

## Pathogenesis of Chronic Pancreatitis

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### Introduction

Chronic pancreatitis is a progressive inflammatory disorder characterized by loss of functional pancreatic tissue, fibrous tissue conversion and ultimately loss of endocrine and exocrine function. However, although morphologic and clinical features have been well described, the pathogenesis of chronic pancreatitis is incompletely understood. There is no single etiology that inevitably leads to chronic pancreatitis and it is rather considered as a complex disease with several contributing factors (40, 74). Currently development of chronic pancreatitis is considered to be the result of a pathology involving pancreatic acinar, ductal and stellate cells. Our current understanding of the pathogenesis arises from experimental animal models as well as epidemiological and genetic studies in humans. Therefore this section will address both pathophysiological mechanisms, including results from established animal models, and known genetic and etiological factors that are associated with chronic pancreatitis.

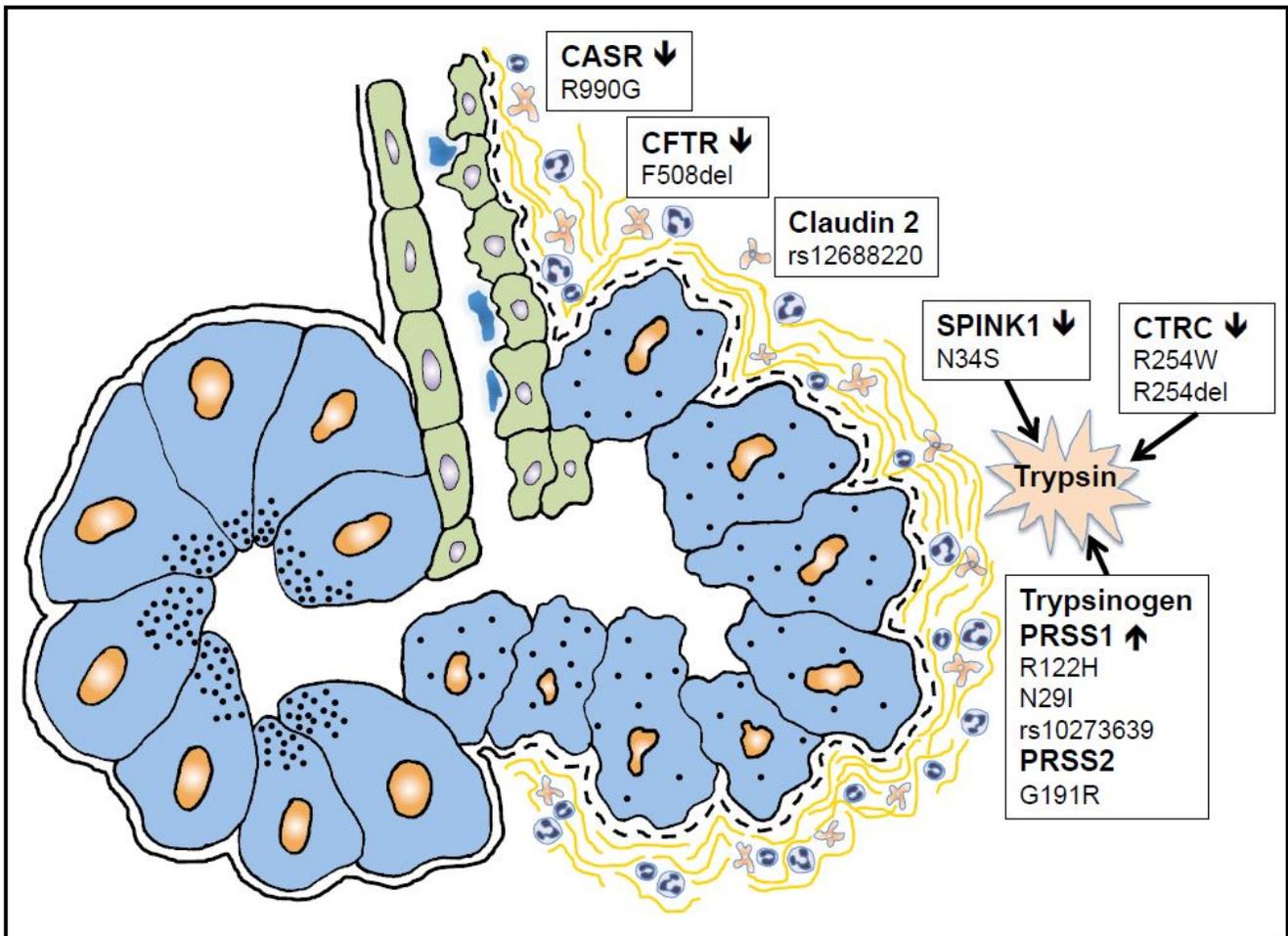
### 1. Pancreatic Cells and cellular components that promote fibrosis

From a histological point of view the pancreas consists of three critical cell lineages: acinar, ductal and endocrine. In addition terminal end

ductal cells that interface with acini are called centroacinar cells (49). Adjacent to the basolateral part of acinar cells and to a minor extent around small pancreatic ducts and blood vessels are pancreatic stellate cells, that compromise around 4-7% of all parenchymal cells (3, 5).

Each cell type that is found in the pancreas – either acinar or ductal or stellate cells - is suspected to contribute to chronic pancreatitis. In addition the extra-pancreatic environment including inflammatory cells contributes to the progress of both acute and chronic inflammatory pancreatic diseases.

There is much evidence that pancreatic proteases and their premature intracellular activation are responsible for the pathogenesis of *acute* pancreatitis. Therefore these enzymes also might be of interest for *chronic* pancreatitis, not least because findings of genetic studies in humans underline their critical role. Six major genes have been identified that target either acinar cells through a trypsin-dependent pathway (PRSS1, PRSS2, CTSC, CASR, SPINK) or duct cells (CFTR). Below, these cellular constituents will be introduced more comprehensively. Both (extra-) pancreatic cell types and frequently cited genes and their aberrations are summed up in **Figure 1**.



**Figure 1: Elements for the pathogenesis of chronic pancreatitis.** Components for the pathogenesis involve acinar, ductal and pro-fibrotic cells. In addition an invasion of immune cells occurs. Some very frequently cited proteins and their genomic aberrations are listed here.

