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Genetic Risk Factors for Pancreatic Disorders

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Abstract

A combination of genetic, environmental, and metabolic factors contribute to development and recurrence of acute and chronic pancreatitis; information on all of these is required to manage patients effectively. For example, variants that affect regulation of the *protease, serine* (*PRSS*)*1-PRSS2* and *claudin* (*CLDN*)*2* loci, rather than their coding sequences, interact with other genetic and environmental factors to affect disease development. New strategies are needed to use these data and determine their contribution to pathogenesis, because these variants differs from previously studied, rare variants in exons (coding regions) of genes such as *PRSS1, SPINK1, cystic fibrosis transmembrane conductance regulator (CFTR), chymotrypsin (CTR)C*, and *calcium-sensing receptor* (*CASR*). Learning how various genetic factors affect pancreatic cells and systems could lead to etiology-based therapies, rather than treatment of symptoms.

Keywords

cystic fibrosis; alcoholism; hereditary pancreatitis; complex traits; chronic disease; personalized medicine

Introduction

Genetic analysis will soon be central to management of patients with complex pancreatic disorders [1]. The effects of genetic variations, however, cannot be understood in isolation; they must be considered in the context of environmental, metabolic, epigenetic, and other genetic factors that influence the dynamics of disease activity. A combination of all these factors should be considered in development of disease interventions.

In 1996, a breakthrough in understanding recurrent acute pancreatitis (RAP) and chronic pancreatitis (CP) came with the discovery that gain-of-function mutations in the gene that encodes cationic trypsinogen (*PRSS1*) cause hereditary pancreatitis [2, 3], a syndrome characterized by RAP and later CP. Using candidate gene approaches, mutations in the coding regions of 4 additional genes linked to the control of trypsin in the pancreas have been associated with pancreatitis. RAP and CP have also been associated with loss-of-function mutations in genes that encode the serine peptidase inhibitor Kazal type 1 (*SPINK1*) and the cystic fibrosis transmembrane conductance regulator (ATP-binding

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cassette sub-family C, member 7; *CFTR*). Mutations in the chymotrypsin C (caldecrin) gene (*CTRC*) and the calcium-sensing receptor gene (*CASR*) (see recent reviews [4-8]) were associated with smaller increases in risk. These genetic factors have greater levels of association with early-onset pancreatitis than alcohol- and smoking-induced pancreatitis.

More recent breakthroughs are helping us to understand the complex risk factors for RAP and CP. These were led by the discovery that variants in non-coding regions of the *PRSS1-PRSS2* and *CLDN2* loci affect risk for sporadic and alcoholic pancreatitis [9]. In this review, we integrate the knowledge gained from these recent advances into our understanding and management of pancreatitis.

Genetics of Pancreatitis

The pancreas is a simple retroperitoneal digestive gland that secretes zymogens to digest intraluminal nutrients, bicarbonate to neutralize gastric acid, and hormones to regulate nutrient assimilation and utilization. Located in the back of the abdomen, the pancreas is well protected from mechanical injury and from direct interactions with the environment, toxins, and infectious agents. As a result, pancreatic diseases are relatively rare, with the exception of type 1 diabetes mellitus (T1DM), which results from autoimmune-associated destruction of the pancreatic islet β cells [10]. AP and CP are the most common disorders of the exocrine pancreas, and in contrast to T1DM, the mechanism of injury or destruction is generally not autoimmune-mediated. The major risk for the development of these diseases lies with the risk of premature activation of trypsin, followed by zymogen activation, tissue auto-digestion, and the generation of a robust immune response and its sequelae [11].

AP and CP are clinical syndromes, [12] defined by signs and symptoms that together form a pathologic condition. The problem with these clinical-pathologic definitions, however, is that they are descriptive rather than mechanistic, which limits the diagnosis of mild and early-stage disease and the design of targeted treatments. Additional challenges for physicians include the variability and unpredictability of disease onset, severity, complications, and clinical course. As a result, therapies are primarily directed at symptoms, rather than pathogenic mechanisms. A new approach is needed to better understand and manage patients with pancreatic diseases (reviewed in [1]).

Models

In a simple model, CP results from 2 general hits. [13, 14]. The first hit, AP, activates the immune system and starts the process[14, 15]. Susceptibility to pancreatic injury is linked to premature activation of trypsin—either in acinar cells or pancreatic ducts. In addition, there are trypsin-independent mechanisms of injury that can activate the inflammatory process, such as direct trauma, toxic agents, or immune-mediated mechanisms, such as in autoimmune pancreatitis (AIP). Factors that determine the severity of AP will not be addressed here.

Inflammation is a normal response to injury that usually leads to tissue repair and regeneration. The second hit that contributes to CP appears to be modification of the normal inflammatory response, leading to sustained activation of pancreatic stellate cells and fibrosis (or other irreversible structural or functional changes; see reviews [13, 14]). So the first hit comes from factors that cause injury, whereas the second hit involves factors that promote inflammation and inflammation-associated complications, such as fibrosis and sclerosis, failed acinar cell regeneration, distorted tissue architecture, progressive loss of the normal parenchyma, metaplasia, dysplasia, and pain syndromes. This second hit could include various responses of the immune and autonomic and sensory nervous systems, acinar and duct cell stress responses, cell regeneration and trans-differentiation, tissue

remodeling, dysplasia, altered anatomy, and other factors [15] (Figure 1). Different genetic variants can affect each of these systems.

