# Invited Review for the 100<sup>th</sup> Anniversary of the TJEM

# A New Insight into Chronic Pancreatitis

# Tooru Shimosegawa<sup>1</sup>

<sup>1</sup>South Miyagi Medical Center, Shibata-gun, Miyagi, Japan

Chronic pancreatitis (CP) is a pancreatic disease with poor prognosis characterized clinically by abdominal pain, morphologically by pancreatic stones/calcification, duct dilatation and atrophy, and functionally by pancreatic exocrine and endocrine insufficiency. CP is also known as a risk factor for the development of pancreatic cancer. CP has long been understood based on a fixed disease concept deduced from the clinical and morphological features of the end-stage disease. However, identification of causal genes for hereditary pancreatitis and success in the isolation and culture of pancreatic stellate cells have advanced the understanding of the underlying pathological mechanisms, the early-stage pathophysiology, and the mechanisms behind pancreatic fibrosis. These advances have led to moves aimed at improving patient prognosis through prevention of disease progression by early diagnosis and early therapeutic intervention. The strategy for preventing disease progression has included a proposal for diagnostic criteria for early CP and introduction of a new definition of CP in consideration of the pathological mechanisms. Our group has been committed deeply to these studies and has provided a large amount of information to the world.

**Keywords:** chronic pancreatitis; early chronic pancreatitis; hereditary pancreatitis; mechanistic definition; pancreatic stellate cell

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#### Introduction

CP is a progressive inflammatory disease of the pancreas with poor prognosis characterized histologically by destruction of parenchyma and replacement with strong fibrosis, which results in exocrine and endocrine insufficiency in the end-stage. According to the results of a nationwide survey, the total estimated number of CP patients under treatment in 2011 in Japan was 66,980, and the number has been increasing in the past 2 decades (Hirota et al. 2014). CP develops predominantly in middleaged to elderly male people with a male to female ratio of 4.3 and a peak of age in the 60s in both men and women. CP is characterized by the high rate of alcohol for the etiology, which accounts for the cause of 67.5% of total patients and 75.7% of total male CP patients (Hirota et a. 2014). Although the pathological mechanisms and pathophysiology have long been largely unknown, this disease has become understood from a new point of view recently thanks to several breakthroughs in the past two decades. In this special review, I would like to focus on "chronic pancreatitis (CP)," a traditional research topic in our department of gastroenterology and to introduce our research efforts and achievements in an attempt to develop further the succession of traditions inherited from our predecessors.

# **Footsteps of Predecessors**

Tohoku University was established in 1907, near the end of the Meiji era. The medical department was founded 8 years later, in 1915, as the Medical College of Tohoku Imperial University. The third department of Internal Medicine, the origin of our department, began a series of lectures in 1918. The first professor was Shotaro Yamakawa (1918-1941), who received instruction at the Medical Department led by Professor Tanemichi Aoyama at Tokyo Imperial University, and later went on to conduct research on dietetics and carbohydrate metabolism. The second professor was Toshio Kurokawa (1941-1960), who studied X-ray examinations of the stomach during his studies in Germany and subsequently introduced this technique to Japan. He established a gastric cancer screening procedure known as the "Miyagi System" and was later awarded the Order of Cultural Merit for his contribution to saving the lives of patients with gastric cancer, which was one of the most important medical issues in Japan at that time. The third professor was Shoichi Yamagata (1960-1976), who studied the technique of gastroendoscopy in Germany before introducing it to Japan for wide dissemination. During the era of Professor Yamagata, the Department of Gastroenterology produced an excellent body of research and witnessed a flourishing

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period in which it was known as the "Mecca of gastroenterology in Japan." The fifth professor, Yoshio Goto (1976-1988), specialized in diabetology and is known for the development of a spontaneous rat model of diabetes mellitus (DM), known as "Goto-Kakizaki rats" (Goto et al. 1976). The sixth professor, Takayoshi Toyota (1988-2000), studied the pathological mechanisms underlying type 1 DM and contributed to the progress in the diagnosis and treatment of DM. Following a governmental policy which increased the priority of the graduate school, the third department of internal medicine was reorganized into two divisions: The Division of Gastroenterology, for which I served as professor for 19 years from 1998 to 2017.

## **Traditions of Pancreatitis Research**

Although a diverse area of gastroenterology research was covered in the department led by Professor Yamagata, his own lifework was the elucidation of the cause and pathophysiology of pancreatitis. He joined the "Kurokawa Department" and started his research career by developing a method to measure serum amylase. He later made a short trip to the United States to observe their medical education system, and on the occasion, he learned a direct method for a pancreatic exocrine function test, which enabled pancreatic exocrine function to be evaluated by measuring the volume, amylase output, and bicarbonate concentration of duodenal juice collected under secretin stimulation. He introduced this method to his department and placed it at the center of pancreas research, as an especially important method for the diagnosis and assessment of the pathological condition of CP (Takebe et al. 1976). A stream of associated research in the "Yamagata Department" gave birth to creative research outcomes, such as the assessment of exocrine pancreatic function through the maximum stimulation of the pancreas (Takebe et al. 1978), the measurement of sweat chloride concentrations in CP patients (Hanawa et al. 1978) and a comparison between secretin-stimulated exocrine pancreatic function and pathological change of the pancreas in CP patients (Saito et al. 1984). These achievements provided a unique interpretation on the clinical and pathophysiological conditions of CP (Yamagata and Takebe 1967) which played an important role in the formation of the disease concept in Japan.