## 2. Acinar cells

### Cationic and anionic trypsinogen (PRSS1/PRSS2)

The smallest functional units of the exocrine pancreas are acinar cells whose primary function is the synthesis, storage and secretion of digestive enzymes (zymogens). One of them is trypsin, a serine proteinase that is stored as its inactive precursor, trypsinogen, in zymogen granules. Under physiologic conditions these enzymes remain inactive during intra-cellular transport, secretion and passage through the pancreatic duct until they reach the duodenum where trypsinogen is activated by the brush border enzyme enterokinase. This activation leads to a cascade-like activation of other

pancreatic protease precursors (25). There are three isoforms of trypsinogen in the human pancreas and on the basis of their electric charge these are cationic trypsinogen (PRSS1), anionic trypsinogen (PRSS2) and mesotrypsinogen (PRSS3). Cationic and anionic trypsinogen represent the overwhelming part of the whole trypsinogen content whereas mesotrypsinogen only makes up of about 5% (57, 74).

From acute pancreatitis we already know that one of the key events is the intracellular and premature activation of pancreatic digestive enzymes that ultimately leads to organ injury and autolysis (12). Trypsinogen plays a pivotal role in the beginning of acute pancreatitis as it further activates other zymogens intracellularly such as chymotrypsin, elastase, carboxypeptidase A2 or

phospholipase (28, 35, 37). Since enterokinase is absent inside the pancreas other activators for trypsinogen must exist. According to the co-localization hypothesis this activation occurs by the lysosomal protease cathepsin B: lysosomal hydrolases and digestive zymogens co-localize during experimental pancreatitis with accumulation of cathepsin B to a zymogen enriched subcellular fraction (55, 72), furthermore in-vitro experiments showed that cathepsin B directly activates trypsinogen by proteolytic cleavage (21) and deficiency of cathepsin B markedly reduced trypsinogen activation in a mouse model (26).

There is much evidence coming from genetic studies in humans that trypsinogen is an important pathogenetic factor for chronic pancreatitis as well. In 1996 Whitcomb and co-workers identified a gain-of-function mutation of the cationic trypsinogen (p.R122H) gene in patients with hereditary pancreatitis rendering to an inappropriate trypsin activation due to a higher resistance to hydrolysis of this mutated form of trypsin (76). A second mutation of the cationic trypsinogen was found in the same exon (p.N29I) in patients with recurrent acute and chronic pancreatitis just one year later (24). Meanwhile other PRSS1 mutations have been identified in association with hereditary pancreatitis (20, 63, 69, 82). A complete loss-of-function mutation was found by Witt et al. in the anionic trypsinogen gene (PRSS2) that was significantly underrepresented in patients with chronic pancreatitis compared to healthy control subjects (G191R in exon 4) (85). These results indicate that first not only PRSS1 mutations are associated with idiopathic or hereditary chronic pancreatitis and secondly inactivating mutations of trypsinogen can modify the susceptibility to chronic pancreatitis as well.

Taken together these observations support the critical role of the protease/antiprotease system, in particular trypsin, in the pathogenesis of pancreatitis. However, it should be mentioned that less than 60% of patients with hereditary chronic

pancreatitis and less than 20% of patients with idiopathic chronic pancreatitis harbour mutations in the PRSS1 gene so that an inappropriate trypsinogen cannot be the only causative factor for chronic pancreatitis (14, 15, 47).

In an attempt to mimic the role of trypsinogen in vivo, deduced from the observations in human genetics, mouse strains carrying overexpressed forms of mutated trypsinogen (R122H and N29I) as well as wildtype human PRSS1 were created (4). All three strains not only developed more severe acute pancreatitis upon caerulein treatment but also spontaneously displayed characteristics of chronic pancreatitis including vacuolization, inflammatory infiltrates and fibrosis, as it was expected from human genetics data. Interestingly phenotype between the transgenic strains didn't differ significantly. One underlying reason might be that human trypsinogen has a higher propensity for auto-activation compared to trypsinogens from other species (30).

Recent experimental animal models questioned the detrimental effects of trypsinogen in chronic pancreatitis. Mice lacking trypsinogen 7 (T-/-) didn't show pathologic intracellular trypsinogen activation during caerulein-induced acute pancreatitis but surprisingly still developed chronic pancreatitis showing indistinguishable histomorphologic features like wildtypes. Previous works indicated that a genetic deletion of trypsinogen isoform 7 in mice led to a 60% reduction of pancreatic trypsinogen content (16). Similar to these observations cathepsin B deficient mice - that fail to activate trypsinogen by limited proteolysis - developed chronic pancreatitis (54). Both knockout and wildtype mice showed comparable intracellular transcriptional activation as demonstrated by activation of nuclear factor (NF)- $\kappa$ B and similar COX-2 overexpression. Both COX-2 and NF- $\kappa$ B are key mediators for chronic inflammation (53). More studies and most probably the application of alternative mouse models will be necessary to further question the trypsin-centered theory of chronic pancreatitis.

Interactions between genetic and environmental factors in patients with recurrent acute and chronic pancreatitis have recently been investigated in the North American Pancreatitis Study 2 (NAPS2) using genome-wide association studies (GWAS) (78). A single nucleotide polymorphism (SNP) was detected in the PRSS1-PRSS2 locus in the 5'-promoter region of PRSS1 and might affect expression of the trypsinogen gene. Another SNP was found at the CLDN2 locus. CLDN2 encodes Claudin-2, a tight junction protein physiologically expressed between duct cells and endocrine islets but also found in an atypical localization along the basolateral membrane of acinar cells in chronic pancreatitis (77). The findings of the PRSS1-PRSS2 and CLDN2 variants were replicated in a large European cohort with alcoholic and non-alcoholic chronic pancreatitis and a strong association was found in subjects with alcohol pancreatitis (17). When compared to patients with alcoholic liver cirrhosis there was no significant association suggesting that these variants are not susceptibility factors for alcoholism per se or for fibrosing disorders associated with alcohol abuse in general. More studies will be necessary for complete understanding the underlying cellular events.

### **Serine protease inhibitor Kazal type 1 (SPINK1)**

As trace amounts of trypsinogen normally get activated within the pancreas there are protective mechanisms that prevent the digestive enzyme activation cascade (75). One of them is the serine protease inhibitor Kazal type 1 SPINK1 (synonyme: pancreatic secretory trypsin inhibitor, PSTI) (OMIM 167790), which acts as an intracellular inhibitor for intrapancreatic active trypsin (81). SPINK1 is synthesized inside the acinar cells and stored in zymogen granules, the same compartment as trypsinogen. It is also secreted into the pancreatic juice where it protects from trypsinogen activation inside the pancreatic ducts and besides its physiological function in the pancreas it presumably has additional functions

because this protein is also detected in sera and various malignant tissues (29). The active site of SPINK1 binds covalently to the catalytic serine residue of trypsin and is considered to inhibit approximately 20% of total trypsin activity inside the acinar cell (46).

