Review Article

Framework for Interpretation of Trypsin–antitrypsin Imbalance and Genetic Heterogeneity in Pancreatitis

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ABSTRACT

Early intracellular premature trypsinogen activation was interpreted as the key initiator of pancreatitis. When the balance in the homeostasis of trypsin and antitrypsin system is disequilibrated, elevated aggressive enzymes directly attack the pancreatic tissue, which leads to pancreatic destruction and inflammation. However, trypsin alone is not enough to cause complications in pancreatitis, which may play a crucial role in modulating signaling events in the initial phase of the disease. NFκB activation is the major inflammatory pathway involved in the occurrence and development of pancreatitis and it can be induced by intrapancreatic activation of trypsinogen. Synthesis of trypsinogen occurs in endoplasmic reticulum (ER), and ER stress is an important early acinar cell event. Components of ER stress response are known to be able to trigger cell death as well as NFκB signaling cascade. The strongest evidence supporting the trypsin-centered theory is that gene mutations, which lead to the generation of more trypsin, or reduce the activity of trypsin inhibitors or trypsin degradation, are associated with pancreatitis. Thus, trypsin–antitrypsin imbalance may be the first step leading to pancreatic autodigestion and inducing other pathways. Continued experimental studies are necessary to determine the specific relationships between trypsin–antitrypsin imbalance and genetic heterogeneity in pancreatitis. In this article, we review the latest advances that contributed to the understanding of the basic mechanisms behind the occurrence and development of pancreatitis with a focus on the interpretation of trypsin–antitrypsin imbalance and their relationships with other inflammation pathways. We additionally highlight genetic predispositions to pancreatitis and possible mechanisms associated with them.

Key Words: Genetic heterogeneity, pancreatitis, trypsin–antitrypsin imbalance

Received: 24.01.2015, Accepted: 20.02.2015
How to cite this article: Lin K, Gao F, Chen Q, Liu Q, Chen S. Framework for interpretation of trypsin-antitrypsin imbalance and genetic heterogeneity in pancreatitis. Saudi J Gastroenterol 2015;21:198-207.

The pancreas is an endocrine gland, which secretes insulin into the blood stream, and can also act as an exocrine gland that synthesizes digestive zymogens. The exocrine pancreas is composed of acinar cells and ductal structures. The acinar cells are responsible for the synthesis, storage, and secretion of digestive zymogens. These zymogens are produced as precursor molecules packaged into granules, and they will not be activated until they are delivered into small intestine. Intrapancreatic premature zymogens activation can be vital. Trypsinogen is one of the most important digestive zymogens as it plays a central role in the activation of other digestive enzymes. Owing to its physiological functions, acinar cells are subjected to a high risk of autodigestion.[1-3] Under normal conditions, defensive coping mechanisms are sufficient to fully protect acinar cells from inappropriate activation of digestive zymogens. When stimuli overwhelm compensatory mechanisms or compensation reactions become excessive, pancreatic destruction and disease ensue.

Pancreatitis may result from a complex mix of reasons. The etiological risk factors of pancreatitis are various, and include genetic background, environmental factors (eg., alcohol consumption, high-fat diets, hypercalcemia, drugs),[1] biliary obstruction and autoimmune factors.[2-4] It is presumed that exposure to these risk factors may result in premature enzyme activation that cause acute injury of acinar cells, duct cells, or interstitial mesenchymal cells, which if continued, leads to inflammatory responses with the activation of pancreatic stellate cells, resulting in extracellular matrix deposition and

Access this article online
Quick Response Code:
Website: www.saudijgastro.com
DOI: 10.4103/1319-3767.161643

mediating fibrosis.[5] Chronic pancreatitis (CP) is believed to be the result from repeated attacks of acute pancreatitis (AP), and is characterized by persistent inflammation, acinar cell destruction as well as irreversible fibrotic replacement, leading to impaired endocrine and exocrine functions.[6] CP may also increase the probability of genetic instability and the risk of developing pancreatic cancer.[7-9]

