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The Course of Genetically Determined Chronic Pancreatitis

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ABSTRACT

Context The clinical course of chronic pancreatitis in patients with mutations of cationic trypsinogen and the trypsin inhibitor *SPINK1* has not yet been characterized.

Setting Cationic trypsinogen (*PRSS1*) and the serine protease inhibitor, Kazal type 1 (*SPINK1*), were analyzed in patients with pancreatitis of unclear origin.

Patients Eighty subjects with trypsinogen mutations (21x N29I, 59x R122H) and 59 patients with the *SPINK1* N34S variant (11 homozygous, 48 heterozygous) were included in the study.

Main outcome measures In patients with mutations of *PRSS1* (N29I, R122H) and *SPINK1* (N34S) the parameters such as calcification, dilatation of the main pancreatic duct, diabetes mellitus, hospital treatments, and surgery were recorded.

Design Case control studies were performed to compare both mutational groups, and the follow-up time served as a matching criterion. The Kaplan-Meier analysis was used to estimate the time course of the symptoms.

Results Ten years after the onset of the disease, the probability (±SE) of symptoms in patients with *PRSS1* mutations was as follows: 1st hospital stay: 86±4%; calcification: 21±4%; duct dilatation: 26±9%; surgery: 19±5%; diabetes: 6±5%. After 25 years, we found the following data: 1st hospital stay: 96±3%; calcification: 38±8%; duct dilatation: 38±8%; surgery: 37±10%; diabetes: 28±8%. A case-control-study of 38 pairs of patients with either *PRSS1* or *SPINK1* mutations showed that the probability of duct dilatation, diabetes and calcification was slightly higher in patients having a *SPINK1* mutation. There was no difference between those subjects with a homozygous or heterozygous *SPINK1* mutation. In comparison to alcoholic chronic pancreatitis patients, the *PRSS1* associated disease revealed a lower frequency of calcification and diabetes.

Conclusions The progression of chronic pancreatitis was slightly more rapid in patients with *SPINK1* mutations than in patients with cationic trypsinogen mutations, but was much less than in those having alcoholic chronic pancreatitis.

INTRODUCTION

Chronic pancreatitis is an inflammatory disease that may lead to the destruction of the exocrine and the endocrine tissue resulting in maldigestion and diabetes mellitus. Several large studies of its natural course described a continuous progression of individual signs and clinical symptoms such as endocrine or exocrine insufficiency, calcification and duct dilatation [1, 2, 3, 4]. Heavy alcohol

consumption represents the main cause of the disease, but the pathophysiological mechanisms by which alcohol induces chronic pancreatic inflammation are poorly understood. In about 10-30% of patients, no causal factor of pancreatitis can be identified and these patients are labeled as having idiopathic chronic pancreatitis. A previous study suggested that the extent and the progression of pancreatic dysfunction differs in various etiologies [2].

In the recent years, several genetic risk factors for chronic pancreatitis have been identified. In families with an autosomal-dominant trait, two mutations in the cationic trypsinogen (*PRSS1*), N29I and R122H, were frequently found [5, 6]. Further enzyme variants such as A16V, D22G, K23R, and R116C have been described, but their significance and inheritance pattern is not yet clear [7, 8, 9, 10, 11]. Mutations in the serine protease inhibitor, Kazal type 1 (*SPINK1*), an important pancreatic trypsin inhibitor, have been associated with idiopathic chronic [12, 13] and alcoholic chronic pancreatitis [14], and were also found in up to 50% of patients with tropical calcific pancreatitis [15, 16]. Furthermore, an increased frequency of mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene in patients with chronic pancreatitis has been described by two independent groups [17, 18] and has been confirmed by others [19, 20, 21].

In contrast to alcoholic pancreatitis, a detailed analysis of the course of inherited pancreatitis is still lacking, since the underlying molecular defects were only identified recently. Only a few studies reported clinical data on a small number of families with cationic trypsinogen mutations [22, 23, 24]. Investigating the symptom pattern in 101 *PRSS1* mutated pancreatitis subjects, the majority were either without symptoms or suffered from mild disease [25]. In contrast to the recently published smaller studies, the phenotype did not differ between patients with an N29I and an R122H mutation. These data, however, were only descriptions of the actual symptoms and did not investigate the course of the disease. To this end, we reviewed the clinical data of patients with *PRSS1* and *SPINK1* mutations and analyzed the course of their disease symptoms.

