Insights into the genetic risk factors for the development of pancreatic disease

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Abstract: Diseases of the exocrine pancreas such as recurrent acute pancreatitis (RAP), chronic pancreatitis (CP) and pancreatic ductal adenocarcinoma (PDAC) represent syndromes defined according to traditional clinicopathologic criteria. The failure of traditional approaches to identify primary mechanisms underlying these progressive disorders illustrates a greater problem of failure of the germ theory of disease for complex disorders. Multiple genetic discoveries and new complex disease models force consideration of a new paradigm of 'precision medicine', requiring a new mechanistic definition of CP. Recognizing the advances in understanding complex gene and environment interactions, as well as the development of new strategies that limit or prevent the development of devastating end-stage diseases of the pancreas may lead to substantial improvements in patient care.

Keywords: pancreatic disease, precision medicine, chronic pancreatitis, genetic, modeling, acute pancreatitis, diabetes, exocrine pancreatic insufficiency

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The pancreas

The pancreas serves two primary functions. The exocrine pancreas, made up of acinar cells and duct cells, serves a central role in digestion. Acinar cells synthesize zymogens that pass into the intestines where they become active pancreatic enzymes and digest complex nutrients. Duct cells generate a sodium bicarbonate-rich juice that protects the pancreas from premature zymogen activation and neutralizes gastric acid in the duodenum. The endocrine pancreas consists of specialized hormone-producing cells within the islets of Langerhans, and plays a critical role in metabolism by switching energy systems from catabolism to anabolism in anticipation of digestion and absorption of a meal. Both the exocrine and endocrine pancreas demonstrate variable susceptibilities to noninfectious inflammation resulting in serious chronic disorders. Different physician specialists manage exocrine and endocrine disorders, resulting in narrow and limited attention to the overlapping conditions.

Acute, recurrent, and chronic pancreatitis

Acute and chronic pancreatitis (CP) are the most common disorders of the exocrine pancreas

defined as clinical syndromes, disease states defined by specific combinations of signs and symptoms, rather than being defined by underlying disease mechanisms.¹ There is broad consensus on the definition and diagnosis of acute pancreatitis, while the definition and diagnosis of CP remains controversial, especially in the early stages. The challenges and controversies surrounding the definition and diagnosis of CP resulted in the development and acceptance of a new mechanistic definition of CP,² as discussed below.

Acute pancreatitis describes a clinical syndrome of sudden onset of abdominal pain associated with an elevation of pancreatic digestive enzymes such as amylase and lipase in the blood, typically resulting in evidence of pancreatic gland edema and inflammation on abdominal imaging.³ The syndrome reflects injury from any etiology, followed by an acute inflammatory response. The syndrome is typically self-limited, although progression to systemic inflammation and organ dysfunction can be life threatening, and severe damage to the pancreas results in prolonged morbidity.

Recurrent acute pancreatitis (RAP) is defined as more than one episode of acute pancreatitis. To

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date, no explanation exists for the observation that, after the first, or sentinel acute pancreatitis event (SAPE), the pancreas appears to become sensitized to further attacks.

Most consensus conferences have defined CP by following the definition of the Cambridge conference in 1984.⁴ The participants of that conference defined CP as ‘a continuing inflammatory disease of the pancreas, characterized by irreversible morphological change, and typically causing pain or permanent loss of function’. However, many experts recognize that, based on this definition, a diagnosis of CP may be delayed for over 5 years from the onset of symptoms,⁵ and patients with inflammation and pain but without significant fibrosis (minimal change CP)⁶ may never be correctly diagnosed by these criteria. Thus, the clinicopathologic definitions of CP continued to be challenging, primarily because the diagnosis required identification of CP at an advanced stage, reflecting significant pathology. Thus, ‘early CP’ cannot be diagnosed, and presumably, early CP is the stage where diagnostic certainty is most important, and targeted treatment will be most effective.

A paradigm shift

Our ability to manage complex syndromes defined by clinicopathologic definitions and diagnostic criteria has reached its limits, and a new paradigm of disease definition and classification, based on the underlying mechanisms must replace the current approach. Genetic testing represents a new tool for determining whether a patient has underlying pathogenic variants and therefore possesses risk of developing gene-related diseases. Thus, genetic testing serves an increasingly important part of understanding and managing patients with complex pancreatic disorders, but this approach is not sufficient in isolation since risk does not indicate the presence of disease.⁷ Rather, the effects of genetic variation should be understood in the context of the many factors that contribute to the complex pathogenesis and natural history of pancreatic disease.