The development of models is important for studying and identifying treatment strategies for disorders that arise based on dozens of variables. Modeling allows for the organization of known risk factors and disease-modifying factors into compartments, which in turn provides information about how multiple risk factors interact. Additionally, modeling organizes sequential events, in which responses depend on initiating conditions, facilitating the anticipation of potential effects when initial conditions are met. Finally, risk categories can constructed based on therapeutic approaches, linking risk factors with specific therapies.

Modeling provides a framework for understanding complex diseases[1] and therefore a basis for personalized medicine. Treatment approaches must be specifically designed for disorders that have multiple etiologies with similar pathologies, those that cause organ dysfunction without tissue pathology, and those with unpredictable behaviors, such as RAP and CP.

Susceptibility to AP and RAP

Disorders of the pancreas can be congenital; acquired through trauma, infection, or gallstones; linked to alcohol, smoking, or genetic factors; or result from unknown factors (idiopathic disease). The fact that most people do not have pancreatic disease indicates that the system is robust and has multiple adaptive and protective mechanisms in place.

One approach to understanding complex genetics is to focus on the specialized cells that mediate organ function and response to injury. This approach is similar to reverse engineering, in which individual components are studied, and the information is used to determine the mechanisms of a larger system. Acinar and duct cells are the primary effector cells of the pancreas and the likely initiators of AP.

Acinar Cells

The acinus, comprising acinar cells and upstream duct cells, are the primary functional unit of the exocrine pancreas (Figure 2). Acinar cells rapidly produce large amounts of pancreatic digestive pro-enzymes (zymogens) that are delivered to the duodenum, where they are activated to digest ingested nutrients. Trypsin is a protease and digestive enzyme that breaks peptide chains at arginine (Arg, R) or lysine (Lys, L) residues; it activates all of the other pancreatic zymogens in the duodenum under normal conditions. Like the mast cell enzyme tryptase, trypsin can also initiate a prolonged immune response if it is activated in the wrong location as in pancreatitis[16]. Therefore, it is important for pancreatic homeostasis (and human health) that trypsin remain in its zymogen form (trypsinogen) until its activity is needed.

Calcium signaling is an important regulator of acinar cell function [17]. Release of calcium from internal stores is required for excitation to be coupled with secretion and expulsion of zymogen granule contents from inside the acinar cells to the duct lumen. Calcium levels are tightly regulated; ATP is required to pump calcium into internal stores and out of the cell [18]. Excessive alcohol intake can damage mitochondria, which normally produce large amounts of ATP [19, 20], and thereby disrupt the energy source for calcium regulation in acinar cells.

Multiple lines of evidence indicate that premature activation of trypsin in acinar cells damages the cells to cause an inflammatory response, recognized clinically as acute pancreatitis (see reviews [18, 19, 21-23]). The most abundant forms of trypsinogen in the pancreas are cationic trypsinogen (PRSS1) and anionic trypsinogen (PRSS2). PRSS1 is more easily activated, from trypsinogen to trypsin; [24] this occurs via cleavage of trypsinogen

activation peptide (TAP), an 8 amino acid N-terminus extension that forms a calcium binding site [25-27]. When the calcium concentration increases, the activation site is stabilized, allowing cleavage of the TAP. Therefore, activation of trypsin is also regulated by the calcium concentration [25]. Super-physiological concentrations of calcium in acinar cells have been associated with trypsin activation and initiation of pancreatitis [19, 20].

Cleavage of TAP alone does not activate trypsin—its N-terminus must fold back into the globular molecule and interact with the internal sites [28]. This process is pH dependent [29], with highest levels of activation occurring at pH values between 7 and 8 [29, 30]. Low acinar cell pH (<7.15), observed, also promotes activation of trypsin, and can lead to pancreatitis as observed in diabetic patients with ketoacidosis [31-33], possibly in conjunction with activation of cathepsin B [34].

Acinar and duct cells have many mechanisms that protect against trypsin activation, including trypsin self destruction (autolysis), in which other trypsins and CTRC (another proteolytic zymogen activated by trypsin) digest trypsinogen and trypsin at specific sites (Arg-122–Val-123 and Leu-81–Glu-82) to inactivate them [35, 36]. However, there is a second calcium-binding site on the trypsin molecule, near the 2 cleavage sites; when calcium concentrations increase, the cleavage sites are blocked, and trypsin is protected [26, 36, 37]. Furthermore, during the inflammatory response, the trypsin inhibitor SPINK1 is activated [38-40]. Therefore, pancreatitis can arise from defects in acinar cell regulation of trypsin activity or calcium concentration, changes in pH, or alterations in trypsin structure.

