An Evaluation of Factors Associated With Pathogenic *PRSS1*, *SPINK1*, *CTFR*, and/or *CTRC* Genetic Variants in Patients With Idiopathic Pancreatitis

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OBJECTIVES: **We evaluated factors associated with pathogenic genetic variants in patients with idiopathic pancreatitis.**

METHODS: **Genetic testing (*PRSS1, CFTR, SPINK1*, and *CTRC*) was performed in all eligible patients with idiopathic pancreatitis between 2010 to 2015. Patients were classified into the following groups based on a review of medical records: (1) acute recurrent idiopathic pancreatitis (ARIP) with or without underlying chronic pancreatitis; (2) idiopathic chronic pancreatitis (ICP) without a history of ARP; (3) an unexplained first episode of acute pancreatitis (AP)<35 years of age; and (4) family history of pancreatitis. Logistic regression analysis was used to determine the factors associated with pathogenic genetic variants.**

RESULTS: **Among 197 ARIP and/or ICP patients evaluated from 2010 to 2015, 134 underwent genetic testing. A total of 88 pathogenic genetic variants were found in 64 (47.8%) patients. Pathogenic genetic variants were identified in 58, 63, and 27% of patients with ARIP, an unexplained first episode of AP <35 years of age, and ICP without ARP, respectively. ARIP (OR: 18.12; 95% CI: 2.16–151.87; *P*=0.008) and an unexplained first episode of AP<35 years of age (OR: 2.46; 95% CI: 1.18–5.15; *P*=0.017), but not ICP, were independently associated with pathogenic genetic variants in the adjusted analysis.**

CONCLUSIONS: **Pathogenic genetic variants are most likely to be identified in patients with ARIP and an unexplained first episode of AP<35 years of age. Genetic testing in these patient populations may delineate an etiology and prevent unnecessary diagnostic testing and procedures.**

Am J Gastroenterol 2017; 112:1320–1329; doi:10.1038/ajg.2017.106; published online 25 April 2017

INTRODUCTION

Acute pancreatitis (AP) is a clinical syndrome reflecting an acute inflammatory response to injury, characterized by the sudden onset of upper abdominal pain, elevation of serum pancreatic enzyme levels as well as pancreatic edema and inflammation on abdominal imaging studies. Each episode of AP has serious potential medical and surgical consequences, and acute recurrent pancreatitis (ARP) confers a significant risk of progression to chronic pancreatitis. Thus, a major goal of evaluating patients during an attack of AP is to determine, and when possible, eliminate the underlying etiology to prevent ARP and CP.

In ~10–30% of cases of pancreatitis, no etiology can be found following a comprehensive evaluation (1,2). These cases are referred to as idiopathic pancreatitis. Over the last two decades, there has been growing recognition of the causative role of genetic variants in the development of pancreatitis; however, there is still uncertainty with regards to which patients with idiopathic pancreatitis should undergo genetic testing (3). As genetic testing is costly and

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Received 30 October 2016; accepted 13 March 2017

carries implications for both patients and their family members, any effort to better define which patients are most likely to harbor pathogenic genetic variants will help prevent indiscriminate use of this technology.

There are currently four genes that can be commercially sequenced in patients with pancreatitis. These include the cationic trypsinogen gene (*PRSS1*), cystic fibrosis transmembrane conductance regulator gene (*CFTR*), the serine protease inhibitor Kazal type 1 gene (*SPINK1*), and chymotrypsin C gene (*CTRC*) (4–7). Prior studies have shown that the prevalence of pathogenic genetic variants in acute recurrent idiopathic pancreatitis (ARIP) and idiopathic chronic pancreatitis (ICP) ranges from 30 to 60% and 12 to 43%, respectively (8–16). These studies had many limitations including a focus on evaluating only 1–2 gene(s) (9–11), inclusion of genetic variants of unknown significance (8,11), variability in the definition of CP (8–12), and limited evaluation for other etiologic factors (12), particularly smoking, whose causative role has been more firmly established (17,18).

The primary aim of this study was to evaluate the factors associated with pathogenic genetic variants using a four gene panel (*PRSS1, CFTR, SPINK1*, and *CTRC*) in patients with idiopathic pancreatitis.

METHODS

Study design and data collection

This retrospective study was approved by the Johns Hopkins Institutional Review Board for Human Research and complied with Health insurance Portability and Accountability Act (HIPPA) regulations.

All adult idiopathic pancreatitis patients referred to the multidisciplinary pancreatitis clinic at Johns Hopkins Hospital from 2010 to 2015 were offered genetic testing after counseling and underwent commercially available genetic testing (*PRSS1, CFTR, SPINK1*, and *CTRC*). In some cases, testing was not completed because insurance coverage denied payment before genetic testing and the patient could not independently pay for testing, they were lost to follow up, or those with advanced age and/or extensive comorbidities as the results of genetic testing would not alter their clinical management. All patients who underwent genetic testing at outside facilities before referral were also included in the study. The medical records of all patients were reviewed for demographic and clinical data. All abdominal imaging and endoscopic examinations were reviewed to evaluate pancreatic morphology.

