SMART System Review

SPINK1 Genetic Variants in Pancreatitis.

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Abstract: Genetic variants disrupting the expression or function of the Serine Protease Inhibitor, Kazal Type 1 (*SPINK1*) gene—encoding the pancreatic secretory trypsin inhibitor (PSTI, also called SPINK1)—elevate the risk of pancreatitis and pancreatic cancer. This review elucidates SPINK1's role as a key inhibitor of active trypsin, detailing its protective mechanism alongside other regulatory factors. We emphasize human *SPINK1* variants, spotlighting the high-risk haplotype tagged by p.N34S (Table 1) and other risk, predisposing or pathogenic variants (Table 2). Additionally, we explore the conditions and co-factors—genetic and environmental—that trigger acute pancreatitis (AP) and progression to chronic pancreatitis (CP), providing a comprehensive framework for understanding SPINK1's clinical significance

Key Words: Pancreatitis, acute pancreatitis, chronic pancreatitis, genetics, SPINK1, PRSS1, trypsin inhibitor, genetic counseling, medical genetics, precision medicine.

Outline:

1. Background

Chronic pancreatitis (CP) is a progressive inflammatory disease of the pancreas, characterized by irreversible morphological changes and gradual loss of pancreatic function.(1) It is a debilitating condition that poses significant challenges in clinical practice due to its complex etiology, variable clinical presentations and unpredictable clinical course. Multiple genes and variants are associated with acute pancreatitis (AP) and progression to chronic pancreatitis, especially linked to a trypsin-dependent pathway(2), (See Trypsin control pathway image Hyperlink).

Trypsin is the master enzyme of pancreatic zymogens as it is responsible for activating all of the inactive pancreatic digestive enzymes in the duodenum.(3) Acute pancreatitis is caused by premature activation of trypsin in the acinar cell or ducts, and perhaps in the interstitial space as well. Therefore, regulation of trypsin by controlling activation and inhibition is key to pancreatic health. While there are at least ten mechanisms for controlling trypsin, the contribution of SPINK1 ranks among the most important.

2. SPINK1 Biology

SPINK1 (Serine Protease Inhibitor, Kazal Type 1) encodes a trypsin-selective inhibitor protein (pancreatic secretory trypsin inhibitor, PSTI). The *SPINK1* gene is located on chromosome 5q33 and consists of four exons. *SPINK1* is primarily expressed in the pancreas (Link to *SPINK1* Gene Expression [section 2, GTEx]). SPINK1 is synthesized in the pancreatic acinar cell as is trafficked to the zymogen granules for secretion into the pancreatic duct. PSTI (also called

Abbreviations used in this paper: AAP, acute alcoholic pancreatitis; AP, Acute Pancreatitis; CNV, copy number variants; CP, Chronic Pancreatitis; HTRZ, heterozygous; PSTI, pancreatic secretory trypsin inhibitor; RAP, Recurrent AP; SNV, Single Nucleotide Variants; RER, rough endoplasmic reticulum SPINK1, Serine Protease Inhibitor, Kazal Type 1; SRP, *SPINK1*-related Pancreatitis.

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SPINK1) is an acute phase protein that is upregulated during injury and inflammation.[4]

The primary function of SPINK1 in the pancreas appears to be as a "suicide inhibitor" of active trypsin, even though the inhibition is slowly reversible (Figure 1).

Three-dimensional images can be found at these websites:

3D images of trypsin and SPINK1

- https://www.ncbi.nlm.nih.gov/Structure/pdb/1CGJ.
- https://www.ncbi.nlm.nih.gov/Structure/pdb/1V2Q

