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REVIEW

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Genetics and clinical implications of *SPINK1* in the pancreatitis continuum and pancreatic cancer

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Abstract

Serine peptidase inhibitor, Kazal type 1 (SPINK1), a 56-amino-acid protein in its mature form, was among the first pancreatic enzymes to be extensively characterized biochemically and functionally. Synthesized primarily in pancreatic acinar cells and traditionally known as pancreatic secretory trypsin inhibitor, SPINK1 protects the pancreas by inhibiting prematurely activated trypsin. Since 2000, interest in SPINK1 has resurged following the discovery of genetic variants linked to chronic pancreatitis (CP). This review provides a historical overview of SPINK1's discovery, function, and gene structure before examining key genetic findings. We highlight three variants with well-characterized pathogenic mechanisms: c.-4141G > T, a causative enhancer variant linked to the extensively studied p.Asn34Ser (c.101A > G), which disrupts a PTF1L-binding site within an evolutionarily conserved HNF1A-PTF1L *cis*-regulatory module; c.194 + 2T > C, a canonical 5′ splice site GT > GC variant that retains 10% of wild-type transcript production; and an *Alu* insertion in the 3′-untranslated region, which causes complete loss of function by forming extended double-stranded RNA structures with pre-existing *Alu* elements in deep intronic regions. We emphasize the integration of a full-length gene splicing assay (FLGSA) with SpliceAI's predictive capabilities, establishing *SPINK1* the first disease gene for which the splicing impact of all possible coding variants was prospectively determined. Findings from both mouse models and genetic association studies support the sentinel acute pancreatitis event (SAPE) model, which explains the progression from acute pancreatitis to CP. Additionally, *SPINK1* variants may contribute to an increased risk of pancreatic ductal adenocarcinoma (PDAC). Finally, we discuss the therapeutic potential of *SPINK1*, particularly through adeno-associated virus type 8 (AAV8)-mediated overexpression of *SPINK1* as a strategy for treating and preventing pancreatitis, and highlight key areas for future research.

Keywords Acute pancreatitis, Chronic pancreatitis, Gene therapy, Genetic variants, Pancreatic ductal adenocarcinoma, PDAC, Pancreatic secretory trypsin inhibitor, *SPINK1* gene

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Background

SPINK1 (serine peptidase inhibitor, Kazal type 1; OMIM #167790) encodes pancreatic secretory trypsin inhibitor, one of the earliest enzymes to be thoroughly characterized biochemically and functionally. Renewed interest in *SPINK1* has surged since 2000, following the discovery of gene variants linked to chronic pancreatitis (CP). This review provides a comprehensive examination of *SPINK1*, beginning with a brief overview of its protein discovery, function, gene structure, and disease association. We then explore genetic findings, focusing on selected disease-associated *SPINK1* variants and highlighting a prospective approach that assessed the splicing effects of all potential *SPINK1* coding variants. Additionally, we discuss the involvement of *SPINK1* variants in the pancreatitis continuum and pancreatic cancer. Finally, we evaluate the potential of *SPINK1* in disease treatment and prevention before highlighting several areas for future research.

Overview of SPINK1: protein discovery and function, gene structure, and disease association

Trypsin, a key enzyme in protein digestion, is produced in the pancreas as an inactive precursor called pretrypsinogen. This precursor is processed in the rough endoplasmic reticulum and Golgi complex to become trypsinogen. Trypsinogen is then stored in secretory granules, released into the pancreatic duct, and activated by enteropeptidase in the duodenum, which triggers the activation of other digestive enzymes [1].

Premature activation of trypsinogen within the pancreas poses a significant threat due to its potential role in pancreatic autodigestion, driven by its autoactivation properties [2]. To mitigate this risk, pancreatic acinar cells synthesize trypsin inhibitors, including SPINK1 [3, 4], alongside trypsinogen. In healthy individuals, SPINK1 can inhibit up to 13% of trypsin potential [5], underscoring its crucial role as a protective mechanism against premature trypsin activation in the pancreas (Fig. 1).

The *SPINK1* mRNA sequence was first identified from a human pancreatic cDNA library in 1985 [6], and the genomic sequence structure of *SPINK1* was unveiled in

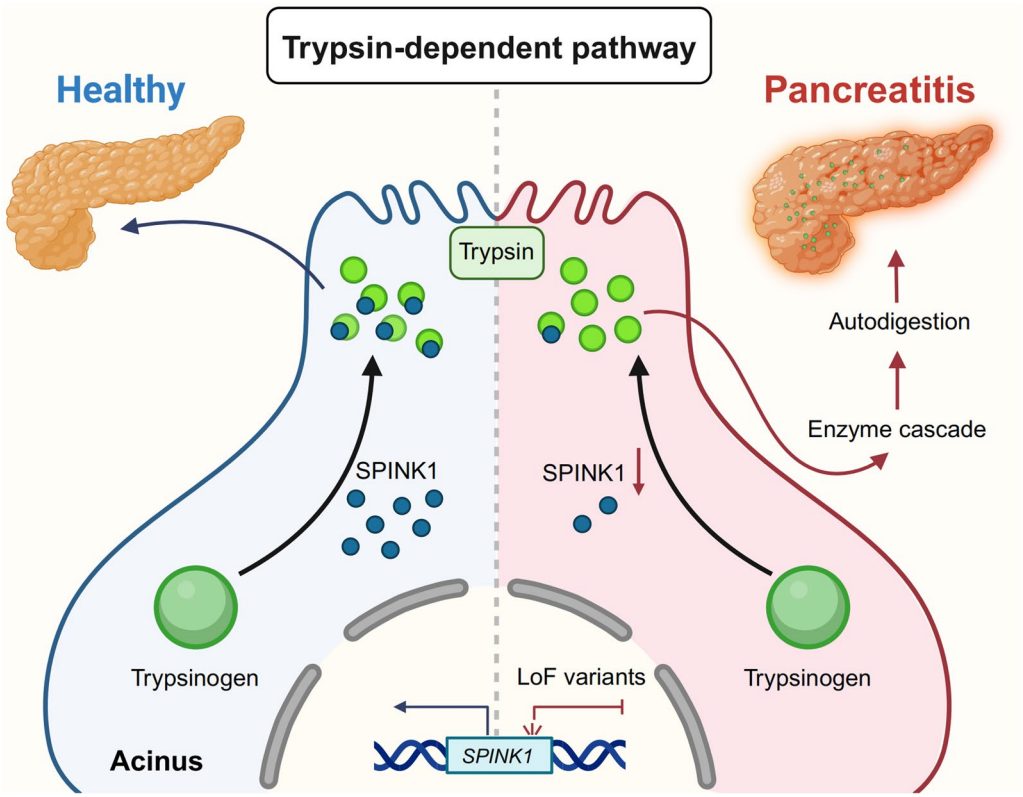

Fig. 1 The role of SPINK1 in protecting against pancreatitis. Normally, SPINK1 inhibits prematurely activated trypsin within the pancreas. However, reduced expression or function of SPINK1, due to loss-of-function (LoF) variants in the *SPINK1* gene, disrupts the trypsin activation/inhibition balance, thereby predisposing individuals to pancreatitis

