

# Genetic background of pancreatitis

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Trypsin activity is properly suppressed by pancreatic secretory trypsin inhibitor (PSTI), which is also known as serine protease inhibitor Kazal type 1 (SPINK1), thereby preventing damage to pancreatic acinar cells as a first line of defence. However, if trypsin activation exceeds the capacity of PSTI/SPINK1, a subsequent cascade of events leads to the activation of various proteases that damage cells. Five mutations (R122H, N29I, A16V, D22G and K23R) in cationic trypsinogen and two mutations (N34S and M1T) in the PSTI/SPINK1 gene have been found to correlate significantly with the onset of pancreatitis. From analyses of hereditary pancreatitis and the phenotype of PSTI/SPINK1 (Spink3) knockout mice, we showed that the imbalance of trypsin activation and its inhibition by PSTI/SPINK1 would lead to the development of pancreatitis.

The main mechanism in the onset of pancreatitis is believed to be the autodigestion of pancreatic structural cells by various proteases that are activated in response to the ectopic (intrapancreatic) activation of trypsinogen (trypsin production).

A relationship between the trypsinogen gene mutations and the onset of pancreatitis was initially reported in 1996.<sup>1</sup> The effect of mutations in the pancreatic secretory trypsin inhibitor (PSTI) gene, which is known as the serine protease inhibitor Kazal type 1 (SPINK1) gene, on the onset of pancreatitis was reported in 2000.<sup>2</sup> Recently, a more detailed understanding of the mechanism of the effects of trypsin and PSTI/SPINK1 on the onset of pancreatitis has been attained.<sup>3</sup> In this paper, we review the genetic background of pancreatitis.

## ACTIVATION OF TRYPSINOGEN (TRYPSIN PRODUCTION) AND INHIBITION OF TRYPSIN ACTIVITY BY SPINK1/PSTI

Pancreatic digestive enzymes are stored as inactivated precursors (ie trypsin as trypsinogen) in pancreatic zymogen granules. Under normal conditions, activation is strictly controlled (box 1) to prevent autodigestion of the pancreas (ie pancreatitis). However, in certain circumstances, excessive amounts of pancreatic trypsinogen are activated to trypsin (ectopic activation), activating other downstream zymogens, and leading to autodigestion of the pancreas.

Triggers for the activation of trypsinogen to trypsin in the pancreas include excessive pancreatic exocrine stimulation, reflux of bile or

duodenal fluid, disturbance of pancreatic duct flow, and inflammation. Enterokinase is the most efficient activator, but other molecules that activate trypsinogen include trypsin (trypsin-catalysed autoactivation), lysosomal enzyme cathepsin B and neutrophilic enzymes. Also, activation of trypsin from trypsinogen can also occur without enzyme involvement (non-enzymatic autoactivation). Calcium inhibits the degradation (autolysis) of activated trypsin, whereas bile acids promote the autoactivation of trypsin.

PSTI/SPINK1 (the main trypsin inhibitor in the pancreas) is synthesised in acinar cells of the pancreas and is thought to inhibit up to 20% of the trypsin activity in the pancreas by binding to its catalytic site. However, pancreatitis can ultimately develop, if pancreatic activation of trypsinogen is too high or the trypsin-binding ability of PSTI/SPINK1 is too low (fig 1). For example, in PSTI/SPINK1 knock-out mice, autophagic cell death triggered by intake of milk results in the pancreas disappearing within a few days of birth.<sup>4</sup> Thus, similar to the offensive and defensive relationship between a spear and a shield, the balance between trypsin and PSTI/SPINK1 activities protects the pancreas from autodigestion.

