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Is genetic analysis helpful for diagnosing chronic pancreatitis in its early stage?

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Diagnosis of chronic pancreatitis in its early stage is an extremely difficult task. If the genetic predispositions are identified, it may help make possible the earlier diagnosis of chronic pancreatitis or the detection of patients at risk. There are two major hypotheses about the pathogenesis of chronic pancreatitis known as the "necrosis–fibrosis" and "pancreatic stone protein" hypotheses. Recent molecular and genetic evidence suggests that both pathways contribute to the pathogenesis of chronic pancreatitis. Chronic pancreatitis may be caused by either increased proteolytic activity [the cationic trypsinogen (*PRSS1*) gene] or decreased protease inhibition (the pancreatic secretory trypsin inhibitor (*PSTI*) gene]. The impaired pancreatic duct function [cystic fibrosis transmembrane conductance regulator (*CFTR*) gene] may also be involved in the pathogenesis of the disease. Except for *PRSS1* mutations, the known genetic risk for chronic pancreatitis is not high. The high individual variability and low incidence of chronic pancreatitis suggest that yet unidentified genetic and environmental factors are important. Further genetic analysis is necessary for understanding the pathogenesis of chronic pancreatitis, which may be helpful for the earlier diagnosis of the juvenile- or young-onset disease.

Key words: chronic pancreatitis, necrosis–fibrosis hypothesis, pancreatic stone protein hypothesis, *PRSS1* gene, *PSTI* gene, *CFTR* gene

Introduction

Diagnosis of chronic pancreatitis in its early stage is an extremely difficult task. When a patient visits a clinic for the first time, we cannot distinguish acute pancreatitis from an initial attack of chronic pancreatitis. The majority are diagnosed several years later, when unequivocal evidence, such as pancreatic stones, an abnormal pancreatogram, and exocrine and endocrine insufficiency, appears (Fig. 1). The progression of the disease varies considerably among individuals.[1–4] Some patients have a very long asymptomatic period, or never experience pain, while others have pancreatic stones at the initial attack of pain. It is not known why the progression of this disease is so variable. Clinically, alcohol is the leading cause of chronic pancreatitis. However, only a limited number of alcoholics (2%–3%) develop alcoholic pancreatitis.[5] The high individual variability and low incidence of chronic pancreatitis strongly suggest that yet unidentified genetic and environmental factors are important for the pathogenesis of this disease. It is expected that once the genetic predispositions are identified, it may help in earlier recognition of chronic pancreatitis or patients at risk.

Pathogenesis of chronic pancreatitis

There are two major hypotheses for the pathogenesis of chronic pancreatitis: the "necrosis–fibrosis" and "protein plug" hypotheses (Fig. 2). The necrosis–fibrosis hypothesis is that chronic pancreatitis is the result of repeated episodes of acute pancreatitis.[6] Sarles et al.,[7] on the other hand, have claimed that chronic pancreatitis begins within the lumen of the pancreatic ducts, that is, the formation of protein plugs and stones from pancreatic stone protein (PSP).[8] Chronic contact of the stones with pancreatic duct cells produces epithelial damage, resulting in stenosis, cyst formation, and parenchymal atrophy distal to the obstructed ducts. Recent molecular and genetic evidence suggests that both pathways can contribute to the pathogenesis of chronic pancreatitis and that the two theories are not mutually exclusive.

