HHS Public Access

Author manuscript

Pancreatology. Author manuscript; available in PMC 2021 December 01.

Published in final edited form as:

Pancreatology. 2020 December ; 20(8): 1648–1655. doi:10.1016/j.pan.2020.10.030.

The Histopathology of *SPINK1*-associated Chronic Pancreatitis

Terrell E. Jones, MD[1], **Melena D. Bellin, MD**[2,3], **Dhiraj Yadav, MD**[4], **Martin L. Freeman, MD**[5], **Sarah J. Schwarzenberg, MD**[2], **Adam Slivka, MD, PhD**[4], **Jennifer S. Chennat, MD**[4], **Gregory J. Beilman, MD**[3], **Srinath Chinnakotla, MD**[3], **Timothy L. Pruett, MD**[3], **Varvara Kirchner, MD**[3], **Abhinav Humar, MD**[6], **Martin Wijkstrom, MD**[6], **Amer H. Zureikat, MD**[6], **Marina N. Nikiforova, MD**[1], **Abigail I. Wald, PhD**[1], **David C. Whitcomb, MD, PhD**[4], **Aatur D. Singhi, MD, PhD**[1]

[1]Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA

[2]Department of Pediatrics, University of Minnesota Medical School, Minneapolis, MN

[3]Department of Surgery, University of Minnesota Medical School, Minneapolis, MN

[4]Department of Medicine, University of Pittsburgh Medical Center, Pittsburgh, PA, USA

[5]Department of Medicine, University of Minnesota Medical School, Minneapolis, MN

[6]Department of Surgery, University of Pittsburgh Medical Center, Pittsburgh, PA, USA

Abstract

Background: The identification of genetic risk factors for chronic pancreatitis, such as *PRSS1*, *CFTR* and *SPINK1*, provides the opportunity to define key pathologic hallmarks and etiologic-specific changes. For example, pancreata from *PRSS1* and *CFTR* patients exhibit progressive lipomatous atrophy without significant fibrosis. Considering the pathology of *SPINK1*-associated pancreatitis is ill-defined, we examined the pancreata of *SPINK1* patients with chronic pancreatitis.

Methods: Histologic sections after total pancreatectomy with islet autotransplantation and associated clinicopathologic data were collected from 28 patients with *SPINK1* germline alterations. Clinical findings, germline data, anatomic anatomic anomalies and pathologic findings were descriptively evaluated.

Results: Patients ranged in age from 5 to 48 years (median, 21.6 years) with abdominal pain between 2 and 25 years (median, 5.8 years). Most patients were *SPINK1* heterozygous and 14 (50%) had co-occurring *CFTR* (n=12) and *CTRC* (n=2) mutations. Other pancreatitis risk factors

Correspondence to: Aatur D. Singhi, MD PhD, UPMC Presbyterian Hospital, Department of Pathology, 200 Lothrop Street, Room A616.2, Pittsburgh, PA 15213, Phone: 412-864-1508, singhiad@upmc.edu.

Author Contributions

Study Concept and Design: TEJ, MDB, DY, ADS

Acquisition of Data: TEJ, MDB, DY, MLF, SJS, AS, JSC, GJB, SC, TLP, VK, AH, MW, AHZ, MNN, AIW, DCW, ADS

Analysis and Interpretation of Data: TEJ, MDB, DY, ADS

Drafting of the Manuscript: TEJ, MDB, DY, ADS

Disclosure/conflict of interest: The authors have no conflicts of interest to declare.

included anatomic anomalies (n=9) and tobacco use (n=1). Overall, 24 (86%) patients had additional pancreatitis-associated germline alterations, *SPINK1* homozygosity, anatomic anomalies or environmental factors. Examination of pancreata revealed a sequential pattern of exocrine parenchymal loss and replacement by prominent fibrosis, dependent on the duration of abdominal pain. No malignancies were identified, but low-grade pancreatic intraepithelial neoplasia was present for 2 cases.

Conclusions: Within this descriptive study, *SPINK1*-associated pancreatitis is characterized by parenchymal fibrosis and suggests divergent pathophysiologic mechanisms from *PRSS1* and *CFTR*-associated pancreatitis. Moreover, *SPINK1* patients frequently had additional etiologic factors that did not impact the development of pancreatic fibrosis and may implicate *SPINK1* as a disease modifier gene.

Keywords

alcohol; fibrosis; pancreatitis; genetics; pathology

Introduction

Chronic pancreatitis is a progressive condition that leads to irreversible damage to the pancreas and is frequently accompanied by chronic, disabling pain. However, rather than a single entity, chronic pancreatitis represents a heterogenous group of disorders with protean clinical and pathologic findings.[1] The etiology of chronic pancreatitis is often multifactorial and known risk factors include chronic alcohol consumption, smoking, autoimmune conditions, pancreatic duct obstruction and anatomic anomalies. Genetics is also recognized as a risk factor and, in certain instances, influences disease penetrance and severity. Germline alterations strongly associated with chronic pancreatitis include those involving *PRSS1*, *CFTR* and *SPINK1* and, to a lesser extent, *CTRC* and *CASR*.[2] Importantly, the identification of germline carriers allows the opportunity to define the pathologic hallmarks and etiologic-specific changes of chronic pancreatitis. Further, the presence of distinctive histology could provide information on the pathogenesis of chronic pancreatitis associated with other etiologic factors.

Recently, the clinicopathologic features of *PRSS1*-associated chronic pancreatitis were reported. Germline alterations in *PRSS1* are autosomal dominant with a 40% to 96% penetrance.[3–6] Patients suffer from recurrent episodes of acute pancreatitis, which often progresses to chronic pancreatitis. *PRSS1*-associated chronic pancreatitis usually begins in early childhood, but onset can vary from infancy to the sixth decade of life. Histologically, the pancreata from *PRSS1* carriers develop lipomatous atrophy that leads to pancreatic insufficiency.[7] Similar observations have also been made with *CFTR* patients, and, thus, alterations in both genes may follow a common pathophysiologic mechanism.[8, 9]

However, among the genes strongly associated with chronic pancreatitis, the histopathologic features of pancreata from *SPINK1* carriers have not been characterized. Therefore, we have collected pancreatic specimens from 28 *SPINK1* patients of various ages with a reported history of chronic pancreatitis and who underwent total pancreatectomy with islet autotransplantation. We describe the patient demographics, clinical history, presence of

anatomic anomalies, co-occurring germline alterations and the pancreatic pathology of this unique cohort.

