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Factors associated with prior acute pancreatitis episodes among patients with chronic pancreatitis

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ABSTRACT

Background: The relationship between chronic pancreatitis (CP) and acute pancreatitis (AP) is complex and not well understood. CP could be preceded by antecedent episodes of AP.
Aims: The aim of this study was to explore both genetic and environmental factors associated with AP episodes before the diagnosis of CP.
Methods: This was a cross-sectional study including 1022 patients. Detailed demographic, genetic, and clinical data were collected. Based on the presence of AP episode(s) before diagnosis of CP, patients were divided into AP group (further classified into single episode of AP group and recurrent AP group) and non-AP group. Related factors among these groups were assessed using multivariate logistic regression model.
Results: Before diagnosis of CP, 737 patients (72.1%) had a history of AP. Smoking($P=0.005$) and heavy alcohol consumption($P=0.002$) were risk factors for AP while age at CP onset($P<0.001$), harboring the *SPINK1* mutation($P<0.001$), diabetes($P<0.001$) and steatorrhea($P<0.001$) were protective factors. Further, alcoholic CP($P=0.019$) was the only independent risk factor for recurrent AP attacks while age at onset of CP($P<0.001$), pancreatic stones($P=0.024$). and pseudocysts($P=0.018$) served as protective factors.
Conclusions: *SPINK1* mutations served as protective factor for AP episodes, suggesting *SPINK1* mutation might play a pathogenic role in CP occurrence with occult clinical manifestations.

Introduction

Chronic pancreatitis (CP) is a progressive fibro-inflammatory disease of the pancreas that eventually leads to irreversible loss of pancreatic exocrine and endocrine functions [1]. Acute pancreatitis (AP), which is characterized by acute onset of persistent, severe epigastric pain and increased amylase [2], is common in the course of CP. The sentinel AP event (SAPE) hypothesis, which was first proposed by Whitcomb in 1999 and is widely accepted as the pathogenesis of CP, proposes a two-hit model where CP is preceded by a sentinel attack of AP causing infiltration of inflammatory cells and activation of stellate cells, with subsequent ongoing injury or stress promoting fibrosis [3]. Based on this hypothesis, many studies have focused on the progression from AP to CP. A meta-analysis concluded that approximately 10% of patients with a single episode of AP will develop CP, while this rate was 36% for patients with recurrent AP (RAP) [4]. In addition, smoking and alcohol consumption are recognized risk factors for progression from AP to CP [5–7].

However, not all patients have a history of AP episodes when diagnosed with CP [8,9]. The clinical manifestations of CP are highly variable; while some patients will have episodes of AP, others may have chronic pain or may even be asymptomatic [10]. The reasons for the different types of presentations are still poorly understood. Genetic, environmental, and even emotional factors have been shown to contribute to the variability in presentation [11].

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To the best of our knowledge, few studies have explored the prevalence of AP as the initial event for patients with CP, and the potential associated factors. A recent study by Hori et al. comprising 499 patients reported that CP was associated with prior AP in only 50% of patients; however, the authors did not conduct a multivariate analysis of the related factors [12]. Another study by Olesen et al. of 334 patients with CP confirmed the above findings and further indicated that age at diagnosis of CP, pain, and exocrine pancreatic insufficiency were associated with prior AP attacks [13]. However, it should be noted that the factors examined in the above study were limited and the potential influence of genetic factors was not considered; this is important as genetic factors have been widely demonstrated to influence the course of CP. Therefore, the present study aimed to determine the prevalence of AP attacks before the diagnosis of CP and to further identify the potential genetic and environmental factors associated with AP episodes.

Method

Patients

This was a cross-sectional study conducted at a large CP center in China (Shanghai Changhai Hospital). Consecutive patients with a diagnosis of CP were enrolled between January 2011 and December 2015. All patients provided written informed consent. The following data were obtained from each patient: demographic data, smoking and alcohol history, family disease history, clinical course of CP (age at disease onset and diagnosis, etiologies, complications present before diagnosis of CP), pain profile before diagnosis of CP, and laboratory and imaging tests. All enrolled patients agreed to provide a blood sample for genetic tests. The study was approved by the institutional review board of Changhai Hospital. All authors had access to the study data and reviewed and approved the final manuscript.