## ONSET OF PANCREATITIS FROM TRYPSINOGEN GENE MUTATIONS

Using microsatellite markers from a family with a history of hereditary pancreatitis, the disease gene for hereditary pancreatitis was found by sequence analysis in 1996, and is located on the long arm of chromosome 7 (7q35).<sup>5,6</sup> At the same time, the human genomic sequence of the T cell receptor  $\beta$  chain gene locus in 7q35 was reported, and it was found that this multigene family cluster includes eight trypsinogen genes and a pseudogene.<sup>7</sup> Among these eight trypsinogen genes, three generate functional proteins: the cationic trypsinogen gene, the anionic trypsinogen gene and the mesotrypsinogen gene. Of these three proteins, cationic trypsinogen is most abundant in humans and is readily activated, but slowly degraded.

Whitcomb *et al*<sup>1</sup> determined the sequence of five exons of the cationic trypsinogen and anionic trypsinogen genes using genomic DNA from patients with hereditary pancreatitis. They discovered a point mutation in exon 3 of the cationic trypsinogen gene (365G→A: R122H) that results in an amino acid substitution in the autolytic domain of trypsin. Thus, the

**Abbreviations:** CFTR, cystic fibrosis transmembrane conductance regulator; PSTI, pancreatic secretory trypsin inhibitor; SPINK1, serine protease inhibitor Kazal type 1

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### Protective mechanisms against pancreatitis

1. Packaging of pancreatic secretory trypsin inhibitor (PSTI)/serine protease inhibitor Kazal type 1 (SPINK1) in zymogen granule
2. Synthesis of enzymes as inactive zymogens
3. Segregation of enzymes in membrane-bound compartments
4. Restriction of enterokinase in the duodenum

mutation blocks autolysis and results in continuous trypsin activity.<sup>1,8</sup> Two additional groups of investigators<sup>9,10</sup> discovered an alternative mutation in exon 2 (85A→T: N29I) in the DNA from other families with a history of hereditary pancreatitis. Other mutations related to hereditary pancreatitis, including -28delTCC in exon 1, A16V, D22G, K23R and P36R in exon 2, and E79K, G83E, K92N, D100H, L104P, R116C, R122C, V123M and C139F in exon 3 have also been identified. Families with a history of hereditary pancreatitis carrying R122H and N29I have also been found in Japan.<sup>11</sup> Among all these mutations, five (R122H, N29I, A16V, D22G and K23R) have been found to correlate significantly with the onset of pancreatitis;<sup>12</sup> insignificant data are presently available to evaluate the other mutations. Interestingly, no mutations related to pancreatitis have yet been found in anionic trypsinogen and mesotrypsinogen genes.<sup>13</sup>

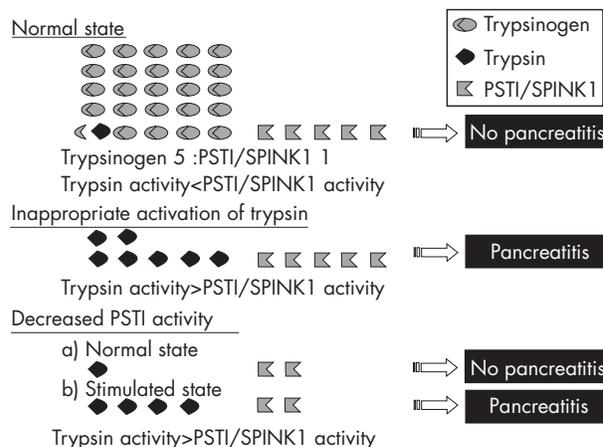
The cationic trypsinogen gene, also known as the protease serine type 1 (PRSS1) gene, is located on chromosome 7 (7q35). The cationic trypsinogen gene is approximately 3.6 kb in length and encodes five exons. Hereditary pancreatitis associated with a mutation in the cationic trypsinogen gene is inherited as an autosomal dominant trait, with an estimated penetrance of 80%.