Because SPINK1 keeps a balance of active and inhibited trypsin inside the pancreas its inactivation is considered to be an important factor for the development of inflammatory pancreatic disorders, including chronic pancreatitis. In accordance with its biological role there are several studies describing the association of mutations of the SPINK1 gene and pancreatitis. The most commonly observed mutation leads to an exchange of asparagine to serine of codon 34 (p.N34S) and was observed in idiopathic and hereditary chronic pancreatitis as well as in alcoholic chronic and tropical pancreatitis (10, 83, 84). However, it is worth mentioning that the incidence of this mutation is around 0.5-2.5% in the common population indicating that it cannot be the only causative factor for chronic pancreatitis and probably it rather acts as a disease modifier (46, 58). Novel polymorphisms were identified in patients with chronic pancreatitis such as the D50E mutation and a variety of intronic polymorphisms but their frequency is extremely low and some were found in single patients (47).

Functional analysis of N34S recombinant SPINK1 did not show any reduced trypsin inhibitor capacity in-vitro so that the exact pathophysiologic action of this mutation has not been clarified so far. Maybe the impaired function of SPINK1 is based on an intronic mutation rather than the N34S itself as N34S is usually associated with intronic sequence variants (33, 81, 84). Further research on the exact function of SPINK1 exonic and intronic mutations is inevitable to gain deeper knowledge on its pathophysiologic role in chronic pancreatitis.

### **Chymotrypsin C (CTRC)**

Chymotrypsin C appeared to be identical to Rinderknecht's enzyme Y that he initially found in the pancreatic juice (50) and has seemingly ambivalent functions on trypsinogen. The prevailing  $\text{Ca}^{2+}$  concentration regulates the balance between activation and degradation of cationic trypsinogen. At high calcium concentrations it facilitates autoactivation by limited proteolysis of the trypsinogen activation peptide (TAP) (43, 65). On the other hand, in a milieu with low  $\text{Ca}^{2+}$  concentration it selectively cleaves a peptide bond in the calcium-binding loop of trypsinogen, which results in its degradation (67). Since higher calcium concentrations ( $>1$  mM) arise in the upper small intestine trypsinogen is activated more easily as it is also designated for digestion of proteins. In the lower small intestine along with falling  $\text{Ca}^{2+}$  levels trypsinogen degradation is predominating (67). Intracellular calcium signaling is important for acinar cell physiology and regulates secretion of enzymes, too. In pathologic condition a release of  $\text{Ca}^{2+}$  from intracellular stores into the cytosol, especially in the apical cell pole and for a prolonged time ( $>100$  sec), is considered to account for premature intracellular protease activation (32, 71).

Mutations of the cationic trypsinogen gene interfere with the cleaving effects of CTRC that could be shown in in-vitro studies (23, 41, 66). Absence of chymotrypsin C increased the autoactivation of R122H mutated trypsinogen only slightly whereas addition of CTRC drastically increased activation leading to high trypsin levels (23). In contrast to the human situation the R122H mutation did not have a relevant effect on autoactivation of T8 trypsinogen by CTRC in mice (42). Again introduction of known human mutations into mouse trypsinogen isoforms can have different effects than in humans. Therefore mutagenesis techniques might have limitations when investigating chronic pancreatitis in animal models.

Several genetic variations have been found in the chymotrypsin C gene and were associated to

chronic pancreatitis. Variants of the CTRC gene are detected in 3.3% of individuals with idiopathic or hereditary chronic pancreatitis. The most frequent variants were the c.760C>T (p.R254W) mutation and a deletion on exon 7 (p.K247\_R254del) (52). These CTRC variants were associated with a reduced enzymatic activity and were secreted to a lesser extent and thus are considered to be loss-of-function mutations. In a Chinese population additional CTRC variations were detected in chronic pancreatitis patients, however the overall frequency was 2.3% and thus much lower than in the German study (11). Taken together these data support the importance of the protease/antiprotease system for pathogenesis of chronic pancreatitis as shown above for cationic/anionic trypsinogen and SPINK1. In this context CTRC variants may represent an extra risk factor.

### **Calcium-sensing Receptor (CASR)**

Besides digestive proteases millimolar quantities of calcium ions are secreted from the zymogen granules that, when precipitated, cause intraductal pancreatic stones. The  $\text{Ca}^{2+}$  sensing receptor (CASR) is capable of monitoring changes of extracellular calcium concentrations. Besides its expression in the parathyroid gland, kidney and small intestine this molecule was found on the luminal side of ductal cells and more diffusely distributed inside acinar cells (8). Functional studies showed that CASR regulates hydrogen carbonate ( $\text{HCO}_3^-$ ) efflux into the ducts and thus ensuring a milieu with sufficient fluid secretion to prevent calcium stone precipitation.

Mutations in the CASR gene have been associated with chronic pancreatitis. Recent studies reported that CASR gene mutations in combination of SPINK1 N34S mutation increased the risk of chronic pancreatitis (19, 74). Furthermore the CASR exon 7 polymorphism R990G was associated with chronic pancreatitis and this association was stronger in individuals who reported moderate or heavy alcohol consumption (39). Presumably subjects with considerable alcohol abuse represent a risk group

in which the addition of another risk factor (CASR mutation) enhances the overall risk for development of chronic pancreatitis (39).

### 3. Ductal cells

#### **Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)**

The cystic fibrosis transmembrane conductance regulator (CFTR) gene encodes for an ABC (acronym for ATP-binding cassette) transporter protein that is expressed on epithelial cells and thus also on pancreatic ductal epithelium. It functions as a Cl<sup>-</sup> selective channel and permits chloride-anions and water to enter the ductal lumen which finally allows highly concentrated pancreatic secretory proteins (including trypsinogen) secreted by the acinar cells to remain in a soluble state (70, 79). Cystic fibrosis is quite common in people of Northern European descent, affects approximately one in 2500 births among whites and is characterized by a heterogenous clinical course. The exocrine pancreas is invariably affected in cystic fibrosis with signs of chronic pancreatitis and exocrine insufficiency (34). In 1989 the CFTR gene was found to be located at chromosome 7 (7q31) (51). More than 1000 different mutations have been reported in cystic fibrosis patients. The most common aberration is a deletion found at position 508 (p.F508del) which results in a deletion of phenylalanine (73). In 1998 an association of CFTR mutations and chronic pancreatitis was discovered in patients with idiopathic chronic pancreatitis and alcoholic chronic pancreatitis (13, 61). In a comprehensive genetic analysis of the CFTR gene including all 27 exons including the flanking intronic regions abnormal CFTR alleles were found to be twice as frequent in patients with idiopathic chronic pancreatitis than in healthy controls (18.6% vs. 9.2%,  $p < 0.05$ ) (73).

