The N34S mutation of SPINK1 (PSTI) is associated with a familial pattern of idiopathic chronic pancreatitis but does not cause the disease


**Background:** Mutations in the PRSS1 gene explain most occurrences of hereditary pancreatitis (HP) but many HP families have no PRSS1 mutation. Recently, an association between the mutation N34S in the pancreatic secretory trypsin inhibitor (SPINK1 or PSTI) gene and idiopathic chronic pancreatitis (ICP) was reported. It is unclear whether the N34S mutation is a cause of pancreatitis per se, whether it modifies the disease, or whether it is a marker of the disease.

**Patients and methods:** A total of 327 individuals from 217 families affected by pancreatitis were tested: 152 from families with HP, 108 from families with ICP, and 67 with alcohol related CP (ACP). Seven patients with ICP had a family history of pancreatitis but no evidence of autosomal dominant disease (f-ICP) compared with 87 patients with true ICP (t-ICP). Two hundred controls were also tested for the N34S mutation. The findings were related to clinical outcome.

**Results:** The N34S mutation was carried by five controls (2.5%; allele frequency 1.25%), 11/87 (13%) HP patients (p=0.0013 v controls), and 6/7 (86%) affected HP patients (p=0.001 v controls), in 3/27 (11%) with wild-type and in 1/81 (1%) with mutant PRSS1, and 4/67 ACP patients (all p>0.05 v controls). The presence of the N34S mutation was not associated with early disease onset or disease severity.

**Conclusions:** The prevalence of the N34S mutation was increased in patients with ICP and was greatest in f-ICP cases. Segregation of the N34S mutation in families with pancreatitis is unexplained and points to a complex association between N34S and another putative pancreatitis related gene.

Hereditary pancreatitis (HP) is characterised by recurrent attacks of painful acute pancreatitis from an early age eventually resulting in chronic pancreatitis, with loss of pancreatic exocrine and endocrine function. HP is an autosomal dominant condition with a penetrance of approximately 80%. Causative mutations have been identified in the PRSS1 (PRoteaSe Serine 1) gene which encodes cationic trypsinogen. The two mutations most frequently identified are the N34S mutation, which was found in 4/108 affected HP patients (p=0.724 v controls), and in 3/27 (11%) with wild-type and in 1/81 (1%) with mutant PRSS1, and 4/67 ACP patients (all p>0.05 v controls). The presence of the N34S mutation was not associated with early disease onset or disease severity.

The N34S mutation was carried by five controls (2.5%; allele frequency 1.25%), 11/87 (13%) HP patients (p=0.0013 v controls), and 6/7 (86%) affected HP patients (p=0.001 v controls), in 3/27 (11%) with wild-type and in 1/81 (1%) with mutant PRSS1, and 4/67 ACP patients (all p>0.05 v controls). The presence of the N34S mutation was not associated with early disease onset or disease severity.

Acknowledgements: This study was supported by EUROPAC, European Registry of Hereditary Pancreatitis and Familial Pancreatic Cancer.
five (9.1%) of 55 patients with HP and in 23 (40.4%); seven homozygotes, 14 heterozygotes, and two compound heterozygotes) of 37 patients with ICP. Thus whether an N34S mutation should be considered a causative factor of pancreatitis per se or simply a disease modifier is uncertain.24–26 Multiple SPINK1 sequence variants other than N34S have been identified but their clinical significance is even less certain.14–15 To investigate this further, large groups of patients with HP ICP, and alcohol related chronic pancreatitis (ACP) were tested for the N34S mutation.

METHODS

Patients

Patients and family members with HP and some individuals with ICP were recruited through the European Registry of Hereditary Pancreatitis and Familial Pancreatic Cancer (EUROPAC). Other patients with ICP and all patients with ACP were recruited from the Pancreas Unit based at the Royal Liverpool University Hospitals, Liverpool, UK.