Despite the widely accepted trypsin-centered theory, the exact mechanisms of pancreatitis is not completely understood. A study showed that only rapid inductions with high levels of active trypsin (in homozygotes) was sufficient to cause acute pancreatitis, whereas gradual repetitive inductions or milder expression (in heterozygote) was not sufficient.[10] Furthermore, premature trypsinogen activation is not sufficient to induce the systemic complications in pancreatitis. Inflammation plays a critical role in tipping the protease–antiprotease balance toward protease excess. NFκB is a transcription factor regulating genes involved in inflammation, and has been shown to be the major inflammatory pathway in pancreatitis. NFκB can increase the infiltration of neutrophils, cause local pancreatic damage, and initiate systemic inflammatory response.[11] Early and sustained NFκB activation is responsible for local and systemic inflammatory response in acute pancreatitis, and contributes to the development of fibrosis and stellate cell activation that leads to CP. The activation of trypsinogen and NFκB pathways might be independent of each other or, alternatively, the activation of one might be dependent on the activation of the other.[12]

In recent years, scientific advances have provided us with more comprehensive and objective insights into the pathophysiology of pancreatitis. The first trypsinogen gene (*PRSS1*) mutations in hereditary pancreatitis were discovered in 1996, after that, a variety of gene defects associated with pancreatitis have been reported. Mutations that led to the generation of more trypsin, or reduced the activity of trypsin inhibitors or trypsin degradation, have been reported to be associated with pancreatitis, either alone or in combination. This has broadened the horizon to understand the mechanisms of the disease, and has helped identify those who are at risk of developing pancreatitis. For many years, inappropriate intraacinar trypsinogen activation has prevailed as key initiator of pancreatitis, although the evidence is not direct or concrete. Now we know that many other mechanisms can independently trigger pancreatic pathology. We mainly focused on the interpretation of trypsin–antitrypsin imbalance and their relationships with inflammation pathways. We also highlighted genetic predispositions to pancreatitis and the possible mechanisms associated with them.

PREMATURE TRYPSINOGEN ACTIVATION IN THE ONSET OF PANCREATITIS

Chiari attributed the mechanism of pancreatitis to pancreatic autodigestion in 1896. Premature activation of trypsinogen and subsequent activation of other digestive enzymes were considered as the initiator of the autodigestive process. This century-old trypsin-centered theory remains widely accepted because pathological intra-acinar activation of trypsinogen occurs in most models of both acute and chronic pancreatitis.[13] In contrast, a study that suggested that cathepsin L (cleaves trypsin) knockout mice, which have higher trypsinogen activation, exhibit reduced disease severity, suggesting that trypsinogen activation may have a protective role.[14]

Mechanisms of trypsinogen activation

Although trypsinogen activation is critical in acute pancreatic injury, its trigger remains elusive. The activation by cathepsin B and trypsinogen autoactivation are the two major candidate mechanisms. Premature trypsinogen activation takes place in membrane-bound compartments such as autophagic vesicles within which zymogen and lysosomal contents are co-localized.[15,16] In these co-localization vacuoles, cathepsin B (the major lysosomal hydrolase) activates trypsinogen. Cathepsin B knockout mice exhibit inhibition of trypsinogen activation with reduced pancreatic injury. Thus, autophagy exerts devastating effects in acinar cells by activation of trypsinogen to trypsin through delivering trypsinogen to lysosome.[17] Trypsinogen activation is greatly reduced in the absence of autophagy.[18] However, some described a selective autophagy as a protective response mediated by different pathways.[19] Abnormal cathepsin activation because of an imbalance between cathepsin B (activates trypsinogen) and cathepsin L (cleaves trypsin) leads to lysosomal dysfunction and impaired autophagy, which result in intra-acinar accumulation of active trypsin.[20,21] Autophagy is an intracellular lysosome-driven bulk degradation system, and is considered to have a central role in pancreatitis. Moreover, deficient autophagy may be a dominant mechanism for increased intra-acinar trypsin in pancreatitis.[21]

Sustained global Ca^{2+} rise is one of the pathologic signals linked to the initiation of acute pancreatitis. Intracellular Ca^{2+} rise can be induced by bile acids reflux or alcohol metabolites. A study demonstrated how deletion of transient receptor potential isoform 3 (TRPC3), a membrane Ca^{2+} influx channel, had markedly reduced intra-acinar zymogen activation and pancreatitis severity.[22] Ca^{2+}-activated phosphatase and proteases are the targets of acinar cell Ca^{2+} signaling generated during pancreatitis.[2] Calcineurin inhibitors can result in decreased zymogen activation. The first step of AP is characterized by Ca^{2+}-mediated enzymatic

activation, followed by a systemic inflammatory response as a second step.[23] Furthermore, aberrant intra-acinar Ca^{2+} can result in both trypsinogen and NFκB activation (major inflammatory player in pancreatitis).[12]