Five mechanisms have been proposed to explain how mutations in the cationic trypsinogen gene can lead to increases in trypsin activity. Using rat and human mutant cationic trypsinogen proteins, it has been shown that the R122H mutation prevents inactivation (autolysis) of activated trypsin<sup>14</sup> and also leads to an increase in the autoactivation of trypsinogen.<sup>15</sup> The N29I mutation is hypothesised to change the higher-order structure of trypsin, resulting in decreased PSTI/SPINK1 binding, increased stability and increased autoactivation. As it has been shown that changes in the sequence of the cationic trypsinogen N-terminal peptide increase its rate of degradation, mutations A16V, D22G and K23R (all of which change the signal peptide cleavage site of trypsin) may lead to increased autoactivation of trypsinogen to trypsin. The -28delTCC has been hypothesised to enhance cationic trypsinogen transcription, thereby increasing activity. On the basis of these mechanisms, any of these gene mutations can increase trypsin activity in the pancreas.

### ONSET OF PANCREATITIS BY PSTI/SPINK1 GENE MUTATIONS

The PSTI/SPINK1 gene is located on chromosome 5. The mRNA is approximately 7.2 kb in length and encodes four exons.<sup>16</sup> PSTI/SPINK1, which binds rapidly to trypsin derived from trypsinogen and inhibits its activity, is an important factor in the onset of pancreatitis. It is hypothesised that mutations in the PSTI/SPINK1 gene that affect PSTI/SPINK1 binding to trypsin will contribute to the onset of pancreatitis.

There have been many reports of mutations in the PSTI/SPINK1 genes of patients with pancreatitis, and several hypothesised roles of these mutant proteins in pancreatitis.<sup>2,16-20</sup> A mutation in amino acid 34, encoded in exon 3, substitutes Ser (AGT) for Asn (AAT; 101 A→G: N34S). PSTI/SPINK1



**Figure 1** Importance of intrapancreatic balance between trypsin and pancreatic secretory trypsin inhibitor (PSTI)/serine protease inhibitor Kazal type 1 (SPINK1) activities.

mutations are also found in normal people, and both homozygotes and heterozygotes are attributable to the development of pancreatitis. On the basis of these facts, some authors recognise the role of the PSTI/SPINK1 mutation as a disease modifier. However, the frequency of the N34S mutation occurring in patients with pancreatitis was considerably higher than that in people without pancreatitis.<sup>21</sup> Also, the rate of association of pancreatitis in people with the homozygotic N34S mutation was assumed to be high (98%; 49/50).<sup>21</sup> This high rate of association of pancreatitis in people with the homozygotic N34S mutation suggests that this mutation may be a recessive inherited trait.

Interestingly, the N34S mutation always co-segregates with mutations in intron 1 (IVS1-37 T→C) and intron 3 (IVS3-69 insTTTT, T5 becomes T9). Witt *et al*<sup>2</sup> found that the N34S mutation also co-segregates with IVS2+268 A→G and IVS3-604 G→A. According to the results of functional analysis of the recombinant PSTI/SPINK1 protein with amino acid substitution, mechanisms other than the conformational change, such as abnormal splicing due to intronic mutations, are suggested to underlie the predisposition to pancreatitis in patients with the N34S mutation.<sup>22</sup>

The onset of pancreatitis conferred by a mutation in the cationic trypsinogen gene is a dominant trait. By contrast, on the basis of the data collected from past reports, the N34S mutation of PSTI/SPINK1 has been found to correlate with a high rate of onset of pancreatitis in people with the homozygotic mutation, and may therefore be recessive. Witt *et al*<sup>2</sup> have also identified the MIT mutation in familial pancreatitis, which eliminates the PSTI/SPINK1 start codon, leading to an overall loss of PSTI/SPINK1 activity. Family members carrying this mutation have pancreatitis as a dominant trait, indicating that the absence of one functional PSTI/SPINK1 allele (ie half the normal level of PSTI/SPINK1) leads to a high rate of onset of pancreatitis. Thus, the rate of onset of pancreatitis due to PSTI/SPINK1 gene mutations seems to correlate with the relative effect of each mutation on the activity of the trypsin inhibitor.