Western medicine has long relied on the germ theory of disease to approach and treat illness. This model sees a single pathogenic factor, like a microorganism, as the root cause of a particular disease syndrome. But in recent years, the scientific community has begun to see this framework

as inadequate to describe many complex diseases. The rise of the ‘two-hit’ hypothesis of oncogenesis marked the beginning of this shift for cancers and a similar effect can be seen as we recognize other disorders as the end results of a dynamic interaction between metabolic, environmental, genetic, and epigenetic factors.

This shift in thinking requires a new approach to diagnosis and treatment of complex disease, such as ‘personalized’, or ‘precision’ medicine. This paradigm recognizes the often complicated gene–environment interactions that produce a specific disease syndrome, allowing for a more tailored approach to diagnosis and treatment of the syndrome to manage symptoms and prevent progression to the next stage of a multi-stage disorder.

In this article we will review the genetic factors that contribute to the pathogenesis and natural history of pancreatic diseases within the context of a diverse and complex environment. We will review the mechanism of normal pancreatic physiology, leading into a discussion of the genetic changes that can produce acute and CP. We will conclude with a discussion of pancreatic ductal adenocarcinoma (PDAC) and identifying those patients at risk for developing it.

Early detection of CP

The nature of a symptom-based diagnosis like that of CP restricts the physician’s ability to recognize and treat mild or early-stage disease. It directs and limits the physician’s focus on improving symptoms rather than removing the underlying pathogenic mechanism. Because of this, a different model of thinking is needed when approaching acute and CP.

The two-hit model is useful beyond the concepts of oncogenesis. This paradigm can also be used in other disorders such as CP. A new model for pancreatic disease called the ‘SAPE’ model was developed to organize and classify risk and progression from birth to end-stage disease.^{8,9} In this model, the first hit is pancreatic injury, triggered by the premature activation of trypsin, causing widespread zymogen activation to generate pancreatic digestive enzymes within the pancreas, followed by tissue autodigestion and an acute inflammatory response. Clinicians recognize this process as acute pancreatitis, although subclinical acute pancreatitis also occurs. Acute pancreatitis

can arise independently of premature trypsin activation, as in the cases of direct trauma, exposure to toxins, or autoimmune disease. All of these scenarios involve a response to injury that normally includes inflammation followed by tissue repair. The second hit that leads to CP is a deviation from the normal course of inflammation and tissue regeneration. The inflammatory response is sustained, leading to fibrosis and other structural and functional changes to the pancreas. This can include unique response of the acinar and duct cells to inflammation, dysplasia, altered cell regeneration, or distorted anatomy.⁹ There are distinct genetic variants that can affect each of these factors and alter the course of pancreatic disease.⁹ Identifying and responding to knowledge of these factors represents the paradigm shift; one that moves away from symptom response and towards removal of key pathogenic factors to focusing on the underlying risk mechanisms.

New mechanist definition of CP

In the germ theory of disease an infectious condition was defined by the etiology (e.g. a bacteria), and the characteristics of the disorder (e.g. signs and symptoms of a urinary tract infection). The problem with this approach is that in many cases no etiology or mechanism could be identified, and the definition contained only a list of syndrome characteristics. Unfortunately, this clinical syndrome definition of a disease is woefully inadequate, since multiple disorders share the same clinical features, such as inflammation, fibrosis, organ dysfunction and pain. These clinicopathologic definitions fail to describe the underlying mechanisms, or ‘essence’ of the disorder, precluding definitive diagnostic criteria and the ability to distinguish different disorders with similar features at early stages.

A group of international experts was commissioned to develop a new, international consensus definition of CP. The result is a definition with two parts.² For the essence, ‘CP is a pathologic fibro-inflammatory syndrome of the pancreas in individuals with genetic, environmental or other risk factors who develop persistent pathologic responses to parenchymal injury or stress.’ For the characteristics, ‘Common features of established and advanced CP include pancreatic atrophy, fibrosis, pain syndromes, duct distortion and strictures, calcifications, pancreatic exocrine

dysfunction, pancreatic endocrine dysfunction and dysplasia.’ Thus, the new definition focused on the normal and abnormal response to the injury → inflammation → resolution → regeneration sequence, spread across all of the systems that affect pancreatic function, and that respond to injury and stress signals. Furthermore, it excludes fibrosis from other etiologies (e.g. desmoplastic reaction to PDAC), and allows nonfibrosis features of CP to be independently assessed and classified. This definition also opens the door to improved definition of ‘early CP’ based on a clinical and mechanistic context, and probability estimates that help guide therapies (which have their own set of risk, consequence and costs). This new definition, and framework (below) allows the components of the system to be considered and modeled based on gene × environment interactions.