The diagnosis of chronic pancreatitis was based on clinical and radiological criteria and exocrine pancreatic function tests.25 Following written informed consent both affected individuals and their referring clinicians completed detailed questionnaires relating to clinical history, radiology, pancreatic function tests, interventions, and history. Clinically unaffected individuals from relevant families were rigorously screened for symptoms of pancreatitis by questionnaire. The diagnosis of diabetes mellitus was based on standard tests and/or the requirement for insulin, and the diagnosis of exocrine failure was based on standard diagnostic tests and/or the use of pancreatic enzyme supplements. ACP was diagnosed in a patient with chronic pancreatitis and an alcohol consumption of ≥80 g/day.25 ICP was defined as chronic pancreatitis occurring in an individual for which there was no known cause after a detailed history, biochemical testing, and radiology, including that of both the biliary and pancreatic ducts, and in the absence of other affected individuals within that family.25 A diagnosis of HP was made on the basis of two first degree relatives or three or more second degree relatives, in two or more generations with recurrent acute pancreatitis, and/or chronic pancreatitis for which there were no precipitating factors. Cases in which the criteria for HP were not met, yet in whom there was a familial trend (mainly in cases in which there was more than one affected individual within the same generation), were subclassified as having familial ICP (f-ICP). The other subgroup were those with the true form of ICP (t-ICP) in which there was only one known family member affected. A multidisciplinary professionally experienced committee accepted or rejected individuals and families onto the register and agreed on the relevant diagnostic category after consideration of all of the relevant information.

At the censor date, EUROPAC had studied 152 individuals and families ACP members of ICP members of HP families. At time of study.

Table 1 Characteristics of the study population

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>HP (affected)</th>
<th>Unaffected members of HP families</th>
<th>mt-PRSS1 HP</th>
<th>wt-PRSS1 HP</th>
<th>ACP (affected)</th>
<th>Unaffected members of ICP families</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M:F)</td>
<td></td>
<td>108</td>
<td>44</td>
<td>81</td>
<td>27</td>
<td>94</td>
<td>14</td>
</tr>
</tbody>
</table>

HP, hereditary pancreatitis; mt, mutant; wt, wild-type; ICP, idiopathic chronic pancreatitis; ACP, alcohol related chronic pancreatitis. *At time of study.

N34S mutation detection

Restriction fragment length polymorphism (RFLP) analysis

Primers were designed to amplify exon 3 based on the published nucleotide sequence (GenBank, NM-003122). The forward and reverse primer sequences were 5'-TTGTTTAAATCGCTTACCAT-3' and 5'-GGGCTTTTATCATACAAGTGACCTCT-3', respectively. The primers were designed to introduce a PstI endonuclease restriction site for

Table 1

Characteristics of the study population

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>HP (affected)</th>
<th>Unaffected members of HP families</th>
<th>mt-PRSS1 HP</th>
<th>wt-PRSS1 HP</th>
<th>ACP (affected)</th>
<th>Unaffected members of ICP families</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M:F)</td>
<td></td>
<td>108</td>
<td>44</td>
<td>81</td>
<td>27</td>
<td>94</td>
<td>14</td>
</tr>
</tbody>
</table>

HP, hereditary pancreatitis; mt, mutant; wt, wild-type; ICP, idiopathic chronic pancreatitis; ACP, alcohol related chronic pancreatitis. *At time of study.
Table 2