MATERIALS AND METHODS

Inclusion Criterion

Only patients in whom either one of the cationic trypsinogen mutations N29I or R122H or the *SPINK1* variant N34S was found were included in the study. Patients exhibiting one of the most frequent mutations of the CFTR gene found in Germany were excluded. Chronic pancreatitis was diagnosed when one of the following signs or symptoms was present: calcification, dilatation of the main pancreatic duct or the typical histology of chronic pancreatitis found in an operative specimen. In addition to the year of birth, at what age calcification, duct dilatation or diabetes were first noted or when surgery due to complications of pancreatitis was performed were recorded. Furthermore, hospital stays longer than 1 week due to pancreatitis were recorded. The follow-up was defined as the time elapsed after the first signs or symptoms of pancreatitis were noted. Patients and their physicians were asked and hospital reports were reviewed in order to obtain these data. In one patient, pancreatic cancer was found and this patient was excluded from the study.

Contact to Patients

The patients included in our study were selected from 780 subjects with chronic pancreatitis of unclear origin. Samples from these patients were sent to our referral centers in Leipzig and Berlin in order to carry out genetic testing for inherited pancreatitis. Common causes of pancreatitis such as alcohol consumption, anatomical abnormalities or metabolic diseases were not found.

Analysis of DNA

Leukocyte DNA was extracted from anticoagulated blood specimens. The coding regions of cationic trypsinogen were amplified by PCR and analyzed by direct DNA sequencing as described recently [7, 10]. The *SPINK1* variant N34S was detected by melting curve analysis using fluorescence resonance energy transfer (FRET) probes and the LightCycler (Roche Diagnostics, Basel, Switzerland) as described previously [12].

Case Control Studies

Two case-control studies were performed using follow-up time as a matching criterion. Pairs were regarded as appropriate when the difference in follow-up time was 2 years or less. Patients with *PRSS1* were matched with patients with *SPINK1* mutations (38 pairs) and those with a homozygous *SPINK1* N34S mutation (11 patients) were matched (1 vs. 3) with 33 heterozygous *SPINK1* N34S mutation patients.

Comparison to Alcoholic or Idiopathic Chronic Pancreatitis

Data from two recent studies with a similar follow-up time [1, 2] were used to compare the results of the present study to the results obtained from patients having alcoholic or idiopathic chronic pancreatitis.

ETHICS

Patients were asked for informed consent to document their clinical data. The study was approved by the local ethics committees of the Universities of Leipzig and Berlin.

STATISTICS

Kaplan-Meier analysis was performed to estimate the probability of symptoms in the various groups and the level of significance was evaluated by the log-rank test. Actuarial life tables were used to compare the data of the present study with those previously published. For all other parameters, non-parametric tests were conducted (Mann-Whitney, sign, and Fisher's exact test). Values were either shown as mean±SE (or mean±SD, when indicated), and, when appropriate, as median and 95% confidence intervals (95% CI). Two-tailed P values of less than 0.05 were regarded as statistically significant. Statistical analyses were performed by means of the Prism® version 3 (GraphPad Software Inc., San Diego, USA).

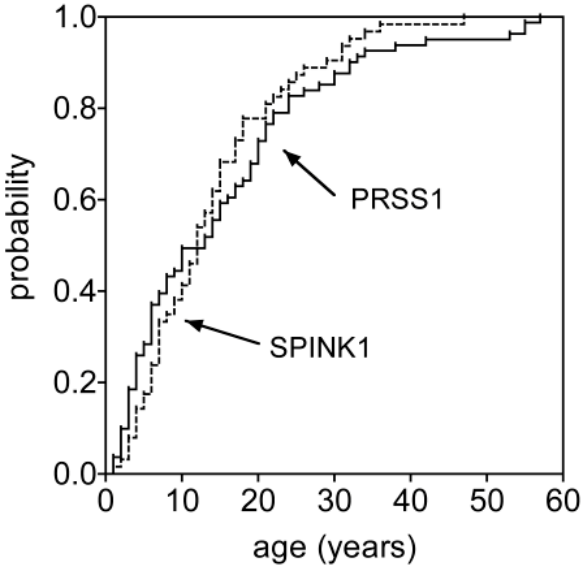

Figure 1. Probability of manifestation of chronic pancreatitis in patients with *PRSS1* mutations (N29I and R122H) and with the *SPINK1* mutation N34S depending upon age (*PRSS1*, solid line; *SPINK1*; broken line).

