the major isoform of trypsinogen in human pancreatic juice affects normal activation of other digestive enzymes in the pancreas. Gain-of-function mutations in the *PRSS1* gene (OMIM 276000; 7q35) result in an enhanced autoactivation of trypsinogen to trypsin. Mutations in the gene generally show an autosomal dominant mode of inheritance and were identified as a cause of hereditary pancreatitis (OMIM 167800) (3), with over 20 mutations detected to date (http://www.uni-leipzig.de/pancreasmutation/db.html).

The serine protease inhibitor Kazal-type 1 SPINK1 (OMIM 167790) is a potent antiprotease that is thought to be an important inactivation factor of intrapancreatic trypsin activity. Loss-of-function *SPINK1* mutations (OMIM 167790), lead to decreased inhibitory capacity and show a complex mode of inheritance (7, 21, 22).

The purpose of our study was to evaluate the hypothesis that mutations of the *CFTR*, *PRSS1* and/or *SPINK1* genes are associated with ARP/ICP.

Materials and methods

Patients and controls

A total of 25 Greek patients (10 men) aged 3–45 years (23.36 ± 11.38) with ICP/ARP participated in the study. They were recruited from the Gastroenterology Outpatient Clinic of the Fourth Department of Pediatrics of the Aristotle University of Thessaloniki and the CF Center of the St Sophia's Children's Hospital, Athens. The criteria for the diagnosis of ARP were based on at least two attacks of acute pancreatitis presenting with abdominal pain and threefold increased serum amylase. Exclusion criteria included the presence of factors such as alcohol, gallstones, trauma, medication, infection or metabolic disorders and a report of positive family history. None of the patients had a prior diagnosis of typical CF or pulmonary involvement.

Sweat chloride measurements were performed by pilocarpine iontophoresis according to the National Committee for Clinical Laboratory Standards (23) for all patients. Fifteen had normal sweat Cl^- (<40 mEq/l) and 10 increased (≥60 mEq/l).

Fecal pancreatic elastase (E1) was determined by enzyme-linked immunosorbent assay (SheBo Tech, Wettenberg, Germany), and pancreatic function was considered normal when fecal pancreatic elastase was at least 200 μg/g stools.

The healthy control population was of the same ethnic origin as the patients and included healthy relatives of ARP/ICP patients ($n = 25$), screened for the *CFTR*, *PRSS1*, and *SPINK* genes and 211 healthy partners or general Greek population subjects who came to the Department of Medical Genetics for *CFTR* gene screening.

Finally, the frequency of CFTR mutations in the ARP/ICP patients and general Greek population were compared to a group of 426 classic CF patients who came to the Department of Medical Genetics for CF mutation characterization from 1998 to 2005.

The Hospital Review Board approved the study and all participants provided informed consent.

Mutation analysis

Genomic DNA was obtained from 3 ml of peripheral blood, using the BioRobot® M48 System (Qiagen, Hilden, Germany) and the commercially available kit MagAttract® DNA Blood Midi M48 Kit (Qiagen).

All exons and neighboring intronic regions of the three genes were assessed by denaturing gradient gel electrophoresis (DGGE) after simple or multiplex polymerase chain reaction of patient DNA (21, 24, 25) (Table S1, supplementary material online). The intron 8 poly(T) variants of the *CFTR* gene were analyzed as described (26). DNA samples showing a shift in DGGE mobility and not presenting a pattern of a known mutation were sequenced using the VISGEN Long Read Tower (Visible Genetics Inc., Toronto, Canada) (24). Sequencing primers for the *PRSS1* and *SPINK1* genes were designed for this study (Table S2, supplementary material online).

Statistical analysis

The frequency of mutations was determined by counts of patients. Differences between proportions were compared by the chi-square statistic or Fisher's exact test. All p values were based on two-sided comparison and values of less than 0.05 were considered to indicate statistical significance.

Results

The patients included came from all parts of Greece. Age of onset of symptoms varied from 3 to 45 years and all patients had 2–9 (mean 4) episodes of pancreatitis at first investigation. Abdominal sonographic findings were non-specific and pseudocysts were not detected. The episodes of acute pancreatitis in 10 of the patients (six women; 14–36 years old), were the initial manifestation which led to the suspicion of CF. None of the patients had a combination of two severe CF mutations as would be expected from a cohort from which all individuals with classic CF had been excluded.