### ANALYSIS OF THE MECHANISM OF ONSET OF PANCREATITIS USING GENETICALLY ALTERED MICE

To analyse the importance of trypsinogen activation (trypsin production) and its regulation by PSTI/SPINK1 in the onset of pancreatitis, we generated the PSTI/SPINK1 gene (SPINK3 gene in mice) knockout mice by gene targeting and analysed their phenotype.<sup>4</sup> The pancreatic acinar cells in knock-out mouse embryos showed vacuolar degeneration and disappeared

completely after birth. The type of cell death in this phenomenon was not necrotic or apoptotic, but autophagic, indicating that PSTI/SPINK1 molecules are important in maintaining the integrity of pancreatic acinar cells.

Autophagy is also found in acinar cells of human acute pancreatitis and rodent experimental pancreatitis, but its pathophysiological role has not been well understood. Two theoretical mechanisms of trypsinogen activation in pancreatic acinar cells have been proposed. In the "cathepsin B theory",<sup>23</sup> trypsinogen is activated by erroneous sorting of the lysosomal enzyme, cathepsin B. In the "autoactivation theory",<sup>24</sup> trypsinogen is transferred to an acidic environment in a subcellular compartment and activated by certain serine protease activity or independently of protease activity. We propose a third hypothetical mechanism of trypsinogen activation, involving autophagy.

The vacuoles we observed by histological examination correspond to autophagosomes, which are the hallmark of autophagy and contain zymogen granules in which trypsinogen exists physiologically. The autophagosome fuses with a lysosome to form an autophagolysosome.<sup>25</sup> In unpublished observations of PSTI/SPINK1 gene (SPINK3) knockout mice, we observed "a failure in control trypsin activity" in pancreatic acinar cells.<sup>26</sup> As trypsin activity is controlled by PSTI/SPINK1 in wild-type mice, there should be an absence of regulation of trypsin activity in the PSTI/SPINK1 (SPINK3) knockout mice. Autophagy could be a result of uncontrolled trypsin activity, or activation of trypsinogen may occur once autophagy is initiated, the first corresponding more to the "autoactivation theory" and the second to the "cathepsin B theory". In the autoactivation theory, autophagy would be a defensive mechanism for eliminating trypsin molecules that have escaped control. In the cathepsin B theory, autophagy would be a rogue event leading to trypsinogen activation. Although liver cells are known to contain high concentrations of PSTI/SPINK1, we did not observe any liver cell autophagy in the PSTI/SPINK1 (SPINK3) knockout mice. The observation that autophagy was specific to pancreatic acinar cells suggests that trypsin activity, which is specific to pancreatic acinar cells, plays a part in autophagy induction.

#### OTHER GENES ASSOCIATED WITH PANCREATITIS

Mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene were also associated with chronic pancreatitis.<sup>27–29</sup> CFTR conducts both chloride and bicarbonate, and controls the bulk of pancreatic fluid secretion. Mutations of this gene, together with other genetic and environmental factors, could be risk factors for chronic pancreatitis, by altering the ability to clear digestive enzymes from the pancreatic duct. About 30% of patients with chronic pancreatitis carried at least one CFTR mutation, and several patients were compound heterozygotic for different CFTR mutations, or were trans heterozygotic for a CFTR mutation and a mutation in the PSTI/SPINK1 or cationic trypsinogen.<sup>28–29</sup> Decreased activity of the CFTR protein may interact with mutations in PSTI/SPINK1 or cationic trypsinogen gene.

Ockenga *et al*<sup>30</sup> reported an increased risk of chronic alcoholic pancreatitis in patients with mutations in uridine 5'-diphosphate glucuronosyltransferase. Uridine 5'-diphosphate glucuronosyltransferase belongs to a family of phase II enzymes involved in metabolism, detoxification and other aspects of cellular defence.<sup>30</sup> The alcohol-metabolising enzymes, such as alcohol dehydrogenase, may have some association with alcoholic pancreatitis. There has also been some work on genes regulating the inflammatory response, such as tumour necrosis factor  $\alpha$ , interleukin (IL)1 $\beta$ , IL6 and IL10. However, the association between mutations in these genes and the development of pancreatitis is not apparent.