Experimental and clinical observations also link low pH environments to trypsinogen activation and enhanced Ca^{2+} response. Studies demonstrate that lowering extracellular pH (pHe) alone has no effect, but it can sensitize pancreatic acinar cells to zymogen activation and cell injury.[24] The effects of reduced pHe in acini are mediated by vacuolar ATPase, a proton transporter on the membrane of co-localization vacuoles.[25] A low pH also enhances the activity of cathepsin B to activate trypsinogen.

Multiple factors can modulate zymogen activation process, for example, Protein Kinase C (PKC) isoforms, which is responsible for stimulation of NFκB and zymogen activation.[16,25] While we are focusing on acinar cells, current research suggests that duct cells are not silent bystanders as they can somehow influence intra-acinar zymogen activation.[25]

Pathologic effects of trypsinogen activation

When trypsinogen activation exceeds the intrinsic protective mechanisms, trypsin becomes the initial step in "digesting" acinar cells and causing injury. Recent studies that examined the direct consequences of intracellular trypsin found that activated trypsin decreased trypsinogen secretion and induced pancreatic acinar cell death via apoptosis.[26,27] Promotion of apoptosis may be beneficial, and can reduce necrosis as well as the severity of pancreatitis.[28] Animal models lacking trypsinogen-7 showed no induction of acinar cell death *in vitro*, and led to a 50% reduction in acinar cell necrosis *in vivo*, whereas wild-type mice developed necrosis.[29] Besides, apoptosis occurred with transfection-mediated intra-acinar expression of active trypsin.[30] Apoptosis is known to eliminate damaged cells without eliciting much inflammation, whereas massive cell injury shifts the cell death to necrosis, which leads to the generation of various chemokines and widespread inflammation.[25] Studies have suggested that mild AP is associated with extensive apoptotic acinar cell death, whereas severe AP involves extensive acinar cell necrosis but very little apoptosis.[31] Although both apoptosis and necrosis are mainly induced by active trypsin, the mechanisms behind how trypsin induces cell death are much more complex. The prognosis for pancreatitis not only depends on the degree of pancreatic necrosis but also the intensity of multisystem organ failure generated by the systemic inflammatory response.[32]

Pancreatitis is usually accompanied by local or systemic inflammatory responses. However, the exact mechanisms of how they are activated are not clearly understood. Trypsin can activate protease-activated receptor-2 (PAR-2), which is present at high densities on the luminal surfaces of acinar cells and duct cells. Results of PAR-2 activation are the production of pro-inflammatory cytokines.[33] These cytokines mediate recruitment and activation of inflammatory cells. Various immune cells can determine the death response in pancreatitis; necrotic or apoptotic.[34] Inflammatory responses inhibit apoptosis during acute pancreatitis.[2] A study has shown that neutrophils play a role in the pathologic activation of digestive enzymes in AP through NADPH-oxidase dependent way.[35] Intrapancreatic activation of trypsinogen may also induce NFκB signaling.[36] NFκB activation increases the severity of AP and longer periods of activation lead to CP.[37] The NFκB pathway is extremely complex since it can induce TNFα expression, which is pro-inflammatory in many diseases, and TNFα directly induces premature trypsin activation and necrosis in pancreatic acinar cells.[38]

It is generally accepted that activated trypsinogen and inflammatory pathways play important roles in the pathogenesis of pancreatitis. Intra-acinar activation of trypsinogen is sufficient to induce acute pancreatitis. Nevertheless, trypsin alone is not sufficient to cause fibrosis or CP.[10] Premature activation of pancreatic zymogens may be the first step leading to pancreatic autodigestion and inducing other pathways.[39] Premature activation of trypsinogen is traditionally considered key for both the initiation of AP and the development of CP. Conversely, mice genetically lacking pathologic intra-acinar trypsinogen activation developed CP comparable with that in wild-type mice, suggesting that induction of CP does not require intra-acinar activation of trypsinogen.[40] It challenges the existing trypsin-centered theory of pancreatitis, and further confirmation is needed.

