ABSTRACT

Objective: The cause of acute recurrent pancreatitis (ARP) or chronic pancreatitis (CP) is sometimes difficult to determine in children. In such patients, genetic analysis may prove helpful. This study analyzed mutations of cationic trypsinogen (PRSS1), serine protease inhibitor Kazal type 1 (SPINK1), chymotrypsin C (CTRC), and carboxypeptidase A1 (CPA1) and investigated the clinical features of children with these mutations.

Methods: Genetic analysis of mutations in these 4 genes was conducted in 128 patients with ARP or CP. Characteristics of the patients having the mutations were investigated using medical records.

Results: Fifty of the 128 (39.1%) subjects had at least 1 mutation (median age at first onset, 7.6 years). Abdominal pain was the presenting symptom of pancreatitis in 48 of the 50 patients (96%). Fifteen of those 50 patients (30.0%) had a family history of pancreatitis. Twenty-six patients had gene mutations in PRSS1, 23 in SPINK1, 3 in CTRC, and 5 in CPA1. In the 31 patients with mutations in SPINK1, CTRC, and CPA1, 16 (51.6%) had homozygous or heterozygous mutations with other mutations. Three patients underwent surgery and another 4 patients underwent endoscopy to manage their ARP or CP. Although 3 of the 7 patients complained of mild abdominal pain, none of those 7 patients had any obvious episode of ARP after treatment.

Conclusions: In pediatric patients with idiopathic ARP and CP, genetic analysis is useful for identifying the cause of pancreatitis. Early endoscopic or surgical treatment prevent ARP by extending the interval between episodes of pancreatitis in this population.
Keywords: clinical features; familial pancreatitis; hereditary pancreatitis; genetic mutations

What is Known:

- Mutations in the cationic trypsinogen gene and serine protease inhibitor gene are reportedly related to chronic pancreatitis (CP) of unknown cause.
- A relationship between mutation in the carboxypeptidase A1 gene and early-onset idiopathic CP was identified in 2013.
- Clinical data regarding these genetic risk factors in childhood CP have been limited.

What is New:

- There is a high possibility of the presence of genetic risk factors in Japanese pediatric patients with early-onset idiopathic acute recurrent pancreatitis (ARP) and CP.
- In the symptomatic patients with genetic mutations, early endoscopic or surgical treatment could prevent recurrent pancreatitis by extending the interval between episodes of pancreatitis.
Abbreviations:

*CFTR*: cystic fibrosis transmembrane conductance regulator

*PRSS1*: cationic trypsinogen

*SPINK1*: serine protease inhibitor Kazal type 1

*CTRC*: chymotrypsin C

*CPA1*: carboxypeptidase A1

*ER*: endoplasmic reticulum

*ARP*: acute recurrent pancreatitis

*CF*: cystic fibrosis

*CP*: chronic pancreatitis

*AP*: acute pancreatitis

*INSPPIRE*: International Study Group of Pediatric Pancreatitis: In search for a cure

*FP*: familial pancreatitis

*HP*: hereditary pancreatitis

*MRCP*: magnetic resonance cholangiopancreatography

*ERCP*: endoscopic retrograde cholangiopancreatography
INTRODUCTION

The etiologies of acute recurrent pancreatitis (ARP) and chronic pancreatitis (CP) in children include drugs, anatomic abnormalities, genetic mutations, and other conditions. Despite the use of adequate biochemical and radiological studies, determining the cause of pancreatitis is difficult in some cases [1, 2]. In such patients, genetic testing may prove helpful. Cystic fibrosis transmembrane conductance regulator gene (CFTR) identified in 1989 is well known as a cause of pancreatitis [3-5]. Afterward, as genetic causes of pancreatitis, cationic trypsinogen gene (protease serine 1, PRSS1) was reported [6], followed by serine protease inhibitor gene (Kazal type 1: SPINK1) [7] and chymotrypsin C (CTRC) [8, 9]. Recent data have demonstrated a high prevalence of genetic mutations among pediatric patients with pancreatitis [10-13]. In 2013, carboxypeptidase A1 (CPA1) was identified as a novel pancreatitis susceptibility gene implicated in early-onset pancreatitis in children up to 10 years of age [14]. The mechanism by which a CPA1 mutation confers an increased risk of pancreatitis may involve misfolding-induced endoplasmic reticulum (ER) stress, rather than elevated trypsin activity [14].