RESULTS

We identified a cationic trypsinogen (*PRSS1*) mutation (21x N29I, 59x R122H) in 80 patients with chronic pancreatitis and the *SPINK1* N34S variant in 59 patients (11 of whom were homozygous for the N34S allele). The median age at onset was similar in both groups (*PRSS1*: 13 years, 95% CI: 0.5-28.4; *SPINK1*: 12 years, 95% CI: 0.7-31.3; P=0.381, log rank test) (Figure 1).
As shown in Figure 2, the probability of all symptoms in *PRSS1* mutated patients increased with time. A first hospital stay due to pancreatitis was observed significantly earlier than all other recorded parameters (P<0.001, log rank test) and, after 10 years, its

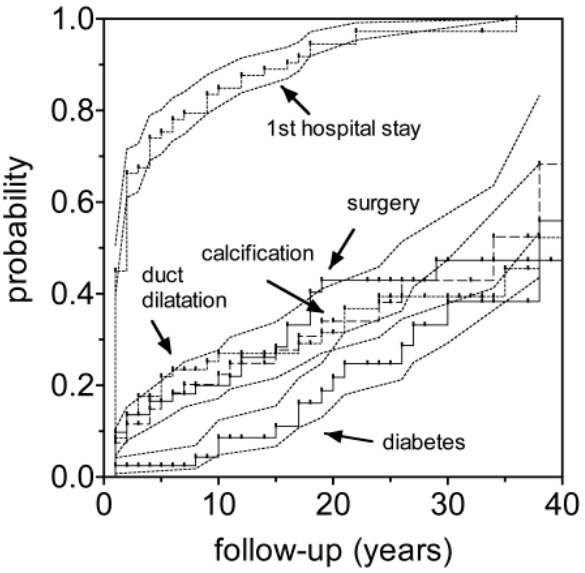

Figure 2. Course of chronic pancreatitis in patients with *PRSS1* mutations. The probability of individual symptoms was calculated according to Kaplan-Meier. 95% confidence intervals (broken lines) were shown for the parameters 1st hospital stay, calcification, and diabetes.

probability was 86±4% (mean±SE; after 25 years: 96±3%). The probability of calcification (after 10 years: 21±4%; after 25 years: 38±8%), duct dilatation (after 10 years: 26±9%; after 25 years: 38±8%) and surgery (after 10 years: 19±5%; after 25 years: 37±10%) increased in parallel. Diabetes developed slowly as, after 10 and 25 years of follow-up, its probability was only 6±5% and 28±8%, respectively (significantly different from calcification: P=0.047, log rank test).

Patients with the *SPINK1* N34S variant also showed a rapidly increasing probability of a first hospital stay and after 5 years it was 98±1% (Figure 3). The corresponding values for surgery, calcification or duct dilatations were 13±4%, 30±6%, and 42±7%, respectively. Only after more than 5 years of follow-up, did the probability of diabetes increase above baseline. Similar to patients with *PRSS1* mutations, the probability of a first hospital stay was significantly different (P<0.001; log rank test) from all other parameters and the frequency of diabetes was different than that of calcifications (P<0.001; log rank test).