Recent functional studies underlined the role of

CFTR in pancreatitis as high levels of alcohol consumption impair the function of CFTR in pancreatic duct cells, thereby disturbing exocrine pancreatic secretion and sensitizing the organ to pathological stimuli (38).

#### **Pancreatic stellate cells (PSCs)**

Although not activated in an un-injured pancreas, stellate cells fulfill important functions for tissue architecture as they control synthesis and degradation of extracellular matrix (2). In particular tissue homeostasis results from secretion of matrix metalloproteinases (MMP) and their inhibitors (tissue inhibitors of matrix metalloproteinases, TIMPs) (48), secondly by phagocytosis of necrotic acinar cells (62). During pancreatic injury stellate cells are activated and secrete a high amount of extracellular matrix proteins (2). These ECM proteins consist of collagens, fibronectin and laminin; furthermore there is an increase of matrix metalloproteinase 2 (MMP2) production. In addition activated PSCs show an increased capacity of cell proliferation and migration. Several activators of PSCs have been identified, including a variety of chemokines, alcohol and its metabolites, fatty acid ethyl esters oxidative stress or endotoxins (3).

### 4. Theories of pathogenesis of chronic pancreatitis

In the past decades multiple theories have emerged that tried to explain the pathogenesis of chronic pancreatitis. Some well-known concepts will be outlined briefly in the following. In a review worth reading by Stevens et al. the traditional and more recent theories are described in more detail including arguments for and against these hypotheses (64).

*Oxidative Stress Theory:* Chronic exposure to oxidative stress leads to fibrosis. Due to an aberrant function of hepatic mixed-function oxidases byproducts of hepatic detoxification such as lipid peroxidation products, free radicals and other toxic compounds are excreted in the bile

and reach the pancreas through reflux in the pancreatic duct (7). Reactive oxygen species further damage cellular membranes, intracellular proteins and DNA. Ethanol is a well-known inducer of oxidative stress and one of the mediators is cytochrome P450 2E1 (CYP2E1). Thereby ethanol serves both as a substrate and an enhancer of enzymatic activity of CYP2E1 that is found overexpressed even in the pancreas after chronic abuse (45).

*Toxic-Metabolic Theory:* Alcohol and its metabolites have a direct toxic effect on acinar cells leading to cellular necrosis, fatty degeneration and eventually fibrosis (6). Alcohol is mainly metabolized by the oxidative pathway including alcohol- and aldehyde-dehydrogenases or to lesser extent enzymes of the microsomal oxidizing system. In an alternative pathway, the non-oxidative pathway, ethanol is esterified with fatty esters that result in the synthesis of fatty acid ethyl esters (FAEE) (80). Although the main site of alcohol metabolism is in the liver, the pancreas is also capable of both oxidative and non-oxidative metabolization causing local damage (18, 22).

*Stone and Ductal Obstruction Theory:* This hypothesis was evoked by the fact that after a variable time most patients with chronic pancreatitis show calcifications and have intraductal pancreatic stones (56). Chronic obstruction leads to local damage and stasis that further enhances stone formation and finally fibrosis. Besides, formation of protein plugs and pancreatic stones are increased by alcohol itself. Experimental animal models show that partial or complete pancreatic duct obstruction - in combination with ethanol feeding (68) or repetitive secretagogue stimulation (86) - markedly increased severity of acute pancreatitis and induced chronic disease as well.

*Necrosis-Fibrosis Theory:* Chronic pancreatitis is considered to be a result of recurrent bouts of acute pancreatitis if they are severe enough (31). An acute inflammation leads to periductal injury and fibrosis that finally compresses the ductal

lumen. This obstruction favors acinar cell atrophy, calculi precipitation due to stasis and further fibrous tissue formation (64). Further support for this theory is given by data from genetic studies and animal experiments: Activating trypsinogen (PRSS1) mutations lead to a gain-of-function associated with unregulated protease activation, acute pancreatitis and lastly chronic pancreatitis. Animal models mimicking chronic pancreatitis make use of induction of recurrent bouts of acute pancreatitis by repeated injections of cholecystokinin analogues such as caerulein. When animals develop acute pancreatitis they either recover completely, once the pathogenic stimuli have been stopped, or they develop atrophy of the organ and fibrosis, especially if the pathogenic stimulus has been given during the recovering period during which animals are extremely susceptible to any harmful event (1, 44).

*Primary Duct Hypothesis:* An autoimmune mechanism has been considered to be causative for chronic pancreatitis. Resembling primary sclerosing cholangitis (PSC) to a certain extent the pancreatic duct is affected by an autoimmune reaction ending up in obliteration of the main and secondary pancreatic ducts (9). A co-incidence of chronic pancreatitis and autoimmune disorders of the gastrointestinal tract has been observed and autoimmune pancreatitis is a form of chronic pancreatitis that is a pancreatic manifestation of IgG4-related diseases (27). This process can be triggered by alcohol consumption, beyond its direct toxic effects on ductal cells as well.

*Sentinel Acute Pancreatitis Event (SAPE) Hypothesis:* In order to create a unifying theory for development of chronic pancreatitis and to include recent advances in pancreatitis and its immunological concepts behind, a new hypothesis was introduced in 1999 (59, 75). The new features of this hypothesis are on the one hand that an initiating event (sentinel event) is necessary for causing acute pancreatitis and acinar cell injury first and on the other hand that anti-inflammatory and pro-fibrotic events enable

the progression to chronic pancreatitis (59). It should be noted that even before the sentinel event occurs the pancreas can be exposed to toxic agents such as alcohol, nicotine, lipids or other compounds that induce chronic metabolic or oxidative stress. During acute pancreatitis an unrestrained trypsinogen activation occurs as described above. Simultaneously with early protease activation there is a pro-inflammatory reaction with invasion of inflammatory cells into the pancreas that perpetuate protease activation and cellular damage. This event is mediated by tumour necrosis factor (TNF)- $\alpha$  in a cathepsin-B and calcium-dependent way (60). In the late phase of acute pancreatitis an anti-inflammatory reaction is observed that usually limits the inflammatory reaction and initiates healing process. During this phase there is an activation of pro-fibrotic cells, including stellate cells. A sustained anti-inflammatory reaction however drives pancreatic fibrosis. This occurs when causative factors such as oxidative stress, alcohol or its metabolites are not removed and thus continuously stimulate pancreatic stellate cells to synthesize components of extracellular matrix causing fibrosis (59, 64).

