Tohoku J. Exp. Med., 2014, **232**, 69-77

Invited Review

Genetics of Pancreatitis: The 2014 Update

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Chronic pancreatitis is a progressive inflammatory disease in which pancreatic secretory parenchyma is destroyed and replaced by fibrous tissue, eventually leading to malnutrition and diabetes. Alcohol is the leading cause in Western countries, but genetic factors are also implicated. Since the identification of mutations in the cationic trypsinogen (*PRSS1*) gene as a cause of hereditary pancreatitis in 1996, we have seen great progress in our understanding of the genetics of pancreatitis. It has been established that mutations in the genes related to the activation and inactivation of trypsin(ogen) such as *PRSS1*, serine protease inhibitor Kazal type 1 (*SPINK1*) and chymotrypsin C (*CTRC*) genes are associated with pancreatitis. In 2013, carboxypeptidase A1 (*CPA1*) was identified as a novel pancreatitis susceptibility gene. Endoplasmic reticulum stress in pancreatic acinar cells resulting from the mis-folding of mutated pancreatic enzymes has been shown to act as a novel mechanism underlying the susceptibility to pancreatitis. In Japan, the nationwide survey revealed 171 patients (96 males and 75 females) with hereditary pancreatitis in 59 families based on the European Registry of Hereditary Pancreatitis and Familial Pancreatic Cancer criteria. Because about 30% of families with hereditary pancreatitis do not carry mutations in any of the known pancreatitis susceptibility genes, other yet unidentified genes might be involved. Next generation sequencers can perform billions of sequencing reactions with a read length of 150-250 nucleotides. Comprehensive analysis using next generation sequencers will be a promising strategy to identify novel pancreatitis-associated genes and further clarify the pathogenesis of pancreatitis.

Keywords: carboxypeptidase; endoplasmic reticulum stress; next generation sequencer; trypsin; whole-exome sequencing

Tohoku J. Exp. Med., 2014 February, **232** (2), 69-77.

Introduction

Chronic pancreatitis (CP) is a progressive inflammatory disease in which pancreatic secretory parenchyma is destroyed and replaced by fibrous tissue, eventually leading to impairment of the exocrine and endocrine functions of the organ (Steer et al. 1995; Witt et al. 2007; Braganza et al. 2011). The main symptom of CP is usually pain, which occurs as attacks that mimic acute pancreatitis or as constant and disabling pain. Alcohol is the leading cause of CP in Western countries, but other factors such as genetic mutations are also implicated. Since the identification of mutations in the cationic trypsinogen (protease, serine, 1; *PRSS1*) gene as a cause of hereditary pancreatitis (HP) in 1996 (Whitcomb et al. 1996), we have seen great progress in our understanding of the genetics of pancreatitis. Genetic studies suggest that pancreatitis results from an imbalance of proteases and their inhibitors within the pancreatic parenchyma. Gain-of-function mutations in the *PRSS1* gene as well as loss-of-function variants in the serine protease inhibitor Kazal type 1 (*SPINK1*) gene and the trypsin-degrading enzyme chymotrypsin C (*CTRC*) increase the risk for CP (Whitcomb et al. 1996; Pfützer et al. 2000; Witt et al. 2000; Rosendahl et al. 2008). Consistent with the proposed pathogenic role of trypsin, a rapidly auto-degrading variant of anionic trypsinogen (*PRSS2*) protects against CP (Witt et al. 2006). In 2013, carboxypeptidase A1 (*CPA1*) was identified as a novel pancreatitis susceptibility gene (Witt et al. 2013). Of note, the underlying mechanism was independent of trypsin activation and the roles of endoplasmic reticulum (ER) stress in pancreatic acinar cells have been highlighted. In this review, I summarize advances in the understanding of the genetics of pancreatitis, especially focusing on data from the Japanese population. In addition, I discuss our attempt to identify novel pancreatitis-associated genes using next generation sequencing.

Received January 8, 2014; revised and accepted January 16, 2014. Published online February 7, 2014; doi: 10.1620/tjem.232.69.

