

Chapter 1

The changing paradigm of chronic pancreatitis

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Summary

Identification of mutations that lead to pancreatitis and its complications has revolutionized our understanding of pancreatic diseases. Integrating the pathologic effect of a gene mutation on the function of key proteins, and understanding the role of these proteins from a systems biology approach will lead to changes in every aspect of medical care. With few exceptions, all forms of pancreatitis are traced back to dysregulation of trypsin. Trypsin controls trypsin, and calcium flips the switches between trypsin mediated activation and inactivation. Mutations in the calcium regulatory domains of trypsinogen (PRSS1) lead to calcium-independent facilitation of activation or prevention of inactivation. Mutations in the SPINK1 gene lower the threshold for unregulated trypsin activation of itself and other zymogens.

Mutations in CFTR, the molecule that regulates pancreatic duct secretion, diminish the ability to flush activated trypsin into the intestine, especially in the presence of distal duct resistance. Mutated PRSS1, SPINK1 and CFTR genes are susceptibility factors for recurrent acute pancreatitis (RAP), and only a subset of these subjects go on to chronic pancreatitis, which should be defined as a complication of RAP. Chronic pancreatitis requires alterations in three domains of risk: Environmental - metabolic stressors, diminished protection from trypsin activation and injury, and an altered immune response resulting in a strong anti-inflammatory response to injury with dominant fibrosis. We propose that patients with genetic susceptibility to recurrent acute pancreatitis be recognized as having RAP with interepisode resolution, or modified RAP with one or more altered or sustained responses to injury including: (A) anti-inflammatory predominant response with accelerated fibrosis, (B) B-type persistent pain, (C) calcific pancreas, and/or (D) diabetes.

Whether this paradigm applies to tropical pancreatitis remains to be determined. However, one possibility is that variations in idiopathic and tropical pancreatitis could be viewed through a new paradigm where tropical calcific pancreatitis (TCP) represents RAP with modifier domains A+B+C whereas fibrocalculous pancreatic diabetes (FCPD) represents RAP with modifier domains A+C+D. Future efforts are being directed toward early molecular diagnostics and developing strategies for intervention and prevention.

Introduction

Our knowledge of human pancreatic diseases arose from comparing clinical symptoms with pathologic abnormalities found at autopsy and abdominal x-ray. Classic studies of Chiari ¹, Comfort ², Zuidema ³ and others led to our first level of understanding, which was expanded, integrated and codified through consensus conferences in Marseille ⁴⁻⁶, Cambridge ^{7,8}, and Atlanta ⁹ which defined and classified acute and chronic pancreatitis based on detailed clinical observations and review of human pathology ¹⁰. The major clinical advances in understanding pancreatic diseases followed the breakthroughs in abdominal imaging with CT scans and ERCPs in the 1980's and MRCP and EUS in the 1990s which provided outstanding images of the pancreas, but did not provide insight into the etiology of the pancreatic diseases, nor long-term prognosis.

Many approaches have been used to determine the etiology of the pancreatic diseases, and each has strengths and limitations. Histology remains the gold standard for defining pathology, but this approach is limited by the danger of pancreatic biopsy ¹⁰. Furthermore, anatomical pathology and histology only provide information about the location and nature of the pathological process (including stage and grade), but this reflects the down-stream results of the cause, (often at the end-stage of the process), and does not provide insight into the etiology unless a foreign body or infectious agent is identified. Imaging studies are surrogates for anatomical pathology. Epidemiology studies have likewise failed to identify all but mild or very weak risk factors, usually reflection of risk with odds ratios (OR) of 3 to 3. For example, alcohol consumption and tobacco smoking are clearly associated with chronic pancreatitis, but the probability of an average person developing chronic pancreatitis after exposure to either factor is very low¹⁰. These limitations have been recognized, and new approaches are needed.

Genetics may help provide *some* answers. Indeed, it has already provided break-through insights into the mechanisms of some forms of recurrent acute and chronic pancreatitis – in some populations - under some conditions. However, these insights have not yet been translated into effective interventions or preventative strategies for individual patients

– and especially those with “tropical pancreatitis”. Progress has been made in understanding “idiopathic” chronic pancreatitis in the United States, and many of the new ideas and perspectives that the data has forced on the author will be presented.

The National Workshop on Tropical Pancreatitis (December 18-19, 2004 Kochi, India) was organized because (a) there is a major national problem with pancreatic diseases (b) there are many interested parties that share a vision of solving this specific problems and (c) new opportunities may be available to determine the scope and mechanism of this medical enigma. This chapter will focus on the challenges of prospectively addressing complex medical problems that have a genetic component, and discuss issues associated with performing studies in complex trait diseases.