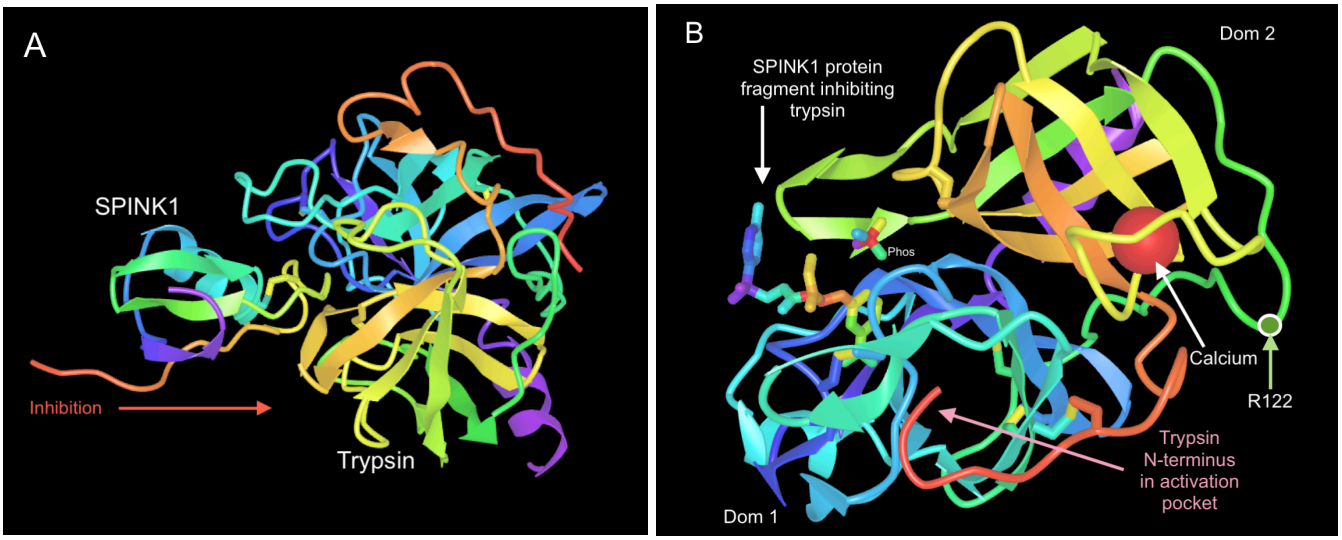

Figure 1. SPINK1 interacting with trypsin as a suicide inhibitor. A. SPINK1 entering the catalytic pocket of trypsin to cause inhibition of protein hydrolysis by blocking entrance into catalytic site. B. Trypsin structure showing a fragment of the SPINK1 molecule at the catalytic site. The activation site has the trypsin activation peptide (TAP) cleaved (not shown) with the N-terminus int the activation pocket. Calcium (red ball) is stabilizing trypsin int the active conformation, including protecting R122H from cleavage. (images from NCBI sites - see text).

3. Loss of SPINK1

The importance of SPINK1 for maintaining acinar cell integrity and prevention of AP, recurrent AP (RAP) and CP is demonstrated by partial or complete loss of SPINK1 function. Loss-of-function genetic variants in *SPINK1* that result in a dysfunctional protein or blunted expression from mutations in the regulatory region result in the risk of uncontrolled trypsin activity – the proximal cause of acute pancreatitis. The effect of high and low expression levels of functional SPINK1 in vivo is illustrated in animal studies as well as human population studies.

Animal Studies

Spink1-deficient (*Spink1*(-/-)) mice exhibit autophagic degeneration of acinar cells, resulting in rapid onset of cell death shortly after birth and death within two weeks.[5] The observed autophagic acinar cell death and impaired tissue regeneration highlight the vital role of trypsin inhibition in maintaining acinar cell integrity and facilitating regeneration. Sun et al demonstrated that introduction of the *Spink1* c.194+2T>C variant causes spontaneous chronic pancreatitis.[6] These findings also suggest ongoing trypsinogen activation to trypsin either at low continuous levels, or with small episodes that are controlled, in part, by a trypsin inhibitor.

Another group made knock-in mice to express mouse PSTI-I in the pancreas under control of the elastase promoter.[7] This resulted in PSTI-expression 190% of normal and decreased the severity of hyperstimulation (caerulein)-induced acute pancreatitis. Furthermore, with repeated caerulein-induced pancreatitis the PSTI transgenic mice had a significant reduction in leukocyte infiltration and markers of fibrosis compared to controls.[8] These findings indicate that trypsin is important to acute and chronic pancreatitis, and that expression of a trypsin inhibitor diminishes trypsin-induced inflammation and its consequences.