Trypsinogen activation and pancreatitis susceptibility genes

The strongest evidence supporting trypsin-centered theory is the discovery of PRSS1 gene mutation in hereditary pancreatitis.[41] Studies demonstrated that most of the mutations in PRSS1 gene such as R122H, N29I, A16V, and R116C, lead to enhanced trypsinogen autoactivation and/or increased trypsin stability, or induce ER stress.[42] Further support comes from mutations in the pancreatic secretory trypsin inhibitor (*SPINK1*) gene (also known as PSTI, a trypsin inhibitor), which is thought to function as the first line of defense against intracellular trypsinogen autoactivation. Loss-of-function mutations or deletion of SPINK1 have been reportedly associated with acinar cell death, impaired regeneration, and CP. Altogether, these experimental findings support the hypothesis that intracellular trypsinogen activation plays a critical role in the disease. Over the last decade, genetic studies of different

forms of pancreatitis (hereditary, idiopathic, familial, or immune) led to the identification of several additional susceptibility genes, such as cystic fibrosis transmembrane conductance regulator (CFTR), calcium-sensing receptor (CASR), and chymotrypsin C (CTRC). We will review these genetic mutations comprehensively in the following sections.

TRYPSIN–ANTITRYPSIN IMBALANCE IN INJURY AND INFLAMMATION

In normal acinar cells, trypsin activity is properly suppressed by SPINK1. If trypsin exceeds the SPINK1 activity owing to excess acinar cell stimulation, acinar cell injury would occur. Alpha-1-antitrypsin (AAT) is a major protease inhibitor in body fluids, which inhibits trypsin by the formation of molar complexes. Numerous reports have associated AAT deficiency with pancreatitis. The trypsin–antitrypsin imbalance results in an excess release of free enzyme and a cascade of continuous activation of other digestive enzymes, which leads to acinar cell destruction. The decreased activity of antitrypsin may lead to trypsinogen activation, which in turn drives inflammatory responses, and further activates the pancreatic stellate cells resulting in fibrosis.[2] This could further alter the balance between the synthesis and degradation of trypsin–antitrypsin system that subsequently leads to CP. We used to regard trypsin only as a protein-degrading enzyme; however, evidence now suggests that AAT is also a signaling molecule that plays an important role in modulating immunity, inflammation, coagulation, and apoptosis.[43,44] Although protease inhibitors, such as gabexate, not only inhibit various pancreatic enzymes, they also inhibit NFκB activation and reduce the inflammatory response, they have not been proven effective in pancreatitis.[39] Thus, the trypsin–antitrypsin balance also suggests an intricate balance between pro-inflammatory cytokine production and an anti-inflammatory response.[45]

Trypsin–antitrypsin imbalance has been traditionally considered as the central event during pancreatitis pathophysiology. Significant advances in the last decade show that NFκB activation is an early acinar cell event that parallels trypsinogen activation. Genetic models utilizing mice lacking trypsinogen-7 or cathepsin B were found to have equivalent local and systemic inflammatory responses to wild-type mice, suggesting that inflammation is minimally affected by trypsin. Additionally, NFκB has been shown to cause severe pancreatitis in the absence of trypsin. The relationship between these two central early cellular events is currently not clearly understood. One study concluded that trypsinogen activation is essential for NFκB activation.[36] Some argue that activation of pro-inflammatory pathways is not the result of zymogen activation.[46] Deletion of PRSS1 in mice does not influence activation of NFκB.[25] In contrast, other studies found that while intracellular trypsin had no significant effect on the activity of NFκB, extracellular trypsin can dramatically increase NFκB activity.[26] Studies showed that although intracellular trypsinogen activation is required for early acinar cell death during acute pancreatitis, it is only responsible for 50% of local pancreatic damage (the other half by NFκB).[29] Experimental evidence points out that trypsin is not required for the progression of inflammatory response in acute pancreatitis, which has been shown to be induced by NFκB activation. However, CP developed independently of trypsinogen activation.[30,40] Sustained NFκB activation, but not persistent intra-acinar expression of active trypsin, was shown to result in CP.[30] NFκB deficient mice demonstrated reduced acinar necrosis and an attenuated pancreatitis proportional to the degree of NFκB inhibition.[47] Both trypsinogen activation and NFκB activation are sufficient to induce AP,[25] but it remains to be explored whether they are prerequisites for pancreatitis.