1987 [7]. Figure 2 illustrates the chromosomal location and mRNA isoforms of *SPINK1*, the primary sequence of the SPINK1 propeptide, and the 3D structure [8] of the mature SPINK1 peptide complexed with human cationic trypsin.

In 1996, a gain-of-function missense mutation (p.Arg122His) in *PRSS1* (OMIM #276000), which encodes cationic trypsinogen, was identified as a cause of autosomal dominant hereditary pancreatitis [9] (for a comprehensive review of disease-associated *PRSS1*

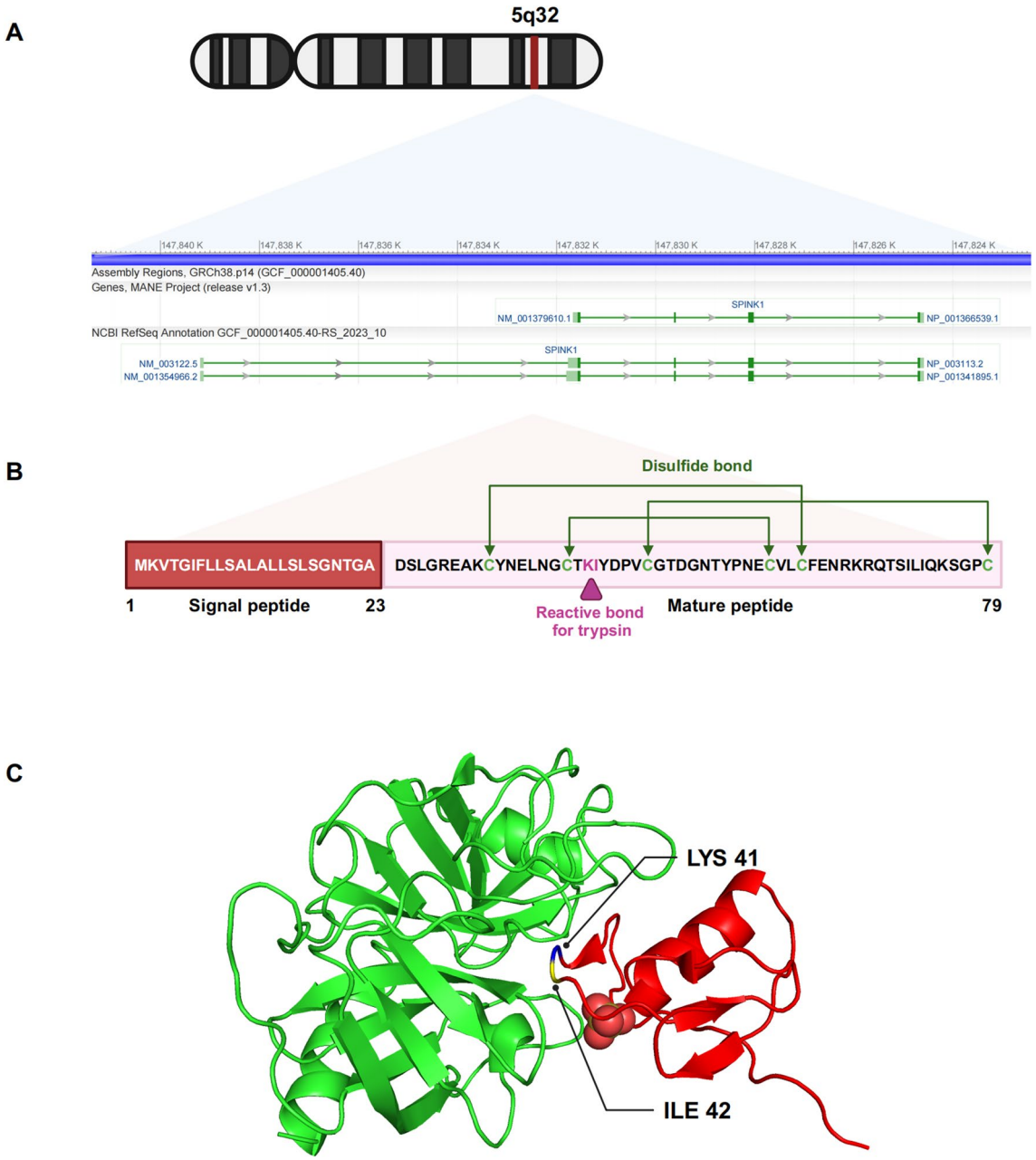

Fig. 2 Overview of *SPINK1* gene structure, protein sequence, and 3D conformation. **A** Chromosomal location and the three mRNA isoforms of the *SPINK1* gene (curated in the UCSC Genome Browser on Human (GRCh38/hg38)). The four-exon mRNA isoform, NM_001379610.1, is expressed in pancreatic tissue. No data is available on the tissues where the other two five-exon isoforms are expressed. All three isoforms have identical coding sequences, encoding the same protein product. Note that *SPINK1* is transcribed from the reverse (minus) strand of chromosome 5. **B** Primary amino acid sequence of the propeptide, indicating the signal peptide sequence, the mature peptide sequence, the Lys41-Ile42 reactive site for binding to trypsin, and the three disulfide bonds. **C** 3D structure of the mature SPINK1 peptide (red) complexed with human cationic trypsin (green). The Lys41 and Ile42 residues in the SPINK1 peptide are indicated. The 3D structure was obtained from the Protein Data Bank [8]

variants, see Masson et al. [10]). Four years later, Witt and colleagues reported an association between *SPINK1* variants and CP by analyzing a German cohort of unrelated children and adolescent patients [11]. This finding has since been corroborated by numerous studies, particularly those conducted between 2000 and 2002 [12–18]. These genetic findings provided unprecedented evidence of SPINK1's essential protective role against pancreatitis, further validated by studies using mouse models [19–25].