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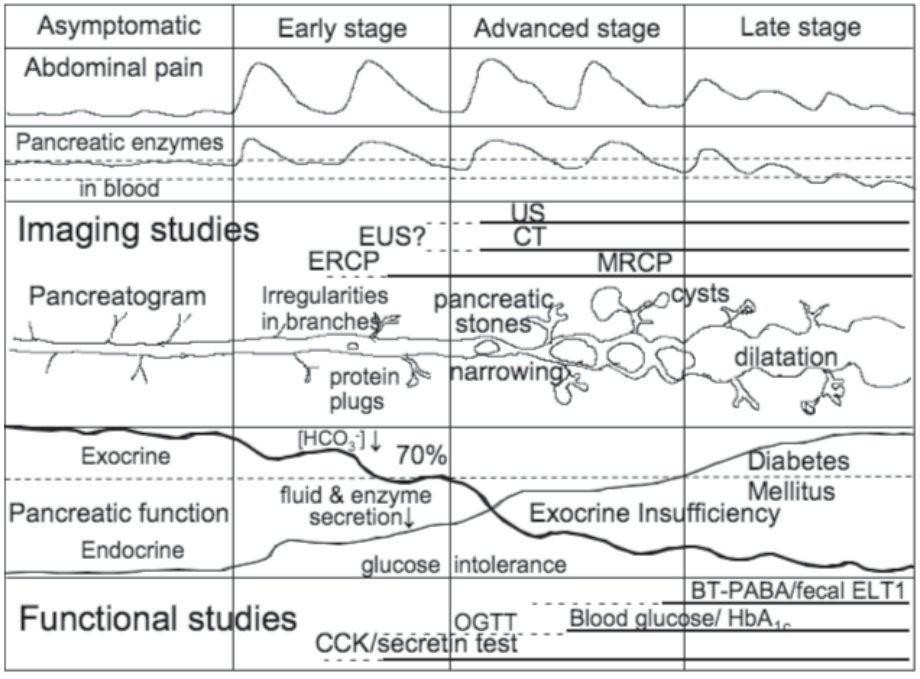

Fig. 1. Progression and diagnosis of chronic pancreatitis by imaging and functional studies. US, ultrasound; *EUS*, endoscopic ultrasonography; *CT*, computed tomography; *ERCP*, endoscopic retrograde cholangiopancreatography; *MRCP*, magnetic resonance cholangiopancreatography; *BT-PABA*, *N*-benzoyl-L-tyrosyl-*p*-aminobenzoic acid; *ELT1*, elastase 1; *OGTT*, oral glucose tolerance test; *CCK*, cholecystokinin

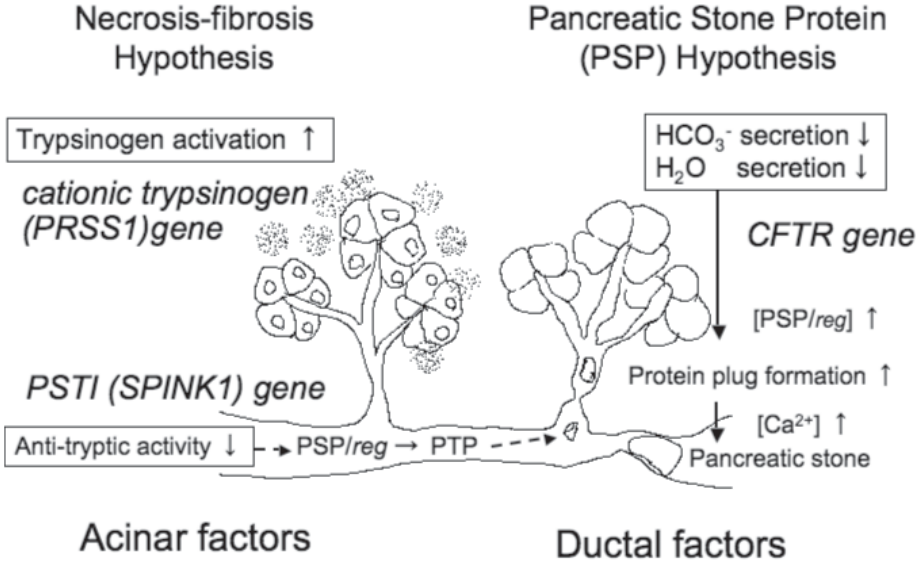

Fig. 2. Pathogenesis of chronic pancreatitis. Both acinar and ductal factors and their related genes are involved

Acinar factors

The discovery of the cationic trypsinogen (*PRSS1*) gene as a cause of hereditary pancreatitis favors the necrosis–fibrosis hypothesis.[9] The R122H mutation, for example, eliminates a key hydrolysis site of trypsin, which is part of the trypsin-inactivation mechanism. Once activated, the mutant trypsin remains active within the pancreas, activates all other digestive enzymes, and leads to autodigestion and inflammation, that is, acute pancreatitis. Chronic pancreatitis is commonly seen in these patients, which suggests that recurrent acute pancreatitis may lead to chronic pancreatitis.