Pain profile

Based on the presence of AP episodes before diagnosis of CP, patients were divided into two groups: patients who experienced pain with a history of AP episodes (AP group) and patients who experienced pain without a prior AP episode (non-AP group). The non-AP group included both patients with no pain and those who experienced abdominal pain but without AP attacks. The patients with a history of AP episodes were classified into a further two groups: those who experienced a single episode of AP and those with RAP (two or more AP episodes).

Genetic testing

The present study examined four major susceptibility genes of CP, i.e., the *SPINK1* (encoding pancreatic secretory trypsin inhibitor), *PRSS1* (encoding cationic trypsinogen), *CTRC* (encoding chymotrypsin C), and *CFTR* (encoding cystic fibrosis transmembrane conductance regulator) genes. The detailed description of procedures for DNA preparation and gene sequencing has been provided previously [14]. The known rare pathogenic variants were included in the final analysis.

Definitions

The diagnosis of CP was established based on the findings of computed tomography, magnetic resonance imaging, or endoscopic ultrasound, in accordance with the Asia-Pacific consensus [15]. The diagnosis of AP was made in accordance with the revised Atlanta international consensus, which requires two of the following three features: pain consistent with AP; amylase or lipase $>$ 3 times the upper normal limit; characteristic findings on imaging [2].

The age at onset of CP was defined as the age of first clinical symptoms attributable to CP (e.g., abdominal pain, diabetes, steatorrhea) or age at diagnosis of CP in asymptomatic patients through regular or incidental check-ups based on abdominal ultrasonography or radiological findings [16]. Smoking status was classified as never (smoked $<$ 100 cigarettes in lifetime) or ever (smoked $\geq$ 100 cigarettes in lifetime). The pattern of alcohol consumption was classified as follows: abstainers, light drinkers ($\leq$ 20 g/d), moderate drinkers (20–80 g/d), and heavy drinkers ($\geq$ 80 g/d).

The etiology of CP was classified as alcoholic CP (ACP), idiopathic CP (ICP), or other. ACP was assigned when alcohol intake was $\geq$ 80 g/d for males and 60 g/d for females for at least two years [17]. Other etiologies of CP included hereditary, abnormal anatomy of the pancreatic duct, traumatic and hyperlipidemia. Hereditary CP was defined as two first-degree relatives or $\geq$ 3 s degree relatives with recurrent AP and/or CP. Abnormal anatomy of the pancreatic duct included pancreas divisum and anomalous pancreatico-biliary junction. Post-traumatic CP was diagnosed when there was a history of abdominal trauma with imaging evidence of pancreatic injury and subsequent ductal dilation. Hyperlipidemia was considered as an etiology when blood triglyceride was $>$ 1000 mg/dL [18,19]. Patients were considered ICP if there were no known causes [20]. CP-related complications included pancreatic stones, diabetes, steatorrhea, pseudocysts, pancreatic portal hypertension, and common bile duct (CBD) stenosis, which were defined in accordance with the definitions described in our previous publications [20–25].

Statistical analyses

For the continuous variables, tests of data normality were carried out using the Shapiro-Wilk test. Normally distributed variables are presented as mean $\pm$ standard deviation (SD) and were compared using Student's t-tests while non-normally distributed variables are presented as median (interquartile range, IQR) and were compared using Mann-Whitney U tests. Categorical variables are presented as frequencies and percentages. Chi-squared analysis or Fisher's exact test were used for comparison of categorical variables. Two-sided P values less than 0.05 were considered statistically significant. Variables with a P value $<$ 0.10 in univariate analyses were included in the multivariate logistic regression models. For multivariate analysis, the likelihood ratio statistic was calculated with a forward logistic regression model in order to identify the independent predictive factors. Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. Data were analyzed using SPSS version 23 (SPSS Inc., Chicago, Illinois, USA).