Part 1 – The changing paradigm of idiopathic chronic pancreatitis in North America and India

What is chronic pancreatitis?

Chronic pancreatitis is a term that reflects the end-stage pathology of inflammation-associated diseases. Chronic pancreatitis should be distinguished from acinar cell hypofunction from Shwachman-Diamond Syndrome^{11,12}, pancreatic atrophy or loss of the pancreatic gland from surgery or other processes. Although end-stage chronic pancreatitis results in pancreatic exocrine insufficiency, all exocrine insufficiency is not caused by chronic pancreatitis. Unfortunately, the term chronic pancreatitis is used clinically to describe a wide variety of disorders with a few similar features of inflammation, destruction and fibrosis, and therefore can lead to confusion when clinical features of totally different diseases that are generally classified as “chronic pancreatitis” are lumped and compared. Our thinking has been further biased by the definitions of the Marseille meetings⁴⁻⁶ which made a distinction between acute pancreatitis and chronic pancreatitis, implying that they are totally different disorders, and that when they are seen together, the acute pancreatitis is a consequence of chronic pancreatitis. This must be rejected. Instead, we should view acute pancreatitis as an *event*, and chronic pancreatitis as an inflammatory cell mediated destructive *process* that is dominated by fibrosis^{13,14}.

What do we actually know about the origin of chronic pancreatitis? First, we know that something happens within a person that causes a normal pancreas to progressively deteriorate into an end-stage, sclerotic pancreatic remnant over some period of time (Figure 1). Historically, this progression has been documented through autopsy studies and surgical biopsies leading to definitions of chronic pancreatitis based on histology^{4,10}. Therefore, our clinical efforts reflect this historical perspective and are directed at *predicting histology* in living subjects using CT, ERCP, MRCP or pancreatic function test.

Our research group is also concerned that investigative studies comparing tissue from normal pancreas with tissue from subjects with chronic pancreatitis using arrays, proteomics or other techniques will never lead to an understanding of etiology, mechanism of progression or substantially improve prognosis (Figure 1)¹³. Comparative molecular and gene expression studies on pancreatic tissue will define histology, not etiology.

Human genetic studies revolutionized our understanding of the etiology and prognosis of chronic pancreatitis. The initial breakthrough came in 1996 with the genetic linkage studies in hereditary pancreatitis kindreds^{15,16} and the molecular identification of mutations in the cationic trypsinogen gene (*PRSS1*) of these families¹⁷. Two additional genetic variations also are strong susceptibility factors for chronic pancreatitis – the serine protease inhibitor, Kazal type 1 gene (*SPINK1*)^{18,19} and the cystic fibrosis transmembrane conductance regulator gene (*CFTR*).^{20,21} However, as we focus on the physiology of these genes and the impact of the common mutations on protein function, we recognize that they are genes that *regulate trypsin activity* during synthesis, storage, secretion or transport out of the pancreatic duct. Trypsin is the key enzyme that regulates the activity of all of the other pancreatic zymogens, and if trypsinogen is activated inside the pancreas, it will lead to pancreatic injury through autodigestion. Theoretically, this should lead to *acute pancreatitis*, not chronic pancreatitis. Indeed, on reexamination, the known risk factors for chronic pancreatitis are also risk factors for recurrent acute pancreatitis. This leads us to advance the following hypothesis: chronic pancreatitis is a complication of recurrent acute pancreatitis (RAP) defined by extensive post-injury fibrosis. The process

of initiating the fibrosis process (SAPE hypothesis model) and the organization of risk factors (TIGAR-O) are presented elsewhere .^{10, 14, 22}

Combining our knowledge of pancreatic physiology and mechanisms controlling intrapancreatic trypsin activity have provided the clues to understand chronic pancreatitis as a complex disease .¹³ The new insights into the molecular mechanisms of acute and chronic pancreatitis have forced us to rethink the organization of information and development of new models for testing new hypotheses.