Another important early acinar cell event during the development of pancreatitis is endoplasmic reticulum (ER) stress. Synthesis of digestive zymogens occurs in the ER, and can be influenced by many variables, including pH environments, Ca^{2+} levels, and oxidative stress. Accumulation of mis-folded proteins and protein overload are the main cause of ER stress, and therefore initiate ER stress response.[48] ER stress response mechanism appear to be involved in both pancreatic physiology and pathophysiology, and they protect cells from surviving various forms of stress.[49] However, components of ER stress response are known to be able to trigger cell death as well as inflammatory pathways such as NFκB signaling cascade.[50] Excessive exposure to chronically elevated ER stress will induce apoptosis, or even necrosis.[48] It is currently unknown whether or not ER stress is trypsin-dependent or is an independent event. The most important message that we can conclude from these studies is that multiple pathways are simultaneously activated during pancreatitis. Trypsin–antitrypsin imbalance is only one important participant that determines the severity of pancreatitis.

MOLECULAR BASIS OF PANCREATITIS

Genetic mutations have emerged as a critical factor in evaluating AP and CP. Identification of critical genetic risk factors for a patient with recurrent acute pancreatitis (RAP) provides clinicians with an opportunity for early intervention to prevent the development of CP.[51] A gain-of-function mutation in the gene that encodes cationic trypsinogen (PRSS1) was first found to be involved in causing hereditary pancreatitis. RAP or CP has also been associated with loss-of-function mutations in genes that encode the serine peptidase inhibitor Kazal type 1 (SPINK1) and the CFTR. Mutations in the CTRC, and CASR genes were associated with a smaller increase in risk of AP and CP.[52] Besides the

mutations mentioned in PRSS1, SPINK1, CFTR, and CASR, variants in CPA1 expression, which encodes carboxypeptidase A1, were recently found to be also strongly associated with early onset of CP.[53] Recent findings have also demonstrated how pancreatitis is a complex disease involving interactions and synergisms between multiple genetic factors.

PRSS1 and PRSS2 mutations

Three different isoforms of trypsinogen have been described in human pancreatic secretions. According to their electrophoretic mobility on isoelectric focusing, they have been designated as cationic trypsinogen (PRSS1), anionic trypsinogen (PRSS2), and mesotrypsinogen (PRSS3).[54]

The first breakthrough in the study of genetic risk factors for pancreatitis was the discovery of mutations in cationic trypsin gene (PRSS1), which has been identified as a cause of autosomal dominant or hereditary pancreatitis.[41] Mutations in the serine protease 1 gene (PRSS1), which encodes cationic trypsinogen, can lead to increased trypsin activity, increased trypsin stability, and increased auto-activation.[41]

Several mechanisms have been proposed that explain how mutations in the PRSS1 can lead to increase in trypsin activity, or pancreatitis. It has been demonstrated that the R122H mutation prevents deactivation of activated trypsin and also leads to an increase in the auto-activation of trypsinogen.[55,56] The N29I mutation is hypothesized to change the higher order structure of trypsin, resulting in decreased PSTI binding, increased stability, and increased auto-activation. These two are the most common mutations causing hereditary pancreatitis. The mutations A16V, D22G, and K23R that cause alterations around the signal peptide cleavage site of trypsin [Figure 1] may lead to increased trypsinogen auto-activation to trypsin.[33] In addition, A16V or IVS 2 +56_60 del CCCAG was suggested to cause type 1 autoimmune pancreatitis (AIP) through increasing the rate of chymotrypsinogen C (CTRC)-mediated trypsinogen activation.[57,58] Another pancreatitis-causing mutation, R116C, was hypothesized to trigger the endoplasmic reticulum stress.[59] Furthermore, copy number mutations, such as 605-kb duplication and triplication,[60,61] or 320-kb complex copy number mutation,[62] involving the human trypsinogen locus results in a gain of trypsin function through gene dosage effect, thus causing the disease.

