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Chronic Pancreatitis in the 21st Century - Research Challenges and Opportunities:

Summary of a National Institute of Diabetes and Digestive and Kidney Diseases Workshop

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Abstract

A workshop was sponsored by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) to focus on research gaps and opportunities in chronic pancreatitis (CP) and its sequelae. This conference marked the 20th year anniversary of the discovery of the cationic trypsinogen (*PRSS1)* gene mutation for hereditary pancreatitis. The event was held on July 27, 2016, and structured into 4 sessions: (1) pathophysiology; (2) exocrine complications; (3) endocrine complications; and (4) pain. The current state of knowledge was reviewed; many knowledge gaps and research needs were identified that require further investigation. Common themes included the need to design better tools to diagnose CP and its sequelae early and reliably, identify predisposing risk factors for disease progression, develop standardized protocols to distinguish type 3c diabetes mellitus from other types of diabetes and design effective therapeutic strategies through novel cell culture technologies, animal models mimicking human disease, and pain management tools. Gene therapy and cystic fibrosis conductance regulator *(CFTR)* potentiators as possible treatments for CP were discussed. Importantly, the need for chronic pancreatitis endpoints and intermediate targets for future drug trials was emphasized.

INTRODUCTION

Chronic pancreatitis (CP) is a syndrome defined by a combination of signs and symptoms that reflect destruction of the pancreas by an inflammatory process. Recent advances in understanding the pathobiology of CP, and the application of old and new models, provide new insights. The NIDDK workshop, *Chronic Pancreatitis in the 21st Century: Research Challenges and Opportunities* was designed to consolidate and integrate new knowledge, and set a course into the future. This effort is in line with other national and international initiatives to develop a mechanistic definition of CP, and develop a framework for future studies.

Historically, the clinical diagnosis of CP was based on the triad of calcifications of the pancreas on imaging, steatorrhea and diabetes mellitus. The diagnosis required evidence of irreversible, end-stage disease.[1–4] Therefore, early diagnosis and etiology-based treatments were not possible. The demonstration that hereditary pancreatitis was caused by a mutation in the cationic trypsinogen gene (*PRSS1*) in 1996 provided a conceptual breakthrough[5]. This discovery led to understanding of at least one type of non-alcoholic pancreatitis, and the opportunity to follow the natural history of disease from acute pancreatitis (AP), to recurrent acute pancreatitis (RAP) and CP. Complications of CP are significant and include exocrine pancreatic insufficiency (EPI), type 3c diabetes mellitus (T3cDM), pain syndromes, and pancreatic cancer.[6–8]

To address the problem of diagnosing CP at an earlier stage, a new "mechanistic" definition of CP was developed by an international group of experts.[9] The new definition of CP has two parts representing the essence and the characteristics of the diseae. First, the essence, c*hronic pancreatitis is a pathologic fibro-inflammatory syndrome of the pancreas in individuals with genetic, environmental and/or other risk factors who develop persistent pathologic responses to parenchymal injury or stress"*. Second, the characteristics, "c*ommon 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"*. This mechanistic definition recognizes the complex nature of CP, separates risk factors from disease activity markers and disease endpoints, and allows for a rational approach to early diagnosis, classification, prognosis and personalized therapy.

The mechanism of CP is linked to a disease model, with five disease stages: A) At risk; B) AP-RAP; C) Early CP; D) Established CP; E) End-Stage CP. It further directs attention to critical systems and cell types that respond differently to injury and inflammation in different people, including acinar cell, duct cell, stellate cell, islet cell, nervous system and DNA repair system. This framework will enable experts to extend the definitions of CP to specific stages by defining the essence of each stage, characteristics, inclusion criteria, and biomarkers of the disease state and disease activity. These states represent the condition of the pancreas, not the etiology, modifying factors, disease trajectory or effects of protective factors or potential treatments. Thus, it can allow data harmonization between individuals with various etiologies, disease states, and predicted outcomes.

The NIDDK workshop comes at a critical time, when a series of international meetings, sponsored by the major pancreas societies, systematically address these issues. Each lecture and session provided expert perspective on one or more stages and conditions of CP, which can now be placed into a well-defined mechanistic framework.

PATHOPHYSIOLOGY OF CHRONIC PANCREATITIS

Overview of the problem

The pathophysiological mechanisms of CP are not well understood. Despite uncertainties about the molecular details underlying the pathophysiology of CP, it is clear that CP is a complex disease. As mentioned above, abnormal genes clearly contribute to the pathogenesis of CP. They contribute to the risk of developing disease and likely contribute to the variability in the clinical course observed in patients with CP. Much evidence suggests that environmental factors also influence the onset and course of CP. A complete understanding of the pathophysiology of CP requires a full delineation of all elements contributing to the disease. Therapy directed at CP will require intermediate and terminal endpoints acceptable to the Food and Drug Administration (FDA) to measure effectiveness of medical treatment interventions.

Competing Models of CP Pathophysiology

Over the years, many competing mechanisms have been proposed. The trypsin-dependent theory was first suggested by Hans Chiari in 1896 who observed inappropriate activation of digestive enzymes within the pancreas, leading to destruction of the gland[10]. Premature trypsin activation has been found in all models of pancreatitis as well as human samples of pancreatitis. However, demonstration of premature trypsinogen activation in the pancreas does not necessarily establish causality for CP.