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HP in Japan

HP is a rare cause of CP. There is no consensus about the diagnostic criteria of HP, but the European Registry of Hereditary Pancreatitis and Familial Pancreatic Cancer (EUROPAC) trial has defined it as two first-degree relatives, or 3 or more second-degree relatives, in 2 or more generations with recurrent acute pancreatitis and/or CP, for which there were no predisposing factors (Howes et al. 2004). The nationwide epidemiological survey of CP in Japan revealed that the estimated number of patients with CP was 47,100 in 2007 (Hirota et al. 2012). The prevalence of CP in Japan was 36.9/100,000, and the number has continued to increase even in recent years. Alcohol is the leading cause of CP, accounting for 70% of the cases, followed by idiopathic causes, which accounted for 21%. HP was only 10 of 1,236 cases reported for the secondary survey, accounting for 0.8% of the cases. Based on this calculation, the prevalence of HP was 0.3/100,000. In the French cohort, on a national basis, the HP prevalence was 0.3/100,000 (Rebours et al. 2009). A cohort study of patients < 30 years of age based on the Danish National Registry included 977 patients in the period from 1977 to 2004 (Joergensen et al. 2010). The prevalence of HP was estimated as 0.57/100,000 for symptomatic HP patients and 0.13/100,000 for carriers of the *PRSS1* mutations. The estimated prevalence of HP in Japan, therefore, appears to be similar to that in Western countries.

To further clarify the current status of HP in Japan, we took a nationwide survey. The target subjects were patients with HP, familial pancreatitis, or juvenile pancreatitis who visited the surveyed hospitals between 2001 and 2011. The first survey was conducted to determine the number of patients, and the second survey was to obtain detailed clinical and genetic information. The questionnaire was sent to 2,290 departments consisting of departments of gastroenterology certified by the Japanese Society of Gastroenterology, departments of gastroenterology and pediatrics in hospitals having more than 400 beds, children's hospitals, and those who sought genetic testing for a patient with pancreatitis from our department (Tohoku University) between 2001 and 2011. Of 2,290 departments, 781 departments responded to the first-stage survey. The response rate was 34.1%. The second survey showed that there were 171 patients (96 males and 75 females) with HP in 59 families based on the EUROPAC criteria. *PRSS1* mutations accounted for about 40% of the families, and the *SPINK1* mutations accounted for about 30%. Importantly, the remaining 30% of the families did not carry mutations in any of the known pancreatitis susceptibility genes (Table 1).

PRSS1 mutations

PRSS1 encodes cationic trypsinogen, the most abundant isoform of trypsinogen in human pancreatic juice. In 1996, Whitcomb et al. (1996) identified the p.R122H (c.365G>A) mutation in the *PRSS1* gene as a cause of HP. In the following year, the p.N29I (c.86A>T) mutation was reported in patients with HP (Gorry et al. 1997). The p.R122H mutation is the most common one, followed by the p.N29I mutation. The p.A16V (c.47C>T) mutation (Grocock et al. 2010) is the third most common one, but has not been reported in Japanese subjects.

Very recently, Schnúr et al. (2014) reported the functional effects of 13 *PRSS1* variants found in sporadic CP. They reported that five mutants, including the p.G208A (c.623G>C), showed reduced secretion, suggesting that these variants might increase the risk of pancreatitis related to mutation-induced misfolding and consequent ER stress. Interestingly, the p.G208A variant had been reported only in Asian subjects: a 12-year-old Asian male with CP, a Korean child with recurrent acute pancreatitis, and a 7-year-old Korean child with necrotizing acute pancreatitis (Keiles and Kammesheidt 2006; Lee et al. 2011). We therefore conducted screening of the *PRSS1* p.G208A (c.623G>C) variant and found an association with pancreatitis in Japan (Masamune et al. 2014). The *PRSS1* p.G208A variant was

Table 1. Genotypes of families with hereditary pancreatitis in Japan.

Type of mutations	Number of families (n = 44)
PRSS1 or *SPINK1* mutation negative	13 (29.5%)
PRSS1 or *SPINK1* mutation positive	31 (70.5%)
PRSS1 mutation positive	18 (40.9%)
p.R122H	13
p.N29I	4
p.R122H/*SPINK1* c.194+2T>C	1
SPINK1 mutation positive	14 (31.8%)
p.N34S	7
c.194+2T>C	5*
p.N34S/c.194+2T>C	1
p.N34S/p.R67C	1

*includes a family carrying the *PRSS1* p.R122H and the *SPINK1* c.194+2T>C mutations.