Results

Patient characteristics

Overall, 1022 patients (717 males, 70.2%) were included in the present study. A detailed description of the characteristics of the enrolled patients is provided in Table 1. The median (IQR) age was 37.5 (25.0–48.0) years at onset of CP and 42.0 (29.0–52.0) years at diagnosis of CP. A total of 481 patients (47.1%) had a history of smoking and 478 patients (46.8%) had a history of alcohol consumption. At least one pathogenic variant of *SPINK1* was identified in 394 patients (38.6%), while variant(s) of *PRSS1* were observed in 133 patients (13.0%), variant(s) of *CTRC* were observed in 20 patients (2.0%), and variant(s) of *CFTR* were observed in 52 patients (5.1%). The CP etiology was classified as idiopathic in the majority of patients (61.9%), while 28.7% were classified as ACP and 9.4% as other.

Table 1
Characteristics of enrolled patients.

Characteristics	Total ($n = 1022$)	Male ($n = 717$)	Female ($n = 305$)	P value
Age at onset of CP/yrs.	37.5 (25.0–48.0)	39.0 (28.0–49.0)	30.0 (18.0–44.0)	< 0.001
Age at diagnosis of CP/yrs.	42.0 (29.0–52.0)	44.0 (33.0–53.0)	36.0 (21.0–49.0)	< 0.001
Smoking status-no. (%)	481 (47.1)	476 (66.4)	5 (1.6)	< 0.001
Drinking status-no. (%)				< 0.001
0	544 (53.2)	254 (35.4)	290 (95.1)	
< 20 g/d	102 (10.0)	94 (13.1)	8 (2.6)	
20–80 g/d	83 (8.1)	81 (11.3)	2 (0.7)	
> 80 g/d	293 (28.7)	288 (40.2)	5 (1.6)	
Variants-no. (%)				
SPINK1	394 (38.6)	243 (33.9)	151 (49.5)	< 0.001
PRSS1	133 (13.0)	83 (11.6)	50 (16.4)	0.036
CTRC	20 (2.0)	13 (1.8)	7 (2.3)	0.611
CFTR	52 (5.1)	34 (4.7)	18 (5.9)	0.440
Etiologies-no. (%)				< 0.001
ICP	633 (61.9)	374 (52.2)	259 (84.9)	
ACP	293 (28.7)	288 (40.2)	5 (1.6)	
Others*	96 (9.4)	55 (7.7)	41 (13.4)	
Complications-no. (%) #				
Stones	739 (72.3)	511 (71.3)	228 (74.8)	0.255
Diabetes	221 (21.6)	163 (22.7)	58 (19.0)	0.187
Steatorrhea	154 (15.1)	119 (16.6)	35 (11.5)	0.036
Pseudocysts	139 (13.6)	105 (14.6)	34 (11.1)	0.136
Portal hypertension	12 (1.2)	8 (1.1)	4 (1.3)	0.758
Common bile duct stenosis	25 (2.4)	22 (3.1)	3 (1.0)	0.048

CP, chronic pancreatitis; ICP, idiopathic chronic pancreatitis; ACP, alcoholic chronic pancreatitis/.
Continuous variables were presented as median (interquartile range).
* Including hereditary CP, abnormal anatomy of pancreatic duct, traumatic, and hyperlipidemia.
# Existing before or at diagnosis of CP.

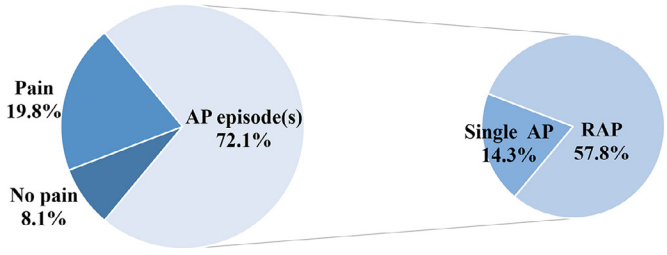

Fig. 1. Pain profiles of enrolled patients.