The inclusion criteria were: (i) Age ≥18 years; (ii) ARIP and/or definite/probable/borderline ICP according to the M-ANNHEIM classification (19); and (iii) Genetic testing (*PRSS1, CFTR, SPINK1*, and *CTRC*) obtained by our multidisciplinary pancreatitis clinic or referring provider before evaluation. All patients were categorized into the following groups based on commonly employed criteria for genetic testing (20–23): (i) ARIP with or without ICP; (ii) ICP without a history of ARP; (iii) An unexplained first episode of AP <35 years of age; and (iv) Family history of pancreatitis. It should be noted that groups 1 and 2 are mutually exclusive but patients in groups 3 and 4 could also be in the first two groups.

Definitions

The electronic medical record of each patient was evaluated for the following clinical characteristics: personal or family history of pancreatitis; ever smokers (defined as a history of smoking ≥100 cigarettes during their lifetime); history of at risk alcohol consumption, as defined in the North American Pancreatitis Study 2 (NAPS2) study (24); age at the first episode of AP; history of cholecystectomy; abnormalities on imaging and endoscopic studies consistent with CP (calcifications, number of endoscopic ultrasound (EUS) features of CP and changes of the pancreatic duct consistent with CP per the Cambridge classification (25) as defined in the M-ANNHEIM classification).

AP was defined according to the revised Atlanta classification (26). ARP was defined as 2 or more episodes of AP with resolution of symptoms between episodes. All medical records were obtained from prior hospitalizations and clinic notes to ensure that patients met criteria for AP and/or ARP. Patient-reported episodes of AP that could not be verified through a review of medical records were not classified as AP.

Idiopathic pancreatitis was defined as a failure to identify an etiology after a thorough history, physical examination, laboratories and imaging resulted in the exclusion of several causes of pancreatitis including biliary (27), heavy alcohol use and/or smoking, use of class 1A/1B drugs (17,28), hypercalcemia, hypertriglyceridemia, and autoimmune (29,30). The presence of pancreas divisum on imaging was recorded but this was not considered a cause of pancreatitis.

Genetic testing was defined as positive if known pathogenic genetic variants were present in at least one of the 4 genes (*PRSS1*, *CFTR*, *SPINK1*, and *CTRC*). Patients with variants of unknown significance with no other pathogenic variants were categorized as pathogenic variant negative. Pathogenic genetic variants were defined according to two databases, the Chronic Pancreatitis Genetic Risk Factors Database and the Clinical and Functional Translation of CFTR (CFTR2) database (31,32).

Statistical analysis

Baseline demographic and clinical characteristics were compared using standard parametric testing. Each factor potentially associated with pathogenic genetic variants was evaluated in a single and multiple logistic regression model, adjusted for smoking, alcohol, pancreas divisum and history of cholecystectomy. Given that the majority of patients with idiopathic AP <35 years of age had ARP (>90%), two separate multiple logistic regression models were developed. This was done to account for the collinearity between AP <35 years of age and ARP in our cohort. The first multiple logistic regression model included: ARP, ICP without a history of ARP and family history of pancreatitis and was adjusted for smoking, alcohol, pancreas divisum, and history of cholecystectomy. The second model included: AP <35 years of age, ICP without a history of ARP and family history of pancreatitis and was adjusted for smoking, alcohol, pancreas divisum, and history of cholecystectomy. All models were evaluated for potential interaction terms. The results of the logistic regression were expressed as odds ratios (OR) with 95% confidence intervals and *P* values.

Two tailed *P* value of <0.05 were considered statistically significant. Statistical analysis was performed using STATA 14 (College Station, TX, USA).

RESULTS

A total of 1,214 patients were evaluated in the multidisciplinary pancreatitis clinic at Johns Hopkins Hospital between July 2010 and December 2015. There were 462 (38.0%) patients with ARP and/or CP. Among the 314 and 148 patients with ARP and/or CP, 154 (49.0%) and 43 (29.0%) were diagnosed with ARIP and/or ICP and were offered genetic testing, respectively. A total of 97 (63.0%) patients with ARIP underwent genetic testing with 47 presenting for a single consultation visit or were lost to follow-up, 5 not tested due to insurance denial, and 5 not tested due to advanced age and/or extensive comorbidities. Among 97 ARP patients, 75 (77.3%) had CP while 22 (22.7%) did not have CP. A total of 26 (60.5%) patients with ICP underwent genetic testing with 15 presenting for a single consultation visit or were lost to follow-up and 2 not tested due to advanced age and/or extensive comorbidities (**Figure 1**). A total of 88 pathogenic genetic variants were detected in 64 (47.8%) patients (**Table 1**). There were no significant differences between patients with and without pathogenic genetic variants with regards to demographic and clinical characteristics. Patients with pathogenic genetic variants were significantly more likely to have a history of AP, ARP, and an unexplained first episode of AP <35 years of age. Patients with ICP without a history of ARP were less likely to have pathogenic genetic variants on testing. There was no difference between the two groups with regards to a family history of pancreatitis (**Table 2**).

