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SHORT REPORT

Mutations in the *SPINK1* gene in idiopathic pancreatitis Italian patients

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Idiopathic chronic and acute recurrent pancreatitis (IP) have been associated with mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. Mutations in the serine protease inhibitor Kazal 1 (*SPINK1*) have been described in some idiopathic chronic patients and it has been suggested that mutations in this gene could be responsible for a loss of trypsin inhibitor function. In this study, the 5′UTR region, and the four exons and exon–intron boundaries of the *SPINK1* gene in 32 IP patients have been analyzed. Three IP patients (9.3%) and one control/100 carried the N34S mutation of the *SPINK1* gene (Fisher's exact test, $P = 0.044$) . No other mutation that could be associated with an altered function of the *SPINK1* protein was observed. The N34S mutation was present in two patients who carried the *CFTR-IVS8 5T* variant and in one who carried the L997F variant in the *CFTR* gene. The association of *SPINK1* with *CFTR* gene mutations in IP patients is statistically significant (3/32 IP cases and 0/100 control individuals carrying mutations in both genes; Fisher's exact test $P = 0.01$).
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Introduction

Chronic pancreatitis (CP) is a continuing or relapsing inflammatory disease of the pancreas. CP follows recurrent attacks of acute pancreatitis with all of the common complications of CP, including unrelenting pain, parenchymal and ductal calcifications, duct distortion, fibrosis, maldigestion, and diabetes mellitus. The etiological factor of about one-third of chronic and acute recurrent pancreatitis patients is unknown and this group of patients is classified as suffering from idiopathic pancreatitis (IP). Mutations of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene have been associated with IP.[1–4] Active trypsin is inhibited by trypsin inhibitors as serine protease inhibitor Kazal type 1 (*SPINK1*), and when trypsin activity exceeds the inhibitor effect of *SPINK1* it will continue to activate trypsinogen and other zymogens, leading to autodigestion of the pancreas and pancreatitis. Mutations in the *SPINK1* gene have been reported in hereditary and idiopathic acute and chronic pancreatitis.[4–7] Witt *et al*[5] reported that 22 out of 96 CP patients carried a *SPINK1* mutation and that 18 of them carried a missense mutation in codon 34 (N34S). Pfützer *et al*[6] found that 26 out of 57 IP patients carried a *SPINK1* mutation and that 23 of them carried the N34S, confirming the association of this mutation with CP and postulating that alterations in the SPINK1 can act as a disease modifier gene in this pathology. Audrézet *et al*[7] demonstrated that four out of 39 IP cases carried the N34S mutation in the *SPINK1* gene.

The aim of this study was to search for mutations in the SPINK1 gene in association with susceptibility to IP in Italian patients and to study the possibility of a

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synergistic effect between *SPINK1* and *CFTR* gene mutations in IP.

Patients and methods

Cases and controls

The population under study consisted of 32 IP Italian subjects (18 males, 14 females, mean age 34.6 years, range 7–59 years), suffering from either idiopathic chronic pancreatitis (17) or recurrent acute pancreatitis (15). The criteria for diagnosis were as follows: for idiopathic recurrent pancreatitis, multiple episodes of usually clinical mild pancreatitis in the absence of the etiological factors most commonly associated with pancreatitis such as colelithiassis, alcohol abuse, pancreas divisum, hypercalcemia, dyslipidemia and viral infections; and for idiopathic chronic pancreatitis: progressive pancreatic inflammatory disease characterized by irreversible morphological changes and typically causing pain and/or permanent loss of function, where none of the possible causes previously mentioned in the other definitions had been detected. A total of 50 control individuals from the same Italian population were analyzed in this study. Mutation N34S was tested in a total of 100 control individuals. The patients had been previously analyzed for mutations in the *CFTR* gene.[8,9]

DNA extraction and analysis

DNA was extracted from peripheral blood leukocytes by standard procedures.

Mutation N34S of the *SPINK1* gene was detected by PCR with modified primers that produce a restriction site during *in vitro* amplification (RG-PCR)[10] using forward primer 5′CAATCACAGTTATTCCCCAG3′, and modified reverse primer 5′TGGTGCATCCATTAAGTGCA3′ which creates a restriction site for endonuclease Bsp1286 I when allele 34S is present. Digestion products were analyzed by 8% acrylamide gel electrophoresis.

The 5′UTR region, the four exons and the exon–intron boundaries of the *SPINK1* gene were amplified as described[11] and denaturing gradient gel electrophoresis (DGGE) analysis of the amplified fragments was performed. Amplified fragments showing an altered migration pattern were directly sequenced using the BigDye terminator Cycle Sequencing kit (Perkin–Elmer) on a 377 ABI PRISM sequencer.

Results and discussion

Mutation N34S was tested in 32 IP, and 100 control individuals. The mutation was present in three IP patients, one of whom was homozygous, and in one control individual (Fisher's exact test, $P = 0.044$) (Table 1).

