



## Research paper

## Natural history of SPINK1 germline mutation related-pancreatitis



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## ABSTRACT

**Background:** The aim was to describe genetic, clinical and morphological features in a large, multicentre European cohort of patients with *SPINK1* related pancreatitis, in comparison with patients with idiopathic pancreatitis (IP).

**Methods:** All *SPINK1* mutation carriers with pancreatic symptoms from two French and one English centers were included. Patients with IP were included in a control group. Genetic, clinical, radiological and biochemical data were collected.

**Findings:** 209 and 302 patients were included in the *SPINK1* and control groups (median follow-up: 8.3 years (3.7–17.4) vs 5.3 (2.5–8.8)).

The median age at onset of symptoms was 20.1 years (17.5–22.8) in the *SPINK1* group versus 41.2 (35.2–45.2). The age of exocrine pancreatic insufficiency (EPI) onset in the *SPINK1* group was 49.5 (44.5–54.6) years vs. 65.2 (62.1–68.3),  $p < 0.001$ . *SPINK1* patients with EPI were 5.3%, 14.7%, 28.3% and 52.4% at 20, 30, 40 and 50 years.

Diabetes occurred 37.7 (33.3–42.1) years following the onset of symptoms in the *SPINK1* group vs. 30.6 (17.3–43.8) ( $p = 0.002$ ). *SPINK1* patients with diabetes were 7.8%, 13.4%, 26.3% and 43.4% at 30, 40, 50 and 60 years.

Seven patients (3.3%) developed pancreatic cancer in the *SPINK1* group (versus 3 (0.99%),  $p = 0.1$ ), at a median age of 60 vs 66 years. The cancer risk was 0.8% before 50 years, 11.9%, 27.7%, 51.8% at 60, 70 and 80 years and was 12 times higher than in controls (Cox HR 12.0 (3.0–47.8),  $p < 0.001$ ).

**Interpretation:** *SPINK1* related pancreatitis is associated with earlier onset and pancreatic insufficiencies. p.N34S *SPINK1* may well be associated with cancer.

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**Abbreviation list:** CFTR, cystic fibrosis transmembrane conductance regulator gene; CP, chronic pancreatitis; DM, diabetes mellitus; EPI, exocrine pancreatic insufficiency; EUS, endoscopic ultrasound; IP, idiopathic pancreatitis; PDAC, pancreatic ductal adenocarcinoma; SPINK1, serine protease inhibitor kazal type 1; SRP, SPINK1-related pancreatitis; CTSC, chymotrypsin C; PRSS1, serine protease 1; CAPS, international cancer of the pancreas screening.

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## Research in context

## Evidence before this study

Advances in the field of clinical genetics and the availability of genetic testing at a larger scale in the general population have led to major progress in understanding the pancreatic inflammatory process and more particularly the causes of pancreatitis. The role of the *SPINK1* gene is to maintain the integrity of exocrine pancreatic tissue by inhibiting prematurely activated intra-pancreatic trypsin, accounting for inhibition of 20% of trypsin activity. If the

*SPINK-1* mutations variant is associated with idiopathic pancreatitis, the natural history of *SPINK-1* related-pancreatitis is currently inadequately described and previous literature reports are still controversial.

#### Added value of this study

This study is a multicentre European study, based on a large cohort of patients recruited on a French and English national basis. In this study, the natural history of *SPINK 1* related-pancreatitis was extensively described and compared with a well-defined control group of patients with idiopathic pancreatitis. *SPINK1* related pancreatitis was associated with earlier onset of pancreatic inflammation. The risk of pancreatic cancer was 12 times higher than in idiopathic pancreatitis. We found a high rate of pancreatic cancer, especially in smokers and in case of multiple pancreatic calcifications.

#### Implications of all the available evidence

Patients with *SPINK-1* mutations related chronic pancreatitis and p.N34S carriers especially should be offered appropriate advice and follow-up in a multidisciplinary specialist pancreatic outpatient clinic and monitoring for symptoms or signs of chronic pancreatitis. A cancer-screening program has to be discussed for patients, especially in case of pancreatic calcifications.

## 1. Introduction

Advances in the field of clinical genetics and the availability of genetic testing at a larger scale in the general population have led to major progress in understanding the pancreatic inflammatory process and more particularly the causes of pancreatitis. The role of genetic variants is now better understood, providing an explanation of why some patients develop chronic pancreatitis early in life or why, on the other hand, only 5% of chronic alcohol drinkers will develop pancreatic disorders. Over the last two decades, several genes and multiple variants have been reported to have an effect on pancreatic homeostasis and act as potential risk factors for pancreatic inflammation. In everyday practice, however, only few genes, serine protease 1 (*PRSS1*), cystic fibrosis transmembrane conductance regulator gene (*CFTR*), serine protease inhibitor Kazal type 1 gene (*SPINK1*), chymotrypsin C gene (*CTRC*) and carboxypeptidase A1 (*CPA1*) are regularly analyzed in cases of idiopathic recurrent acute or chronic pancreatitis. Genetic testing for these cases should be performed within a context of a multidisciplinary approach, including genetic counselling when appropriate. *PRSS1*, encoding for cationic trypsinogen, is responsible for hereditary pancreatitis, an autosomal dominant disease with penetrance up to 93% [1]. Its role in hereditary pancreatitis is well-established and is not a matter of debate. However, the role of heterozygous variants of *CFTR*, *CTRC* and *SPINK1* genes in pancreatitis remains controversial. The prevalence of these variants in the general population is as high as 4% for *CFTR*, suggesting that there are other genetic and environmental modifying factors.

*SPINK1* (Serine Protease Inhibitor Kazal type 1), is a 4-exon gene located on chromosome 5, encoding for a 56 amino-acid protein. Its role is to maintain the integrity of exocrine pancreatic tissue by inhibiting prematurely activated intra-pancreatic trypsin, accounting for inhibition of 20% of trypsin activity [2]. Over 30 *SPINK1* variants have been reported, the p.N34S mutation being the most frequently reported. An association between *SPINK1* variants and pancreatitis was first reported by Witt et al., who described the presence of the p.N34S variant in 18/85 (21%) of children with idiopathic pancreatitis (IP) [3]. Further studies reported *SPINK1* mutations in 6.4% to 43% of patients with idiopathic pancreatitis [4–

8]. However, the prevalence of heterozygous p.N34S mutation in the general population was estimated to be 1–2%, suggesting that the presence of *SPINK1* p.N34S variant alone is not sufficient to explain the development of chronic pancreatitis (CP) [6]. Therefore, the *SPINK1* p.N34S variant can be considered as a disease modifier rather than a causative factor, leading to chronic pancreatitis, when additional risk factors for pancreatic inflammation such as environmental/lifestyle (alcohol or tobacco consumption) or genetic (known or yet unidentified) are present.