Pancreatic function and specialized cells

To better understand the role of genetics in the development of acute and CP we will focus on the primary effector cells of pancreas function, the acinar cells and duct cells.

Acinar cells. The acinar cells of the pancreas produce inactive digestive enzymes, or zymogens, that are then flushed from the pancreas to the duodenum with a sodium bicarbonate-rich fluid produced by the duct cells. Trypsin is a protease that activates these other zymogens in the duodenum under physiologic conditions. Premature activation can lead to an excessive, unregulated inflammatory response in the pancreatic tissue, clinically presenting as acute pancreatitis. Thus, careful regulation of the timing and location of trypsinogen cleavage to trypsin is essential for physiologic homeostasis.

Calcium is used in the coupling of excitation–secretion of zymogen content from the acinar cells to the duct lumen. But calcium also plays a major role in the regulation of acinar cell function through its role in trypsinogen activation.¹⁰ The levels of calcium within the cells are tightly regulated by active removal from the cell using adenosine triphosphate,^{11,12} and alterations in calcium concentration can lead to alterations in pancreatic homeostasis.

Trypsinogen activity. The two most common forms of pancreatic trypsinogen are the cationic

(PRSS1) and anionic (PRSS2) forms. The cationic form is easily activated from trypsinogen to trypsin *via* cleavage of its 8-amino acid N-terminus extension that forms a calcium-binding site.^{13–16} Higher levels of calcium expectedly lead to site stabilization and cleavage of trypsinogen to its active enzyme form. Elevated concentrations of calcium in the acinar cells have been associated with trypsin activation and clinical acute pancreatitis.¹² A variant on the haplotype in the non-coding region of the *PRSS1-PRSS2* locus results in a significant reduction in the expression of PRSS1, which reduces the risk of pancreatitis.¹⁷ Conversely, mutations such as gain-of-function *PRSS1* R122H (hereditary pancreatitis) are a risk factor for acute pancreatitis and, in many cases CP by increasing the likelihood of developing RAP.^{18,19} These mutations underscore the important role of trypsinogen in the pathophysiology of pancreatitis.

Trypsinogen regulation. The typical acinar cells have multiple mechanisms to protect against this excess trypsin activation, and failure of these mechanisms can contribute to the proliferation of active trypsin. During the inflammatory response of the pancreas, *SPINK1* (a specific trypsin suicide inhibitor produced within the acinar cell), is markedly upregulated to minimize the presence of active trypsin. Defects in *SPINK1* expression or function can lead to unchecked trypsin activity. Genetic variants in *SPINK1* such as the N34S haplotype, are associated with increased risk of recurrent pancreatitis.^{20–22}

The *SPINK1* N34S high-risk haplotype is relatively common, being present in 1–3% of people in populations across the world, much higher than the prevalence of CP. It is unlikely that *SPINK1* mutations are major risk factors for SAPEs, but rather, for RAP and progression to CP. A 2010 study found that the prevalence of *SPINK1* polymorphisms in a group with a first attack of acute pancreatitis was no different than a control population. Conversely, the odds ratio (OR) for those patients with the *SPINK1* mutation to develop recurrent attacks was OR = 19.1.²³ Another study found that the *SPINK1* N34S variant increases the risk of tropical CP 19-fold, idiopathic CP, 15-fold, and alcoholic CP, 5-fold.²⁰ This suggests that *SPINK1* is important in protecting the pancreas from abnormally sustained and recurrent trypsin activity, independent of the etiology of trypsinogen

generation. Furthermore it appears that patients with heterozygous *SPINK1* mutations must simultaneously have a mutation in a susceptibility gene associated with recurrent trypsin activation [like *PRSS1*, cystic fibrosis transmembrane conductance regulator (*CFTR*) or calcium sensing receptor gene (*CASR*)] in order to be linked to recurrent acute or CP.^{24–26} Thus heterozygous *SPINK1* mutations do not cause pancreatitis, but rather they exacerbate recurrent pancreatic injury associated with trypsin activation and promote progression to CP.