Pancreatitis-associated *SPINK1* variants

Pancreatitis-associated *SPINK1* variants encompass a wide range of types and numbers. These include canonical splice site variants [11, 26–30], large gene deletions [31–33], small indel variants [26, 34, 35], and experimentally analyzed regulatory [33, 36–39] and missense [40–44] variants that result in functional loss. For a comprehensive review of reported *SPINK1* variants, interested readers are invited to consult the work of Girodon and colleagues [45]. In this section, we highlight three specific *SPINK1* variants selected for their well-characterized and interesting pathogenic mechanisms.

c.-4141G > T: causal variant in linkage disequilibrium with p.Asn34Ser

The p.Asn34Ser (c.101A > G) variant is one of the most extensively studied variants associated with CP, largely due to its high allele frequency in European populations (e.g., 0.01151 in gnomAD non-Finnish Europeans [46]) and its substantial effect size. Notably, three meta-analyses [47–49] and a representative German study [50] have consistently reported an odds ratio (OR) of approximately 10 for CP in individuals carrying this variant.

Identifying the true pathogenic variant underlying this association has been a long and complex process, as highlighted in two commentary papers [51, 52]. Studies have consistently ruled out the possibility that p.Asn34Ser, as a missense mutation, causes functional loss [41, 42, 44, 53, 54]. Furthermore, both minigene and full-length gene splicing assays have demonstrated that neither c.101A > G nor any linked variants significantly affect mRNA splicing or stability [55–57].

Using HaploReg v4.1 to query data from the 1000 Genomes Project Phase 1 for the European population, 25 single nucleotide polymorphisms were identified in strong linkage disequilibrium (LD) with c.101A > G. Among these, only rs142703147:C > A (c.-4141G > T) was found within an evolutionarily conserved and highly accessible chromatin region, predicted to disrupt a putative HNF1A – PTF1L *cis*-regulatory module [39]. HNF1A and PTF1L are essential components of the transcriptional network that regulates exocrine pancreatic function and acinar cell homeostasis [58–60].

Functional studies, including co-transfection transactivation experiments, have shown that c.-4141G > T reduces *SPINK1* gene expression [39]. Consistently, reduced expression of the variant allele has been observed in two pancreatic cancer cell lines heterozygous for the *SPINK1* p.Asn34Ser haplotype [61]. RNA transcript analyses from three individuals heterozygous for p.Asn34Ser similarly revealed significantly fewer transcript reads from the variant allele compared to the wild-type (WT) allele [62].

The identification of c.-4141G > T as the true pathogenic variant is significant, as it highlights a potential target for personalized therapeutic approaches. In line with the recommendations of the ClinGen Low Penetrance/Risk Allele Working Group [63], c.-4141G > T should be prioritized for analysis and reporting.

c.194 + 2T > C: a canonical 5′ splice site GT > GC variant retaining some WT transcript production

Variants that alter canonical splice sites (donor, +1 and +2; acceptor, −1 and −2) are generally assumed to result in a complete loss of WT transcripts [64–67]. However, an increasing number of canonical 5′ splice site + 2T > C (GT > GC) variants challenge this assumption, as they continue to produce some WT transcripts [66, 68–83]. For the molecular mechanisms underlying this phenomenon, readers are referred to Lin et al. [75].

SPINK1 c.194 + 2T > C, located in intron 3, exemplifies such a variant. Homozygotes for this variant produce approximately 10% of normally spliced transcripts compared to normal subjects [74, 84]. This significant, yet incomplete, effect on splicing was confirmed using a full-length gene splicing assay (FLGSA) [27].

SPINK1 variants causing complete functional loss are either absent or extremely rare (allele frequency below 0.0001) in gnomAD [85], reflecting strong selection pressure. By contrast, c.194 + 2T > C displays a higher allele frequency (0.002192) in gnomAD East Asian populations [46], suggesting less stringent selection pressure. This likely explains its frequent detection in both heterozygous and homozygous states in a large Chinese idiopathic CP (ICP) cohort [34]. In this cohort, c.194 + 2T > C heterozygotes and homozygotes (all without pathogenic variants in *PRSS1*, *CFTR* and *CTRC*) were associated with ORs of 30.4 and 162.4, respectively. Furthermore, homozygotes exhibited significantly earlier ages of disease onset and pancreatic stone formation compared to heterozygotes, who in turn showed significantly earlier ages of disease onset and pancreatic stone formation compared to ICP patients lacking pathogenic genotypes in *PRSS1*, *SPINK1*, *CFTR*, and *CTRC* [34] (see also Fig. 3).

The pathogenic effects of c.194 + 2T > C heterozygotes and homozygotes were also modeled in mice. While

