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Genetics of pancreatitis

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Abstract

Purpose of review—Chronic pancreatitis is a syndrome characterized by chronic inflammation of the pancreas, with variable pain, calcifications, necrosis, fatty replacement, fibrosis and scarring and other complications. Disease susceptibility, severity, progression and pain patterns vary widely and do not necessarily parallel one another. Much of the variability in susceptibility to recurrent acute and chronic pancreatitis is now clearly shown to be related to genetic differences between patients. This review highlights recent advances and future directions in genetic research.

Recent findings—The strongest risk factors are associated with genetic variations in *PRSS1, SPINK1, CFTR*, and to a lesser extent, *CTRC* and *CASR*. The latest research suggest that a single factor rarely causes pancreatitis, and the majority of patients with recurrent acute and chronic pancreatitis have multiple variants in a gene, or epistatic interactions between multiple genes, coupled with environmental stressors.

Summary—Pancreatic diseases have a strong genetic component. Rather than a classic Mendelian disorder, recurrent acute and chronic pancreatitis represents truly complex diseases with the interaction and synergism of multiple genetic and environmental factors. The future will require new predictive models to guide prevention and therapy.

Keywords

CASR; CFTR; CTRC; cystic fibrosis; epistasis; pancreas; PRSS1; SPINK1; trypsin; trypsinogen

Introduction

The increasing availability of genetic data has often run far ahead of clinical application – but times are changing. In pancreatitis research, there have not been any recent discoveries of any major pancreatitis susceptibility genes that have a major effect or that have been widely replicated over the past decade. Instead, we have observed a continual reporting of a growing number of case studies showing regional variations on the three major pancreatitis genes (*PRSS1, CFTR* and *SPINK1*). Some candidate genes have been reported, but have not been widely studied in clinical series or replicated because mutations are rare or the independent effect size is small. However, with the advantage of large study populations in

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Conflicts of interest

both the US and Europe, recent advances in the understanding of complex genetics, and new availability of next-generation (Next-Gen) sequencing technology this field is poised for major new discoveries.

The real goal and challenge of genetic research in pancreatic disease is how to determine the genetic basis for sporadic or idiopathic cases, to understand what variations mean for an individual patient and how to apply this knowledge for effective treatment decisions. Answers to these questions are becoming clear through insights from large, adequately powered multicenter cohort studies, pointing to the importance of complex genetics and combinations of genetic factors. Thus, the major focus of this review will be on the complex genetics of chronic pancreatitis that is now emerging with clarity as the data from the North American Pancreatitis Study 2 (NAPS2) are analyzed.

Premise and perspective

To understand the genetics of chronic pancreatitis we must first clarify the pathophysiologic mechanisms of chronic pancreatitis. The key concepts for understanding pathologic mechanisms of chronic pancreatitis are: chronic pancreatitis is a complex syndrome of signs and symptoms – rather than an isolated disease; multiple causative pathways converge into similar phenotypic features; and the process leading to chronic pancreatitis most often begins with acute and recurrent acute pancreatitis. The key concepts about the clinical presentation and clinical course of chronic pancreatitis in an individual patient are: the onset of pancreatitis is unpredictable, the severity of chronic pancreatitis is unpredictable, the complications of chronic pancreatitis are unpredictable, and the rate of progression is unpredictable. The premise of the current review is that genetic and environmental risk factors significantly alter the probability of an event or outcome, and therefore are useful to the clinician in predicting severity, complications, clinical course and the response to treatment in an individual patient. Integration of these factors in an individual patient to develop a treatment plan is a prime example of personalized medicine.

Genetic factors affecting susceptibility

Genetics has emerged as a critical factor in evaluating chronic pancreatitis. The first major feature is that recurrent acute pancreatitis in many cases progresses to chronic pancreatitis. Identification of critical genetic risk factors for chronic disease in a patient with recurrent acute pancreatitis provides the clinician an opportunity for early intervention to prevent the development of chronic pancreatitis, the total replacement of the pancreas with scar tissue and subsequent high risk of pancreatic adenoma.