Witt et al.[10] have found a strong association of mutations in the pancreatic secretory trypsin inhibitor (*PSTI*) or serine protease inhibitor Kazal type 1 (*SPINK1*) gene with idiopathic chronic pancreatitis in children and adolescents. However, *SPINK1* mutations are common in the normal population, and the chronic pancreatitis risk associated with the N34S mutation is not high (~1%), suggesting that these mutations are disease modifiers.[11] Interestingly the G191R mutation in the

PRSS2 gene, which renders the mutant anionic trypsin sensitive to autolysis by introducing a new tryptic cleavage site, appears to protect the carrier against chronic pancreatitis.[12] Thus, chronic pancreatitis may be caused by either increased proteolytic activity or decreased protease inhibition. As the majority of patients with idiopathic or hereditary chronic pancreatitis do not show a *PSTI* or *PRSS1* mutation, other genetic factors must also be involved.

Ductal factors

An association of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene with chronic pancreatitis[13,14] strongly suggests that impaired pancreatic duct function may also be involved in the pathogenesis of this disease. The *CFTR* gene encodes a 1480-amino acid chloride-channel protein that is regulated by cAMP. Bicarbonate ion is secreted into the duct lumen by the action of CFTR in the apical membranes.[15] Water is transported into the lumen via the AQP1 water channel following the osmotic gradient created by bicarbonate transport.[16] In the absence of CFTR, the pancreatic duct cells cannot secrete fluid and bicarbonate and hence cystic fibrosis (CF) of the pancreas develops.

As in CF, plug formation within the intra- and interlobular ducts is one of the earliest findings in chronic pancreatitis. PSP, also known as the regeneration (reg) protein,[17] is the major component of the core protein of pancreatic stones as well as of the protein plugs.[8,18] Based on the assumption that PSP inhibits pancreatic stone formation, Sarles et al.[19] proposed the renaming of PSP as "lithostathine." Although later studies did not support his "lithostathine" hypothesis, the PSP/reg protein has a very unique character. After tryptic cleavage of the N-terminal undecapeptide, the resultant C-terminal peptide of the PSP/reg protein, named pancreatic thread protein (PTP), rapidly polymerizes into insoluble fibrils at pH 5–9.[20,21] The thread protein is highly resistant to a wide spectrum of proteases in pancreatic juice[21] and thus contributes to protein plug formation within the ducts. Upon intraductal activation of trypsinogen, decreased protease inhibition caused by *PSTI* mutations may facilitate conversion of the PSP/reg protein to the insoluble PTP (Fig. 2).

It is not known exactly how the *CFTR* mutations lead to the development of chronic pancreatitis. A partial loss of CFTR function may reduce ductal fluid secretion in the vicinity of acini and thus may increase the concentrations of enzymes and other proteins such as PSP/reg protein. The elevated concentration of Ca^{2+} in pancreatic juice in the early stage of chronic pancreatitis, together with that of hexosamine,[22,23] probably promotes the deposition of $CaCO_3$ within and around the plugs.

Genetic aspects of chronic pancreatitis in the Japanese

The same *PRSS1* (R122H and N29I) and *PSTI* mutations (N34S and R67C) found in the white populations have been identified in Japanese kindred with hereditary pancreatitis[24,25] and in patients with juvenile and familial chronic pancreatitis.[26,27] As CF is extremely rare in Japan, *CFTR* mutations may not be related to Japanese chronic pancreatitis. In an initial screening study, common CF-causing mutations were not identified in either patients with chronic pancreatitis or control subjects.[28] However, non-CF causing mutations (Q1352H and R1453W) are highly associated with chronic pancreatitis. Furthermore, these mutations are on the same allele as the M470V variant, which has low (~60%) intrinsic chloride channel activities.[29] The Q1352H mutation in the M470 background causes a 60%–80% reduction in CFTR-dependent Cl^- currents and completely abolishes them in the M470V variant.[30] Therefore, as in whites, *CFTR* mutations/polymorphisms are likely to be associated with chronic pancreatitis in Japanese, although mutations may differ depending on ethnic origins.