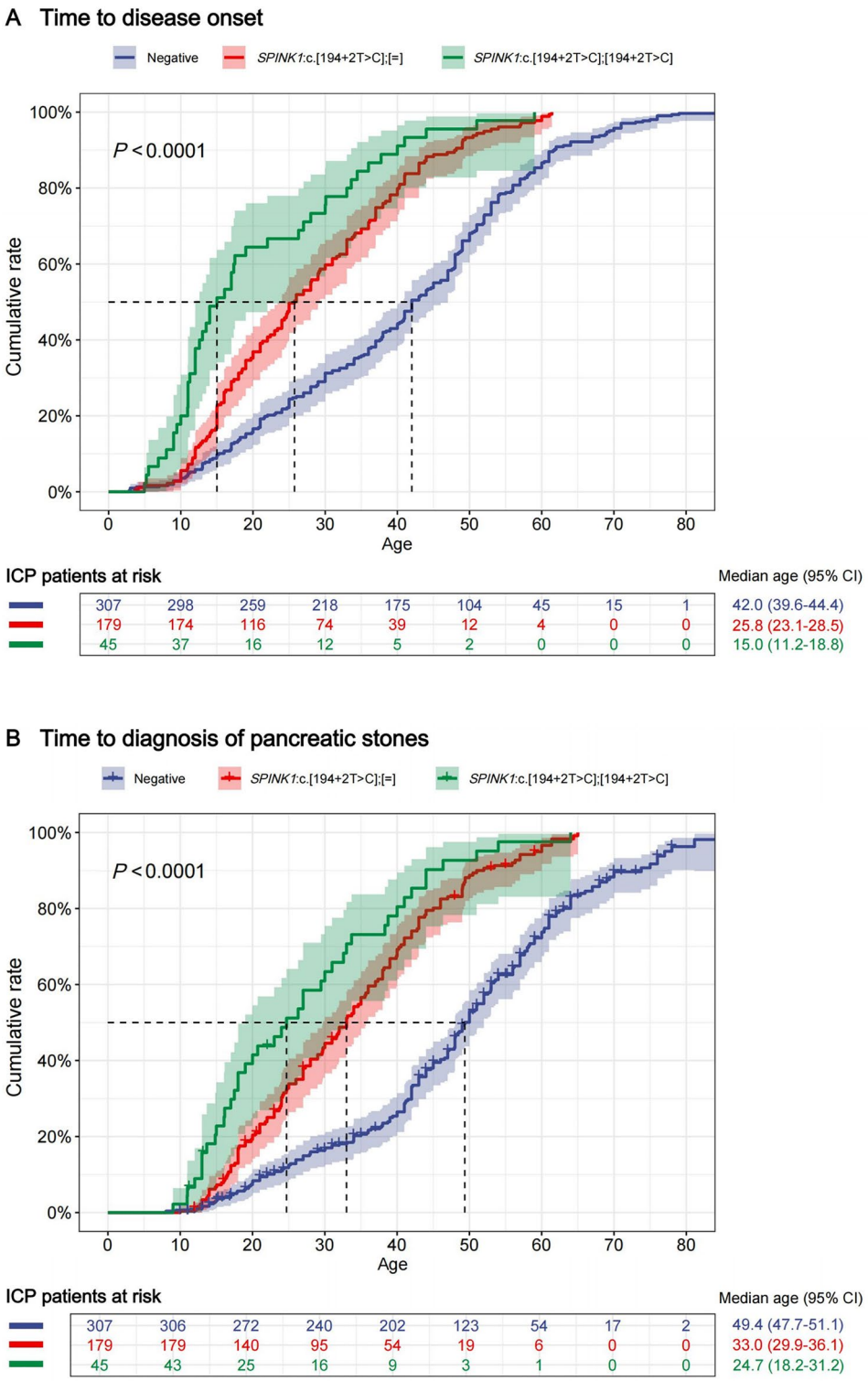

Fig. 3 Quantitatively different clinical outcomes in *SPINK1* c.194 + 2T > C heterozygous and homozygous patients with idiopathic chronic pancreatitis (ICP). **A** Time to disease onset: Kaplan–Meier plots showing differences in the cumulative rate of disease onset between *SPINK1* c.194 + 2T > C heterozygous patients (*SPINK1*:[c.194 + 2T > C];[=], red) and homozygous patients (*SPINK1*:[c.194 + 2T > C];[c.194 + 2T > C], green). **B** Time to diagnosis of pancreatic stones: Kaplan–Meier plots illustrating differences in the cumulative rate of pancreatic stone formation between heterozygous and homozygous patients. Patients without pathogenic genotypes involving *PRSS1*, *SPINK1*, *CFTR*, and *CTRC* (labeled as "Negative") served as controls. The number of patients at risk and the median age at disease onset or pancreatic stone diagnosis (with 95% confidence intervals) are provided in the tables below each panel. Data are redrawn based on the open-access work of Zou et al. [34]