Taken together, the best model of chronic pancreatitis appears to reflect the convergence of three domains of risk (Figure 2) as a complex disorder .^{13,14} In the first domain are the metabolic and environmental factors that increase the risk of trypsinogen activation. This is based on the recognition that patients with *PRSS1*, *SPINK1* or *CFTR* mutations do not have ongoing pancreatic injury, but rather metabolic- or environmental-factor stimulated attacks. The second domain includes genes that are mutated or other factors that limit the capacity of the pancreas to respond to injury. *Thus, the activation force is defined by domain one and the threshold for triggering an attack of acute pancreatitis is defined by domain two.* The frequency of insults that overcome the protective mechanisms therefore equals the frequency of recurrent acute pancreatitis. However, a *different* set of environmental and genetic factors controls fibrosis. This is an immune system-mediated process involving the macrophages, stellate cells, cytokines and related factors. Genetic and environmental factors that influence the immune response fall into domain 3. Thus, the factors that promote fibrosis or retard reabsorption of the matrix proteins determines the rate and severity of fibrosis in patients with recurrent acute pancreatitis.

What is tropical pancreatitis?

Tropical pancreatitis has been defined as a form of "idiopathic chronic pancreatitis", with unique epidemiological and clinical features. In the most simple terms tropical pancreatitis was described by Geevarghese as a disease with "pain in childhood, diabetes in puberty and death at the prime of life". A recent text book describes tropical pancreatitis as a form of chronic pancreatitis characterized by *recurrent abdominal pain*,

pancreatic *calculi*, and *diabetes mellitus*, occurring mostly among poor children and young adults of many developing nations.²³

The initial abdominal pain is reminiscent of typical recurrent acute pancreatitis with “episodes of pain *lasting for days*, not minutes or hours” and “usually *aggravated by small amounts of food* so that the patients refuse all food by mouth. In the early stages, the bouts of pain are *severe* and are associated with *vomiting*.”²³. Some patients develop severe pain late in the course of the disease associated with an inflammatory mass in the head of the pancreas or other features. The characteristic of this type of pain is similar to B type pain described by Ammann et al²⁴ in alcoholic chronic pancreatitis, and remains resistant to all but the most aggressive treatment including major surgery.

Other contributors to this volume describe various clinical and pathological features of tropical pancreatitis in detail. However, it is clear that there remains marked variability in the presentation and clinical course of patients with pancreatic disease in Southern Asia. The clinical features and prognosis are further complicated by reports that are highly biased by referral patterns: the disorder looks very different in patients referred to the gastroenterologists for pain, the endocrinologists for diabetes, the surgeon for management of the most severe structural complications, or the pathologists who often only see the end-stage remnant of the pancreas. The clinical features and presentation of tropical pancreatitis has also changed over the past 50 years^{3, 23, 25}. The consensus now is that the age of onset is older, the character is changing, but some aspects remain unique.

The most striking of these features, in comparison to the patients that are cared for by our group in the United States, is the strong propensity to diabetes mellitus – well before exocrine failure, and marked calcifications in a grossly dilated main pancreatic duct. It is also interesting to recognize that the severity of diabetes appears to correlate with the degree of calcification, suggesting that this represents a clearly different form of pancreatitis and that the pathophysiological mechanisms are linked.

Comparison of tropical pancreatitis and idiopathic chronic pancreatitis

A central question remains unanswered: Is the idiopathic pancreatitis seen in southern Asia only tropical pancreatitis, or is there a mixture of different disorders that have overlapping clinical features and pathologic appearances? As noted, this question remains unanswered. The primary reasons for this situation is that (a) there is no consensus on the distinguishing features of tropical pancreatitis, and (b) the molecular mechanisms have not been determined.

There are some mechanistic similarities between tropical pancreatitis in Southern Asia and idiopathic chronic pancreatitis in Europe and North America. In both cases a significant fraction of subjects have *SPINK1* mutations, and especially the N34S phenotype. Interestingly, this high-risk haplotype was only seen in a subset of children in Germany¹⁸ or families in North America¹⁹ with the phenotype of the heterozygous and homozygous being identical.¹⁹ Since these mutations appear to lower the threshold for intrapancreatic trypsin activation, it appears that trypsin-related injury is a component of each of these disorders. Of even greater interest was the initial finding from Bangladesh in 2001 that *SPINK1* N34S mutations were associated with diabetes predominant tropical pancreatitis (fibrocalculous pancreatic diabetes, FCPD) and calcification/pain associated tropical pancreatitis (tropical calcific pancreatitis, TCP).^{26,27} The association between the various forms of tropical pancreatitis was confirmed in India in 2002 by Chandak et al²⁸, and later by several other additional studies.²⁹⁻³¹ The biggest surprise was that there was a subset of patients with diabetes mellitus but without evidence of exocrine pancreatic disease that also had *SPINK1* mutations in Bangladesh, a finding that does not appear in diabetes populations tested in the United States.³⁰

It should be noted that there is a distinct phenotypic feature of FCPD in Bangladesh that clearly distinguishes it from diabetes caused by the destruction of pancreas in advanced chronic pancreatitis. Rossi et al demonstrated that compared to controls, patients having tropical pancreatitis and no diabetes showed normal plasma C-peptide values at baseline and after arginine stimulation, while FCPD demonstrated a

typical diabetic pattern for plasma C-peptide levels.³² In contrast, pancreatic alpha-cell functioning was preserved in both pancreatitis groups.