The mean follow-up after disease onset in the *PRSS1* mutated group was significantly longer than in the *SPINK1* patient group (*PRSS1*: 14±14 years; *SPINK1*: 6±5 years; mean±SD; P<0.001, Mann-Whitney test). To compare both patient groups directly, a case-control study was performed using the follow-up time as a matching criterion (±2 years). From our data set 38 pairs were selected who fulfilled these conditions. In fact, the matching led to comparable groups (mean±SD follow-up: *PRSS1*: 7.4±4.9 years; *SPINK1*: 7.9±5.1 years; P=0.873, sign test) and the mean±SD age at onset (*PRSS1*; 13.8±13.9 years, *SPINK1*; 17.3±10.0 years; P=0.187, sign test) did not differ in the two groups. As shown in Fig 4a, after a follow-up of 5 years, the probability of duct dilatation in *SPINK1* (42±8%) was significantly higher than in *PRSS1* (19±8%, P=0.040, log rank test). Similarly, diabetes (Figure 4a; P=0.017, log rank test) as well as calcification (Figure 4b; P=0.017, log rank test) was significantly more frequent in *SPINK1* than in *PRSS1*. The frequency of surgery (P=0.513, log rank test) and hospital stays (P=0.237, log rank test) did not vary significantly (data not shown).

Homozygous and heterozygous N34S patients were analyzed in a further case control study. As there were many more N34S

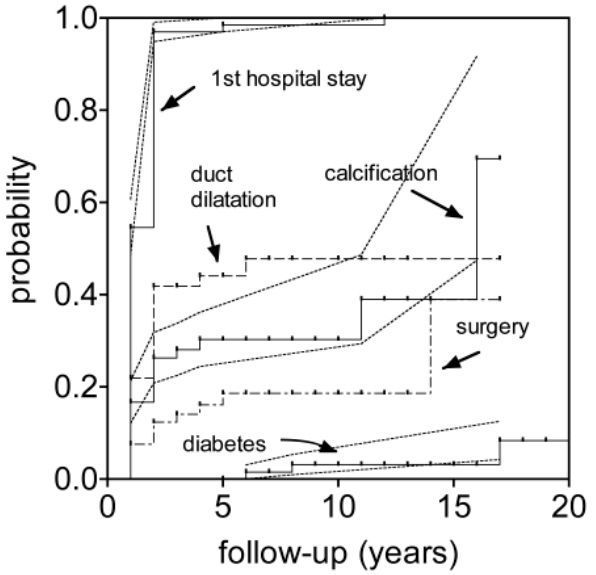

Figure 3. Course of chronic pancreatitis in patients with the *SPINK1* variant N34S. The probability of individual symptoms was calculated according to Kaplan-Meier. 95% confidence intervals (broken lines) were shown for the parameters 1st hospital stay, calcification, and diabetes.