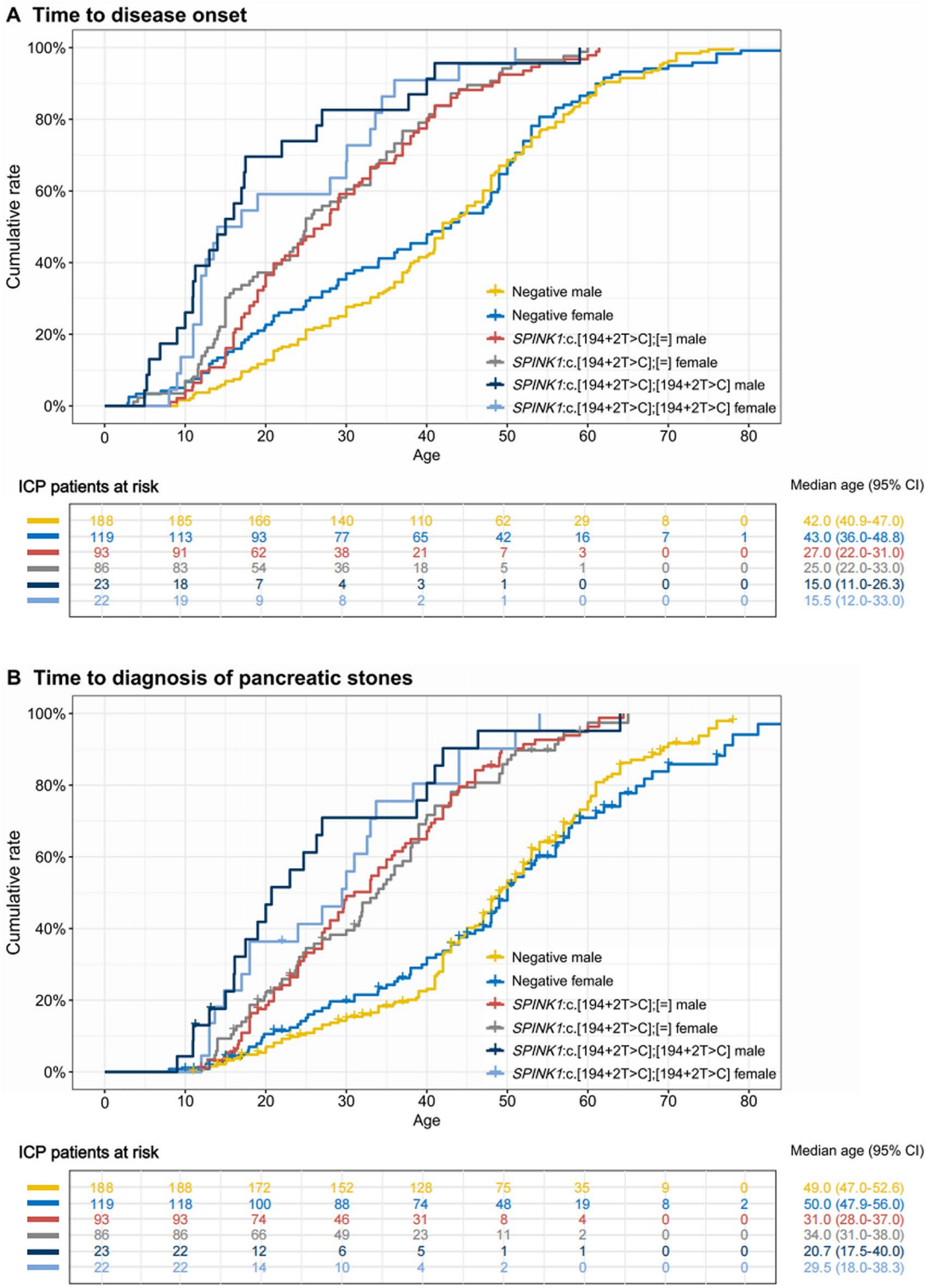

Fig. 4 Sex-specific comparison of *SPINK1* c.194 + 2T > C genotypes on age at disease onset and cumulative rate of pancreatic stone formation in Chinese patients with idiopathic chronic pancreatitis (ICP). **A** Time to disease onset: Kaplan–Meier plots comparing age at disease onset among male and female patients with different *SPINK1* c.194 + 2T > C genotypes: heterozygous (*SPINK1*:[c.194 + 2T > C];[=]) and homozygous (*SPINK1*:[c.194 + 2T > C];[c.194 + 2T > C]) individuals, as well as genotype-negative controls. **B** Time to diagnosis of pancreatic stones: Kaplan–Meier plots showing cumulative rates of pancreatic stone formation across the same genotype and sex groups. The number of patients at risk and the median age at disease onset or pancreatic stone diagnosis (with 95% confidence intervals) are provided in the tables below each panel. Data are based on the original work of Zou et al. [34]

homozygous mice died shortly after birth, approximately 30% of heterozygous mice developed pancreatitis by 14 weeks of age [22, 23].

Interestingly, *SPINK1* c.194+2T>C is also associated with adverse pregnancy outcomes in female Chinese CP patients [86]. Whether this association arises from a direct effect of the variant on pregnancy outcomes or an indirect effect secondary to CP remains unclear. Furthermore, investigating whether c.194+2T>C heterozygotes and homozygotes exhibit quantitatively distinct adverse pregnancy outcomes would be valuable as more such cases become available.

Although *SPINK1* variant-related adverse pregnancy outcomes are restricted to women, differences in pancreatic anatomy, size, and function between men and women may suggest other sex-related differences in the genetic etiology of CP. For example, alcohol and tobacco use are generally considered predominant risk factors in men with CP, whereas idiopathic and obstructive etiologies are more common in women [87]. However, inconsistent observations exist in the literature regarding male-to-female ratios in CP cohorts used for genetic analyses. For instance, a large German case–control study of 660 unrelated CP patients included a slightly higher fraction of women (n=347, 52.6%) [50], while the large Chinese ICP cohort comprised 69.6% men [34]. Such discrepancies make cross-study comparisons of male-to-female ratios in patients with pathogenic variants challenging or essentially irrelevant.

To address this, we tested whether the three genotypes—c.194+2T>C heterozygotes, c.194+2T>C homozygotes, and ICP patients lacking pathogenic genotypes in *PRSS1*, *SPINK1*, *CFTR*, and *CTRC*—differed in age of disease onset or pancreatic stone formation between male and female Chinese patients. As shown in Fig. 4, no significant differences were observed for any of the genotypes between male and female patients.

Finally, it is important to emphasize that the findings related to *SPINK1* c.194+2T>C [27, 74] have led to further investigation into the prevalence of similar variants that maintain the ability to generate WT transcripts. Specifically, data from a meta-analysis of disease-associated+2T>C variants and FLGSA of artificially created+2T>C variants suggest that 15–18% of

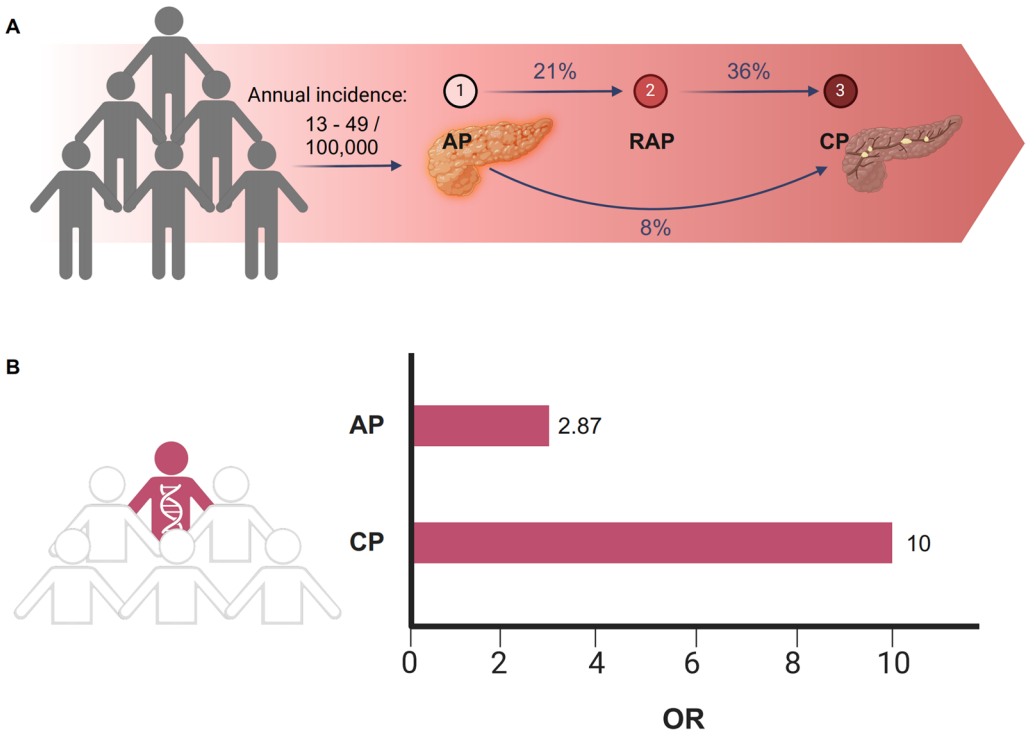

Fig. 5 The role of p.Asn34Ser-containing haplotype in the pancreatitis disease continuum. **A** Progression through the pancreatitis disease continuum: Schematic illustration showing the annual incidence of AP and the stepwise progression of pancreatitis. Specifically, 21% of patients with a first AP episode progress to RAP, and 36% of those with RAP eventually develop CP. This progression corresponds to 8% of patients with a first AP episode ultimately progressing to CP. **B** Risk of AP and CP associated with the *SPINK1* p.Asn34Ser haplotype: Bar graph depicting the OR for AP (2.87) and CP (10) conferred by the p.Asn34Ser haplotype. It is important to note that while the p.Asn34Ser variant is functionally neutral, the true causative variant in linkage disequilibrium is the c.-4141G>T enhancer variant. AP, acute pancreatitis; CP, chronic pancreatitis; RAP, recurrent acute pancreatitis; OR, odds ratio