# What is Chronic Pancreatitis (CP)? Conventional Definition of CP

Comfort et al. (1946) established the idea that CP was a distinct disease entity. They described the features of this disease precisely by investigating the clinical and pathological aspects of 29 cases of CP in detail. Generally speaking, the Cambridge classification definition of 1984 (Sarner and Cotton 1984) is prevalently used in the West: "CP is defined as a continuing inflammatory disease of the pancreas, characterized by irreversible morphological change, and typically causing pain and/or permanent loss of function." Meanwhile, the following definition is favored in Japan: "CP is a condition characterized by chronic pathological changes in the pancreas such as irregular fibrosis, inflammatory cell infiltration, and the loss of parenchyma and granuloma tissue, which result in deteriorated exocrine and endocrine function of the pancreas with progression. It takes mostly an irreversible course." The definition of CP varies widely between countries and academic societies, even in the West, and the clinical course is deduced rather speculatively from the clinical and pathological findings of the end-stage disease with pancreatic calcification and exocrine and endocrine insufficiency. In other words, CP is a miscellany of diseases with a common end stage characterized by the progressive destruction of the pancreas, irrespective of etiology.

# A Breakthrough - Discovery of the Genes Responsible for Hereditary Pancreatitis

Since the introduction of CP by Comfort et al. (1946), no particular progress was made for a long time in regard to the pathophysiological mechanism of the disease, which was therefore expressed as "an enigmatic process of uncertain pathogenesis" (Steer et al. 1995). However, in 1996, a breakthrough was achieved by the discovery of the gene responsible for hereditary pancreatitis by Whitcomb et al. (1996a, b). Hereditary pancreatitis is an autosomal dominant inherited disease, as originally reported in Comfort and Steinberg (1952), who identified a familial aggregation of patients with pancreatitis among the 29 CP cases originally reported by Comfort et al. (1946).

# a. PRSS1 and protective genes against pancreatitis

Whitcomb et al. (1996b) mapped the hereditary pancreatitis gene to chromosome 7q35 by linkage analysis and simultaneously determined the cause as a point mutation of the cationic trypsinogen gene (PRSS1) (resulting in p.R122H) using positional cloning in the family members with hereditary pancreatitis (Whitcomb et al. 1996a). The gene mutation was thought to form abnormal trypsinogen and trypsin, resistant to autolysis, indicating a gain-of-function mechanism (Whitcomb et al. 1996a). On another front, Witt et al. (2000) discovered that mutations were frequently found in the Kazal type 1 (SPINK1) gene, also known as pancreatic secretory trypsin inhibitor (PSTI), an intrinsic trypsin inhibitor that can inactivate trypsin within acinar cells. As the patients with idiopathic CP, who retained gene mutations in the trypsindegradation enzyme chymotrypsin C (CTRC) (Szmola and Sahin-Tóth 2007) were subsequently identified (Rosendahl et al. 2008; Masamune et al. 2013), a molecular mechanism of lowering the threshold for pancreatitis by the imbalance between trypsin as an offensive factor and PSTI and/or chymotrypsin C as the defense mechanism was unveiled. By contrast, rare mutations of the PRSS1 gene suggested another mechanism of pancreatitis caused by stress in the endoplasmic reticulum due to the disturbed secretion and intracellular accumulation of structurally abnormal trypsinogen (Schnúr et al. 2014; Masamune et al. 2014). These findings suggest diversity in the molecular mechanism of pancreatitis (Table 1).

#### b. Abnormality of the CFTR gene

In 1998, Cohn et al. (1998) and Sharer et al. (1998) studied mutations in the cystic fibrosis trans-membrane conductance regulator (CFTR) gene, which is responsible for cystic fibrosis, an inherited autosomal recessive disease commonly seen in Western countries, and reported a high frequency of major *A*F508 heterozygous mutations and some others in CP patients (Table 1). A series of subsequent studies suggested minor mutations of the CFTR gene that partly disturbed its function and trans-heterozygous mutations of CFTR and SPINK1 as risk factors that lowered the threshold for pancreatitis (Schneider et al. 2011; Jalaly et al. 2017). We also conducted a comprehensive analysis of the CFTR gene using next-generation sequencing and reported several novel polymorphisms in Japanese CP patients (Nakano et al. 2015). A molecular mechanism of protein plug and pancreatic stone formation was subsequently proposed. According to the hypothesis, gene mutations cause functional impairments of CFTR, an anion channel for chloride and bicarbonate, which may result in the disturbed alkalization of pancreatic juice and injury to duct epithelium, followed by the aggregation and precipitation of pancreatic juice proteins (Franks 2011).