Prematurely activated trypsin is also controlled by hydrolysis by another trypsin molecule, or a proteolytic enzyme activated by trypsin, called chymotrypsin C (CTRC). These proteolytic enzymes digest trypsinogen and trypsin molecules at specific cleavage sites.^{27,28} Activation and inactivation of trypsin is controlled by local calcium levels, with calcium interacting with two calcium-binding sites within the trypsinogen molecule. The first site is in the activation area, where binding of calcium facilitates trypsinogen activation to trypsin. The second calcium-binding site on the trypsin molecule regulates access of the second trypsin molecule and CTRC to their corresponding cleavage sites so that destruction is prevented. Thus, when calcium levels are low (as in the normal state in the acinar cell) the activation site is disabled and the cleavage sites exposed, resulting in slow activation and rapid destruction of trypsinogen and trypsin in the presence of trypsin activity. When calcium concentrations are high at any site for any reason, the activation site is exposed and the destruction sites blocked, resulting in unrestrained trypsin activity. Mutations in the cationic trypsinogen gene such as *PRSS1*, N29I, N29T, R122C, and R122H disrupt the trypsin destruction mechanism.^{29,30} Furthermore, loss-of-function mutations or variants in the regulatory mechanism of CTRC also affect the trypsin destruction mechanism, increasing the risk of CP.^{29–33}

Genetic mechanisms leading to pancreatitis through the trypsin activation pathway result in different effects and different types of risk. Homozygous and compound heterozygous mutations in CTRC and CASR do not appear to be sufficient to initiate recurrent acute or CP alone. Rather, these variants cluster in patients with CP who also carry variants in *PRSS1*, *CFTR*, or *SPINK1*^{25,34} suggesting that they are disease

modifiers, making mild or subclinical pancreatitis more severe. The most common variant of *CTRC*, marked by the G60G allele, is most strongly associated with progression from RAP to CP, especially in the presence of smoking.³³

The acinar cell is a protein factory, producing enough pancreatic digestive enzymes to digest all of the complex nutrients in all of the meals and snacks of the day. Recent evidence suggests that some of the mutations in the exons or intron–exon boundaries result in protein molecules with abnormal folding and structure, activating the unfolded protein response (UPR). Examples include some of the *PRSS1* mutations,^{35,36} chymotrypsinogen A1 (*CPA1*)³⁷ and likely others. The mechanism linking the UPR and CP remains speculative.

Lipase genes are also associated with CP. Carboxyl ester lipase is a digestive pancreatic enzyme encoded by the *CEL* gene. Families with a rare type of maturity onset diabetes of the young (MODY), type 8 (MODY8) also suffered from exocrine pancreatic insufficiency, suggesting that the diabetes was secondary to destruction of the islets by CP.³⁸ Another *CEL* variant also caused CP, which is a hybrid allele (*CEL-HYB*) originating from a crossover between *CEL* and its neighboring pseudogene, *CELP*.³⁹ In three cohorts of nonalcoholic CP patients the *CEL-HYB* was identified in 3.7% (42/1122) of cases and 0.7% (30/4152) of controls [OR = 5.2; 95% confidence interval = 3.2–8.5; $p = 1.2 \times 10^{-11}$]. Cellular models demonstrated reduced lipolytic activity, impaired secretion, prominent intracellular accumulation and induced autophagy, adding to the UPR model of CP.³⁹

Duct cells. The duct cells of the pancreas play an important role in the organ's function. These specialized cells secrete a bicarbonate-rich fluid that flushes zymogens from the pancreas and into the duodenum where gastric acid is neutralized and pancreatic zymogens are activated into digestive enzymes. Failure to clear the pancreas of these zymogens, linked with zymogen activation, can lead to recurrent, acute and CP.

The normal duct cell expresses luminal and intraductal molecules that regulate duct cell function. Under normal conditions the duct cells respond to neurohormonal signals on the basolateral surface to initiate ion transport, and thus

fluid secretion. The primary receptors include muscarinic acetylcholine (ACh) receptors that respond to release of ACh from post-ganglionic vagal (parasympathetic) nerves, and hormone receptors, especially receptors for secretin and vasoactive intestinal polypeptide. Second messengers from these receptors include both calcium signals and generation of cyclic AMP, which result in *CFTR* activation. The result is transcellular transport of bicarbonate molecules, with the paracellular transport of sodium and water.