all potential + 2T > C variants in human genes are capable of generating 1%-84% normal transcripts [75]. This recognition is crucial for disease diagnosis and variant interpretation, as it implies that + 2T > C variants may not always be pathogenic and can account for unexpectedly mild disease expressions. Moreover, predicting whether a + 2T > C variant will produce WT transcripts remains challenging with current in silico tools [75, 84].

Alu insertion in *SPINK1*'s 3′-UTR: a novel mechanism leading to complete functional loss

Alu elements, approximately 300 base pairs long, make up about 11% of the human genome, with over a million copies distributed throughout [88]. *Alu* insertions, mobilized by LINE-1 [89, 90], have been implicated in various human diseases [91–94].

A rare *Alu* insertion in the 3′-untranslated region (3′-UTR) of *SPINK1* was discovered in an individual with severe infantile isolated exocrine pancreatic insufficiency (SIIEPI) [33]. This mutation, found in the homozygous state, was shown to completely abolish *SPINK1* expression, as demonstrated by both a cell culture-based full-length gene expression assay and RT-PCR analysis using lymphocytes from the affected individual [33]. Similarly, another SIIEPI patient was identified with a complete homozygous deletion of *SPINK1*. Both individuals exhibited severe exocrine pancreatic insufficiency from early infancy and developed diffuse pancreatic lipomatosis, without significant dysfunction in other organs [33]. These symptoms closely resemble the pancreatic acinar cell necrosis observed in homozygous *Spink1* knockout mice, which typically results in perinatal death [19].

The detrimental effect of the *Alu* insertion has recently been attributed to its ability to form extended double-stranded RNA structures with pre-existing *Alu* elements in *SPINK1*'s intron 3, revealing a previously unknown pathogenic mechanism. The key evidence for this mechanism includes the fact that the two pre-existing intronic *Alu* sequences are oriented oppositely to the *Alu* insertion and that *SPINK1* mRNA expression was restored when all three *Alu* elements were aligned in the same orientation [95]. Considering that new *Alu* insertions can potentially occur at nearly any genomic location and that *Alu* elements are widely dispersed throughout the human genome, these findings carry substantial implications for variant detection and interpretation.

SPINK1: the first gene with prospectively determined splicing impacts of all possible coding variants

Variants previously considered neutral, such as missense or silent mutations, can contribute to disease by affecting splicing [96]. This is critical in precision medicine, where personalized treatments for specific variants may fail if splicing alterations are overlooked [97]. Therefore, accurately determining the splicing impact of coding variants, ideally in a prospective manner, is essential [98, 99].

Unlike the common practice of using minigene assays to assess splicing impacts, as seen in some recent publications [100–104], we routinely used FLGSA to characterize the splicing effect of *SPINK1* variants. In our FLGSA system, the pcDNA3.1/V5-His TOPO vector served as the backbone, with the approximately 7 kb genomic sequence of *SPINK1*, including all exons and introns, inserted. This approach preserves the gene's natural genomic context, which is crucial given the complexities of splicing regulation [105]. The accuracy and reliability of our FLGSA assay were validated using known *SPINK1* variants [27, 28, 57, 106–108].

Leveraging the FLGSA assay and the predictive capabilities of SpliceAI [109], a deep neural network tool for splicing prediction, we recently succeeded in prospectively interpreting the splicing effects of all potential coding single-nucleotide variants (SNVs) within *SPINK1*. We began with a retrospective analysis of 27 previously FLGSA-assessed *SPINK1* coding SNVs, followed by a prospective analysis of 35 new SNVs. In total, we examined 67 *SPINK1* coding SNVs, representing 9.3% of the 720 possible coding SNVs [110].

Among the 67 SNVs analyzed, 12 were found to impact splicing. By comparing the FLGSA results with SpliceAI predictions, we inferred that the remaining 653 untested coding SNVs in *SPINK1* are unlikely to significantly affect splicing. Therefore, it was concluded that less than 2% of potential coding SNVs in *SPINK1* are likely to influence splicing [110].

SPINK1 variants and the SAPE model of pancreatitis

Genetic research into pancreatitis risk factors typically begins with studies on CP. However, it is now widely recognized that there is a continuum between acute pancreatitis (AP), recurrent AP (RAP), and CP [111–113]. To explain this progression, the sentinel acute pancreatitis event (SAPE) model was proposed [114]. This model posits that an initial episode of AP serves as a sentinel event, sensitizing the pancreas and making it more susceptible to RAP. Repeated stress and inflammation from RAP can eventually lead to CP. Epidemiological data, including the annual incidence

of AP [115] and progression rates from a first AP episode to CP [116], are shown in Fig. 5A.

Mouse studies supporting the SAPE model

Recent studies using mouse models have provided direct support for the SAPE model. In one study, a cerulein-induced AP mouse model demonstrated that an initial AP episode exacerbated the severity of subsequent attacks. Persistent macrophage infiltration was identified as a key mechanism driving enhanced injury and more severe inflammatory responses during successive episodes [117].

In a second study, SAPE attacks were induced in both WT and *Spink1* c.194 + 2T > C mutant mice via cerulein injections. The *Spink1* c.194 + 2T > C mutant mice exhibited a more severe AP phenotype within 24 h after the SAPE attack and developed a significantly more severe CP phenotype during the chronic phase compared to their WT counterparts. Proteomic analysis revealed elevated IL-33 levels in the mutant mice, and subsequent in vitro experiments demonstrated that IL-33 promoted M2 macrophage polarization and pancreatic stellate cell activation [25].