The natural history of *SPINK1*-related pancreatitis (SRP) is currently inadequately described and previous literature reports are still controversial. This is due to a variety of reasons including: limited number of small studies describing cohorts of 6 to 48 patients; genetic variants of unknown significance were included; evaluation for other risk factors is sometimes limited to tobacco consumption; finally, the frequent coexistence of other potential causes of pancreatitis [6,8,9] did not allow for clear conclusions to be drawn regarding the role of *SPINK1* variants in pancreatitis. Furthermore the association between coexistent *SPINK1* mutations and other germline mutations in genes involved in pancreatic diseases (trans-heterozygosity) needs to be further elucidated [10].

The aim of this study was to describe epidemiological, genetic, clinical and pancreatic morphological features in a large, multicentre European cohort of patients with chronic pancreatitis who carry the minor allele of *SPINK1* codon 34, and to compare these characteristics with a control population consisting of patients with idiopathic pancreatitis who carry wild-type *SPINK1*.

## 2. Patients and methods

### 2.1. Patients

All consecutive *SPINK1* mutation carriers with pancreatic symptoms from two French centers (Beaujon University Hospital, Clichy, and Rangueil University Hospital, Toulouse) and from the Royal Liverpool University Hospital, England, were identified from the local prospective cohorts dated from January 1st 2000 to June 1st 2018 and were included in this study.

#### 2.1.1. *SPINK1*-related pancreatitis group

##### 2.1.1.1. Inclusion criteria.

- Presence of at least one *SPINK1* germline mutation
- Pancreatic-related manifestations defined as acute or recurrent acute pancreatitis, chronic pancreatic pain, chronic pancreatitis or exocrine or endocrine pancreatic insufficiency with no other evident cause of pancreatitis.
- Extensive workup performed in order to rule out other causes of pancreatic disorders including clinical examination, biochemical analysis and imaging investigations including transabdominal ultrasound, CT scan, MRI (including MRCP, MR pancreas-specific protocol, and secretin-stimulated MRCP when indicated) or endoscopic ultrasound (EUS).

2.1.1.2. *Indications for genetic testing.* Genetic screening was performed when one or more of the following conditions were met: 1) Acute and recurrent acute pancreatitis with no identifiable cause following extensive investigations, 2) Chronic pancreatic pain, 3) Morphological abnormalities consistent with chronic pancreatitis and no identified cause 4) First attack of acute pancreatitis in young patients (< 35 years); or 5) In the case of a family history of pancreatitis.

Genetic testing included search for mutations in genes known to be associated with pancreatic inflammation: *SPINK1* gene, cationic trypsinogen gene (*PRSS1*) and cystic fibrosis transmembrane regulator (*CFTR*). *CTRC* gene analysis was routinely performed only at the Beaujon and Toulouse centers.

### 2.1.1.3. Exclusion criteria.

- All patients with potential causes of pancreatitis were excluded: chronic alcohol consumption (daily alcohol intake > 3 units (24 g of pure alcohol) per day in men, or > 2 units (16 g of pure alcohol) per day in women; hypercalcaemia >3 mmol/L; hypertriglyceridemia >10 mmol/L; ductal obstruction (ampullary adenoma, intraductal papillary mucinous neoplasm, cystic pancreatic neoplasm); post-traumatic duct stenosis or autoimmune pancreatitis.
- Presence of a *PRSS1* mutation, because of the high penetrance, hence it would be adequate as a sole risk factor to explain the phenotype.

Patients with *CFTR* or *CTRC* mutations or pancreas divisum were not excluded.

**2.1.1.4. Control group, idiopathic pancreatitis.** The control group consisted of all patients with IP from Beaujon and Liverpool Hospitals.

Inclusion criteria were the same as for the SRP group but without any causes of pancreatitis after comprehensive workup including genetic testing for the *PRSS1*, *SPINK1*, *CFTR* and *CTRC* genes.

## 2.2. Data collection

The records of all included patients were retrospectively reviewed for data collection. The following data were collected:

- General characteristics (age, gender, smoking status and number of pack-years, alcohol consumption as number of units of alcohol consumed per day, family history of PDAC, recurrent acute or chronic pancreatitis).
- Genetic characteristics (presence and type of *SPINK1*, *PRSS1*, *CFTR* or *CTRC* mutations).
- Clinical characteristics: date of first clinical manifestation (i.e. acute pancreatitis, pancreatic pain, exocrine or endocrine pancreatic insufficiency, cholestasis or PDAC); date of detection of imaging abnormalities including parenchymal or ductal calcifications, other ductal abnormalities, mass lesions, pseudocysts, porto-venous thrombosis; date of SRP diagnosis; date of diabetes mellitus (DM) diagnosis and type of treatment required; date of exocrine insufficiency diagnosis; date and cause of death.
- Endoscopic or surgical procedures performed for the management of acute and chronic pancreatitis-related complications.

## 2.3. Definitions

The diagnosis of CP was based on the presence of, at least, one of the following: pancreatic calcifications diagnosed by CT scan or endoscopic ultrasonography (EUS), moderate to marked pancreatic ductal abnormalities on gadolinium-enhanced MRI with magnetic resonance cholangiopancreatography or EUS or presence of exocrine pancreatic insufficiency. Isolated mild CP stigmata on EUS (heterogeneity of the pancreatic parenchyma, hyperechoic foci without shadowing) were also considered as CP.

Acute pancreatitis was defined by acute abdominal pain with increased serum pancreatic enzyme levels over three times the upper limit of normal values.

Pancreatic pain was defined as typical pancreatic pain without elevation of serum pancreatic enzyme or typical pancreatitis lesion at imaging procedure. It was described either as continuous (with no pain-free intervals) or episodic.

Exocrine pancreatic insufficiency (EPI) was diagnosed in the case of clinical steatorrhea, faecal elastase-1 concentration lower than 100  $\mu\text{g/g}$  of stool or a need for long-term oral pancreatic enzyme supplements.

Diabetes mellitus was diagnosed if two venous blood fasting glucose concentrations were recorded  $\geq 126$  mg/dL (6.99 mmol/l) or if one was recorded  $\geq 11$  mmol/l post prandially. Insulin requirement was defined by the inefficacy of adequate diet (diet without sugar) and oral drugs (biguanides, sulfonylurea, alpha-glucosidase inhibitors) in preventing hyperglycemia.

Cholestasis was defined as increased alkaline phosphatase levels 1.5 times above the upper limit of normal values associated with a dilated common bile duct.

Pseudocyst was defined according to the Atlanta classification, as a round and ovoid collection of pancreatic fluid enclosed by a wall of fibrous or granulation tissue, which arises as a result of acute or chronic pancreatitis, occurring at least 4 weeks after onset of symptom [11].

Acute venous thrombosis was defined as the presence of a cruoric thrombus in the porto-mesenteric system or the splenic vein as evidenced by imaging procedures.

PDAC were all histologically proven.

The number of cigarettes smoked per day and duration of smoking were expressed as pack-years.