According to the M-ANNHEIM criteria, patients with and without pathogenic genetic variants had similar numbers with definitive, probable and borderline CP and the morphological features of CP patients did not differ between these two groups (**Table 3**).

Among patients with ARIP, 56 out of 97 (57.7%) patients had one or more of the following pathogenic genetic variants including: 48 *CFTR*, 22 *SPINK1*, 5 *PRSS1*, and 2 *CTRC*. Out of 75 patients with ARP and CP, 42 (56.0%) had pathogenic genetic variants while 12 (54.5%) out of 22 ARP without CP had identifiable pathogenic genetic variants. Among patients with definitive or probable ICP without a history of ARP, 7 out of 26 (26.9%) had an identifiable pathogenic genetic variants, including: 6 *CFTR* and 2 *SPINK1*. In patients with an unexplained first episode of AP<35 years of age, 37 out of 59 patients (62.7%) had one or more pathogenic genetic variants, including: 27 *CFTR*, 21 *SPINK1*, 4 *PRSS1*, and 2 *CTRC*. Only 12 patients had a family history of pancreatitis, among whom 6 (50%) patients were found to have one or more pathogenic genetic variants, including: 2 *CFTR*, 1 *SPINK1* and 3 *PRSS1*.

Among the 64 patients with a positive pathogenic genetic variant, 12 were categorized as trans-heterozygotes, as they had a pathogenic variant in more than one gene. Out of 12 patients, 10 had both a CFTR and SPINK1 mutation, one had both a SPINK1 and PRSS1 mutation, and one had both a SPNIK1 and CTRC mutation. All 12 trans-heterozygote patients had ARIP and 10 of these patients had an unexplained first episode of AP <35 years of age. (**Table 4**).

In the unadjusted analysis, ARIP (OR: 6.24; 95% CI: 2.60–15.01; *P*<0.001) and an unexplained first episode of AP <35 years of age (OR: 2.04; 95% CI: 1.02–4.09; *P*=0.045) were both significantly associated with pathogenic genetic variants (**Table 4**). Conversely, a history of ICP without a history of ARP was associated with a lower incidence of a pathogenic genetic variants in the unadjusted analysis (OR: 0.25; 95% CI: 0.10–0.63; *P*=0.004). A family history of pancreatitis (OR: 1.02; 95% CI: 0.98–1.06; *P*=0.28) did not appear to influence the results of genetic testing in the unadjusted or adjusted analysis. In the adjusted analysis, only ARIP (OR: 18.12; 95% CI: 2.16–151.87; *P*=0.008) and an unexplained first episode of AP<35 years of age (OR: 2.46; 95% CI: 1.18–5.15; *P*=0.017) were independently associated with pathogenic genetic variants. There were no significant interaction terms in either model.

DISCUSSION

The present study has attempted to define the subgroups of patients with idiopathic pancreatitis who are more likely to harbor pathogenic genetic variants. Our primary finding is that patients with ARIP and an unexplained first episode of AP<35 years of age are significantly more likely to harbor pathogenic *PRSS1*, *CFTR*, *SPINK1*, and/or *CTRC* gene variants than patients with ICP but without a history of ARP. In addition, a family history of pancreatitis does not appear to predict pathogenic variants on genetic testing.

The four genes in the genetic testing panel are mechanistically liked to control of trypsin activity within the pancreas. The prototype susceptibility factor is *PRSS1* with two well established gain-of-function variants, p.N29I and p.R122H, that lead to hereditary pancreatitis. This autosomal dominant Mendelian disorder is characterized by ARP, with only a subset of subjects progressing to classic CP, usually years after the initial AP event (33). Indeed, ARP remains one of the strongest drivers of CP, as demonstrated by multiple investigators (34–38). SPINK1 is linked to trypsin as a specific trypsin inhibitor, CTRC as a trypsin degradation enzyme, and CFTR as ductal anion channel responsible for generation of fluid secretion to flush prematurely activated trypsin in the ducts out of the pancreas. Thus, association between these pathogenic variants in these trypsin-associated genes and ARP is expected. The reported prevalence of pathogenic variants in these four genes varies in the general population. CFTR is one of the most common genetic variants with a carrier frequency of 13% in healthy controls in the NAPS 2 study (39). SPINK1 mutations are found in ~1–2% of the general population (40). Pathogenic variants in PRSS1 and CTRC are less commonly found in the general population, with frequencies of 0.003% and 0–0.3%, respectively (41,42).