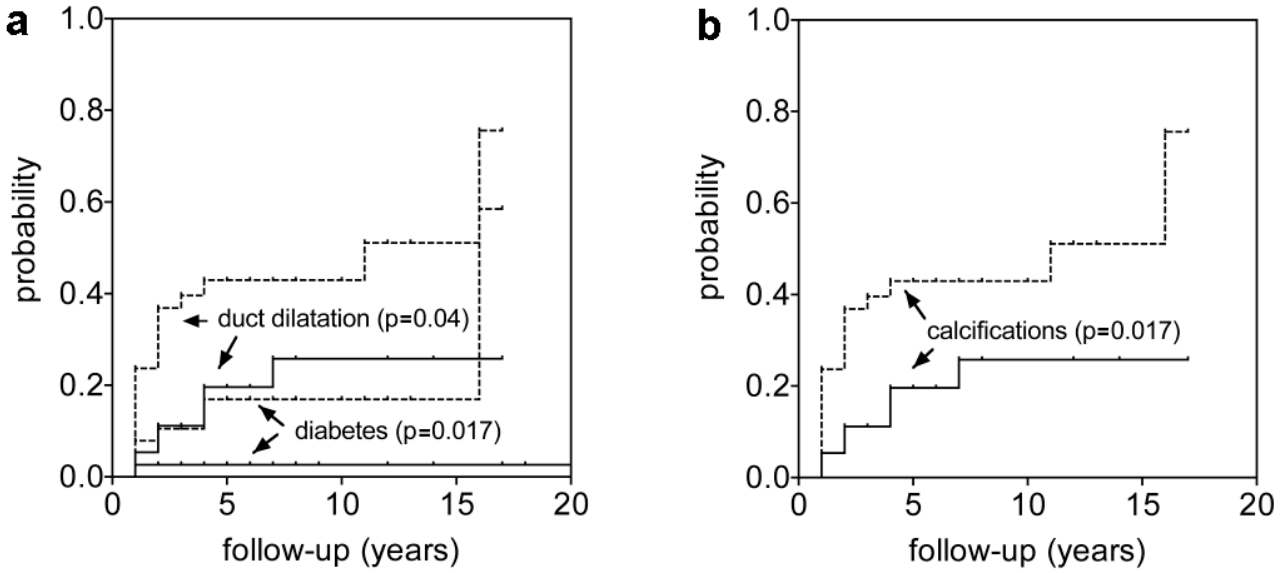

Figure 4. Comparison of the course of chronic pancreatitis associated with *PRSS1* (solid line) and *SPINK1* (broken line) mutations. Data of a case-control study with 38 pairs were analyzed by Kaplan-Meier and the log-rank-test.

heterozygotes, one homozygote N34S was matched to every 3 heterozygous patients. Table 1 shows that none of the clinical parameters differ significantly in the two groups.

A comparison of *PRSS1* associated disease to chronic pancreatitis of other origin (alcoholic, early onset idiopathic) was performed with data taken from two earlier studies [1, 2]. Progression of early onset idiopathic pancreatitis was similar to the course in trypsinogen mutated patients (Figure 5). In both independent studies of alcoholic pancreatitis, the probability of calcification and diabetes was much higher than in *PRSS1*-associated chronic pancreatitis. Although a statistical evaluation of these differences cannot be performed, the actuarial curves of alcoholic pancreatitis were clearly above the 95% confidence intervals of the *PRSS1* patients (Figure 5).

DISCUSSION

In this study, we analyzed the natural course of chronic pancreatitis associated with *PRSS1* and *SPINK1* mutations. As the mutations had only recently been identified, a retrospective study had to be performed. The principle disadvantage of such an approach is that the precision of the data may be questionable. To minimize this problem, only those patients in whom all requested clinical parameters on the course of the disease were available in written form were included (hospital recordings, technical investigations). Only those signs of chronic pancreatitis such as duct dilatation, calcification or typical histology were used as inclusion criterion which can be easily detected by standard methods. We did not evaluate the exocrine insufficiency as this parameter is not well-defined. Mainly mild or moderate insufficiency is not reliably detected

Table 1. Comparison of symptoms in patients with a homozygous or heterozygous *SPINK1* N34S mutation.

	Homozygous (n=11)	Heterozygous(n=33)	P value
Follow-up (years±SE)	5.0±2.7	5.0±2.6	0.921
Age at onset (years±SE)	11.0±5.5	13.0±9.3	0.159
Diabetes	0 (0%)	2 (6%)	1.000
Calcification	3 (27%)	9 (27%)	1.000
Duct dilatation	3 (27%)	15 (45%)	0.480
Operation	2 (18%)	7 (21%)	1.000
Hospital stay	11 (100%)	32 (97%))	1.000

Case-control study: matching criterion was: difference in follow-up time ±2 years or less.
The Mann Whitney test was used to analyze follow-up and age at onset; for the other parameters, we employed the Fisher's exact test.