Duct cells also express danger-sensing receptors like protease-activated receptor (PAR) on the basolateral and luminal membranes, especially PAR1 and PAR2 that sense trypsin activity.^{40,41} Analogously, the purinergic receptors (P2Y2, P2X4, and P2X7) sense injury and destruction of neighboring cells that release phosphorylated adenosine (e.g. ATP) or uridine (e.g. UTP).⁴²

The duct cells also express intracellular chloride receptors, such as WNK lysine deficient protein kinase 1 (WNK1). During active secretion the intracellular chloride concentrations may fall, resulting in activation of WNK1 (and other WNKs) that phosphorylates other kinases, or directly modifies multiple ion transporters, antiporters and channels, including *CFTR*.^{43,44} These intracellular receptors play key roles in optimizing duct cell function during the dynamic changes in ion concentrations while transitioning from resting to active states, and back to rest.

CFTR. CFTR is a regulated, epithelial cell anion channel. Severe mutations of the *CFTR* gene on both alleles (autosomal recessive) result in cystic fibrosis (CF) of the pancreas. The cyst and fibrosis of CF describe the pathologic appearance of the pancreas in infants that died of CF, few other consistent pathologic features are present at this early stage. Over 2000 genetic variants of *CFTR* have been identified, but the clinical and functional implications of most of these variants remain unclear. For those variants with known effects, they can be classified clinically as mild-to-severe depending on the degree of pancreatic dysfunction. On a molecular level, *CFTR* mutations can be classified based on the degree of disruption in *CFTR* function. Class I–III variants refer to severe perturbations and Class IV–V variants correspond to milder or variable dysfunction. These variants in *CFTR* determine the risk of pancreatitis in patients with CF.⁴⁵

Proper duct cell function relies on normal CFTR activity, and variants in the genes coding for *CFTR* are associated with susceptibility to pancreatitis. Prior studies from around the globe have shown that patients with idiopathic pancreatitis and some with alcohol-associated CP have higher rates of *CFTR* variations than would be expected by chance.^{25,46,47} It follows that these *CFTR* variations place patients at a higher lifetime risk of pancreatitis.

CFTR is a regulated anion channel with regard to both the open/closed state, and in anion selectivity. In 2010, Park and colleagues⁴⁴ demonstrated active WNK1 (with SPAK) caused CFTR to change from preferentially conducting chloride to preferentially conducting bicarbonate. Since bicarbonate conductance of CFTR is important for pancreatic function, as well as function of several other organs, we tested the hypothesis that genetic variants in *CFTR* that altered the ability of the CFTR channel to transform to a bicarbonate channel would increase the risk of RAP and CP.⁴⁸ Over 80 CF-causing *CFTR* variants and variants previously identified in genotyping studies of RAP or CP were evaluated in nearly 1000 CP patients from the NAPS2 study. A total of nine variants, which do not typically cause lung disease were identified. These variants were cloned into wild type (WT) *CFTR*, and electrophysiology studies confirmed normal conductance of chloride but diminished conductance of bicarbonate in the presence of WNK1-SPAK. However, the mutations were spread throughout the molecule, so the mechanism was not obvious. Construction of a three-dimensional model, with molecular dynamic simulation demonstrated four mechanisms for altering bicarbonate conductance.⁴⁸ The two primary mechanisms included physical obstruction of the channel lumen and variants altering the hinge region (Figure 1). As a further test of the clinical significance of these variants, we compared the rates of chronic sinusitis and male infertility in CP subjects with and without the CFTR bicarbonate-defective (*CFTR*^{BD}) variants. As predicted, we found an increased risk of both rhinosinusitis (OR 2.3, $p < 0.005$) and male infertility (OR 395, $p < 0.0001$).⁴⁸ The nine *CFTR*^{BD} variants provided not only important insights into a CFTR-related syndrome, but also provided critical insights into the mechanism of channel ion specificity.⁴⁹

The importance of CFTR in regulating duct function is also illustrated by the synergistic

effects of CFTR variants, which diminish bicarbonate secretion, with other factors that either diminish generation of pancreatic juice flow or increase distal duct resistance. The most important examples are combined pathogenic *CFTR* variants and smoking,⁵⁰ and pancreatitis with pathogenic *CFTR* variants and pancreas divisum.^{51–53}

The example of pathogenic *CFTR* variants and pancreatitis illustrates the importance of the duct cells for maintaining pancreatic homeostasis. Indeed, disruption of normal activity in either acinar or duct cell types can lead to clinical pancreatitis. In complex disorders such as RAP and CP, it appears that combinations of risk factors plus stressful events are the etiological rule rather than the exception.

