Clinical Characterization of Patients with Hereditary Pancreatitis and Mutations in the Cationic Trypsinogen Gene

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PURPOSE: We determined the clinical manifestations of hereditary pancreatitis in nearly 30 families.

PATIENTS AND METHODS: The two trypsinogen mutations N29I and R122H were identified in a group of 550 patients with chronic pancreatitis of unclear origin. The following criteria were used to characterize the severity of chronic pancreatitis (one point each): calcifications, cysts, dilation of the pancreatic duct, diabetes, hospital treatment, and operation. Stages were defined as stage 0 (no points), stage 1 (one to two points), stage 2 (three to four points), and stage 3 (more than four points). Smoking and drinking habits were also recorded.

RESULTS: Six families with the N29I mutation (25 subjects with the mutation) and 21 families with the R122H mutation (76 subjects with the mutation) were identified. The median ages for the onset of disease were 11 years in N29I and 10 years in R122H patients. The severity of chronic pancreatitis and symptoms were similar for both mutations. About 26% (n = 26) of the 101 subjects carrying a mutation were asymptomatic, and 42% (n = 42) had mild disease (stage 1). Twenty-nine percent (n = 29) had moderate disease (stage 2), and only 4% (n = 4) had severe disease (stage 3).

CONCLUSIONS: Symptoms of patients with the N29I or R122H trypsinogen mutation were generally similar. The majority of subjects with trypsinogen mutations had mild disease or was asymptomatic.

MATERIAL AND METHODS

Nomenclature
There has been confusion concerning the nomenclature of the trypsinogen mutations because of the two different schemes (chymotrypsin and trypsin systems) in use. For clarity and in accordance with recent recommendations (22,23), only the trypsin nomenclature is used. Thus, N21I is called N29I, and R117H is called R122H.

Laboratory Techniques
We analyzed more than 550 blood samples that were sent to our referral center for analysis of cationic trypsinogen gene mutations in patients who had chronic pancreatitis of unclear origin. After obtaining informed consent, leukocyte deoxyribonucleic acid (DNA) was extracted from anticoagulated blood specimens (11) using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany). DNA was stored in Tris-EDTA buffer pH 8.0 at 4°C. Exons of the cationic trypsinogen were amplified by polymerase chain reaction (PCR). Cycling conditions consisted of an initial 2-minute denaturation step at 94°C, followed by 35 cycles at 94°C for 20 seconds, 61°C for 30 seconds, 72°C for 45 seconds, and a 7-minute elongation step at 72°C after the last cycle (24). DNA sequencing of PCR products was performed using the sense primer on an ABI 377 DNA sequencer and the ABI PRISM Dye Terminator Cycle Sequencing Reaction Kit (PE Biosystems, Weiterstadt, Germany).

After identification of trypsinogen mutations N29I or R122H in a patient’s sample, we asked respective family members to contribute to our study by sending us their blood samples and answering a questionnaire. The clinical characterization of those with trypsinogen mutations included the year of birth, sex, and the age at onset of symptoms. For clinical staging, the following parameters were recorded (one point each): documented episode of acute pancreatitis (elevated serum lipase level, or pancreatitis proven by computed tomography or sonography), calcification, cysts, dilation of the main pancreatic duct, diabetes, hospital treatment longer than 1 week because of pancreatitis (nonoperative), and operation. Chronic pancreatitis was defined as mild (stage 1) when one or two of the above criteria were present, moderate (stage 2) for three or four, and severe (stage 3) when more than four criteria were noted. Those carrying a trypsinogen mutation, but without any signs or symptoms typical of chronic pancreatitis, were classified as stage 0.

Patients were asked about their drinking and smoking habits (number of drinks per week, and age at onset of smoking and drinking). Smoking or alcohol intake was considered to be disease associated when consumption started at least 2 years before onset of symptoms. The follow-up time was defined as the time elapsed since the first symptoms of pancreatitis were noted. Statistical analysis was performed using either a chi-squared test or a nonparametric sign test. A value of P less than 0.05 was regarded as statistically significant.