Anionic trypsinogen (PRSS2) shares the same physiological activity with cationic trypsinogen (PRSS1) but is synthesized in lower amounts and auto-activates less but autolyzes more rapidly.[63] Until now, no pancreatitis causing PRSS2 mutations have been found.[64] On the contrary, a single mutation, the G191R variant of PRSS2, mitigates intrapancreatic trypsin activity and thereby appears to protect against CP.[65]

SPINK1 mutation

The serum protease inhibitor, Kazal type 1 gene (SPINK1), encodes pancreatic secretory trypsin inhibitor (PSTI), which is one of the key defensive mechanisms against prematurely activated trypsin within the pancreatic acinar cells.[66] Mutations in the SPINK1 gene interfere with the protective function and predispose individuals to pancreatitis. Multiple clear or experimentally demonstrated loss-of-function variations in the SPINK1 gene have been found in patients with CP.[42,67]

An A > G transition at 101 nucleotide position in the SPINK1 gene that leads to substitution of asparagine by serine at codon 34 (N34S) (c. 101A > G) in exon 3 containing haplotype is of the most common SPINK1 haplotypes to be associated with CP.[68] In spite of being one of the strongest predictors and an important risk factor for the pathogenesis of CP, the mechanism behind N34S SPINK1 mutations contributing to disease phenotype remains elusive.[69]

Next to the most prevalent p.N34S mutation, there are also other missense mutations, such as the p.N55S, p.D50E, p.Y54H, p.R65Q, p.R67C, and p.G48E, found to be associated with chronic pancreatitis, which were described by Boulling's group.[70] They went further to analyze eight additional missense mutations in the SPINK1 gene. Five missense mutations (N64D, K66N, R67H, T69I, and C79F) caused a complete loss of PSTI expression and were therefore identified as disease-predisposing changes. Two other missense mutations, S10N and N37S, did not cause a statistically significant loss of PSTI expression and were therefore considered of no pathogenic relevance. The last missense mutation, Q68R, caused a surprisingly significant increase in PSTI expression. Interestingly, it co-existed with the disease-causing PRSS1 N29I missense mutation in the respective patient. Two hypotheses were proposed to explain this finding. Q68R may itself be a protective variant, whose effect is however surpassed by PRSS1 N29I's strong predisposing effect. Alternatively, the changes of PSTI structure induced by the Q68R mutation may have lead to a lower binding affinity with prematurely activated trypsin within the pancreas, resulting in a loss-of-function effect.[71]

Boulling's group also assessed the potential functional impact of 11 SPINK1 promoter variants by means of both luciferase reporter gene assay and electrophoretic mobility shift assay (EMSA), using human pancreatic COLO-357 cells as an expression system. They found that only three promoter variants, -53C > T, -142T > C, and -147A > G, which are associated with a loss-of-function, were likely to be contributing to CP.[72]

CFTR mutation

The third major gene to be implicated in CP is the CFTR, which is expressed in the ductal and centroacinar cells.[42]