The identification of a trypsinogen (*PRSS1*) mutation pR122H and pI21N in hereditary pancreatitis in 1996[11] further supported the trypsin-dependent theory. Since then, more than 30 additional *PRSS1* (cationic trypsinogen) mutations have been reported in the context of pancreatitis.[12] These gain-of-function mutations result in the production of trypsinogen or trypsin that is resistant to degradation or autolysis. While a delay in degradation of trypsinogen has no apparent detrimental effect, the delay in autolysis of trypsin can potentially cause autodigestion of pancreatic tissue leading to pancreatitis.[13–16]

Current models have not confirmed increased intra-acinar trypsin activation as the disease-causing mechanism of the mutations associated with hereditary pancreatitis. In transgenic mice, intracellular activation of trypsinogen led to acute but not chronic pancreatitis.[17] The role of trypsin in pancreatic inflammation is further challenged by the work in trypsinogen-7 gene (T−/−) knockout mice.[18] The model explores the role of intra-acinar trypsinogen activation and allows the investigation of potential trypsin-independent pathways and their roles in CP. In $T^{-/-}$ mice, pathologic trypsinogen activation was important in initial pancreatic injury but was not a pre-requisite for CP. In this model, nuclear factor kappa beta (NFκB) activation played an important role in CP and occurred independently of trypsinogen activation. A study by Marrache et al[19] further strengthens the role of NF-kB in CP when human interleukin-1β (IL-1 β) is overexpressed in murine pancreas. IL-1β is a known downstream target of the NF-kB pathway. The results of this study demonstrates that overexpression of IL-1β in the murine pancreas leads to development of CP, highlighting the potential importance of IL-1β/NFkB signaling in the pancreas.

Endoplasmic reticulum (ER) stress may also be sustained in CP, independent of trypsin.[18] T −/− mice, which lack intra-acinar trypsinogen activation, had comparable levels of the unfolded protein response (UPR), indicating a minimal role of trypsin in ER stress during CP. ER stress leads to activation of the UPR components: activating transcription factor 4 (ATF4), CCAAT/enhancer binding protein (C/EBP) homologous protein (CHOP), 78 kDa glucose-regulated protein (GRP78), and X-box binding protein-1 (XBP1). Therefore, although trypsin activation or persistence has an important role in initiating the pancreatitis cascade, other factors may be contributing to continuing or recurrent inflammatory events which result in CP. Further investigations are greatly needed to define the mechanistic role of trypsin in CP.

Genetic Influences and the Value of Testing

The mechanism whereby genetic factors contribute to the onset and course of disease remains unclear. The *PRSS1* mutations associated with hereditary pancreatitis are hardly

ever detected in the general population and convey an autosomal dominant inheritance pattern of pancreatitis (with 80% penetrance). Other genetic mutations associated with pancreatitis must be treated quite differently. They represent mere risk factors without Mendelian inheritance and a significant percentage of the healthy population will carry these single nucleotide polymorphisms without ever developing pancreatitis. Among patients, the proportion of carriers is simply higher[20]. These genetic changes affect the genes for carboxypeptidase A1 (*CPA1*, the risk ratio [RR] for which increases 30-fold but prevalence is rare), the pancreatic secretory trypsin inhibitor *SPINK1* (20-fold and common), carboxyl-ester lipase (*CEL*, 6-fold and rare), chymotrypsinogen C (*CTRC*, 5-fold and common), the cystic fibrosis transmembrane conductance regulator (*CFTR*, 2–3-fold and common), the blood type ABO locus (2,7-fold for blood type B versus O and common) and the calcium sensing receptor (*CASR*, 1.9-fold and common).

Because some of the identified genes that increase risk for CP have potential interactions with trypsin, their identification has been used to support trypsin-dependent models of CP pathophysiology. For instance, polymorphisms in *SPINK1* and *CFTR* in alcoholic and non-hereditary forms of CP are associated with increased trypsinogen activation. Furthermore, many of these genes have additional functions that may explain their role as a risk factor. In particular, *SPINK1* may be involved in basic cellular processes such as cell death regulation[21], autophagy[22], growth[23] and inflammation[24], other than trypsinogen activation.

The motivation to test for these genetic risk factors is often born out of a) scientific curiosity, b) to dispel the allegation of underlying alcohol abuse, c) to find an explanation for pancreatitis in a child or young adult with no apparent risk factors, or d) to assess the risk for developing pancreatic cancer (70% in hereditary *PRSS1*-mutation-associated pancreatitis versus, 1–2% in sporadic pancreatitis). Currently, knowledge of the genetic mutations has little effect on treatment choices and are therefore not generally recommended in routine care. *PRSS1* mutations are an exception because of the high cancer risk; patients should be tested with unexplained pancreatitis under the age of 25 and/or when they have first degree relatives with pancreatitis[25]. Although knowledge about the presence or absence of these genetic factors holds great promise for personalized medicine approaches, they can and should be collected in IRB-approved research studies.

Environmental Risk Factors

Even in patients with genetic risk factors, environmental factors contribute to the overall risk and course of disease. Several studies have described the risk and environmental factors affecting the progression from AP to RAP and CP[9,26–29]. Data from experimental studies support these observations, suggesting that AP, RAP and CP likely represent a disease continuum. These observations are important for clinical management and research opportunities, by offering avenues for primary and secondary prevention.

As mentioned above, a new definition for CP has been proposed, which provides a framework to better define this relationship from a translational and mechanistic standpoint. This understanding may open doors for detection of disease at an earlier stage, or therapies that may alter or prevent disease progression, beyond the traditional recommendations for avoidance of exposure to alcohol and tobacco.

In the past few years, several advances have been made in our understanding of the epidemiology and role of environmental factors in the pathophysiology of CP. The epidemiology of CP seems to be changing, likely related to earlier detection of disease from increased utilization and improvements in imaging techniques and a better understanding of the role of genetic factors. Estimates for CP are available from some populations, but from other populations it is important to provide a context of geographic variability and its reasons. In two meta analyses, the role of alcohol in susceptibility to pancreatitis has been examined. While this relationship at higher levels is unquestionable, the role of lower amounts of drinking remains unclear. A recent study reported provocative data that smaller amounts of alcohol consumption even may be protective - a finding that needs to be further examined[30]. The role of smoking in pancreatitis is well established, and smoking cessation may prevent or slow disease progression.