<table>
<thead>
<tr>
<th></th>
<th>Total N34S</th>
<th>wt-N34</th>
<th>Total N34S</th>
<th>wt-N34</th>
<th>Total N34S</th>
<th>wt-N34</th>
<th>Total N34S</th>
<th>wt-N34</th>
<th>Total N34S</th>
<th>wt-N34</th>
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<tbody>
<tr>
<td>Population</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total population</td>
<td>417 (527)</td>
<td>27 (42)</td>
<td>6%</td>
<td>390 (485)</td>
<td>94%</td>
<td>269 (217)</td>
<td>25 (22)</td>
<td>9%</td>
<td>244 (198)</td>
<td>91%</td>
</tr>
<tr>
<td>All ICP</td>
<td>91 (108)</td>
<td>15 (27)</td>
<td>16%</td>
<td>76 (81)</td>
<td>84%</td>
<td>94 (91)</td>
<td>17 (15)</td>
<td>18%</td>
<td>77 (78)</td>
<td>82%</td>
</tr>
<tr>
<td>t-ICP</td>
<td>87 (92)</td>
<td>11 (11)</td>
<td>13%</td>
<td>76 (81)</td>
<td>87%</td>
<td>87 (87)</td>
<td>11 (11)</td>
<td>13%</td>
<td>76 (76)</td>
<td>87%</td>
</tr>
<tr>
<td>f-ICP</td>
<td>4 (16)</td>
<td>4 (16)</td>
<td>100%</td>
<td>0</td>
<td>0%</td>
<td>7 (4)</td>
<td>6 (4)</td>
<td>86%</td>
<td>1 (2)</td>
<td>14%</td>
</tr>
<tr>
<td>All HP</td>
<td>59 (152)</td>
<td>3 (6)</td>
<td>5%</td>
<td>56 (146)</td>
<td>95%</td>
<td>108 (59)</td>
<td>4 (3)</td>
<td>4%</td>
<td>104 (57)</td>
<td>96%</td>
</tr>
<tr>
<td>mt-PRSS1</td>
<td>45 (117)</td>
<td>1 (2)</td>
<td>2%</td>
<td>44 (115)</td>
<td>98%</td>
<td>81 (45)</td>
<td>1 (1)</td>
<td>1%</td>
<td>80 (44)</td>
<td>99%</td>
</tr>
<tr>
<td>wt-PRSS1</td>
<td>14 (35)</td>
<td>2 (4)</td>
<td>14%</td>
<td>12 (31)</td>
<td>86%</td>
<td>27 (14)</td>
<td>3 (2)</td>
<td>11%</td>
<td>25 (15)</td>
<td>74%</td>
</tr>
<tr>
<td>All familial</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HP+f-ICP</td>
<td>63 (168)</td>
<td>7 (22)</td>
<td>11%</td>
<td>56 (146)</td>
<td>89%</td>
<td>115 (63)</td>
<td>10 (7)</td>
<td>9%</td>
<td>105 (59)</td>
<td>91%</td>
</tr>
<tr>
<td>ACP</td>
<td>67 (67)</td>
<td>4 (4)</td>
<td>6%</td>
<td>63 (63)</td>
<td>94%</td>
<td>67 (67)</td>
<td>4 (4)</td>
<td>6%</td>
<td>63 (67)</td>
<td>94%</td>
</tr>
<tr>
<td>Controls</td>
<td>200 (200)</td>
<td>5 (5)</td>
<td>2.5%</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

ICP, idiopathic chronic pancreatitis; t-ICP, true idiopathic chronic pancreatitis; f-ICP, familial idiopathic chronic pancreatitis; HP, hereditary pancreatitis; ACP, alcohol related chronic pancreatitis; mt, mutant; wt, wild-type.