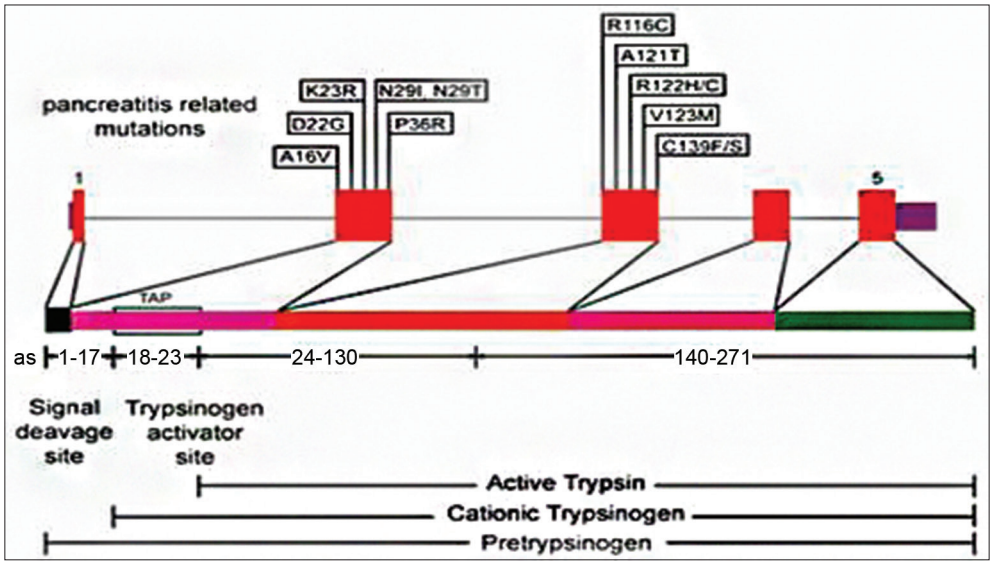

Figure 1: The structure of *PRSS*1 gene and its known mutated forms

The major function of CFTR in the pancreas is believed to be to dilute and alkalinize the protein-rich acinar secretions, thereby preventing the formation of protein plugs that predispose to pancreatic injury.[42] Mutations cause a defect in the CFTR protein, which causes abnormal sodium and chloride transport, leading to defective pancreatic secretion. The Whitcomb group has previously reported that the CFTR variant p.R75Q causes a selective defect in bicarbonate conductance and increases the risk of pancreatitis. Coinheritance of p.R75Q or cystic fibrosis (CF) causing CFTR variants with SPINK1 variants significantly increases the risk of idiopathic CP.[73] However, the role of the trans-heterozygosity for CFTR R75Q and SPINK1 variants in patients with CP was challenged by Witt and colleagues, who argued that the overall risk contribution of CFTR variants to the pathogenesis of CP seems to be lower than previously reported.[71]

CTRC mutation

This gene was initially studied as a candidate gene for pancreatitis because it appears to be able to promote trypsin degradation, and therefore protects the pancreas against trypsin-related injury.[74] Taking into consideration the biochemical activities of CTRC and the functional properties of CTRC mutants, Zhou and Sahin-Tóth[75] hypothesized that three mutually nonexclusive models might explain why CTRC mutations increase the risk of CP: (1) Impaired trypsinogen and/or trypsin degradation; (2) impaired activation of A-type carboxypeptidases; and (3) induction of ER stress. Rosendahl *et al*.[76] analyzed the gene encoding of CTRC in a large European cohort of over 900 German subjects with idiopathic or hereditary CP and found that two alterations in this gene, namely, p.R254W and p.K247_R254del, were significantly overrepresented in the pancreatitis group, being present in 30 of 901 (3.3%) affected individuals but only 21 of 2804 (0.7%) controls. The significance of CTRC variants was also shown through analyzing 71 individuals who were affected with tropical pancreatitis and 84 controls of Indian origin. They did find more CTRC variants in affected individuals than in controls, suggesting that CTRC was a susceptible gene. In a French study, by analyzing all eight exons of the CTRC gene for conventional genetic variants in a total of 287 French white patients, Masson *et al*.[77] also found rare variants more frequently in the ICP patients than in the controls (12.0% combined frequency in the ICP patients and 1.1% in the controls, respectively). However, no CTRC mutation was found in Chinese children with ICP, indicating that CTRC mutations may vary geographically or ethnically.[78] It also indicates that, to prove that CTRC is a susceptible gene may require a very large study population with unusually common rare variants, and to confirm the potential role of CTRC in other populations would require DNA sequencing of a large number of both patients and controls.[51]

CASR mutations

The CASR is a plasma membrane-bound G-protein-coupled receptor that senses extracellular calcium levels.[79] CASR may be a monitor and regulator of pancreatic juice calcium concentration by triggering ductal electrolyte and fluid secretion when levels are elevated.[80] This action would wash out duct fluid with high concentrations of calcium, which increases risk of trypsinogen activation and stabilization of trypsin that in turn causes acute pancreatitis.[51] Over 200 mutations in the CASR gene, CASR, have been reported (see http://www.casrdb.mcgill.ca). Of note, only p.L173P, p.V477A, p.A986S, p.R990G, and p.Q1011E have been reported in more than one patient with pancreatitis.[51]

Is there a new gene?

Despite these recent advances, many individuals with CP do not appear to carry mutations in any of the known susceptibility genes, suggesting that there is an involvement of other yet to be identified genes in this process. Witt *et al.* analyzed encoding carboxypeptidase A1 (CPA1) in subjects with nonalcoholic CP and controls both from Europe and non-Europe areas. They found that loss-of-function CPA1 variants are strongly associated with nonalcoholic CP, especially early-onset disease. They hypothesized that the mechanism by which CPA1 variants confer increased pancreatitis risk may involve "misfolding-induced endoplasmic reticulum stress" rather than elevated trypsin activity, as seen with other genetic risk factors of this disease. The identification of functionally impaired CPA1 variants in both European and non-European sample collections establishes its global role in the pathogenesis of CP.[53]