PRSS1

The first breakthrough was the discovery of mutations in cationic trypsin gene (*PRSS1*) identified to be the cause of autosomal dominant or hereditary pancreatitis [1]. These patients make up about 2–3% of chronic pancreatitis in the US. About 20 mutations are known to be gain-of-function mutations, mainly clustered around calcium-binding sites which regulate trypsin activation and inactivation [2] and are identified in approximately 80% of HP families. This discovery was important because it demonstrated [1] that recurrent acute pancreatitis leads to chronic pancreatitis [2,3] – a genetic cause of pancreatitis that was identical in phenotypic features to other forms of chronic pancreatitis (demonstrating the need for genetic testing in clarifying the cause) [3], that trypsin was a key molecule in the pathophysiology of acute and chronic pancreatitis, and [4•] due to the location of pathologic mutations within the trypsinogen molecule, the role of calcium as a critical regulator of trypsin, particularly within the acinar cell. Furthermore, this focus on trypsinogen activation has implications on duct cell pathophysiology. Once trypsinogen is secreted from the acinar

cells into the pancreatic duct the calcium concentrations are so high that the trypsinogen-binding sites favor trypsinogen activation and protect from trypsin degradation. Inside the pancreatic duct the pH is kept high by secretion of bicarbonate from the duct cells through the cystic fibrosis transmembrane conductance regulator (CFTR) and at high pH trypsin remains inactive even if the trypsinogen activation peptide (TAP) is cleaved from trypsinogen, transforming it into trypsin.

These facts about the trypsinogen gene have been known for over a decade. The most significant new study related to PRSS1 genetics came recently from the European Registry of Hereditary Pancreatitis and Pancreatic Cancer, and reported that *PRSS1* A16V is a common mild variant that increased the risk of pancreatitis, but has a much lower penetrance compared to major mutations such as *PRSS1* R122H or N29I [4•]. This study demonstrates that even in the critical *PRSS1* gene, mutations may result in variable clinical consequences and care must be taken when reporting and interpreting rare or novel mutations.

SPINK1

The serum protease inhibitor, Kazal type 1 gene (*SPINK1*), codes for the pancreatic secretory trypsin inhibitor, which is an acute-phase protein and specific trypsin inhibitor. *SPINK1* is expressed in the pancreatic acinar cells in the context of ongoing inflammation. It is therefore a critical feedback inhibitor of trypsin in the case of pancreas injury in inflammation, which is typically initiated by trypsin activation. The importance of *SPINK1* mutations was demonstrated by Witt *et al.* [5] in children in 2000 and verified in idiopathic pancreatitis by Pfutzer *et al.* [6] in 2000. An intriguing observation was that homozygous *PRSS1* N34S mutations appear to cause familial recurrent acute and chronic pancreatitis, but there was an increased number of cases of heterozygous *PRSS1* variants in these patient groups as well [6]. Complete sequencing of the *SPINK1* gene and promoter region failed to reveal additional polymorphisms that would be consistent with a complex heterozygous genotype and autosomal recessive disease model. The fact that *SPINK1* is normally not expressed in the acinar cells (except at very low levels [7]) suggests that SPINK1 is not the primary risk factor for developing recurrent acute and chronic pancreatitis, but rather represents failed feedback inhibition of recurrent trypsin activation [6,8]. A novel way of evaluating this was published by Aoun *et al.* [9] in which a series of meta-analyses were conducted in order to test the hypothesis that *SPINK1* mutations were a stronger risk factor in cases of chronic pancreatitis associated with recurrent trypsin activation than they were in pancreatitis caused by other mechanisms, for example, alcohol and smoking. Indeed, it was discovered that idiopathic chronic pancreatitis was significantly more strongly associated with *SPINK1* mutations than alcoholic pancreatitis, suggesting that the cause of idiopathic pancreatitis was primarily through a trypsin activation mechanism [9].

Molecular mechanisms behind *SPINK1* high-risk variants have been investigated and whereas rare mutations suspected to cause autosomal dominant pancreatitis have clear mechanisms of dysfunction, such as RNA splicing deficiency or impaired secretion [10,11] the most frequent disease associated variant N34S still has no known molecular basis [12].

