

# Pancreatitis Risk in Primary Hyperparathyroidism: Relation to Mutations in the *SPINK1* Trypsin Inhibitor (N34S) and the Cystic Fibrosis Gene

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- OBJECTIVE:** Primary hyperparathyroidism (pHPT)-related hypercalcemia is considered to represent a risk factor for the development of pancreatitis. We therefore explored whether mutations in genes that were previously identified to increase the risk for pancreatitis coexist in a cohort of 826 patients with pHPT prospectively studied between 1987 and 2002.
- METHODS:** Among 826 patients with pHPT, 38 patients were identified with pancreatitis (4.6%). DNA was available from 25 patients (13 women/12 men, 16 acute pancreatitis/9 chronic pancreatitis). These individuals and 50 patients with pHPT without pancreatitis were analyzed for mutations in the serine protease inhibitor Kazal type I (*SPINK1*) gene (N34S) and the cationic trypsinogen gene (*PRSS1*) (N29I, R122H) by melting curve analysis and DNA sequencing. Sequence analysis of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene was carried out for the detection of 36 mutations and the Tn polymorphism.
- RESULTS:** Four of 25 patients with pHPT and pancreatitis carried the N34S missense mutation in the *SPINK1* gene (16%), while all 50 controls (pHPT without pancreatitis) showed no mutation in *SPINK1* or *PRSS1* genes ( $P < 0.05$  vs controls,  $P < 0.001$  vs general population). CF-causing *CFTR* mutations were present in four patients ( $P < 0.05$  vs general population), while one patient carried a 5T allele. One patient was transheterozygous (*SPINK1*: N34S/*CFTR*: R553X). Mean serum calcium levels in pancreatitis patients (3.1 mmol/L) did not differ significantly from the mean of the entire cohort (3.0 mmol/L) or pHPT patients without pancreatitis (3.1 mmol/L).
- CONCLUSION:** Pancreatitis risk is approximately 10-fold elevated in pHPT, but pancreatitis occurs infrequently. This indicates an existing but minor impact of pHPT-related hypercalcemia. If pancreatitis occurs, it seems associated with genetic risk factors such as mutations in the *SPINK1* and *CFTR* genes. In contrast, a combination of both hypercalcemia and genetic variants in *SPINK1* or *CFTR* increases the risk to develop pancreatitis in patients with pHPT.

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## INTRODUCTION

Despite decades of research, the pathophysiology of acute (AP) and chronic (CP) pancreatitis is still far from being completely understood. One key element in the induction of pancreatitis is altered trypsin activation that can be promoted by various factors (*i.e.*, genetic alterations). Hypercalcemia is a widely accepted risk factor for AP and CP (1). On a cellular basis it has been described that abnormal elevation of cytosolic  $\text{Ca}^{2+}$  triggers acute pancreatitis (2).

Primary hyperparathyroidism (pHPT) is associated with longstanding hypercalcemia. However, the prevalence of pancreatitis in pHPT patients varies widely (1.5–6.8%, Table 1) (3–8). Whereas most studies reported a positive association of pHPT and pancreatitis, analysis of 2 large cohorts of pHPT patients revealed a prevalence rate for pancreatitis of 1.5% that did not differ from the frequency reported in the normal population (3). These controversial results suggest that additional disease-modifying factors may be important for the development of pancreatitis in pHPT patients.

Therefore, we explored, in a large cohort of patients with clinically defined pHPT, whether there is a high prevalence

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**Table 1.** Studies Investigating the Association of pHPT-Related Hypercalcemia and Pancreatitis

Study	Years	Patients With pHPT	Pancreatitis	Prevalence	Conclusion
Bess <i>et al.</i> (3)	1950–1975	N = 1,153	N = 17	1.5%	No association
van Lanschot and Bruining (4)		N = 686	N = 10	1.5%	Association cannot be excluded
Ronni-Sivula (5)	1956–1979	N = 240	N = 8	3.3%	
Koppelberg <i>et al.</i> (6)	1987–1992	N = 234	N = 13	5.6%	Association
Carnaille <i>et al.</i> (7)		N = 1,224	N = 40	3.2%	Association
Agarwal <i>et al.</i> (8)	1991–2003	N = 87	N = 6	6.8%	Association
<b>Mean of these studies</b>		$\sum$ N = 3,624	$\sum$ N = 94	<b>X = 2.6%</b>	

of pancreatitis, and analyzed additional genetic risk factors that may promote the development of pancreatitis. Mutations in the cationic trypsinogen gene (*PRSSI*) have been shown to cause hereditary pancreatitis (9). The N34S mutation in the serine protease inhibitor Kazal type I (*SPINK1*) gene has been identified with a high prevalence in cohorts with idiopathic chronic pancreatitis (ICP) (25%), tropical pancreatitis (44%), and alcoholic pancreatitis (2%) (10–16), and mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene have also been found in patients with ICP (17–21).