Patients with HP due to a PRSS1 gene mutation or relapsing pancreatitis due to a SPINK1 gene mutation can subsequently develop pancreatic exocrine insufficiency and diabetes; these patients represent a high-risk group for pancreatic cancer [15-17]. In young patients, early treatment prevents episodes of pancreatitis [18] and reduces the possibility of the future development of diabetes or cancer due to pancreatitis [15, 19]. Despite the importance of this topic, few genetic studies on childhood pancreatitis have been conducted in Japan.

Cystic fibrosis (CF) is the most common hereditary disease in Caucasians, but few
reports have described findings for Japanese populations [20, 21]. As result of the fact, the aim of this study was to analyze mutations in the PRSS1, SPINK1, CTRC, and CPA1 genes in Japanese children with ARP or CP and to clarify the clinical features.

METHODS

Study population

Participants comprised 128 patients (50 girls, 78 boys), all of whom were under 16 years old and had ARP or CP. Study cases were identified from 62 hospitals in Japan between 2007 and 2015. Acute pancreatitis (AP), ARP, and CP in children were characterized according to the ‘The International Study Group of Pediatric Pancreatitis: In Search for a Cure (INSPPIRE) criteria’[2]. AP was defined by at least two of the following: abdominal pain; serum amylase or lipase increased to ≥3 times the upper limit of normal; or imaging findings of AP. ARP was defined as ≥2 distinct episodes of AP with an intervening return to baseline. CP required one of the following: 1) abdominal pain consistent with pancreatic origin and imaging findings suggestive of chronic pancreatic damage; 2) evidence of exocrine pancreatic insufficiency and imaging findings suggestive of pancreatic damage; 3) evidence of endocrine pancreatic insufficiency and imaging findings suggestive of pancreatic damage; or 4) surgical or pancreatic biopsy demonstrating histopathological features compatible with CP.

This study was approved by the institutional review board at Juntendo University (approval number 2012171). Informed consent was obtained from the parents of each subject prior to enrollment in the study. The study was also in compliance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards (as revised in Edinburgh 2000). Patient information in this study was extracted in
conformity with the guidelines proposed in 2001 on genetic testing of pancreatitis in children [22].

**Clinical Assessment**

We retrospectively reviewed the following information from the medical records of each subject: age at onset; concomitant pancreatic or biliary disorders; and family history of pancreatitis. Change of form and abnormalities of the pancreatic duct were demonstrated by ultrasonography, computed tomography, magnetic resonance cholangiopancreatography (MRCP), or endoscopic retrograde cholangiopancreatography (ERCP). All study patients underwent at least one of these studies. The severity of clinical pancreatitis was determined according to the Atlanta classification [23].

**Genetic analysis**

Exon primers of the PRSSI, SPINK1, CTRC, and CPA1 genes were designed based on the published nucleotide sequence (PRSSI: GenBank: NM_002769.3; SPINK1: GenBank: NM_003122.3; CTRC: GenBank: NM_007272.2; CPA1: GenBank: NM_001868.2) [7, 8, 24]. DNA samples were extracted for analysis from peripheral blood leukocytes. We performed polymerase chain reaction (PCR) using 0.75 U of AmpliTaq Gold Polymerase (Applied Biosystems, Darmstadt, Germany), 2 mM of deoxynucleoside triphosphate in 25 mM of MgCl₂ solution, and 0.5 μM of each primer in a total volume of 25 μL in an automated thermal cycler. Cycle conditions were as follows: initial denaturation for 4 min at 94°C, 35 cycles of 30 s denaturation at 94°C, 30 s annealing at 60°C, 60 s primer extension at 72°C, and a final extension step for 7
min at 72°C. PCR products were refined using an Amicon Ultra Centrifugal Filter (Merck Millipore, Darmstadt, Germany). Cycle sequencing was performed using the BigDye Terminator Mix v1.1 (Applied Biosystems) 3 µL, BigDye Terminator Buffer v1.1 v3.1 5 µL, and 0.08 mM of forward or reverse primer 1 µL in an automated thermal cycler. Cycle conditions were as follows: initial denaturation for 1 min at 96°C, 25 cycles of 10 s denaturation at 96°C, 5 s annealing at 50°C, and 240 s primer extension at 60°C. The reaction products were purified with ethanol precipitation and loaded into the 3130 Genetic Analyzer (Applied Biosystems). Sequenced data were analyzed using SeqScape version 2.5 (Applied Biosystems).