The follow-up period was defined as the interim between the date of the first symptom related to pancreatic disease and the date of the last visit or death.

## 2.4. Genetic data

Mutation screening of the *SPINK1* and *PRSS1* gene was performed by pyrosequencing, denaturing High Performance Liquid Chromatography (DHPLC) as previously described [12,13], or by High Resolution Melting (HRM) analysis: four primer pairs (Table 1 supplementary data) were designed to amplify the four exons and their immediate flanking sequences. PCR was performed in a 10  $\mu\text{l}$  reaction mixture containing 4  $\mu\text{l}$  of LightScanner® Master Mix (Idaho Technology Inc., Salt Lake City, UT), 0.3  $\mu\text{M}$  of each primer (Table 1 supplementary data) and 50 ng of DNA. Cycling conditions consisted of an initial denaturation step at 94 °C for 2 min, followed by 40 cycles of 30 s at 94 °C, 30 s at a specific hybridization temperature (Table 1 supplementary data), 30 s at 72 °C, and a final cycle of 30 s at 94 °C and 30 s at 25 °C. After amplification, melting analysis was performed on LightScanner® System (Idaho Technology Inc.). Samples showing a positive profile were sequenced using the BigDye™ Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) to identify mutations [12,13].

Exons and exon/intron junctions of the *CFTR* gene were screened by DHPLC [14] or high-resolution DNA melting analysis [15] as previously described. Samples showing abnormal profiles were sequenced as previously described [14,15].

All exons of the *CTRC* gene and their immediate flanking sequences were analyzed by direct sequencing as previously described [16].

## 2.5. Statistical analysis

Continuous variables were expressed as median interquartile range [IQR]) and compared using the Mann–Whitney test. Qualitative variables were expressed as frequencies (percentages) and compared using Chi<sup>2</sup> test or Fisher's exact test, whichever most appropriate.

The age of onset and the prevalence of pancreatitis-associated symptoms and complications were compared between the SRP and the control groups.

The actuarial risks of occurrence of chronic pancreatitis, exocrine pancreatic insufficiency, diabetes mellitus and cancer were estimated using the Kaplan–Meier method, and were compared between the SRP and control groups using a log-rank test. Durations

**Table 1**  
Population characteristics and pancreatic complications in both groups.

	SPINK1-related Missing values	pancreatitis group N=209	Idiopathic pancreatitis group Missing values	N=302	p- value
Male gender, n (%)		107 (51.2)		145 (48)	0.48
Follow-up from the date of first symptoms*	8	8.3 (3.7–17.4)	1	5.3 (2.5–8.8)	<0.001
Associated mutations, n (%)					
CFTR	2	44 (21.3)			
CTRC	154	5 (9.1)			
Alcohol consumption, n (%)	3	33 (16)	2	158 (52.3)	<0.001
Tobacco consumption, n (%)	8	68 (33.8)	2	139 (46.3)	0.005
Number of pack-year*	19	8 (0.5–80)	25	15 (0.5–140)	<0.0001
Acute pancreatitis, n (%)	4	167 (81.5)		230 (76.2)	0.15
Acute pancreatitis with organ failure, n (%)	9	10 (5)	20	24 (8.5)	0.14
Number of acute pancreatitis*	19	8 (5–15)	25	15 (8–30)	<0.001
Pancreatic pain, n (%)	1	166 (79.8)		189 (62.6)	<0.001
Type of pancreatic pain, n (%)					0.08
Episodic		131 (78.9)		134 (70.9)	
Continuous		35 (21.1)		55 (29.1)	
Chronic pancreatitis, n (%)	3	144 (69.9)	2	162 (54)	<0.001
Ductal abnormalities, n (%)	5	103 (72.5)	55	53 (48.6)	<0.001
Calcifications, n (%)	3	107 (74.3)		105 (64.8)	0.07
Pseudocyst(s), n (%)	4	34 (16.6)	53	55 (22.1)	0.14
Thrombosis, n (%)	2	19 (9.2)	2	34 (11.3)	0.44
Cholestasis, n (%)	2	14 (6.8)	101	30 (15.9)	0.008
Steatorrhea, n (%)	1	77 (37)	2	110 (36.7)	0.97
Diabetes mellitus, (%)	1	36 (17.3)	2	69 (23.0)	0.12
Diabetes mellitus with insulin requirement, n (%)		25 (69.4)	21	29 (60.4)	0.39
Endoscopic treatment, n (%)	1	32 (15.4)	0	42 (13.9)	0.64
Surgical treatment, n (%)	1	35 (16.8)	0	53 (17.5)	0.83
Pancreatic ductal adenocarcinoma, n (%)	0	7 (3.3)	0	3 (0.99)	0.10
Pancreatic cancer and tobacco consumption		5/7		3/3	<0.0001

\* Expressed as median and range.

and survivals were expressed as their medians with 95% confidence intervals.

Genotype-phenotype correlations were analyzed by comparing the risk of developing pancreatic symptoms or complications in heterozygous versus homozygous p.N34S patients and in heterozygous p.N34S patients with co-mutations (*CFTR* and *CTRC*) versus heterozygous p.N34S patients without co-mutations (*CFTR* and *CTRC*), using univariate Cox regression hazard models.

All analyses were two-sided. Any *p*-value <0.05 was considered significant. All analyses were performed using Prism® (version 6, Graphpad™) and SPSS® (version 20, IBM™).

### 3. Results

#### 3.1. Population

Overall, 209 patients (male, 51.2%) were included in the SRP group and 302 patients with idiopathic pancreatitis (male, 48.0%) in the control group (Table 1). The median follow-up was 8.3 years (3.7–17.4) in the SRP group vs 5.3 years (2.5–8.8) in the control group (*p* < 0.001). The percentage of smokers and ex-smokers was higher in the control group than in the SRP group (46.3% vs. 33.8%, *p* = 0.005), as well as the median number of pack-years smoked (15 vs. 8, *p* < 0.0001).

The median age at last contact or death was 34.5 years (24.4–48.3) in the SRP group versus 50.1 years (34.9–61.5) in controls.

#### 3.2. Genetic characteristic

Within the SRP group, most patients had missense mutations (200/209, 95.7%) and 6 patients had deletions (2.9%). Finally, 3 patients (1.4%) had nonsense mutations due to frameshift, including 2 unrelated patients who had a previously undescribed exon 3 mutation (p.C61FfsX2).

Of the 200 patients with specific missense mutations, exon 1 was involved in 4 patients (2%), exon 3 in 182 patients (91%), exon

4 in 5 patients (2.5%), intron 2 in 3 patients (1.5%), intron 3 in 10 patients (5%). Among them, 5 had compound heterozygous mutations. The most frequent specific mutation was p.N34S (exon 3), which was heterozygous or homozygous in 159 patients and 22 patients, respectively (79.5% and 10.5%). One patient had a previously undescribed exon 1 mutation (p.S16R). Other mutations, less frequent, were found in 28 patients. All data are showed in the supplementary data section, Table 2.