found in 8 patients with alcoholic CP, 9 patients with idiopathic CP, and one patient with hereditary CP (Table 2). Of the 9 patients with idiopathic CP carrying the p.G208A variant, 7 patients developed pancreatitis before the age of 20 (the age at onset in these 7 patients was 1, 2, 7, 11, 12, 15, and 19 years old). Two patients (a patient with alcoholic CP whose age at onset was 28 years and a patient with idiopathic CP whose age at onset was 12 years) carrying the p.G208A variant also had the *CTRC* p.R29Q (c.86G>A) mutation (Masamune et al. 2013). One patient with idiopathic CP carrying the p.G208A variant also had the *SPINK1* p.N34S (c.101A>G) mutation. The frequency of the p.G208A variant was higher in patients with CP than in the controls (Table 2). The differences were still significant even if the patients were stratified based on the etiology. Our results showed that the *PRSS1* p.G208A variant is associated with CP in Japan. To our knowledge, this is the first *PRSS1* variant found in association with alcoholic CP. The risk conferred by the p.G208A variant seemed lower than that by the *PRSS1* "classic mutation" p.R122H and the p.G208A variant might need other (genetic, alcoholic, or environmental) factors to reach the threshold for the development of CP. This notion might be supported by the association of this variant with alcoholic CP and by the presence of the *CTRC* p.R29Q and the *SPINK1* p.N34S mutations in some patients. On the other hand, this variant might be a unique genetic background of pancreatitis in Asia.

SPINK1 mutations

SPINK1, also known as pancreatic secretory trypsin inhibitor, provides the first line of defense against premature trypsinogen activation by inhibiting up to 20% of trypsin activity within the pancreas (Rinderknecht 1986). In 2000, Witt and coworkers reported that 22 out of 96 patients with juvenile CP had mutations in the *SPINK1* gene (Witt et al. 2000). Of note, 18 patients had the p.N34S (c.101A>G) mutation. The following studies have shown that mutations in the *SPINK1* gene are associated with CP of various etiologies including idiopathic, familial, and tropical (Witt et al. 2000; Pfützer et al. 2000; Chen et al. 2001; Bhatia et al. 2002; Kume et al. 2005; Aoun et al. 2008). The p.N34S mutation has been found worldwide in CP patients and healthy controls, with an average allele frequency of 9.7% and 1%, respectively (Witt et al. 2000; Pfützer et al. 2000; Chen et al. 2001; Bhatia et al. 2002; Kume et al. 2005; Aoun et al. 2008). In addition to CP, we and others have reported that the p.N34S mutation is strongly associated with recurrent acute pancreatitis, but does not increase the risk of the first or sentinel acute pancreatitis event (Aoun et al. 2010; Masamune et al. 2011). The second most common mutation c.194+2T>C (IVS3+2T>C) has been reported in patients with idiopathic, familial, and alcoholic CP (Witt et al. 2000; Pfützer et al. 2000; Bhatia et al. 2002; Kume et al. 2005; Oh et al. 2009; Ota et al. 2010; Sun et al. 2013).

Table 3 shows the frequency of the *SPINK1* mutations in Japanese patients with CP. The frequency of the p.N34S mutation was higher in patients with idiopathic, familial, and hereditary pancreatitis than in controls. The frequency of the c.194+2T>C mutation was higher in patients with idiopathic and hereditary CP than in controls. The characteristic feature of the *SPINK1* mutations in East Asia including Japan is a high frequency of the c.194+2T>C mutation. In China, this intronic mutation was found in 45% of the patients with idiopathic CP (Sun et al. 2013). Similarly, in Korea, this mutation was found in 19% of the patients with idiopathic CP (Oh et al. 2009). In India, the p.N34S mutation is frequently found, but the c.194+2T>C mutation is very rare (Midha et al. 2010). In Europe and the United States, the c.194+2T>C mutation was present in 1 to 3 percent of the cases (Witt et al. 2000; Pfützer et al. 2000).

It has been suggested that *SPINK1* mutations might result in altered interaction between SPINK1 and trypsin, thus affecting the protease/antiprotease balance within the pancreas (Witt et al. 2000). But the underlying mechanism linking the *SPINK1* p.N34S mutation and pancreatitis remains unknown. The p.N34S variation had no effect on the secretion of SPINK1 protein from transfected cells and the trypsin inhibitory activity of the mutant protein was also unchanged (Kuwata et al. 2002; Király et al. 2007). The roles of co-segregating four intronic alterations have been excluded (Masamune et al. 2007; Kereszturi et al. 2009a). On the other hand, we have shown that the c.194+2T>C

Table 2. Frequency of the *PRSS1* p.G208A (c.623G>C) variant in Japanese patients with CP.