CFTR

The third major gene to be implicated in chronic pancreatitis is *CFTR.* CFTR is an anion channel that allows the movement of either chloride or bicarbonate across the apical (luminal) membrane from inside of the duct cell into the duct in which it is responsible for increasing pH, as well as initiating and driving pancreatic juice flow. It has long been known that two severe CFTR mutations lead to cystic fibrosis, which is characterized with severe chronic pancreatitis beginning *in utero*, as well as dysfunction of the lung, sweat glands, and other organs. In 1998, two groups led by Sharer [13] and Cohn [14] demonstrated that

severe *CFTR* variants were more commonly seen in chronic pancreatitis patients without lung disease than expected. Because CFTR is expressed in the pancreatic duct cell, this indicates that CFTR-related pancreatic disease is initiated in the pancreatic duct cell rather than pancreatic acinar cell, even though the phenotypic features are the same as other forms of chronic pancreatitis. CFTR is a cAMP-regulated ion channel and is also a regulator of other ion channels, such as the epithelial sodium channel (ENaC) and a family of bicarbonate transporters (SLC26), raising the significant possibility that CFTR-mediated bicarbonate conductance is physically due to another channel. However, an important study by Park *et al.* [15•] demonstrated that in pancreatic duct cells from humans the secretion of bicarbonate is clearly through the CFTR molecule and other membrane transporters (e.g. SLC26A3 and SLC26A6) are inhibited during the act of secretion making CFTR the primary molecule in bicarbonate conductance. A second very important feature of the pancreatic duct cells is that they do not express chloride transporters on the basal lateral membrane, so chloride cannot enter on the basal lateral surface, and thus duct cells can only secrete bicarbonate.

A number of distinguished studies have detailed the frequency of CFTR mutations (such as F508del) among various chronic pancreatitis cohorts [13,16,17], providing recurrent evidence that carrier status of well known CF causing mutations significantly increases risk of pancreatitis. In a similar vein, a large patient study out of Toronto recently reported that pancreatic sufficient CF patients, that is those with two common, well defined CFTR mutations were highly susceptible to pancreatitis attacks (23%) and that the class and severity of CFTR mutation were critical to this risk (Ooi Gastroenterology 2001 PMID: 20923678); however, the effect of atypical and rare CFTR variants has been more difficult to determine. A recent study from the NAPS2 consortium and the Pittsburgh Hereditary Pancreatitis Study, published in *Gastroenterology* in January 2011, demonstrated that the *CFTR* R75Q variant increased risk for chronic pancreatitis but does not increase risk for lung disease [18••]. Analysis of both familial and sporadic nonalcoholic pancreatitis cases identified both F508del and R75Q as the most frequent CFTR variants over-represented in patients. Functional studies also demonstrated that the R75Q variant specifically disrupted bicarbonate but not chloride secretion. The implication is that in the pancreatic ducts, secretion is markedly altered because neither chloride nor bicarbonate can be transported across the duct epithelium. Chloride cannot get in on the basal lateral side, and bicarbonate cannot get out on the apical side with a net result of markedly diminished fluid secretion. Of note, this does not mean that there is zero pancreatic fluid secretion, since heterozygosity of CFTR variants infers isolated pancreatitis disease risk as opposed to homozygous mutations, and there are several other cell types (including the acinar cells) that can secrete fluid via sodium chloride transport. However, the critical level of bicarbonate secretion, which protects the pancreas but maintaining a high duct lumen pH, is lost. This finding suggests that there may be an entire class of CFTR variants that were thought to be benign since they do not cause classic cystic fibrosis but do cause pancreatic disease.

The finding of *CFTR* variants leads to several clinically actionable considerations. Since many of the *CFTR* variants result in reduced, but not absent function, maximizing stimulation of pancreatic secretion and minimizing distal resistance represent possible targets for therapy. Avoiding proton pump inhibitors should increase duodenal acidification and activate secretin release, which stimulates pancreatic duct flow. Reducing distal resistance, for example treatment of pancreas divisum, would be of theoretic benefit. The development of CFTR ‘correctors’, drugs that restore CFTR function [19], is an exciting future direction that has not yet been tested in CFTR-associated pancreatitis.