The cystic fibrosis transmembrane conductance regulator, CFTR, plays a major role in the pathogenesis of pancreatitis in North America and Northern Europe. Initial studies from India suggest that in tropical pancreatitis, mutations in the CFTR gene are rare. However, it appears that both CFTR-associated chronic pancreatitis¹⁴ and tropical pancreatitis are duct drainage problems. In CFTR-associated pancreatitis the problem is in generating proximal flow resulting in low head pressure. In tropical pancreatitis the primary problem appears to be in the main duct, where mucus (?) and large stones appear to cause distal resistance with high proximal pressures. This hypothesis is also supported by the observation that tropical pancreatitis, in some cases, is associated with pancreatic atrophy – similar to what is seen with other forms of pancreatitis duct obstruction. However, until the phenotype is clearly defined this hypothesis will remain theoretical.

Genes and environment

A number of environmental factors have been suggested to contribute to tropical pancreatitis. The most interesting is diet, including both protein and carbohydrate content, and consumption of cassava. Although a review of environmental factors associated with tropical pancreatitis is beyond the scope of this chapter, it should be remembered that the increased risk of tropical pancreatitis is of the order of 1.2 to 3 fold. Our studies in North America suggest that combined genetic factors confirm risk in the 200-500-fold range.³³ Thus the primary factor is genetic, while secondary factors are environmental. This is NOT to say that environmental factors are not important. For example, smoking cigarettes increases the risk of pancreatic cancer 1.5- to 2-fold. If the risk of population for pancreatic cancer is 0.8%, then 1.6% of smokers will generally get pancreatic cancer. However, in subjects with hereditary pancreatitis the risk of pancreatic cancer is about 50 times higher than the average risk of 0.8%³⁴ and about 40% of subjects will get pancreatic cancer. If smoking doubles this risk, then the impact of smoking is great.³⁵ Knowing both the genetic and environmental risk

in the future will be important because the environmental risks are the easiest to change!

Taken together, it appears that the similarities between idiopathic chronic pancreatitis and TCP or FCPD are that the initial injury to the pancreas is trypsin-related. The difference is that the *response* to repeated injury differs between these groups. This hypothesis can be illustrated by considering Figure 2. In this case the third domain, the response to RAP that leads to fibrosis, could be replaced by other modifying factors that predispose to B-type pain, calcifications or diabetes (Table 1). Thus, all of the major features that define chronic pancreatitis are actually complications of RAP, with the most dominant features reflecting underlying genetic or environmental factors. This is a hypothesis that can be tested, and may further change the paradigm for understanding pancreatic disease.

Part 2 – Strategies to resolve complex genetic traits in India

The nation or the patient: What is the question?

As a physician who cares for individual people I want to know the problems that the *individual* patient faces, that threatens their health and well-being. At that moment I am less concerned with the percent of the population that has this or that disease; I want to know why this person is having symptoms, what underlying disorders are causing the specific symptoms, if they are at risk of developing additional problems, and how I can prescribe a special treatment or intervention that will address the symptoms and prevent any disease from progressing.

This approach is in sharp contrast to the questions and interest of allied health professionals. What they do is very important, and indeed essential to society. The epidemiologists are interested in factors that affect populations, the scientists are interested in the details of general biological principles and mechanisms, the pharmacologists are interested in agents that target specific biological pathways, the pharmaceutical companies want to provide effective agents to many people over many years, and the government is interested in the general well-being of the country, with special interest in public health initiatives that offer the greatest benefit to the most people for the lowest cost.

A special group is the physician-scientists in academic medicine. Their primary goal is to discover new knowledge about specific diseases, to integrate this knowledge into the correct context, and to communicate the insights gained from this new knowledge to all of the other interested health care-associated professionals, with special emphasis on training future physicians.