Materials and Methods

Case Selection

Study approval was obtained from the institutional review boards at the University of Pittsburgh and University of Minnesota. A search for all patients undergoing total or complete pancreatectomy with islet autotransplantation at the University of Pittsburgh Medical Center and the University of Minnesota that harbored a *SPINK1* gene mutation was performed between 2004 and 2018. All patients are participants in a prospective observational study collecting laboratory, medical records, radiographic/endoscopic imaging, intraoperative findings and pathology reports. Informed parental consent and assent or informed consent, as age appropriate, was obtained for all participants. Upon identification of 28 *SPINK1* carriers, germline testing reports were reviewed for each patient to confirm the presence of *SPINK1* alterations and the identification of other germline variants. Germline testing data included reports from Ambry Genetics (Aliso Viejo, CA), Associated Regional and University Pathologists (Salt Lake City, UT), Quest Diagnostics (Madison, NJ) and the Molecular and Genomic Pathology Laboratory at the University of Pittsburgh Medical Center (Pittsburgh, PA). For all testing platforms, extended testing of not only *SPINK1* was performed, but also *PRSS1* and *CFTR*. A germline analysis for *CTRC* was done for 19 (68%) patients and an evaluation of *CASR* was performed for 10 (36%) patients.

Histopathologic analysis

For each surgical specimen, the relevant gross findings were documented to include color, texture, lobularity, concretions, cysts and mass lesions. Prior to islet cell isolation, representative sections of the pancreas were obtained and formalin-fixed and paraffin-embedded (FFPE). For each specimen, FFPE sections ranged between 1 to 9 (mean, 4 sections; median, 4 sections) with at least 1 section taken from the head of the pancreas. Hematoxylin-and-eosin stained slides for each case were independently reviewed by 2 surgical pathologists (T.E.J. and A.D.S.) that were blinded to clinical data. Particular attention was paid to document the number, distribution, and changes of pancreatic acini, ducts, and islets of Langerhans. Furthermore, the amount and location of both acute and chronic inflammation, presence of concretions, extent and distribution of parenchymal fibrosis and fat, and neoplasia were noted. Histopathologic findings were reviewed independently by both surgical pathologists and then were discussed and re-reviewed collectively with correlation to patient age, disease onset, abdominal pain duration, social history, germline genetics, and anatomic anomalies. As accepted guidelines for the interpretation of pancreata for chronic pancreatitis and associated changes are not available, the histopathologic findings were documented (Supplementary Table 1) as previously reported for patients with genomic alterations in *PRSS1*.[7] The presence of fibrosis was semi-quantitatively graded as mild, moderate or severe with respect to intralobular, interlobular and perilobular parenchymal locations.[10]

Results

The study cohort consisted of 28 patients with a germline mutation in *SPINK1*. For each patient, the clinical features, pancreatobiliary/gastrointestinal tract anatomic anomalies and genomic findings are summarized in Table 1. At the time of total pancreatectomy, the patients ranged in age from 5 to 48 years (mean, 21.9 years; median, 20.5 years) and the majority were female (n = 17, 61%). All patients reported a history of intermittent abdominal pain with an age of onset ranging from infancy to 44 years and a duration of symptoms that ranged between 2 and 25 years (mean, 8.8 years; median, 5.7 years), which did not correlate with patient age. Other risk factors for pancreatitis, including alcohol consumption (>5 drinks per day), were absent; but, a history of tobacco smoking was identified for 1 patient. Of note, 9 (31%) patients were remarkable for pancreatobiliary or gastrointestinal tract anatomic anomalies that included pancreatic divisum (n = 6), pancreatobiliary maljunction (n = 1), ampullary stenosis (n = 1) and intestinal malrotation (n = 1). In addition, a history of exocrine insufficiency was documented 15 (54%) patients and more likely to be seen in patients with longstanding duration of symptoms.

Germline testing for *SPINK1*, *PRSS1* and *CFTR* was performed in all cases. In addition, 19 (68%) patients had germline testing for *CTRC*. A heterozygous mutation in *SPINK1* was identified in 25 patients, while the remaining 3 patients had a *SPINK1* homozygous mutation. Detailed genomic information was available for 25 patients and the most prevalent *SPINK1* alteration was the p.N34S missense mutation (n = 24, 96%). The remaining patient was reported to harbor a heterozygous *SPINK1* p.R67H missense mutation. Among *SPINK1* heterozygous carriers, 14 (56%) patients also had a heterozygous mutation in either *CFTR* (n = 12) or *CTRC* (n = 2).

Upon microscopic examination of representative sections from patients' pancreata, a sequential pattern of histologic changes became apparent based on the duration of reported abdominal pain and could be subdivided into four pathologic stages of progressive chronic pancreatitis (Supplementary Table 1). The pancreata of *SPINK1* carriers with a history of abdominal pain of < 4 years (n = 10) exhibited mild atrophy that was characterized by decreasing size and variable shape of the pancreatic lobules (Figure 1A and 1B). These histologic findings were often associated with multifocal, cytoplasmic vacuolization of the acinar parenchyma (n = 6) (Figure 1C) and the presence of mild intralobular fibrosis at the periphery of the pancreas (n = 7) (Figure 1D). Intralobular (n = 3) and extralobular (n = 4) lipomatous infiltration into the pancreatic parenchyma was also seen but focal in distribution. The presence of lipomatous infiltration was distinctly seen among *CFTR* carriers.