RESULTS

Baseline characteristics of patients
Median age at first onset of AP was 7.6 years (range, 2-15). Among the 128 study patients with CP or ARP, 50 patients (39.1%) carried a mutation in 1-3 of the 4 genes analyzed (PRSS1, SPINK1, CTRC, and CPA1). Fifteen of those 50 patients (30.0%) had a family history of ARP or CP.

Clinical Aspects
Abdominal pain was the presenting symptom of pancreatitis in 48 of the 50 patients (96%). Table 1 shows the characteristics and clinical features of the patients with those mutations. Patients with a family history of mutations included 10 patients with R122R/H, 2 patients with G208A/G, 4 patients with multiple SPINK1 mutations, 1 patient with CTRC mutation, and no patients with CPA1 mutations. Abnormal findings on ERCP and/or MRCP were detected in 24 of the 50 patients (48.0%). Among the 50
patients in this study, 3 received longitudinal pancreaticojejunostomy, and 4 underwent stent placement or papillotomy via endoscopy. Although 3 of the 7 patients complained of mild abdominal pain and serum amylase and lipase levels were elevated beyond the upper limit of normal, none of those 7 patients experienced any episodes of recurrent pancreatitis within 2 years after the treatment. All three patients who received longitudinal pancreaticojejunostomy required the substitution of pancreatic enzymes because of measured exocrine insufficiency by BT-PABA (N-benzoyl-L-tyrosyl-p-aminobenzoic acid) test presenting steatorrhoea. None of the 7 children have presented with endocrine insufficiency to date.

In the present study, 2 patients were judged as having severe pancreatitis according to the Atlanta criteria. The 1 patient with CTRC R29G/R required mechanical ventilation and hemodialysis therapy to treat severe AP at the third hospital admission. ERCP findings revealed a meandering main pancreatic duct and pancreas divisum after recovery from AP. The patient underwent endoscopic minor papillotomy to prevent recurrence of the pancreatitis. No further episodes of abdominal pain or pancreatitis were seen during the follow-up period of 31 months. Another patient showed underlying disease (neurofibromatosis, medication with phenobarbital, food allergy, and malnutrition) and was diagnosed with severe pancreatitis. He died due to respiratory dysfunction and renal failure.

**Genetic analysis**

*PRSS1 mutations*

Twenty-six (14.8%) of the 128 subjects with ARP or CP displayed mutations in the *PRSS1* gene. We identified the heterozygous R122H/R mutation in 19 of those patients.
and G208A/G in 7 patients. Three of the 7 patients having G208A/G had additional mutations: N34N/S (1), IVS3+2T>C (2) and N34N/S and A137A/G (3) (Table 1, Figure 1).

**SPINKJ mutations**

*SPINKJ* mutations were identified in 23 of the 128 (18.0%) study patients with CP or ARP. The N34N/S mutation was observed in 19 of these 23 patients, the N34S homozygous mutation in 1 patient, and the N34N/S heterozygous mutation in 6 patients. The IVS3+2T>C mutation was seen in 12 of the 23 patients. One patient had the IVS3+2T>C heterogeneous mutation, and 9 patients were compound heterozygotes for N34N/S and IVS3+2T>C. In the remaining 6 patients, combinations of these mutations were detected as follows: G208A/G+N34N/S (1), G208A/G and IVS3+2T>C (2), G208A/G, N34N/S, and A137A/G (3), IVS3+2T>C and Q223H/Q (4), IVS3+2T>C and A137A/G (5), and N34N/S and c.1332+8delC (6) (Table 1, Figure 1).

**CTRC mutations**

*CTRC* mutations were identified in 3 patients (2.4%) with ARP or CP. The mutations R29G/R, Q223H/Q, and A28A/T in the *CTRC* gene were each observed in 1 patient. In patients with Q223H/Q, compound heterozygous mutation with N34N/S (4) was detected (Table 1, Figure 1).

**CPAI mutations**

*CPAI* mutations were identified in 5 patients (3.9%) with ARP or CP. The A137A/G mutation in the *CPAI* gene was observed in 4 of these 5 patients, and c.1332+8delC (6) was observed in 1 patient. Two of the patients with A137A/G mutations were compound heterozygotes for N34N/S and G208G/A (3) and IVS3+2T>C (5) (Table 1, Figure 1).
DISCUSSION

In the present study, patients with *PRSS1* R122R/H mutations did not have any other detectable mutations. In contrast, 3 of the 7 patients had the *PRSS1* G208A/G mutation together with other mutations. This mutation occurs through mutation-induced protein misfolding and results in ER stress [25]. The G208A/G mutation has been reported in Asian subjects, including a 12-year-old Korean patient with CP with recurrent AP, and a 7-year-old Korean patient with necrotizing AP [26]. One report stated that the G208A/G mutation is found in association with pancreatitis [27]. The isolated risk from the G208A/G mutation is considered to be lower than the risk from the R122R/H mutation. Patients with the G208A/G mutation might need other factors such as other genetic factors before pancreatitis will develop.