Of the 209 SRP patients only 207 and 55 patients were tested for *CFTR* and *CTRC* mutations respectively. A *CFTR* or *CTRC* mutation was found in 44 (21.3%) and 5 (9.1%) patients respectively. Of note, 2 patients from a same family (mother and son) had a triple *SPINK1-CFTR-CTRC* mutation. Among patients with *CFTR* mutations, mutations were mild (*n* = 17), severe (*n* = 6), composite heterozygote severe /mild (*n* = 3), composite heterozygote with 2 severe mutations (*n* = 1), uncertain (*n* = 2).

#### 3.2.1. Onset of symptoms

The median age at onset of symptoms was 20.1 years (95% CI, 17.5–22.8) in the SRP group and 41.2 years (95% CI, 35.2–45.2) in the IP group (*p* < 0.001). In the SRP group, the proportion of symptomatic patients (i.e. pancreatic pain, steatorrhea etc.) was 49.8% at 20 years, 71.1% at 30 years, 85.1% at 40 years and 92.0% at 50 years (Fig. 1). The median delay between the onset of symptoms and the diagnosis of *SPINK1* mutation was 3.7 years (1–11.3).

Among the SRP patients, the existence of a p.N34S homozygous mutation was associated with a statistically non-significant tendency for earlier onset of symptoms compared to p.N34S heterozygous mutation (HR = 1.47, 95% CI [0.93–2.33], *p* = 0.10). Among the group of p.N34S heterozygous patients, the existence of a co-mutation on the *CFTR* or *CTRC* genes tended to be associated with a younger age at onset of symptoms (HR = 1.31, 95% IC [0.89–1.92], *p* = 0.18).

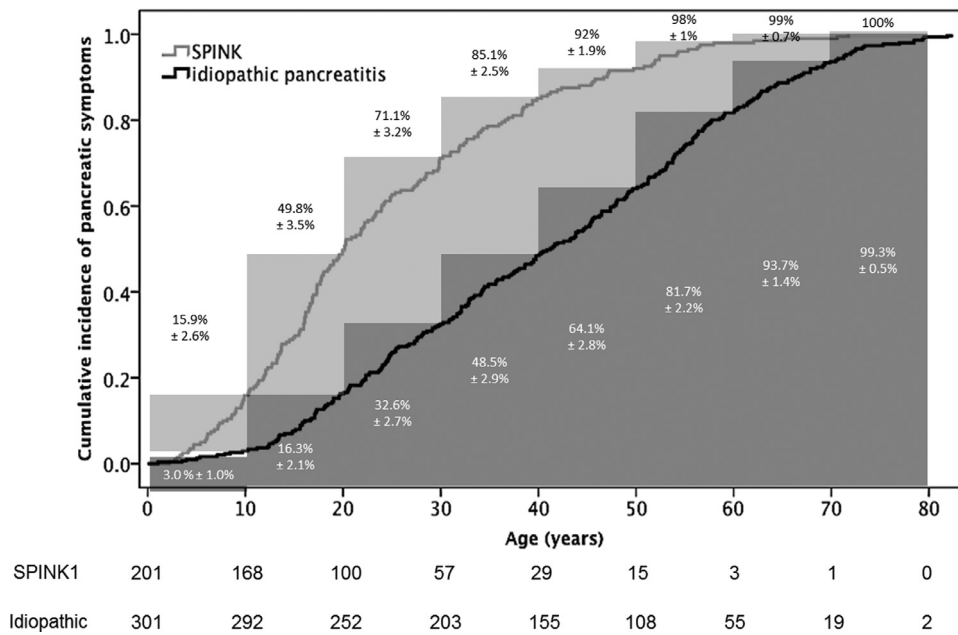


Fig. 1. Cumulative incidence of symptoms depending on age.

3.2.2. Acute pancreatitis and pancreatic pain

Regarding the presentation of pancreatitis, the proportion of patients who had at least one episode of acute pancreatitis was similar in both groups, although the median number of acute pancreatitis episodes was higher in the SRP group, with no difference in terms of severity (Table 1).

The proportion of patients with pancreatic pain was higher in the SRP group than in the IP group (79.8% vs. 62.6%,  $p < 0.001$ ), but there was no difference regarding the type of pain, i.e., episodic vs. continuous (Table 1).

3.2.3. Chronic pancreatitis

About 70% of SRP patients had signs of CP, versus 54% in the IP group ( $p < 0.001$ ). The SRP patients more frequently developed ductal abnormalities (72.5% vs. 48.6%,  $p < 0.001$ ) and pancreatic calcifications (74.3% vs 64.8%) but this result didn't reach statistical significance ( $p=0.07$ ). The median age of CP diagnosis was younger, of approximately 20 years in the SRP group [34.3 (95% CI, 30.8–37.8) years vs. 57.3 (95% CI, 53.7–60.9) years,  $p < 0.001$ ]. The cumulative incidence of CP signs in SRP patients was 16.1% at 20 years, 38.5% at 30 years, 56.7% at 40 years and 76.0% at 50 years (Fig. 2a).

The median delay between the first symptoms and the diagnosis of CP was similar in both groups [6.9 years in the SRP group (95% CI, 4.5–9.3) vs. 5.9 years (95% CI 4.2–7.6) years in the IP group]. Conversely to the IP group, tobacco consumption in SRP group was not associated with earlier CP diagnosis (Fig. 2b). The proportion of patients with intra-abdominal spleno-portal venous thrombosis and/or pseudocysts was similar (Table 1).

The median age at which CP was diagnosed was 34.6 years (95% CI, 31.7–37.4) and 28.7 years (95% CI, 12.4–45.0) in heterozygous and homozygous p.N34S patients, respectively (HR=1.50, 95% CI [0.89–2.52],  $p=0.13$ ). Among the p.N34S heterozygous patients, the presence of co-mutations did not influence the occurrence of CP (HR = 1.25, 95% IC [0.78–2.0],  $p=0.36$ ).

3.2.4. Exocrine pancreatic insufficiency

At the end point, the proportion of patients with steatorrhea was similar in both groups. The median delay between the first symptoms and the diagnosis of EPI was longer in the SRP group

than in the control group [25.0 (95% IC, 20.2–29.8) years vs. 15.0 (95% IC, 10.3–19.7) years,  $p=0.007$ ]. However, the age of onset of EPI in the SRP group was about 15 years younger than in the control group [median age, 49.5 (95% IC, 44.5–54.6) years vs. 65.2 (95% IC, 62.1–68.3) years,  $p < 0.001$ ]. As shown in Fig. 3a, the proportion of SRP patients with EPI was 5.3% at 20 years, 14.7% at 30 years, 28.3% at 40 years and 52.4% at 50 years.

Conversely to the control group, tobacco consumption in SRP group was not associated with earlier onset of EPI (Fig. 3b).