Among *SPINK1* patients with a history of abdominal pain ranging from 4 to < 7 years (n = 6), their pancreata showed a progressive loss of acinar parenchyma. Further, mild interlobular (n = 6), mild intralobular (n = 5) and mild perilobular (n = 5) fibrosis was frequently identified and composed of thick bundles of collagen, fibroblasts and scattered islands of neutrophilic inflammation (Figure 2A and 2B). In fact, neutrophilic inflammation was seen in the majority of cases (n = 4) and present within and in between the pancreatic lobules (Figure 2C and 2D). In addition, fat necrosis and saponification were found within

the pancreatic periphery for 3 cases. Extralobular lipomatous infiltration was seen in 2 patient that were also heterozygous for *CFTR*.

Pancreatic specimens from patients with a 7-to-15-year history of abdominal pain (n = 8) displayed increasing loss of not only acinar cell epithelium, but also intralobular ducts. These findings coincided with a moderate amount of intralobular, interlobular and perilobular fibrosis (Figure 3A and 3B). Main duct and interlobular ductal alterations were identified (n = 7) and included ductal ectasia (n = 6), squamous metaplasia (n = 5) and intraductal concretions (n = 5) (Figure 3C). The periphery of the pancreas was remarkable for complete loss of acinar and ductal epithelium, but preservation of islets of Langerhans and surrounding nerves (Figure 3D) and, for 5 cases, the presence of fat necrosis and saponification. Of note, 3 patients within this group were *SPINK1* homozygous and did not show appreciable differences from the remaining 6 *SPINK1* heterozygous patients (Figure 3E and 3F). Lipomatous infiltration within an extralobular distribution was focally present in 2 patients with a germline alteration in *CFTR*.

Finally, pancreata from patients with a history of abdominal pain that ranged between 21 and 25 years (n = 4) exhibited near complete to complete loss of acinar and ductal epithelium, and extensive or severe parenchymal fibrosis (Figure 4A and 4B). The remnant exocrine pancreas consisted primarily of a dilated main pancreatic duct and associated interlobular ducts (Figure 4C). Three cases also had intraductal concretions/calcifications and, for 2 cases, scattered foci of reactive and hyperplastic intralobular ducts were also identified (Figure 4D). Pancreatic intraepithelial neoplasia (PanIN) of the highest grade PanIN-2 was also seen for 2 cases. The borders of the pancreata were ill-defined and the only clue were the presence of residual islets of Langerhans embedded in fibrosis (Figure 4E and 4F). Fat necrosis and saponification were absent. With regards to the presence of anatomic anomalies or germline status in not only *SPINK1*, but *CFTR*, *CTRC* and *PRSS1*, no significant pathologic differences that deviate from those associated with the duration of abdominal pain were identified. Of note, the presence of lipomatous infiltration was distinctly absent, even among the 2 *CFTR* patients within this group.

Discussion

The serine protease inhibitor Kazal type 1 (SPINK1) protein is an antiprotease secreted by pancreatic acinar cells and is hypothesized to function by delaying the autoactivation cascade of converting trypsinogen to trypsin.[11] This delay is important as it allows for the digestive proteases, such as trypsin, to transit from the pancreas to the duodenum in an inactive form and, thus, preventing autodigestion of the gland.[12] Although the underlying molecular mechanisms between *SPINK1* and pancreatitis are unclear, mutations in *SPINK1* may lead to diminished inhibition of trypsin activity.[13] Several mutations in *SPINK1* have been documented, but the most common is the p.N34S haplotype and may increase the risk of chronic pancreatitis by up to 23%.[14–17] However, this mutation is also a common polymorphism in the general population at a frequency of approximately 2%, which suggests that it may play a role as a disease modifier rather than a causative agent.[18–20] In fact, when inherited in a heterozygous form, as is the case for the majority of patients with

chronic pancreatitis, other inherited germline alterations, environmental factors and/or anatomic anomalies have been reported.[21–26]

Herein, we collected pancreatic specimens from patients with pathogenic *SPINK1* variants and chronic pancreatitis. Among the reported *SPINK1* variants, the p.N34S mutation was the most prevalent within our study cohort and were predominantly heterozygous. Furthermore, consistent with its potential status as a disease modifier, the majority of *SPINK1* heterozygote patients (23 of 25, 92%) had either co-occurring alterations in other genes associated with chronic pancreatitis, anatomic anomalies and/or a history of tobacco use. Additional etiologic factors were not identified among patients with *SPINK1* homozygous mutations. Examination of pancreata from *SPINK1* carriers of various ages revealed a progressive loss of primarily exocrine parenchyma and replacement by prominent fibrosis. These findings do not seem dependent on patient age, but the duration of reported abdominal pain or clinical chronic pancreatitis.

The presence of progressive parenchymal fibrosis within the pancreas is intriguing and contrasts histopathologic findings reported for other germline variants associated with chronic pancreatitis, such as *PRSS1* and *CFTR*. *PRSS1* encodes for cationic trypsinogen and gain-of-function mutations lead to premature trypsinogen activation.[27] As a result, *PRSS1* patients exhibit chronic pancreatitis at a young age and develop progressive exocrine insufficiency.[28] Previously, we published the histopathology of *PRSS1*-associated chronic pancreatitis and found these patients develop pancreatic lipomatous atrophy and not parenchymal fibrosis.[7] Similarly, dysfunctional germline alterations in *CFTR*, which leads to retention of trypsinogen within the pancreas and, consequently, pancreatitis, also results in fatty replacement of the pancreas.[8, 9] Pancreatic parenchymal fibrosis has not been described in *CFTR* patients. *SPINK1* carriers with co-occurring mutations in *CFTR* exhibited prominent fibrosis of the pancreas and only focal intralobular and/or extralobular lipomatous infiltration into the pancreatic parenchyma. In addition, the presence of lipomatous atrophy was not seen in longstanding cases of *SPINK1*-associated chronic pancreatitis. Our observations would suggest that the pathophysiologic mechanisms of *SPINK1*-associated pancreatic parenchymal fibrosis are not only distinct from those described in *PRSS1* and *CFTR* patients, but overcome those associated with lipomatous atrophy.