> **The SPINK1 gene, critical for regulating intracellular trypsin activity, plays a vital role in protecting the pancreas from acute and chronic pancreatitis by preventing trypsin-related autodigestion and inflammation.**

4. Effects of genetic variants on SPINK1 expression and function.

Classifying SPINK1 mutations using the ACMG framework[9]—pathogenic, likely pathogenic, uncertain significance, likely benign, or benign—poses challenges despite the known link between SPINK1 variants and pancreatitis. As a trypsin inhibitor, SPINK1's 'pathogenicity' hinges on upstream injury and variable trypsin activation, meaning even severe variants, like gene deletions, aren't inherently disease-causing without this trigger.

While many pancreatitis-associated variants linked to the *SPINK1* gene are known, attempts to classify them using the standard ACMG categories of "pathogenic, likely

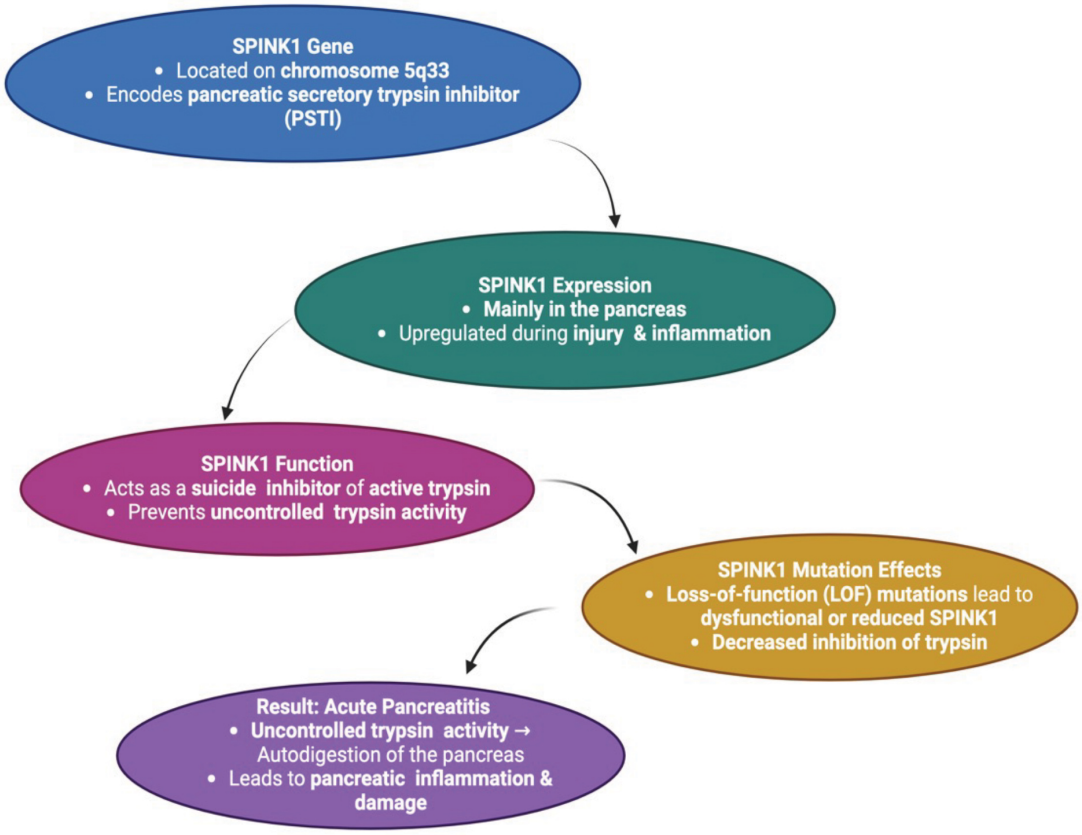

Figure 2. Flowchart summarizing the role of SPINK1 in pancreatitis

pathogenic, unknown significance, likely benign, benign" are difficult to apply. The reason is that, as an inhibitor, "pathogenicity" is fully dependent on an upstream injury linked to variable levels of trypsin activation. Thus, even the worst variants (e.g. gene deletion) are not, by themselves, disease causing. An important attempt to classify pancreatitis-associated genetic variants was proposed by Masson *et. al.* for PRSS1 variants.[10] Here, we have attempted to grade *SPINK1*-associated variants based on the degree of dysfunction or reduction in gene expression compared to expected, since the true effect on a human varies widely based on coexisting factors.. For an excellent discussion on the epidemiology and clinical impact of SPINK1 variants, see Wang, et al.[11]

SPINK1 risk haplotypes: Example, p.N34S

Dozens of genetic variants linked to *SPINK1* have been associated with AP, recurrent AP (RAP) and CP.[12] The initial association was linked to a missense mutation, p.N34S (p.Asn34Ser, c.101A>G, rs17107315T>C)[13-15] This is the most common *SPINK1* mutation in people of European and South Asian ancestry. However, recombinant SPINK1 p.S34 was as effective as p.N34 in inhibiting trypsin.[16] Other experiments, including minigene analysis indicated that DNA containing the p.N34S variant had no significant effect on gene expression.[17] Further studies identified the pathogenic mechanism linked to a haplotype (a group of DNA variations that are physically close to each other on a chromosome that are inherited together from one parent. see NHGRI hyperlink). Indeed, there are dozens of variants in this haplotype, with multiple variants affecting the regulatory regions for expression SPINK1 in the pancreatic acinar cells when needed (see HaploReg Hyperlink) and enter rs17107315 in rsID box and examples in **Figure 1**).