While ARP is felt to be a prerequisite for CP in the majority of cases, a small proportion of patients can present with CP in the absence of ARP (35,36). The apparent lower prevalence of genetic variants in ICP may be affected by number of explanations. First, the definition of CP relied on the demonstration of irreversible morphologic features of the pancreas which was known to be

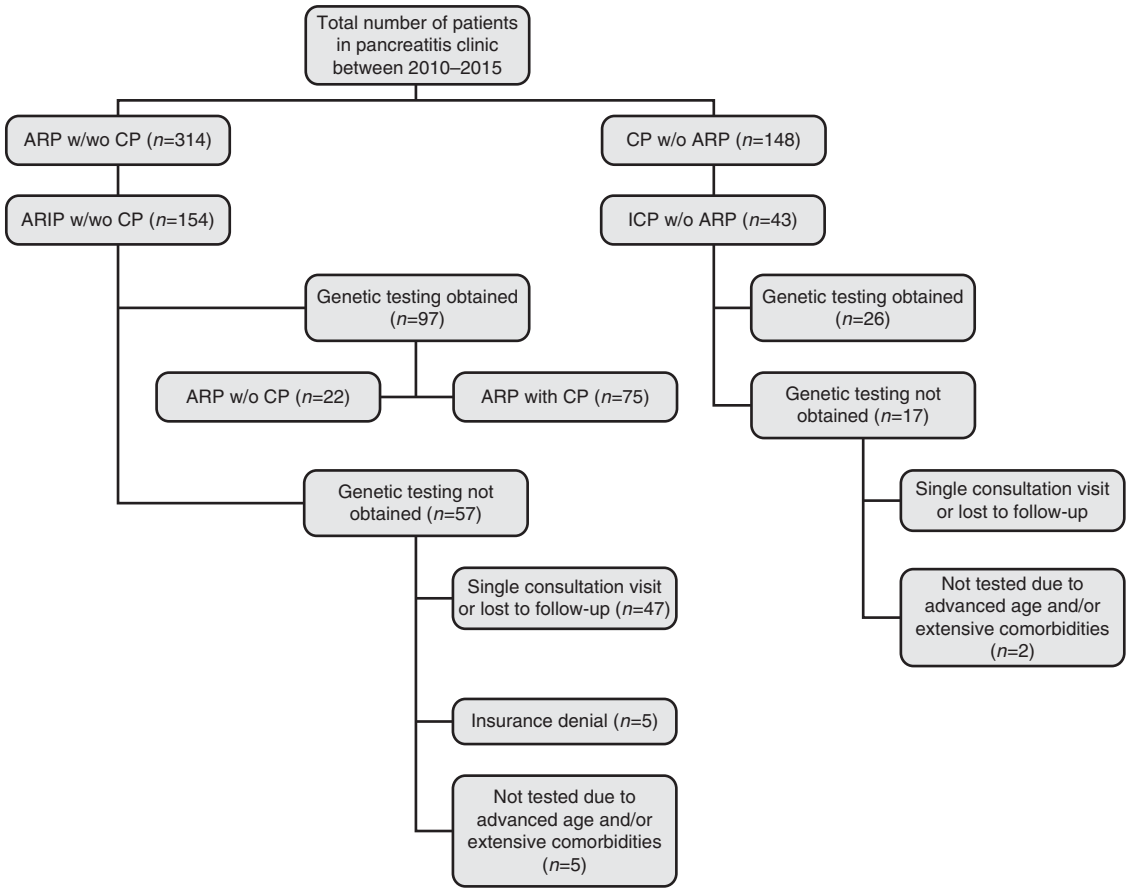

Figure 1. Description of study cohort.

associated with fibrosis since 1984 (25). At the time, abdominal imaging technology was rudimentary and insensitive compared to current technologies, so that only advanced disease could be reliably detected. Newer technologies, including CT, MRI, and EUS are so sensitive that changes of "early CP" are being detected in patients who have other conditions that can result in fibrosis such as diabetes mellitus (43) or even aging (44–46). Thus, it is possible that some patients diagnosed with CP by imaging criteria have other diseases that are not associated with the genetic tests being evaluated. On the other hand, in the setting of abdominal pain, which is far more prevalent that CP, there is a real potential to falsely diagnose patients with ICP. Second, commercially available genetic testing only evaluates four out of more than a dozen genes that have been shown to be associated with pancreatitis; therefore, many pathogenic genetic variant negative patients may harbor pathogenic genetic variants which have different natural histories compared to the four genes evaluated in this study (47). Third, there are likely other genetic or environmental factors that, alone or in combination, result in or influence the development of CP (48). Fourth, the lack of a generally accepted definition of CP results in this diagnosis being even made in patients with EUS score of 3 or more who are classified as borderline CP by the M-ANNHEIM classification. Many of the borderline ICP included in our study underwent genetic testing at outside facilities. Finally, it is possible that some ICP patients had episode(s) of AP that were not documented, obtained for review or for which the patient did not seek medical attention. However, we found that 27% of patients with ICP harbor pathogenic genetic variants, which is consistent with prior studies that have reported rates of ~30–37% (5,8).