A total of 20 changes were found: 14 in the *CFTR* gene, two in the *PRSS1* gene and four in the *SPINK1* gene (Tables 1–3).

Molecular findings for the *CFTR* gene

Eight patients were carriers (16%): three with p.R1070Q and five with p.F508del, p.G576A, p.F1052V, CFTRdel2,3 (21 kb), and c.2752−15G/C, each, representing a heterozygote frequency 2.1-fold higher than that found in the 211 general population controls (7.6%, $p < 0.0001$). Nine patients (36%) were compound heterozygotes for two CFTR mutations, both mild (class IV or V): p.I148T/p.R75Q, c.2789+5G>A/p.R75Q or mild and severe: three with p.F508del/p.R334W and four with c.444delA/p.R334W, p.E822X/c.2789+5G>A, p.E822X/p.R347H and p.F508del/c.3272−26A>G, each. The most common mutation F508del was found in 10% of patients and only in 0.47% of the controls ($p < 0.0001$).

The exon 9 missplicing, low chloride activity CFTR protein haplotypes V470-TG11-T5 and V470-TG12-T7 were found in four patients (8%), two in combination with CFTR mutations (Table 1).

Molecular findings for the *PRSS1* gene

No mutations in the *PRSS1* gene were detected in the patients while promoter polymorphism −36G>A was found once in the controls.

Synonymous variant p.D162D was found in nine patients (18%) and in 10 controls (20%). Both groups included eight heterozygotes and two homozygotes for the T allele. Polymorphism p.N246N in exon 5 of the gene was found in nine patients (18%) and in 11 controls (22%), again both groups included two homozygotes for the minor T allele (Table 3).

Molecular findings for the *SPINK1* gene

One mutation and three variants were detected in the *SPINK1* gene in our patient cohort.

Mutation p.N34S was found in two patients (4%) and in none of the controls. One patient was additionally transheterozygote for CFTR mutation p.F1052V. The p.N34S was linked to intronic variant IVS1−37T/C in both cases (Tables 1 and 3).

The c.272C>T variant in the 3′ untranslated region (UTR) was found in two patients (4%), in combination with p.N34S in one of them and in none of the controls. The −253T/C polymorphism in the promoter region was found in four patients (8%), including one homozygous for the minor C allele, and in five controls (10%) (Table 3).

Discussion

By screening the whole coding sequence of the three genes (*CFTR*, *PRSS1* and *SPINK1*) using