Site in sequences containing the N34S mutation and a BsrDI endonuclease restriction site in wild-type sequences. A polymerase with 3' to 5' proof reading activity was chosen to eliminate PCR error. PCR was performed using 50 ng genomic DNA template, 0.5 U Pfu DNA polymerase (Promega, Southamptom, UK), 2 mmol/l MgCl2, 200 µmol/l of each deoxynucleotide triphosphate (Roche Diagnostics, East Sussex, UK), and 200 nmol/l of each primer in a 50 µl reaction volume. Thirty five cycles of PCR were performed (30 seconds at 94°C, 30 seconds at 60°C, and 60 seconds at 72°C). The PCR products were then digested with restriction endonucleases PstI and BsrDI. PCR product (25 µl) was added to 10 U PstI (New England Biolabs), 1× digest buffer (New England Biolabs), and 1× bovine serum albumin (BSA) (New England Biolabs) to a final volume of 50 µl and incubated at 37°C for one hour. The remaining 25 µl of PCR product was added to 10 U BsrDI (New England Biolabs), 1× digest buffer (New England Biolabs), 1× BSA (New England Biolabs) to a final volume of 50 µl and incubated at 55°C for one hour. The digestion reactions were heat inactivated by incubation at 80°C for 15 minutes. The products were analysed by agarose gel electrophoresis using a 3% (w/v) NuSieve 3:1 agarose (Flowgen, Leicestershire, UK) gel, 1× TAE buffer, and 0.5 µg/ml ethidium bromide. Undigested amplification products were 320 bp in length. After digestion with PstI a product of 286 bp was obtained from mutant sequences and an identical result was achieved from wild-type sequences after digestion with BsrDI. Heterozygote samples produced both products of 320 bp and 286 bp after digestion with both endonucleases (fig 1). To validate the RFLP analysis further, the PCR reactions were repeated and the amplification products purified by ethanol precipitation prior to digestion. The digestion reactions were then incubated for 16 hours. The results obtained were identical in both experiments.

Sequence analysis
Primers were designed from intronic sequences flanking exon 3, based on the published nucleotide sequence. The forward and reverse primer sequences were 5'-AATGAGGCGACATGGACTTA-3' and 5'-AATCCAGGTCTCGACTATT-3', respectively (designed from GenBank AF286028). PCR was performed using 50 ng genomic DNA template, 0.5 U Pfu DNA polymerase (Promega), 2 mmol/l MgCl2, 200 µmol/l each deoxynucleotide triphosphate (Roche Diagnostics), and 200 nmol/l of each primer in a 50 µl reaction volume. Thirty five cycles of PCR were performed (30 seconds at 94°C, 30 seconds at 60°C, 60 seconds at 72°C). Cycle sequencing was performed on the products directly using the ABI Prism Big Dye Terminator Cycle Sequencing kit (PE Biosystems). Sequencing reactions consisted of 100 ng template, 3.2 pmol primer, 4 µl of Cycle Sequencing premix, and 4 µl of sequencing buffer in a total volume of 20 µl. Reaction products were purified by isopropanol precipitation and run on an ABI Prism 377 DNA Analyser (PE Biosystems).

Statistical analysis
All statistical analysis was carried out using the software Statview 5 (SAS Institute Inc., Cary, North Carolina, USA, 1998). Categorical data were analysed by Fisher's exact probability test. The principal contingency analyses were the prevalence of the N34S mutation in patients with ICP, HP, and ACP compared with controls; the remaining analyses were performed for consistency. Continuous data were analysed by the Kruskall-Wallis and Mann-Whitney U tests and interactions by analysis of variance (ANOVA). The cumulative incidence was calculated by the Kaplan-Meier method and statistical comparisons were made by Weibull or log rank (Mantell-Cox) analysis. Significance was set at p<0.0025 (Bonferroni correction).
RESULTS
N34S mutation screening technique
The RFLP screening technique detected 31 individuals with the N34S mutation (heterozygous in 29 cases and homozygous in two cases) and was confirmed by direct sequencing in each case. In addition, 80 cases with no N34S mutation by the RFLP technique were shown to have wild-type sequences in exon 3 of the SPINK1 gene by direct sequencing. Thus the PstI and BsrDI restriction digests were shown to identify the presence or confirm the absence of the N34S mutations with 100% accuracy.

Frequency of the N34S mutations in the study groups
The frequencies of the N34S mutation in the different groups are summarised in tables 2–4 and the results of contingency analysis are given in table 5. Four of 100 (4%) blood donors from Liverpool, UK, and one of 100 (1%) blood donors from Münster, Germany, carried the N34S mutation and all were heterozygous. This gave an average prevalence of the N34S mutation among controls of 2.5% and an allele frequency of 1.25%.