Thus, the frequencies of mutations in the *PRSSI*, *SPINK1*, and *CFTR* genes were determined in patients with pHPT and pancreatitis.

## MATERIALS AND METHODS

### Subjects

Between April 1987 and December 2002, a total of 826 patients underwent surgery for pHPT in the Department of Visceral, Thoracic, and Vascular Surgery at the University-

Hospital Marburg. Patients were prospectively documented for clinical data, laboratory data, and symptoms (details are shown in Table 2). Surgical treatment of pHPT consisted of bilateral exploration and identification of all four parathyroid glands and removal of enlarged glands both in sporadic cases and in multiple endocrine neoplasia (MEN). The control group (pHPT without pancreatitis) was recruited from the same cohort and consisted of 50 patients with isolated pHPT without any signs of acute pancreatitis or episodes of abdominal pain in their medical history.

### Methods

Genomic DNA was prepared from tissue which was cryopreserved or from EDTA whole blood samples in the Department of Visceral, Thoracic, and Vascular Surgery, University-Hospital Marburg. Informed consent was obtained from all subjects and the study was approved by the Ethical Committee of the Medical Faculty, Ruhr-University of Bochum. Coded DNA samples of the patients were analyzed for *PRSSI* and *SPINK1* mutations using melting point curve analysis and DNA sequencing.

**Table 2.** Characterization of the Cohort and Patients With pHPT and Pancreatitis

	pHPT Cohort	pHPT Controls	pHPT and Pancreatitis
Patients	826	50	25
MEN syndrome	24	1	1
Age at surgery	59 ± 0.5	60 ± 13	57 ± 2.5
Sex (male/female)	245/581	25/25	12/13
Parathormone [ $<80$ pg/mL]	212 ± 11	210 ± 82	226 ± 71
Phosphate [0.8–1.5 mmol/L]	0.76 ± 0.1	0.8 ± 0.27	0.98 ± 0.15
*Calcium [2.2–2.6 mmol/L]	3 ± 0.1	3.0 ± 0.33	3.1 ± 0.1
Renal stones	371	10	15
Peptic ulcer	77	3	5
Bone tenderness	289	8	10
Pancreatitis	38	0	25
Acute	16	0	10
Acute hemorrhagic	2	0	1
Acute recurrent	6	0	5
Chronic calcificated	14	0	9
†Lipase [ $<190$ U/L]	51 ± 4	57 ± 43	227 ± 61
[Lipase in AP]			[1,593 ± 759]
Amylase [8–65 U/L]	32 ± 1	33 ± 15	143 ± 43
Alkaline phosphatase [40–190 U/L]	141 ± 9	139 ± 101	192 ± 23

Data are reported as mean ± SEM or frequencies.

\*Calcium level was accurately given by 753 patients.

†pHPT and pancreatitis have been mostly diagnosed in peripheral hospitals; presented are laboratory data from the day of admission and from the patients with acute pancreatitis [in brackets] in which lipase has been documented in the peripheral hospitals.

For *CFTR* analysis, samples were tested with two validated commercial kits (INNO-LiPA *CFTR* 19 and INNO-LiPA *CFTR* 17 Tn polymorphism, Innogenetics N.V., Ghent, Belgium), which simultaneously detect 36 mutations and the Tn polymorphism.

#### **Polymerase Chain Reaction, Melting Curve Analysis, and DNA Sequencing**

Primers flanking the designated coding regions of the *PRSSI* and *SPINK1* gene were designed according to the nucleotide sequences that have been published before (*PRSSI* GenBank #U66061, *SPINK1* GenBank #AF286028) and which have been described previously (10, 22).

Melting curve analyses for the R122H and N29I *PRSSI* and N34S *SPINK1* mutations were performed using specific pairs of fluorescence resonance energy transfer (FRET) probes synthesized by TIB MOLBIOL (Berlin, Germany) and the Light Cycler (Roche Diagnostics, Mannheim, Germany), which have also been described previously (22).