At diagnosis of CP, 83 patients (8.1%) reported no pain while 202 patients (19.8%) reported abdominal pain without AP attacks. All of the above patients were classified into the non-AP group. The other 737 patients (72.1%) had prior AP episodes, with 146 patients (19.8%) experiencing a single episode of AP and 591 patients (80.2%) experiencing RAP (Fig. 1).

AP group vs. non-AP group

In the univariate analyses, the factors found to be associated with AP episodes before diagnosis of CP included male sex ($P < 0.001$), age at CP onset ($P = 0.017$) and diagnosis ($P = 0.021$), smoking status ($P < 0.001$), drinking status ($P < 0.001$), variants of *SPINK1* ($P < 0.001$), etiology of CP ($P < 0.001$), pancreatic stones ($P < 0.001$), diabetes ($P < 0.001$), and steatorrhea ($P < 0.001$) (Table 2). Collinearity between the onset and diagnosis age of CP was found in collinearity test. Considering that the diagnosis of CP is affected by many factors (i.e., medical standard discrepancies in different regions, patients' attention to health, etc.), only onset age of CP was included in the further multivariate analysis. In the final multivariate logistic regression model, smoking status (OR, 1.77; 95% CI, 1.19–2.62; $P = 0.005$) and heavy alcohol consumption (OR, 2.02; 95% CI, 1.29–3.15; $P = 0.002$) were identified as independent risk factors for AP episodes. Further, age at CP onset (OR, 0.97; 95% CI, 0.96–0.98; $P < 0.001$), harboring a *SPINK1* mutation (OR, 0.45; 95% CI, 0.32–0.63; $P < 0.001$), diabetes (OR, 0.373; 95% CI, 0.26–0.53; $P < 0.001$), and steatorrhea (OR, 0.44; 95% CI, 0.30–0.66; $P < 0.001$) were protective factors for AP episodes (Fig. 2).

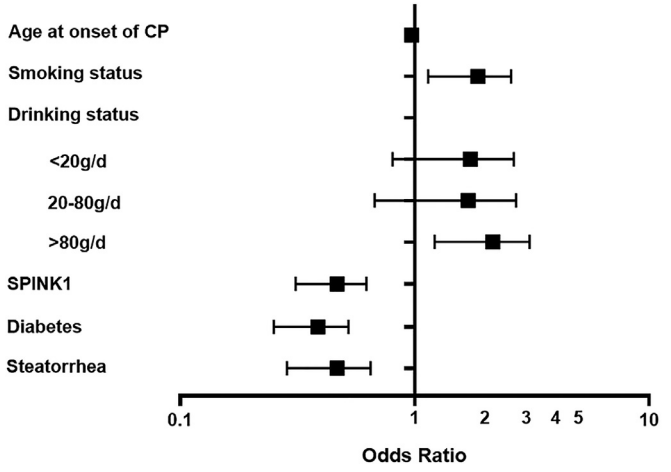

Fig. 2. Forest plot of the multivariable logistic regression model for the presence of acute pancreatitis in patients with chronic pancreatitis.

Single episode of AP group vs. RAP group

Among the patients with AP episodes, the factors found to be closely associated with RAP in univariate analyses included age at CP onset ($P < 0.001$) and diagnosis ($P = 0.007$), variants of PRSS1 ($P = 0.066$), etiologies of CP ($P = 0.079$), pancreatic stones ($P = 0.017$), diabetes ($P = 0.018$), and pseudocysts ($P = 0.008$). Because of the collinearity between onset and diagnosis age of CP, only onset age of CP was included in the multivariate analysis. In the multivariate logistic regression analysis, ACP (OR, 1.66; 95% CI, 1.09–2.53; $P = 0.019$) was the only independent risk factor for RAP while age at onset of CP (OR, 0.98; 95% CI, 0.97–0.99;

Table 2
Univariate analysis of factors affecting the presence of AP episodes among patients with chronic pancreatitis.