The median age at EPI onset was similar in heterozygous and homozygous p.N34S patients (HR = 1.08, 95% CI [0.51–2.29],  $p=0.84$ ). No difference was observed due to concomitant mutations in CFTR or CTSC genes.

3.2.5. Diabetes mellitus (DM)

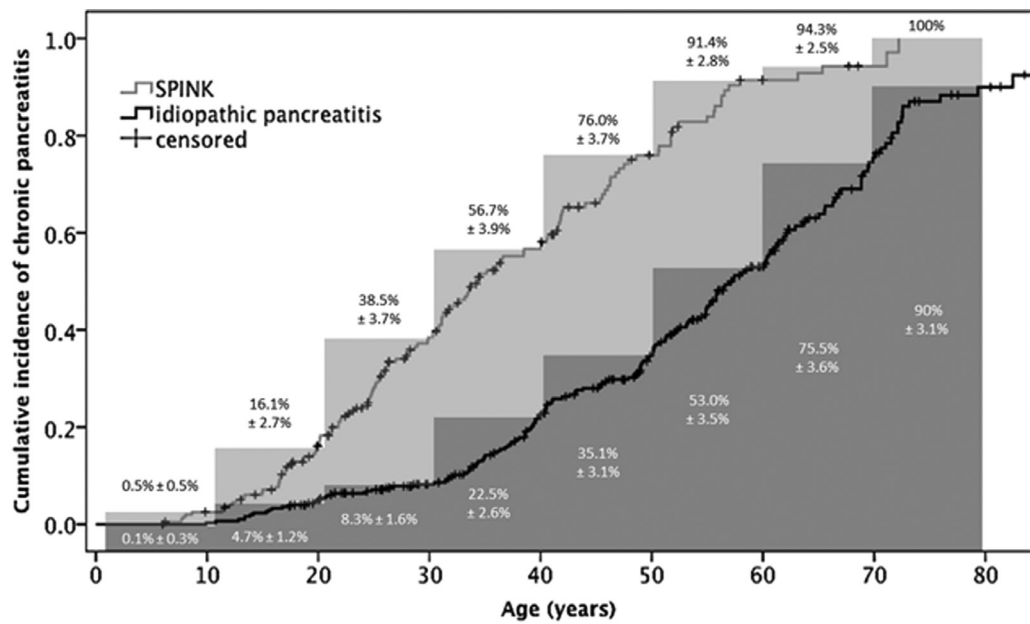
At the end of follow-up, there was a similar proportion of patients with DM in the SRP (17.3%) and in the IP (23%) groups (Table 1). The severity of DM was similar, with a comparable proportion of patients requiring insulin.

DM occurred after a median delay of 37.7 (95% IC, 33.3–42.1) years following the onset of symptoms in the SRP group vs. 30.6 (95% IC, 17.3–43.8) years in the IP group ( $p=0.002$ ). The age of DM onset was approximately 15 years younger in the SRP group than in the control group [68.5 (95% CI, 56.2–80.7) vs. 82.3 (95% IC, 68.4–96.2),  $p=0.018$ ]. The proportion of SRP patients with DM was 7.8% at 30 years, 13.4% at 40 years, 26.3% at 50 years and 43.4% at 60 years (Fig. 4a).

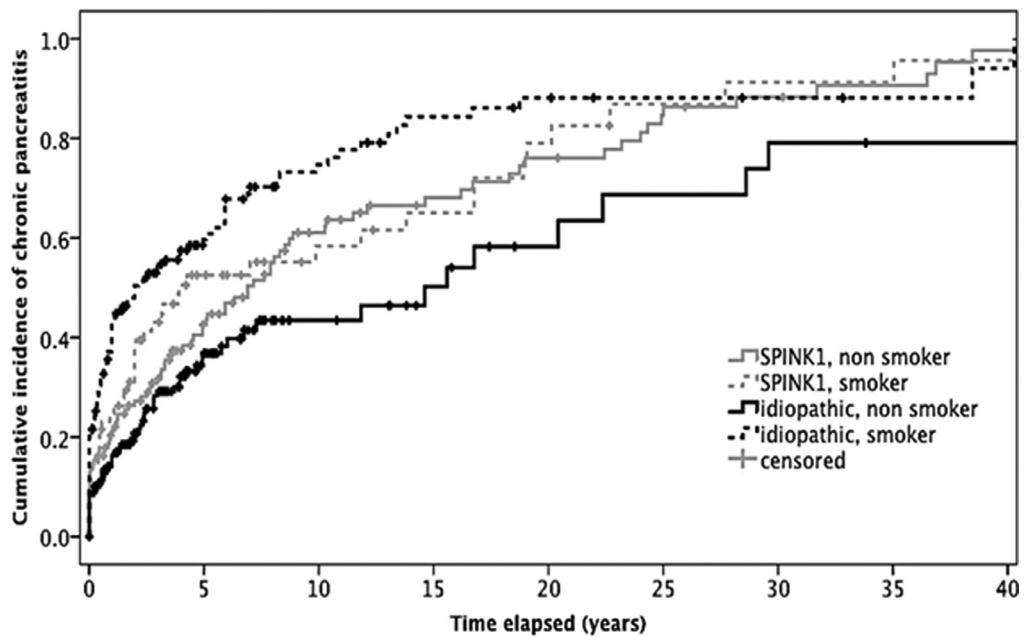
Conversely to the control group, tobacco consumption in the SRP group was not associated with earlier DM onset (Fig. 4b). Homozygous carriers for p.N34S mutation did not appear to be at increased risk of diabetes, in comparison with heterozygous carriers for p.N34S mutation (HR=0.27, 95% CI [0.04–2.02],  $p=0.20$ ). Among heterozygous p.N34S patients, the presence of concomitant mutations in CFTR or CTSC genes did not influence the occurrence of diabetes either (HR = 1.56, 95% IC [0.60–4.04],  $p=0.36$ ).

3.2.6. Pancreatic ductal adenocarcinoma

Seven patients (3.3%) and 3 patients (0.99%) developed PDAC in the SRP and IP groups respectively ( $p=0.1$ ), at a median age of 60 vs 66 years. In both groups, all patients with PDAC had morpho-