The *challenge* in academic medicine is to coordinate the varied interests of all of these parties so that there is efficient and effective cooperation among the different groups and that appropriate resources are made available to solve the major unknown questions that impact the patient. The strength of the leading academic physician-scientists is also their weakness – they are independent thinking and hold strong convictions based on their own ideas and interpretation. This is a critically important characteristic, because there can be no progress in medicine unless someone challenges the way medicine is currently practiced as being relatively ineffective, and proposing bold new approaches based on new insights developed by independent thinkers. The reason that the bold independence of the academic physician-scientists is a weakness is that they do not work well together as a group. The opportunity that physician-scientists recognize is that by investing a significant amount of their own time and effort into helping others with interest that are different than their own they can obtain critical data that is unique, important to their own interests, and cannot be otherwise obtain.

At this time, the primary question is: “How can pancreatic disease be prevented in individual patients that live in tropical and non-tropical regions of the countries in which we live?” The challenge will be to develop a consortium of interested parties that will provide sufficient support so that a committed core of focused physician-scientists can lead an effective working group to achieve a great thing that is otherwise impossible.

Planning to answer the major question about tropical pancreatitis

During the National Workshop on Tropical Pancreatitis a number of very important questions were raised. Some are epidemiological, and some are mechanistic. In planning a major study, multiple factors must be

considered and entered into the overall protocol or protocols so that at the end of the study, the major questions will be answered. I am most interested in complex genetic traits, and critical information needed to resolve disorders such as tropical pancreatitis should be obtained.

Complex trait genetics

On first glance, the challenges of resolving the interacting factors that make up a complex trait are daunting. There appears to be an infinite number of potential environmental factors and about a billion possible genetic mutations. However, the problem can be solved using insights from genetic linkage studies, epidemiology studies and systems biology (physiology).

Designing a study:

Several questions must be considered in designing a study. What is the question, what are the specific aims, what are the resources and (in the case of human studies) are there enough patients to answer the question if the specific aims are achieved? The problem of complex genetic traits compounds the difficulty in using power calculations to answer a question.

In the United States, as in Asia, it is becoming increasingly clear that diseases of the pancreas result in a broad spectrum of clinical signs and symptoms. The variables include age of onset, presence or absence of acute attacks of inflammation, severity of attacks, degree of fibrosis, degree, nature and severity of pain, degree and location of calcifications, diabetes with or without insulin and/ or glucagon deficiency, and risk of pancreatic cancers. In addition, the exposure to metabolic or environmental risk factors (e.g. hormones, alcohol, tobacco smoking) appears to influence phenotypic expression.

Spectrum of pancreatic diseases in Southern Asia

The problem faced by investigators, including epidemiologists and geneticists, is that chronic pancreatitis is not a specific disorder, but rather a syndrome composed by multiple disorders with many *identical*

pathologic features, and not all individuals have all of the clinical features that are commonly seen in the disease.³⁶ Unfortunately, the current clinical “phenotype” is based on historical clinical criteria and has little to do with pathological mechanisms. Furthermore, if the phenotype is a syndrome, then it is by definition a group of signs and symptoms that tend to be seen together. In the case of inflammatory diseases of the pancreas, the syndrome is often based on pathologic features or abdominal imaging appearance.

The solution to this dilemma is to throw a broad net, to recruit ALL patients with any sign of recurrent acute or chronic pancreatitis. There should be minimal classification of patients into disease subtypes, especially if the phenotype is in doubt. Instead, there should be a very careful and comprehensive ascertainment of all of the signs and symptoms found in the individual patient, all of the laboratory and testing information, personal and family history, environmental exposures, and response to any therapies. In addition to collecting blood for DNA analysis, the radiographic images and pathologic specimens should be obtained. All of this information becomes critical as each feature of the complex trait is teased out of the overall disorder using a candidate gene approach. In addition, the same information should be obtained from a spouse or friend, and another family member so that the frequency of each candidate gene polymorphism can be compared with the frequency of the polymorphisms in a relevant population.

There also continues to be ongoing debate on exactly how many subjects are needed to have valid association studies³⁷. Unfortunately, the current thinking is highly biased by epidemiological studies that require studies of over 1000 subjects in each group in order to have confidence that a variable with a small effect (e.g. OR 1.5) is truly associated with the phenotypic feature. In complex genetics that focuses on pathologic mechanisms, the primary genetic features will have combined effects with risk into the hundreds. The real issue in these studies is not *study size*, but *study power*, in which study size is considered as one component. However, since environmental features remain a very important consideration, large studies will be needed to clearly determine any association. Thus, from a molecular epidemiology and complex genetic trait perspective a study of 1000 subjects

and 1000-2000 controls will be needed. This could be accomplished within a couple of years if 20 dedicated centers each contributed 50 cases and appropriate controls, as we have demonstrated in the North American Pancreatitis Study 2 (NAPS2). Indeed, using much of the format and information of the NAPS2 study in an all-India study would be important for future comparison of subjects across the world.