Genetic Risk for Acinar Cell Susceptibility

Several genetic factors that affect risk for pancreatitis affect acinar cell function. Mutations in the trypsinogen gene (such as *PRSS1* N29I, N29T, V39A, R122C, and R122H; see http://www.pancreasgenetics.org) prevent cleavage of CTRC by trypsin. CTRC is likely to function in acinar cells, and loss-of-function mutations in CTRC are associated with pancreatitis [41, 42]. Finally, mutations in the trypsin inhibitor SPINK1 (such as N34S) are associated with increased risk of pancreatitis [43-45]. SPINK1 is synthesized by acinar cells [46] and follows trypsinogen from synthesis to secretion, so it is likely to be involved in protecting both acinar cell and the duct from prematurely activated trypsin.

A new finding is that a variant on a haplotype in the non-coding region of the *PRSS1-PRSS2* locus significantly reduces expression of PRSS1 and reduces the risk of pancreatitis [9]. A common haplotype in linkage with rs10273639T (present in about 40% of population alleles) is associated with partial protection against pancreatitis. This finding again indicates the importance of the trypsinogen genes in RAP and CP.

Alcohol

Multiple studies have linked alcohol consumption with an increased risk of developing AP and CP (see reviews [14, 47, 48]). Animal studies have indicated that the increased risk is related to a lower threshold for hyperstimulation-associated pancreatic injury [49, 50]. Other studies have indicated that chronic alcohol consumption alters the neuro-hormonal mechanisms of pancreatic activation, with hyperstimulation occurring during alcohol withdrawal (disinhibiting excitatory nerves that adapted to alcohol-associated inhibition) and nutrient feeding (resulting in hyperstimulation) [51], consistent with clinical observations [52]. However, the conditions needed for alcohol to initiate AP are so specific that alcoholic AP is uncommon even among heavy drinkers. Genetic risk factors that link alcohol to susceptibility to AP have not been clearly defined.

Epidemiology studies have provided additional insight. Less than 3% of heavy users of alcohol develop CP [53], and the risk of alcoholic pancreatitis, when adjusted for smoking in

regression analysis, is low [54]. Furthermore, a threshold of >5 drinks a day (or 35 drinks a week) must be reached before there is an associated risk of pancreatitis [14, 54]. These data indicate that alcohol intake is a weak susceptibility factor (first hit), but could be a strong modifier factor (second hit), especially with smoking[54] and the *CLDN* risk variant[9].

Duct Biology and Risk

The primary function of duct cells is to secrete a bicarbonate-rich fluid that flushes the zymogens out of the pancreas into the duodenum. The most important molecule within the duct is CFTR, an anion channel that transports chloride and bicarbonate. The electrochemical mechanism of pancreatic chloride and bicarbonate secretion has been well defined in animal and mathematical models [55, 56].

Risk for pancreatitis is linked with the zymogens within the duct rather than within the duct cell. The duct cells express multiple sensors on the luminal surface that detect trypsin activity (such as protease activated receptors PAR 1 and PAR2 [57, 58]) and are therefore protective, whereas other molecules (such as the purinergic receptors P2Y2, P2X4, and P2X7 [59]) sense calcium concentration and ATP release as signals of injury. Activation of these sensors results in opening of CFTR channels, secretion of bicarbonate-rich fluid, and flushing of the duct contents into the duodenum [59]. There are also sensors inside duct cells that change the permeability of CFTR to promote conductance of bicarbonate when intracellular concentrations of chloride are low [55, 60]. In addition, there are multiple types of duct cells, with different characteristics—for example, the duct cells nearest the acinar cells have the highest concentration of CFTR molecules [55]. The duct also contains mucus-secreting cells that protect the pancreatic duct from zymogens. So, there are different sensors and mechanisms that protect the pancreas from different types of risk; defects in 1 protective mechanism might be only relevant to 1 type of stress or injury, but not others.

Duct Cell Dysfunction

Mutations in *CFTR* cause cystic fibrosis, an autosomal recessive disease characterized by the development of chronic pancreatitis (beginning in utero), demonstrating the importance of CFTR in pancreatic physiology. Nearly 2000 variants in *CFTR* have been identified (http://www.genet.sickkids.on.ca/app), but little is known about their functional effects (CFTR2 project - http://www.cftr2.org). The more-common variants are classified clinically as severe or mild, based on their effect on pancreatic function. An accepted molecular classification strategy organizes the variants by their effects on CFTR function, with Class I–III variants causing severe dysfunction, and Class IV–V causing reduced or altered function [61, 62]. Class IV–V variants have mild–variable effects on the pancreas and other organs, often leaving sufficient function for basic physiological needs but not enough to handle stress. The effects of *CFTR* Class IV–V variants have been reported on borderline pancreatic exocrine sufficiency in patients with CF, and their risk of pancreatitis [63].

In 1998, 2 studies reported that patients with idiopathic pancreatitis, and some with alcohol-associated CP, had more variants in *CFTR* than could be explained by chance [64, 65]. These findings have been replicated in studies from the United States (US) [66], Europe [67-70], India [71], and China and Taiwan [72, 73]. What was not clear was whether these patients had mild or atypical CF, or whether they had *CFTR*-related disorders with complex, pancreas-specific mechanisms [63, 74-76]. What is clear is that proper CFTR activity is required for normal duct cell function, and that variants in *CFTR* are associated with susceptibility to pancreatitis.