SPINK1	199	191	152	96	58	24	5	1	0
Idiopathic	299	298	276	234	185	131	71	25	5

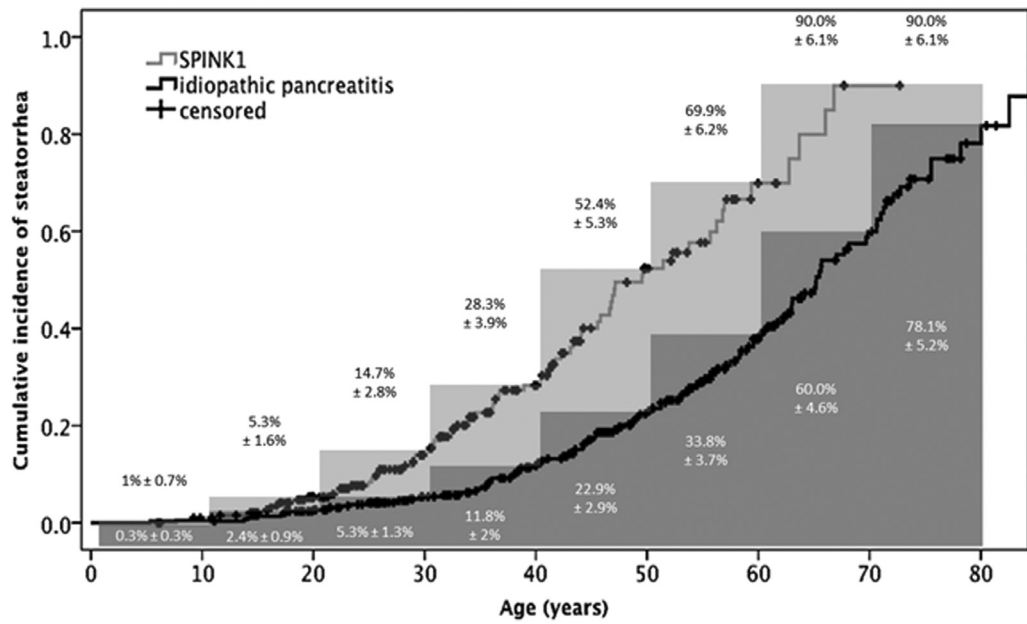


SPINK1, non smoker	124	54	30	20	15	9	6	4	1
SPINK1, smoker	65	22	13	10	6	3	2	2	1
Idiopathic, non smoker	159	51	20	13	8	6	4	3	3
Idiopathic, smoker	139	35	17	9	6	4	3	2	1

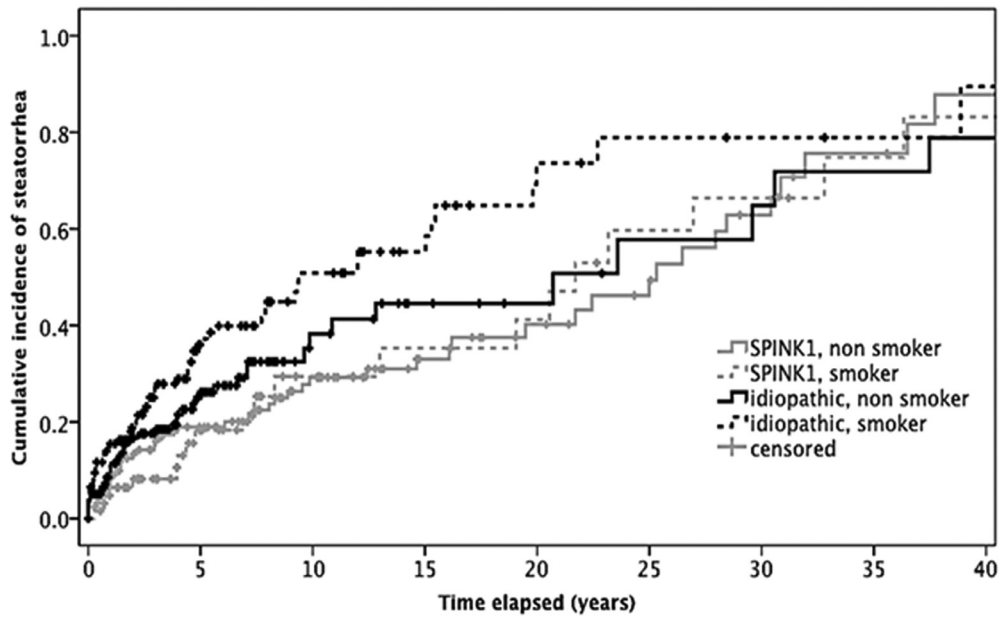
Fig. 2. Cumulative incidence of chronic pancreatitis depending on age (2a) and according to tobacco status (2b).

logical signs of chronic pancreatitis (missing data for one patient in each group). All but one patient had calcifications (one missing data) in the SRP group, none in the control group. All patients in the control group were smokers (3/3) versus 5/7 in SRP group. In the SRP group, the median tobacco consumption was higher in pa-

tients with PDAC (15 pack-years) than in the whole group (8 pack years). The actuarial risk of developing PDAC was 0.8% before 50 years, 11.9% at 60 years, 27.7% at 70 years and 51.8% at 80 years in the SRP group and was significantly higher than in the control group ( $p < 0.001$ ) (Fig. 5). Patients in the SRP group had a risk of



SPINK1	202	196	163	114	70	31	8	1	0
Idiopathic	298	297	279	236	193	138	75	33	6



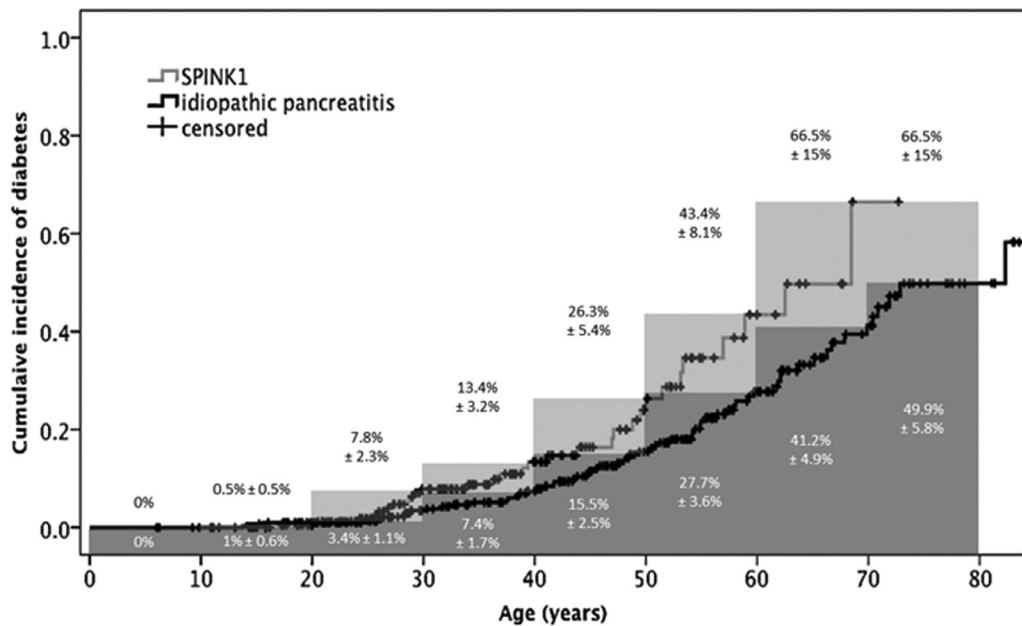
SPINK1, non smoker	125	76	49	31	22	16	10	5	2
SPINK1, smoker	64	29	15	11	10	6	5	3	2
Idiopathic, non smoker	158	61	21	12	9	6	5	4	3
Idiopathic, smoker	138	49	25	14	6	4	3	2	1

Fig. 3. Cumulative incidence of steatorrhea depending on age (3a) and depending on tobacco status (3b).

