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. **Am J Med. 2001;111:622–626.** ©2001 by Excerpta Medica, Inc.

Since its description by Comfort and Steinberg in 1952 (1), hereditary pancreatitis, an autosomal dominant disease, has been characterized mainly by using a phenomenologic definition of the disease (2–4). Based on these data, a penetrance of 80% (2) and increased risk of pancreatic cancer (5) were described. A genetic defect was first identified by Whitcomb et al. (6), who described the R117H mutation (R122H in the trypsin nomenclature) in exon 3 of the cationic trypsinogen gene. A second mutation, called N21I (N29I in the trypsin nomenclature), on exon 2 of the same gene is also associated with the disease (7–9). Additional mutations of this gene have been described; these are only weakly associated with pancreatitis, such as A16V (10), or found only in single families, such as D22G (11) or K23R (12). In addition, mutations in other genes have been associated with familial pancreatitis (13–15).

The pathogenesis of hereditary pancreatitis was not known until Sahin-Toth et al. (16) reported a reduced rate of autolysis in the R122H trypsinogen mutation. Increased autolytic stability is also suspected to be the cause of N29I-associated disease. In contrast, there is facilitated activation of the recombinant proenzyme (17), as well as an increased hydrolysis rate of the activation peptides for D22G and K23R trypsinogens (11). A unifying concept for the pathogenesis of hereditary pancreatitis has been proposed, suggesting that increased activity of trypsin within the pancreatic acinar cell is responsible. The increased trypsin activity may be the result of increased activation of trypsinogen (trypsinogen variants D22G, K23R, and N29I), reduced autolysis of trypsin (trypsinogen variant R122H), or compromised binding of the trypsin inhibitor (15).

A characteristic feature of hereditary pancreatitis is an increased risk rate of pancreatic cancer (18), which is estimated to be 53-fold higher after the age of 50 years than in a control population (19). Hereditary pancreatitis is therefore the strongest known risk factor for pancreatic cancer, although the explanation for the increased risk is not known.

Although the genetic defects N29I and R122H were identified more than 4 years ago (6–9), a comparison of their clinical expressions has been performed in only a limited number of families (20,21). Thus, we analyzed the data from the German Registry of Hereditary Pancreatitis to identify risk factors and to correlate the clinical findings with the genetic background in these patients.

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Supported in part by Grant Ke 347/8-1 from the Deutsche Forschungsgemeinschaft.

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Manuscript submitted August 3, 2000, and accepted in revised form July 31, 2001.

0002-9343/01/$–see front matter
PII S0002-9343(01)00958-5

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.

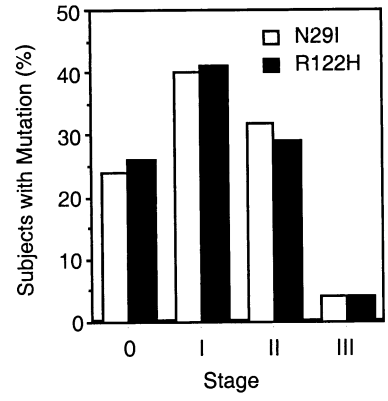

Figure 1. Stages of hereditary pancreatitis associated with the N29I (n = 25 subjects) and R122H (n = 76 subjects) mutations in the trypsinogen gene. The numbers of subjects (N29I, R122H) were stage 0 (6, 20), stage 1 (10, 32), stage 2 (8, 21), and stage 3 (1, 3).

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

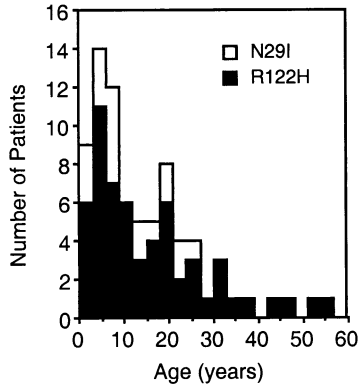

Figure 2. Age at onset of symptoms among 75 subjects with trypsinogen gene mutations (n = 19 with the N29I mutation; n = 56 with the R122H mutation).

subjects will develop symptoms, for an estimated penetrance 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 symptom-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 alcohol 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 3. Cumulative probability of pancreatitis among patients with mutations in the trypsinogen gene.