Table 1. CFTR, PRSS1 and SPINK1 genotypes in patients with pancreatitis

Patient ID	Sex/age	CFTR allele 1	CFTR allele 2	CFTR variants		PRSS1		SPINK1		
1523[a]	M18	p.R1070Q		5T/7T-TG11/TG10	V470	—		—		
1546[a]	F19	—	—	7T/7T-TG11/TG10	M470	p.D162D (c.486C/T)	p.N246N (c.738C/T)	—		
1650[a]	F30	—	—	5T/7T-TG11/TG11	M470V c.4521G/A; c.875+40A/G	p.D162D (c.486C/T)	p.N246N (c.738C/T)	—		
2851[a]	F39	p.G576A	—	7T/7T-TG10/TG11	M470V c.2134C/T	—		−253T/C		
1948[a]	M45	—	—	7T/9T-TG11/TG10	M470V c.1001+11C/T	p.D162D (c.486C/T)	p.N246N (c.738C/T)	—		
2718[a]	F25	p.R1070Q	—	7T/7T-TG11/TG11	M470V	p.D162D (c.486C/T)		−253T/C		
2872[a]	F30	—	—	7T/7T-TG12/TG10	V470	p.D162D (c.486C/T)	p.N246N (c.738C/T)	c.272C/T (3′UTR)		
2888[a]	F23	p.R1070Q	—	9T/7T-TG12/TG10	M470V c.4002A/G	—		−253T/C		
2901[a]	F42	—	—	7T/7T-TG10/TG10	M470V	p.D162D (c.486C/T)	p.N246N (c.738C/T)	—		
3301[a]	M30	—	—	7T/7T-TG11/TG11	V470	—		—		
3358[a]	M11	—	—	7T/7T-TG11/TG11	M470V	p.D162D (c.486C/T)		p.N34S	c.272C/T (3′UTR)	IVS1−37T/C
2978[a]	F19	—	—	7T/7T-TG11/TG11	V470	p.D162D (c.486C/T)		—		
3534[a]	M4	p.F1052V	—	9T/7T-TG10/TG11	M470	p.N246N (c.738C/T)		p.N34S		IVS1−37T/C
3785[a]	M3	p.I148T	p.R75Q	7T/7T/TG11/TG11	M470V	c.486T/T		—		
3740[a]	F12	—	c.2752−15G/C	7T/7T-TG11/TG11	M470V	—		—		
177[b]	F18	c.444delA	p.R334W	7T/7T-TG11/TG11	M470V	—	—			
254[b]	M18	p.F508del	p.R334W	9T/7T-TG10/TG11	M470V	—	—			
673[b]	F19	c.2789+5G>A	p.R75Q	7T/7T-TG11/TG11	M470V c.2694T/G; c.4521G/A; c.3041−52C/G	—	—			
1640[b]	F14	CFTRdel2,3 (21 kb)	—	7T/7T-TG10/TG11	M470V c.874+40A/G; c.2694T/G; c.4521G/A	—	—			
3575[b]	F22	p.E822X	c.2789+5G>A	7T/7T-TG11/TG11	M470V	p.N246N (c.738T/T)	—			
3563[b]	F35	p.F508del	p.R334W	9T/7T-TG10/TG11	M470V	p.N246N (c.738C/T)	−253C/C			
3412[b]	F36	p.E822X	p.R347H	7T/7T-TG11/TG11	V470	—	—			
3576[b]	M23	p.F508del	p.R334W	9T/7T-TG11/TG10	M470V	—	—			
3956[b]	M37	p.F508del	c.3272−26A>G	9T/7T-TG11/TG10	M470	p.N246N (c.738T/T)				
758[b]	M12	p.F508del	—	7T/9T-TG10/TG11	M470V	—	—			

UTR, untranslated region.
[a]Patients with pancreatitis with normal sweat test level (<40 mEq/l).
[b]Patients with pancreatitis with increased sweat chloride level (>60 mEq/l).

Table 2. Mutations and variants in the *CFTR* gene

CFTR mutation/variant	Patients with pancreatitis, $n = 25$ (%)	Controls[a], $n = 211$ (%)	Classic patients with CF, $n = 426$ (%)	p *vs* controls	p *vs* patients with CF
p.F508del	5 (10)	2 (0.47)	465 (54.6)	**<0.0001**	**<0.0001**
p.R334W	4 (8)	—	7 (8.2)	**0.00011**	**0.0019**
c.444delA	1 (2)	—	1 (0.1)		
c.2789+5G>A	2 (4)	—	11 (1.3)	**0.011**	
CFTRdel2,3 (21 kb)	1 (2)	—	2 (0.2)		
c.E822X	2 (4)	—	12 (1.5)	**0.011**	
p.R347H	1 (2)	—	—		**0.055**
p.R1070Q	3 (6)	1 (0.24)	7 (0.8)	**0.004**	**0.013**
p.G576A	1 (2)	—	1 (0.1)		
p.F1052V	1 (2)	4 (0.95)	1 (0.1)		
p.I148T	1 (2)	—	1 (0.1)		
c.3272−26A>G	1 (2)	—	7 (0.82)		
p.R75Q	2 (4)	4 (0.95)	1 (0.1)		**0.0086**
c.2752−15G/C	1 (2)	4 (1)	5 (0.6)		
TG11T7	26 (52)	286 (67.7)	ND		
TG11T5	2 (4)	5 (1.18)	ND		
TG10T7	8 (16)	79 (18.7)	ND		
TG10T9	8 (16)	14 (3.3)	ND	**0.0005**	
TG12T7	2 (4)	8 (1.9)	ND		
M470	6 (12)	48 (11.4)	ND		
V470	8 (16)	166 (39.3)	ND	**0.008**	

CF, cystic fibrosis; ND, not determined. p values with statistical significance only shown. p value in all mutations in patients with pancreatitis *vs* controls: >0.000001 (shown in bold).
[a]Additional mutations found in the controls: p.R1162L (1.66%), p.D565G (0.47%), p.A120T (0.47%) and 0.24% each for p.R297Q, p.L997F, p.E826K, p.I807M, p.S495Y and p.C491S.