CTRC

Identification of additional genes involved in chronic pancreatitis has depended on both bio-molecular processes (as in interaction with trypsin) as well as extensive genotyping of relatively unexplored genetic regions. These principles are illustrated by studies in the chymotrypsin C gene (*CTRC*). The gene was initially studied as a candidate gene for pancreatitis because it appears to be able to degrade trypsin, and therefore protect the pancreas from trypsin-related injury [20]. Rosendahl *et al.* [21], using a large European cohort of over 900 patients (mostly German), discovered that the p.R254W and p.K247_R254del variants were significantly overrepresented in the pancreatitis group, being present in 30 of 901 (3.3%) affected individuals but only 21 of 2804 (0.7%) controls (i.e. less than 1% of the population). They could not directly replicate this finding in a cohort from India, but they did find more *CTRC* variants in affected individuals than in controls, suggesting that *CTRC* was a susceptibility gene. In a French study Masson *et al.* [22] were also not able to replicate the German study, but it did identify 18 variations in *CTRC* that were either very rare, or absent in the French control population. Thus, to discover that *CTRC* was a susceptibility gene required a very large population that happened to have unusually common rare variants, and to confirm the potential role of CTRC in other populations required DNA sequencing of a large number of both patients and controls.

CASR

The CaSR is a plasma membrane-bound G-protein-coupled receptor that senses extracellular calcium levels [23]. The CaSR is expressed in the parathyroid gland, bone, intestine, kidney, brain, and both acinar and duct cells of the pancreas [23–25]. Elevations in extracellular calcium concentrations activate the CaSR which increases intracellular phospholipase A and C, promoting calcitonin production and urinary calcium excretion while suppressing the synthesis and secretion of parathyroid hormone (PTH), thereby lowering the calcium concentration [23,25,26]. In the pancreas the effects of activating the CaSR on the acinar cells have relatively minor effects [24]. However, CaSR is also expressed on the luminal side of the ducts and appears to be tightly linked with an increase of cAMP and activation of bicarbonate secretion [24] (see cell-specific second-messenger signaling discussion by Riccardi and Brown [23]). Thus, CaSR may be a monitor and regulator of pancreatic juice calcium concentration by triggering ductal electrolyte and fluid secretion when levels are elevated [27]. This action would wash out duct fluid with high concentrations of calcium, which increases risk of trypsinogen activation and stabilization of trypsin, which in turn causes acute pancreatitis.

Over 200 mutations in the CaSR gene, *CASR*, have been reported [25,28,29] (see http://www.casrdb.mcgill.ca). Inactivating mutations result in familial hypocalcuric hypercalcemia (FHH), whereas activating mutations result in autosomal dominant hypoparathyroidism (ADH) ([29,30]: Varghese, 2011 #4177).

In 2003 Felderbauer *et al.* [31] reported chronic pancreatitis in a FHH family with hypercalcemia and chronic pancreatitis. The FHH was linked to *CASR* p.L173P but the only family members with chronic pancreatitis also had *SPINK1* N34S mutations. Since then, four additional studies have confirmed an association between *CASR* variants and pancreatitis, with or without *SPINK1* mutations, with and without family histories of either FHH or chronic pancreatitis [32••,33–35]. From these studies more than a dozen variants have been identified (Table 1). Of note, only p.L173P, p.V477A, p.A986S, p.R990G and p.Q1011E have been reported in more than one patient. The last three variants are well studied polymorphisms with the p.A986S and p.Q1011E minor alleles being inactivating variants that increase serum calcium levels [29,36,37].

In the case of *CASR*-inactivating mutations it is conceivable that mild or transient hypercalcemia increases the risk of trypsinogen activation to trypsin, and that the presence of failed feedback inhibition of trypsin by deficient SPINK1 potentiates the risk of chronic pancreatitis. This hypothesis is supported by studies of patients with primary hyperparathyroidism (pHPT) related hypercalcemia in which the risk of pancreatitis is nearly 5%, and independent of *CASR* mutations [30].