Environmental risk factors for RAP and CP

The TIGAR-O etiological list was designed to identify all of the major risk factors for recurrent acute and CP within a single individual so that synergistic combinations and subtypes of etiologies could be recognized.¹ The TIGAR-O list classifies the common risk factors by etiologies and frequency beginning with Toxic metabolic factors (e.g. alcohol, smoking, triglycerides, hypercalcemia), Idiopathic (young and old), Genetic (monogenic and complex) Autoimmune, Recurrent or severe acute pancreatitis, and Obstructive. This approach continues to be valuable for classifying patients, and considering treatments. Here, we will briefly review alcohol and smoking.⁵⁴

Alcohol. Acute alcoholic pancreatitis remains uncommon among most heavy drinkers, and progression to CP is seen in <3% of heavy drinkers.⁵⁵ However, depending on the study and sophistication of diagnostic techniques, the majority of patients with CP were believed to have an alcohol etiology.

The link between alcohol and pancreatitis is complex. Heavy alcohol use results in morphologic changes in the pancreas, but this ‘pancreatopathy’ appears to be different than CP. According to the SAPE Model,^{8,9,54} the immune system must first be activated, resulting in immune-mediated damage. Indeed, patients with ‘true’ alcoholic CP have RAP and multiple changes of CP, including gross morphologic changes and pain.^{56,57}

Figure 1. Molecular modeling and simulations of CFTR WT and variants.⁴⁸

The WT CFTR dimer is seen from the top (panel a) and bottom (panel b) views with the nucleotide-binding domains and membrane-spanning domains labeled and the lipid bilayer seen as the color grey. The black represents subunit 1 of CFTR with residues 1–859 and the blue represents subunit two with residues 860–1480. The red are the CFTR variants that were studied. Residues R170 and L967 are the hinge region. The role of R117 remains unknown. Panels c–f demonstrate the simulations comparing channel diameters of WT and mutants, with channel diameter decreasing at the pore region near the L997F amino acid substitution.

CFTR, cystic fibrosis transmembrane conductance regulator; WT, wild type.

In 2009, three studies suggested that alcohol consumption was only associated with the risk of pancreatitis after a rather large threshold was exceeded.^{58–60} These data suggested that a threshold of >5 drinks per day was required to link alcohol with an increased risk of developing acute and CP. Taken together, these observations

suggest that alcohol may be a weak susceptibility factor (first hit) but a strong modifier factor (second hit).⁹ In rat studies we found that low and high-dose alcohol feeding had no gross morphologic effects on the pancreas, although evidence of mitochondrial damage and metabolic stress was present.^{61,62} Instead, 1, and especially >1

episode of AP (e.g. RAP) markedly changed the effects of alcohol on the pancreas, resulting in rapid progression to CP,⁶² and changed the response to injury to favor inflammation-associated pancreatic necrosis rather than fibrosis.⁶³ Studies from Finland provided further insights, by demonstrating that in the majority of patients with their first attack of acute alcoholic pancreatitis, the symptoms begin during the early withdrawal period.⁶⁴ This also matches our rat studies that demonstrated pancreatic hyperstimulation during the alcohol withdrawal period,⁶⁵ with hyperstimulation being a primary mechanism of triggering pancreatitis.

In 2012, the first pancreatitis genome-wide association study identified a major risk factor for alcoholic CP. There is a particular genetic locus that has been associated with a significant increase in the risk of alcoholic RAP and CP. The *CLDN2* risk allele T is an X-linked variant seen in 25.8% of control men and 6.9% of control women.¹⁷ In those patients with nonalcohol-related pancreatitis, there was a single-copy frequency of 38.5% (26% expected frequency) in men and a homozygous frequency of 10% in women (7% expected frequency). With a 16% prevalence of at-risk alcohol consumption in males and a 25.8% prevalence of *CLDN2* risk allele T, only 4% of men would be expected to have both at-risk levels of alcohol consumption and the risk allele. However, the *CLDN2* risk allele T was found in 47.6% of men with alcohol-associated CP. This finding has been replicated in other populations from Europe, India and Japan.^{66–68} The X-linked nature of this risk allele may partially explain the higher prevalence of alcoholic pancreatitis among men compared with women.

If alcohol is a driver of the progression from AP to CP, then alcohol cessation should slow or stop the process. Several reports in human studies suggest that this hypothesis is true. In Finland^{69,70} and Japan,⁷¹ the cessation of alcohol appears to alter the natural history of alcoholic pancreatitis. Thus, major efforts to address the use of alcohol immediately after the first episode of acute pancreatitis appear justified.