	AP group ($n=737$)	Non-AP group ($n=285$)	P Value
Male-no. (%)	541 (73.4)	176 (61.8)	**< 0.001**
Age at onset of CP-yrs.	37.0 (24.0–47.0)	39.0 (27.0–50.0)	**0.017**
Age at diagnosis of CP-yrs.	42.0 (28.0–51.0)	44.0 (33.0–53.0)	**0.021**
Smoking status-no. (%)	380 (51.6)	101 (35.4)	**< 0.001**
Drinking status-no. (%)			**< 0.001**
0	359 (48.7)	185 (64.9)	
< 20 g/d	79 (10.7)	23 (8.1)	
20–80 g/d	63 (8.5)	20 (7.0)	
> 80 g/d	236 (32.0)	57 (20.0)	
Variants-no. (%)			
SPINK1	251 (34.1)	143 (50.2)	**< 0.001**
PRSS1	100 (13.6)	33 (11.6)	0.397
CTRC	16 (2.2)	4 (1.4)	0.427
CFTR	33 (4.5)	19 (6.7)	0.148
Etiologies-no. (%)			**< 0.001**
ICP	429 (58.2)	204 (71.6)	
ACP	236 (32.0)	57 (20.0)	
Others*	72 (9.8)	24 (8.4)	
Complications-no. (%) #			
Stones	510 (69.2)	229 (80.4)	**< 0.001**
Diabetes	119 (16.1)	102 (83.9)	**< 0.001**
Steatorrhea	80 (10.9)	74 (26.0)	**< 0.001**
Pseudocysts	106 (14.4)	33 (11.6)	0.241
Portal hypertension	6 (0.8)	6 (2.1)	0.105
Common bile duct stenosis	19 (2.6)	6 (2.1)	0.661

AP, acute pancreatitis; CP, chronic pancreatitis; ICP, idiopathic chronic pancreatitis; ACP, alcoholic chronic pancreatitis.
Continuous variables were presented as median (interquartile range).
* Including hereditary CP, abnormal anatomy of pancreatic duct, traumatic, and hyperlipidemia.
# Existing before or at diagnosis of CP.

$P < 0.001$), pancreatic stones (OR, 0.61; 95% CI, 0.39–0.94; $P=0.024$), and pseudocysts (OR, 0.56; 95% CI, 0.35–0.91; $P=0.018$) were protective factors (Table 3).

Discussion

In the present study, 72.1% of well-phenotyped Chinese patients with CP had a history of AP, with 80.2% of these patients presenting with recurrent AP episodes. Furthermore, smoking and heavy alcohol consumption were independent risk factors for prior AP episodes, while late-onset of CP, diabetes, steatorrhea, and in particular, harboring the *SPINK1* mutation, were protective factors.

The relationship between AP and CP is complex. AP is common in patients with CP. A recent review reported that approximately 70% of adult patients with CP experience at least one episode of AP and 50% experience RAP during the clinical course of CP [9]. Although many hypotheses explaining this relationship, typified by SAPE, consider AP as an initial event that leads to CP, the finding that AP might be absent or occur nearly at the same time or even after the diagnosis of CP blurs the causal link between AP and CP. A recently published study on a mouse model also found that low-level inflammation can cause progressive CP without overt acute attacks [26]. Therefore, the SAPE hypothesis is not sufficient to clarify the complex course of CP. Identification of the potential factors associated with AP attacks among patients with CP might reveal new information about key concepts and mysteries regarding the relationship between AP and CP.