One of the challenges of the SAPE hypothesis is its intention to combine divergent etiologies for chronic pancreatitis through a common pathway leading to the same endpoint, i.e. chronic pancreatitis. This hypothesis also gives an explanation why some individuals with mutations in the trypsin-dependent pathway such as PRSS1, SPINK1 or CTSC as well as the majority

of alcoholics do not eventually develop acute or chronic pancreatitis because they lack the sentinel event, a condition sine qua non for initiation of chronic pancreatitis.

## 5. Conclusions

Recent knowledge on the pathogenesis of chronic pancreatitis has been gained by both genetic linkage analyses and experimental in-vitro and animal studies. There are multiple genetic susceptibility factors, which mostly involve the protease/antiprotease system of the exocrine pancreas. Recent genome wide association studies have identified genetic variants affecting proteins seemingly unrelated to the “trypsin-centered pathway” whose underlying cellular mechanisms are still unclear. From animal models we can learn that at least two principal mechanisms seem to predispose to the development of chronic pancreatitis which are recurrent pathologic stimuli on the pancreas or one single severe event such as an obstruction of the bile or pancreatic duct (36). Furthermore new theories postulate that a sequence of two events is essential for the pathogenesis of chronic pancreatitis.

It can be assumed that not a single but rather a combination of different pathologic stimuli, including immune mediated processes, will eventually be necessary to develop chronic pancreatitis. The pathogenesis of this disease is too complex to get reduced to one single event.

## 6. References

1. **Adler G, Hupp T and Kern HF.** Course and spontaneous regression of acute pancreatitis in the rat. *Virchows Arch A Pathol Anat Histo* 382(1): 31-47, 1979. [PMID: 157597.](#)
2. **Apte M, Pirola R and Wilson J.** The fibrosis of chronic pancreatitis: new insights into the role of pancreatic stellate cells. *Antioxid Redox Signal* 15(10): 2711-2722, 2011. [PMID: 21728885.](#)
3. **Apte MV, Pirola RC and Wilson JS.** Pancreatic stellate cells: a starring role in normal and diseased pancreas. *Front Physiol* 3: 344, 2012. [PMID: 22973234.](#)
4. **Athwal T, Huang W, Mukherjee R, Latawiec D, Chvanov M, Clarke R, et al.** Expression of human cationic trypsinogen (PRSS1) in murine acinar cells promotes pancreatitis and apoptotic cell death. *Cell Death Dis* 5: e1165, 2014. [PMID: 24722290.](#)
5. **Bachem MG, Schneider E, Gross H, Weidenbach H, Schmid RM, Menke A, et al.** Identification, culture, and characterization of pancreatic stellate cells in rats and humans. *Gastroenterology* 115(2): 421-432, 1998. [PMID: 9679048.](#)