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

Characteristic	Early Onset (n = 49)	Late Onset (n = 26)	*P* Value
	Number (%), Median (Interquartile Range), or Mean ± SD		
Age at onset (years)	6 (3–10)	24 (20–33)	
Follow-up (years)	14 (6–29)	13 (5–23)	0.69
Severity score*	2.2 ± 1.4	2.1 ± 1.3	0.76
Duct dilation	16 (33)	12 (31)	0.86
Cysts	12 (25)	3 (12)	0.15
Calcifications	17 (35)	5 (19)	0.16
Diabetes	11 (23)	9 (35)	0.33
Hospital treatment	47 (96)	23 (88)	0.08
Operation	16 (33)	9 (35)	0.86

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

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 trypsinogen variants N29I and R122H, as well as those of their relatives. Our estimate of about a 78% penetrance is consistent with prior estimates of about 80% (1,8). Most subjects with the mutations were asymptomatic or had mild disease, although the scoring system that we used for chronic pancreatitis has not been validated.

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

Characteristic	N29I Mutations (n = 19)	R122H Mutations (n = 56)	*P* Value
	Number (%), Median (Interquartile Range), or Mean ± SD		
Severity score	2.5 ± 1.3	2.3 ± 1.0	0.49
Age at onset (years)	11 (5–23)	11 (4–21)	0.80
Follow-up (years)	16 (5–27)	17 (3–28)	0.84
Calcifications	4 (21)	17 (30)	0.43
Cysts	5 (26)	11 (20)	0.54
Duct dilation	6 (32)	17 (30)	0.91
Diabetes	3 (16)	15 (27)	0.33
Hospital treatment	16 (84)	52 (93)	0.26
Operation	7 (37)	17 (30)	0.60

Based on the number of relatives or affected subjects, the R122H mutation was about three to four times more common than the N29I 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 N29I 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 (Rückersdorf), K. Jobke (Rückersdorf), S. Koletzko (München), E. Pahlov-Nejad (Emsbüren), H. J. Peters (Sögel), R. Renz (Weiden), and M. Rünzi (Essen).