#### EPILOGUE

The role of trypsin and trypsin inhibitor, especially as they relate to the onset of pancreatitis, have been described. From analyses of hereditary pancreatitis and the phenotype of the PSTI/SPINK1 (SPINK3) knockout mice, we showed that activation of trypsinogen and PSTI/SPINK1 regulate the onset of pancreatitis.

Recent epidemiological studies estimate that the relative risk of pancreatic cancer in patients with hereditary pancreatitis due to mutations in cationic trypsinogen is 50–70-fold higher than average, and that about 40% of the patients with hereditary pancreatitis will develop pancreatic cancer by the age of 70 years.<sup>31–32</sup> A similar situation may exist in patients with mutations in the PSTI/SPINK1 gene,<sup>33</sup> as repeated chronic inflammation from youth seems to lead to the eventual development of pancreatic cancer.

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#### REFERENCES

- Whitcomb DC, Gorry MC, Preston RA, *et al*. Hereditary pancreatitis is caused by a mutation in the cationic trypsinogen gene. *Nat Genet* 1996;**14**:141–5.
- 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. *Nat Genet* 2000;**25**:213–16.
- Hirota M, Kuwata K, Ohmuraya M, *et al*. From acute to chronic pancreatitis: the role of the mutations in the pancreatic secretory trypsin inhibitor gene. *JOP* 2003;**4**:83–8.
- Ohmuraya M, Hirota M, Araki M, *et al*. Autophagic cell death of pancreatic acinar cells in serine protease inhibitor Kazal type 3-deficient mice. *Gastroenterology* 2005;**129**:696–705.
- Le Bodic L, Bignon JD, Reguenes O, *et al*. The hereditary pancreatitis gene maps to long arm of chromosome 7. *Hum Mol Genet* 1996;**5**:549–54.
- Whitcomb DC, Preston RA, Aston CE, *et al*. A gene for hereditary pancreatitis maps to chromosome 7q35. *Gastroenterology* 1996;**110**:1975–80.
- Rowen L, Koop BF, Hood L. The complete 68-kilobase DNA sequence of the human  $\beta$ T cell receptor locus. *Science* 1996;**272**:1755–62.
- Varallyay M, Graf L, Toth M. Two mutations in the rat trypsin confer resistance against autolysis. *Biochem Biophys Res Commun* 1998;**243**:56–60.
- Gorry MC, Ghabbaizadeh D, Furey W, *et al*. Mutations in the cationic trypsinogen gene are associated with recurrent acute and chronic pancreatitis. *Gastroenterology* 1997;**113**:1063–8.
- Teich N, Mossner J, Keim V. Mutations of the cationic trypsinogen gene in hereditary pancreatitis. *Hum Mutat* 1998;**12**:39–43.
- Nishimori I, Kamakura M, Fujiwara-Adachi K, *et al*. Mutation in exons 2 and 3 of the cationic trypsinogen gene in Japanese families with hereditary pancreatitis. *Gut* 1999;**44**:259–63.
- Lerch MM, Albrecht E, Ruthenberger M, *et al*. Pathophysiology of alcohol-induced pancreatitis. *Pancreas* 2003;**27**:291–6.
- Chen LM, Audrezet MP, Mercier B, *et al*. Exclusion of anionic trypsinogen and mesotrypsinogen involvement in hereditary pancreatitis without cationic trypsinogen gene mutations. *Scand J Gastroenterol* 1999;**34**:831–2.
- Sahin-Toth M, Graf L, Toth M. Trypsinogen stabilization by mutation Arg117-to-His: a unifying pathomechanism for hereditary pancreatitis? *Biochem Biophys Res Commun* 1999;**264**:505–8.
- Sahin-Toth M, Toth M. Gain-of-function mutations associated with hereditary pancreatitis enhance autoactivation of human cationic trypsinogen. *Biochem Biophys Res Commun* 2000;**278**:286–9.
- Kuwata K, Hirota M, Sugita H, *et al*. Genetic mutations in exon 3 and 4 of pancreatic secretory trypsin inhibitor in patients with pancreatitis. *J Gastroenterol* 2001;**36**:612–18.
- Pfutzer RH, Barmada MM, Brunskill APJ, *et al*. SPINK1/PSTI polymorphisms act as disease modifiers in familial and idiopathic chronic pancreatitis. *Gastroenterology* 2000;**119**:615–23.
- Chen JM, Mercier B, Audrenet M, *et al*. Mutations of the pancreatic secretory trypsin inhibitor (PSTI) gene in idiopathic chronic pancreatitis. *Gastroenterology* 2000;**120**:1061–4.
- Kaneko K, Nagasaki Y, Furukawa T, *et al*. Analysis of the human pancreatic secretory trypsin inhibitor (PSTI) gene mutations in Japanese patients with chronic pancreatitis. *J Hum Genet* 2001;**46**:193–7.
- Kuwata K, Hirota M, Nishimori I, *et al*. Mutational analysis of the pancreatic secretory trypsin inhibitor (PSTI) gene in familial and juvenile pancreatitis in Japan. *J Gastroenterol* 2003;**38**:365–70.