Seventeen (18%) of 94 patients with ICP carried the N34S mutation (p<0.0001 v controls), two of whom were homozygous for the mutation. No other cases of homozygosity for N34S were identified in our cohort. Thus the prevalence of N34S in ICP patients was higher than in HP patients with PRSS1 mutations but the difference was only of marginal statistical significance (3/27 wild-type PRSS1 patients compared with 1/81 patients with a PRSS1 mutation; p=0.047). Furthermore, the prevalence of N34S in HP patients with no PRSS1 mutation grouped with f-ICP patients was significantly higher than in controls (9/34 compared with 5/200; p<0.0001).

Ten individuals from families with wild-type PRSS1 carried the SPINK1 mutation. Two of these were affected homozygotic twins; as no other family member had pancreatitis, including both parents, these were considered as f-ICP. Two families (one with HP and one with f-ICP) had two affected individuals, both with N34S. The latter, f-ICP family (fig 2), has been described above. The remaining three patients came from separate families, which in two families (one HP and one f-ICP) included another affected family member who did not have the N34S mutation.

Only one (1.9%) of 53 patients with the R122H mutation in PRSS1 had the N34S mutation. None of the 28 patients with other PRSS1 mutations carried the N34S SPINK1 mutation (N29I=23; A16V=5). Only one of 58 unaffected relatives of patients with pancreatitis from 31 families carried the N34S mutation (table 4). Finally, there were four (6%) of 67 patients with ACP who carried the N34S mutation (p=0.235 v controls).

Table 3

<table>
<thead>
<tr>
<th>Population</th>
<th>Affected individuals (families)</th>
<th>N34S</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Heterozygous Homozygous %*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All ICP</td>
<td>94 [91]</td>
<td>17</td>
<td>15</td>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td>t-ICP</td>
<td>87 [87]</td>
<td>11</td>
<td>9</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>f-ICP</td>
<td>7 [4]</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>86</td>
</tr>
<tr>
<td>All HP</td>
<td>108 [59]</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>All mt-PRSS1</td>
<td>81 [45]</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>PRSS1: R122H</td>
<td>53 [29]</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PRSS1: N29I</td>
<td>23 [13]</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PRSS1: A16V</td>
<td>2 [3]</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>All wt PRSS1</td>
<td>27 [14]</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>ACP</td>
<td>67 [67]</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>6</td>
</tr>
</tbody>
</table>

*Percentage of affected individual cases.

ICP, idiopathic chronic pancreatitis; t-ICP, true idiopathic chronic pancreatitis; f-ICP, familial idiopathic chronic pancreatitis; HP, hereditary pancreatitis; ACP, alcohol related chronic pancreatitis; mt, mutant; wt, wild-type.

Figure 1
N34S mutation restriction fragment length polymorphism analysis. Gel electrophoresis (3% Nusieve) of PstI and BsrDI digestion products obtained from a wild-type individual, a heterozygote, and a homozygote. Lanes 1, 4, 7: undigested polymerase chain reaction product. Lane 2: wild-type individual—PstI. Lane 3: wild-type individual—BsrDI. Lane 5: heterozygous individual—PstI. Lane 6: heterozygous individual—BsrDI. Lane 8: homozygous individual—PstI. Lane 9: homozygous individual—BsrDI. Lane 10: negative control.
The presence of the N34S mutation was not associated with early disease onset or any aspect of disease severity. Median (IQR) age at symptom onset in patients with HP was 12 (6.0–19.0) years and 12 (7.5–20.5) years for patients with ICP (log rank p value after Kaplan-Meier analysis; p=0.981). Comparisons of patients with and without the N34S mutation revealed no significant differences in terms of age of disease onset, including all patient groups together (log rank, p=0.279) or any of the groups separately (all p>0.05, data not shown).