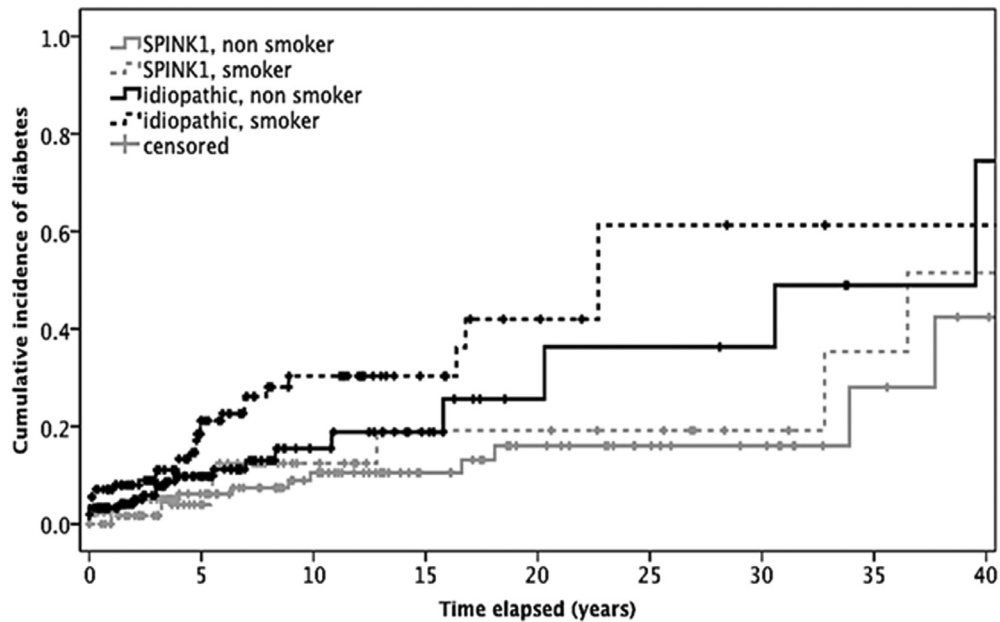
developing PDAC 12 times higher than the patients in the control group (Cox HR 12.0 (3.0–47.8),  $p < 0.001$ ).

Among the SRP with PDAC, 5 had p.N34S heterozygous mutation (including one with *CTRC* and *CFTR* co-mutations) and 2 had a *SPINK1* deletion.

The presence of co-mutations did not influence the occurrence of PDAC (HR = 1.56, 95% IC [0.60–4.04],  $p = 0.36$ ).



SPINK1	196	192	166	113	70	32	10	1	0
Idiopathic	294	294	277	230	194	139	76	34	8



SPINK1, non smoker	124	81	56	36	25	17	13	6	3
SPINK1, smoker	62	35	18	12	12	10	6	4	3
Idiopathic, non smoker	152	71	26	15	7	6	5	2	2
Idiopathic, smoker	125	57	29	15	8	4	3	2	2

Fig. 4. Cumulative incidence of mellitus diabetes depending on age (4a) and depending on tobacco status (4b).

3.2.7. Death

Seven patients died in SRP group (4 of PDAC, one of cardiac amyloidosis, 2 of unknown cause) and 20 in the control group. There was no increased risk of death from any cause among the SRP patients (HR = 0.48, 95% CI [0.19–1.18], p = 0.11).

4. Discussion

We described herein one of the largest cohorts of patients with SRP. By the age of 50 years old, 76% of patients with SRP had morphological signs of CP, 52.4% had EPI and 26.3% had DM. In



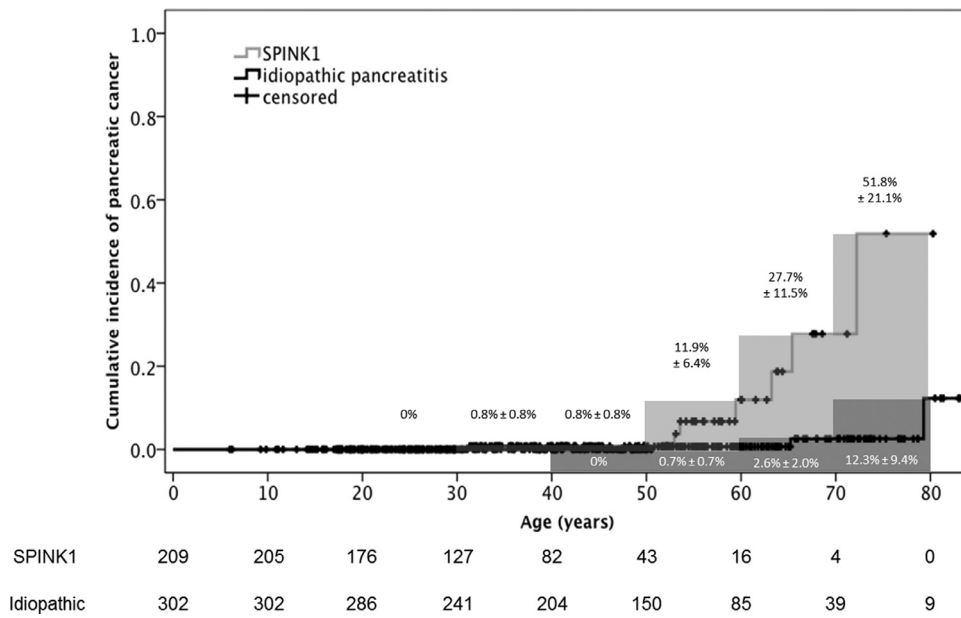


Fig. 5. Cumulative incidence of pancreatic ductal adenocarcinoma cancer depending on age.

comparison with patients with idiopathic pancreatitis, patients in the SRP group presented with more episodes of acute pancreatitis, more pancreatic pain, had more frequent morphologic signs of CP. In addition, they had a significantly higher actuarial risk of developing chronic pancreatitis (Fig. 2a), EPI (Fig. 3a), DM (Fig. 4a) and PDAC (Fig. 5). These results might have been influenced by the longer follow-up in the SRP group and by the mismatch of disease proportions between the two groups. The presence of more patients with chronic pancreatitis in the SPINK1 related-pancreatitis group should have lead to an increased incidence of pancreatic insufficiencies and cancer. However, these clinical complications, except from PDAC, systematically occurred from 15 to 20 years earlier in the SRP group than in the control group. However, some authors [6,8] previously reported no clinical or morphological difference between patients with SRP and patients with ICP. Sandhu et al. [9] reported an earlier onset and more frequent acute bouts of pancreatitis in 13 patients with SRP compared to 35 patients with ICP. However, these studies included limited populations and SRP patients frequently had other causes of CP.