Etiology	Frequency (%)	OR (95% CI)	*P* value (vs. Controls)*
Alcoholic	8/232 (3.4)	14.6 (1.8-117.8)	0.002
Idiopathic	9/198 (4.5)	19.5 (2.5-155.2)	< 0.001
Hereditary	1/34 (2.9)	12.4 (0.7-203.2)	0.15
Familial	0/20 (0)	1.0 (1.0-1.0)	> 0.99
Total CP	18/484 (3.7)	15.8 (2.1-119.2)	< 0.001
Controls	1/411 (0.2)	—	—

*Fisher's exact test.
CP, chronic pancreatitis; OR, Odds ratio; CI, confidence interval.
The data are from the paper by Masamune et al. (2014).

Table 3. Frequency of the *SPINK1* mutations in Japanese patients with CP and controls.

Etiology		*SPINK1* p.N34S			*SPINK1* c.194+2T>C		
		Positive (hm)	Frequency	*P* value*	Positive (hm)	Frequency	*P* value*
Alcoholic	*n* = 129	0 (0)	0%	> 0.99	4 (1)	3.1%	> 0.99
Idiopathic	*n* = 85	9 (2)	10.6%	< 0.001	10 (2)	11.8%	< 0.001
Hereditary	*n* = 19	2 (0)	10.5%	0.006	4 (0)	21.1%	< 0.001
Familial	*n* = 11	4 (1)	36.4%	< 0.001	0 (0)	0%	> 0.99
Others	*n* = 36	1 (0)	2.8%	0.18	0 (0)	0%	> 0.99
CP total	*n* = 280	16 (3)	5.7%	< 0.001	18 (3)	6.4%	< 0.001
Control	*n* = 540	2 (0)	0.4%		0 (0)	0%	

CP, chronic pancreatitis; hm, homozygote.
*Fisher's exact test.

Table 4. Frequency of the *PRSS2* p.G191R variant in Japanese patients with CP.

Etiology		*PRSS2* p.G191R variant		
		Positive (hm)	Frequency	*P* value*
Alcoholic	*n* = 129	1 (0)	0.8%	0.009
Idiopathic	*n* = 85	0 (0)	0%	0.01
Hereditary	*n* = 19	0 (0)	0%	0.62
Familial	*n* = 11	0 (0)	0%	> 0.99
CP total	*n* = 244	1 (0)	0.4%	< 0.001
Control	*n* = 402	26 (2)	6.5%	

CP, chronic pancreatitis; hm, homozygote.
*Fisher's exact test.

mutation affects the consensus splicing donor site, resulting in the skipping of exon 3 (Kume et al. 2006). Because the trypsin-binding site is located on exon 3, the skipping of exon 3 results in the loss of the trypsin-binding site, leading to a disturbance in the protease/anti-protease balance within the pancreas. Along this line, we have recently shown that the serum SPINK1 concentration was low in patients carrying the c.194+2T>C mutation, further supporting the notion that functional SPINK1 is decreased in patients carrying this mutation (Kume et al. 2012).

PRSS2 variants

PRSS2 is another major trypsinogen isoform constituting the bulk of secreted trypsinogen in humans (Kukor et al. 2003). CP and alcoholism lead to a characteristic reversal of the isoform ratio, and anionic trypsinogen becomes the predominant zymogen secreted (Rinderknecht et al. 1979). It has been reported that the *PRSS2* p.G191R (c.571G>A) variant was less frequent in patients with CP [32/2,466 (1.3%)] than in controls [220/6,459 (3.4%)] in Europe (Witt et al. 2006). Upon activation by enterokinase or trypsin, purified recombinant p.G191R protein showed a complete loss of trypsin activity owing to the introduction of a new tryptic cleavage site that renders the enzyme hypersensitive to autocatalytic proteolysis (Witt et al. 2006). Therefore, the *PRSS2* p.G191R variant mitigates the intra-pancreatic trypsin activity and thereby protects against CP. In Japan, the updated frequency of the *PRSS2* p.G191R variant was 1/244 (0.4%) in patients with CP, while it was 26/402 (6.5%) in the control population (Table 4) (Kume et al. 2009). The differences were still significant even if the patients were stratified based on the etiology ($P = 0.009$ for alcoholic CP vs. Controls, and $P = 0.01$ for idiopathic CP vs. Controls). Thus, the *PRSS2* p.G191R variant may protect against CP in the Japanese population, as well.