RESULTS

Of more than 550 patients with suspected hereditary pancreatitis, 6 with the N29I mutation and 21 with the R122H variant were identified. A family history of chronic pancreatitis was evident in all 27 patients. We received blood samples from 52 relatives of the N29I patients and from 96 relatives of the R122H patients. A complete set of clinical data was available from a total of 25 subjects with the N29I mutation and from 76 subjects with the R122H mutation who are included in this report. Among the 75 subjects with clinically manifest pancreatitis (stages 1 to 3), the ages of onset (N29I: median, 11 years; interquartile range, 6 to 21 years; R122H: median, 10 years; interquartile range, 4 to 20 years) were similar in the two mutations. Three subjects with the N29I mutation were without any gastrointestinal symptoms, and 3 complained of occasional and uncharacteristic abdominal pain without evidence of acute or chronic pancreatitis. The median age in this group was 43 years (range, 10 to 71 years). Among those with the R122H mutation, 16 of 76 had no symptoms and 4 had slight abdominal pain without signs of pancreatitis. Their median age was 35 years (range, 7 to 73 years). The penetrance rate was therefore 75% (75 of 101). Most subjects either were without any symptoms or had a mild disease. Only a few patients had severe disease (Figure 1).

Most affected patients were symptomatic by an early age (Figure 2), usually before 30 years (Figure 3). Based on these data and the ages of the unaffected subjects with the mutation, we estimate that approximately 4 more
subjects will develop symptoms, for an estimated pen-
etrance of 78%. When patients with early-onset disease
(17 years of age or younger) were compared with those
with late-onset pancreatitis (18 years of age or older),
there were no substantial differences between the two
groups (Table 1). There was also no difference in clinical
characteristics between patients with the N29I or R122H
mutations (Table 2).

Smoking and drinking habits in the 26 patients with
late-onset disease were compared with those in symp-
tom-free adults with a trypsinogen mutation. Only those
subjects who started drinking or smoking at least 2 years
before symptoms were included. Age (median, 43 years;
interquartile range, 37 to 52 years) and alcohol intake
(median, one drink per week; interquartile range, 0 to 1
drink per week) in symptom-free adults were similar to
those in patients with late-onset pancreatitis (median age,
41 years; interquartile range, 33 to 56 years; median alco-
hol intake, one drink per week; interquartile range, 1 to 2
drinks per week). Eighteen of the 26 patients (69%) with
late-onset disease were smokers, compared with 5 of the
12 asymptomatic subjects (42%; P < 0.1).

Figure 2. Age at onset of symptoms among 75 subjects with
trypsinogen gene mutations (n = 19 with the N29I mutation;
n = 56 with the R122H mutation).

Pancreatic cancer was diagnosed in 3 patients with the
R122H mutation, a median of 23 years (range, 10 to 33
years) after the onset of pancreatitis, a rate of about 1 per
1200 person-years among affected patients.

DISCUSSION
We evaluated the clinical characteristics of patients with
hereditary pancreatitis associated with the two trypsino-
gen variants N29I and R122H, as well as those of their
relatives. Our estimate of about a 78% penetrance is con-
sistent with prior estimates of about 80% (1,8). Most sub-
jects with the mutations were asymptomatic or had mild
disease, although the scoring system that we used for
chronic pancreatitis has not been validated.

Table 1. Comparison of Patients with Early-onset and Late-
onset Hereditary Pancreatitis

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Early Onset (n = 49)</th>
<th>Late Onset (n = 26)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at onset (years)</td>
<td>6 (3–10)</td>
<td>24 (20–33)</td>
<td></td>
</tr>
<tr>
<td>Follow-up (years)</td>
<td>14 (6–29)</td>
<td>13 (5–23)</td>
<td>0.69</td>
</tr>
<tr>
<td>Severity score</td>
<td>2.2 ± 1.4</td>
<td>2.1 ± 1.3</td>
<td>0.76</td>
</tr>
<tr>
<td>Duct dilation</td>
<td>16 (33)</td>
<td>12 (31)</td>
<td>0.86</td>
</tr>
<tr>
<td>Cysts</td>
<td>12 (25)</td>
<td>3 (12)</td>
<td>0.15</td>
</tr>
<tr>
<td>Calcifications</td>
<td>17 (35)</td>
<td>5 (19)</td>
<td>0.16</td>
</tr>
<tr>
<td>Diabetes</td>
<td>11 (23)</td>
<td>9 (35)</td>
<td>0.33</td>
</tr>
<tr>
<td>Hospital treatment</td>
<td>47 (96)</td>
<td>23 (88)</td>
<td>0.08</td>
</tr>
<tr>
<td>Operation</td>
<td>16 (33)</td>
<td>9 (35)</td>
<td>0.86</td>
</tr>
</tbody>
</table>

* On a zero-point to seven-point scale.