#### c. Other gene abnormalities

Statistically higher rates of mutations in various genes have been reported in patients with idiopathic and/or alcoholic CP (Table 1). These mutations include the genes encoding carboxypeptidase A1 (CPAI), an enzyme abundant in the pancreatic juice (Witt et al. 2013), the calcium sensing receptor (*CASR*) protein (Felderbauer et al. 2003), carboxyl ester lipase (*CEL*) (Fjeld et al. 2015; Zou et al. 2016) and the tight junction protein claudin-2 (*CLDN2*) (Whitcomb et al. 2012; Masamune et al. 2015). However, it is not always clear why these genetic abnormalities cause pancreatitis.

#### d. Molecular mechanisms of CP

It was fortunate for us to retain a large familial aggregation of CP patients in our department. In the wake of the discovery of the gene responsible for hereditary pancreatitis, we performed genetic analyses of these pedigrees and identified the PRSS1 p.R122H mutation (Otsuki et al. 2004). In addition, we found multiple families with hereditary pancreatitis with the PRSS1 gene mutation in Japan (Otsuki et al. 2004). We conducted a parallel investigation of genetic abnormalities in the SPINK1 gene among Japanese CP patients, finding the occurrence of not only the p.N34S mutation that is prevalent in Western countries but also the intronic mutation of c.IVS3 + 2T>C (Kaneko et al. 2001; Kume et al. 2005; Shimosegawa et al. 2006). This finding attracted special attention because the latter occurred at the splice donor site for the formation of mature mRNA in PSTI. We later verified that the intronic mutation caused alternative splicing with exon 3 skipping, resulting in the generation of a truncated form of PSTI without trypsin inhibitor capacity (Kume et al. 2006). That report provided the first molecular evidence that a loss of function of PSTI could be a cause of pancreatitis (Masamune et al. 2007). Our finding of the high frequency of the c.IVS3 + 2T>C mutation in the SPINK1 gene in Japanese CP patients contrasted findings in the Western population, in which the intronic mutation was minor, suggesting ethnic differences in SPINK1 gene

molecule	gene	mutations	mechanism	category
TRY	PRSS1	p,R122H, p,N29I p,A16V, p,D22G, p,K23R, etc. p,D100H, p,G208A	trypsin-dependent trypsin-dependent ER stress	hereditary (au, dominant) familial/idiopathic idiopathic
	PRSS2	p.G191R	trypsin-dependent	idiopathic/alcoholic
PSTI	SPINK1	p.N34S c.IVS3+2T>C trypsin-depend		hereditary/familial/idiopathic idiopathic
CTRC	CTRC	p.R254W, p.K247_R254del	trypsin-dependent	idiopathic
CFTR	CFTR	ΔF508, p.R117H, poly T, p.Q493X, p.R560T, p.R553X, p.N1303K, p.I336K, p.R75Q, p.Q1352H, p.L1156F, etc.	channel function	idiopathic
others	CPA1 CLDN2-MORC4 CEL CASR	p.V251M Rs7057398, rs12688220 CEL-HYB p.L173P	ER stress unknown ER stress calcium sensing	idiopathic alcoholic familia1/idiopathic/alcoholic familia1

Table 1. The Gene Mutations Associated with CP.

TRY, trypsinogen; PSTI, pancreatic secretory trypsin inhibitor; CTRC, chymotrypsin C; CFTR, cystic fibrosis trans-membrane conductance regulator.

mutation patterns. This idea was supported by subsequent reports from Korea (Oh et al. 2009) and Taiwan (Chang et al. 2009) that showed the dominancy of the c.IVS3 + 2T>C mutation in Korean and Chinese idiopathic and familial CP patients.

Even when focusing on the *PRSS1* gene, most of the mutations do not show a clear inheritance pattern, occurring even in solitary and sporadic cases, except for p.R122H and p.N29I mutations, which can form large families of pancreatitis patients because of autosomal dominant inheritance. Moreover, as *SPINK1* and other various minor mutations can be detected only in some of the patients with idiopathic and alcoholic CP, and as comprehensive gene analysis does not always confirm these mutations in CP patients, it is considered that the genetic abnormality is not a sole determinant, and that the interaction of genetic and environmental factors such as alcohol abuse (Aghdassi et al. 2015; Kume et al. 2015) and smoking (Lin et al. 2000) is important for disease onset (Keim 2008; Shelton and Whitcomb 2014; Kleeff et al. 2017) (Fig. 1).



Fig. 1. Genetic and Environmental Interaction for CP development.

The relative risk of the combination of genetic abnormalities (*PRSS1*, *SPINK1*, *CFTR* and *CLDN2-MORC4*) and environment factors (heavy drinking and smoking) for development of CP is shown according to the order of risk grade.

CFTR, cystic fibrosis trans-membrane conductance regulator.

#### **CP and Pancreatic Cancer**

#### a. Epidemiological evidence

The notion that CP is a risk factor for pancreatic cancer was made clear by epidemiological surveys (Lowenfels et al. 1993, 1997, 2001; Talamini et al. 1999; Malka et al. 2002; Wang et al. 2003; Howes et al. 2004) (Table 2). In a multicenter historical cohort study of many CP patients recruited from six countries, Lowenfels et al. (1993) showed that the risk of development of pancreatic cancer was 16.5 times higher in CP patients compared with controls. Another cohort study of hereditary pancreatitis patients registered in 10 countries revealed that the pancreatic cancer risk was 53 times higher in CP patients than in controls (Table 2) (Lowenfels et al. 1997), and that pancreatic cancer developed about 20 years earlier in patients with compared with those without a history of current or former cigarette smoking (Lowenfels et al. 2001).