CTRC variants

CTRC specifically degrades all human trypsin and trypsinogen isoforms, and serves as a second line of defense against premature activation of trypsinogen isoforms (Rosendahl et al. 2008). It has been reported that the *CTRC* variants in exons 3 and 7 are associated with CP in Europe and India (Rosendahl et al. 2008; Masson et al. 2008; Beer et al. 2013; Paliwal et al. 2013). In European cohorts, the micro-deletion variants p.K247_R254del (c.738_761del24) and p.R254W, both located in exon 7, are the most common *CTRC* variants (Rosendahl et al. 2008; Masson et al. 2008; Beer et al. 2013). In India, these variants are rare, and the p.A73T (c.217G>A) variant in exon 3 and the p.V251I (c.703G>A) variant in exon 7 are the most common ones

Table 5. Frequency of the *CTRC* variants in Japanese patients with CP and controls.

CTRC missense variants	Total CP (%)	Alcoholic CP (%)	Non-alcoholic CP (%)	Controls (%)	*P* value (Total CP vs. Controls)*
Exon 2					
p.R29Q (c.86G>A)	2/506 (0.4)	1/244 (0.4)	1/262 (0.4)	0/274 (0)	0.54
Exon 6					
p.I209M (c.627C>G)	0/506 (0)	0/244 (0)	0/262 (0)	1/274 (0.4)	0.35
Exon 7					
p.S239A (c.715T>G)	1/506 (0.2)	0/244 (0)	1/262 (0.4)	0/274 (0)	> 0.99
p.S239C (c.716C>G)	1/506 (0.2)	1/244 (0.4)	0/262 (0)	0/274 (0)	> 0.99
p.K247E (c.739A>G)	3/506 (0.6)	1/244 (0.4)	2/262 (0.8)	0/274 (0)	0.56
p.R254W (c.760C>T)	1/506 (0.2)	0/244 (0)	1/262 (0.4)	0/274 (0)	> 0.99
CTRC missense variants total	8/506 (1.6)	3/244 (1.2)	5/262 (2.0)	1/274 (0.4)	0.17

CP, chronic pancreatitis.
*Fisher's exact test.
All missense variants were in a heterozygous form.
The data are from the paper by Masamune et al. (2013). These missense variants except for the p.R254W had not been previously reported.

Table 6. Frequency of the functionally impaired *CPA1* variants in multiple populations.

Cohorts	Non-alcoholic CP (%)	Controls (%)	*P* value
German	29/944 (3.1)	5/3,938 (0.1)	1.5×10^{-16}
European	8/600 (1.3)	9/2,432 (0.4)	0.01
Indian	6/239 (2.5)	1/340 (0.3)	0.02
Japanese	5/247 (2.0)	0/341 (0)	0.013

This table summarizes the results published in the paper of Witt et al. (2013).
The functionally impaired (< 20%) *CPA1* variants are p.R27X, p.R27P, p.L50_E127del78, p.T164M, p.R181Q, p.G225S, p.T229M, p.R237H, p.K238M, p.V251M, p.P253R, p.R255M, p.N256K, p.P270R, p.C271R, p.G277S, p.A280D, p.E283K, p.Y308H, p.Y314C, p.Y318X, p.E328K, p.D330IfsX51, p.Y358CfsX5, p.G362E, p.T368_Y369ins20, p.S376P, p.R382W, p.A406V and p.N416IfsX9.

(Rosendahl et al. 2008; Paliwal et al. 2013). These common variants in Europe and India are very rare in Japanese subjects and only one patient with idiopathic CP had the p.R254W variant (Masamune et al. 2013) (Table 5). On the other hand, we found 5 novel missense variants including p.R29Q (c.86G>A). Our results show that the spectrum and distribution of the *CTRC* variants in Japanese subjects were different from those reported from Europe and India.