There have been no genetic factors associated with duct obstruction, sphincter of Oddi dysfunction, or pancreatic divisum. The effect of genetic factors on formation of pancreatic

duct stones, gallstones, and pancreatic neoplasia have not been fully evaluated in patients with pancreatitis.

Genetic Risk of Autoimmune Disease

AIP is identified based on imaging analysis of the pancreatic parenchyma and duct, serologic analysis of immunoglobulin (Ig)G4 levels, features of other organs, histologic analysis of the pancreas, and sometimes response to steroid therapy [77]. Two types of AIP have been described: [77, 78] Type 1 AIP is more prevalent in Japan and Asia that in the United States and Europe, and occurs at an older age that Type 2, which is associated with higher levels of IgG4 and more proximal biliary, retroperitoneal, renal, or salivary disease. Type 2 AIP is rarely observed in Asians, is more often associated with inflammatory bowel disease, and has unique pathology features [77, 79].

Differences in the racial and geographic distribution of AIP indicate that environmental or genetic risk factors are important. To date, no compelling evidence has been reported to establish or refute either type of risk. Challenges in doing so include the complex interaction of AIP with the involvement of other organs and immune-mediated syndromes and the relatively small number of patients with this disease. Human and animal models have focused on the immune system, including the HLA region [80, 81] and other loci linked with autoimmunity in rodent models [82], but little is known about the pathogenesis of AIP.

Progression from RAP to CP

There is no single etiology for CP. It could be considered as a disorder that progresses over years from an initial injury to complete gland destruction under the influence of 1 or more etiologic pathways, so treatments might be selected to interrupt the process and allow healing and regeneration. The Sentinel Acute Pancreatitis Event (SAPE) model [13, 14, 83], recently tested in a population-based study from Allegheny County (Pittsburgh), PA [84] (Figure 3), proposes that patients with AP progress to CP at different rates, based on etiology, with alcohol etiology having the highest risk (Figure 3A), and that the mechanism for progression is linked to RAP (Figure 3B).

The SAPE model is built on the observation that individuals live for many years with multiple risk factors for CP—then suddenly the process begins and CP develops [13, 14]. The premise is that the development of CP requires activation of the immune response, as happens during an episode of AP. The outcome of an episode of AP can be either complete recovery, necrosis–fibrosis following severe AP, or the initiation of progressive inflammation and fibrosis, leading to CP over time. During the interval between AP and CP (Figure 1), there is opportunity to intervene and stop progression. The approach selected to prevent RAP and/or progression should be based on etiology, such as a cholecystectomy for patients with gallstone pancreatitis but not for patients with alcohol or genetic etiologies. Risk factors for RAP and progression of fibrosis must therefore be addressed.

Trypsin Activation and Disease Progression

Studies in patients with hereditary pancreatitis have shown how a major susceptibility factors, such as *PRSS1* R122H, become risk factors for AP and CP through RAP [2, 85, 86]. On the average, carriers of *PRSS1* R122H or N29I have their first episode of AP at a median age of 10 years, with evidence of CP developing within the next decade. This provides some of the most compelling data that RAP could eventually lead to CP.

SPINK1 variants could contribute to pancreatic disease via different mechanisms than *PRSS1* or *CFTR* variants. Heterozygous mutations or variants are likely to modify CP development, rather increase susceptibility [43, 87]. This is based on the observation that the

SPINK1 N34S high-risk haplotype is common (1%–3% of most populations), whereas CP is uncommon (42/100,000 persons [88]). However, the *SPINK1* N34S variant increases the risk of alcoholic CP 5-fold, idiopathic CP 15-fold, and tropical CP 19-fold[45]. It is unlikely that *SPINK1* mutations are a susceptibility factor for AP, but rather for RAP and CP [89, 90]. SPINK1 could be effective in controlling the effects of recurrent intra-pancreatic trypsin activation from a variety of etiologies, but mutations in SPINK1 might allow recurrent trypsin-associated injury to lead to development of fibrosis (Figure 4).

In support of this hypothesis, increasing evidence indicates that heterozygous *SPINK1* mutations are only associated with RAP or CP when patients have a mutation in a susceptibility gene associated with recurrent trypsin activation, such as *PRSS1, CFTR, CASR,* or *CTRC* [66, 69, 71]. Thus, heterozygous *SPINK1* mutations do not cause pancreatitis, but instead exacerbate the clinical outcome of patients with recurrent pancreatic injury from trypsin activation. Of note, *SPINK1* mutations are only slightly more common in patients with alcoholic pancreatitis patients than in the general population [91-94]. The progression from pancreatic injury to CP therefore appears to differ from that of alcohol-associated CP [45, 95] (Figure 4).

Complex genetics—CTRC and CASR

Mutations in *CTRC* and *CASR* are associated with CP [7, 8]. Unlike *CFTR* and *SPINK1* mutations, homozygous or compound heterozygous mutations in *CTRC* and *CASR* have not been found in family clusters of CP or patients with sporadic pancreatitis, indicating that, independently, they are not sufficient to cause RAP or CP. Instead, variants in *CTRC* and *CASR* genes identified in patients with CP almost always occur with heterozygous variants in *PRSS1, CFTR,* or *SPINK1* [7, 69, 96], indicating that combinations of genetic factors are required to increase the risk for RAP and CP. Table 1 summarizes various combinations of genetic risk factors and clinical phenotypes.