Recurrent pancreatitis by protein plugs may lead to chronic pancreatitis

As discussed above, protein plugs are one of the earliest findings in alcoholic chronic pancreatitis. There are several reports that protein plug formation may be responsible for recurrent pancreatitis in adolescence, which leads to histologically proven chronic pancreatitis without calcification of the plugs.[31] Figure 3 illustrates test results for a 53-year-old man with alcoholic chronic pancreatitis. He consumed 75–110 g/day of ethanol for over 30 years and had his first acute pancreatitis attack at the age of 47. After several attacks of pain, he developed a cyst in the pancreatic head and a narrowing of the main pancreatic duct and dilatation of the distal duct. He underwent pylorus-preserving pancreatoduodenectomy. Although he had no X-ray-positive pancreatic stone, the histology showed chronic pancreatitis with visible protein plugs in the branches of the duct. They were composed mainly of protein (>98%) and fatty acid calcium. He continued drinking after the operation and developed acute pancreatitis again (Fig. 3A). Magnetic resonance cholangiopancreatography (MRCP) during the first admission revealed a round defect near the orifice of the pancreas (Fig. 3B). The next MRCP was taken 4 months later, during the second attack of acute pancreatitis. The orifice of the duct was completely obstructed, and the main pancreatic duct was dilated (Fig. 3C). Recurrent pain that continued after admission disappeared after intravenous administration of secretin. Four

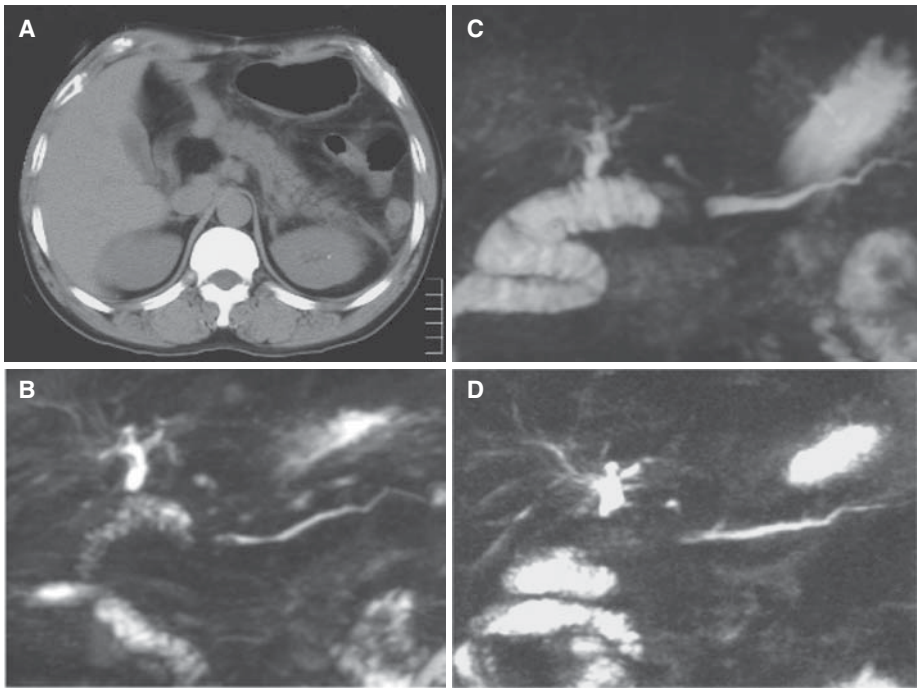

Fig. 3. A Abdominal CT of a 53-year-old man with alcoholic chronic pancreatitis who suffered a relapse of acute pancreatitis after pylorus-preserving pancreatoduodenectomy. The pancreas was edematous with surrounding effusions. There was no calcification in the pancreas. **B** MRCP obtained after recovery from the first relapse (**A**). **C** MRCP obtained during the second relapse (4 months after **B**). **D** MRCP obtained 4 months after the second relapse (**C**)

months later, the dilatation of the main duct disappeared but a round defect remained within the duct (Fig. 3D). His genetic analysis revealed the R1453W mutation in the *CFTR* gene but none in the *PSTI* gene. Although the relationship of *CFTR* mutations and protein plug formation remains to be elucidated, the protein plugs appear to cause chronic pancreatitis after repeated attacks of acute pancreatitis.