Even in Japan, a multicenter study conducted by the Research Committee of Intractable Pancreatic Diseases (RCIPD) supported by the Ministry of Health, Labour and Welfare, Japan (chairman: Tooru Shimosegawa) estimated the risk of developing pancreatic cancer to be 11.8 times higher in CP patients compared with controls, and the cumulative risk of pancreatic cancer to be 2.6%, 5.6%, 8.8% and 12.2% at 10, 15, 20, and 25 years after the diagnosis of CP, respectively (Ueda et al. 2013). In addition, very interestingly, the results also showed that the cumulative risk of pancreatic cancer was significantly higher in patients with compared with those without calcification in the pancreas; the risk was also significantly higher in patients who were current alcohol drinkers compared with those who abstained, and the risk decreased significantly in patients who underwent compared with those who did not undergo surgical treatment for CP (Ueda et al. 2013).

We previously reported on the siblings of patients with chronic calcifying pancreatitis with the SPINK1 mutation p.N34S, of whom, the younger sister developed pancreatic cancer (Masamune et al. 2004). We were inspired by these cases and therefore decided to investigate the association between the occurrence of the SPINK1 gene mutation in CP patients and the rate of cancer development. The results revealed that the rate of cancer development was 18.8% in CP patients with p.N34S, which was significantly higher than the rate of 2.3% in patients without the mutation (Shimosegawa et al. 2009). Moreover, the SPINK1 mutation p.N34S was detected in 37.5% of the pancreatic cancer patients with a background of CP, whereas it was found at a significantly lower rate of only 1.9% in patients with ordinary pancreatic cancer without CP (Shimosegawa et al. 2009). As no clear association was found between the type of gene mutation and the incidence of pancreatic cancer, it was considered that inflammation of the pancreas itself may be an important factor promoting pancreatic carcinogenesis.

	study	study type	pop.size	2-year lag period ES (95% Cl)	5-year lag period ES (95% Cl)			
CP	Lowenfels et al. 1993	cohort	1,552	16.50 (11.10, 23.70)	14.40 (8.50, 22.80)			
	Talamini et al. 1999	cohort	715	18.50 (10.00, 30.90)	13.30 (6.40, 24.50)			
	Malka et al. 2002	cohort	373	26.70 (7.30, 68.30)				
	Wang et al. 2003	cohort	420	27.20 (7.40, 69.60)				
	Ueda et al. 2013	cohort	506	11.80 (7.10, 18.40)				
	study	study type	pop.size	SIR (95% CI)				
HP	Lowenfels et al. 1997	cohort	246	53 (23, 105)				
	Howes et al. 2004	cohort	418	67 (50, 82)				

Table 2. CP/hereditary pancreatitis (HP) and the Risk of Pancreatic Cancer.

#### b. Animal model

The somatic K-ras gene mutation can be detected in the cancer tissues of more than 90% of pancreatic cancer patients (Yachida and Iacobuzio-Donahue 2013). The activation of K-ras by the gene mutation occurs even in the precancerous condition called pancreatic intraepithelial neoplasias (PanINs), suggesting that the K-ras mutation is an important molecular mechanism from the most initial stage of pancreatic cancer (Hruban et al. 2000). Mice genetically manipulated to express the K-ras gene mutation in the pancreas frequently develop multiple PanIN lesions, but rarely develop invasive cancer (Hingorani et al. 2003). However, the induction of pancreatitis in these mice by cerulein administration promoted the development of invasive cancer, suggesting a model of "inflammation and carcinogenesis" (Guerra et al. 2007). The introduction of the K-ras mutation in the pancreas leads to the expression of p.16, a master molecule that triggers senescence, whereas the induction of pancreatitis inhibits this process. Therefore, the animal model explains the inflammationinduced inhibition of senescence as an important molecular mechanism for carcinogenesis of the pancreas (Guerra et al. 2011).

#### History of the Diagnostic Criteria for CP

# a. Diagnostic criteria in the Western world

The first definition of pancreatitis was the Marseille classification in 1963, which classified it into four categories: acute, acute recurrent, chronic recurrent and CP (Sarles 1965). In Europe and the U.S., a series of diagnostic criteria for CP were subsequently proposed. These include the Cambridge classification (Sarner and Cotton 1984) and the revised Marseille classification in 1984 (Singer et al. 1985), the Marseille-Rome classification in 1988 (Sarles et al. 1989), the etiology-based TIGAR-O system in 2001 (Etemad and Whitomb 2001), and the M-ANNHEIM classification in 2007 (Schneider et al. 2007), which enabled assessments of the cause, clinical stage and severity of this disease. In 2014, the American

Pancreatic Association (APA) proposed new diagnostic criteria, in which CP could be diagnosed comprehensively based on the findings of various imaging modalities, pancreatic exocrine function and clinical symptoms (Conwell et al. 2014).