DGGE and sequencing we were able to detect both common and uncommon mutations/variants and additionally assess the risk contribution of variant haplotypes on ICP/ARP development.

In summary, 13 patients had mutations/variants in the *CFTR* gene alone, six were transheterozygotes for *CFTR* and *SPINK1* while one carried both molecular defects in the *SPINK1* gene.

It is of interest that a distinct subset of CF mutations or with frequencies differing from classic CF was found in our pancreatitis cohort. It could additionally be argued that specific mild mutations are pancreatitis pre-disposing. This could especially apply for p.R334W, p.R347H, p.R1070Q, p.R75Q and c.2789+5G>A, for which the difference in mutation frequency between patients and classic CF cohort, reached statistical significance (Table 2). It is especially of note that p.I148T in our patient cohort was not found linked to c.3199del6, which has been considered the classic CF causing allele (27).

Mutation CFTRdel2,3 (21 kb) considered a severe mutation and found once in our patient cohort, has been previously reported in an otherwise healthy 43-year-old woman, who presented with mild relapsing pancreatitis and had R117H in trans (28). Our patient has no other molecular defect (Tables 1 and 2).

Mutation p.G576A, found in transheterozygote state with SPINK1 −253T/C promoter variant in our patient, has previously been reported in a CP patient, although the previous study did not screen the *SPINK1* gene as well (16).

The function modulating *CFTR* haplotypes (V470-TG11-T5 and V470-TG12-T7) were found either alone (patient 1650) or more commonly either in compound heterozygosity with p.R1070Q (patient 1523) or in trans-heterozygous state in two cases with c.272C/T *SPINK1* variant (Table 1).

Previous studies have identified variants in the *SPINK1* gene, and in particular the p.N34S

Table 3. Mutations and variants in the *PRSS1* and *SPINK1* genes

Gene	Mutation	Patients with pancreatitis, $n = 25$ (%)	Controls, $n = 25$ (%)
PRSS1	c.486C/T (p.D162D)	8 (16)	8 (16)
	c.486T/T	1 (2)	2 (4)
	c.738C/T (p.N246N)	7 (14)	9 (18)
	c.738T/T	2 (4)	2 (4)
	−36G/A	—	1 (2)
SPINK1	−253T/C	3 (6)	5 (10)
	−253C/C	1 (2)	—
	c.272C/T (3′UTR)	2 (4)	—
	p.N34S	2 (4)	—
	IVS1−37T/C	2 (4)	—

UTR, untranslated region.

mutation with high frequency in CP patients. We detected two heterozygotes (4%) for p.N34S mutation, cosegregating with IVS1−37T>C one was additionally transheterozygote with CFTR mutation p.F1052V. This frequency is somewhat lower than the 9–11% carrier frequency found in previous studies for p.N34S (1, 7, 21, 22, 29) (Table 1).

Promoter variant −253T/C was detected in three patients all trans-heterozygotes with CFTR: two with p.R1070Q, and one with p.G576A. The homozygous −253C/C minor allele genotype was observed in one patient already CFTR compound heterozygous (p.F508del/p.R334W) (Table 1). This variant located in the promoter region of the gene could affect gene transcription and in combination with CFTR mutations may contribute to ARP/CP (30).

Variant c.272C/T in the 3′UTR of *SPINK1* was found in two patients (one with V470-TG12-T7 *CFTR* missplicing variant). Variants in the 3′UTR of genes could be involved in regulating gene expression and determine such properties as mRNA stability, nuclear export, subcellular localization and translation efficiency thus modifying disease susceptibility in combination with other genetic and environmental factors. A functional *in vitro* analysis of c.272C/T could elucidate its effects on SPINK1 mRNA expression and/or stability (31).

Previous studies have shown a combination of PRSS1 and a CFTR mutation (7, 16), PRSS1 plus SPINK1 (1, 6, 32) or CFTR and SPINK1 mutations (1, 16). Our results demonstrate that 80% (20/25) of patients labeled as ARP/CP had in fact a genetic defect in either or both of the *CFTR* and *SPINK1* genes (Table 1).