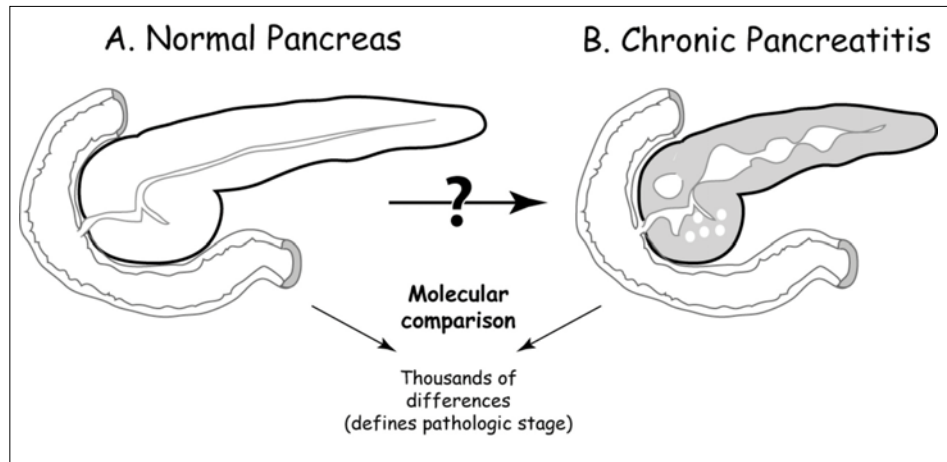


Figure 1: Comparison studies and etiology: Comparison of tissue form normal pancreas (A) with end stage chronic pancreatitis (B) is valuable for defining the histologic and pathologic features. Addition of molecular techniques provides information on thousands of differences between A and B. However, these approaches are more valuable in defining molecular pathology and staging than for determining etiology or prognosis. (From Whitcomb (13) with permission)

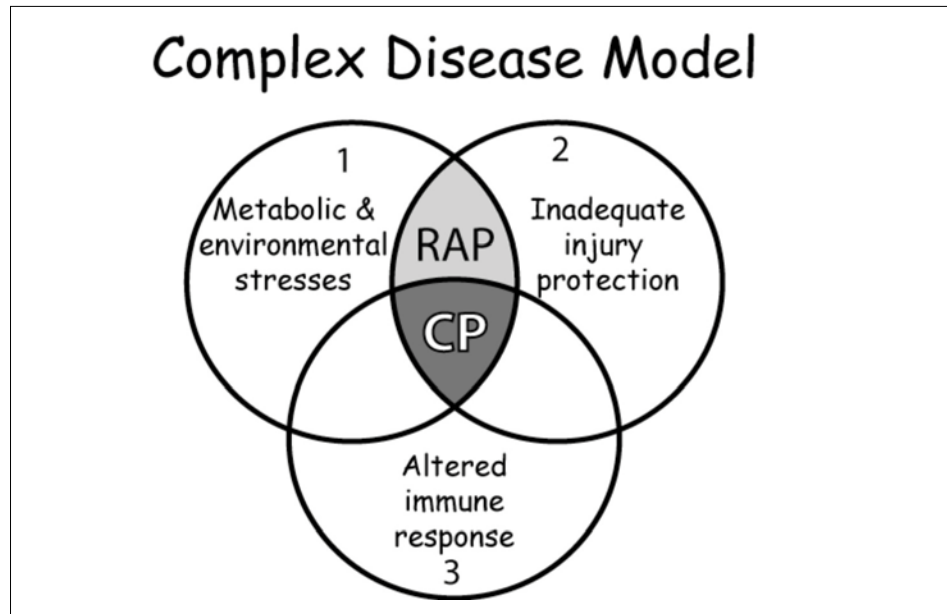


Figure 2: Three domains of chronic pancreatitis risk : Chronic pancreatitis is modeled as a complex trait in which one or more factors must be present in each of at least three domains before chronic pancreatitis develops. The three major genes with mutations that increase susceptibility to chronic pancreatitis (*PRSS1*, *SPINK1* and *CFTR*) are all in the domain of “inadequate injury protection” and lead to recurrent acute pancreatitis (RAP) in the presence of a sufficiently strong metabolic or environmental stressor. Only the subset of patients with an altered immune response favoring fibrosis develop chronic pancreatitis (CP), but this response requires RAP to direct it to the pancreas rather than other organs. (Modified from Whitcomb (13) with permission)

Table 1: Chronic pancreatitis as a complication of recurrent acute pancreatitis

(A) If the common underlying lesion in all cases of chronic pancreatitis is trypsin activation, then the spectrum of signs and symptoms of chronic pancreatitis could be considered as complication of recurrent acute pancreatitis, with the specific features reflecting underlying genetic and environmental modifying factors. (B) This could be the primary distinction between TCP and FCPD, in which both have SPINK1 mutations but different clinical profiles.