SPINK1* **p.N34S risk haplotype**.

Chr	Pos (hg38)	Variant (rsID)	Alt
5	147817967	rs145959667	A
5	147818851	rs17107296	C
5	147819449	rs145479930	C
5	147825312	rs149882377	T
5	**147828115**	**rs17107315**	**C**
5	147829331	rs17107316	C
5	147829667	rs17107318	G
5	*147835718*	*rs142703147*	*A*
5	147838808	rs148911734	T
5	147845072	rs142120065	A

Table 1. Example of SNPs in tight linkage with the *SPINK1* p.N34S variant. p.N34S is bold. Chr. Chromosome number; pos (38) position on the chromosome build 38; rsID is the reference variant in dbSNP. Alt is the minor allele nucleotide at the rsID locus. *Italics indicates the gene rather than the protein.

Analysis of mRNA expression in human pancreatic tissue finally demonstrated that the risk haplotype that includes p.N34S reduces SPINK1 expression with S-allele expression 57% lower than N-allele.[18] Thus, this variant is a haplotype that fails to respond with normal SPINK1 expression when it is needed during pancreatic injury and inflammation.

SPINK1 splice-site variants: Example, IVS3+2T>C (c.194+2T>C, rs148954387)

Splice sites are the locations within a gene where introns are removed and exons linked together to make mRNA (Hyperlink to Ward-Cooper Review).[19] The c.194+2T>C variant affects the 5' splice site in intron 3 causing exon skipping, and complete failure of functional *SPINK1* expression.[17, 20, 21] This is a more severe variant than p.N34S and, to our knowledge, no subject with homozygous IVS3+2T>C variants and *without* pancreatitis has been reported.[17] This is the most common pancreatitis-associated SPINK1 mutation in East Asia, although it has been seen throughout the world.

A recent study by a leading French group systematically evaluated all known variants in the *SPINK1* gene and predicted that 1.67% of potential *SPINK1* coding SNVs exert a discernible impact on splicing outcomes, making them pathogenic because of alternate splicing rather than protein misfolding or loss of functional or regulatory amino acids.[22]

SPINK1 functional missense mutations: *Examples, p.G48E, p.D50E, p.Y54H, and p.R67C*

Although the p.N34S (and p.P55S) variants in protein structure have minimal effects on reducing trypsin inhibition, other variants that alter protein folding are associated with CP. Examples include p.G48E, p.D50E, p.Y54H, and p.R67C, that have been evaluated in laboratory test.[23, 24] Thus, some misfolding mutations not only block the inhibitory effect of SPINK1 on trypsin, but also cause accumulation of proteins in the rough endoplasmic reticulum (RER) to generate an unfolded protein response and can worsen an inflammatory response. Other variants cause frame shifts (e.g. c.162del, p.Asn56fs) or truncation mutations (e.g. c.174C>A p.Cys58Ter) that also eliminate translation of viable SPINK1 proteins.