While the histopathologic findings of *SPINK1*-associated chronic pancreatitis may seem unique, they are reminiscent of those seen among chronic pancreatitis patients with documented heavy alcohol consumption. Within a large autopsy series, Suda et al. found pancreata from patients with chronic alcohol abuse exhibited a combination of intralobular and perilobular fibrosis.[29] Intralobular fibrosis was characterized by a uniform distribution of fibrosis within the pancreas that imparts a micronodular arrangement around atrophic lobules. In comparison, perilobular fibrosis is often dense with ductal ectasia and intraductal calcifications. In advanced cases, the pancreatic parenchyma may be completely replaced by extensive fibrosis. These histopathologic findings are analogous to pancreata from *SPINK1* patients and, similarly, may represent a sequence of changes that correlates with duration of heavy alcohol consumption and clinical chronic pancreatitis. In fact, the reported prevalence of *SPINK1* mutations in alcohol-induced chronic pancreatitis is higher than the general population at 5.6% to 6%.[19, 24, 30, 31] It is interesting to speculate that there may be a

common pathophysiologic mechanism shared between *SPINK1*-associated and alcohol-induced chronic pancreatitis.

In addition to defining the histopathologic features of chronic pancreatitis among *SPINK1* carriers, our observations are also significant as they can be used to determine the validity of current and future animal models of *SPINK1*-associated chronic pancreatitis. Targeted disruption of *Spink3* (the murine homologue of human *SPINK1*) results in is severe pancreatic damage and death within two weeks after birth.[32] The histopathologic changes are restricted to pancreatic acinar cells, which are filled with numerous autophagic vacuoles with enhanced trypsin activity. These vacuoles closely resemble the vacuolated acinar parenchyma of *SPINK1* patients with a history of chronic pancreatitis of < 4 years. Mosaic expression of human SPINK1 in *Spink3* knockout mice rescued the perinatal lethality, but these mice gradually developed chronic pancreatitis, manifested by loss of acinar cells, intralobular fibrosis and dilatation of intralobular ducts, which bears resemblance to human *SPINK1*-associated chronic pancreatitis.[33] However, in humans, homozygous *SPINK1* mutations do not result in embryonic lethality, and heterozygous mutations are frequently present in the general population with development of chronic pancreatitis typically associated with the co-occurrence of other genetic alterations, anatomic anomalies and environmental factors. Hence, while current mouse models emulate several aspects of *SPINK1*-associated chronic pancreatitis, they likely do not recapitulate human disease.

It is worth noting that there are several limitations to our study. Although it is the first to assess the histopathologic features of *SPINK1*-associated chronic pancreatitis, the number of cases within our cohort is relatively small and a control cohort was not available for comparative histopathologic review. In addition, most of our patients were heterozygous for *SPINK1*, which as discussed previously is common within the general population, and had co-occurring genomic, anatomic and/or environmental risk factors. Thus, one could infer that the histopathologic findings described herein may be unrelated to *SPINK1* status. However, our study cohort included 3 patients that were homozygous for *SPINK1* and had no additional risk factors. The pancreata from these 3 patients exhibited similar histopathologic findings as *SPINK1* heterozygotes within the reported timeframe of abdominal pain. Another limitation of this study is that we did not use a histopathologic scoring system for chronic pancreatitis, but rather graded the presence of fibrosis semi-quantitatively.[10] While clinical, imaging and functional scoring systems have been developed for chronic pancreatitis, an accepted and reproducible method of evaluating the microscopic findings of chronic pancreatitis does not exist.[34–36] In fact, within a recent joint publication by the International Association of Pancreatology, the American Pancreatic Association, the Japan Pancreas Society and the European Pancreatic Club, there was strong agreement that there currently exists no reproducible or universally accepted histological grading system to assess the severity of chronic pancreatitis.[37] Moreover, there was strong agreement that a three-tiered semi-quantitative scoring system is typically used to evaluate the pancreas.[37] Therefore, we chose to report the clinical, genomic and histopathologic findings of *SPINK1*-associated chronic pancreatitis within a descriptive manner. As additional cases of *SPINK1*-associated chronic pancreatitis are evaluated, it is likely that the histopathologic features described herein may prove to represent a continuum of findings rather than discrete stages of chronic pancreatitis. Further, the duration of abdominal pain

and subsequent time periods were determined retrospectively at the time of total pancreatectomy with islet autotransplantation and, thus, was dependent upon patient recall. Our study also suffers from selection bias because only patients undergoing total pancreatectomy with islet autotransplantation were evaluated. Finally, the isolation of islets during this procedure precludes complete histologic analysis of the pancreas. Therefore, only representative sections of the pancreas were reviewed.

In summary, *SPINK1*-associated chronic pancreatitis is characterized by progressive parenchymal fibrosis. These findings contrast the reported findings of lipomatous atrophy observed is *PRSS1* and *CFTR* patients, suggesting divergent pathophysiologic mechanisms. Further, patients with *SPINK1*-associated chronic pancreatitis frequently have additional etiologic factors for pancreatitis, such as alterations in *CFTR*, that did not impact the development of pancreatic fibrosis and may indicate a role for *SPINK1* as a disease modifier gene. While this study enhances our current understanding of chronic pancreatitis among *SPINK1* carriers, certainly additional questions emerge regarding the pathogenesis of *SPINK1* mutations and its interaction with other etiologic factors. However, as germline testing for chronic pancreatitis becomes more widespread, future studies should provide greater insight into this disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding support: None