A) In patients with recurrent acute pancreatitis

- Normal response = healing
- Factor "A" Anti-inflammatory immune response = fibrosis
- Factor "B" = B-type - severe, continuous pain
- Factor "C" = Calcifications
- Factor "D" = Diabetes mellitus

B) Types of "tropical pancreatitis"

- RAP + A+B+C = tropical *calcific* pancreatitis (TCP)
- RAP + A+C+D = fibrocalculous pancreatic *diabetes* (FCPD)

References

1. Chiari H. Ueber selbstverdauung des menschlichen pankreas. Zeitschrift fur Heilkunde 1896;17:69-96.
2. Comfort M, Gambill D, Baggenstoss A. Chronic relapsing pancreatitis. Gastroenterology 1946;6:239-285.
3. Zuidema PJ. Calcification and cirrhosis of the pancreas in patients with deficient nutrition. Doc. Med. Geograph. Trop. Ansterdam 1955;5:229.
4. Sarles H. Pancreatitis: Symposium of Marseille, 1963. Basel: Karger; 1965.
5. Sarles H. Proposal adopted unanimously by the participants of the Symposium, Marseilles 1963. Bibliotheca Gastroenterologica 1965;7:7-8.
6. Sarles H, Adler G, Dani R, Frey C, Gullo L, Harada H, et al. The pancreatitis classification of Marseilles, Rome 1988. Scand J Gastroenterol 1989;24:641-641.

7. Sarner M, Cotton PB. Classification of pancreatitis. *Gut* 1984;25:756-759.
8. Sarner M, Cotton PB. Definitions of acute and chronic pancreatitis. *Clinical Gastroenterology* 1984;13:865.
9. Bradley EL, 3rd. A clinically based classification system for acute pancreatitis. Summary of the International Symposium on Acute Pancreatitis, Atlanta, Ga, September 11 through 13, 1992. *Arch Surg* 1993;128(5):586-90.
10. Etemad B, Whitcomb DC. Chronic pancreatitis: Diagnosis, classification, and new genetic developments. *Gastroenterology* 2001;120:682-707.
11. Mack DR, Forstner GG, Wilschanski M, Freedman MH, Durie PR. Shwachman syndrome: exocrine pancreatic dysfunction and variable phenotypic expression. *Gastroenterology* 1996;111(6):1593-602.
12. Boockock GR, Morrison JA, Popovic M, Richards N, Ellis L, Durie PR, et al. Mutations in SBDS are associated with Shwachman-Diamond syndrome. *Nat Genet* 2003;33(1):97-101.
13. Whitcomb DC. Advances in understanding the mechanisms leading to chronic pancreatitis. *Nat Clin Practice, Gastro Hepatol* 2004;1(1):46-52.
14. Whitcomb DC. Value of genetic testing in management of pancreatitis. *Gut* 2004;53(11):1710-7.
15. Le Bodic L, Bignon JD, Ragueneas O, Mercier B, Georgelin T, Schnee M, et al. The hereditary pancreatitis gene maps to long arm of chromosome 7. *Human Molecular Genetics* 1996;5(4):549-54.
16. Whitcomb DC, Preston RA, Aston CE, Sossenheimer MJ, Barua PS, Wong-Chong A, et al. A gene for hereditary pancreatitis maps to chromosome 7q35. *Gastroenterology* 1996;110(6):1975-80.
17. Whitcomb DC, Gorry MC, Preston RA, Furey W, Sossenheimer MJ, Ulrich CD, et al. Hereditary pancreatitis is caused by a mutation in the cationic trypsinogen gene. *Nature Genetics* 1996;14(2):141-5.
18. Witt H, Luck W, Hennies HC, Classen M, Kage A, Lass U, et al. Mutations in the gene encoding the serine protease inhibitor, Kazal type 1 are associated with chronic pancreatitis. *Nature Genetics* 2000;25(2):213-6.
19. Pfützer RH, Barmada MM, Brunskil APJ, Finch R, Hart PS, Neoptolemos J, et al. SPINK1/PSTI polymorphisms act as disease modifiers in familial and idiopathic chronic pancreatitis. *Gastroenterology* 2000;119:615-623.