SPINK1 gene deletions

Some people with pancreatitis have loss of *SPINK1* expression from one of the chromosomes due to deletion of a major portion, or the entire *SPINK1* gene. These may be

Chr	pos (38)	rsID	c Nom	p Nom	PMID/ClinVar	Effect - comment
5	145197355-148541511	ID: 147500	Del 3,344,157 bp	CNV*1	CV ID: 147500	Pathogenic (gene deletion)
5	147164969-149315489		Del 2,150,520 bp	CNV*1	CV ID: 1808574	Pathogenic (gene deletion)
5	147824641-147831792		Del 7151 bp	CNV*1	CV ID: 583901	Pathogenic (gene deletion)
5	147824702	rs515726208	c.199C>T	**p.Arg67Cys**	PMID: 17568390	Complex effects (haplotype)
5	147828022	rs141634296	c.194G>A	p.Arg65Gln	PMID: 28320769, 17525091, 17568390, 15980664, 11368029	Risk (altered regulatory sites may reduce gene expression)
5	147828020	rs148954387	**c.194+2T>C**	intron	PMID: 16849362	Pathogenic (Splice Donor)
5	147828041	rs781162491	c.175G>A	p.Val59Met	CV ID: 1392176	Missense Variant (VUS)
5	147828042	rs35737774	c.174C>T	**p.Cys58=**	PMID: 25741868 / CV ID: 258913	Risk (linked to p.N34S)
5	147828042	rs35737774	c.174C>A	p.Cys58Ter	CV ID: 1779356	Pathogenic (stop site gained)
5	147829598	rs1554089895	c.162del	p.Asn56fs	CV ID: 547787	Pathogenic (splice donor)
5	147828053	rs111966833	c.163C>T	p.Pro55Ser	PMID:>6 CV ID: 36778	Benign-Likely-Benign
5	147828056	rs515726207	c.160T>C	p.Tyr54His	PMID: 17568390	Missense – uncertain effect
5	147828113	rs2480199266	c.103G>T	p.Glu35Ter	CV ID: 1773780	Pathogenic (nonsense)
5	147828115	rs17107315	c.101A>G	**p.Asn34Ser**	PMID:>15	Key Risk haplotype
5	147829598	rs1554089895	c.87+1G>A	CV ID: 547787	CV ID: 547787	Pathogenic (splice donor)
5	147830562-147831897		c.-191-129_56-932del		CV: ID: 13766	Pathogenic (splice acceptor)
5	147831537	rs104893939	c.41T>C	p.Leu14Pro	PMID: 17274009, 10835640	Pathogenic - Retained protein
5	147831542	rs35877720	c.36G>C	**p.Leu12Phe**	PMID: 17274009, 17568390	clinVar = Benign-Likely-Benign (African variant)
5	147831551	rs1554089895	c.27del	p.Ser10fs	CV ID: 36780	Pathogenic (Splice donor)
5	147831576	rs104893938	c.2T>C	p.Met1Thr	PMID 10835640	Pathogenic (start site)
5	147831792	rs191068215	c.-215G>T	5' utr	CV ID: 13763	Pathogenic (regulatory site)
5	147831792-147824661		Del 7131 pb	CNV*1	CV ID: 830855	Pathogenic (deletion)
5	147835718	rs142703147	c.-4141G>T	**intron**	PMID: 34828289	Pathogenic (regulator site)

Table 2. List of pathogenic and risk variants linked to the *SPINK1* gene. The two variants in red represent the most common variants in human studies. Bold variants are part of the p.N34S risk haplotype (see Table 1). Chr. Chromosome number; pos (38) position on the chromosome build 38; c Nom, position based in the cDNA transcript number (not shown); p. Nom, position based on amino acid number; PMID, manuscript PubMed number; CV ID, ClinVar variant identification number.

classified as copy number variants (CNV). The frequency of gene deletions in SPINK1 and pancreatitis is unknown but is thought to be unusual. Deletion of one copy of the *SPINK1* gene is not sufficient to cause pancreatitis, but it is a major risk factor linked to other pancreatitis-associated gene variants such as *CFTR*.[25]

5. *SPINK1* variants and pancreatitis.