CPA1 variants

In 2013, there was a "break-through" in this area. Carboxypeptidases are pancreatic metalloproteases that hydrolyze C-terminal peptide bonds in dietary polypeptide chains (Vendrell et al. 2000). Three different isoforms have been described in human pancreatic juice. A-type carboxypeptidases (CPA1 and CPA2) act on aromatic and aliphatic amino acid residues exposed by the action of chymotrypsins and elastases, whereas the B-type carboxypeptidase (CPB1) hydrolyzes C-terminal lysine and arginine residues generated by tryptic cleavages (Szmola et al. 2011). Activation of human proCPA1 to CPA1 is catalyzed by the sequential action of trypsin and CTRC, which cleave and degrade the propeptide. After trypsinogens, proCPA1 is the second largest component of pancreatic juice, contributing about 16% of the total protein (Scheele et al. 1981). An internationally collaborative team, including our group, showed that functionally defective *CPA1* variants are associated with pancreatitis (Witt et al. 2013). In the initial German cohort of 944 individuals with non-alcoholic CP and 3,938 control subjects, 30 missense variants, 1 nonsense variant, 1 frame-shift variant, and 1 splice-site variant were identified, and 3 variants were significantly enriched in patients. Functional analysis demonstrated that 17/33 (52%) variants resulted in a marked (> 80%) loss of apparent CPA1 activity. Of note, 14 out of 17 (82%) functionally impaired variants were found exclusively in patients, including the most frequent p.N256K (c.768C>G) variant. Collectively, *CPA1* variants with less than 20% apparent activity were found in 29/944 (3.1%) of patients with non-alcoholic CP and in 5/3,938 (0.1%) of the control subjects (Table 6). The association between functionally defective *CPA1* variants and non-alcoholic CP was confirmed in rep-

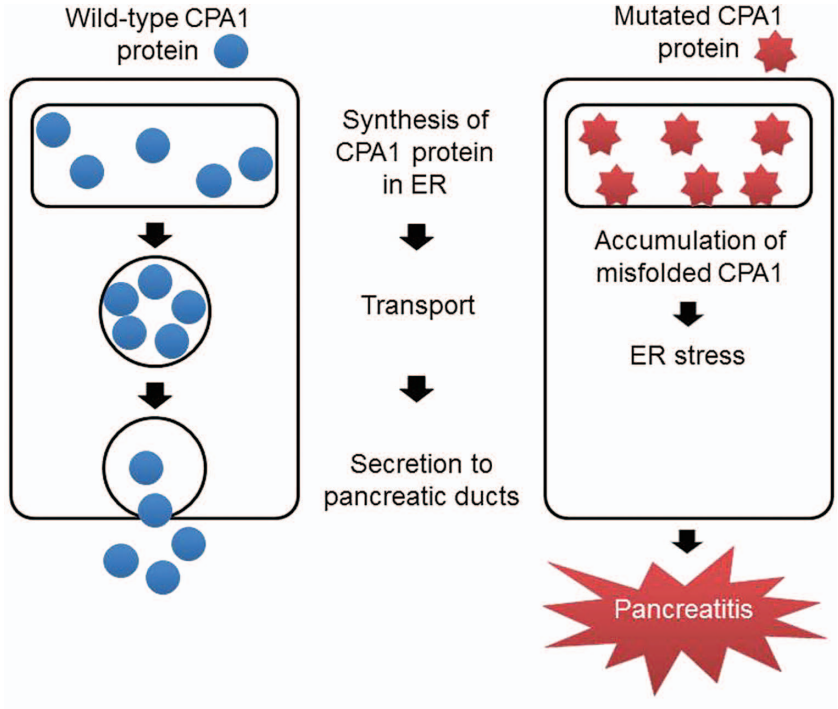

Fig. 1. Schematic mechanism underlying *CPA1* mutation-associated pancreatitis.
In pancreatic acinar cells, wild-type CPA1 protein is synthesized in endoplasmic reticulum (ER), transported and secreted to the pancreatic duct. *CPA1* mutants generate misfolded protein in ER. The accumulation of misfolded CPA1 protein causes ER stress, leading to the development of pancreatitis.

lication sets from Europe, India and Japan. Importantly, carrying a functionally defective *CPA1* variant in German cohorts increased the risk for pancreatitis 38-fold in patients younger than 20 years old and 84-fold in patients younger than 10 years old.