Fibrosis: Pathophysiology, Prevention and Reversal

The development of novel therapies requires knowledge about the factors that determine the clinical and pathological course. Fibrosis, the progressive accumulation of excess extracellular matrix proteins within the parenchyma of the pancreas, is a defining feature of CP and understanding the pathophysiology of fibrosis is key to developing new therapies. As in other organs, progressive accumulation of fibrosis compromises organ function, impairs regeneration and predisposes to the development of cancer. In the case of CP, pancreatic fibrosis also compromises ductal patency and secretion and contributes to chronic pain.

A facile mouse model of CP based on repeated acute injury from caerulein results in substantial pancreatic fibrosis and organ atrophy over 10 days in female C57BL/6 mice. This model was used to test the ability of a novel integrin inhibitor to block or reverse fibrosis. Integrins are heterodimeric transmembrane proteins that interact with extracellular matrix components. The αV-β1 integrins participate in the extracellular conversion of latent TGF-β1 into active TGF-β1, a key cytokine responsible for activation of pancreatic stellate cells (PSCs) which are responsible for fibrogenesis.

Treating mice with the αV integrin inhibitor CWHM-12 both prevented pancreatic fibrosis and reversed established pancreatic fibrosis in the repetitive injury model[31]. An inactive stereoisomer had no effect. The drug did not reduce acute injury or prevent the effects of repetitive injury on pancreatic atrophy and thus the effect appeared to be fairly specific at preventing fibrogenesis and reversing fibrosis. Cell culture experiments provided mechanistic confirmation that PSCs convert inactive TGF-β1 to the active form and that this activation is inhibited by CWHM-12.

Research Gaps and Opportunities

Future research should be directed at better understanding the contribution of genetic variants in pancreatic inflammation, development of CP and its complications and role of environmental risk factors in this process with the goal to test hypotheses in several clinical and pre-clinical models using solid end-points. Specific priorities for research in regards to improving our understanding of the pathophysiology of CP include:

- There is an urgent need to develop or validate animal models that recapitulate human disease, particularly disease associated with genetic polymorphisms. The availability of reliable, validated animal models will facilitate the evaluation of drugs for treatment of fibrosis, and prevention of CP.
- To assess the utility of new therapies, clinically relevant end points and intermediate drug targets are needed.
- Gaps in the epidemiology of RAP and CP need to be addressed. Estimates for the incidence, and health and economic burden of RAP and CP are limited and have not been studied in large parts of the world.
- Occult or subclinical CP, as likely present in patients with diabetes, needs to be defined and better methods for diagnosis need to be developed.
- There is opportunity to quantitate the individual susceptibility from defined genetic risk factors in large scale genetic studies of existing and new longitudinal cohorts.
- Efforts to identify genetic risk factors by non-candidate gene approaches (i.e. functional genomics) should be undertaken.
- The pancreatic cancer risk associated with individual genetic pancreatitis predispositions should be assessed in well-characterized cohorts.
- Biomarker strategies for cancer prevention and early detection in pancreatitis patients need to be prioritized.

EXOCRINE COMPLICATIONS OF CHRONIC PANCREATITIS

Overview of the problem

The normal pancreas produces more than 900,000 USP units of lipase with each meal, along with much larger amounts of various proteases and amylase. Exocrine pancreatic insufficiency (EPI) and steatorrhea occurs when less than 10% of this amount is present and active in the duodenum. A number of pancreatic conditions are commonly associated with EPI: CP, cystic fibrosis (CF), loss of pancreatic tissue due to resection or necrosis, pancreatic adenocarcinoma, pancreatic duct obstruction, and other rarer conditions. EPI may also be associated with acute or acute relapsing pancreatitis without necrosis, longstanding diabetes, advanced age, malnourished individuals, previous intestinal surgery, and duodenal mucosal diseases. Controversial associations include non-alcoholic steatohepatitis (NASH), irritable bowel syndrome (IBS), smoking, chronic renal failure, unexplained osteoporosis, and others.

There is a great need to assess and accurately diagnose EPI before end-organ damage develops in conditions such as RAP. Current methods can only diagnose advanced disease and lack the ability to predict disease progression. Standardization of current tests and adoption of standard protocols are needed. Furthermore, there are no drugs that can slow or retard disease progression. Recent advances in CF treatment and knowledge of the

interaction of ductal and acinar cell secretion hold promise for targeted therapeutic intervention.

Assessing Acinar and Duct Cell Function: Gaps and Opportunities

Two related diagnostic challenges exist. The first involves accurate diagnosis of early CP prior to the development of EPI, while the second is the accurate and convenient diagnosis of EPI in more advanced CP and other pancreatic conditions associated with EPI. The diagnosis of EPI requires the documentation of maldigestion, along with evidence that reduced pancreatic function is the cause of maldigestion. The documentation of maldigestion with a 72-hour fecal fat analysis is not feasible in the outpatient setting. While breath tests (C^{13} or C^{14} mixed triglyceride) of digestion have shown some promise[32], they remain largely unavailable. Tests of basal pancreatic secretion of enzymes (fecal elastase or serum trypsin) are prone to error[33]. Hormone-stimulated tests of bicarbonate or enzyme secretion, requiring tube or endoscopic collection, are most suited for early diagnosis[34], but have not gained widespread acceptance and are only available at a few centers. There is a need for the development of new and simple diagnostic tests that document maldigestion, acinar and duct cell secretion and function under stimulated conditions.