To address the problem of defining CP by imaging criteria alone a new "mechanistic" definition has been proposed that seeks to define CP by both the essence of the pathogenic process, and the common (nonspecific) end-stage clinical features (49). The essence of CP was defined as "a pathologic fibro-inflammatory syndrome of the pancreas in individuals with genetic, environmental, and/or other risk factors who develop persistent pathologic responses to parenchymal injury or stress". This definition limits the diagnosis of CP to the sequelae of persistent pancreatic injury from a stereotypic type of inflammation that is typically linked to recurrent episodes of AP and eventually results in the classic features of CP (50). ARP and CP are therefore linked, so that preceding history of ARP strongly increases the probability that the features of CP seen on imaging studies is from CP (36,38). This is the most likely explanation as to why pathogenic genetic variants conferring susceptibility to ARIP are also commonly found in patients with ICP.

Table 1. Distribution of specific genetic variants results according to clinical factors for genetic testing

Clinical indication for genetic testing	Pathogenic genic variants				Pathogenic gene variant negative
	CFTR	SPINK1	PRSS1	CTRC	
Acute recurrent idiopathic pancreatitis	*29 (26.8%)*	*10 (10.3%)*	4 (41.2%)	1 (1.0%)	41 (42.3%)
	R117H(2)	*N34S(8)*	*K23R(1)*	*494-1G>C(1)*	
	F508del(11)	*R67H(1)*	*R122H(3)*		
	R352Q(1)	*c.194+184T>A(1)*			
	Poly T (10)				
	L997F(2)				
	W1282X(3)				
	R1162X(1)				
	G576A(1)				
	R668C(1)				
	R117C(1)				
	G542X(1)				
	S1235R(1)				
	1898+3A>G(1)				
	2789+2insA(2)[a]				
	c.164+12T>C(1)[a]				
	c.3468+6T>C(1)[a]				
	c.-812T>G(1)[a]				
	Q1042R(1)[a]				
	112622+1G>T(1)[a]				
	Trans	***Trans***	***Trans***	***Trans***	
	R117H+N34S(1)	*R122C+ N34S (1)*	*R122C+ N34S (1)*	*N34S+R254W(1)*	
	F508del+N34S(2)	*N34S+R254W(1)*			
	R117H+c.27delC(1)	*R117H+N34S(1)*			
	R117H,F508del+N34S(1)	*F508del+N34S(2)*			
	F508del,S1235R+N34S(1)	*R117H+c.27delC(1)*			
	621+3A->G+c.14G>A[a]*(1)*	*R117H,F508del+N34S(1)*			
	Poly T+N34S(1)	*F508del,S1235R+N34S(1)*			
	Poly T+194+2T>C+ N34S(1)	*621+3A->G+c.14G>A*[a]*(1)*			
	Poly T+c.1429C>T[a] *+N34S (1)*	*Poly T+N34S(1)*			
		Poly T+194+2T>C+ N34S(1)			
		Poly T+c.1429C>T[a] *+N34S (1)*			
Idiopathic chronic pancreatitis without acute recurrent pancreatitis	5 (19.2%)	2 (7.7%)	0	0	19 (73%)
	G576A(1)	*N34S (2)*			
	R668C(1)				
	Poly T (1)				
	E92X(1)				