CASR p.R990G is a common polymorphism (~15%in the USA) that has been identified in chronic pancreatitis patients reported by Felderbauer *et al.* [33] and Muddana *et al.* [34]. *CASR* p.R990G is a functional polymorphism, with the high-risk G allele associated with lower serum calcium levels [36] and lower PTH levels [37] and increased renal stones (associated with hypercalciuria) [38], suggesting that this is an activating mutation. Vezzoli *et al.* [26] transfected the 990G allele into HEK-293 cells and found that the extracellular calcium concentration producing the half-maximal intracellular calcium response was lower in those transfected with the 990G allele than in those transfected with the wild-type allele ($P = 0.0001$). However, 990G may have only a partial activating effect with phospholipase A activated more than phospholipase C [26]. Furthermore, the receptor has significant post-translational modification and cell-specific effects that may further affect genotype–phenotype effects. As noted by Muddana *et al.* [34], the actual changes in reported serum ionized calcium level with p.R990G minor allele are very small, and lower serum calcium levels would be predicted to lower the risk of RAP and chronic pancreatitis, rather than raise the risk. Another interesting observation what that the risk was greater for chronic pancreatitis than RAP, and specifically associated with moderate alcohol drinking rather than abstainers, light drinkers or very heavy, and that it was not tightly linked to *SPINK1* mutations. These observations suggest that the increased risk of chronic pancreatitis is through a mechanism that does not primarily involve recurrent trypsinogen activation (see Aoun *et al.* [9] for discussion). An alternative hypothesis is that *CASR* p.R990G could facilitate the fibrosis process through an unknown mechanism that is potentiated by alcohol (and/or smoking – which correlates with drinking [39]). Of note, *CASR* p.R896H, which was identified in a chronic pancreatitis patient by Felderbauer *et al.* [33], is also an activating mutation through increased transport of CaSR to the plasma membrane [40•].

Taken together, it is clear that *CASR* variants confer risk of pancreatitis, either through increasing extracellular ionized calcium levels, or other yet-to-be-defined mechanisms. The high rate of rare mutations seen in chronic pancreatitis patients argues for the use of Next-Gen sequencing, since the common SNPs only identify a subset of the inactivating mutations. Furthermore, it appears that there is significant epistasis between the inactivating *CASR* variants and the *SPINK1* variants, as has also been reported for mild, pancreas-targeting *CFTR* variants [18••] (see below). Identification of these variants may be important for disease control or prevention as new allosteric modulators of the CaSR are developed to treat HFF [23].

Complex genetics and epistasis

The clinical phenotype of RAP and chronic pancreatitis is pancreatic inflammation. It was previously shown by Shrikhande *et al.* [41] that it was impossible to distinguish the cause of chronic pancreatitis by viewing the histology. From a pathological phenotyping perspective the appearance of alcoholic, tropical pancreatitis and idiopathic pancreatitis was identical. This suggests that the mechanism of pancreatic injury is different between individuals but that the pathologic pathway is (at some point) the same. Since susceptibility to injury cannot be accounted for by common environmental factors (with the exception of very heavy alcohol drinking with smoking [39]), or common metabolic factors (with the rare exception

of chronic hypercalcemia or chronic hypertriglyceridemia), there must be at least some genetic basis for susceptibility to RAP and chronic pancreatitis.

The problem is as follows. The discovery of a genetic susceptibility factor is driven by statistical inferences. The probability that the factor being tested is the true cause of a difference in a measured outcome is based on a number of factors, which, for discussion, will be linked to the power calculation. For clinical studies the difficulty (and cost) is linked to study number, which is low if the factor being evaluated has a major effect (such as high penetrance *PRSS1* mutations R122H or R122C), and the variance is low (the study group is known to be homogenous). Another issue arises if one does a series of tests to determine which one of many factors causes the outcome. In the case of chronic pancreatitis, the major discoveries were possible because in hereditary pancreatitis the effect size was huge, causing an autosomal dominant disorder with high penetrance. In *CFTR* the variance was low (e.g. the cystic fibrosis phenotype was homogenous, allowing the *CFTR* gene to be discovered) and for both *CFTR* in chronic pancreatitis and *SPINK1* in chronic pancreatitis, the effect size was relatively large, the number of tests was small (i.e. one gene with one dominant SNP – *CFTR* F508del, *SPINK1* N34S), and the large effect mutations were common (~3% and ~2% of the primary populations being studied). However, for a pathologic SNP to be common in a large population, it had to either occur hundreds of generations ago (i.e. a founder effect) or be in a mutational hot-spot. On a case-by-case basis, however, it is possible that an affected individual either has a relatively new mutation in a different location of a known risk gene, or a high-risk mutation in an important gene that is not commonly mutated, or they may have a combination of mutations that alone do not confer much risk, but together have a multiplicative effect (i.e. epistasis). Recent studies in large chronic pancreatitis cohorts demonstrate that all three mechanisms are important as risk factors for chronic pancreatitis.