Mechanisms and Biomarkers for Progression from Recurrent Acute Pancreatitis to Chronic Pancreatitis

The detection of early-stage CP remains elusive due to a poor understanding of pathogenic mechanisms and scarcity of morphological changes on radiologic and endoscopic imaging. Ideally, the diagnosis of CP should be made prior to the development of EPI. This task is difficult, given the lack of a specific diagnostic feature[9], and by the frequent development of pancreatic fibrosis in subjects without clinical pancreatitis (such as the aged or those with longstanding diabetes). Detection of early-stage CP before irreversible damage represents a necessary step for any intervention aimed at modifying or retarding disease progression. This is a major gap in our current knowledge. There is also a critical need to identify appropriate patients who might be considered for therapies such as total pancreatectomy with islet auto-transplantation (TP-IAT). As pancreatic biopsy is not a viable option, early diagnosis currently largely rests on imaging and endoscopic testing. Current imaging techniques, primarily MRI/MRCP with secretin stimulation, lack sensitivity and specificity[34]. Endoscopic imaging with EUS does not appear to have sufficient accuracy for early diagnosis[35]. Pancreatic function testing (tube-based or endoscopic-based) under supra-physiologic stimulation remains the most accurate test for early CP[34], but is too complex for routine use.

Recent experimental efforts using refined biospecimen processing workflows have explored molecular signatures in proximal biological fluid (pancreatic juice, duodenal aspirate) and in more systemic/distal biological fluids such as urine and blood. These more easily obtainable distal biological specimens would be ideal for screening for CP followed by confirmatory testing, if needed, in proximal pancreas fluid.

Thus, current research using biomarkers obtained in serum, pancreatic juice, or urine hold significant promise to develop panels of markers that are easily available, more accurate, and

with the ability to rule out disease in those with low-moderate pre-test probability[36]. Future research should focus on the development of more accurate, useful, and innovative approaches for biomarkers that detect early disease, likely using hormone-stimulated conditions and collection of serum or urine rather than pancreatic secretions.

Pancreatic enzyme replacement therapy: treating exocrine pancreatic insufficiency and beyond

After the diagnosis of EPI in patients with CP or other pancreatic conditions (e.g. after pancreatic surgery), there is widespread under-utilization of pancreatic enzyme replacement therapy (PERT)[37,38]. The frequency and impact of EPI in the other non-pancreatic conditions noted above, and the potential benefit of PERT, remain unknown. The clinical impact of inadequately treated EPI includes malnutrition and osteoporosis, as well as fat soluble vitamin and other vitamin and trace element deficiency[39,40]. Even in those prescribed PERT at the proper dose and timing, maldigestion may not be reversed owing to the relative ineffectiveness of current porcine products. Future research will require better diagnostic tests for CP and for EPI; to be able to identify those at risk for EPI, identify barriers to effective use of PERT, identify complications of maldigestion, define the role of diet, and develop more effective enzyme products[41].

New approaches to restoration of pancreatic secretion

CP damages the exocrine pancreas resulting in impairment of both acinar cell enzyme secretion and duct cell water and bicarbonate secretion. Therefore, restoration of pancreatic secretion will only be possible via restoration of cells and/or proteins that control the secretory process. This requires a better understanding of the interactions between the exocrine and endocrine pancreas. Recent studies, using CFTR correctors, demonstrate proof of principal that restoration of secretion is possible[42–44]. Novel and minimally invasive gene manipulation techniques developed in animal models can be translated to humans[45]. Finally, cell based therapies and CRISPR/Cas9 technology explored in animal models of diabetes and pancreatic cancer[46–49], may prove to be useful in CP. Research focused on preservation of acinar and ductal cells, and restoration of function, will likely involve co-culture models, better mechanistic understanding of the role of inflammatory, immune, stellate, and stem cells, genetic manipulation, and the design of novel therapies that target individual cell populations.

Research Gaps and Opportunities

Future research should be directed at improving diagnostic methods for early detection and prognostication of CP, accurate diagnosis and treatment of EPI secondary to CP, nutritional monitoring and novel approaches to restore acinar and duct cell function in CP. Specific research priorities include the following:

- Improve methods for reliable, reproducible, and accurate assessment of maldigestion and EPI.
- Establishment of simpler, less-invasive tools to measure acinar and ductal cell function from more easily obtained biological specimens such as urine or blood to screen for pancreatic disease.

- Develop RAP and CP biomarkers that can be used to better define the stage, determine prognosis, assess severity and stratify patients for medical or surgical intervention utilizing the mechanistic definition framework.
- Evidence-based recommendations for proper dietary intake and the requirements for PERT (initiation, dose, timing, follow-up).
- Develop enzyme products requiring fewer pills and with better compliance and potency.
- Develop long-term primary acinar and ductal epithelial cell culture models.
- Explore co-culture models (e.g., acinar-duct; duct-islet; acinar-islet) to identify factors which regulate exocrine cell function and restitution.
- Define mechanisms by which gene mutations/variants cause pancreatic inflammation, ductal cell malfunction and acinar cell loss.
- Design novel therapies that target restoring pancreatic acinar cells and/or manipulate ductal cells (i.e. gene and cell-based therapies, CRISPR/Cas9, CFTR correctors and potentiators).
- Develop experiments to determine the critical age and time for intervention to reestablish appropriate stem cell niches for cell-based therapies in diseases that damage the exocrine pancreas.

ENDOCRINE COMPLICATIONS OF CHRONIC PANCREATITIS

Overview of the Problem

Despite the fact that the islets comprise only 2% of the mass of the normal pancreas, the maintenance of the endocrine compartment of the pancreas is crucial for normal health. Endocrine failure is a late event in CP due to the resilience and reserve capacity of the islets, but islet function is affected by the exocrine compartment, indirectly by the absorption of nutrients and by the action of gut hormones. The mechanisms and features of endocrine failure caused by CP remain unclear, however, and a better understanding of the cross-talk of endocrine and exocrine cells holds promise for new treatment approaches.