Table 1 continued on following page

Table 1. Continued

Clinical indication for genetic testing	Pathogenic genic variants				Pathogenic gene variant negative
	CFTR	SPINK1	PRSS1	CTRC	
	S1235R(1)				
	F508del(1)				
	K68E(1)[a]				
	c.1731C>T(1)[a]				
An unexplained first episode of AP <35 years of age	14 (23.7%)	9 (15.2%)	3 (5.0%)	1 (1.7%)	22 (37.3%)
	F508del(6)	*N34S(8)*	*K23R(1)*	*494-1G>C(1)*	
	1898+3A>G(1)	*c.194+184T>A(1)*	*R122H(2)*		
	S1235R(1)	*R67H(1)*			
	Poly T (4)				
	R352Q(1)				
	G576A(1)				
	R668C(1)				
	G542X(1)				
	R1162X(1)				
	c.-812T>G(1)[a]				
	2789+2insA(2)[a]				
	Q1042R(1)[a]				
	Trans	***Trans***	***Trans***	***Trans***	
	F508del,S1235R+N34S(1)	*N34S+R254W(1)*	*N34S+R122C(1)*	*N34S+R254W(1)*	
	F508del+N34S(2)	*N34S+R122C(1)*			
	R117H,F508del+N34S(1) R117H+c.27delC(1)	*F508del,S1235R+N34S(1)*			
	Poly T+c.1429C>T[a]*+N34S (1) Poly T+N34S(1)*	*F508del+N34S(2)*			
	Poly T+194+2T>C, N34S(1)	*R117H,F508del+N34S(1) R117H+c.27delC(1) Poly T+c.1429C>T*[a]*+N34S (1)*			
		Poly T+N34S(1)			
		Poly T+194+2T>C, N34S(1)			
Family history of pancreatitis	1(8.3%)	1 (8.3%)	3 (25%)	0	6 (50%)
	F508del (1)	*N34S(1)*	*R122H(2)*		
	K68E(1)[a]		*365G>A (1)*		
	Trans	***Trans***			
	F508del+N34S(1)	*F508del+N34S(1)*			

AP, acute pancreatitis.
[a]Genetic variants of unknown significance.

The fact that ARP is a strong risk factor of CP does not preclude the possibility that CP can develop through trypsin-independent pathways (51). Indeed, families with CP linked to the carboxyl ester lipase gene (*CEL*) rarely report AP (52). The mechanism of disease appears to be generation of stress signals from unfolded protein responses, a mechanism shared by other CP-associated genes including carboxypeptidase A1 gene (*CPA1*) (53) and most of the trypsinogen (*PRSS1*) variants besides p.N29I and p.R122H

Table 2. Comparison of demographic and clinical characteristics in patients with and without pathogenic genetic variants in *PRSS1*, *CFTR*, *SPINK1*, and/or *CTRC*

	Pathogenic genetic variant positive (*N*=64)[a]	Pathogenic genetic variant negative (*N*=70)[a]	*P* value
Demographics			
Age at time of genetic testing	39.83 (1.9)	35.54 (3.5)	0.30
Race White	59 (92.2%)	60 (85.7%)	0.24
Non-white	5 (7.8%)	10 (14.3%)	–
Male	22 (34.4%)	30 (42.9%)	0.31
Clinical characteristics			
Smoking	26 (40.6%)	28 (40.00%)	0.94
History of heavy/very heavy alcohol use	5 (7.8%)	4 (5.7%)	0.63
Cholecystectomy	47 (73.4%)	56 (80%)	0.37
Pancreas divisum	18 (25.7%)	10 (15.6%)	0.15
M-ANNHEIM criteria			
Definite	42 (65.6%)	37 (52.9%)	0.133
Probable	9 (14.1%)	13 (18.6%)	0.482
Borderline	13 (20.3%)	20 (24.6%)	0.268
Clinical factors for gene testing			
ARP	56 (87.5%)	41 (58.6%)	<0.001
CP[b]	51 (79.7%)	50 (71.4%)	0.27
CP without a history of ARP[b]	7 (10.9%)	19 (27.1%)	0.002
History of AP	60 (93.7%)	44 (62.9%)	<0.001
Age of unexplained first episode of AP, (years) (*n*=108)	29.37 (2.1) (*n*=58)	34.59 (2.7) (*n*=46)	0.13
Age <35 at unexplained first episode of AP	37 (57.8%)	22 (31.4%)	0.04
Family history of pancreatitis	6 (9.4%)	6 (8.6%)	0.87
Age <35 first episode of AP and family history of pancreatitis	4 (6.2%)	0	0.034

AP, acute pancreatitis; ARP, acute recurrent pancreatitis; CP, chronic pancreatitis.
[a]Mean±s.d. or *n* (%).
[b]Defined according to the definitive and probable ductal criteria within the M-ANNHEIM Criteria for chronic pancreatitis.

Table 3. Comparison of the morphologic features of chronic pancreatitis in patient with and without pathogenic genetic variants in *PRSS1*, *CFTR*,*SPINK1* and/or *CTRC*

	Pathogenic genetic variant positive (*N*=64) (%)[a]	Pathogenic genetic variant negative (*N*=70) (%)[a]	*P* value
Calcifications	23 (35.9)	25 (35.7)	0.98
Severe/moderate Cambridge ductal criteria for CP on ERCP/MRCP	28 (43.7)	25 (35.7)	0.34
Mild Cambridge ductal criteria for CP by ERCP/MRCP	13 (39.4)	20 (60.6)	0.27
EUS score(*n*=118)			0.88
<3	15 (26.8)	19 (30.6)	–
3–4	21 (37.5)	23 (37.1)	–
≥5	20 (35.7)	20 (32.3)	–

CP, chronic pancreatitis; EUS, endoscopic ultrasound; ERCP, endoscopic retrograde cholangiopancreatography; MRCP, magnetic resonance cholangiopancreatography.
[a]Mean±s.d. or *n* (%).
Defined according to the definitive and probable ductal criteria within the M-ANNHEIM criteria for chronic pancreatitis.