Recently, a large Chinese series aimed to assess the risk of chronic pancreatitis and complications in case of rare pathogenic variants in the *CFTR*, *SPINK1*, *PRSS1* and *CTRC* genes. Authors included 1061 Chinese CP patients divided into three subgroups (idiopathic CP, alcoholic CP and tobacco related CP) and 1196 controls. Mutation-positive patients had earlier median ages at disease onset and at diagnosis of diabetes mellitus and steatorrhea than mutation-negative IP patients. Pathogenic variants were present in 57.1, 39.8, and 32.1% of the following subgroups: idiopathic, alcohol and tobacco. Even if this cohort is large and well explored by genetic testing, some issues have to be addressed. The definitions of the subgroups can be debated. A CP was considered as tobacco related if the intake was up to 2 pack-years; tobacco intake was not excluded in the alcohol group and patients with idiopathic pancreatitis could have alcohol consumption under 80g/day. No specific analysis was performed according to the genetic type and for patients with *SPINK1* variants especially. In our study, we had very selective criteria to obtain the more characterized populations with no potential cofounded factors as alcohol intake for example. [17]

We found limited genotype-phenotype correlations. There was no influence of the genetic alterations (homozygous vs heterozy-

gous p.N34S mutations, presence of co-mutations) on the age of onset of pancreatic complications although there was statistical tendency for earlier onset of symptoms in patients with homozygous p.N34S mutation or co-mutations (*CFTR* and *CTRC* in addition to *SPINK1*). This result is consistent with those reported by Drenth et al. [18] describing a population of 14 *SPINK1* mutation carriers including two patients with homozygous p.N34S mutation.

The patients included in this SRP cohort had no other known causes of pancreatic disease (especially we excluded patients with a consumption of alcohol greater than 3 units per day), which strongly suggests that their pancreatic disease were related to the *SPINK1* mutations. This is consistent with a recent Italian meta-analysis which reported that the presence of p.N34S mutation increases nine times the overall CP risk in a European population (OR 9.7, 95% CI [7.9–11.9]) [19].

However, *SPINK1* mutations are frequent in the general population (1–2%) but not constantly associated with pancreatitis [4]. The pattern of inheritance and the reasons explaining the development of SRP in only a small proportion of *SPINK1* mutation carriers is unclear. This is also underlined by the fact that the p.N34S mutation may not have functional consequences in vitro [20]. Hence, yet unidentified environmental or genetic factors are likely to be associated with *SPINK1* mutations for the development of pancreatic symptoms. Among the patients in the SRP group, 21.3% and 9.1% had a co-mutation involving *CFTR* or *CTRC*, which are well recognized causative or facilitating mutations for the development of CP [21,22]. These rates are much higher than in the general population (4% for *CFTR*). This advocates that mutations in the *SPINK1* gene might facilitate – rather than cause – pancreatic disease, when associated with other cofactors, some of them being still unidentified. Similarly, Sofia et al. [10] recently reported compound heterozygosity, involving various genes including *SPINK1*, enhanced the risk for CP in patients with cystic fibrosis.

Surprisingly, tobacco consumption was not associated with an earlier onset of CP, EPI or DM in the SRP group in comparison with the control group. One explanation could be that there was quantitatively and qualitatively significantly less smokers in the SRP group than in the control group. Moreover, the influence of tobacco exposure on the onset of the first symptoms could not be evaluated. Indeed, one could imagine that following SRP diagnosis,

patients with medical follow-up were most likely strongly advised to quit smoking which finally ends up in a better natural history of the disease. Tobacco then falsely appears as a protective factor.

Conversely to PRSS1 mutations, for which the risk of PDAC is now clearly identified [1,23], the association between the presence of a *SPINK1* mutation and the development of PDAC has been controversial. Several studies reported that *SPINK1* did not significantly increase the risk of PDAC [24–26]. Nevertheless, those studies did not have the sufficient statistical power nor follow-up to estimate life-long actuarial risk. In the present study, the risk of PDAC was 12 times higher in the SRP patients than in the IP patients and the actuarial risk of PDAC was also higher in the SRP patients than in the IP patients ( $p < 0.001$ , Fig. 5b). Although this estimation relied on a low number of PDAC, it may well be that p.N34S is associated with cancer.

This study has several limitations, such as its retrospective design. Moreover as the role of *SPINK1* mutations in pancreatitis is controversial (facilitating gene more than causative) it would have been interesting to test other susceptibility genes (ie: CEL-HYB, CTRB [27,28]) which was impossible in this retrospective study.

We were unable to explain why the number of deaths was higher in the control group than in the SRP group because causes of deaths in the first group were not known.

*SPINK1* mutations are considered as strong candidates for contributing to the pathogenesis of tropical calcific pancreatitis [29], an entity for which the risk of PDAC is considered elevated [30,31]. In our study the identification of risk factors for the development of PDAC was not possible because of a limited number of patients with PDAC. However, we observed that all the patients who developed PDAC had morphological signs of CP and a heavy smoking consumption (median 15 pack-years), which are two major risk factors of PDAC. Finally, the only patient who developed PDAC before 50 years had a first-degree familial history of PDAC. Large prospective cohorts would be necessary to be precise, in the population of *SPINK1* mutation carriers, the risk factors for PDAC in order to identify a subgroup of patients who could benefit from PDAC screening. It is highly possible that the development of PDAC is a consequence of pancreatic inflammation (partly assessed by the presence of calcifications) rather than the *SPINK1* mutation itself.

Given the results of this study we would propose a close surveillance of patients over 50 year old, smokers, with morphological signs of severe CP, or in any patients with a familial history of PDAC, as for other germline mutations predisposing to PDAC. The modalities of such a screening is yet to be determined but should be performed accordingly to the International Cancer of the Pancreas Screening (CAPS) guidelines [32].

#### Declaration of Competing Interest

No conflicts of interest exist.

#### CRediT authorship contribution statement

**Nelly Muller:** Conceptualization, Data curation, Formal analysis, Methodology, Supervision, Writing - original draft. **Ioannis Sarantitis:** Data curation, Formal analysis, Writing - original draft. **Marie Rouanet:** Data curation, Writing - review & editing. **Louis de Mestier:** Data curation, Formal analysis, Writing - original draft. **Christopher Halloran:** Data curation, Formal analysis, Writing - original draft. **William Greenhalf:** Data curation, Formal analysis, Writing - original draft. **Claude Férec:** Data curation, Writing - review & editing. **Emmanuelle Masson:** Data curation, Writing - review & editing. **Philippe Ruszniewski:** Writing - review & editing. **Philippe Lévy:** Writing - review & editing. **John Neoptolemos:**

Writing - review & editing. **Louis Buscail:** Writing - review & editing. **Vinciane Rebours:** Conceptualization, Data curation, Formal analysis, Methodology, Supervision, Writing - original draft.

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#### Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ebiom.2019.09.032.

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