Type 3c Diabetes: Diagnosis, Prevention, and Therapy

Type 3c diabetes (T3cD) is a surrogate term for pancreatogenous or pancreatogenic diabetes and refers specifically to diabetes mellitus (DM) that results from exocrine pancreatic disease[50]. T3cD comprises 0.5–8% of all diabetes in the U.S. and western developed countries, and 75–80% of T3cD is attributable to CP[51]. Up to 75% of patients with CP will develop T3cD in their lifetime[52–55].

T3cD is diagnosed based on plasma glucose and/or hemoglobin A1c (HbA1c) values according to standard American Diabetes Association criteria in the setting of known or suspected pancreatic exocrine disease[56]. Expert consensus recommends yearly screening of fasting glucose and HbA1c levels in patients with established CP, and to consider oral

glucose tolerance testing when either level is in pre-diabetic range[57]. Currently, T3cD is distinguished from type 1 diabetes (T1D) and type 2 diabetes (T2D) based on clinical picture, although meal-stimulated pancreatic polypeptide (PP) may distinguish T3cD (with low PP levels) from T2D (with high PP levels)[57].

The hallmark of T3cD is insulin deficiency resulting from destruction of the islets in the milieu of exocrine pancreatic injury and fibrosis. Patients with CP have progressive deficits in insulin secretion which are most pronounced in CP with T3cD[58,59]. In addition, pancreatic inflammation may contribute to beta cell dysfunction. Pancreatic-specific expression of interferon-gamma (INF-γ) is highly elevated in CP with T3cD and associated with both impaired nuclear translocation of pancreatic duodenal homeobox-1 (PDX-1) and with impaired *in vitro* glucose stimulated insulin secretion[60,61]. In addition to insulin deficiency, hepatic insulin resistance has emerged as an important contributor to T3cD, which can be reversed by PP infusion[62,63]. Pancreatic enzyme replacement therapy (PERT) can increase post-prandial secretion of the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotopic polypeptide (GIP) as well as post-prandial insulin and C-peptide[64].

Treatment of T3cD includes appropriate nutritional management, PERT, and standard diabetes therapies. Clinical trials for directed treatment of T3cD are completely lacking and represent an important research/knowledge gap. First line antidiabetic agents in T3cD are most often insulin and/or metformin. Insulin therapy is the treatment of choice for malnourished patients or those with highly elevated HbA1c. For obese patients, those with mild hyperglycemia, or features of metabolic syndrome, metformin may be an appropriate first line therapy to address the hepatic insulin resistance[57]. Metformin also has a theoretical advantage in that it has been shown to reduce the risk of pancreas cancer in case control studies of T2D[65,66], and can be co-administered with insulin. The role for other anti-diabetic therapies is unclear. There are theoretic concerns with use of sulfonylurias (beta cell “burn out”[67]), thiazolidinediones (increased bone fracture risk[68,69]), and the incretin therapies, GLP-1 analogs and DPP-4 inhibitors (possible associations with pancreatic cancer and/or pancreatitis and GI intolerance[70]).

Prevention of T3cD likely will stem from adequate control of the underlying CP, from inhibition of mediators of the inflammatory process or from inhibition of pancreas stellate cell (PSC) activation. At the current time, treatments specifically to prevent progression to T3cD do not yet exist, and represent a critical research need.

Exocrine Effects on Endocrine Cells: Lessons from Cystic Fibrosis-related Diabetes (CFRD)

Diabetes is a frequent comorbidity of CF, with ~80% of individuals carrying *CFTR* mutations developing diabetes by middle age[71]. CFRD occurs primarily due to deficient insulin secretion, especially first phase insulin secretion, with a lesser but variable contribution of insulin resistance. Historically, deficient insulin secretion in CFRD was assumed to be due to collateral damage to islets secondary to the severe exocrine pancreatic disease that occurs in CF. Those with CF who remain exocrine-sufficient are far less likely to develop diabetes[72]. Individuals with CFRD have an ~50% loss of beta cells postmortem,

however this degree of beta cell loss alone is likely insufficient to cause diabetes. Furthermore, late-phase and secretagogue-induced insulin secretion remain intact in many CFRD patients long after exocrine pancreatic function declines. These points raise the possibility that there is intrinsic beta cell dysfunction in CF islets independent of active exocrine pancreatic disease.

Recent work in CF animal models also suggests that CF induces a degree of beta cell dysfunction independent of major structural pancreatic damage. For example, newborn CF ferrets exhibit loss of first phase insulin secretion and impaired *in vitro* glucose stimulated insulin secretion, despite a lack of pancreatic structural damage[73]. Furthermore, CF pigs are born with abnormal glucose tolerance and impaired insulin secretion despite sparing of islet mass[74]. Thus, the relative contribution of intrinsic islet dysfunction versus indirect dysfunction due to exocrine pancreatic disease remains unclear in CF.

The relationship between the natural history of glycemia in CF and the progression of exocrine pancreatic disease is presently unknown. In CF ferrets, exocrine pancreatic disease begins at birth and by one month of age there is extensive pancreatic fibrosis, ductal dilation, and marked loss of pancreatic acini. As CF animals age, pancreatic adipogenesis increases and islets remodel within fibrotic areas surrounding large ducts[75]. These histologic changes are nearly identical to human CF pancreas pathology. CF ferrets experience a glycemic crisis with spontaneous diabetic-level hyperglycemia at 1 month of age. This occurs during a spike in pancreatic inflammation that is preceded by pancreatic fibrosis and loss of beta cell mass. Surprisingly, there is spontaneous normalization of glucose levels at 2–3 months, with intermediate hyperglycemia thereafter. Glucose intolerance was not detected until 4 months when insulin secretion in response to hyperglycemia and to arginine was impaired. Insulin sensitivity, measured by euglycemic hyperinsulinemic clamp, was normal. Pancreatic inflammation rapidly diminished after 2 months of age during a period where beta cell mass rose and gene expression of islet hormones, PPARγ, and adiponectin increased[76]. Therefore, active CF exocrine pancreatic inflammation adversely affects beta cells but is followed by islet resurgence. Very young humans with CF may experience a transient glycemic crisis, and it is postulated that pancreatic inflammatory-to-adipogenic remodeling may facilitate islet adaptation in CF. Further research in the effects of exocrine disease on islet function may elucidate mechanisms of islet dysfunction and recovery.