Smoking. Multiple studies over the past 20 years demonstrated that smoking increased the risk for CP.^{72–75} Unlike alcohol, which had a threshold for effect, smoking appears to confer a dose-dependent effect.^{54,58,76} Also unlike alcohol, the risk of

smoking on the pathogenesis of CP was under-recognized by physicians until recently.⁷⁷

The effects of smoking on CP are significant, but the effect of both alcohol and smoking are synergistic risk factors.⁵⁸ Furthermore, recent genetic studies demonstrated the pathogenic variants in the *CTRC* gene (G60G haplotype) markedly increase the risk of progression from RAP to CP, and the primary effect appears to be in smokers.³³ As with alcohol cessation, early smoking cessation appears to be of benefit to the patients with early pancreatitis.⁷⁵

Clinical implications

Pancreatic disease is uncommon and likely requires a random event to trigger pancreatic inflammation and initiate the destructive pathway. Thus, screening of asymptomatic people for pathogenic variants is not recommended, with the exception of a few cases under the direction of a genetic counselor. Indeed, the complexity of the genetic basis of RAP, CP and their complications requires new approaches to counseling.^{78,79}

The detection of pathogenic genetic variants in patients with RAP or features of CP serves multiple purposes in helping to manage clinical patients. The most mature area is detection and classification of Mendelian disorders. This is important for at least two reasons. First, it provides clear answers as to the etiology of nonspecific signs and symptoms of abdominal or pancreatic diseases, thus focusing attention on the correct diagnosis and precluding a prolonged, inconvenient, invasive and expensive workup of many possibilities in a differential diagnosis. Secondly, determining that a patient has a specific syndrome, often detected initial by pancreatic dysfunction, directs the clinician and patient to well defined disease evaluation and management plans, such as referral to a CF center for definitive diagnosis with sweat chloride, and immediate treatment regimens targeted and optimizing nutrition and minimizing pulmonary disease and other manifestations. Finally, comprehensive and high quality gene sequencing panels that include detection of copy number variants can nearly rule out major disorders in the majority of patients with ambiguous signs and symptoms.

Testing for the major Mendelian pancreatic syndromes such as hereditary pancreatitis and atypical CF identifies the etiology of pancreatic disease in a

Figure 2. Conceptual model of CP progression. A total of five progressive states are represented. (A) At-risk patients appear completely normal, and can remain so for years. A random (stochastic) injury triggers the inflammation (SAPE) resulting in (B). Acute pancreatitis. Mild AP completely resolves while more severe AP results in some changes suggestive of (C). Early CP. While AP or early CP may appear to resolve, the pancreas is markedly more sensitive to episodes of AP (RAP, dashed line). (D). Established CP requires two or more systems within the pancreas to develop inflammation-associated, 'permanent' morphologic and/or functional damage. (E). End-stage CP one or more systems fail, requiring supportive treatment (enzyme replacement, insulin replacement) or radical interventions (partial or total pancreatectomy for pain) or PDAC. T3cDM, diabetes mellitus from destruction of the islet cells from inflammation.

AP, acute pancreatitis; CP, chronic pancreatitis; DM, diabetes mellitus; PDAC, pancreatic ductal adenocarcinoma; RAP, recurrent acute pancreatitis; SAPE, sentinel acute pancreatitis event.

minority of adult patients. In pediatric patients the likelihood of finding major pathogenic genetic variants is much higher, in part environmental etiologies (excessive alcohol and cigarette smoking) is uncommon, and because the strong effect of the mutations results in early age of onset. Recently Kumar and colleagues⁸⁰ reported that among 301 children in the INSPPIRE study cohort,⁸¹ at least one gene mutation in pancreatitis-related genes was found in 48% of pediatric patients with RAP and 73% of patients with CP. We expect that the genetic burden is even higher since key genes such as *CPA1*, *CEL*, *SBDS*, *CASR*, *GGT1*, *UGT1* and others were not included. Early evidence of the major contribution of pathogenic variants to CP is provided by new whole exome sequencing experiments. Evaluation of 70 genes linked to calcium regulation, enzyme processing and other pathways revealed significant mutations in 80% of patients with 'idiopathic CP'.⁸² While many of these findings await replication and functional studies, future directions are clear.