6. **Bordalo O, Goncalves D, Noronha M, Cristina ML, Salgado A and Dreiling DA.** Newer concept for the pathogenesis of chronic alcoholic pancreatitis. *Am J Gastroenterol* 68(3): 278-285, 1977. [PMID: 596358.](#)
7. **Braganza JM.** Pancreatic disease: a casualty of hepatic "detoxification"? *Lancet* 2(8357): 1000-1003, 1983. [PMID: 6138545.](#)
8. **Bruce JI, Yang X, Ferguson CJ, Elliott AC, Steward MC, Case RM, et al.** Molecular and functional identification of a Ca<sup>2+</sup> (polyvalent cation)-sensing receptor in rat pancreas. *J Biol Chem* 274(29): 20561-20568, 1999. [PMID: 10400686.](#)
9. **Cavallini G and Frulloni L.** Autoimmunity and chronic pancreatitis: a concealed relationship. *JOP* 2(2): 61-68, 2001. [PMID: 11867865.](#)
10. **Chandak GR, Idris MM, Reddy DN, Bhaskar S, Sriram PV and Singh L.** Mutations in the pancreatic secretory trypsin inhibitor gene (PSTI/SPINK1) rather than the cationic trypsinogen gene (PRSS1) are significantly associated with tropical calcific pancreatitis. *J Med Genet* 39(5): 347-351, 2002. [PMID: 12011155.](#)
11. **Chang MC, Chang YT, Wei SC, Liang PC, Jan IS, Su YN, et al.** Association of novel chymotrypsin C gene variations and haplotypes in patients with chronic pancreatitis in Chinese in Taiwan. *Pancreatology* 9(3): 287-292, 2009. [PMID: 19407484.](#)
12. **Chiari H.** Über die Selbstverdauung des menschlichen Pankreas. *Z. Heilkunde* 17: 69-96, 1896.
13. **Cohn JA, Friedman KJ, Noone PG, Knowles MR, Silverman LM and Jowell PS.** Relation between mutations of the cystic fibrosis gene and idiopathic pancreatitis. *N Engl J Med* 339(10): 653-658, 1998. [PMID: 9725922.](#)
14. **Creighton J, Lyall R, Wilson DI, Curtis A and Charnley R.** Mutations of the cationic trypsinogen gene in patients with chronic pancreatitis. *Lancet* 354(9172): 42-43, 1999. [PMID: 10406366.](#)
15. **Dasouki MJ, Cogan J, Summar ML, Neblitt W, 3rd, Foroud T, Koller D, et al.** Heterogeneity in hereditary pancreatitis. *Am J Med Genet* 77(1): 47-53, 1998. [PMID: 9557894.](#)
16. **Dawra R, Sah RP, Dudeja V, Rishi L, Talukdar R, Garg P, et al.** Intra-acinar trypsinogen activation mediates early stages of pancreatic injury but not inflammation in mice with acute pancreatitis. *Gastroenterology* 141(6): 2210-2217 e2212, 2011. [PMID: 21875495.](#)
17. **Derikx MH, Kovacs P, Scholz M, Masson E, Chen JM, Ruffert C, et al.** Polymorphisms at PRSS1-PRSS2 and CLDN2-MORC4 loci associate with alcoholic and non-alcoholic chronic pancreatitis in a European replication study. *Gut*. [PMID: 25253127.](#)
18. **Edenberg HJ.** The genetics of alcohol metabolism: role of alcohol dehydrogenase and aldehyde dehydrogenase variants. *Alcohol Res Health* 30(1): 5-13, 2007. [PMID: 17718394.](#)
19. **Felderbauer P, Klein W, Bulut K, Ansorge N, Dekomien G, Werner I, et al.** Mutations in the calcium-sensing receptor: a new genetic risk factor for chronic pancreatitis? *Scand J Gastroenterol* 41(3): 343-348, 2006. [PMID: 16497624.](#)
20. **Ferec C, Ragueneas O, Salomon R, Roche C, Bernard JP, Guillot M, et al.** Mutations in the cationic trypsinogen gene and evidence for genetic heterogeneity in hereditary pancreatitis. *J Med Genet* 36(3): 228-232, 1999. [PMID: 10204851.](#)
21. **Figarella C, Mischuk-Jamska B and Barrett AJ.** Possible lysosomal activation of pancreatic zymogens. Activation of both human trypsinogens by cathepsin B and spontaneous acid. Activation of human trypsinogen 1. *Biol Chem Hoppe Seyler* 369 Suppl: 293-298, 1988. [PMID: 3202969.](#)
22. **Fjeld K, Weiss FU, Lasher D, Rosendahl J, Chen JM, Johansson BB, et al.** A recombined allele of the lipase gene CEL and its pseudogene CELP confers susceptibility to chronic pancreatitis. *Nat Genet* 47(5): 518-522, 2015. [PMID: 25774637.](#)
23. **Geisz A, Hegyi P and Sahin-Toth M.** Robust autoactivation, chymotrypsin C independence and diminished secretion define a subset of hereditary pancreatitis-associated cationic trypsinogen mutants. *FEBS J* 280(12): 2888-2899, 2013. [PMID: 23601753.](#)
24. **Gorry MC, Gabbaizedeh D, Furey W, Gates LK, Jr., Preston RA, Aston CE, et al.** Mutations in the cationic trypsinogen gene are associated with recurrent acute and chronic pancreatitis. *Gastroenterology* 113(4): 1063-1068, 1997. [PMID: 9322498.](#)
25. **Halangk W and Lerch MM.** Early events in acute pancreatitis. *Clin Lab Med* 25(1): 1-15, 2005. [PMID: 15749229.](#)
26. **Halangk W, Lerch MM, Brandt-Nedelev B, Roth W, Ruthenbueger M, Reinheckel T, et al.** Role of cathepsin B in intracellular trypsinogen activation and the onset of acute pancreatitis. *J Clin Invest* 106(6): 773-781, 2000. [PMID: 10995788.](#)
27. **Hart PA, Zen Y and Chari ST.** Recent Advances in Autoimmune Pancreatitis. *Gastroenterology*, 2015. [PMID: 25770706.](#)

28. **Hofbauer B, Saluja AK, Lerch MM, Bhagat L, Bhatia M, Lee HS, et al.** Intra-acinar cell activation of trypsinogen during caerulein-induced pancreatitis in rats. *Am J Physiol Gastrointest Liver Physiol* 275(2 Pt 1): G352-362, 1998. [PMID: 9688663.](#)
29. **Horii A, Kobayashi T, Tomita N, Yamamoto T, Fukushima S, Murotsu T, et al.** Primary structure of human pancreatic secretory trypsin inhibitor (PSTI) gene. *Biochem Biophys Res Commun* 149(2): 635-641, 1987. [PMID: 3501289.](#)
30. **Kereszturi E and Sahin-Toth M.** Intracellular autoactivation of human cationic trypsinogen mutants causes reduced trypsinogen secretion and acinar cell death. *J Biol Chem* 284(48): 33392-33399, 2009. [PMID: 19801634.](#)
31. **Kloppel G and Maillet B.** Chronic pancreatitis: evolution of the disease. *Hepatogastroenterology* 38(5): 408-412, 1991. [PMID: 1765357.](#)
32. **Kruger B, Albrecht E and Lerch MM.** The role of intracellular calcium signaling in premature protease activation and the onset of pancreatitis. *Am J Pathol* 157(1): 43-50, 2000. [PMID: 10880374.](#)
33. **Kuwata K, Hirota M, Shimizu H, Nakae M, Nishihara S, Takimoto A, et al.** Functional analysis of recombinant pancreatic secretory trypsin inhibitor protein with amino-acid substitution. *J Gastroenterol* 37(11): 928-934, 2002. [PMID: 12483248.](#)
34. **Lebenthal E, Lerner A and Rolston DDK.** The pancreas in cystic fibrosis. The pancreas: biology, pathobiology, and disease. Go VLW, DiMagno EP, Gardner JD, Lebenthal E, Reber HA and Scheele GA. New York, Raven Press: 1041-1081, 1993.
35. **Lerch MM and Gorelick FS.** Early trypsinogen activation in acute pancreatitis. *Med Clin North Am* 84(3): 549-563, viii, 2000. [PMID: 10872413.](#)
36. **Lerch MM and Gorelick FS.** Models of acute and chronic pancreatitis. *Gastroenterology* 144(6): 1180-1193, 2013. [PMID: 23622127.](#)
37. **Lerch MM, Saluja AK, Dawra R, Ramarao P, Saluja M and Steer ML.** Acute necrotizing pancreatitis in the opossum: earliest morphological changes involve acinar cells. *Gastroenterology* 103(1): 205-213, 1992. [PMID: 1612327.](#)
38. **Malet J, Balazs A, Pallagi P, Balla Z, Kui B, Katona M, et al.** Alcohol disrupts levels and function of the cystic fibrosis transmembrane conductance regulator to promote development of pancreatitis. *Gastroenterology* 148(2): 427-439 e416, 2015. [PMID: 25447846.](#)
39. **Muddana V, Lamb J, Greer JB, Elinoff B, Hawes RH, Cotton PB, et al.** Association between calcium sensing receptor gene polymorphisms and chronic pancreatitis in a US population: role of serine protease inhibitor Kazal 1 type and alcohol. *World J Gastroenterol* 14(28): 4486-4491, 2008. [PMID: 18680227.](#)
40. **Muniraj T, Aslanian HR, Farrell J and Jamidar PA.** Chronic pancreatitis, a comprehensive review and update. Part I: epidemiology, etiology, risk factors, genetics, pathophysiology, and clinical features. *Dis Mon* 60(12): 530-550, 2014. [PMID: 25510320.](#)
41. **Nemeth BC and Sahin-Toth M.** Human cationic trypsinogen (PRSS1) variants and chronic pancreatitis. *Am J Physiol Gastrointest Liver Physiol* 306(6): G466-473, 2014. [PMID: 24458023.](#)
42. **Nemeth BC, Wartmann T, Halangk W and Sahin-Toth M.** Autoactivation of mouse trypsinogens is regulated by chymotrypsin C via cleavage of the autolysis loop. *J Biol Chem* 288(33): 24049-24062, 2013. [PMID: 23814066.](#)
43. **Nemoda Z and Sahin-Toth M.** Chymotrypsin C (caldecrin) stimulates autoactivation of human cationic trypsinogen. *J Biol Chem* 281(17): 11879-11886, 2006. [PMID: 16505482.](#)
44. **Neuschwander-Tetri BA, Burton FR, Presti ME, Britton RS, Janney CG, Garvin PR, et al.** Repetitive self-limited acute pancreatitis induces pancreatic fibrogenesis in the mouse. *Dig Dis Sci* 45(4): 665-674, 2000. [PMID: 10759232.](#)
45. **Norton ID, Apte MV, Haber PS, McCaughan GW, Pirola RC and Wilson JS.** Cytochrome P450E1 is present in rat pancreas and is induced by chronic ethanol administration. *Gut* 42(3): 426-430, 1998. [PMID: 9577353.](#)
46. **Ohmuraya M and Yamamura K.** Roles of serine protease inhibitor Kazal type 1 (SPINK1) in pancreatic diseases. *Exp Anim* 60(5): 433-444, 2011. [PMID: 22041280.](#)
47. **Pfutzer RH, Barmada MM, Brunskill AP, Finch R, Hart PS, Neoptolemos J, et al.** SPINK1/PSTI polymorphisms act as disease modifiers in familial and idiopathic chronic pancreatitis. *Gastroenterology* 119(3): 615-623, 2000. [PMID: 10982753.](#)
48. **Phillips PA, McCarrroll JA, Park S, Wu MJ, Pirola R, Korsten M, et al.** Rat pancreatic stellate cells secrete matrix metalloproteinases: implications for extracellular matrix turnover. *Gut* 52(2): 275-282, 2003. [PMID: 12524413.](#)
49. **Reichert M and Rustgi AK.** Pancreatic ductal cells in development, regeneration, and neoplasia. *J Clin Invest* 121(12): 4572-4578, 2011. [PMID: 22133881.](#)