Endocrine Effects on Exocrine Cells: Protective Effects of Insulin on Acinar Cells During Pancreatitis

AP is characterized by pancreatic inflammation, auto-digestion, and necrosis. CP is a progressive deterioration and fibrosis of the pancreas following mild attacks of AP. Therefore, understanding the earliest triggering events of AP, how these can be prevented and the mechanisms responsible for pancreatic regeneration are critical in understanding CP. The major causes of pancreatitis include bile acid reflux from gallstones, fatty acid/ethanol metabolites from excessive alcohol, and fat consumption. Impaired metabolism and cytotoxic Ca^{2+} ($[Ca^{2+}]i$) overload in pancreatic acinar cells are central events regardless of the cause[77]. Metabolism and $[Ca^{2+}]i$ are linked by the ATP-driven plasma membrane Ca^{2+}

pump (PMCA), which prevents cytotoxic Ca^{2+} overload. Therefore, restoration of acinar cell metabolism and protection of PMCA function represents an attractive therapeutic strategy.

Although pancreatitis can cause diabetes due to collateral pancreatic beta cell injury, clinical and animal studies suggest that diabetes may worsen pancreatitis and that insulin is protective and facilitates pancreas regeneration. Diabetes increases the mortality in patients with CP[78] and T2D patients have an ~ 3 fold increased risk of developing AP[79]. However the incidence of AP is reduced among insulin-treated patients[80]. Streptozotocin-induced diabetes aggravates caerulein-induced pancreatitis and delays pancreatic regeneration in mice; exogenous insulin abolishes these effects[81].

Caerulein-induced pancreatitis is worse in the Akita mouse model of T1D[82], but it was difficult to separate the confounding effects of hyperglycemia or reduced insulin from the loss of a protective effect of insulin on acinar cells. This was achieved using Pancreatic Acinar Conditional Insulin Receptor Knock Out (PACIRKO) mice, in which the insulin receptor (IR) was specifically deleted in pancreatic acinar cells. Similar to Akita T1D mice, pancreatitis was worse in PACIRKO mice, either induced by caerulein or by fatty acid/ethanol, and the recovery of the pancreas 7 days following pancreatitis was also impaired[82].

Cellular models of pancreatitis revealed that insulin markedly attenuated palmitoleic acid (POA)-induced ATP depletion, PMCA inhibition, cytotoxic Ca^{2+} overload and necrotic cell death[82,83]. This protection was Akt-dependent and due to a metabolic switch from mitochondrial to glycolytic metabolism sufficient to maintain cytosolic ATP to fuel PMCA and thus prevent cytotoxic Ca^{2+} overload, even in the face of impaired mitochondria.

Collectively these data suggest that endogenous insulin directly protects pancreatic acinar cells during pancreatitis and that the loss of acinar IRs aggravates pancreatitis and impairs pancreatic recovery. This provides a strong mechanistic link between both T1D and T2D and pancreatitis (acute and chronic) that could be exploited therapeutically. Future research should focus on a) testing the protective effect of insulin therapy, and b) dissecting the downstream molecular mechanism for insulin's protective effects to develop novel therapeutic strategies.

Bone Disease in Chronic Pancreatitis: Prevalence, Prevention and Therapy

There is a high risk of malnutrition and nutrient deficiency in CP, driven in part by poor dietary intake secondary to pain and gastrointestinal symptoms, maldigestion and malabsorption of food, and for some, excess alcohol consumption. The effects of nutrient deficiency are varied, and include weight loss, muscle weakness, osteoporosis, neurological abnormalities and visual defects. Of particular concern is bone health. Patients with CP have a higher-than-normal risk of developing low bone mineral density and in a systematic review of 513 CP patients who had undergone dual x-ray absorptiometry (DXA) in a series of studies, 65% had osteoporosis or osteopenia[39]. Crucially, this high osteoporosis risk translates into a higher prevalence of low-trauma fractures compared to healthy controls[68,69,84]. The reasons for premature bone demineralization in CP are multifactorial, and low serum vitamin D levels due to impaired absorption of fat-soluble vitamin D, poor dietary intake, heavy smoking, low physical activity, chronic inflammation, and

malabsorption all contribute. Basic preventative measures should be advised for all CP patients, including adequate calcium and vitamin D intakes, regular weight-bearing exercise, smoking and alcohol avoidance, and PERT[85]. Sunshine exposure to optimize serum vitamin D levels should also be encouraged. Where there is a diagnosis of osteopenia, a DXA should be repeated every 2 years, and for those with confirmed osteoporosis (or who have vertebral fractures), appropriate medication (such as bisphosphonates) and a referral to a bone specialist may be required.

Research Gaps and Opportunities

Future research should be directed at a number of knowledge gaps in type 3c diabetes, exocrine-endocrine interactions in the pancreas, and pancreatitis related bone disease. Critical areas of research include:

- Standardizing diagnostic protocols that distinguish type 3c diabetes from type 1 and type 2 diabetes.
- Clinical trials comparing therapeutic protocols for treatment of type 3c diabetes which target hepatic insulin resistance.
- Investigating the role of metformin (alone or in combination with insulin) in prevention of pancreatic cancer in the setting of underlying CP and type 3c diabetes.
- Identifying therapies that halt the progression of pancreatic inflammation and fibrosis, or reverse beta cell loss/dysfunction, to prevent the development of type 3c diabetes in patients with cystic fibrosis and CP.
- Examining the therapeutic effects of insulin or insulinomimetic agents in acute and recurrent acute pancreatitis.
- Longitudinal studies of oral calcium, vitamin D, and pancreatic enzyme replacement therapy to reduce the incidence of osteoporosis and bone fracture in patients with hereditary and acquired CP.