#### b. Diagnostic criteria in Japan

The first and most primitive diagnostic criteria for CP was compiled by The Japanese Society of Pancreatic Disease (1971). In 1983, the Japanese Society of Gastroenterology (JSGE) proposed clinical diagnostic criteria that consisted of five items: 1) pathological findings of the pancreas, 2) calcification of the pancreas, 3) pancreatic exocrine function, 4) imaging findings of the pancreas including the duct structure, and 5) upper abdominal pain and/or tenderness with persistent elevation of serum pancreatic enzymes (The Criteria Committee for Chronic Pancreatitis of the Japanese Society of Gastroenterology 1983). Shoichi Yamagata, the second professor of our department, played a key role in compiling these criteria. A point worthy of special mention is the classification of CP into two categories, group I and II, according to the reliability of diagnosis. Group I refers to patients with a more reliable diagnosis of CP, whereas group II refers to patients possibly at the early stage of the disease. However, the concept of group II was later considered obsolete because it was not accepted in foreign countries, and because no disease progression was observed in the patients within this category. In 1995, the Japan Pancreas Society (JPS) compiled clinical diagnostic criteria that enabled the comprehensive diagnosis of CP utilizing various imaging modalities and exocrine pancreatic function tests (The Criteria Committee for Chronic Pancreatitis of the Japan Pancreas Society 1995; Homma et al. 1997). Thereafter, the JPS made a minor revision by including magnetic resonance cholangiopancreatography (MRCP) findings in the 1995 diagnostic criteria and proposed new criteria in 2001 (The Criteria Committee for Chronic Pancreatitis of the Japan Pancreas Society 2001).

# Proposal of Diagnostic Criteria for Early CP

#### a. The need to diagnose early-stage CP

The results of a prognostic survey of CP patients conducted by the RCIPD (chairman: Makoto Otsuki) revealed that the average life-span of male CP patients was 67.2 years, 10.5 years younger than that of the general Japanese male population, while that of female CP patients was 68.7 years, 16 years younger than that of the general Japanese female population (Otsuki and Fujino 2008). The most frequent cause of death among CP patients was a malignant tumor (43.1%), and the incidence of pancreatic cancer was especially high, with a standardized mortality rate of 7.33 (Otsuki and Fujino 2008). It is usually quite difficult to diagnose pancreatic cancer complicated by CP because strong fibrosis and calcification in the pancreas makes the interpretation of imaging findings complicated; in most cases, this results in a delayed diagnosis, even at advanced stages. To improve the long-term prognosis of CP patients, it is indispensable to diagnose CP in the early stage and prevent its progression through therapeutic interventions.

# b. Disease concept of early CP

In Japan, the clinical course of CP is typically classified into three stages: compensated, transitional and decompensated (Ito et al. 2016). In the "compensated stage," the pancreatic parenchyma retains a sufficient volume with no obvious impairment in exocrine or endocrine function, and the major symptom is recurrent abdominal pain due to acute-on-chronic pancreatitis. In the "decompensated stage," the pancreatic parenchyma starts becoming diminished with extension and the progression of inflammation and fibrosis, leading to the appearance of exocrine and endocrine insufficiency, with episodes of abdominal pain gradually subsiding. Since the boundary between the two stages is not clear, the transition period from the compensated to the decompensated stage is referred to as the "transitional stage." Early CP corresponds to the time when CP has already started with clinical symptoms and signs of pancreatic injury; however, characteristic morphological changes of the pancreas have still not been detected clearly on imaging modalities (Fig. 2). Theoretically early CP is considered to be a reversible pathological condition (Ito et al. 2016).

# c. Clinical diagnostic criteria for early CP

In 2009, the RCIPD (chairman: Tooru Shimosegawa), JPS and JSGE collaborated in revising the "Diagnostic Criteria of CP 2001," and published the new "Clinical Diagnostic Criteria 2009" (The Research Committee of the Intractable Pancreatic Diseases supported by the Ministry of Health, Labour and Welfare of Japan (RCIPD) 2009; Shimosegawa et al. 2010). The criteria classified "early CP" in the category of CP together with the "definite" and "probable" diseases, and proposed its diagnostic criteria for the first time anywhere the world. The clinical and pathological findings of the early stage of hereditary pancreatitis were referred to in order to draft the diagnostic criteria for early CP.

Under these criteria, early CP is diagnosed in patients suspected of pancreatic injury based on their clinical symptoms, laboratory test results, and lifestyle, and when they show minor morphological changes on imaging that are suggestive of chronic inflammation of the pancreas (Fig. 3). Regarding the findings suggestive of pancreatic injury, the following four items were cited: 1) repeated abdominal pain attacks, 2) abnormal levels of serum or urine pancre-



Fig. 2. Clinical Course of CP.

The clinical course of CP patients is schematically shown with the major clinical symptom (abdominal pain; blue line) and signs (exocrine and endocrine dysfunction; red line) according to the 3 stages (compensated, transitional and decompensated). The severity of imaging findings is also shown below from light changes in the left (parenchymal changes and branch duct dilatation) to severe changes in the right (calcification, MPD dilatation and atrophy). The rough time range corresponding to early CP is shown by brown shadow.



Fig. 3. Clinical Diagnostic Criteria of Early CP.