The mechanism by which loss-of-function *CPA1* variants predispose to CP appears to be different from other currently known pancreatitis susceptibility genes. CPA1 had no detectable effect on trypsinogen activation, trypsin activity or trypsinogen degradation by CTRC, indicating that *CPA1* variants do not exert their effect via increasing the intrapancreatic trypsin activity. On the other hand, most of the loss-of-function *CPA1* variants exhibit markedly reduced secretion, raising the possibility that *CPA1* variants undergo misfolding in the ER and cause ER stress, as demonstrated previously for some *PRSS1* and *CTRC* mutants (Kereszturi et al. 2009b; Beer et al. 2013). Indeed, the expression of p.N256K variant in AR42J rat pancreatic acinar cells resulted in ER stress, as evidenced by increased splicing of X-box binding protein 1 and elevated mRNA levels of the chaperons BiP and calreticulin. Considering that CPA1 is one of the most abundant proteins synthesized by the pancreas, ER stress induced by misfolding seems a plausible mechanism to explain the clinical effect of *CPA1* variants (Fig. 1).

Whole-exome sequencing (WES) using next generation sequencing

Despite these recent advances, we still find that many patients do not carry mutations in any of the known pancreatitis susceptibility genes, suggesting the involvement of other yet unidentified genes. A new approach that uses massive parallel sequencing called next generation sequencing is becoming standardized, and the cost is rapidly dropping (Metzker 2010). Using the ultimate platforms, such systems are able to perform billions of sequencing reactions with a read length of 150-250 nucleotides. WES is the application of the next-generation technology to determine variations in all the coding regions or exons of known genes. WES provides coverage of more than 95% of the exons, which contain 85% of the disease-causing mutations in Mendelian disorders and many disease-predisposing single nucleotide polymorphisms throughout the genome (Rabbani et al. 2013). The roles of more than 150 genes have been revealed by means of WES, and the number is rapidly increasing. We conducted WES in 3 patients of a family with HP and 4 patients of 2 families with familial pancreatitis using the Illumina HiSeq2000 (Kume et al. 2013). We picked up 1,348 candidate variants and validated their authenticity based on their functions, expression patterns and known association with diseases. We also identified the *CPA1* p.V251M (c.G751A) mutation in all of the 3 subjects of the family with HP, suggesting that WES

might be useful to identify novel pancreatitis susceptibility genes.

One problem with WES is that there are too many DNA variations with unpredictable meaning and incidental findings of mutations with high clinical importance, i.e. those in cancer-susceptibility genes that are detected at a considerable frequency (Johnston et al. 2012). Targeted capture of custom-designed regions followed by sequencing of selected genomic regions of interest provides an attractive, cost-effective alternative. HaloPlex™ target enrichment technology is a selective circularization-based method that is a further development of the principle of selector probes (Berglund et al. 2013). In the HaloPlex™ technology, genomic DNA is fragmented by restriction enzyme digestion and circularized by hybridization to probes whose ends are complementary to the target fragments. Compared to hybrid capture methods, the HaloPlex™ system requires smaller amounts of starting DNA, has higher specificity, and provides more uniform genome coverage. In an attempt to identify novel pancreatitis susceptibility genes and perform the screening of known pancreatitis susceptibility genes, we have developed the HaloPlex™ for 70 genes, including pancreatic digestive enzymes, those highly expressed in the pancreas and those related to ER stress. Using the bench-top Illustra MiSeq machine, comprehensive analysis of many samples can be done. This system will be useful to screen the known pancreatitis susceptibility genes simultaneously, some of which have more than 20 exons.

Conclusions

I reviewed the recent progress concerning the genetics of pancreatitis, especially focusing on the data from our laboratory. Comprehensive analyses using next generation sequencers will be a promising strategy to identify novel pancreatitis-associated genes and further clarify the pathogenesis of pancreatitis.

Acknowledgements

The author is grateful to Professor Tooru Shimosegawa for his great mentorship and to colleagues and collaborators, especially Drs. Kiyoshi Kume and Eriko Nakano, for their outstanding contributions. This work was supported in part by Grant-in-Aid from Japan Society for the Promotion of Science (23591008 and 23659392), by the Pancreas Research Foundation of Japan, by the HIROMI Medical Research Foundation, by the Mother and Child Health Foundation and by the Research Committee of Intractable Pancreatic Diseases provided by the Ministry of Health, Labour, and Welfare of Japan.

Conflict of Interest

The author declares no conflict of interest.

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