PAIN COMPLICATIONS OF CHRONIC PANCREATITIS

Overview of the problem

Abdominal pain is the most disabling symptom in people with CP, leading to multiple hospital visits, endoscopic procedures, surgical procedures, and causes significant socioeconomic burden. Despite increasing evidence that the pain of CP is more related to the “wiring” versus the “plumbing” of the pancreas, there are currently no simple, inexpensive, and widely available tool(s) that clinicians can use to accurately differentiate neuropathic and visceral pain. Novel approaches are needed to adequately assess pain in CP and guide therapy.

Biomarkers of Pain

A biomarker is an objective measurement of a pathobiological phenomenon that can help in the diagnosis, prognosis or treatment of an illness. Further, biomarkers can also serve as

more convenient or more verifiable surrogates for clinical endpoints in trials. Objective biomarkers that could complement the subjective assessment of pain would therefore be a major breakthrough in pain medicine. In contrast to many other diseases, there is no good biomarker for pain in CP, which is not surprising since the pain is subjective and biomarkers are objective in nature. Nevertheless one can speak of biomarkers for nociception (which is the neural process activated during pain) rather than a pain biomarker itself.

Several studies from the same group of investigators suggest a correlation between inflammatory and neuropathological expression of certain biological factors and severity of pancreatic pain[86]. However, this is based on access to resected pancreatic tissue and so is not a practical biomarker. On the other hand, there is some promise in analyzing proximal biological fluid obtained at endoscopy for cytokines and small molecules; elevated levels of prostaglandin E2 (PGE2) have recently been found in the pancreatic secretions of patients with early CP who have significant pain but no clear structural abnormalities[87]. Further studies are needed to validate this and other molecules (e.g., TGFβ) in a broad spectrum of patients. Finally, there are several central nervous system (CNS) markers that may be affected in CP and perhaps provide a so-called biomarker for pain in this condition. Electroencephalography (EEG) in patients with CP shows functional changes suggesting a maladaptive pain response[88]. The CNS may also be affected as seen by microstructural changes in cingulate and prefrontal cortices with differences noted in brain imaging between patients with episodic versus continuous pain as well as overall correlations between MRI findings and pain scores[89]. Modalities such as quantitative sensory testing, which are specific to nociception, clinically practical, and respond to treatment show promise as putative biomarkers. There is an emerging need for prospective studies that use these biomarkers to follow the disease course and response to treatment.

Pain Pathogenesis: Is it all about Central Sensitization?

Traditionally, the understanding of pain in CP has been based on the assumption that it is generated by increased pressure in the pancreatic ductal system or the parenchyma. This mechanical understanding of pain has been the theoretical rationale for most interventions with different surgical and endoscopic drainage procedures. There is, however, little evidence to support that increased pressure or structural changes are associated with pain or the effects of invasive treatment[90,91]. On the contrary increasing information has supported that inflammation and progressive replacement of the normal pancreatic tissue with fibrosis can lead to changes in function and morphology of intrapancreatic nerves with neuropathic-like changes[92]. This may lead to peripheral sensitization, which increases the afferent barrage to the CNS. An augmented signaling of noxious stimuli to the spinal cord induces increased responsiveness of central pain transmitting neurons and thereby increases the gain in the whole pain system. This phenomenon is known as central sensitization and once established these changes may persist even though the peripheral drive returns to normality[93,91]. There is major evidence from human studies to support central sensitization in patients with pain due to CP such as decreased pain thresholds and increased areas of referred somatic pain, and augmented visceral pain sensitivity including postprandial pain. Descending inhibition is also impaired in CP and has implications for treatment[91].

Several studies have indicated that deafferentation, chronic pain, and hyperalgesia in many diseases are all associated with functional reorganization of the brain. In the same way we have shown that patients with severe pain due to CP have both dynamic and structural changes in the brain[91]. For example analysis of the networks between active centers in the limbic system during pain demonstrated that these are abnormal, and the pathological changes are associated with clinical pain intensity[94]. Such findings are very similar to those found in patients with phantom pain after amputations. This is supported by the findings that ketamine, which blocks the N-methyl-D-aspartic acid receptor having a key involvement in central sensitization, has been shown to reverse hyperalgesia associated with CP. Furthermore, pregabalin that is normally used to treat neuropathic pain is also effective in CP.

Taken together there is solid evidence that the pain in many patients with CP is related to central sensitization and should be treated as such. There may, however, still be a fraction of patients where the peripheral input is a prerequisite for maintaining the central pain, and theoretically these patients may benefit from invasive therapy. Therefore the most important research priorities are studies where patients have a thorough neurophysiological and imaging workup, and subsequent treatment is guided by mechanisms rather than local traditions and beliefs without scientific support.

Clinical and Etiologic Factors Affecting Pain Outcomes After Intervention

The presence of disabling pain and abnormal pancreatic morphology continue to be the primary patient selection criteria for medical, endoscopic and surgical therapies. In particular, the majority of endoscopic and surgical therapies focus on treating pain by alleviating pancreatic duct obstruction. While short-term complete and partial pain relief rates of these interventions are high[95], long-term results are disappointing. Similar long-term outcomes are seen with total pancreatectomy, an operation conceived on the premise that removal of the primary nociceptive focus should lead to higher pain relief rates, with one large series reporting 51% of patients requiring opioids at 2 years[96]. However, studies that have evaluated these interventions have many limitations.