#### **Statistical Analysis**

Statistical analysis was performed using SPSS (SPSS Inc., Chicago, IL). Data concerning *SPINK1* mutations (pHPT pancreatitis patients vs pHPT controls) were compared using Fisher's exact test. *P* values lower than 0.05 were considered statistically significant. As a secondary analysis, prevalence ratios for observed versus expected frequencies (*CFTR* mutation and *SPINK1/CFTR* transheterozygous state) were compared using one-sample binominal confidence intervals and binomial tests. The expected frequency for *SPINK1* mutations was applied under the assumption that the probability of the appearance of a heterozygous N34S *SPINK1* mutation in the control group equals the probability in the normal population, which has been reported to be less than 2% (11, 23–27). For *CFTR* mutations, the expected frequency (1/25) was assumed according to the suspected frequency of CF-causing mutations (4%) in the general German population (28).

## **RESULTS**

#### **Patients With pHPT and Pancreatitis**

The pHPT databank contained 826 patients (1987–2002). Thirty-eight of them had been clinically diagnosed to have pancreatitis (4.6%) (Table 2). Thirty-seven patients with pancreatitis had a diagnosis of sporadic pHPT, while one patient had MEN. DNA was available from 25 of 38 patients. The diagnosis acute or chronic pancreatitis was made according to standard clinical criteria (AP: acute attack of abdominal pain (1), elevated blood levels of pancreatic enzymes (lipase or amylase  $\geq 3$ -fold above the upper reference level (29), abnormal diagnostic imaging (3); CP: according to the Marseilles-Rome classification (30). Three of 25 patients in whom DNA was available had a reported medical history of gallstones (only one had gallstones when pancreatitis had been diag-

nosed but a biliary origin could be excluded; two developed pancreatitis years after cholecystectomy had been performed) and two reported limited alcohol use. In respect to age, sex, calcium, and parathormone levels, the pancreatitis subgroup did not differ significantly from the entire cohort or the controls, as shown in Table 2. The follow-up of the patients with pancreatitis after successful surgical treatment of pHPT did not reveal further episodes of pancreatitis.

#### **PRSSI Mutations**

*PRSSI* gene mutations (N29I, R122H) were absent in all patients with pHPT and pancreatitis and in all control individuals (Table 3).

#### **SPINK1 Mutations**

Four of 25 patients (16%) with pHPT and pancreatitis carried a heterozygous N34S *SPINK1* mutation whereas no N34S mutation was identified in the controls ( $P < 0.05$ , Fisher's exact test). This is also significantly more than in the general German population ( $P < 0.001$ , Table 4). All 4 patients had moderate hypercalcemia comparable to the wild types (3.2 vs 3.1 mmol/L, n.s.) (Table 5). Parathormone levels were lower compared to *SPINK1* wild-type patients (123 vs 243 pg/mL) but without statistical significance. No patient carrying the N34S *SPINK1* mutation reported a family history that would support a hereditary cause of the disease, especially hereditary CP, and only one reported seldom alcohol use.

**Table 3.** Mutations Detected in the *PRSSI*, *SPINK1*, and *CFTR* Genes in 25 Patients With pHPT and Pancreatitis

Patient	<i>PRSSI</i> N29I/R122H	<i>SPINK1</i> N34S	<i>CFTR</i> Mutant	<i>CFTR</i> Poly T
1	– #	–	–	7T/7T
2	–	–	F508del	9T/7T
3	–	–	–	7T/7T
4	–	–	–	7T/7T
5	–	N34S	–	7T/7T
6	–	N34S	–	7T/7T
7	–	–	–	9T/7T
8	–	–	–	7T/7T
9	–	–	ND*	ND*
10	–	–	–	7T/7T
11	–	–	–	7T/7T
12	–	–	–	7T/5T
13	–	–	–	7T/7T
14	–	–	–	9T/7T
15	–	–	–	7T/7T
16	–	N34S	–	7T/7T
17	–	–	F508del	9T/7T
18	–	–	R553X	7T/7T
19	–	–	–	7T/7T
20	–	–	–	7T/7T
21	–	N34S	R553X	7T/7T
22	–	–	–	ND*
23	–	–	–	ND*
24	–	–	–	ND*
25	–	–	–	ND*

\*ND, not done due to absence of DNA. #, indicates two wild-type alleles.