Although the mechanisms by which CTRC protects against pancreatitis are established [35, 97], the importance of *CTRC* variants in terms of risk for RAP and CP is less clear. Rosendhal et al. [42] demonstrated that the rare p.R254W and p.K247_R254del variants were significantly overrepresented in pancreatitis cases from Germany but not among individuals with CP in India. Masson et al. [41] did not associate p.R254W or p.K247_R254del with CP in patients in France, but did associate them with multiple rare, newly identified mutations in CTRC. Chang et al. [98] evaluated a Chinese cohort of CP cases and associated additional mutations with CP, but did not replicate the previous findings. Paliwal et al. reported multiple variants in CTRC in among patients with CP in India, with p.V235I being the most prevalent[96].

In the North American Pancreatitis Study (NAPS)-2, CP in the US cohort identified a few of the variants in exons 2, 3, or 7 that were seen in Germany, but these were too rare in the NAPS-2 population for the finding to be statically significant (D. Whitcomb, unpublished observations, 2009, 2012). However, functional studies have shown that rare mutations do affect CTRC function[35, 97] and therefore are likely of clinically important based on mechanistic evidence rather than statistical evidence alone.,. Taken together, it has been difficult to prove a role for specific CTRC variants in CP using population-based genetic studies, even though mechanistic studies have shown how defects in CTRC could contribute to the disease. If CTRC variants can be associated with CP risk, they can be used to design personalized treatment approaches.

Mutations in the CASR gene have also been associated with CP [7]. The association was initially reported in patients with familial hypocalciuric hypercalcemia, a condition caused by loss-of-function mutations in *CASR*. Felderbauer et al. [99] identified 2 patients with CP

that each carried the *CASR* and *SPINK1* mutations. A screen of 19 families with idiopathic CP revealed multiple additional cases with loss-of-function variants in *CASR* together with *SPINK1* mutations [100]. In a study of 338 subjects, Muddana et al. [101] associated pancreatitis with the gain-of-function R990G variant of CASR [102]. Risk of CP was not associated with *SPINK1* variants, but was associated with moderate to heavy alcohol consumption.

Over 70 *CASR* variants have been classified and are included in the calcium-sensing receptor locus-specific database (CASRdb); they have been associated with familial (benign) hypocalciuric hypercalcemia, neonatal severe hyperparathyroidism, and autosomal dominant hypocalcemia [103]. There is evidence that that loss-of-function variants of *CASR*, in association with *SPINK1* and *CFTR* variants, affect duct cell function (Figure 2), whereas gain-of-function variants in *CASR* that are associated with alcoholic pancreatitis affect acinar cell functions. Further research is needed to test this hypothesis.

Alcoholic Pancreatitis and Progression

Patients with alcoholic AP are at high risk of RAP [104] and progression from RAP to CP, if they continue to drink alcohol and/or smoke [84, 105-107]. In the NAPS2, the prevalence of *CFTR* variants was equal among subjects with RAP and CP suggesting that CFTR variants increase susceptibility to pancreatic injury, yet only 25% of RAP and 46% of CP subjects drank amounts of alcohol associated with disease risk [54]. This could mean that alcohol (especially with smoking) is a disease modifier rather than a typical susceptibility factor and is associated with rapid transition from RAP to CP. The predicted result would be lowering the prevalence of alcohol-associated pancreatitis among individuals with RAP while increasing it among those with CP. Several recent discoveries provide some insight as to how alcohol might promote this transition.

Alcohol Causes RAP

Recurrent pancreatitis itself promotes fibrosis (Figure 3). In humans, this initially became evident in patients with hereditary pancreatitis, [2, 3] and was supported by data from animal studies [108, 109]. Alcohol could affect regulation of calcium by acinar cells by injuring the mitochondria and limiting the ability of the acinar cells to pump calcium out of the cytoplasm [19, 20]. In support of this concept, gain-of-function mutations in *CASR* the risk increase risk for alcoholic pancreatitis [7, 101]. Preventing RAP through cessation of alcohol and smoking [105, 106] is important in limiting progression to CP.

Alcohol Alters the Immune Response

Alcohol increases the rate of fibrosis development in animal models beyond what is expected from RAP alone [110, 111]. The mechanism appears involve alterations in the immune response to recurrent injury[110]. In humans, preliminary studies link alcohol-associated CP, but not other types of CP, with the T-helper (Th)17 cell response. Mutations in the interleukin (IL)-23 receptor that reduce its function protect against Crohn's disease, [112, 113] were also reported to protect against alcoholic pancreatitis [114].

The CLDN2 Locus and Risk for Alcoholic Pancreatitis

Recently, a genetic locus was found to be associated with a marked increase in the risk of alcohol-related RAP and CP [9]. In population controls, 25.8% of men carried a single copy of the X-linked *CLDN2* risk allele T (rs12688220) and 6.9% of women were homozygous for the variant. The variant was found to have a significant effect of risk of pancreatitis with a non-alcohol etiology, with a single-copy frequency in men of 38.5% (expected 26%) and homozygous frequency in women of 10.0% (expected 7%). Only 4% of men are expected to have both at-risk levels of drinking (about 16% prevalence[115]) and the *CLDN2* risk allele T

(25.8% prevalence in male controls). However, 47.6% of men with alcohol-associated CP were found to have the *CLDN2* risk allele T, indicating that these factors, in combination, increase risk for CP. Furthermore, the X-chromosome linkage could partially explain the higher prevalence of alcohol-associated pancreatitis among men.