The identification of heterozygous *CFTR* or *SPINK1* mutations does not fully explain the disease in a subject nor does it predict pancreatitis risk because obligate *CFTR* mutation carriers do not have an excess incidence of pancreatitis; however, compound heterozygosity of mild/mild or severe/mild CFTR mutations may predispose to pancreatitis. Furthermore, the coexistence of *SPINK1* mutations may increase the risk of pancreatitis.

Genetic testing and long-term follow-up might be useful as it would improve our understanding of the polygenic/complex nature of ARP/CP and it might also identify atypical cases in which episodes of pancreatitis have preceded the diagnosis of CF.

Databases

Cystic Fibrosis Genetic Analysis Consortium Database: http://www.genet.sickkids.on.ca/cftr.

Pancreatitis mutation database: http://www.uni-leipzig.de/pancreasmutation/db.html.

GenBank for each of the three genes: *CFTR* NM_000492.2; *PRSS1* NM_002769; *SPINK1* NM_003122.2.

ESE finder program: rulai.cshl.edu/tools/ESE.

Supplementary material

Table S1. Primers used for DGGE analysis.

Table S2. Sequencing primers for *PRSS1* and *SPINK1* genes.

Supplementary materials are available as part of the online article at http://www.blackwell-synergy.com

Acknowledgements

The authors wish to thank Effie Daniel for technical assistance. This work was partly funded by an Athens University Research Grant (ELKE no. 70/4/1661).

References

1. Audrezet MP, Chen JM, Le Marechal C et al. Determination of the relative contribution of three genes – the cystic fibrosis transmembrane conductance regulator gene, the cationic trypsinogen gene, and the pancreatic secretory trypsin inhibitor gene-to the etiology of idiopathic chronic pancreatitis. Eur J Hum Genet 2005: 10: 100–106.
2. Mitchell RMS, Byrne MF, Baillie J. Pancreatitis. Lancet 2003: 361: 1447–1455.
3. Whitcomb DC, Gorry MC, Preston RA et al. Hereditary pancreatitis is caused by a mutation in the cationic trypsinogen gene. Nat Genet 1996: 14: 141–145.
4. Werlin SL, Kugathasan S, Frautschy BC. Pancreatitis in children. J Pediatr Gastroenterol Nutr 2003: 37: 591–595.
5. De Boeck K, Weren M, Proesmans M, Kerem E. Pancreatitis among patients with cystic fibrosis: correlation with pancreatic status and genotype. Pediatrics 2005: 115: 463–469.
6. Grigorescu M, Grigorescu MD. Genetic factors in pancreatitis. Rom J Gastroenterol 2005: 14: 53–61.
7. Pfutzer RH, Barmada MM, Brunskill AP et al. SPINK1/PST1 polymorphisms act as disease modifiers in familial and idiopathic chronic pancreatitis. Gastroenterology 2000: 119: 615–623.
8. Cohn JA. Reduced CFTR function and the pathobiology of idiopathic pancreatitis. J Clin Gastroenterol 2005: 39: S70–S77.
9. Whitcomb DC, Ermentrout GB. A mathematical model of the pancreatic duct cell generating high bicarbonate concentrations in pancreatic juice. Pancreas 2004: 29: 30–40.
10. Rosenberg MF, Kamis AB, Alexandrov LA, Ford RC, Riordan JR. Purification and crystallization of the cystic fibrosis transmembrane conductance regulator (CFTR). J Biol Chem 2004: 279: 39051–39057.
11. Morinville V, Slivka A. Cystic fibrosis transmembrane regulator mutations and pancreatic disease: closing the gap between genotype and phenotype. Gastrointest Endosc 2006: 63: 240–242.
12. Zielenski J. Genotype and phenotype in cystic fibrosis. Respiration 2000: 67: 117–133.
13. Noone PG, Knowles MR. ''CFTR-opathies'': disease phenotypes associated with cystic fibrosis transmembrane regulator gene mutations. Respir Res 2001: 2: 328–332.