**Table 4.** Prevalence Ratios for N34S *SPINK1* and *CFTR* Mutations in pHPT and Pancreatitis

Genotypes	Frequencies				
	*Expected	Observed	O/E ratio	95% CI	P Value
Abnormal <i>SPINK1</i> allele					
N34S	2/100	4/25	8.0	1.0–15.2	<0.001
Abnormal <i>CFTR</i> alleles					
CF <sup>sev</sup> (severe)	<sup>a1</sup> 1/25	4/24	4.2	0.4–7.9	0.002
5T allele	5/100	1/20	1.0	0.02–2.0	1.0
<i>CFTR</i> heterozygous + N34S heterozygous <sup>†</sup>	1/16,400	1/25	656	13.3–1,295.6	<0.001

\*The frequency of N34S *SPINK1* mutations reported in the normal German population is less than 2% (0.36% (10) to 1.6% (28)). The expected allele frequency of severe *CFTR* mutations was estimated <sup>a1</sup> assuming that the mean carrier frequency for CF-causing mutations is 1/25 (23). The expected frequency for the 5T allele (most common CF mutation) is 5% (41). The expected allele frequency for the N34S *SPINK1* mutation and the combination of both *CFTR* and *SPINK1* is cited according to a similar calculation made by Noone *et al.* (20) combining data from three prior studies (10, 11, 42).

<sup>†</sup>The combination of both mutations was also included in the calculations for *CFTR* and *SPINK1* frequencies, respectively.

Mean age for the first episode of pancreatitis was 48 yr. While in 3 patients, pHPT was diagnosed directly after pancreatitis occurred (2 with CP), one patient was reported to have the first episode of AP at 18 (transheterozygous patient). Both patient groups did not differ significantly in all other clinical parameters.

#### CFTR Mutations

Four of 24 patients (17%) with pHPT and pancreatitis carried a severe heterozygous *CFTR* mutation ( $P < 0.05$  compared to general population, Table 4) (DNA of one patient had run out completely after *PRSS1* and *SPINK1* testing; in another four patients, the analysis for Tn polymorphism could not be performed due to insufficient DNA amounts). One patient had a 5T allele. Laboratory results (Table 5) did not differ significantly from the controls or the wild type. As for the *SPINK1* mutational carriers, no patient carrying the *CFTR*

mutation reported a family history of cystic fibrosis or any pulmonary disorders.

#### Transheterozygous State of a Patient (*SPINK1*: N34S/*CFTR*: R553X) With pHPT and Pancreatitis

The patient with a combination of both mutations had the first episode of pancreatitis at 18 yr and although a resection of the pancreatic head had been performed at 28, the abdominal symptoms persisted. In this patient, a three-and-a-half gland resection was done due to multiglandular disease 4 yr after diagnosis of pHPT.

#### DISCUSSION

For more than two decades, the association of primary hyperparathyroidism and pancreatitis has evoked much controversy. Prevalence rates of pancreatitis in pHPT patients range

**Table 5.** Characterization of the Patients With pHPT and Pancreatitis (Mutation Carriers vs Wild Type)

	pHPT and Pancreatitis ( <i>SPINK1</i> Wild Type)	pHPT and Pancreatitis ( <i>SPINK1</i> N34S Heterozygous)	pHPT and Pancreatitis ( <i>CFTR</i> Wild Type*)	pHPT and Pancreatitis ( <i>CFTR</i> Mutation)
Patients	21	4	19	5
MEN syndrome	1	0	1	0
Age at surgery	58 ± 2	52 ± 8	59 ± 2	49 ± 8
Sex (male/female)	9/12	2/2	8/11	3/2
Parathormone [ $<80$ pg/mL]	243 ± 82	123 ± 43	245 ± 98	203 ± 53
Phosphate [0.8–1.5 mmol/L]	1 ± 0.2	0.74 ± 0.2	1 ± 0.2	0.8 ± 0.1
Calcium [2.2–2.6 mmol/L]	3.1 ± 0.1	3.2 ± 0.3	3.1 ± 0.1	3.1 ± 0.2
Renal stones	12	3	11	3
Peptic ulcer	4 <sup>‡</sup>	1	5	0
Bone tenderness	10	0	8	1
Pancreatitis	21	4	19	5
Acute	8	2	7	3
Acute hemorrhagic	1	0	1	0
Acute recurrent	5	0	5	0
Chronic calcificated	7	2	6	2
<sup>‡</sup> Lipase [ $<190$ U/L]	201 ± 65	209 ± 124	171 ± 39	325 ± 266
[AP]	[528 ± 236]	[2,236 ± 1,029]	689 ± 509]	[1,480 ± 725]
Amylase [8–65 U/L]	108 ± 27	321 ± 226	156 ± 54	103 ± 53
Alkaline phosphatase [40–190 U/L]	195 ± 25	178 ± 63	206 ± 29	144 ± 21

Data are reported as mean ± SEM or frequencies.