A "pathogenic" mutation in *SPINK1* is neither necessary nor sufficient to cause pancreatitis. Table 3 gives the prevalence of the common *SPINK1* p.N34S variant in various populations, revealing that heterozygous carriers (HTRZ) are ~2% or higher in Europeans, Asians, Other Asians and South Asians (right column). In contrast, the prevalence of homozygous risk alleles in these populations (Alt HMOZ) is low – about 20 per 100,000 people. Furthermore, the mechanism of action is a protective one, such that risk of pancreatitis through some other mechanism is required.

Pathogenicity is conditional.

Population	Sample Size	Ref Allele	Alt Allele	Alt HMOZ	HTRZ
Total	304528	T=0.990654	C=0.009346	0.00023	0.018231
European	258604	T=0.990217	C=0.009783	0.00024	**0.019087**
African	13086	T=0.99733	C=0.00267	0	0.005349
African American	12612	T=0.99722	C=0.00278	0	0.00555
Asian	6852	T=0.9907	C=0.0093	0.000292	**0.018097**
East Asian	4900	T=0.9978	C=0.0022	0	0.00449
Other Asian	1952	T=0.9728	C=0.0272	0.001025	**0.052254**
Latin American 1	1322	T=0.9947	C=0.0053	0	0.01059
Latin American 2	2538	T=0.9961	C=0.0039	0	0.00788
South Asian	366	T=0.986	C=0.014	0	**0.027322**

Table 3. Frequency of the SPINK1 p.N34S variant in major ancestral groups. See text. From https://www.ncbi.nlm.nih.gov/snp/rs17107315 , accessed March 13, 2025

Fire extinguisher illustration of the pathogenicity of SPINK1 and trypsin activation.

Think of SPINK1 as a fire extinguisher in a home—the pancreas. It sits quietly until a fire, or trypsin overactivation, ignites. Before the blaze, you can't tell if it works fully, partially, or not at all. For a small fire, a good extinguisher stops it early, just as SPINK1 halts trypsin to prevent pancreatitis. But a raging inferno overwhelms even the best extinguisher, mirroring widespread trypsin activity leading to full-blown pancreatitis. In a city, more faulty extinguishers mean more homes burn, though other factors matter too. Likewise, defective *SPINK1* variants raise pancreatitis rates, but they're not the whole story—environmental and genetic co-factors also drive the risk.

Figure 3. Illustration of an uncontrolled fire because of an empty, non-functional fire extinguisher. Image created by DCW using ChatGPT

6. Impact of combined *SPINK1* Genetic Variants and Environmental Factors

SPINK1 and tropical pancreatitis

The tropical pancreatitis is a notion born in the 1960s and 1970s based on the observation of a high prevalence of calcifying pancreatitis in the Indian Subcontinent. Multiple explanations were proposed and environmental causes especially (i.e, Protein calorie malnutrition and cassava consumption). None of them were confirmed. In a pooled analysis of patients with so called "tropical pancreatitis", an increased frequency of both homozygous and heterozygous forms of the N34S variant was observed[26]

indicating a significant genetic susceptibility to this condition in Indian populations. Conversely, within the general population, the occurrence of the homozygous N34S variant was remarkably low. This suggests that while this variant may be a potential risk factor for pancreatitis in specific environments, its presence alone does not confer a significant risk for the development of the disease in the broader population.