**DISCUSSION**

This study confirms an association between the SPINK1 N34S mutation and ICP. The prevalence of N34S was higher in patients with ICP (18%) than in normal individuals (2.5%) and much higher in f-ICP patients (86%) than in t-ICP patients (12.6%). Moreover, we identified N34S in unaffected individuals: in family members of patients with pancreatitis as well as in controls. The prevalence of N34S was not significantly elevated in HP patients with a causative PRSS1 gene mutation (1%).

The combined prevalence of N34S in our control populations is consistent with the prevalence of 1.58% in individuals from the USA and 1.5% from France but contrasts with the lower prevalence rate of 0.36% from the Berlin area. Thus the prevalence of N34S is much more geographically varied than originally perceived.

The prevalence of N34S compares with a much lower prevalence of ICP (~0.0066%). These observations argue against the notion that N34S is causative of ICP in an autosomal dominant manner (since an extremely low penetrance would be required) and support the previous linkage study. Witt and colleagues reported on a unique pancreatitis pedigree with a SPINK1 mutation (M1T) disrupting the start codon that conceivably could have had a dominant pattern of inheritance. Thus much more extensive genetic studies are needed to clarify the role of the N34S mutation in chronic pancreatitis.

### Table 4

**Summary of the distribution of the N34S mutation in unaffected relatives of affected individuals**

<table>
<thead>
<tr>
<th>Population</th>
<th>Unaffected individuals (families)</th>
<th>N34S</th>
<th>Total</th>
<th>Heterozygous</th>
<th>Homozygous</th>
<th>%*</th>
</tr>
</thead>
<tbody>
<tr>
<td>All ICP</td>
<td>14 (4)</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>hICP</td>
<td>5 (3)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>fICP</td>
<td>9 (1)</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>All HP</td>
<td>44 (27)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>All mtPRSS1</td>
<td>36 (22)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>PRSS1: R122H</td>
<td>16 (12)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>PRSS1: N291</td>
<td>7 (7)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>PRSS1: A16V</td>
<td>13 (3)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>All wtPRSS1</td>
<td>8 (5)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Total family members</td>
<td>58 (31)</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>All controls</td>
<td>200 (200)</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>Liverpool</td>
<td>100 (100)</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Münster</td>
<td>100 (100)</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

*Percentage of individual cases.

ICP, idiopathic chronic pancreatitis; hICP, true idiopathic chronic pancreatitis; fICP, familial idiopathic chronic pancreatitis; HP, hereditary pancreatitis; mt, mutant; wt, wild-type.

### Table 5

**Contingency table comparing the incidence of N34S mutations between different study population groups**

<table>
<thead>
<tr>
<th>Group A</th>
<th>Group B</th>
<th>p Value (Fisher’s exact test)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>All ICP affected (17/94)</td>
<td>All controls (5/200)</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>hICP affected (11/87)</td>
<td>All controls (5/200)</td>
<td>0.0013*</td>
</tr>
<tr>
<td>hICP affected (6/7)</td>
<td>All controls (5/200)</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>fICP affected (11/87)</td>
<td>HCP affected (6/7)</td>
<td>0.001*</td>
</tr>
<tr>
<td>HP affected: all (4/108)</td>
<td>HP affected: wtPRSS1 (3/27)</td>
<td>0.724</td>
</tr>
<tr>
<td>HP affected: mtPRSS1 (1/81)</td>
<td>All controls (5/200)</td>
<td>0.677</td>
</tr>
<tr>
<td>HP affected: wtPRSS1 (3/27)</td>
<td>All controls (5/200)</td>
<td>0.056</td>
</tr>
<tr>
<td>All familial pancreatitis (HP plus ICP) affected (10/115)</td>
<td>All controls (5/200)</td>
<td>0.024</td>
</tr>
<tr>
<td>Wt PRSS1 (hICP and HP) affected (9/34)</td>
<td>All controls (5/200)</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>ACP (4/67)</td>
<td>ACP (4/67)</td>
<td>0.235</td>
</tr>
<tr>
<td>ICP affected (17/94)</td>
<td>HP and ICP affected: all (2/108)</td>
<td>0.009</td>
</tr>
<tr>
<td>ICP affected (17/94)</td>
<td>HP and ICP affected: wtPRSS1 (3/27)</td>
<td>0.539</td>
</tr>
<tr>
<td>ICP affected (17/94)</td>
<td>HP and ICP affected: wtPRSS1 (1/81)</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>HP and ICP affected: mtPRSS1 (1/81)</td>
<td>All controls (5/200)</td>
<td>0.047</td>
</tr>
<tr>
<td>Wt PRSS1 (hICP and HP) affected (9/34)</td>
<td>HP and ICP unaffected (1/17)</td>
<td>0.334</td>
</tr>
<tr>
<td>HCP affected (6/7)</td>
<td>Wt PRSS1 (hICP and HP) unaffected (1/17)</td>
<td>0.135</td>
</tr>
<tr>
<td>HCP unaffected (1/9)</td>
<td>Wt PRSS1 (hICP and HP) unaffected (1/17)</td>
<td>0.0087</td>
</tr>
<tr>
<td>Liver resistance controls (4/100)</td>
<td>Münster controls (1/100)</td>
<td>0.369</td>
</tr>
</tbody>
</table>