Epistasis

An exciting and powerful approach to understanding complex trait genetics has been recognition of genetic epistasis. Genetic epistasis refers to the effect of one gene to modify the effect of another gene. As noted above, we initially suspected that *SPINK1* mutations were acting as disease severity modifiers [6,8], but were not sure which risk factor(s) it was modifying. Using multiple meta-analyses we found that the strongest evidence of epistasis for *SPINK1* was in ‘idiopathic’ chronic pancreatitis, and the lowest was in alcoholic chronic pancreatitis [9]. The hypothesis that *SPINK1* was modifying heterozygous *CFTR* mutation effects to target the pancreas was raised by Noone *et al.* [42] who observed that the severe *CFTR* genotypes (e.g. F508del) and *SPINK1* genotypes (e.g. N34S) occurred together at a rate that had an extremely low probability of occurring by chance.

Using the knowledge that *SPINK1* variants represent failed inhibition of recurrent trypsin activation, the combination of either *PRSS1* variants or *CFTR* variants was explored in the large NAPS2 cohort and unexplained familial pancreatitis kindreds in the Pittsburgh hereditary pancreatitis study [18••]. We found that *PRSS1* variants were not commonly associated with *SPINK1* variants, probably because these mutations have such a strong effect that the feedback inhibitory mechanism is incapable of preventing RAP and chronic pancreatitis. The question as to whether *SPINK1* variants were in epistasis with *CFTR* variants would require a large study with complete sequencing of the 27 exons in a significant number of patients in order to detect rare variants. This study has now been completed.

Using the NAPS2 cohort and hereditary pancreatitis familial study project we found that there were several genetic combinations that put a patient at very high risk of pancreatitis [18••]. What Schneider *et al.* [18••] demonstrated was that both *CFTR* severe (functional

class I–III) and *CFTR* mild (class IV) 'benign' and unclassified variants were associated with pancreatitis cases, especially if the patient had a concurrent *SPINK1* mutation. It is critical to note that although the individual risk of pancreatitis among either SPINK1 or CFTR single mutation carriers is 2–4-fold, carrier frequency is 2–5% each and 99% of carriers are healthy. However, with concurrent multiple *SPINK1/CFTR* variants, the risk is synergistic, mutation specific, and healthy carriers of multiple mutations are exceptionally rare. This highlights the basic genetic concept that complex disease is frequently multigenic, with common mild variants conferring minimal risk unless carried concurrently.

Environmental factors

Excessive alcohol intake has been a long recognized factor in pancreatitis, and for a subset of patients, alcohol is the only risk factor considered. However, given that the vast majority of severe alcoholics do not develop any kind of pancreatic disease, alcohol-induced pancreatitis must still be due in part to genetic predisposition. A small limited study tested a single mild *CFTR* mutation among alcoholic patients, finding that cases were statistically over-represented compared to alcoholic controls but not a healthy control group, but thus far the data have not been replicated [43]. This underscores the difficulty of collecting a 'healthy alcoholic' control group as well as the limited understanding of the complex genetic factors. Other small studies have had negative results for the standard genetic risk factors [44], but there have been some advances in identifying novel genetic risk factors in alcohol-induced pancreatitis [45]. Common polymorphisms in alcohol metabolism genes, such as ALDH1, ALDH3, and ADH, have been implicated as either protective or damaging factors for alcoholic pancreatitis in Europe and Asia [46,47], whereas North American similar studies are ongoing.

Fewer studies have been reported on the combined effects of genetic variation and smoking [48], but tobacco use has been emerging as an even more critical risk factor in pancreatitis than ever [49••].