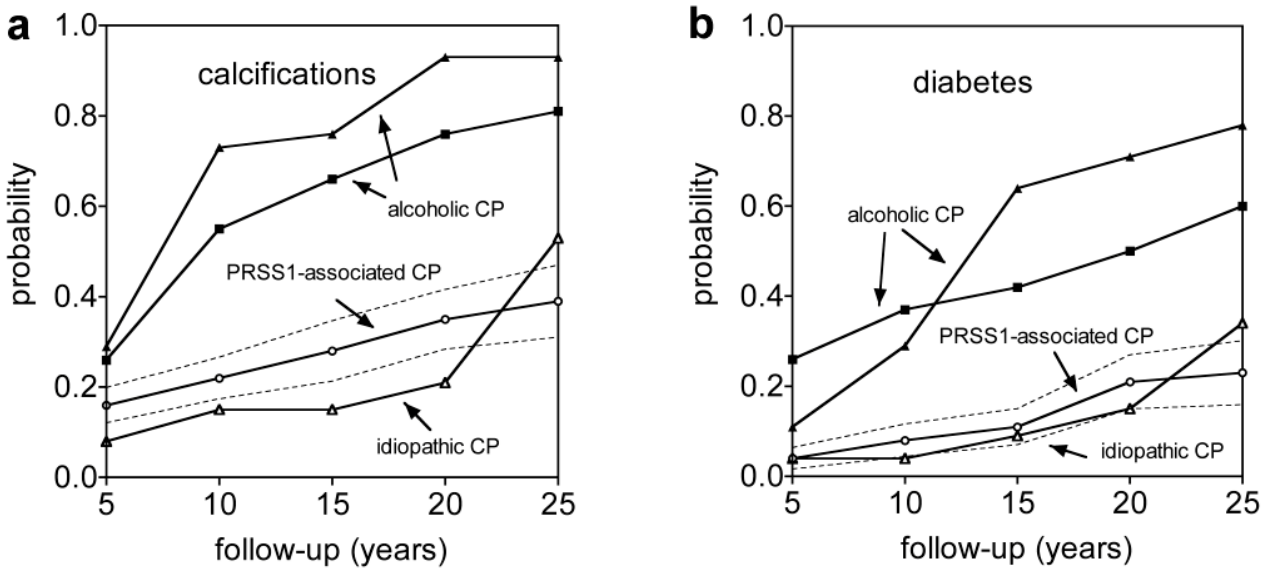

Figure 5. Comparison of alcoholic, idiopathic early-onset, and *PRSS1*-associated chronic pancreatitis (CP). Data on alcoholic and idiopathic early-onset chronic pancreatitis were taken from Ammann *et al.* [1] and Layer *et al.* [2]. The broken lines represent the 95% confidence intervals of patients with *PRSS1* mutations.
Open triangles (idiopathic pancreatitis): data from Layer *et al*. [2]
Closed triangles (alcoholic pancreatitis): data from Amman *et al*. [1]
Closed squares (alcoholic pancreatitis): data from Layer *et al*. [2]
Open circles: present study

by indirect pancreatic function tests and a secretin-cerulein test was not performed in any of the subjects. Pain was not recorded as this symptom cannot be reliably determined in a retrospective set-up.

As we have shown in a recent study [25], there were only minor differences in the clinical presentation of patients with mutations N29I or R122H of cationic trypsinogen. Consequently, the data from both mutational groups were combined for the present analysis.

In a Kaplan-Meier estimate, *PRSS1* as well as *SPINK1*-associated chronic pancreatitis both progress in 3 phases (Figures 2 and 3). The first phase of the disease consists of an initial hospital stay due to pancreatitis. This suggests a severe acute attack in the early course of genetically determined chronic pancreatitis which leads to treatment in a hospital. At this point in time, definite signs of chronic pancreatitis (duct dilatation, calcification) were found only rarely. This is in striking contrast to alcoholic pancreatitis in which a significant number of patients already display duct alterations at the initial hospital stay [26]. In genetically determined chronic pancreatitis, these definite signs of chronic pancreatitis progressively appear in the second stage of the disease which is also characterized by increased probability of a first surgery. The probability of diabetes increased late, 5-10 years from the initial symptoms.

A direct comparison of genetically determined chronic pancreatitis to other etiologies of the disease (namely, chronic alcoholic) is impaired by the different follow-up times in the respective patient groups. In two studies, however, the mean duration of observation was similar to ours in *PRSS1*-associated chronic pancreatitis (14 years), i.e. 17 years [1] or 18 years [2] in alcoholic pancreatitis or 14 years in early onset idiopathic pancreatitis [2]. This comparison revealed that both calcification and diabetes were more frequent in alcoholic pancreatitis than in idiopathic or *PRSS1*-associated pancreatitis (Figure 5). Though it is impossible to perform a statistical comparison of studies from different investigators, the actuarial curves for alcoholic pancreatitis were clearly above the 95% confidence intervals of those patients with trypsinogen

mutations. The progression of chronic alcoholic pancreatitis seems to be much faster than in the genetically determined disease.