References

1. Kleeff J, Whitcomb DC, Shimosegawa T, et al. Chronic pancreatitis. Nat Rev Dis Primers 2017;3:17060. [PubMed: 28880010]
2. Zhan W, Shelton CA, Greer PJ, et al. Germline Variants and Risk for Pancreatic Cancer: A Systematic Review and Emerging Concepts. Pancreas 2018;47:924–936. [PubMed: 30113427]
3. Sossenheimer MJ, Aston CE, Preston RA, et al. Clinical characteristics of hereditary pancreatitis in a large family, based on high-risk haplotype. The Midwest Multicenter Pancreatic Study Group (MMPSG). Am J Gastroenterol 1997;92:1113–6. [PubMed: 9219780]
4. Howes N, Lerch MM, Greenhalf W, et al. Clinical and genetic characteristics of hereditary pancreatitis in Europe. Clin Gastroenterol Hepatol 2004;2:252–61. [PubMed: 15017610]
5. de las Heras-Castano G, Castro-Senosiain B, Fontalba A, et al. Hereditary pancreatitis: clinical features and inheritance characteristics of the R122C mutation in the cationic trypsinogen gene (PRSS1) in six Spanish families. JOP 2009;10:249–55. [PubMed: 19454815]
6. Rebours V, Boutron-Ruault MC, Schnee M, et al. The natural history of hereditary pancreatitis: a national series. Gut 2009;58:97–103. [PubMed: 18755888]
7. Singhi AD, Pai RK, Kant JA, et al. The histopathology of PRSS1 hereditary pancreatitis. Am J Surg Pathol 2014;38:346–53. [PubMed: 24525505]
8. Tham RT, Heyerman HG, Falke TH, et al. Cystic fibrosis: MR imaging of the pancreas. Radiology 1991;179:183–6. [PubMed: 2006275]
9. Soyer P, Spelle L, Pelage JP, et al. Cystic fibrosis in adolescents and adults: fatty replacement of the pancreas--CT evaluation and functional correlation. Radiology 1999;210:611–5. [PubMed: 10207457]

10. Kloppel G, Maillet B. Pseudocysts in chronic pancreatitis: a morphological analysis of 57 resection specimens and 9 autopsy pancreata. Pancreas 1991;6:266–74. [PubMed: 1862065]
11. Kazal LA, Spicer DS, Brahinsky RA. Isolation of a crystalline trypsin inhibitor-anticoagulant protein from pancreas. J Am Chem Soc 1948;70:3034–40. [PubMed: 18882536]
12. Pubols MH, Bartelt DC, Greene LJ. Trypsin inhibitor from human pancreas and pancreatic juice. J Biol Chem 1974;249:2235–42. [PubMed: 4818832]
13. Kume K, Masamune A, Ariga H, et al. Do genetic variants in the SPINK1 gene affect the level of serum PSTI? J Gastroenterol 2012;47:1267–74. [PubMed: 22526274]
14. Witt H, Luck W, Hennies HC, et al. Mutations in the gene encoding the serine protease inhibitor, Kazal type 1 are associated with chronic pancreatitis. Nat Genet 2000;25:213–6. [PubMed: 10835640]
15. Teich N, Bauer N, Mossner J, et al. Mutational screening of patients with nonalcoholic chronic pancreatitis: identification of further trypsinogen variants. Am J Gastroenterol 2002;97:341–6. [PubMed: 11866271]
16. Schneider A, Barmada MM, Slivka A, et al. Clinical characterization of patients with idiopathic chronic pancreatitis and SPINK1 Mutations. Scand J Gastroenterol 2004;39:903–4. [PubMed: 15513391]
17. Truninger K, Kock J, Wirth HP, et al. Trypsinogen gene mutations in patients with chronic or recurrent acute pancreatitis. Pancreas 2001;22:18–23. [PubMed: 11138965]
18. Pfutzer RH, Barmada MM, Brunskill AP, et al. SPINK1/PSTI polymorphisms act as disease modifiers in familial and idiopathic chronic pancreatitis. Gastroenterology 2000;119:615–23. [PubMed: 10982753]
19. Threadgold J, Greenhalf W, Ellis I, et al. The N34S mutation of SPINK1 (PSTI) is associated with a familial pattern of idiopathic chronic pancreatitis but does not cause the disease. Gut 2002;50:675–81. [PubMed: 11950815]
20. Chandak GR, Idris MM, Reddy DN, et al. Absence of PRSS1 mutations and association of SPINK1 trypsin inhibitor mutations in hereditary and non-hereditary chronic pancreatitis. Gut 2004;53:723–8. [PubMed: 15082592]
21. Schneider A, Larusch J, Sun X, et al. Combined bicarbonate conductance-impairing variants in CFTR and SPINK1 variants are associated with chronic pancreatitis in patients without cystic fibrosis. Gastroenterology 2011;140:162–71. [PubMed: 20977904]
22. Witt H Chronic pancreatitis and cystic fibrosis. Gut 2003;52 Suppl 2:ii31–41. [PubMed: 12651880]
23. Whitcomb DC. Value of genetic testing in the management of pancreatitis. Gut 2004;53:1710–7. [PubMed: 15479696]
24. Aoun E, Chang CC, Greer JB, et al. Pathways to injury in chronic pancreatitis: decoding the role of the high-risk SPINK1 N34S haplotype using meta-analysis. PLoS One 2008;3:e2003. [PubMed: 18414673]
25. Shimosegawa T, Kume K, Masamune A. SPINK1 gene mutations and pancreatitis in Japan. J Gastroenterol Hepatol 2006;21 Suppl 3:S47–51. [PubMed: 16958672]
26. Reddy DN, Prasad SS. Genetic basis of chronic pancreatitis in Asia Pacific region. J Gastroenterol Hepatol 2011;26 Suppl 2:2–5.
27. Sahin-Toth M, Toth M. Gain-of-function mutations associated with hereditary pancreatitis enhance autoactivation of human cationic trypsinogen. Biochem Biophys Res Commun 2000;278:286–9. [PubMed: 11097832]
28. Whitcomb DC, Gorry MC, Preston RA, et al. Hereditary pancreatitis is caused by a mutation in the cationic trypsinogen gene. Nat Genet 1996;14:141–5. [PubMed: 8841182]
29. Suda K, Takase M, Takei K, et al. Histopathologic study of coexistent pathologic states in pancreatic fibrosis in patients with chronic alcohol abuse: two distinct pathologic fibrosis entities with different mechanisms. Pancreas 1996;12:369–72. [PubMed: 8740404]
30. Drenth JP, te Morsche R, Jansen JB. Mutations in serine protease inhibitor Kazal type 1 are strongly associated with chronic pancreatitis. Gut 2002;50:687–92. [PubMed: 11950817]
31. Witt H, Luck W, Becker M, et al. Mutation in the SPINK1 trypsin inhibitor gene, alcohol use, and chronic pancreatitis. JAMA 2001;285:2716–7.