A number of clinical factors have been studied as predictors of poor pain relief after intervention including on-going alcohol/smoking addiction, duration (>3 years), a continuous pattern of pain, and preoperative opioid analgesic use[97]. Morphologic factors that result in poor pain relief after intervention include advanced stage disease and small (non-dilated) duct disease[98,99]. Procedural factors that influence pain relief focus on the use of adjunctive therapies to endoscopy, such as extracorporeal shockwave lithotripsy, and whether partial resection is pursued in additional to ductal drainage at the time of surgery.

There are emerging data that etiology also influences pain relief rates after intervention. A recent study of 60 patients, who underwent surgery for painful CP, concluded that the only factor associated with higher pain relief was a toxic (alcohol and/or smoking) etiology[100]. This study also showed that the rates of severe fibrosis and calcifications were significantly higher in patients with toxic versus idiopathic etiology. There is increasing recognition that the majority of patients with idiopathic CP harbor pathogenic gene mutations and the associated “field defect” may be an explanation for why interventions that simply address

ductal hypertension may lead to suboptimal pain relief rates[100]. Total pancreatectomy (with islet auto-transplantation) for the initial management of refractory pain in patients with hereditary or idiopathic disease may be the preferred intervention in selected cases[101].

Emerging Therapies to Improve Quality of Life and Manage Pain

CP is associated with high disease burden across the lifespan and recurring abdominal pain is the most prevalent and distressing symptom. Pain severity reduces quality of life (QOL) for individuals with CP[102]. In particular, CP pain is associated with increased fatigue, sleep disturbance, anxiety and depressive symptoms, lower general health status, and reduced physical and role functioning. Children with CP represent an important subgroup that experiences frequent abdominal pain, reduced school attendance, poor QOL, and high health care utilization and costs[103–105]. The complexity of CP pain warrants a biopsychosocial approach to assessment.

Therapy of pain is normally restricted to drugs, endoscopy and/or surgery, but it is outside the scope of this paper to discuss in detail and here the focus is cognitive-behavioral therapies. Self-management is defined as actions taken by the patient (or family) to manage or minimize the impact of a chronic condition on physical health and to manage psychosocial problems that result from the condition. Cognitive-behavioral interventions are a type of self-management that include interventions such as education and goal setting, building self-efficacy in coping with symptoms through behavioral strategies (e.g., relaxation strategies), teaching new ways to think about pain (e.g., cognitive skills), and promoting physical activity and healthy lifestyle (e.g., behavior activation, sleep interventions). In other chronic painful conditions including gastrointestinal disorders, self-management interventions have been effective for reducing pain and pain impact in pediatric[106] and adult populations[107]. Despite their relevance self-management interventions have not been evaluated in individuals with CP.

Even when effective treatments are developed, such as pain self-management interventions, major barriers exist for individuals to access care due to the geographical distance from treatment centers, scheduling constraints, and long wait lists, leaving a significant unmet clinical need. Availability of information and communication technology has expanded opportunities for intervening with individuals remotely. An emerging evidence base now exists for internet-delivered pain self-management interventions in both adult and pediatric populations[108] with patients showing improvements in pain and disability. As one example, in a large multicenter randomized controlled trial with 273 adolescents with chronic abdominal, headache, or musculoskeletal pain, Palermo et al[109] found improvements in daily physical functioning, depressive symptoms, and parent perceived impact of pain in families receiving an 8-week internet pain self-management program plus parent coaching compared to an education control group.

Research Gaps and Opportunities

Future research should be directed at developing tools to assess pain in CP, biomarker discoveries and novel treatment approaches. Critical areas of research include:

- A simple and practical quantitative sensory testing approach should be developed for assessment of pain chronification and guidance of treatment.
- Prospective surveys with assessment of pain biomarkers (quantitative sensory testing, clinical and psychological variables, biopsies, pancreatic juice and urine, genetics, pharmacological challenge etc.) are needed to predict treatment outcome.
- Clinical tools to differentiate between pancreatic and non-pancreatic abdominal pain need to be developed to guide treatment of peripheral and central sensitization.
- Longitudinal pain studies which include pain-free patients should be done where biomarkers are used to predict medical and surgical treatment.
- Studies shall explore opioid use, dependence, and other complications like opioid induced bowel dysfunction and systemic hyperalgesia.
- Develop of new treatments shall be the focus of the pancreas community among them technology-delivered pain self-management interventions to improve pain, associated brain manifestations, and quality of life.
- Outcome studies of treatment approaches to pain in CP should assess the comparative effects of acquired versus hereditary/idiopathic CP to further explore the evidence that the etiology of the CP, and not the morphology of the CP, should determine the best treatment.

CONCLUSIONS

The workshop examined all aspects of CP from basic pathophysiology to clinical presentation, available diagnostics and therapeutics and identified several critical gaps in our understanding of this disease. The participants repeatedly emphasized that without addressing these gaps, it would not be possible to advance the field and offer therapies to people with CP, a disease that causes significant clinical burden and socioeconomic impact in both adults and children. The lack of appropriate animal models that mimic human disease is identified as a critical need as well as cell culture models to examine the complex interactions between exocrine and endocrine pancreas. Biomarkers are urgently needed for early detection of CP and assessment of pain as well as epidemiological studies and robust databases to better define CP and predict outcomes. Likewise, better methods are needed to measure the exocrine pancreatic function in a reliable, reproducible and non-invasive manner. Protocols need to be standardized to distinguish type 3c diabetes mellitus from other types of diabetes. Development of better therapies to treat or prevent complications of CP will depend on better understanding the intricate crosstalk at a cellular level in the pancreas as well as animal model approaches, defining solid endpoints for clinical studies, nutritional considerations, and examining similar disease models such as cystic fibrosis-pancreatic disease and other chronic pain syndromes.

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