- 21 **Hirota M**, Kuwata K, Ohmuraya M, *et al.* From acute to chronic pancreatitis: the role of mutations in the pancreatic secretory trypsin inhibitor gene. *JOP* 2003;**4**:83–8.
- 22 **Kuwata K**, Hirota M, Shimizu H, *et al.* Functional analysis of recombinant pancreatic secretory trypsin inhibitor protein with amino-acid substitution. *J Gastroenterol* 2002;**37**:928–34.
- 23 **Steer ML**, Meldolesi J. The cell biology of experimental pancreatitis. *N Engl J Med* 1987;**316**:144–50.
- 24 **Gorelick FS**, Modlin IM, Leach SD. Intracellular proteolysis of pancreatic zymogens. *Yale J Biol Med* 1992;**65**:407–20.
- 25 **Mizushima N**, Ohsumi Y, Yoshimori T. Autophagosome formation in mammalian cells. *Cell Struct Funct* 2002;**27**:421–9.
- 26 **Ohmuraya M**, Hirota M, Baba H, *et al.* Trypsin activity was enhanced in *Spink3* deficient pancreatic acinar cells. *Pancreas*, 2006.in press..
- 27 **Taylor CJ**. Chronic pancreatitis and mutations of the cystic fibrosis gene. *Gut* 1969;**44**:8–9.
- 28 **Audrezet MP**, Chen JM, Le Marechal C, *et al.* Determination of the relative contribution by these 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;**10**:100–6.
- 29 **Noone PG**, Zhou Z, Silverman LM, *et al.* Cystic fibrosis gene mutations and pancreatitis risk: relation to epithelial ion transport and trypsin inhibitor gene mutations. *Gastroenterology* 2001;**121**:1310–19.
- 30 **Ockenga J**, Vogel A, Teich N, *et al.* UDP glucuronosyltransferase (UGT1A7) gene polymorphism increase the risk of chronic pancreatitis and pancreatic cancer. *Gastroenterology* 2003;**124**:1802–8.
- 31 **Haber PS**, Wilson JS, McGarity BH, *et al.* Alpha 1-antitrypsin phenotypes and alcoholic pancreatitis. *Gut* 1991;**32**:945–8.
- 32 **Lowenfels AB**, Maisonneuve P, DiMagno EP, *et al.* Hereditary pancreatitis and the risk of pancreatic cancer. International Hereditary Pancreatitis Study Group. *J Natl Cancer Inst* 1997;**89**:442–6.
- 33 **Masamune A**, Mizutamari H, Kume K, *et al.* Masamune A, Mizutamari H, Hereditary pancreatitis as the premalignant disease: a Japanese case of pancreatic cancer involving the SPINK1 gene mutation N34S. *Pancreas* 2004;**28**:305–10.

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