32. Ohmuraya M, Hirota M, Araki M, et al. Autophagic cell death of pancreatic acinar cells in serine protease inhibitor Kazal type 3-deficient mice. Gastroenterology 2005;129:696–705. [PubMed: 16083722]

33. Sakata K, Araki K, Nakano H, et al. Novel method to rescue a lethal phenotype through integration of target gene onto the X-chromosome. Sci Rep 2016;6:37200. [PubMed: 27845447]

34. Schneider A, Lohr JM, Singer MV. The M-ANNHEIM classification of chronic pancreatitis: introduction of a unifying classification system based on a review of previous classifications of the disease. J Gastroenterol 2007;42:101–19. [PubMed: 17351799]

35. Conwell DL, Lee LS, Yadav D, et al. American Pancreatic Association Practice Guidelines in Chronic Pancreatitis: evidence-based report on diagnostic guidelines. Pancreas 2014;43:1143–62. [PubMed: 25333398]

36. Tirkes T, Shah ZK, Takahashi N, et al. Reporting Standards for Chronic Pancreatitis by Using CT, MRI, and MR Cholangiopancreatography: The Consortium for the Study of Chronic Pancreatitis, Diabetes, and Pancreatic Cancer. Radiology 2019;290:207–215. [PubMed: 30325281]

37. Esposito I, Hruban RH, Verbeke C, et al. Guidelines on the histopathology of chronic pancreatitis. Recommendations from the working group for the international consensus guidelines for chronic pancreatitis in collaboration with the International Association of Pancreatology, the American Pancreatic Association, the Japan Pancreas Society, and the European Pancreatic Club. Pancreatology 2020;20:586–593. [PubMed: 32414657]

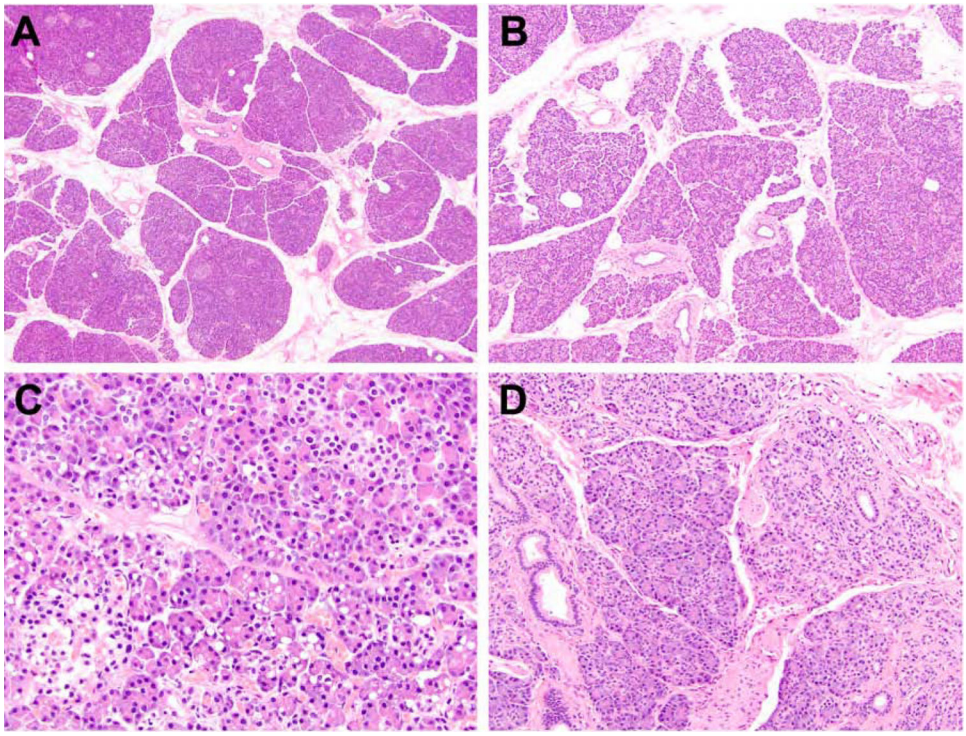

Figure 1.

The pancreata of *SPINK1* carriers with a history of abdominal pain of < 4 years. An examination of pancreatic specimens identified mild atrophy that was characterized by decreasing size and variable shape of the pancreatic lobules (A and B). For a subset of cases, multifocal, cytoplasmic vacuolization of the acinar parenchyma (C) and focal intralobular fibrosis at the periphery of the pancreas (D) were seen.

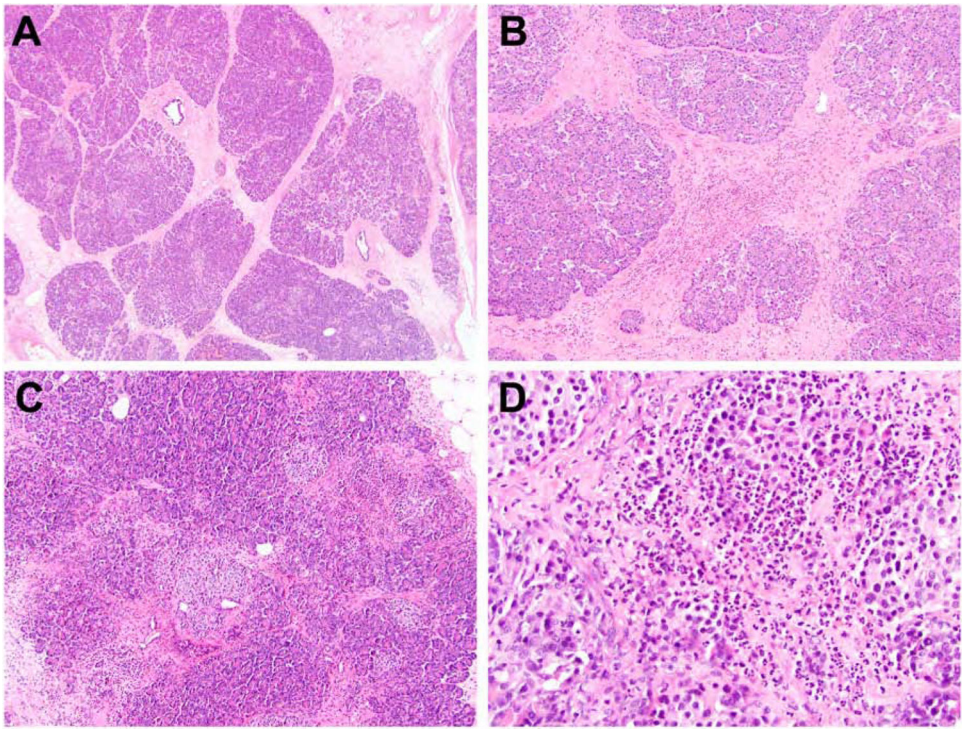

Figure 2.