Association with other mutations

The p.N34S risk haplotype of SPINK1, while a significant genetic marker, is not sufficient alone to induce CP, as evidenced by its occurrence in the control population. Furthermore, the interaction of the p.N34S risk haplotype with other mutations, particularly mutations in the CFTR gene, suggests a complex gene-gene interplay that contributes to the disease's etiology. [27-29]

Association with environmental factors

Environmental factors such as tobacco and alcohol use, dietary habits, obesity, diabetes, and gut microbiota also play a role in modifying the risk and progression of SPINK1-related chronic pancreatitis. In a study based on a cohort of 798 patients with CP followed for 10.5 years, it was determined that gene mutations in major susceptibility genes of chronic pancreatitis were not correlated with the development of pancreatic insufficiency. However, environmental factors (either alcohol consumption or smoking) markedly accelerated the disease progression.[30] A comparative analysis of the *SPINK1* gene mutation prevalence across different cohorts revealed that its impact varied in the context of chronic alcohol consumption. It concluded that SPINK1 mutations were not an independent risk factor for acute alcoholic pancreatitis (AAP). However, in a subgroup of individuals with excessive alcohol use, it was found to be independently associated with AAP**,** highlighting the gene-environment interaction in the pathogenesis of pancreatitis.[31] (Figure 1). This calls for a complex understanding of chronic pancreatitis as an interplay between genetic susceptibilities and environmental factors rather than a condition driven by single-gene mutations. Rosendahl et al. critically re-examined the role of CFTR mutations in chronic pancreatitis, demonstrating the complexity of genetic interactions in CP and a minor influence of *CFTR* alterations in CP development.

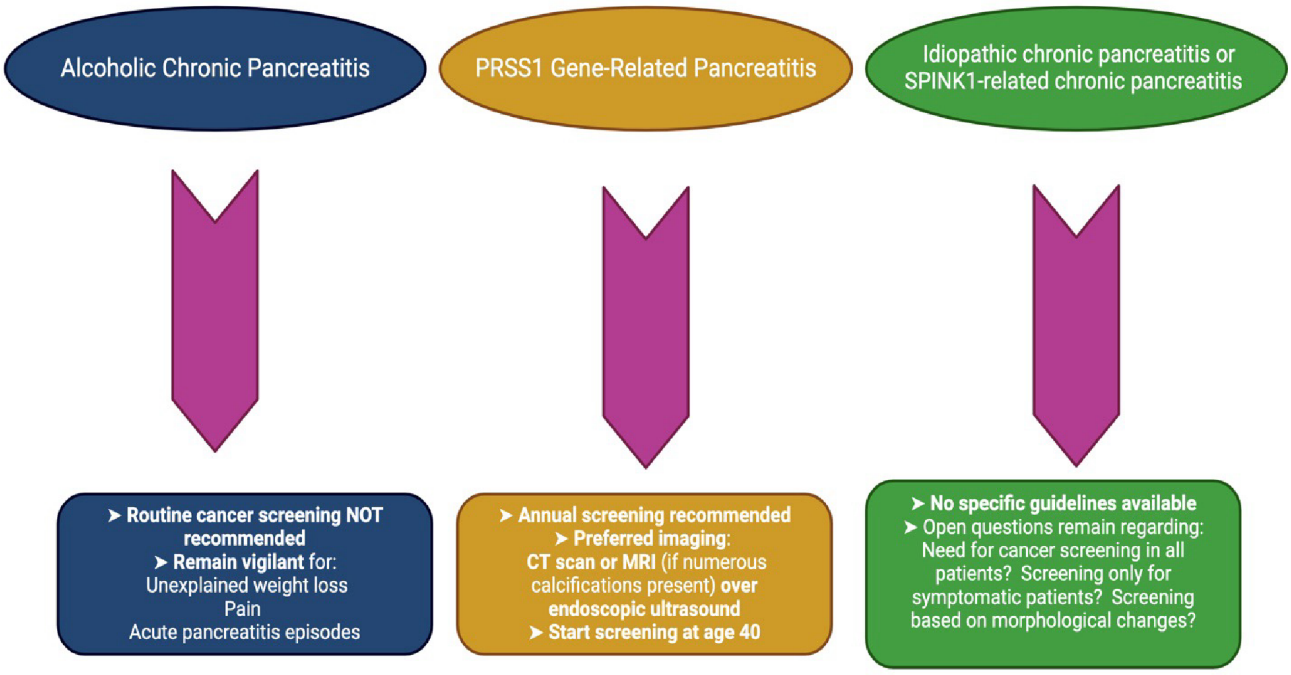

Figure 4. Screening and Surveillance Recommendations for early pancreatic cancer detection. Note that many experts avoid using CT scan for surveillance due to radiation exposure.