These studies provided complementary insights: the first [117] offered general proof-of-concept evidence supporting the SAPE model, while the second [25] offered genetic proof-of-concept evidence, linking the *Spink1* c.194 + 2T > C mutation to the SAPE-driven progression of pancreatitis.

p.Asn34Ser: linking AP to CP

The first study that identified a significant link between *SPINK1* variants and AP analyzed 371 AP patients and 459 controls [118]. Specifically, it found a significantly higher prevalence of the p.Asn34Ser variant among AP patients (7.8%) compared to controls (2.6%) ($P < 0.001$). A meta-analysis encompassing nine studies, with a combined total of 1493 cases and 2595 controls, revealed a significant association between the p.Asn34Ser variant and a heightened risk of AP (OR 2.87, 95% confidence interval (CI) 1.89–4.34; $P < 0.001$) [119]. Additionally, p.Asn34Ser appears more frequently in moderate to severe AP cases than in mild cases, suggesting it predisposes individuals to more severe disease progression (OR 2.05, 95% CI 0.85–5.1; $P < 0.005$) [120].

Subsequent studies have shown that the *SPINK1* p.Asn34Ser variant is linked to RAP rather than the initial AP episode [121, 122]. However, individuals with this variant experiencing a sentinel AP episode are approximately 19 times more likely to develop RAP [121]. Long-term follow-up of children with RAP over 25.5 months showed that the presence of p.Asn34Ser was significantly associated with progression to CP ($P = 0.01$) [123].

Three meta-analyses [47–49] have consistently demonstrated an OR of approximately 10 for CP risk associated with the p.Asn34Ser haplotype. By contrast, the *SPINK1* p.Asn34Ser haplotype is associated with a lower OR of 2.87 for AP [119] (Fig. 5B). This difference in ORs may reflect the variant's role in accelerating progression from AP to CP, as suggested by the SAPE model [114] and evidence from the *Spink1* c.194 + 2T > C mouse study [25]. Unlike p.Asn34Ser, no other *SPINK1* variant has similarly extensive association data for both AP and CP.

In summary, the strong association of the *SPINK1* p.Asn34Ser haplotype (with c.-4141G > T identified as the true causative variant [39]) with both AP and CP supports the SAPE model. Given its high frequency in European populations and its significant impact on mRNA expression [62], c.-4141G > T stands out as a key inherited genetic factors driving progression from AP to CP.

SPINK1 variants and PDAC

Pancreatic ductal adenocarcinoma (PDAC) represents approximately 95% of all pancreatic cancer cases [124]. With a five-year survival rate of only 8%, PDAC is one of the deadliest cancers [125]. By 2030, it is predicted to be the second leading cause of cancer-related deaths [126].

CP is a major risk factor for PDAC, with a standardized incidence ratio of 22.61 (95% CI 14.42–35.44) according to a recent meta-analysis of 12 studies [127]. Given the pivotal role of SPINK1 in modulating CP risk, it is logical to investigate whether *SPINK1* variants associated with CP also contribute to an elevated risk for PDAC. However, early case–control studies often yielded inconclusive findings. For instance, a meta-analysis of six case–control studies, involving 929 pancreatic cancer cases and 1890 healthy controls, found no significant difference between the groups (OR 1.52, 95% CI 0.67–3.45; $P = 0.315$) [128]. The lack of statistical significance in these findings can primarily be attributed to small sample sizes, particularly considering the multifactorial nature of pancreatic cancer [129, 130] and the fact that only about 5% of CP patients develop pancreatic cancer over a 20-year period [131].

A recent analysis of 1009 Chinese PDAC patients from the Nanjing cohort provided compelling evidence, identifying 21 cases heterozygous for the c.194 + 2T > C variant. This yielded an OR of 3.2 (95% CI 1.8–5.7; $P < 0.001$) when compared to 4327 East Asian controls from the ExAC cohort [132]. However, no statistically significant difference would be achieved if we use the 1196 Chinese controls analyzed in Zou et al. [34] as controls (21 heterozygotes of 1,009 cases [132] *versus* 13 heterozygotes of 1,196 controls [34]; OR 1.93; 95% CI 0.96–3.88;

$P=0.059$). This case illustrates the importance of large sample sizes (either case or control) in achieving statistical significance.

Further exploration was conducted in two additional studies from different perspectives. The first, a large multicenter European cohort study, compared 209 individuals with *SPINK1*-related pancreatitis to 302 individuals with idiopathic pancreatitis. Although the incidence of PDAC did not differ with statistical significance between these groups (3.3% [7 individuals] of the *SPINK1*-related pancreatitis cohort *versus* 0.99% [3 individuals] of the idiopathic pancreatitis cohort; $P=0.1$), the former group had a 12-fold higher risk of developing PDAC compared to the latter group (Cox Hazard Ratio [HR] 12.0, 95% CI 3.0–47.8; $P<0.001$) [133]. The second study, a prospective observational study, followed 965 Chinese CP patients for 11 years. This study found a lower risk of pancreatic cancer among patients with *SPINK1* variants compared to those without (Cox HR 0.39, 95% CI 0.14–1.04; $P=0.059$) [134]. These controversial findings underscore the complexity of interpreting such data, particularly given CP's well-established role as a risk factor for PDAC, independent of the underlying etiology of CP.

To effectively assess the relationship between *SPINK1* variants and PDAC, incorporating a mechanistic perspective into patient selection is justified. Case–control studies should ideally compare the frequency of *SPINK1* variants in PDAC individuals with a prior diagnosis of CP against healthy controls. Reanalysis of data from the aforementioned two studies supports this approach: In the European cohort, 70% (7 out of 10) of the PDAC individuals were heterozygous for either the p.Asn34Ser mutation or large *SPINK1* deletions [133]; in the Chinese cohort, 17% (4 out of 24) of the pancreatic cancer patients carried the c.194+2T>C variant [134]. These frequencies significantly exceed those observed in the corresponding normal populations, reinforcing the link between LoF *SPINK1* variants and an increased risk of PDAC.

In summary, LoF *SPINK1* variants are likely linked to an increased risk of PDAC. This elevated risk may stem from variant-induced CP, which creates a pro-inflammatory, tumorigenic microenvironment that promotes cancer initiation and progression, consistent with the well-established role of inflammation in cancer [135, 136].