With regard to the N34N/S mutation, the mechanism of pancreatitis remains unclear [28]. The IVS3+2T>C mutation affects the consensus splicing donor site, resulting in the skipping of exon 3 [21]. In exon 3, SPINK1 possesses a reactive site that serves as a specific target substrate for trypsin. *SPINK1* mutations have been suggested to result in altered interactions between SPINK1 and trypsin, thus affecting the protease/antiprotease balance within the pancreas [29]. In patients with *SPINK1* mutations together with other factors, excluding 1 patient with the N34S homozygous form, 15 showed other gene mutations. N34N/S and IVS3+2T>C mutations were observed in 9 patients, and 6 of those with N34N/S or IVS3+2T>C had other mutations. In 5 of the 9 patients with N34N/S and IVS3+2T>C mutations, the father and mother had either of the heterogeneous mutations N34N/S or IVS3+2T>C. This compound heterozygous form may thus be associated with ARP, and may also be related to early onset of pancreatitis.
In analysis of the *CTRC* gene, R29G/R, Q223H/Q, and A28A/T mutations were detected. One of the 3 patients had both Q223H/Q and N34N/S mutations. Q223H/Q and A28A/T mutations have not been reported previously. Lee et al. reported a relatively high incidence of mutations in patients with pancreas divisum and suggested that patients with genetic mutations combined with pancreas divisum tend to develop CP at an early age [12]. In our study, a patient with the R29G/R mutation combined with pancreas divisum was diagnosed as having severe AP, and early endoscopic treatment was useful in preventing the development of CP and ARP. Similarly, remaining 6 patients who required stent placement, papillotomy, or abdominal surgery have not developed recurrent pancreatitis. Eventually, 4 of the 7 patients had pancreatic divisum or pancreaticobiliary maljunction indicated that combination of these genetic mutations and anatomical abnormalities may led to early-onset uncontrollable pancreatitis. Since obvious ARP episode was not seen in any of those 7 patients after the treatment, these vigorous interventions could be useful for preventing CP and ARP. This result was the same as that reported by Kargl et al., in which a therapeutic step-up strategy including early treatment and intervention proved effective in extending the interval between episodes of pancreatitis [18].

We weighed the findings from past reports against this study (Table 2) [10-13]. These studies seem in broad accord, and the age of onset was around 5-10 years. Abdominal pain was the most frequent and structural abnormality was found in 20-40% of children as a factor contributing to the etiology of ARP or CP in these series. Although the statistical analysis in frequency of *CTRC, CFTR, and CPA1* mutations among our study and other 4 previous studies could not calculate because of lack of complete data, there were no significant differences in those of both *PRSSI1* and *SPINK1* gene mutations.
between ours and previous ones (The Fisher’s exact test were used to identify significant changes with IBM SPSS Statistics version 20; Statistical Package for Social Science Japan, Inc., Tokyo, Japan). Differences in the ethnicity or genetic background of patients would be related to the incidence of each genetic variation. To date, few reports have described \textit{CPAI} mutations [14, 30]. The frequency and clinical features of pancreatitis in patients with \textit{CPAI} mutations remain unclear. \textit{CPAI} mutations with less than 20\% apparent activity of the CPA1 protein have been observed to be significantly overrepresented in patients with chronic pancreatitis [14]. In the present study, \textit{CPAI} mutations were detected as A137A/G and c.1332+8delC in 5 patients. Activity of the \textit{CPAI} protein with the A137A/G mutation was 52\% [14] compared to the wild type; the A137A/G mutation might thus need other mutations before pancreatitis develops. The c.1332+8delC mutation has not been previously reported. Further research should focus on the perseverance of \textit{CPAI} mutations in pediatric ARP and CP patients.