A flow to diagnose early CP is schematically shown. The diagnosis of early CP can be made in the patient who had 2 or more clinical findings (CF) from 1 to 4, and when the patient satisfied the imaging criteria (IF) for EUS or ERCP. The diagnosis of suspicious early CP is given to the patient who had only 1 CF, and when the patient satisfied the imaging criteria for EUS or ERCP, and when other diseases are ruled out. The diagnosis of suspicious CP is made in the patient who had two or more CFs but lack IF, and when other diseases are ruled out.

hc, honeycombing; MPD, main pancreatic duct; EUS, endoscopic ultrasonography; ERCP, endoscopic retrograde cholangiopancreatography.

atic enzyme, 3) impaired pancreatic exocrine function, and 4) history of continuing heavy drinking of more than 80 g/ day (equivalent to pure ethanol). A diagnosis of early CP can be made in patients with two or more of these four items and when they show minor changes in the pancreatic parenchyma or branch ducts detected by endoscopic ultrasonography (EUS) or endoscopic retrograde cholangiopancreatography (ERCP). Regarding EUS findings, the following seven features were adopted: 1) lobularity with honeycombing, 2) lobularity without honeycombing, 3) hyperechoic foci without shadowing, 4) stranding, 5) cysts, 6) dilated side branches, and 7) hyperechoic main pancreatic duct (MPD) margin. The EUS criteria for early CP involve two or more of the seven features, including at least one from 1) to 4); these four features are the findings reported to be more specific to pancreatic fibrosis based on a comparison between EUS findings and histological evaluations of the pancreas (Catalano et al. 1998, 2009; Kahl et al. 2002; Varadarajulu et al. 2007). The ERCP criteria for early CP adopted the irregular dilatation of three or more branch ducts, which corresponds to the ERCP criteria of "mild change" in the Cambridge classification (Axon 1989). Based on the concern of post-ERCP pancreatitis, EUS is recommended first for diagnosis, and the application of ERCP should be considered carefully (Fig. 3).

#### *d. Cases of early CP*

We previously reported two patients initially diagnosed with early CP based on the "Clinical Diagnostic Criteria 2009" who later progressed to definite CP (Hirota et al. 2012). The first case was a 71-year-old male patient who was a social drinker with a history of cigarette smoking for over 20 years. He experienced light acute pancreatitis attacks, first in his 40s, and then twice more in his early 60s, and was diagnosed with early CP at the age of 64 after a detailed examination. Multiple stones appeared in the pancreas head 1 year after the fourth attack at the age of 68, and a diagnosis of definite CP was made. The second case was a 59-year-old male patient who was a heavy drinker, having a history of persistent drinking of 60 g/day of ethanol from the age of 20 years, which increased to 120 g/ day after reaching the age of 40 years. He had also persistently smoked a pack of cigarettes/day from the age of 20 years. He was hospitalized for the treatment of diabetic ketoacidosis at the age of 56 years, and subsequently diagnosed with early CP by examination at the hospital. Soon after discharge, he resumed drinking, and the appearance of a few pancreatic stones was noticed on computed tomography (CT) 1 year later. He experienced his first attack of pancreatitis when he was 58 years old, which triggered the rapid progression of imaging findings such as diffuse calcification and irregular MPD dilatation

the following year. As seen in these two cases, the progression of early CP shows various courses depending on the patient.

#### e. Nationwide survey of early CP patients

In 2014, the RCIPD (chairman: Yoshifumi Takeyama) conducted a nationwide survey of patients with early CP, targeting those diagnosed in 2011 using the "Clinical Diagnostic Criteria 2009." The results of the first-round survey revealed an estimated total number of the patients under treatment of 5,410 (95% confidence interval [CI]: 3,675-6,945) and an incidence rate of 1,330 [95% CI: 1,058-1,602] (Masamune et al. 2017). Since the total number of CP patients (definite and probable) in 2011 in Japan was estimated to be 66,980 [95% CI: 59,743-74,222] (Hirota et al. 2014), the number of patients with early CP was equivalent to about 8.1% of the total.

#### f. Prospective follow-up of early CP patients

Following the announcement of the diagnostic criteria for early CP, the RCIPD (chairman: Tooru Shimosegawa) performed a multi-institutional joint 2-year follow-up of early CP patients (Ito et al. 2015). According to the data obtained from 52 patients who completed the 2-year follow-up, the average number of clinical items in the criteria decreased significantly from  $2.50 \pm 0.58$  at registration to  $1.44 \pm 1.062$  years later, whereas the average number of EUS features increased significantly from 2.67  $\pm$ 1.02 at registration to  $2.96 \pm 1.27$  at the end of the 2-year follow-up. Five (9.6%) of the 52 patients with early CP, showed progression to established (definite or probable) CP, whereas 15 (28.8%) did not show any change in the diagnosis and 32 (61.5%) showed disappearance of symptoms or downgrading of the diagnosis to suspicious early CP. The five patients who showed progression were all alcoholics, and four (80%) of whom had continued drinking (Ito et al. 2015).