SPINK1 patients with a history of abdominal pain ranging from 4 to < 7 years. The pancreata exhibited a progressive loss of acinar parenchyma with coinciding mild interlobular and mild perilobular fibrosis. Fibrosis was typically composed of thick bundles of collagen, fibroblasts and scattered islands of neutrophilic inflammation (A and B). A brisk acute inflammatory infiltrate was also seen present within the pancreatic lobules (C and D).

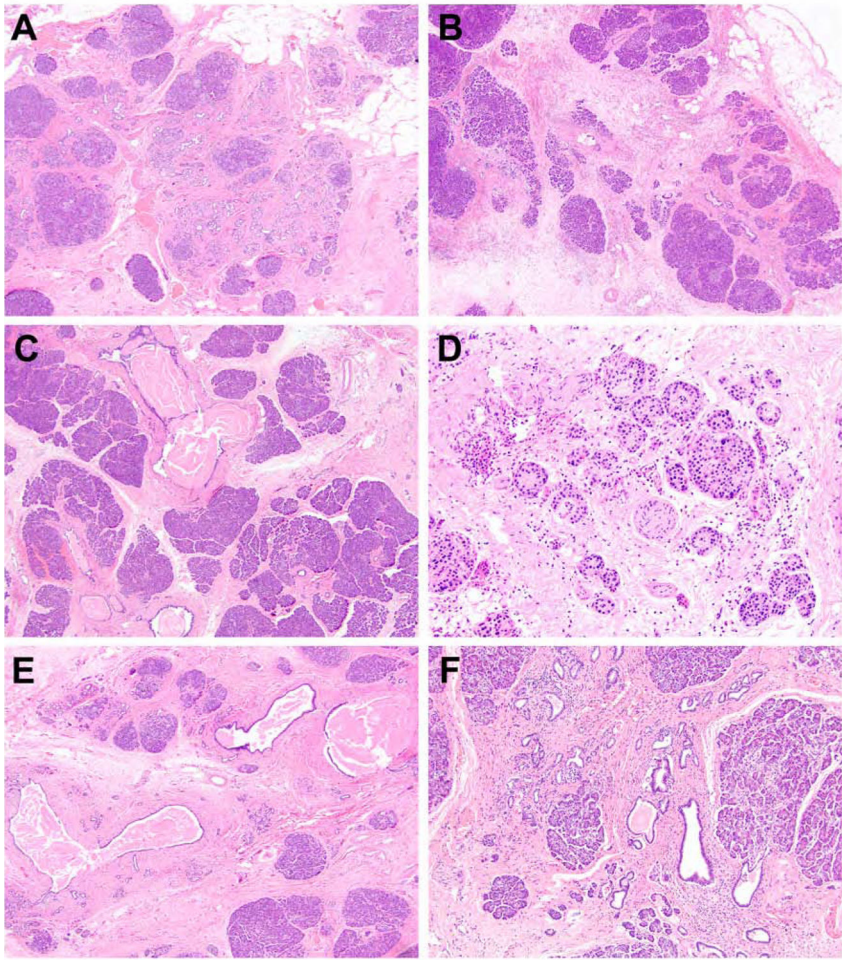

Figure 3.

Pancreatic specimens from *SPINK1* patients with a 7-to-15-year history of abdominal pain. The pancreata demonstrated increasing loss of acinar cell epithelium and intralobular ducts, which coincided with intralobular, interlobular and perilobular fibrosis (A and B). Ductal ectasia, squamous metaplasia and intraductal concretions were also present (C). The periphery of the pancreas was remarkable for complete loss of acinar and ductal epithelium, but preservation of islets of Langerhans and surrounding nerves (D). In addition, while the majority of patients were heterozygous for *SPINK1*, 3 patients were *SPINK1* homozygotes with reported abdominal pain between 8.1 to 10 years and their pancreata exhibited similar histopathologic features as their *SPINK1* heterozygous counterparts with ductal ectasia and intraductal concretions (E) and both intralobular and interlobular fibrosis (F).