CLDN2 is normally expressed at low levels by the duct cells of the exocrine pancreas and by the islets. It differs from other claudins in the pancreas in that it forms low-resistance, cation-selective ion and water channels between endothelial cells[116, 117], which facilitate sodium and water movement into the duct lumen. Variants in the coding region of *CLDN2* have not been associated with pancreatitis. However, immunohistolochemical analysis of pancreatic tissues from patients with CP, collected during pancreatic surgery, found increased levels and altered localization of CLDN2 in acinar cells of patients with the high-risk *CLDN2* genotype. Expression of CLDN2 increases in in porcine acinar cells during development of AP [118], linking acinar cell stress to CLDN2 levels. The *CLDN2* promoter includes a binding site for the nuclear factor-κB [119], and *CLDN2* expression increases in other cells under conditions of injury or stress[120-122]. In inflamed tissues, acinar cells might interact with or be injured by M2-type macrophages that express CLDN2,[123] which promote the inflammatory process. Further studies are needed to fully define the functional variant in the *CLDN2*-locus and the mechanisms by which it could contribute to CP pathogenesis.

Future Directions

Insights gained from studies of genetic variations and/or environmental factors have transformed our understanding of CP. We now know that CP begins with an injury that activates the immune response, and progression occurs with immune system activation, in the context of additional risk and disease modifiers, which leads to fibrosis and other complications. Pathogenesis of CP is multi-factorial, and involves altered activities of acinar cells, duct cells, pancreatic ducts, and the immune system (autoimmunity). Treatment will therefore likely require a combination of different therapeutic approaches. In the future, patients with AP or early signs of pancreatic disease will undergo a systematic evaluation that includes genetic analysis, to provide information about disease etiology, and then receive therapies selected to target specific factors, based on the genetic information. A model pancreas clinic has already been developed [1] and will continue to integrate new findings from genetic analyses into therapeutic approaches for pancreatic disorders. Someday, the effectiveness of treatment could be monitored with biomarkers; therapeutics could be continually adjusted to maintain pancreatic function and prevent progression of disease.

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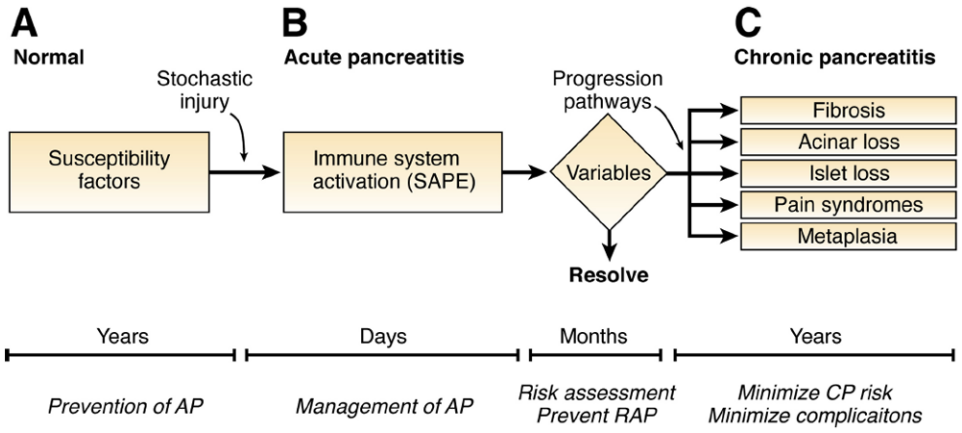

Figure 1. General Models of Inflammation

Chronic pancreatitis is illustrated as a syndrome that develops over time (left to right). A. Individuals may live for years with multiple susceptibility factors. Patients with very high risk may be candidates for new prevention strategies such as avoiding alcohol. B. When a stochastic event leads to pancreatic injures it initiates the Sentinel Acute Pancreatitis Event (SAPE) with intra-pancreatic immune system activation.

Management includes identification of actionable risk and prevention of RAP. C. Factors such as alcohol, smoking and genetic mutations or presently unidentified factors affect the specialized cells of the pancreas and infiltrating cells to cause various complications. Research efforts are focusing on identifying risk, mechanisms and biomarkers of the progression pathways.