14. Kanavakis E, Tzetis M, Antoniadi Th, Pistofidis G, Milligos S, Kattamis C. Cystic fibrosis mutation screening in CBAVD patients and men with obstructive azoospermia or severe oligospermia. Mol Hum Reprod 1998: 4: 333–337.
15. Tzetis M, Efthymiadou A, Strofalis S et al. CFTR gene mutations – including three novel nucleotide substitutions – and haplotype background in patients with asthma, disseminated bronchiectasis and chronic obstructive pulmonary disease. Hum Genet 2001: 108: 216–211.
16. Casals T, Aparisi L, Martinez-Costa C et al. Different CFTR mutational spectrum in alcoholic and idiopathic chronic pancreatitis? Pancreas 2004: 28: 374–379.
17. Ahmed N, Corey M, Forstner G et al. Molecular consequences of cystic fibrosis transmembrane regulator (CFTR) gene mutations in the exocrine pancreas. Gut 2003: 8: 1159–1164.
18. Cuppens H, Lin W, Jaspers M et al. Polyvariant mutant cystic fibrosis transmembrane conductance regulator genes. The polymorphic (Tg)m locus explains the partial penetrance of the T5 polymorphism as a disease mutation. J Clin Invest 1998: 101: 487–496.
19. Groman JD, Hefferon TW, Casals T et al. Variation in a repeat sequence determines whether a common variant of the cystic fibrosis transmembrane conductance regulator gene is pathogenic or benign. Am J Hum Genet 2004: 74: 176–179.
20. Azlami WM, Fogel EL, Schmidt S et al. ERCP findings in idiopathic pancreatitis: patients who are cystic fibrosis gene positive and negative. Gastrointest Endosc 2006: 63: 234–239.
21. Chen JM, Mercier B, Audrezet MP, Ferec C. Mutational analysis of the human pancreatic secretory trypsin inhibitor (PST1) gene in hereditary and sporadic chronic pancreatitis. J Med Genet 2000: 37:67–69.
22. Witt H, Luck W, Hennies HC, Classen M, Kage A, Lass U. Mutations in the gene encoding the serine protease inhibitor, Kazal type 1 are associated with chronic pancreatitis. Nat Genet 2000: 25: 213–216.
23. NCCLS. Sweat testing: sample collection and quantitative analysis; approved guideline, 2nd edn. Wayne, PA: NCCLS, 2000 [NCCLS document C34-A2 (ISBN 1-56238-407-4)].
24. Kanavakis E, Efthymiadou A, Strofalis S, Doudounakis S, Traeger-Synodinos J, Tzetis M. Cystic fibrosis in Greece: molecular diagnosis, haplotypes, prenatal diagnosis and carrier identification amongst high-risk individuals. Clin Genet 2003: 63: 400–409.
25. Ferec C, Raguenes O, Salomon R et al. Mutations in the cationic trypsinogen gene and evidence for genetic heterogeneity in hereditary pancreatitis. J Med Genet 1999: 36: 228–232.
26. Friedman KJ, Heim R, Knowles MR, Silverman LM. Rapid characterization of the variable length polythymidine tract in the cystic fibrosis (CFTR) gene: association of the 5T allele with selected CFTR mutations and its incidence in atypical sinopulmonary disease. Hum Mutat 1997: 10: 108–115.
27. Rohlfs EM, Zhou Z, Sugarman EA et al. The I148T CFTR allele occurs on multiple haplotypes: a complex allele is associated with cystic fibrosis. Genet Med 2002: 4: 319–323.
28. Lamprecht G, Mau UA, Kortum C et al. Relapsing pancreatitis due to a novel compound heterozygosity in the CFTR gene involving the second most common mutation in Central Eastern Europe [CFTRdele2, 3(21kb)]. Pancreatology 2003: 269: 1–5.
29. Kaneko K, Nagasaki Y, Furukawa T et al. Analysis of the human pancreatic secretory trypsin inhibitor (PST1) gene mutations in Japanese patients with chronic pancreatitis. J Hum Genet 2001: 46: 293–297.
30. Bernardino ALF, Guarita DR, Mott CB et al. CFTR, PRSS1 and SPINK1 mutations in the development of pancreatitis in Brazilian patients. JOP 2003: 4: 169–177.
31. Chen JM, Ferec C, Cooper DN. A systematic analysis of disease-associated variants in the 3′ regulatory regions of human protein-coding genes I: general principles and overview. Hum Genet 2006: 120: 1–21.
32. Teich N, Ockenga J, Keim V, Mossner J. Genetic risk factors in chronic pancreatitis. J Gastroenterol 2002: 37: 1–9.