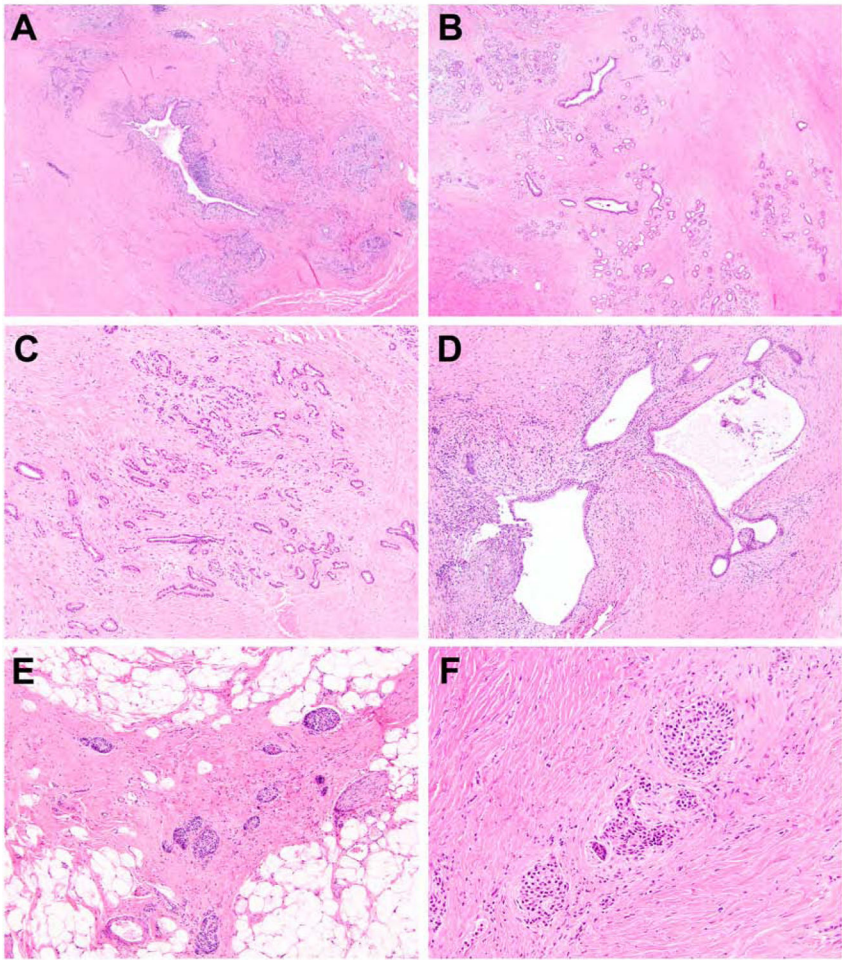

Figure 4.

SPINK1 carriers with a history of abdominal pain that ranged between 21 and 25 years. Pancreatic specimens exhibited near complete to complete loss of acinar and ductal epithelium, and extensive parenchymal fibrosis (A and B). The remnant exocrine pancreas consisted primarily of a dilated main pancreatic duct and associated interlobular ducts (C). Scattered foci of reactive and hyperplastic intralobular ducts were also identified (D). At higher magnification, residual islets of Langerhans were found scattered throughout the pancreas but embedded in dense fibrosis (E and F).

Table 1.

Clinical features, anatomic anomalies and genomic findings of 28 patients with *SPINK1*-associated chronic pancreatitis.

Case	Age (years)	Sex	Duration of Pain (years)	Tobacco Use & Alcohol History*	Exocrine Insufficiency (Age [years] of Diagnosis)	Pancreatobiliary/ Gastrointestinal Tract Anatomic Anomalies	Genotype
1	26	F	2.1	None	No	Pancreatic divisum	*SPINK1* N34S heterozygote; *CFTR* T1299A heterozygote
2	22	F	2.5	None	No	Pancreatobiliary maljunction	*SPINK1* N34S heterozygote
3	35	F	2.7	None	No	Pancreatic divisum	*SPINK1* N34S heterozygote; *CFTR* G576A and R668C heterozygote
4	5	M	2.8	None	Yes (5 y/o)	None	*SPINK1* N34S heterozygote
5	18	F	3.0	None	No	None	*SPINK1* N34S heterozygote; *CFTR* M470V heterozygote
6	48	F	3.3	45 pack year smoking history	Yes (45 y/o)	None	*SPINK1* N34S heterozygote
7	12	M	3.3	None	No	None	*SPINK1* N34S heterozygote; *CFTR* deltaF508 heterozygote
8	8	M	3.3	None	No	Pancreatic divisum	*SPINK1* N34S heterozygote
9	11	F	3.4	None	No	None	*SPINK1* heterozygote**; *CFTR* heterozygote**
10	6	F	3.9	None	Yes (4 y/o)	None	*SPINK1* R67H heterozygote; *CTRC* G103VfsX31 heterozygote
11	26	F	4.2	None	No	Ampullary stenosis	*SPINK1* N34S heterozygote
12	27	M	4.3	None	Yes (Unknown)	None	*SPINK1* N34S heterozygote
13	45	F	4.6	None	Yes (43 y/o)	None	*SPINK1* N34S heterozygote; *CFTR* R117H heterozygote
14	14	F	5.7	None	No	Pancreatic divisum	*SPINK1* N34S heterozygote
15	25	F	5.8	None	No	Intestinal malrotation	*SPINK1* N34S heterozygote
16	11	F	6.5	None	No	None	*SPINK1* N34S heterozygote; *CFTR* deltaF508 heterozygote
17	25	F	7.9	None	Yes (Unknown)	None	*SPINK1* N34S heterozygote; *CFTR* IV8–5T heterozygote
18	18	M	8.1	None	Yes (Unknown)	None	*SPINK1* N34S homozygote
19	18	M	9.5	None	Unknown	None	*SPINK1* N34S homozygote
20	27	M	10.0	None	Yes (Unknown)	None	*SPINK1* N34S homozygote
21	15	F	12.1	None	No	None	*SPINK1* N34S heterozygote; *CTRC* V235I heterozygote
22	20	M	14.8	None	Yes (19 y/o)	Pancreatic divisum	*SPINK1* N34S heterozygote; *CFTR* L997F heterozygote
23	17	M	14.8	None	Yes (12 y/o)	None	*SPINK1* N34S heterozygote; *CFTR* 394delTT heterozygote
24	20	F	15.0	None	Yes (19 y/o)	None	*SPINK1* N34S heterozygote; *CFTR* A1285V heterozygote
25	21	M	21.0	None	Yes (17 y/o)	None	*SPINK1 heterozygote*; *CFTR heterozygote***

Case	Age (years)	Sex	Duration of Pain (years)	Tobacco Use & Alcohol History*	Exocrine Insufficiency (Age [years] of Diagnosis)	Pancreatobiliary/ Gastrointestinal Tract Anatomic Anomalies	Genotype
26	41	F	22.4	None	Yes (41 y/o)	None	*SPINK1* N34S heterozygote
27	24	M	24.0	None	Yes (23 y/o)	None	*SPINK1* heterozygote**; *CFTR* heterozygote**
28	29	F	24.8	None	Yes (Unknown)	Pancreatic divisum	*SPINK1* N34S heterozygote

Abbreviations: F, female; M, male.

*Heavy alcohol consumption was defined by more than 5 drinks per day.

**Information regarding the exact genomic alteration identified was not available.