Complex interaction of multiple genes

CP is a complex multigenic disease, in which the patients often carry mutations in several disease-associated genes. A single factor rarely causes pancreatitis, and the majority of patients with recurrent acute and CP have multiple variants in a gene, or epistatic interactions between multiple genes.[51] Genetic epistasis refers to the effect of one gene to modify the effect of another gene.[51] SPINK1 mutations were initially suspected to be acting as disease severity modifiers of other risk factors.[81,82] It is hypothesized that SPINK1was modifying heterozygous CFTR mutation effects to target the pancreas.[83] Schneider *et al.* demonstrated that both severe and mild 'benign' CFTR and unclassified variants were associated with pancreatitis cases, especially if the patient had a concurrent SPINK1 mutation. With concurrent multiple SPINK1/CFTR variants, the risk is synergistic and mutation specific, while healthy carriers of multiple mutations are exceptionally rare.[51] Rosendahl *et al.*[76] found that among 30 German patients with idiopathic or hereditary pancreatitis carrying a disease-associated CTRC variant, nine also carried a heterozygous SPINK1 p.N34S mutation. In their tropical pancreatitis cohort, 27.3% of the p.N34S heterozygotes were also found to carry a CTRC variant. However, none of the patients who were homozygous for SPINK1p.N34S carried a CTRC variant.

AUTOIMMUNE PANCREATITIS

Little is known about the pathogenic mechanisms of AIP, an increasingly recognized, immune-mediated form of chronic pancreatitis. Moreover, AIP have similar clinical manifestations to pancreatic cancer in imaging, thus AIP is often misdiagnosed as pancreatic cancer,[84] resulting in unnecessary surgical intervention. AIP not only poses a diagnostic challenge for clinicians, but can also lead to irreversible injury to patients.[85-88] It has been reported that genetic factors play an important role in the pathogenesis of AIP. In a Japanese population, the proportion of haploid DRBI*0405-DQBl*0401 in patients with AIP is higher than that in patients with other types of chronic pancreatitis.[89,90] And it is now clear that there are two histological types (type 1 and type 2) of AIP. The histological substance of type 1 AIP is known as lymphoplasmacytic sclerosing pancreatitis (LPSP) or traditional AIP, and type 2 AIP is characterized by a distinct histology called idiopathic duct centric pancreatitis (IDCP).[3,58] Serum IgG4 increase is considered a marker for type 1 AIP. Far less is known about type 2 as it lacks predicting markers, which can easily lead to missed diagnosis and misdiagnosis. A recent study showed that type 2 AIP might be a result of multiple gene mutations, including PKHD1, which encodes fibrocystin/polyductin (FPC), a type I membrane protein. It has been proposed that primary cilia sense and transduct multiple stimuli, such as fluid flow, signals initiated by hormones, morphogenes, growth factors, and other physiologically active substances are present. FPC is localized to primary cilia and acts as a receptor-like protein. This protein is present in fetal and adult kidney cells, and it is also present at low levels in pancreas. PKHD1 gene mutations may be changing the expression of beta-galactosidase, and results in pancreatic cysts and pancreatitis.[91]

CONCLUSION AND FUTURE DIRECTIONS

There is convincing evidence for the hypothesis that pancreatitis is merely an autodigestive phenomenon and early activation of trypsinogen to trypsin within the pancreas. The history of research in the pathogenesis of pancreatitis has taken major steps forward in understanding the disease. Genetic risk factors have shed light to the path for physicians to understand pancreatitis. We are at the very beginning of understanding this multifactorial disease, and links between trypsin and antitrypsin signaling systems are still not well established. Similarly, only a minor subset of susceptibility genes for pancreatitis has been identified. The interplay of trypsin–antitrypsin imbalance and genetic heterogeneity are often difficult to characterize, which is why continued experimental studies are necessary to determine the underlying causes of pancreatitis and drive the exploration of new therapeutic options.

ACKNOWLEDGMENTS

This work was supported by a grant from the National Natural Science Foundation of China (no. 81201362 and no. 81201590), a grant from the Natural Science Foundation of Fujian (no. 2013J01302), a grant from Fujian Medical Innovations (no. 2012-CXB-21), and a grant from Outstanding Youth Foundation of Fujian High Education (no.JA12133) and by the National key Technology R and D Program of China (no. 2012AA022604).

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Source of Support: Nil, **Conflict of Interest:** None declared.