SPINK1 and pancreatitis treatment and prevention

CP is a chronic fibroinflammatory disease characterized by persistent abdominal pain, recurrent episodes of AP, irreversible morphological changes, and progressive pancreatic dysfunction. The disease leads to substantial declines in quality of life [137]. With a global prevalence ranging from 13.5 to 52.4 cases per 100 000, and an observed increase over the past two decades, CP represents a significant health burden [138]. There is currently no cure for CP, highlighting the need for effective therapeutic and preventive strategies.

The critical role of *SPINK1* in pancreatitis protection suggests the potential of using external trypsin inhibitors for managing and preventing pancreatitis. This potential has been previously explored in animal models of pancreatitis [139–141], in the treatment of pediatric AP [142], and in reducing pancreatic damage during endoscopic procedures [143, 144]. Additionally, early experiments with mice expressing rat or human *SPINK1* have shown promise for treating CP [20, 21, 145]. More recently, a custom AAV8 vector was modified to express human *SPINK1* (h*SPINK1*) for therapeutic use in mouse models of pancreatitis, including pancreatic duct ligation, caerulein-induced pancreatitis, and the *Spink1* c.194+2T>C mutation [23]. AAV8-h*SPINK1* selectively targeted the pancreas, exhibiting minimal tropism for other organs such as the heart, liver, and kidneys. Optimal expression of h*SPINK1* was achieved at a dose of 2×10^{11} viral genomes per animal. Therapeutic effects peaked four weeks post-administration and persisted for at least eight weeks. A single dose of AAV8-h*SPINK1* significantly reduced the severity of pancreatitis, slowed fibrosis progression, and decreased pancreatic apoptosis and autophagy, thereby accelerating recovery.

Berke and Sahin-Tóth [146] have noted that the AAV8 approach is still limited by the relatively low levels of *SPINK1* expression achievable in the pancreas. Since increasing the viral dose could lead to a higher risk of side effects, an alternative strategy might be to use a *SPINK1* expression vector that inherently expresses higher *SPINK1* levels. Interestingly, there is a phenomenon known as intron-mediated enhancement of gene expression [147, 148], and the insertion of a short intronic sequence into human *SPINK1* cDNA has been shown to significantly boost mRNA expression in cell culture models [55, 146]. This approach holds promise for improving the efficacy of *SPINK1*-based therapies in the treatment of pancreatitis.

Conclusions and prospects

The findings on *SPINK1* since its genetic discovery in 2000 are impressive, but many areas remain to be explored. Leveraging the FLGSA assay [110], a similar prospective approach could be applied to analyze all intronic variants of *SPINK1*. Additionally, the association between *SPINK1* variants and PDAC requires validation through large-scale studies.

Gene-environment interactions are crucial but underexplored [49, 149]. The spontaneous occurrence of CP in some *Spink1* c.194+2T>C$^{+/-}$ or *Spink1*$^{+/-}$ mice [22, 24] makes these models ideal for studying these interactions. For instance, comparing *Spink1* expression levels and CP incidence rates in these mice under different alcohol exposure levels could reveal how alcohol treatment influences CP development.

Genetic studies of pancreatitis have identified both trypsin-dependent [150] and misfolding-dependent [151] pathways in its pathogenesis. Investigating the combined effects of genetic variants in these pathways could provide insights into the complex genetic interactions of pancreatitis. Previous studies have shown synergistic effects between variants in the same pathway in mice [24, 152], but there are no reports on variants in different pathways. Crossing *Spink1* c.194+2T>C$^{+/-}$ mice [22] with *CEL-HYB1*$^{+/-}$ mice [153] could generate offspring carrying both variants, offering a model to study these interactions.

The *SPINK1* c.194+2T>C and c.-4141G>T variants, with notable allele frequencies and significant but partial functional loss, are frequently detected in pancreatitis patients. These variants, correlating with their functional effects, serve as valuable models for exploring pancreatitis pathways. Bulk or single-cell RNA sequencing (RNAseq) analysis of global gene expression patterns in pancreatitis patients carrying these variants could identify specific genes and molecular markers differentially expressed, potentially informing the development of SPINK1-related therapies. Given the difficulty in obtaining pancreatic tissues, RNAseq analysis could be performed on more accessible tissues or fluids such as blood, pancreatic juice, or fine-needle aspiration samples.

Exploring CRISPR/Cas9 gene-editing technology to correct *SPINK1* variants (e.g., c.-4141G>T and c.194+2T>C) in pancreatic cells or organoids could offer a potential therapeutic avenue, paving the way for personalized medicine strategies targeting specific genetic mutations in pancreatitis patients.

In conclusion, while significant progress has been made in uncovering the role of *SPINK1* variants in pancreatitis, many opportunities for further research remain. Addressing these gaps will enhance our understanding of the genetic and environmental factors driving the pancreatitis continuum as well as PDAC, ultimately aiding in the development of more effective prevention and treatment strategies.

Abbreviations

AP	Acute pancreatitis
CI	Confidence interval
FLGSA	Full-length gene splicing assay
HR	Hazard Ratio
ICP	Idiopathic chronic pancreatitis
LD	Linkage disequilibrium
LoF	Loss-of-function
OR	Odds ratio
PDAC	Pancreatic ductal adenocarcinoma
RAP	Recurrent acute pancreatitis
RNAseq	RNA sequencing
RT-PCR	Reverse transcription-PCR
SAPE	Sentinel acute pancreatitis event
SIIEPI	Severe infantile isolated exocrine pancreatic insufficiency
SNVs	Single-nucleotide variants
3′-UTR	3′-Untranslated region
WT	Wild-type

Author contributions

Q.W.W. wrote the review and prepared all figures. W.B.Z. conceived and wrote the review, and prepared the figures. E.M. and C.F. critically revised the review, providing important intellectual input. Z.L. conceived the review and critically revised it with important intellectual input. J.M.C. conceived and wrote the review, and assisted in preparing the figures. All authors reviewed and approved the final manuscript.

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Availability of data and materials

All supporting data are available within the article. No datasets were generated or analysed during the current study.

Declarations

Declaration of generative AI in scientific writing

During the preparation of this work the authors used ChatGPT 4o in order to improve readability and language. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

Jian-Min Chen serves as an Associate Editor for Human Genomics but was not involved in the editorial review process or the decision to publish this article. All remaining authors declare that they have no competing interests.

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