Conclusion

Over the past couple of years there have been major advances in understanding the clinical importance of genetic mutations in understanding idiopathic pancreatic disease. The clinical diagnosis of pancreatitis is a syndrome which incorporates a number of different diseases that have different causes and mechanisms leading to recurrent, acute, and chronic pancreatitis. Since recurrent acute pancreatitis is a very high-risk factor for chronic pancreatitis, it may be important to identify underlying cause so that chronic pancreatitis with all of its complications can be avoided. Our own view is that this is important for managing patients because patients with moderate dysfunction of CFTR should not have acid suppression if possible because acid suppression reduces release of secretin from the duodenum which is important in stimulating duct cells to secrete fluid. Theoretically, a partial dysfunction of CFTR conductance and a reduction in duct cell stimulation would aggravate pancreatic dysfunction and duct cell dysfunction. Therefore, maximum stimulation of pancreatic secretion in patients with marginal *CFTR* variants is important. Patients with two *CFTR* variants in which there is no possibility of stimulating pancreatic secretion, do require acid suppression since the duodenum is not able to buffer the gastric acid, so in cystic fibrosis, proton pump inhibitor therapy is strongly indicated. The third problem is one in the acinar cells associated with calcium regulation. Patients with *PRSS1* variants in which the molecule behaves as if one of the two calcium-binding sites is always occupied appear to be very sensitive to fluctuations in calcium. Thus, in these patients minimizing stimulation of the pancreas by providing pancreatic enzyme supplementation

and/or acid suppression may be beneficial in limiting the episodes of maximal pancreatic stimulation.

Environmental analysis has been thus far segregated from genetic studies in pancreatitis, but it is evident that both genetic and environmental risks are significant in the development and progression of disease. It is also clear that large case studies are necessary to identify additional genes responsible for pancreatitis, either alone or in epistasis. These shortcomings in the field are currently being addressed by the North American and European large multicenter studies as mentioned above, to ultimately eliminate the concept of 'idiopathic' chronic pancreatitis in favor of clear specific diagnoses with evidence-based treatment options.

It is impossible to classify patients into potential treatment categories by looking at the clinical parameters of recurrent acute pancreatitis or chronic pancreatitis since the phenotype is identical between all disease causes. The history of research in the genetics of pancreatic disease has taken major steps forward in that what was once an unknown series of pathology and prognosis, current genetic data shed light onto the path physicians should take to treat and protect each individual patient.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest

•• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 489–490).

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Key points

- PRSS1, SPINK1, CFTR, CTRC and CASR are confirmed as chronic pancreatitis-associated genetic factors.
- Many idiopathic chronic pancreatitis patients have rare variants in the five established genes.
- Chronic pancreatitis appears to be a multigenetic disorder in many cases.
- Alcohol alone appears to confer only marginal risk for chronic pancreatitis, with risk increasing with smoking and possibly genetic variants.
- New treatments targeting *CFTR* and *CASR* function could provide new targets for treating patients with early chronic pancreatitis.

Table 1

Genetic variants in CASR with or without SPINK1 variants

Case population	Country	*CASR*	*SPINK1*	Comments	Reference
FHH family with CP	Europe	p.L173P	N34S	FHH family. Both affected p.L173P had N34S	[31]
19 Idiopathic CP families (*n* = 170 cases)	Europe	p.F391F	N34S	All affected patients had SPINK1 mutations.	[33]
		p.R896H	N34S	p.A986S in 8 of 17 CP. P.R990G in 5 of 17 CP.	
		p. A986S **p.R990G**	N34S	p.Q1011E in 2 of 17 CP	
		p.Q1011E	N34S		
			N34S		
Tropical pancreatitis (*n* = 35)	India	p.P163R	0	Exons 2–5 only	[35]
		p.I427S p.D433H	0	pV477A seen twice, both with SPINK1, no variants in 35 controls	
		p.V477A	0		
			N34S		
Sporadic RAP and CP	United States	p.E191E	N34S	Only p.R990G seen more than once (*n* = 52). Alcohol increased risk from OR 2.0 to 3.1	[34]
	(NAPS2)	p.Y440C p.A746A **p.R990G**	N34S		
			N34S		
			+/− N34S		
Case report	USA	p.P682L	IVS3+2TtoC	Alcohol at 50 g/week	[32••]

The variants in bold represent gain-of-function mutations.