One limitation of the present study was that the \textit{CFTR} gene was not analyzed. CF is well known cause of pancreatitis in Western countries [4, 5]. In contrast, CF is a very rare disease in Japanese, with a frequency of about 1/350,000 [20, 21]. We also did not analyze the cationic trypsinogen, protease serine 2 (\textit{PRSS2}), [31, 32] carboxypeptidase A2 (\textit{CPA2}), carboxypeptidase B1 (\textit{CPB1}) [33, 34], or claudin-2 (\textit{CLDN2}) [35] genes, because it remains unclear whether these gene mutations are associated with pancreatitis. In present study, since the cause of pancreatitis is still unclear in about 60\% of patients (Figure 1), genetic analysis except \textit{PRSSI}, \textit{SPINK1}, \textit{CTRC} and \textit{CPAI} genes may be useful to determine it.
In conclusion, there is a high possibility of the presence of genetic risk factors in Japanese patients with early-onset idiopathic ARP and CP. For symptomatic ARP and CP in children with genetic mutations, early endoscopic or surgical treatment could prevent recurrent pancreatitis by extending the interval between episodes of pancreatitis.

REFERENCES


28. Aoun E, Muddana V, Papachristou GI, et al. SPINK1 N34S is strongly associated with recurrent acute pancreatitis but is not a risk factor for the first or sentinel acute


FIGURE LEGENDS

Figure 1. Genetic evaluation of patients with chronic and recurrent pancreatitis of unknown etiology (n=128).

PRSS1: cationic trypsinogen; SPINK1: serine protease inhibitor Kazal type 1; CTRC:
chymotrypsin C; CPA1: carboxypeptidase A1
<table>
<thead>
<tr>
<th>Gene mutations</th>
<th>No. of patients (%) (n=128)</th>
<th>No. with family history</th>
<th>ERCP and/or MRCP findings</th>
<th>Endoscopic or surgical management</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRSS1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R122R/H</td>
<td>19 (15.0%)</td>
<td>10</td>
<td>Stones, meandering main pancreatic duct, and duct dilatation (n=1) Stones and duct dilatation (n=1) Pancreaticobiliary maljunction and duct dilatation (n=1) Pancreas divisum and duct dilatation (n=2)</td>
<td>Stent placement by endoscopy (n=1) Papillotomy by endoscopy (n=1) Longitudinal pancreaticojejunostomy (n=1)</td>
</tr>
<tr>
<td>G208A/G</td>
<td>4 (3.1%)</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>G208A/G with other mutations +N34N/S: (1) +IVS3+2T&gt;C: (2) +N34N/S and A137A/G: (3)</td>
<td>3 (2.4%)</td>
<td>1</td>
<td>Stones (n=2) (1, 3) Stones, meandering main pancreatic duct and duct dilatation (n=1) (2)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>26 (20.3%)</td>
<td>12</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>SPINK1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N34S homozygous</td>
<td>1 (0.8%)</td>
<td>0</td>
<td>Pancreas stone (n=1)</td>
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</tr>
<tr>
<td>N34N/S heterogeneous mutation</td>
<td>6 (4.7%)</td>
<td>0</td>
<td>Pancreas divisum, stones, and duct dilatation (n=1) Pancreas divisum and duct dilatation (n=1)</td>
<td>0</td>
</tr>
<tr>
<td>IVS3+2T&gt;C single mutation</td>
<td>1 (0.8%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>N34N/S and IVS3+2T&gt;C</td>
<td>9 (7.1%)</td>
<td>2</td>
<td>Stones, meandering main pancreatic duct, and duct dilatation (n=4) Stones and duct dilatation (n=1) Meandering pancreatic duct and duct dilatation (n=1) Duct dilatation (n=1)</td>
<td>Stent placement by endoscopy (n=1) Longitudinal pancreaticojejunostomy (n=2)</td>
</tr>
<tr>
<td>N34N/S or IVS3+2T&gt;C with other mutations (1), (2), (3), +Q223H/Q: (4) +A137A/G: (5) +c.1332+8delC: (6)</td>
<td>6 (4.7%)</td>
<td>2 (1, 4)</td>
<td>Stones (n=4) (1, 3, 4, 6) Stones, meandering main pancreatic duct, and duct dilatation (n=2) (2, 5)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>23 (18.0%)</td>
<td>4</td>
<td>16</td>
<td>3</td>
</tr>
<tr>
<td>CTRC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R29G/R</td>
<td>1 (0.8%)</td>
<td>0</td>
<td>Pancreas divisum, meandering main pancreatic duct (n=1)</td>
<td>Stent placement by endoscopy and minor papillotomy by endoscopy (n=1)</td>
</tr>
<tr>
<td>A28A/T single mutation</td>
<td>1 (0.8%)</td>
<td>0</td>
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<td>0</td>
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<tr>
<td>Q223H/Q with other mutation (4)</td>
<td>1 (0.8%)</td>
<td>1</td>
<td>Stones (n=1)</td>
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<tr>
<td>Total</td>
<td>3 (2.4%)</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>CPAI</td>
<td></td>
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<tr>
<td>A137A/G</td>
<td>2 (1.6%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A137A/G with other mutations (3), (5)</td>
<td>2 (1.6%)</td>
<td>0</td>
<td>Stones (n=1) (3) Stones, meandering main pancreatic duct, and duct dilatation (n=1) (5)</td>
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</tr>
<tr>
<td>c.1332+8delC with other mutation (6)</td>
<td>1 (0.8%)</td>
<td>0</td>
<td>Stones (n=1)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>5 (3.9%)</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Total number of patients</td>
<td>50* (39.1%)</td>
<td>15*</td>
<td>24*</td>
<td>7</td>
</tr>
</tbody>
</table>