Table 2. Comparison of Symptomatic Subjects with Mutations in the Trypsinogen Gene

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N29I Mutations (n = 19)</th>
<th>R122H Mutations (n = 56)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severity score</td>
<td>2.5 ± 1.3</td>
<td>2.3 ± 1.0</td>
<td>0.49</td>
</tr>
<tr>
<td>Age at onset (years)</td>
<td>11 (5–23)</td>
<td>11 (4–21)</td>
<td>0.80</td>
</tr>
<tr>
<td>Follow-up (years)</td>
<td>16 (5–27)</td>
<td>17 (3–28)</td>
<td>0.84</td>
</tr>
<tr>
<td>Calcifications</td>
<td>4 (21)</td>
<td>17 (30)</td>
<td>0.43</td>
</tr>
<tr>
<td>Cysts</td>
<td>5 (26)</td>
<td>11 (20)</td>
<td>0.54</td>
</tr>
<tr>
<td>Duct dilation</td>
<td>6 (32)</td>
<td>17 (30)</td>
<td>0.91</td>
</tr>
<tr>
<td>Diabetes</td>
<td>3 (16)</td>
<td>15 (27)</td>
<td>0.33</td>
</tr>
<tr>
<td>Hospital treatment</td>
<td>16 (84)</td>
<td>52 (93)</td>
<td>0.26</td>
</tr>
<tr>
<td>Operation</td>
<td>7 (37)</td>
<td>17 (30)</td>
<td>0.60</td>
</tr>
</tbody>
</table>

Figure 3. Cumulative probability of pancreatitis among pa-
tients with mutations in the trypsinogen gene.
Based on the number of relatives or affected subjects, the R122H mutation was about three to four times more common than the N291 mutation. However, symptoms of chronic pancreatitis in the two mutations were similar, in contrast to a recent report that suggests that a greater proportion of patients with the R122H mutation have severe pancreatitis (21). In that study, nine families were compared, and genetic analysis revealed that the majority of these families were related to each other. In our study, we analyzed 27 families and more than 100 subjects from different parts of Germany. No common ancestor among the families was found by questioning of the subjects, suggesting that our results may be more representative.

The age at onset of pancreatitis in our subjects suggests a bimodal distribution (Figure 2), with peaks at ages 6 years and 18 to 24 years. Although a similar pattern was observed by Sibert (4), it is difficult to draw any conclusions from our observation, as the second peak was based on only a few patients. Sibert suspected that alcohol consumption that began in puberty could lead to late-onset pancreatitis (4). In the present study, we did not find any difference in alcohol intake between patients with late-onset pancreatitis and those remaining asymptomatic. Alcohol consumption, however, was rather low (approximately one drink per week), so it remains possible that greater levels of consumption might affect the manifestations or course of the disease.

We found that more subjects with late-onset disease were smokers (compared with symptom-free subjects), although the difference was not statistically significant. This finding is consistent with recent reports in which cigarette smoking was associated with a more severe course in alcoholic or idiopathic chronic pancreatitis (25,26) and an earlier development of pancreatic cancer (27).

In contrast with an earlier study (2), a recent investigation reported that pancreatic cancer was more common in hereditary pancreatitis than in alcohol-induced chronic pancreatitis (5). We identified 3 patients, all with the R122H mutation, who died of histologically proven pancreatic cancer. This represents one case of pancreatic cancer per 1200 patient-years, a rate more than four times greater than that published recently (6).

Our major finding was that the majority of subjects with trypsinogen gene mutation N291 and R122H were free of symptoms or had only mild disease. Symptoms did not differ by the type of mutation.

ACKNOWLEDGMENT

We thank Susanne Kistner for her expert technical assistance. The help of the patients affected by the disease and their physicians are acknowledged. The following colleagues in Germany contributed to the study: R. Becker (Kempten), C. Behnen (Sögel), M. Bertele (München), R. Geyer (Rüdersdorf), K. Jobke (Rückersdorf), S. Koletzko (München), E. Pahlov-Nejad (Emsbüren), H. J. Peters (Sögel), R. Renz (Weiden), and M. Rünzi (Essen).

REFERENCES