50. **Rinderknecht H, Adham NF, Renner IG and Carmack C.** A possible zymogen self-destruct mechanism preventing pancreatic autodigestion. *Int J Pancreatol* 3(1): 33-44, 1988. [PMID: 3162506.](#)
51. **Riordan JR, Rommens JM, Kerem B, Alon N, Rozmahel R, Grzelczak Z, et al.** Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* 245(4922): 1066-1073, 1989. [PMID: 2475911.](#)
52. **Rosendahl J, Witt H, Szmola R, Bhatia E, Ozsvari B, Landt O, et al.** Chymotrypsin C (CTRC) variants that diminish activity or secretion are associated with chronic pancreatitis. *Nat Genet* 40(1): 78-82, 2008. [PMID: 18059268.](#)
53. **Sah RP, Dawra RK and Saluja AK.** New insights into the pathogenesis of pancreatitis. *Curr Opin Gastroenterol* 29(5): 523-530, 2013. [PMID: 23892538.](#)
54. **Sah RP, Dudeja V, Dawra RK and Saluja AK.** Cerulein-induced chronic pancreatitis does not require intracinar activation of trypsinogen in mice. *Gastroenterology* 144(5): 1076-1085 e1072, 2013. [PMID: 23354015.](#)
55. **Saluja A, Hashimoto S, Saluja M, Powers RE, Meldolesi J and Steer ML.** Subcellular redistribution of lysosomal enzymes during caerulein-induced pancreatitis. *Am J Physiol Gastrointest Liver Physiol* 253(4 Pt 1): G508-516, 1987. [PMID: 2821825.](#)
56. **Sarles H, Bernard JP and Gullo L.** Pathogenesis of chronic pancreatitis. *Gut* 31(6): 629-632, 1990. [PMID: 2199345.](#)
57. **Scheele G, Bartelt D and Bieger W.** Characterization of human exocrine pancreatic proteins by two-dimensional isoelectric focusing/sodium dodecyl sulfate gel electrophoresis. *Gastroenterology* 80(3): 461-473, 1981. [PMID: 6969677.](#)
58. **Schneider A.** Serine protease inhibitor kazal type 1 mutations and pancreatitis. *Clin Lab Med* 25(1): 61-78, 2005. [PMID: 15749232.](#)
59. **Schneider A and Whitcomb DC.** Hereditary pancreatitis: a model for inflammatory diseases of the pancreas. *Best Pract Res Clin Gastroenterol* 16(3): 347-363, 2002. [PMID: 12079262.](#)
60. **Sendler M, Dummer A, Weiss FU, Kruger B, Wartmann T, Scharffetter-Kochanek K, et al.** Tumour necrosis factor alpha secretion induces protease activation and acinar cell necrosis in acute experimental pancreatitis in mice. *Gut* 62(3): 430-439, 2013. [PMID: 22490516.](#)
61. **Sharer N, Schwarz M, Malone G, Howarth A, Painter J, Super M, et al.** Mutations of the cystic fibrosis gene in patients with chronic pancreatitis. *N Engl J Med* 339(10): 645-652, 1998. [PMID: 9725921.](#)
62. **Shimizu K, Kobayashi M, Tahara J and Shiratori K.** Cytokines and peroxisome proliferator-activated receptor gamma ligand regulate phagocytosis by pancreatic stellate cells. *Gastroenterology* 128(7): 2105-2118, 2005. [PMID: 15940641.](#)
63. **Simon P, Weiss FU, Sahin-Toth M, Parry M, Nayler O, Lenfers B, et al.** Hereditary pancreatitis caused by a novel PRSS1 mutation (Arg-122 --> Cys) that alters autoactivation and autodegradation of cationic trypsinogen. *J Biol Chem* 277(7): 5404-5410, 2002. [PMID: 11719509.](#)
64. **Stevens T, Conwell DL and Zuccaro G.** Pathogenesis of chronic pancreatitis: an evidence-based review of past theories and recent developments. *Am J Gastroenterol* 99(11): 2256-2270, 2004. [PMID: 15555009.](#)
65. **Szabo A and Sahin-Toth M.** Determinants of chymotrypsin C cleavage specificity in the calcium-binding loop of human cationic trypsinogen. *FEBS J* 279(23): 4283-4292, 2012. [PMID: 23035638.](#)
66. **Szabo A and Sahin-Toth M.** Increased activation of hereditary pancreatitis-associated human cationic trypsinogen mutants in presence of chymotrypsin C. *J Biol Chem* 287(24): 20701-20710, 2012. [PMID: 22539344.](#)
67. **Szmola R and Sahin-Toth M.** Chymotrypsin C (caldecrin) promotes degradation of human cationic trypsin: identity with Rinderknecht's enzyme Y. *Proc Natl Acad Sci U S A* 104(27): 11227-11232, 2007. [PMID: 17592142.](#)
68. **Tanaka T, Miura Y, Matsugu Y, Ichiba Y, Ito H and Dohi K.** Pancreatic duct obstruction is an aggravating factor in the canine model of chronic alcoholic pancreatitis. *Gastroenterology* 115(5): 1248-1253, 1998. [PMID: 9797381.](#)
69. **Teich N, Ockenga J, Hoffmeister A, Manns M, Mossner J and Keim V.** Chronic pancreatitis associated with an activation peptide mutation that facilitates trypsin activation. *Gastroenterology* 119(2): 461-465, 2000. [PMID: 10930381.](#)
70. **Wang Y, Wrennall JA, Cai Z, Li H and Sheppard DN.** Understanding how cystic fibrosis mutations disrupt CFTR function: from single molecules to animal models. *Int J Biochem Cell Biol* 52: 47-57. [PMID: 24727426.](#)
71. **Ward JB, Petersen OH, Jenkins SA and Sutton R.** Is an elevated concentration of acinar cytosolic free ionised calcium the trigger for acute pancreatitis? *Lancet* 346(8981): 1016-1019, 1995. [PMID: 7475553.](#)
72. **Watanabe O, Baccino FM, Steer ML and Meldolesi J.** Supramaximal caerulein stimulation and ultrastructure of rat pancreatic acinar cell: early morphological changes during development of experimental pancreatitis. *Am J Physiol Gastrointest Liver Physiol* 246(4 Pt 1): G457-467, 1984. [PMID: 6720895.](#)