20. Sharer N, Schwarz M, Malone G, Howarth A, Painter J, Super M, et al. Mutations of the cystic fibrosis gene in patients with chronic pancreatitis. *New England Journal of Medicine* 1998;339(10):645-652.
21. Cohn JA, Friedman KJ, Noone PG, Knowles MR, Silverman LM, Jowell PS. Relation between mutations of the cystic fibrosis gene and idiopathic pancreatitis. *New England Journal of Medicine* 1998;339(10):653-658.
22. Whitcomb DC. Hereditary Pancreatitis: New insights into acute and chronic pancreatitis. *Gut* 1999;45:317-322.
23. Pitchumoni CS, Mohan V. Pancreatitis: Juvenile Tropical Pancreatitis. In: Walker WA, Goulet O, Kleinman RE, Sherman PM, Shneider BL, Sanderson IR, editors. *Pediatric Gastrointestinal Disease: Pathophysiology, Diagnosis, Management*. Hamilton (ON): BC: BC Decker; 2004. p. 1598-1605.
24. Ammann RW, Muellhaupt B, Group ZPS. The natural history of pain in alcoholic chronic pancreatitis. *Gastroenterology* 1999;116:1132-1140.
25. Mohan V, Premalatha G, Pitchumoni CS. Tropical chronic pancreatitis: an update. *J Clin Gastroenterol* 2003;36(4):337-46.
26. Rossi L, Pfützer RL, Parvin S, Ali L, Sattar S, Azad Kahn AK, et al. SPINK1/PSTI mutations are associated with tropical pancreatitis in Bangladesh: A preliminary report. *Pancreatology* 2001;1(3):242-245.
27. Pfutzer RH, Whitcomb DC. SPINK1 mutations are associated with multiple phenotypes. *Pancreatology* 2001;1(5):457-60.
28. Chandak GR, Idris MM, Reddy DN, Bhaskar S, Sriram PV, Singh L. Mutations in the pancreatic secretory trypsin inhibitor gene (PSTI/SPINK1) rather than the cationic trypsinogen gene (PRSS1) are significantly associated with tropical calcific pancreatitis. *J Med Genet* 2002;39(5):347-51.
29. Bhatia E, Choudhuri G, Sikora SS, Landt O, Kage A, Becker M, et al. Tropical calcific pancreatitis: strong association with SPINK1 trypsin inhibitor mutations. *Gastroenterology* 2002;123(4):1020-5.
30. Schneider A, Suman A, Rossi L, Barmada MM, Beglinger C, Parvin S, et al. SPINK1/PSTI mutations are associated with tropical pancreatitis and type II diabetes mellitus in Bangladesh. *Gastroenterology* 2002;123(4):1026-30.
31. Hassan Z, Mohan V, Ali L, Allotey R, Barakat K, Faruque MO, et al. SPINK1 is a susceptibility gene for fibrocalculous pancreatic diabetes in subjects from the Indian subcontinent. *Am J Hum Genet* 2002;71(4):964-8.

32. Rossi L, Parvin S, Hassan Z, Hildebrand P, Keller U, Ali L, et al. Diabetes mellitus in Tropical Chronic Pancreatitis Is Not Just a Secondary Type of Diabetes. *Pancreatology* 2004;4(5):461-467.
33. Schneider A, Barmada MM, Lamb J, Slivka A, money ME, Brand RE, et al. CFTR and SPINK1 mutations cause early onset idiopathic chronic pancreatitis. *Nat Genet* 2005;(in press).
34. Lowenfels A, Maisonneuve P, DiMagno E, Elitsur Y, Gates L, Perrault J, et al. Hereditary pancreatitis and the risk of pancreatic cancer. *Journal of the National Cancer Institute* 1997;89(6):442-446.
35. Lowenfels AB, Maisonneuve P, Whitcomb DC, Lerch MM, DiMagno EP. Cigarette smoking as a risk factor for pancreatic cancer in patients with hereditary pancreatitis. *Journal of the American Medical Association* 2001;286(2):169-170.
36. Tandon RK, Sato N, Garg PK. Consensus. Chronic pancreatitis: Asia-Pacific consensus report. *J Gastroenterol Hepatol* 2002;17:508-518.
37. Ioannidis JP, Trikalinos TA, Ntzani EE, Contopoulos-Ioannidis DG. Genetic associations in large versus small studies: an empirical assessment. *Lancet* 2003;361(9357):567-71.