Genetic testing in both pediatric and adults is clinically useful and valuable for additional reasons. The new mechanistic definition of CP provides a rationale for considering multiple contextual variables to help determine the probability that RAP or features of early CP are arising from a known mechanism of acinar- or duct-associated

pancreatitis susceptibility mechanism in patients with signs and symptoms of pancreatic inflammation. The mechanistic definition was presented in the context of a conceptual model of the progressive nature of CP over a lifetime, beginning with no pancreatitis but 'at risk' (stage A) and progressing to 'end-stage CP' (stage E), Figure 2. Thus, at any point in time the patient is in one, and only one stage. The inflammatory process begins with injury, typically (but not always) resulting in acute pancreatitis (stage B). Stage C represents the beginning of true CP, but can only be established with certainty retrospectively.⁸³ Currently, CP is established with 'irreversible' clinical, morphological and functional changes to the pancreas (stage D). Genetic testing is most valuable in stages B–D where disease mechanisms can be identified, and serve to guide treatment targets aimed at preventing progression. The combination of risk factors (e.g. present in stage A, especially genetic risk) and immune system activation (stage B) with symptoms of ongoing inflammation or early morphologic changes suggestive of fibrosis or established CP can be used to establish a much higher probability that the patient has early CP (stage C), and initiate treatments to minimize progression.

Recognizing the patient's current stage (and previous stages at specific times), disease activity and

factors driving progression provide context to the patient's state and should, in the near future, provide a rationale for treatment strategies (precision medicine). The addition of susceptibility factors associated with specific features, such as fibrosis, pancreatic exocrine insufficiency, diabetes mellitus, pain syndromes and cancer will provide data to evolving disease models and predictors of outcomes in the future, with or without specific therapies.

Pancreatic cancer

PDAC represents the most feared acquired pancreatic disease. As with CP, the etiology appears to be complex, with multiple gene–environmental interactions rather than any major risk factor yet to be discovered. It is clear that CP is among the strongest risk factors for PDAC,^{84–86} especially longstanding inflammation, as found in hereditary pancreatitis.^{85,87} Furthermore, CP and PDAC share risk factors; smoking tobacco confers a two-fold relative risk for PDAC^{84,88,89} and alcohol increases the likelihood by a factor of ~1.5.⁹⁰ The late age of onset also suggests that multiple random events must occur as part of the oncogenic process, and perhaps in a certain order. It is hypothesized that, over a lifetime, inflammation, toxin exposure, and failed DNA repair together result in the progressive accumulation of somatic mutations in pancreatic cells that can ultimately lead to PDAC. This can be further enhanced by pre-existing oncogenic germline mutations. A review of the specific risk factors, the evidence that they contribute to PDAC, issues of missing hereditary and a model of risk factor interactions leading to cancer has recently been published.⁹¹ From the standpoint of clinical practice, familial risk for pancreatic cancer must be highlighted.

Familial risk for PDAC

At least 5–10% of PDACs can be attributed to a pathogenic sequence variant in familial cancer genes.⁹² Common features that should make the clinician suspicious for a hereditary cancer predisposition include: early onset before 50 years of age, multiple primary cancers, multiple affected family members, particular ethnicities (e.g. Ashkenazi Jewish descent), and unusual or rare malignancies, like male breast cancer. Obtaining a careful family history is essential in assessing a patient's risk for a predisposition to pancreatic cancer. According to recent guidelines, an individual is considered at risk of PDAC if they have:

an identified genetic syndrome that is known to be associated with PDAC; two or more relatives with PDAC with at least one being a first-degree relative; or three or more relatives with PDAC.⁹³ If there are one or two affected first-degree relatives, this confers a 4–7% risk for developing PDAC in a familial pancreatic cancer kindred. With three or more first-degree relatives, this increases the risk to 17–32%.^{94,95} The management of these patients remains challenging, and these families should consider evaluation by expert centers where new screening approaches and other approaches typically first become available before becoming a part of standard practice.

Conclusion

Pancreatic disease, including AP, RAP and CP, as well as PDAC, results from a complex interplay of genetic predisposition, environmental exposures and random stressful events. The germ theory of medicine for pancreatic disease has failed. As we begin to transform our understanding of pancreatic disease for a traditional clinicopathologic framework to modeling and simulation of personalized 'precision medicine', we will need to develop new disease definitions, diagnostic criteria, and therapeutic approaches to limit or prevent these disorders. This process has already begun. An appreciation of advances in understanding the genetic basis of pancreatic diseases and an anticipation of effective diagnostic and treatment strategies represents the next major step in controlling these complex pancreatic disorders.

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Conflict of interest statement

DCW serves as a consultant to AbbVie, Ariel Precision Medicine (Pittsburgh, PA) and Regeneron (Tarrytown, NY). He is a cofounder of Ariel Precision Medicine.

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