Both smoking and alcohol consumption were identified as risk factors for AP in the present study. Previous studies have shown that ACP patients are more likely to experience AP attacks, which affect the quality of life of these patients [17, 27–29]. It is generally assumed that distinct fibrosis and protein plugs in the small ducts contribute to alcohol-dependent AP attacks in the context of clinically latent CP [30]. Furthermore, a randomized trial demonstrated the benefit of repeated counselling against alcohol consumption in reducing the risk of recurrent attacks of pancreatitis and hospitalizations [31]. Smoking has already been identified as an independent risk factor for both occurrence and progression of CP [29]. The pathogenic effects of cigarettes on both the pancreas and nervous system might explain the association between smoking and AP attacks, but the exact mechanism requires further investigation. Further, patients with early onset of CP were not only prone to experiencing AP attacks, but also recurrent AP episodes, which is consistent with previous studies [9,13]. The well-preserved pancreatic exocrine function in young people might provide an explanation for the recurrent episodes of AP. Another possible explanation of the association is that patients experiencing AP attacks are prone to agree to abdominal imaging examinations, which assist in the early discovery of CP. However, for patients with no AP attacks (typical clinical manifestation), the discovery of CP might be delayed.

The highlight of the present study was that harboring pathogenic mutations of *SPINK1* was identified as a protective factor for AP episodes, which is still controversial in the literature. Our finding is consistent with a multicenter European study including 511 patients, which found that patients in the *SPINK1*-related pancreatitis group had a lower median number of AP attacks compared with patients not harboring the detected mutations (8 vs. 15, $P < 0.001$) [32]. However, another study in America found the opposite result. In the subgroup analysis of 35 ICP patients, the above study found that patients harboring the *SPINK-1*/p. N34S mutation presented with a higher number of acute attacks per year (11.8 ± 1.5 vs. 4 ± 0.98) [33]. These differences might be explained by the presence of different variants of *SPINK1* in different populations (that is, p.N34S is the most common variant among Caucasians [9] while c.194+2T > C is the most common variant in the Asian population) [14,34], the small sample size, and the different inclusion criteria. The central role of the

Table 3
Univariate and multivariate analysis of factors affecting the presence of RAP among CP patients with AP episodes.

	Single episode of AP Group ($n = 146$)	RAP Group ($n = 591$)	Univariate *P* Value	Multivariate *P* value	Adjusted ORs (95% CIs)
Male-no. (%)	110 (75.3)	431 (72.9)	0.554		
Age at onset of CP-yrs.	41.0 (28.0–51.0)	36.0 (22.0–45.0)	**< 0.001**	<0.001	0.98 (0.97–0.99)
Age at diagnosis of CP-yrs.	44.0 (33.0- 56.8)	42.0 (26.0–50.5)	**0.007**		
Smoking status-no. (%)	79 (54.1)	301 (50.9)	0.491		
Drinking status-no. (%)			0.240		
0	73 (50.0)	286 (48.4)			
< 20 g/d	14 (9.6)	65 (11.0)			
20–80 g/d	18 (12.3)	45 (7.6)			
> 80 g/d	41 (28.1)	195 (33.0)			
Variants-no. (%)					
SPINK1	48 (32.9)	203 (34.3)	0.737		
PRSS1	13 (8.9)	87 (14.7)	0.066	NS	
CTRC	4 (2.7)	12 (2.0)	0.536		
CFTR	9 (6.2)	24 (4.1)	0.271		
Etiologies-no. (%)			0.079	0.029	
ICP	96 (65.8)	333 (56.3)		-	Reference
ACP	41 (28.1)	195 (33.0)		0.019	1.66 (1.09–2.53)
Others*	9 (6.2)	63 (10.7)		0.106	1.85 (0.88–3.89)
Complications-no. (%)#					
Stones	113 (77.4)	397 (67.2)	**0.017**	0.024	0.61 (0.39–0.94)
Diabetes	33 (22.6)	86 (14.6)	**0.018**	NS	
Steatorrhea	18 (12.3)	62 (10.5)	0.523		
Pseudocysts	31 (21.2)	75 (12.7)	**0.008**	0.018	0.56 (0.35–0.91)
Portal hypertension	1 (0.7)	5 (0.8)	1.000		
Common bile duct stenosis	2 (1.4)	17 (2.9)	0.394		

RAP, recurrent acute pancreatitis; AP, acute pancreatitis; CP, chronic pancreatitis; OR, odds ratio; CI, confidence interval; ICP, idiopathic chronic pancreatitis; ACP, alcoholic chronic pancreatitis.
Continuous variables were presented as median (interquartile range).
* Including hereditary CP, abnormal anatomy of pancreatic duct, traumatic, and hyperlipidemia.
# Existing before or at diagnosis of CP.