There is a complex interplay of genetic factors in chronic pancreatitis, including CFTR, SPINK1, CTRC, and PRSS1 variants, as well as their interaction with environmental influences like tobacco and alcohol use, dietary habits, and metabolic conditions.[10]

Pancreatic divisum and pancreatitis

Recent research challenges the notion of Pancreas Divisum (PD) as the sole etiological factor in pancreatitis. The frequency of PD in the general population is around 7-9% and this prevalence is not significantly different in patients with idiopathic pancreatitis compared to controls, indicating that PD alone is not responsible for the condition. However, it has been observed that the frequency of PD is higher in patients with genetic pancreatitis, especially in those with CFTR mutations or polymorphisms, suggesting a cumulative effect of these two cofactors (Table 1).[32]

SPINK 1 variants and associated conditions

In a multicenter European cohort study, the natural progression of conditions associated with the *SPINK1* p. N34S variant was analyzed.[32] It was found that *SPINK1*-related pancreatitis (SRP) was associated with an earlier onset of pancreatic inflammation. Patients in the SRP group presented with more episodes of acute pancreatitis and had more frequent morphologic signs of CP (Table 1). In addition, they had a significantly higher risk of developing chronic pancreatitis, exocrine pancreatic insufficiency (EPI), Diabetes mellitus (DM) and pancreatic ductal adenocarcinoma (PDAC). (Fig. 5) Notably, the incidence of pancreatic cancer was particularly high among smokers and those with numerous pancreatic calcifications.[33]

7. Screening and Surveillance Recommendations for Early Detection of Pancreatic Cancer

The international consensus guidelines for the surveillance of pancreatic cancer in the context of chronic pancreatitis suggest tailored approaches based on the etiology of pancreatitis.[13] (Figure 4)

- For those with alcoholic chronic pancreatitis, routine screening for cancer is not recommended. However, it is advised to remain vigilant for unexplained weight loss, pain, or acute pancreatitis episodes.
- Patients with PRSS1 gene-related pancreatitis should undergo annual screenings, with CT scans or MRI recommended over endoscopic ultrasound if numerous calcifications are present, starting at the age of 40.
- No specific guidelines are provided for idiopathic chronic pancreatitis or SPINK1-related chronic pancreatitis, leaving open questions regarding the need for cancer screening for all patients, only symptomatic patients, or those with morphological changes regardless of symptoms.

8. Conclusions

SPINK1-assocated genetic variants, like the widespread p.N34S risk haplotype, contribute to chronic pancreatitis (CP) but rarely act alone. Their pathogenicity hinges on reduced trypsin inhibition—via lower expression (e.g., p.N34S), splicing defects (e.g., IVS3+2T>C), or protein misfolding (e.g., p.G48E)—compounded by environmental triggers like alcohol and smoking, or coexisting mutations (e.g., *CFTR*). While heterozygous p.N34S carriers face less than a 1% risk without added factors, homozygotes or more severe variants with other risk factors signal higher danger. Ethnicity shapes this risk through genetic prevalence and lifestyle patterns. For clinicians, CP demands a multifactorial approach: test for *SPINK1* (and other genetic variants) in unexplained AP, RAP or early CP, weigh effects of environmental risks, and tailor management of the disease trajectory with awareness of disease complications (e.g. exocrine pancreatic insufficiency), diabetes mellitus, chronic pain syndromes and, after age 40 years, surveillance for early detection of pancreatic cancer.

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Competing Interest

DCW is a consultant and cofounder of Ariel Precision Medicine.