*Patient numbers (1) to (6) were not counted twice.

(1) G208A/G and N34N/S; (2) G208A/G and IVS3+2T>C; (3) G208A/G, N34N/S, and A137A/G; (4) IVS3+2T>C and Q223H/Q; (5) IVS3+2T>C and A137A/G; (6) N34N/S and c.1332+8delC

PRSSI1, cationic trypsinogen; SPINK1, serine protease inhibitor Kazal type 1; CTRC, chymotrypsin C; CPAI, carboxypeptidase A1; MRCP, magnetic resonance cholangiopancreatography; ERCP, endoscopic retrograde cholangiopancreatography.
Table 2: Studies of acute recurrent or chronic pancreatitis in children

<table>
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<tr>
<td>Number of patients</td>
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<tr>
<td></td>
<td>76 (CP patients)</td>
<td>29 (ARP or CP patients)</td>
<td>32 (ARP or CP patients)</td>
<td>78 (ARP patients)</td>
<td>128 (ARP or CP patients)</td>
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<td>Positive for mutations</td>
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<td></td>
<td>51 (67%)</td>
<td>23 (79%)</td>
<td>15 (47%)</td>
<td>26 (33%)</td>
<td>50 (39%)</td>
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<td>Median age at diagnosis (year)</td>
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<td></td>
<td>9.9</td>
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<td>7.6</td>
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<td>Main symptoms</td>
<td>Abdominal pain 77%</td>
<td>Abdominal pain 100%</td>
<td>No mention</td>
<td>Abdominal pain 76.9%</td>
<td>Abdominal pain 96.6%</td>
</tr>
<tr>
<td></td>
<td>Vomiting 74%</td>
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<tr>
<td></td>
<td>Nausea 40%</td>
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<tr>
<td>Frequency of genetic pancreatitis*</td>
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<td></td>
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<tr>
<td>PRSS1</td>
<td>43%</td>
<td>24%</td>
<td>12.5%</td>
<td>4.5%†</td>
<td>20.3%</td>
</tr>
<tr>
<td>SPINK1</td>
<td>19%</td>
<td>27%</td>
<td>34.4%</td>
<td>7.1%†</td>
<td>18.0%</td>
</tr>
<tr>
<td>CTRC</td>
<td>3%</td>
<td>Non-testing</td>
<td>Non-testing</td>
<td>Non-testing</td>
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<tr>
<td>CFTR</td>
<td>14%</td>
<td>48%</td>
<td>Non-testing</td>
<td>Non-testing</td>
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<tr>
<td>CPA1</td>
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<td>Non-testing</td>
<td>Non-testing</td>
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<td></td>
</tr>
</tbody>
</table>

*: Some overlap exists between cases.
†: Genetic testing results were obtained in 44 (PRSS1), 42 (SPINK1), and 53 (CFTR) of the 78 patients.

Figure 1

Negative genetic testing n=78 (60.9%)

- **SPINK1**
  - n=23 (18.0%)

- **PRSS1**
  - n=26 (20.3%)

- **N34S or N34N/S**
  - n=7 (5.5%)

- **IVS3+2T>C**
  - n=10 (7.9%)

- **G208A/G**
  - n=7 (5.5%)

- **R122R/H**
  - n=19 (15.0%)

- **CTRC**
  - n=3 (2.4%)

- **CPAI**
  - n=5 (3.9%)