The four exons and exon–intron boundaries, and the 5′UTR region of the *SPINK1* gene were screened for sequence variations by DGGE analysis in 32 cases of IP, and in 50 control individuals. In the 5′UTR region, polymorphism −253T>C and the −41G>A variant were found. The common polymorphism −253T>C was present in both groups with similar frequencies and the −41G>A variant was present in one IP patient only. In IVS1, variant −37T>C was observed in three IP patients, and it was in linkage disequilibrium with mutation N34S in exon 3. Beside the N34S mutation, we found the following in exon 3 and boundaries: variant 174C>T(C58C) in one IP patient, variants IVS2−23A>T in two controls, and a novel sequence variant IVS3+12T>G in one IP patient. In exons 1 and 4 and their exon–intron boundaries, no sequence alteration was observed. All the DNA variants detected in this study as well as their distribution in both study groups are summarized in Table 2.

Four sequence variations were found in IP patients, and of these only the N34S mutation, which was present in three patients (9.3%), has previously been considered as being associated with IP. This result is similar to that reported by Audrézet *et al*,[7] who reported the presence of the N34S mutation in 10% of ICP patients, and by Ockenga *et al*,[12] who reported a similar prevalence of SPINK1 mutations in a cohort of adult IP patients. A higher frequency of *SPINK1* mutations was described by Witt *et al*[5] and by Pfützer *et al*,[6] where the frequency of N34S in CP patients was 18.75 and 40.3%, respectively.

In this study, no other sequence alteration was observed that could be associated with an altered function of the *SPINK1* protein. The −41G>A variant, which was present in one IP and in no control individual, has been discussed by Audrézet *et al*[7] as having a possible role in transcription efficiency of the protein. However, it was also present in

Table 1 Mutations and polymorphisms in the *SPINK1* gene and distribution among IP, and controls individuals

Location	*Sequence variation*	*IP*	*Controls*
5′ UTR	−253T>C	11/32	16/50 (3 hom)
	−41G>A	1/32	—
Exon 1	—	—	—
Exon–intron boundary	—	—	—
Exon 2		—	—
Exon–intron boundary	IVS1-37T>C	3/32 (1 hom)	—
Exon 3	N34S	3/32 (1 hom)	1/100
	174 C>T (C58C)	1/32	—
Exon–intron boundary	IVS2-23A>T	—	2/50
	IVS3+12T>G[a]	1/32	—
Exon 4	—	—	—
Exon–intron boundary	—	—	—

[a]Novel polymorphism; hom: homozygous.

Table 2 Analysis of mutations in *CFTR* and *SPINK1* mutations in ICP patients

IP patient	CFTR *gene sequence variation*	SPINK1 *gene mutations*
1	F508del, L997F	—
2	F508del	—
3	2789+5G>A	—
4	F508del	—
5	R1162X	—
6	F508del	—
7	R553X	—
8	F508del; 3878delG	174C>T; -41G>A; −253T>C
9	3849+10KbC>T	—
10	*5T*; L997F; S1235R	—
11	L997F	N34S, IVS1 –37T>C
12	*5T*	—
13	*5T*	N34S, IVS1–37T>C
14	—	—
15	*5T*	34S hom; IVS1–37C hom
16	*5T*	—
17	D614G	—
18	—	—
19	—	—
20	R31C	—
21–31	—	—
32	—	IVS3+12T>G

hom: homozygous.

3/190 control individuals in the study by Pfützer *et al*,[6] so its relevance in IP remains to be determined. Interestingly, the N34S mutation was present in two patients who carried the *5T* variant and in one who carried the L997F variant in the *CFTR* gene (Table 2). The patients carrying one CFTR gene and one SPINK1 gene mutation had no family history of IP. No relatives were available for a segregation study of the mutations. The *5T* variant of the *CFTR* gene is one of the three known alleles with a variant number of thymidines (5,7 or 9) in a polythymidyl tract (T-tract) of the acceptor splice site in intron 8. The variant *5T* is associated with low amounts of normally processed mRNA[13] and, although this allele does not cause CF, it can modify the phenotypic effect of other mutations.[14] It can be hypothesized that the added action of a lower level of correctly processed *CFTR* gene product, together with an altered function of the *SPINK1* gene product, could have a deleterious effect upon the pancreatic phenotype. A high frequency of the L997F mutation of the *CFTR* gene has been described in our cohort of IP patients[8] and it has been postulated that this mutation, together with other *CFTR* gene mutations, can cause IP as an atypical, monosymptomatic form of CF. As with the *5T* variant, it can be hypothesized that the L997F mutation combined with a *SPINK1* mutation can have a synergistic effect upon the development of IP. IP patients carrying a *SPINK1* mutation as well as a *CFTR* gene mutation have also been reported by Witt *et al*[15] as unpublished data. Noone *et al*[4] reported nine out of 24 CP patients carrying the N34S *SPINK1* gene mutation. Three of these patients carried the *SPINK1* mutation together with a *CFTR* mutation: two carrying the *5T* variant in the *CFTR* gene, and one carrying two *CFTR* mutations (F508del and H117H). In three other patients carrying the N34S mutation, the authors could not exclude the presence of *CFTR* rare mutations as only the common *CFTR* gene mutations were screened. Ockenga *et al*,[12] revealed double heterozygosity for *CFTR* Y1092X mutation and *SPINK1* R65Q mutation, not only in a patient but also in his unaffected relatives, indicating that other genetic or environmental factors are needed to develop PI. However, the possible relevance of the novel SPINK1 gene mutation R65Q in IP is still unclear.