(ref. 54). Although the diagnostic yield for pathogenic genetic variants in patients with ARIP was 58%, it is quite possible that the yield may be even higher, as the other 42% of patients with ARIP in this study may have genetic variants that either cannot be commercially tested for at the present time or have yet to be discovered. The case is even stronger for ICP, since non-trypsin pathway genes were not tested. The possibility that the lower prevalence of genetic variants detected in ICP may be related to limited genotyping is further supported by the fact that the patients with idiopathic pancreatitis who were pathogenic genetic variant negative had baseline demographic and clinical characteristics as well as morphologic features of CP that were similar to those patients who were pathogenic genetic variant positive.

Genetic testing of a young patient with an unexplained episode of AP and a family history of pancreatitis is often performed to evaluate for hereditary pancreatitis, which is most commonly due to mutations in the *PRSS1* gene. There is an over-reliance on this indication for genetic testing with some insurance companies only covering the costs of genetic testing in these patients (55). However, *PRSS1* mutations are only present in a minority of cases of idiopathic pancreatitis. We found that only 8% of the patients with pathogenic genetic variants had pathogenic *PRSS1* variant(s) which is consistent with prior studies (12,13). Interestingly, a family history of pancreatitis was not associated with pathogenic genetic variants as affected first degree relatives may have had pancreatitis due to more common etiologies including biliary and alcohol. However, patients with an unexplained episode of AP <35 years of age had over a twofold increased yield for pathogenic genetic variants in both the unadjusted and adjusted analysis.

The present study provides additional support for genetic testing in ARIP and, in addition, highlights the yield of genetic testing in young patients (<35 years of age) with a single episode of idiopathic AP. The early identification of pathogenic genetic variants can be useful in clinical practice. If patients are identified early in the natural history of their disease, there can be aggressive risk factor modification, such as for counseling for reducing

Table 4. Unadjusted and adjusted ORs for pathogenic genetic variants (*PRSS1, CFTR, SPINK1* and/or *CTRC*) based on clinical factors

	Unadjusted OR		Adjusted OR	
Clinical factors	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Acute recurrent idiopathic pancreatitis	6.24 (2.6–15.0)	<0.001	18.12[a] (2.1–151.9)	0.008[a]
ICP without a history of ARP[b]	0.25 (0.1–0.6)	0.004	3.38[c] (0.4–32.2)	0.29[c]
An unexplained first episode of AP<35 years of age	2.04 (1.0–4.0)	0.045	2.46[a] (1.2–5.1)	0.017[a]
Family history of pancreatitis	1.02 (0.9–1.0)	0.28	1.10[d] (0.3–4.3)	0.89[d]

AP, acute pancreatitis; ARIP, acute recurrent idiopathic pancreatitis; ICP, idiopathic chronic pancreatitis; OR, odds ratios.

[a]Adjusted ICP without history of ARP, family history of pancreatitis in addition to cholecystectomy, pancreas divisum, smoking and alcohol use.

[b]Defined according to the definitive and probable ductal criteria within the M-ANNHEIM criteria for chronic pancreatitis.

[c]Adjusted ARIP, family history of pancreatitis in addition to cholecystectomy, pancreas divisum, smoking and alcohol use.

[d]Adjusted ARIP, ICP without history of ARP, and unexplained first episode of AP <35 years of age in addition to cholecystectomy, pancreas divisum, smoking and alcohol use.

alcohol intake and smoking cessation, to prevent progression to CP and reduce the risk of pancreatic cancer (56–59). Identification of a pathogenic gene variant(s) can prevent an alcoholic pancreatitis misdiagnosis as well as the negative stigma this carries. If patients are found to have CFTR gene variant(s), particularly severe genotypes, clinicians should consider further screening for cystic fibrosis with sweat chloride testing. In our clinical practice, we have encountered many patients who initially presented with acute recurrent idiopathic pancreatitis but were subsequently diagnosed with cystic fibrosis and found to have non-pancreatic manifestations of disease (for example, lung disease). As patients with idiopathic ARP and CP often undergo a repetitive battery of costly and sometimes invasive diagnostic testing in search of an etiology, those who are defined as having genetic pancreatitis early in their disease course may avoid further unnecessary diagnostic testing. In particular, repetitive ERCPs and ERCP-related complications in patients who are presumed to have ARIP due to either pancreas divisum or pancreatic sphincter of Oddi dysfunction could be avoided which is especially important as the benefits of endoscopic therapy are questionable for these indications (60,61). The rates of disease progression with regards to the development of calcifications, exocrine and endocrine insufficiency, pancreatic cancer and response to endoscopic therapy in patients with pancreatitis can differ based on different genetic variants (56,62,63). Genetic testing will also help select patients for future trials of targeted pharmacologic therapies (for example, CFTR correctors/modulators, calcium channel blockers for PRSS1 mutations) which may treat symptoms and prevent disease progression (64,65).