REFERENCES

1. Comfort MW, Steinberg AG. Pedigree of a family with hereditary chronic relapsing pancreatitis. *Gastroenterology.* 1952;21:54–63.
2. Kattwinkel J, Lapey A, Di Sant'Agnese PA, Edwards WA. Hereditary pancreatitis: three new kindreds and a critical review of the literature. *Pediatrics.* 1973;51:55–69.
3. Appel MF. Hereditary pancreatitis: Review and presentation of an additional kindred. *Arch Surg.* 1974;108:63–65.
4. Sibert JR. Hereditary pancreatitis in England and Wales. *J Med Genet.* 1978;15:189–201.
5. Lowenfels AB, Maisonneuve P, DiMagno EP, et al, and the International Hereditary Pancreatitis Study Group. Hereditary pancreatitis and the risk of pancreatic cancer. *J Natl Cancer Inst.* 1997;89:442–446.
6. Whitcomb DC, Gorry MC, Preston RA, et al. Hereditary pancreatitis is caused by a mutation in the cationic trypsinogen gene. *Nat Genet.* 1996;14:141–145.
7. Gorry MC, Gabbaizedeh D, Furey W, et al. Mutations in the cationic trypsinogen gene are associated with recurrent acute and chronic pancreatitis. *Gastroenterology.* 1997;113:1063–1068.
8. Teich N, Mössner J, Keim V. Mutations of the cationic trypsinogen in hereditary pancreatitis. *Hum Mutat.* 1998;12:39–43.
9. Nishimori I, Kamakura M, Fujikawa-Adachi K, et al. Mutations in exons 2 and 3 of the cationic trypsinogen gene in Japanese families with hereditary pancreatitis. *Gut.* 1999;44:259–263.
10. Witt H, Luck W, Becker M. A signal peptide cleavage site mutation in the cationic trypsinogen gene is strongly associated with chronic pancreatitis. *Gastroenterology.* 1999;117:7–10.
11. Teich N, Ockenga J, Hoffmeister A, et al. Chronic pancreatitis associated with an activation-peptide mutation that facilitates trypsin activation. *Gastroenterology.* 2000;119:461–465.
12. Ferec C, Raguenes O, Salomon R, et al. Mutations in the cationic trypsinogen gene and evidence for genetic heterogeneity in hereditary pancreatitis. *J Med Genet.* 1999;36:228–232.
13. Ravnik-Glavac M, Dean M, di Sant'Agnese P, et al. Evidence that hereditary pancreatitis is genetically heterogeneous disorder. *Pflügers Arch.* 2000;439(suppl):R50–R52.
14. Whitcomb D, Bartness M, Barmada MM, et al. A new hereditary pancreatitis gene maps to chromosome 12. *Digestion.* 1999;60:409.
15. Witt H, Luck W, Hennies HC, et al. Mutations in the gene encoding the serine protease inhibitor, Kazal type 1 are associated with chronic pancreatitis. *Nat Genet.* 2000;25:213–216.
16. Sahin-Toth M, Toth M. Gain-of-function mutations associated with hereditary pancreatitis enhance autoactivation of human cationic trypsinogen. *Biochem Biophys Res Commun.* 2000;278:286–289.
17. Sahin-Toth M. Human cationic trypsinogen: Role of Asn-21 in zymogen activation and implications in hereditary pancreatitis. *J Biol Chem.* 2000;275:22750–22755.
18. Lowenfels AB, Maisonneuve P, DiMagno EP, et al. Hereditary pancreatitis and the risk of pancreatic cancer: International Hereditary Pancreatitis Study Group. *J Natl Cancer Inst.* 1997;89:442–446.
19. Lowenfels AB, Maisonneuve P, Cavallini G, et al. Pancreatitis and the risk of pancreatic cancer: International Pancreatitis Study Group. *N Engl J Med.* 1993;328:1433–1437.
20. Sossenheimer MJ, Aston CE, Preston RA, et al. Clinical characteristics of hereditary pancreatitis in a large family, based on high-risk haplotype. *Am J Gastroenterol.* 1997;92:1113–1116.

21. Creighton JE, Lyall R, Wilson DI, et al. Mutations of the cationic trypsinogen gene in patients with hereditary pancreatitis. *Br J Surg.* 2000;87:170–175.
22. Antonarakis SE. Recommendations for a nomenclature system for human gene mutations: Nomenclature Working Group. *Hum Mutat.* 1998;11:1–3.
23. Teich N, Hoffmeister A, Keim V. Nomenclature of trypsinogen mutations in hereditary pancreatitis. *Hum Mutat.* 2000;15:197–198.
24. Teich N, Mössner J, Keim V. Screening for mutations of the cationic trypsinogen gene: are they of relevance in chronic alcoholic pancreatitis? *Gut.* 1999;44:413–416.
25. Imoto M, DiMagno EP. Cigarette smoking increases the risk of pancreatic calcification in late-onset but not early-onset idiopathic chronic pancreatitis. *Pancreas.* 2000;21:115–119.
26. Lin Y, Tamakoshi A, Hayakawa T, et al. Cigarette smoking as a risk factor for chronic pancreatitis: a case-control study in Japan. Research Committee on Intractable Pancreatic Diseases. *Pancreas.* 2000;21:109–114.
27. Lowenfels AB, Maisonneuve P, Whitcomb DC. Risk factors for cancer in hereditary pancreatitis: International Hereditary Pancreatitis Study Group. *Med Clin North Am.* 2000;84:565–573.