*Statistically significant with Bonferroni’s correction.

ICP, idiopathic chronic pancreatitis; hICP, true idiopathic chronic pancreatitis; fICP, familial idiopathic chronic pancreatitis; HP, hereditary pancreatitis; ACP, alcohol related chronic pancreatitis; wt, wild-type; mt, mutant.

Values are [number of individuals with the N34S mutation/total number of individuals in the group tested]
papain activity is blocked by this inhibitor.

up to 37% of patients with ICP

and the cystic fibrosis transmembrane conductance regulator

1% in HP families with mutant PRSS1. Another possibility is

this study) that was significant compared with their controls

N34S in patients with ACP (similar to the 6% prevalence in

SPINK1

colleagues

onset or any aspect of disease severity. In contrast, Pfützer and

∼

SPINK1 might predispose to pancreatitis given that

type PRSS1 HP.

genotypic differences between patients with ICP and wild-

PRSS1 and N34S were similar (9.1%

21/24 (88%) patients with wild-type PRSS1 (p<0.05). They found

21/24 (88%) patients with wild-type PRSS1 (p<0.05). They found

v

Figure 2

The proposal that patients with chronic pancreatitis may

The N34S mutation was not associated with early disease

It seems logical to postulate that functional mutations of

It seems logical to postulate that functional mutations of

The alternative proposal that the N34S mutation may

The proposal that patients with chronic pancreatitis may

The proposal that patients with chronic pancreatitis may

populations need to be studied with respect to SPINK1 muta-

The N34S mutation was not associated with early disease

The N34S mutation was not associated with early disease

The alternative proposal that the N34S mutation may

The proposal that patients with chronic pancreatitis may

The proposal that patients with chronic pancreatitis may

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FIGURE 2 Family tree of a family with familial idiopathic chronic pancreatitis. Two (II:2 and III:3) of three affected patients and one (II:11) of

nine unaffected individuals were carriers of N34S. Age at symptom onset for the two N34S positive patients was 17 and 12 years,

respectively. The unaffected individual with N34S (II:11) was their 64-year-old mother. The third (II:4) affected individual, who was not tested

for the N34S mutation, was diagnosed with pancreatitis at the age of 12 years. No confirmed cases of pancreatitis have been recorded in
generations I or II. Two individuals on the paternal side of the family have been diagnosed with diabetes mellitus (II:6 was diagnosed at the

age of 57 years and II:10 at 30 years). Neither of these two patients has complained of abdominal pain; I:1 is now deceased and there was no

known diagnosis of pancreatitis. On the maternal side, there was no report of any symptoms consistent with pancreatic disease.
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The N34S mutation of SPINK1 (PSTI) is associated with a familial pattern of idiopathic chronic pancreatitis but does not cause the disease


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