Sheel et al. (2018) recently reported the results of a reassessment of 807 cases who had been diagnosed with CP based on clinical symptoms such as abdominal pain. Among the patients who were not confirmed as having definite CP by pathological and/or imaging findings, 40 showed minor changes equivalent to CP on the EUS examination. Sheel et al. (2018) reported that twelve (30%) of these 40 cases showed progression to definite CP in a 30-month observation period (range: 18.75-36.5 months), and 67% of whom had continued drinking, 83% of whom had a history of smoking, 75% of whom were current smokers, and 58% of whom had a history of acute pancreatitis. These findings were particularly interesting in regard to understanding the risk factors for the progression of CP.

# **Mechanistic Definition of CP**

*a. New definition of CP and its background* Because the pathological mechanism and clinical condition of CP have become clearer as a result of the identification of the causal gene of hereditary pancreatitis and so on, a new definition of this disease based on the latest understanding was required. Whitcomb et al. (2016) selected representative members known for CP research from several countries, including Japan, to examine, closely and comparatively, the past definitions of CP proposed from different countries and academic societies. After enthusiastic discussions, they announced a new definition of CP, referred to as the "mechanistic definition" of chronic pancreatitis, which was created based on the members' consensus (Whitcomb et al. 2016). The "mechanistic definition" can be understood as a definition that considers the pathological mechanism of CP. The new definition is composed of two parts. The first part describes CP as "a pathologic fibro-inflammatory syndrome of the pancreas in individuals with genetic, environmental and/or other risk factors who develop persistent pathologic responses to parenchymal injury or stress." The second part states that the "common features of established and advanced CP include pancreatic atrophy, fibrosis, pain syndromes, duct distortion and strictures, calcifications, pancreatic exocrine dysfunction, pancreatic endocrine dysfunction and dysplasia." The first part represents a paradigm shift in the definition of CP that enables early diagnosis and an etiological classification of this disease, which has never been possible with conventional definitions.

#### b. Conceptual model

The mechanistic definition of CP proposed a conceptual model composed of five clinical stages (Whitcomb et al. 2016) (Fig. 4). According to the conceptual model, CP develops in at-risk patients (At risk) if their pancreas is exposed to injury or stress. The onset of the disease emerges as acute or recurrent acute pancreatitis (AP-RAP), which becomes chronic and progresses to early CP (Early CP). In the conceptual model, early CP can be resolved, and is therefore a reversible pathological condition. If injury or stress occurs repetitively or persistently, dysfunction is observed in various pancreatic components, including the immune system, acinar cells, endocrine function, pathological pain, and dysplastic change of the duct epithelium, resulting in the establishment of CP (Established CP). Further advances of the pathological condition finally reach the end stage, where severe fibrosis, exocrine and endocrine insufficiency, persistent pain and carcinogenesis become evident. This idea closely resembles the interpretation of early CP in Japan. The new definition and clinical criteria for early CP were voted on through the use of 10 clinical questions (CQs) in a bid to reach a consensus by representatives from various societies including the international association of pancreatology (IAP), the APA, the JPS, the PancreasFest and the European Pancreatic Club (EPC). Although consensus was reached for five CQs, complete agreement was not achieved, leaving the issue as a future subject (Whitcomb et al. 2018)



Fig. 4. Conceptual Model of CP.

SAPE, sentinel acute pancreatitis event; RAP, recurrent acute pancreatitis.

## Pancreatic Stellate Cells (PSCs)

# a. Identification and roles of pancreatic stellate cells (PSCs)

CP is characterized pathologically by a loss of pancreatic parenchyma and replacement by fibrosis as the consequence of chronic inflammation in the pancreas. As a result of the identification and separation of the special cells that play important roles in the regulation of fibrosis, the molecular mechanism of fibrosis in CP is becoming increasingly clear. In 1998, Apte et al. (1998) and Bachem et al. (1998) succeeded in the separation and culture of starshaped cells resembling hepatic Ito cells (Ito 1951) from rat pancreas and resected human pancreatic tissues, respectively. The cells referred to as "PSCs" possess several processes and intracellular vitamin A-storing lipid droplets on fluorescence imaging. Separated PSCs are spontaneously activated in serum-added condition medium and transformed into myofibroblast-like cells as they lose lipid droplets and processes and express  $\alpha$ -smooth muscle actin ( $\alpha$  -SMA) in the affluent cytoplasm (Masamune et al. 2009). PSCs are detected as desmin- and glial fibrillary acidic protein (GFAP)-positive cells around acini, blood vessels and ducts within the pancreas. Activated PSCs promote proliferation and migration through stimulation with platelet-derived growth factors (PDGF) (Luttenberger et al. 2000), increase the production of extracellular matrix (ECM) proteins including various types of collagen through the stimulation with transforming growth factor  $\beta$  (TGF- $\beta$ ) (Schneider et al. 2001; Shek et al. 2002), and enhance the expression of chemokines and cell adhesion molecules such as monocyte chemoattractant protein-1 (MCP-1) and intercellular adhesion molecule-1 (ICAM-1) through the stimulation with several cytokines such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-1 $\beta$  (IL-1 $\beta$ ) (Masamune et al. 2002b; Mews et al. 2002). As PSCs enhance the production of not only ECM proteins, but also various matrix metalloproteases (MMPs), ECM degradation enzymes, and tissue inhibitors of MMPs (TIMPs) (Phillips et al. 2003), PSCs are regarded as a key player in regulating the process of pancreatic fibrosis in a dynamic way.