The primary strength of the present study is the use of a well-phenotyped idiopathic pancreatitis cohort whose risk factors for pancreatitis were clearly defined and whose episodes of AP were rigorously confirmed. In addition, all pathogenic genetic variants were classified according to known published databases to ensure that genetic variants of unknown significance were excluded. The small numbers of patients with ICP without ARP was one of the limitations of this study. However, this is likely to be a very small group of patients regardless of the overall sample size, as only a minority of patients with ICP do not have a history of ARP. Another limitation is that our definition of a family history of pancreatitis could include pancreatitis of any etiology and was subject to patient recall. It is possible that a family history of idiopathic pancreatitis would have resulted in a higher yield of pathogenic genetic variants but this can be difficult to ascertain. Although 62 patients did not undergo genetic testing because they presented for only a single consultation visit to our subspecialty clinic or were lost to follow-up, this represents only 1 patient each month for 5 years or 13% (62/462) of the overall ARP/CP cohort. Although a cost effectiveness analysis was not the aim of this study, it does warrant future study. We did inquire and found that patient and insurance charges for sequencing the four gene panel are approximately $2,100 and $3,000, respectively (Ambry genetics, Aliso Viejo, CA, USA). These charges will likely decrease in the near future with new companies entering the field and with advances in gene sequencing technologies.

In conclusion, a significantly higher yield of genetic testing for pathogenic genetic variants was found in patients with ARIP and those with an unexplained first episode of AP <35 years of age. This data supports genetic testing in patients with ARIP and those with an unexplained first episode of AP <35 years of age. The present data is not sufficient to advocate for or against genetic testing in ICP patients without ARP. More studies are needed to evaluate the yield of genetic testing in ICP patients without ARP, particularly as trypsin-independent gene variants are added to existing commercial sequencing panels.

CONFLICT OF INTEREST

Guarantor of the article: Vikesh K. Singh, MD, MSc.

Specific author contributions: Conception or design of the work: Vikesh K. Singh, Niloofar Y. Jalaly, and Robert A. Moran; data collection: Niloofar Y. Jalaly, Robert A. Moran, Farshid Fargahi, Ayesha Kamal, and Christi Walsh; data analysis and interpretation: Niloofar Y. Jalaly, Robert A. Moran; drafting the article: Niloofar Y. Jalaly and Robert A. Moran; critical revision of the article: Mouen A. Khashab, Ayesha Kamal, Anne Marie Lennon, Martin A. Makary, David C. Whitcomb, Dhiraj Yadav, Liudmila Cebotaru, and Vikesh K. Singh. All authors approved the final draft of manuscript.

Financial support: None.

Potential competing interests: Mouen A. Khashab: consultant for Boston Scientific. These financial interests do not pertain directly to this work; David C. Whitcomb: serves as a consultant for AbbVie, Regeneron, and Ariel Precision Medicine, and has equity in Ariel Precision Medicine. These financial interests do not pertain directly to this work; Dhiraj Yadav: received Royalties for review

Up-To-Date, Inc.; Anne Marie Lennon: consultant for Olympus and NovoNordisc. These financial interests do not pertain directly to this work; Liudmila Cebotaru: has a license agreement with the Vertex Corporation for mutant cell lines and has a contract with Acetylon Pharmaceuticals. These financial interests do not pertain directly to this work; Vikesh Singh: consultant to Novo Nordisk, Calcimedica, and Abbvie. Advisory board participant for Nordmark, Ariel, and Akcea. These financial interests do not pertain directly to this work. All the other authors have no disclosures.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE

- ✓ In approximately one third of patients with pancreatitis, no etiology can be found despite an extensive evaluation.
- ✓ Several pathogenic genetic variants, which result in abnormalities of trypsin activation or degradation and/or pancreatic duct cell function can lead to pancreatitis.

WHAT IS NEW HERE

- ✓ Pathogenic genetic variants are more likely to be found in patients with a history of acute recurrent idiopathic pancreatitis.
- ✓ Pathogenic genetic variants are more likely to be found in patients with history of unexplained first episode of acute pancreatitis <35 years of age.

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