SPINK1 protein, which is synthesized in the pancreatic acinar cell, is to protect the pancreas from prematurely-activated trypsin. It has been suggested that *SPINK1* mutations might affect the protease/antiprotease balance within the pancreas. Compared with mutated *PRSS1,* which causes strong trypsinogen activation, the mutated *SPINK1* gene behaves more like a disease modifying factor [35], this is also supported by a recent study which reported that *SPINK1*-associated patients frequently had additional etiologic factors [36]. The present study further indicates that *SPINK1* mutation might play a relatively weak pathogenic role in CP occurrence with occult clinical manifestations. Further investigation of the mechanism underlying the protective effect of *SPINK1* mutation on AP attacks is required.

The complications of CP were also associated with AP attacks in this study. The presence of diabetes or steatorrhea before diagnosis of CP, which represent a loss of pancreatic endocrine and exocrine function, was associated with no AP attacks. Pancreatic insufficiency is the most common initial symptom of painless CP patients [37]; painless CP patients are a notable group who accounted for a significant proportion of our non-AP group. The loss of pancreatic exocrine insufficiency reduces the likelihood of AP episodes, which might reflect the preservation of pancreatic function to some extent. Further, another possibility to consider is that the diagnosis of CP among patients without AP episodes often occurs at an advanced stage (representing loss of pancreatic functions) because the lack of AP attacks highlighted a problem in the pancreas. Therefore, the causal relationship between AP episodes and pancreatic function was uncertain in this study. Further, pancreatic stones and pseudocysts were identified as protective factors for RAP among patients with AP episodes. A possible explanation may be that the typical imaging manifestations are conducive to early diagnosis of CP.

There are several limitations of this study that should be considered. First, this was a cross-sectional study, so that the causality between AP and its related factors was blurred; besides, the evolution of pain throughout the course of CP did not been explored, which warrant a further well-designed prospective study. Second, recall bias may have affected our results, especially in relation to information from the onset of CP to the first visit to our hospital. Thirdly, the present study only involved four major susceptibility genes of CP; other CP-predisposing genes, such as *CPA1* (encoding carboxypeptidase A1) [38] and *CEL-HYB* (a hybrid allele between the carboxyl ester lipase gene [*CEL*] and its pseudogene, *CELP*) [39], were not included in the present study. However, these gene variants were found not to confer a predisposition to CP in Chinese patients in our previous studies [40,41].

In conclusion, 72.1% of patients with CP experienced AP episodes before diagnosis, with 80.2% of these patients presenting with RAP. While smoking and heavy alcohol consumption were independent risk factors for prior AP episode(s), late onset of CP, diabetes, steatorrhea, and in particular, harboring the *SPINK1* mutation, served as protective factors. A detailed understanding of the relationship and mechanism of AP and CP requires further investigation.

Author contributions

Concept and design: Zhuan Liao, Nan Ru and Wen-Bin Zou; acquisition, analysis, or interpretation of data: Nan Ru and Jia-Hui Zhu with substantial contributions from Sheng-Yong Wu, Fei-Fei Yu, Liang-Hao Hu, Jun Pan, Xiao-Nan Xu, Lei Wang, Zi-Jun Yan and Ji-Yao Guo; drafting of the manuscript: Nan Ru, Liang-Hao Hu and Jia-Hui Zhu; critical revision of the manuscript for important intellectual content: all authors; study supervision: Zhuan Liao and Wen-Bin Zou; funding acquisition: Zhuan Liao, Zhao-Shen Li, Wen-Bin Zou and Jun Pan. All the authors approved the final manuscript.

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Declaration of Competing Interest

The authors declare no conflict of interest.

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None.

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