73. **Weiss FU, Simon P, Bogdanova N, Mayerle J, Dworniczak B, Horst J, et al.** Complete cystic fibrosis transmembrane conductance regulator gene sequencing in patients with idiopathic chronic pancreatitis and controls. *Gut* 54(10): 1456-1460, 2005. [PMID: 15987793.](#)
74. **Whitcomb DC, Gorry MC, Preston RA, Furey W, Sossenheimer MJ, Ulrich CD, et al.** Hereditary pancreatitis is caused by a mutation in the cationic trypsinogen gene. *Nat Genet* 14(2): 141-145, 1996. [PMID: 8841182.](#)
75. **Whitcomb DC.** Genetic risk factors for pancreatic disorders. *Gastroenterology* 144(6): 1292-1302, 2013. [PMID: 23622139.](#)
76. **Whitcomb DC.** Hereditary pancreatitis: new insights into acute and chronic pancreatitis. *Gut* 45(3): 317-322, 1999. [PMID: 10446089.](#)
77. **Whitcomb DC, LaRusch J, Krasinskas AM, Klei L, Smith JP, Brand RE, et al.** Common genetic variants in the CLDN2 and PRSS1-PRSS2 loci alter risk for alcohol-related and sporadic pancreatitis. *Nat Genet* 44(12): 1349-1354. [PMID: 23143602.](#)
78. **Whitcomb DC, Yadav D, Adam S, Hawes RH, Brand RE, Anderson MA, et al.** Multicenter approach to recurrent acute and chronic pancreatitis in the United States: the North American Pancreatitis Study 2 (NAPS2). *Pancreatology* 8(4-5): 520-531, 2008. [PMID: 18765957.](#)
79. **Wilschanski M and Novak I.** The cystic fibrosis of exocrine pancreas. *Cold Spring Harb Perspect Med* 3(5): a009746. [PMID: 23637307.](#)
80. **Wilson JS and Apte MV.** Role of alcohol metabolism in alcoholic pancreatitis. *Pancreas* 27(4): 311-315, 2003. [PMID: 14576493.](#)
81. **Witt H, Apte MV, Keim V and Wilson JS.** Chronic pancreatitis: challenges and advances in pathogenesis, genetics, diagnosis, and therapy. *Gastroenterology* 132(4): 1557-1573, 2007. [PMID: 17466744.](#)
82. **Witt H, Luck W and Becker M.** A signal peptide cleavage site mutation in the cationic trypsinogen gene is strongly associated with chronic pancreatitis. *Gastroenterology* 117(1): 7-10, 1999. [PMID: 10381903.](#)
83. **Witt H, Luck W, Becker M, Bohmig M, Kage A, Truninger K, et al.** Mutation in the SPINK1 trypsin inhibitor gene, alcohol use, and chronic pancreatitis. *JAMA* 285(21): 2716-2717, 2001. [PMID: 11386926.](#)
84. **Witt H, Luck W, Hennies HC, Classen M, Kage A, Lass U, et al.** Mutations in the gene encoding the serine protease inhibitor, Kazal type 1 are associated with chronic pancreatitis. *Nat Genet* 25(2): 213-216, 2000. [PMID: 10835640.](#)
85. **Witt H, Sahin-Toth M, Landt O, Chen JM, Kahne T, Drenth JP, et al.** A degradation-sensitive anionic trypsinogen (PRSS2) variant protects against chronic pancreatitis. *Nat Genet* 38(6): 668-673, 2006. [PMID: 16699518.](#)
86. **Yamasaki M, Takeyama Y, Shinkai M and Ohyanagi H.** Pancreatic and bile duct obstruction exacerbates rat caerulein-induced pancreatitis: a new experimental model of acute hemorrhagic pancreatitis. *J Gastroenterol* 41(4): 352-360, 2006. [PMID: 16741615.](#)