#### b. Regulation of PSCs

Since the regulation of PSCs could realize the treatment of fibrosis in CP, we have studied the mechanism actively. In in vitro experiments, we clarified that various chemicals can inhibit the activity of PSCs and reverse activated PSCs to a state of quiescence. These chemicals include peroxisome proliferator-activated receptor (PPAR)-y ligands (Masamune et al. 2002a), inhibitors of mitogenactivated protein (MAP) kinases (Masamune et al. 2003b) and Rho-Rho kinases (Masamune et al. 2003a), epigallocatechin (Masamune et al. 2005a), antioxidant polyphenols such as curcumin (Masamune et al. 2006) and ellagic acid (Masamune et al. 2005b; Suzuki et al. 2009), nicotinamide adenine dinucleotide phosphate (NADPH) oxidase inhibitor (Masamune et al. 2008), and serine protease inhibitors such as gabexate mesillate (Nakamura et al. 2001) and camostat mesilate (Gibo et al. 2005). Some of these have already been confirmed in the inhibition of

In the conceptual model, CP is considered to occur in the patients with genetic, environmental and/or other risks, and progress from preclinical "At Risk" stage to the "End Stage CP" by pathological responses to parenchymal injury and/or stress in the pancreas. "Early-CP" is positioned between "AP-RAP" and "Established CP" and is considered to be a pathological condition, which can be resolved. In the figure, clinical symptoms like SAPE, RAP and pathological pain, and clinical signs like exocrine insufficiency and DM are shown upward, and pathological and imaging findings in the respective stages are shown downward.

fibrosis, even in *in vivo* animal models of pancreatic fibrosis (Gibo et al. 2005; Masamune et al. 2008; Suzuki et al. 2009). As anti-fibrotic or inhibitory effects on the activation of PSCs have also been reported in antihypertensive agents such as angiotensin-converting enzyme (ACE) inhibitors (Kuno et al. 2003) and lipid-lowering drugs such as 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor (Lee et al. 2012) in experimental animals, they could be candidates for use in clinical applications for the treatment of CP together with PPAR-*y* ligands and serine protease inhibitors.

#### c. New treatment strategy for CP

From the point of view of the molecular mechanisms of hereditary pancreatitis and the importance of the imbalance between trypsin as an offensive factor and its defense mechanism for the development of pancreatitis, the oral administration of camostat mesilate, a synthetic trypsin inhibitor with a low molecular weight, could be reasonable to strengthen the intracellular defense mechanism against pancreatitis (Otani et al. 1997). The use of this medicine from the early stage of CP could be especially effective for the prevention of disease progression through antiinflammatory and anti-fibrosis mechanisms (Ito et al. 2016). Together with increasing knowledge about the role of PSCs and the molecular mechanism of their activation, the expanded application of medicines in other fields needs to be considered for the treatment of pancreatic fibrosis. Therefore, diagnosis in the early stage and interventional treatment with the above-mentioned medicines could be a new treatment strategy for CP.

#### **Challenges for Future**

We hope the day is soon coming when the prognosis of CP patients is improved remarkably by solving the following important issues.

According to a secondary analysis of the nationwide survey of early CP in 2011 (Masamune et al. 2017), the patients diagnosed as having early CP by the "Clinical Diagnostic Criteria 2009" showed somewhat different clinical features compared with those diagnosed as having definite CP, such as a higher female to male ratio, a higher age at onset, a lower rate of alcoholic etiology, and a higher frequency of abdominal pain, suggesting a mixture of patients other than just true CP. The most important and urgent tasks are therefore the exploitation of reliable biomarkers and the development of more sensitive and specific imaging modalities for the detection of early CP.

Camostat mesilate is a medicine that is theoretically expected to prevent the onset and progression of pancreatitis by reinforcing intra-acinar defense mechanisms (Otani et al. 1997). This drug has been used for a long time in clinical practice in Japan for the treatment of CP, but there is no solid evidence for its efficacy (Kanoh et al. 1989). It is a promising medicine especially when applied to the early stage of CP (Ito et al. 2016), and should therefore be verified clinically in patients with early CP. In addition, it would also be interesting to see if camostat mesilate could improve the long-term prognosis of patients with hereditary pancreatitis by using it from a younger age.

The separation and culture of PSCs have enabled a clearer understanding of the molecular mechanisms underlying pancreatic fibrosis. Although there have been several reports of promising drugs for the inhibition of activated PSCs, these mostly involve *in vitro* studies or animal experiments; no clinical evidence for their efficacy in CP patients has been presented. Therefore, the long-term clinical effects of candidate drugs need to be confirmed, especially in early CP patients.

Pancreatic cancer is the most challenging disease with the poorest prognosis of patients because of the very high malignant potential and difficulty in the early detection. Since CP, especially the end-stage CP is a confirmed risk factor for the development of pancreatic cancer, diagnosis of early CP and early interventional treatment could be a promising approach to save lives from this malicious disease.

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