Perpetual stimuli are necessary for the development of chronic pancreatitis

There are number of animal models of chronic pancreatitis, both spontaneous and drug-induced, but their pancreatic fibrosis appear to be different from that in humans.[32] Pancreatic fibrosis, which is progressive and irreversible in humans, is usually transient in animal models and decreases after cessation of the chemicals. A complete ligation of the dog main pancreatic duct induces atrophy of the pancreas, while an incomplete one causes pancreatic stone formation and histology similar to that in human chronic pancreatitis.[33] Decline in exocrine and endocrine function in this model is progressive, as in humans.[34] Thus, these animal models tell us that perpetual stimuli, either chemical or mechanical, are necessary for the development of chronic pancreatitis. In humans, continued drinking is a necessary condition, though not sufficient, for the development of alcoholic chronic pancreatitis. In addition, small but constant genetic propensities must be present, though the responsible genes have not been elucidated yet.

Genetic risk for chronic pancreatitis

In children and adolescents, in whom the influence of environmental factors is minimal, genetic factors probably play an important role in determining susceptibility to chronic pancreatitis and its progression. Among carrier individuals with the R122H or N29I mutation in the *PRSS1* gene, approximately 80% develop acute pancreatitis before the age of 20 years, and of these, 40% may develop chronic pancreatitis. Assuming that the genetic risk is constant throughout life, the cumulative incidence of chronic pancreatitis in individuals with this type of genetic background follows curve A in Fig. 4. Environmental factors such as smoking and alcohol drinking may shift the curve to A' by doubling the risk. However, about 20% of the carriers never develop pancreatitis throughout their lives,[35] suggesting that inappropriate activation of trypsinogen within the pancreas is extremely rare in this subset of the population.

Judging from the incidence of chronic pancreatitis in various countries (4–10/100000 persons), the genetic risk of chronic pancreatitis in the general population is very low (curve C in Fig. 4). Therefore, the majority of individuals never develop pancreatitis even if alcohol or smoking raises the risk (curve C'). Even among those who are carriers of *PSIT* or *CFTR* mutations, which

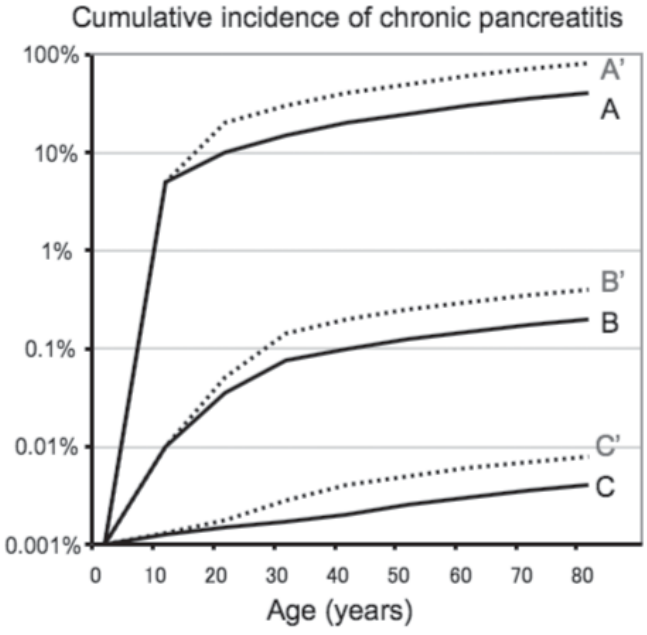

Fig. 4. The genetic risk and cumulative incidence of chronic pancreatitis. The genetic risk is assumed to be constant throughout life. *A*, a model for the *PRSS1* gene. The lifetime cumulative incidence of chronic pancreatitis is 40%. *B*, a model for the *PSIT* or *CFTR* gene. The lifetime cumulative incidence is 0.2%. *C*, a model for the general population. The lifetime cumulative incidence is 0.004%. *A′*, *B′* and *C′*, The risk is increased twofold by environmental factors (smoking and alcohol)

increases the risk 50-fold (curve B) or 900-fold when both genes are affected,[36] the majority never develop chronic pancreatitis. Still, although there is a strong association of *PSIT* mutations with familial and juvenile chronic pancreatitis,[10,11,27] the association of the N34S mutation with early disease onset may be weak or absent.[37] Obviously the pathogenesis of chronic pancreatitis is multifactorial, and at the moment, we can only make a statistical inference regarding the risk of chronic pancreatitis. Further genetic analysis is necessary for understanding the pathogenesis of chronic pancreatitis, which may be helpful for the earlier diagnosis of the juvenile- or young-onset type of the disease.

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