The frequency of double heterozygotes for *SPINK1* and *CFTR* gene mutations among IP patients compared with control individuals is significantly higher (3/32 IP, 0/100 controls, Fisher's exact test $P=0.013$). The presence of patients who carry mutations at the two genes suggests that epistatic interactions in trans-heterozygotes may contribute to disease pathogenesis. The distribution of *SPINK1* mutations in IP patients was not homogeneous, 0/10 patients carrying a CF causing mutation, 3/7 carrying a *CFTR* variant and 0/15 carrying no *CFTR* gene variant (χ^2 after permutation $P=0.037$). Severe CFTR mutations were not identified in this study, possibly because of the low number of observations. Our observation suggests a possible interaction of *CFTR* and *SPINK1* gene variants in determining IP.

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References

1 Cohn JA, Friedman KJ, Noone PG, Knowles MR, Silverman LM, Jowell PS: Relation between mutations of the cystic fibrosis gene and idiopathic pancreatitis. *N Engl J Med* 1998; **339**: 653–658.
2 Sharer N, Schwarz M, Malone G *et al*: Mutations of the cystic fibrosis gene in patients with chronic pancreatitis. *N Engl J Med* 1998; **339**: 645–652.
3 Castellani C, Bonizzato A, Rolfini R, Frulloni L, Cavallini GC, Mastella G: Increased prevalence of mutations in the cystic fibrosis gene in idiopathic chronic and recurrent pancreatitis. *Am J Gastroenterol* 1999; **94**: 1993–1995.
4 Noone PG, Zhou Z, Silverman ML, Jowell PS, Knowles MR, Cohn JA: Cystic fibrosis gene mutations and pancreatitis risk: relation to epithelial ion transport and trypsin inhibitor gene mutations. *Gastroenterology* 2001; **121**: 1310–1319.
5 Witt H, Luck W, Hennies HC *et al*: Mutations in the gene encoding the serine protease inhibitor, Kazal type 1 are associated with chronic pancreatitis. *Nature Genet* 2000; **825**: 213–216.
6 Pfützer RH, Barmada MM, Brunskill APJ *et al*: SPINK1/PST1 polymorphisms act as disease modifier in familial and idiopathic chronic pancreatitis. *Gastroenterology* 2000; **119**: 615–623.

7 Audrézet MP, Chen J-M, Le Maréchal 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* 2002; **2**: 100–106.
8 Gomez-Lira M, Benetazzo MG, Marzari MG *et al*: High frequency of cystic fibrosis transmembrane regulator mutation L997F in patients with recurrent idiopathic pancreatitis and in newborns with hypertrypsinemia. *Am J Hum Genet* 2000; **66**: 2013–2014.
9 Castellani C, Gomez-Lira M, Frulloni L *et al*: Analysis of the entire coding region of the cystic fibrosis transmembrane regulator gene in idiopathic pancreatitis. On line Citation: Human Mutation, Mutation in Brief #436, (2001) On Line. Print citation: **18**: 165–166.
10 Gasparini P, Bonizzato A, Doginini A, Pignatti PF: Restriction site generating polymerase chain reaction (RG-PCR) for the probeless detection of hidden genetic variations: application to the study of some cystic fibrosis mutations. *Mol Cell Probes* 1992; **6**: 1–7.
11 Chen J-M, Bernard M, 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.
12 Ockenga J, Dörk T, Stuhrmann M: Low prevalence of SPINK1 gene mutations in adult patients with chronic idiopathic pancreatitis. *J Med Genet* 2001; **38**: 243–244.
13 Chu CS, Trapnell BC, Curristin S, Cutting GR, Crystal RG: Genetic basis of variable exon 9 skipping in cystic fibrosis transmembrane conductance regulator mRNA. *Nat Genet* 1993; **3**: 152–156.
14 Kiesewetter S, Macek Jr M, Davis C *et al*: A mutation in *CFTR* produces different phenotypes depending on chromosomal background. *Nat Genet* 1993; **5**: 274–278.
15 Witt H, Hennies HC, Becker M: *SPINK1* mutations in chronic pancreatitis. *Gastroenterology* 2001; **120**: 1060–1061.