Several observations could serve as an explanation for this finding. First, initial symptoms may be recorded more carefully in children or young adults than in alcoholics that continue to drink or even consume alcohol to "treat" their abdominal pain. As signs of chronic-alcoholic pancreatitis were already present at the first hospital stay [26], the time from the onset of the disease may be underestimated in the alcoholic group. Furthermore, disease progression as well as the development of diabetes might be accelerated as more than 50% of the patients with alcoholic pancreatitis continue to drink after diagnosis [27]. Mainly endocrine tissue is sensitive to alcohol intoxication as, after abstinence, some recovery of the insulin secretory capacity was described [27, 28]. Finally, nearly all of these alcoholics smoke heavily and this has been shown to lead to more severe disease [29, 30]. Taken together, it can be seen that, due to these co-factors, the course of alcoholic pancreatitis is more severe than the course in the genetically determined form.

The majority of our patients with *SPINK1* or *PRSS1* mutations were young, only a few smoked and none of them drank alcohol in amounts above 20 g/day. In this respect, this group is similar to those with early onset idiopathic pancreatitis [2]. Remarkably, the clinical course of diabetes and calcification in the two groups is rather similar. One may assume that in this historical group of patients with an early onset of idiopathic pancreatitis, several patients with a mutation of cationic trypsinogen or *SPINK1* may be found. By investigating more than 800 patients with chronic pancreatitis, we were able to show that, in patients without a family history, trypsinogen mutations N29I and R122H were very rare [31]. It is therefore unlikely that one of the 25 patients from Layer *et al*. [2] exhibited these trypsinogen mutations. It may be expected, however, that in approximately 20-40% of patients with early onset idiopathic chronic pancreatitis mutations of the *SPINK1* may be present [12, 13]. It is therefore not surprising that the course in patients with early onset idiopathic pancreatitis [2] and in our patients with genetically determined pancreatitis is superimposable. It has to remain open whether there are clinical differences between the patients with N34S and those without any detectable mutation. Our conclusion concerning the rapid progression of alcoholic pancreatitis, however, is not influenced by this fact.

A direct comparison of *PRSS1* and *SPINK1* patients was impossible, as the follow-up times in these two groups were significantly different. In all studies performed up to now, including our own, symptoms increase with follow-up. Therefore, it is reasonable to use this parameter as a matching criterion. In our case-control study, this allocation procedure led to two groups in which both the follow-up time as well as the age at onset of disease was similar. The probability of surgery and first hospital stays were not different in patients with *PRSS1* and with *SPINK1* mutations, whereas the frequency of diabetes, duct dilatation and calcification was significantly higher in the N34S group. This is surprising as N34S is regarded, at least by some authors, as a weak genetic risk factor. Its causal role has recently been questioned and it has been suggested that N34S might only act as a disease modifier [13]. Furthermore, approximately 1% of the general population are heterozygous N34S carriers [14]. The results are even more puzzling as the comparison of heterozygous and homozygous N34S patients revealed no phenotypic difference. Our findings show that N34S-associated chronic pancreatitis is more severe than that in patients with the trypsinogen mutations N29I or R122H. In accord with this, N34S has been found in 50% of patients with tropical calcific pancreatitis, a severe form of chronic pancreatitis characterized by a high rate of calcification and diabetes [15, 16].

In summary, our data suggest that genetically determined chronic pancreatitis progresses in 3 phases: an initial hospital stay, an intermediate stage with an increasing risk of

developing calcification, duct dilatation and undergoing surgery, and a late phase with development of diabetes. The course of *SPINK1*-associated disease was slightly more severe than in subjects with trypsinogen mutations. The comparison of the latter patients to those having alcoholic chronic pancreatitis revealed a more rapid progression in the alcohol-induced disease. Nevertheless, factors that determine manifestation or severity of genetically determined chronic pancreatitis in individual patients are as yet unknown and it will be an important task for the future to determine the role of further environmental or genetic risk factors for disease manifestation and progression.

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Abbreviations CFTR: cystic fibrosis transmembrane conductance regulator; FRET: fluorescence resonance energy transfer; *PRSS1*: cationic trypsinogen; *SPINK1*: serine protease inhibitor, Kazal type 1

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