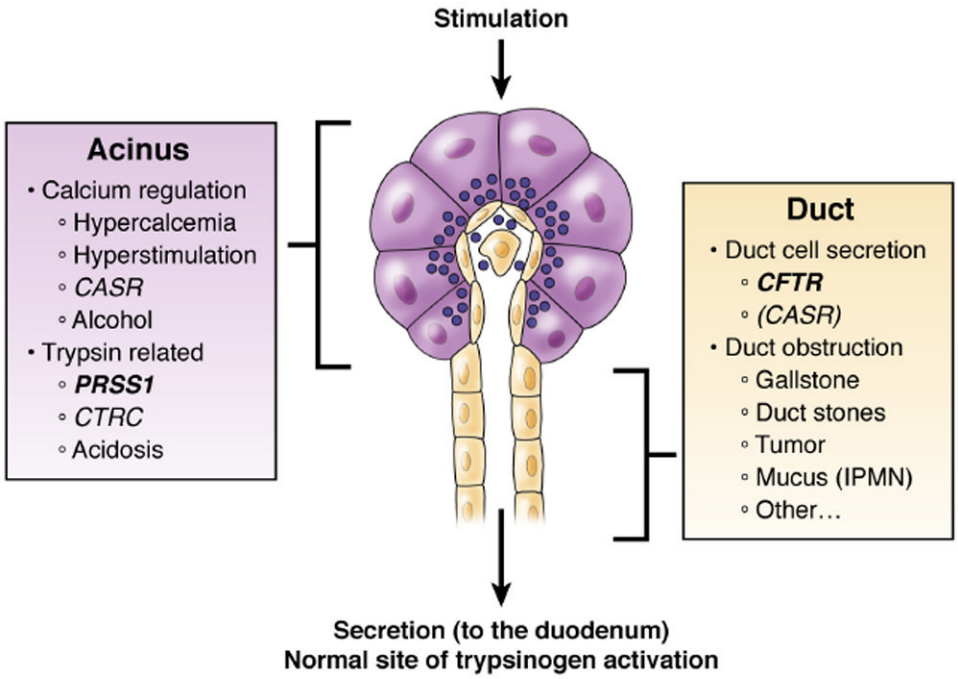

Figure 2. Genetic and Environmental Factors that Affect Acinar Cells or Ducts

Premature trypsin activation may occur within the acinar cell or within the duct to initiate pancreatitis. The majority of known risk factors can be classified as primarily affecting the acinar cells or pancreatic ducts. Understanding the site of likely trypsin activation and mechanism may guide preventative strategies in the future. (from Solomon and Whitcomb[76]).

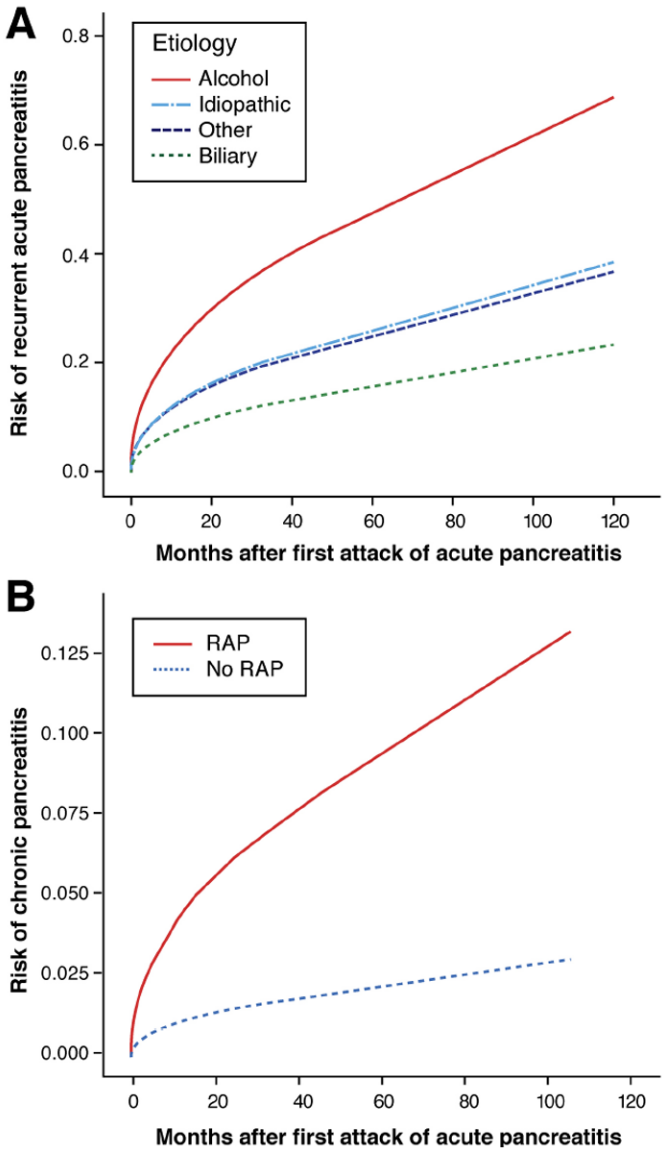

Figure 3. Risks of RAP and CP

Data collected in population-based analysis of 7456 residents of Allegheny County, PA following their first (sentinel) episode of AP. A. Risk of developing RAP based on etiology, B. Risk of CP based on the presence or absence of documented RAP. From Yadav, O'Connell and Papachristou. [84].

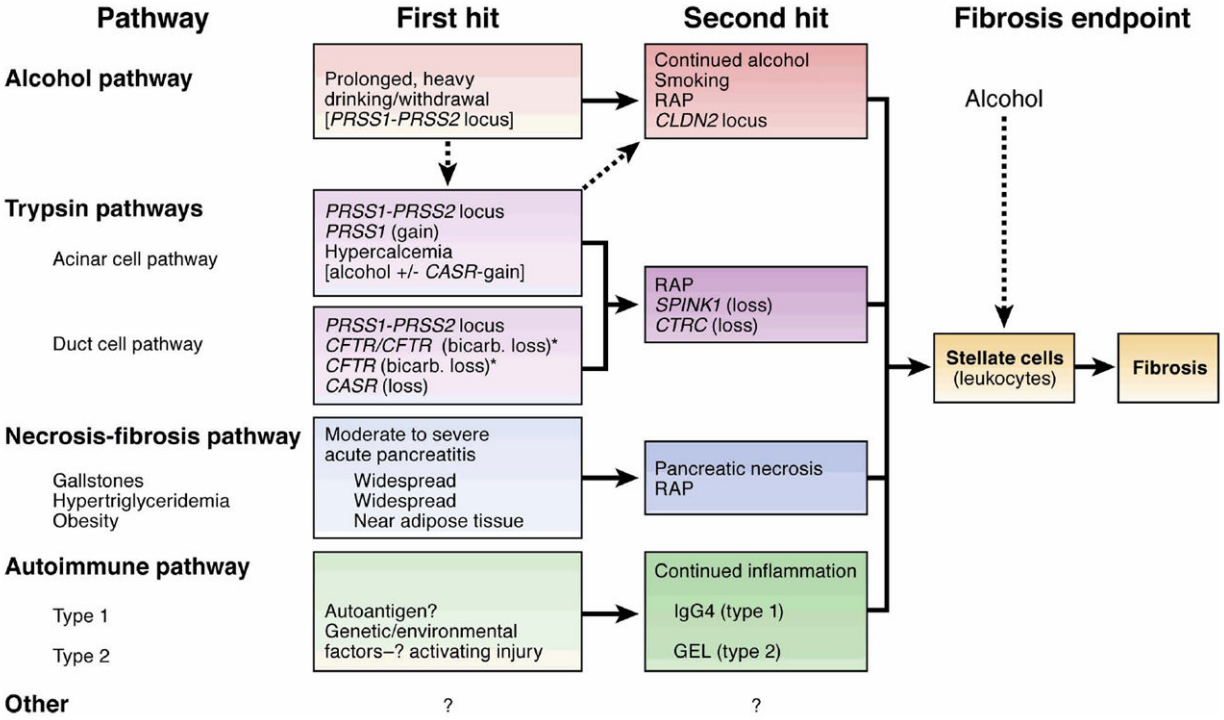

Figure 4. Factors that Contribute to Pancreatic Fibrosis

A combination of factors contribute to pancreatitis. The first hit increases susceptibility to injury, whereas the second hit affects the immune response (and includes leukocytes, such as M2 macrophages) to promote stellate cell-associated fibrosis. Alcohol could injure the pancreas via its effects on acinar cells, but it is probably more important in the second group, as a modifier of the immune response. For example it could promote a Th17-cell response, or act directly on stellate cells (dashed lines). Altered trypsin functions in acinar cells or ducts could also initiate disease. In either case, similar second hit factors then contribute to development of fibrosis (via variants in *SPINK1* and/or *CTRC*). Severe acute pancreatitis involves widespread pancreatic necrosis (about 90% of tissue), which leads directly to scaring and fibrosis in the recovery phase. Obese patients could have areas of adipose tissue that contain local necrosis of acinar tissue, which has been linked to lipotoxicity. Progressive fibrosis could occur in patients with RAP. Other pathways include autoimmune pancreatitis in which the pathway to fibrosis is less well understood.

Table 1

Genotype–Phenotype Correlations in Multi-Organ Syndromes

Genotype (variants)	Phenotype (syndrome)	Comment
PRSS1	HP	Genetic counseling recommended
PRSS1 / any	HP, worse clinical course	Genetic counseling recommended
$CFTR^{sev}/CFTR^{sev}$	CF	Manage with a CF center
$CFTR^{sev}/CFTR^{m\text{-}v}$	atypical CF	Manage with a CF center
SPINK1/ SPINK1	familial pancreatitis	Usually progresses to severe CP
$CFTR^{bicarb}/CFTR^{any}$	Pancreas/Sinus/CBAVD	Newly defined syndrome
$CFTR^{any}$/SPINK1	RAP / CP	Pancreas only
CTRC / SPINK1	RAP / CP	Pancreas only – not well studied
CASR+ / alcohol	RAP/ CP	Pancreas only – not well studied
CASR- / SPINK1	RAP/ CP, familial CP	CP in FHH and sporadic CP
CASR- / CFTR	RAP/ CP	Pancreas only – not well studied

CFTR: sev=severe mutations (typically functional class I-III), m-v = mild-variable mutations, (typically *CFTR* functional class IV), bicarb = bicarbonate conductance disrupting variant (e.g. R75Q), any= either severe, mild-variable or bicarbonate disrupting variants. CASR+, gain of function mutations; CASR-, loss of function mutations; FHH, familial hypocalciuric hypercalcemia; HP, hereditary pancreatitis