\*Only 24 DNA samples were available for *CFTR* analysis.

<sup>‡</sup>One patient had a duodenal ulcer.

<sup>§</sup>pHPT and pancreatitis have been mostly diagnosed in peripheral hospitals; presented are the laboratory data from the day of admission and from the patients with a documented episode of acute pancreatitis [in brackets] in which lipase has been documented in the peripheral hospitals.

between 1.5% and 6.8% (Table 1), indicating that pHPT-associated hypercalcemia may only have a minor impact on the development of pancreatitis. This prospective study comprises 826 pHPT patients recruited during a 16-yr-period. The prevalence of pancreatitis (4.6%, N = 38 patients) was similar to the rates reported in large cohorts before (3, 4, 7). While  $\text{Ca}^{2+}$  serum levels were clearly elevated in all 826 patients (3.1 mmol/L, 2.2–2.6), pancreatitis occurred in only 4.6%, indicating that hypercalcemia may only provide a background for other, possibly genetic, factors to induce pancreatitis. Nevertheless, the relative risk to develop pancreatitis is still increased approximately 10-fold compared to the normal population (31).

*PRSSI* gene mutations (*i.e.*, N29I and R122H) that alter trypsinogen activation have been identified to cause hereditary pancreatitis, but were not present in our cohort. The serine protease inhibitor Kazal type 1 (*SPINK1*) is a specific trypsin inhibitor. Although the mechanistic defect associated with the *SPINK1* N34S haplotype is unknown, carriers of this variant are thought to have a diminished capacity to inhibit trypsin that is prematurely activated within the pancreas (32, 33). There is an ongoing discussion of whether *SPINK1* mutations cause pancreatitis or only act in concert with other factors as disease modifiers (11). The latter is supported by the finding that the N34S *SPINK1* mutation has been identified in cohorts with idiopathic chronic pancreatitis, alcoholic pancreatitis, and tropical pancreatitis (10–16, 34). It is interesting that within the cohorts of idiopathic (25%) (11) and tropical (44%) (14) pancreatitis, the reported prevalence rates differ to a high degree, while they are much higher than the rates published for alcoholic pancreatitis (6.3% (16) and 9.2% (35)). *SPINK1* mutations seem to play an important role in tropical, idiopathic, and pHPT-related pancreatitis, but it has not been explained why their influence on the course of alcoholic pancreatitis seems to be rather limited. N34S *SPINK1* mutations are common in the normal population. Prevalence rates have been described to reach 1.6% in the United States (11, 16), 1.58% in France (26), and 2.6% in Finland (35), whereas they are reported to range in between 0.36% (10) and 1.6% (28) in Germany. The prevalence of 16% in our pancreatitis subgroup significantly exceeds these rates, even if the N34S prevalence rate would be as high as 2%, which has only been reported for one control cohort in Finland (35). In this context, it is interesting to note that the only patient with pHPT and pancreatitis who has ever been tested for a *SPINK1* mutation was positive for the N34S mutation (12). He was part of a group of patients named "miscellaneous" chronic pancreatitis and his result, although only a single case, additionally supports our hypothesis.

The finding that severe *CFTR* mutations were identified in four patients (17%) in this cohort highlights the relevance of additive genetic factors in patients with pHPT and pancreatitis. While it is known since 1998 that cohorts with ICP show *CFTR* mutations in up to 25% (17–21), to date it is not completely understood how heterozygous *CFTR* mutations predispose ICP patients to develop pancreatitis, particularly

with regard to the large number of variants (N > 1,500) and possible different mechanisms in protein function and processing. Noone *et al.*, who measured *CFTR*-mediated ion transport in nasal epithelia in patients with ICP, reported impaired *CFTR*-mediated  $\text{Cl}^-$  conductance in patients with a *CFTR* mutation, indicating that there might also be a pathogenetic effect in the pancreas (20). There is good evidence that most *CFTR* mutations, *e.g.*, the most common  $\Delta\text{F508}$  mutation or the 5T allele, which causes congenital bilateral absence of the vas deferens, are loss-of-function mutations. Patients with cystic fibrosis exhibit abnormal *CFTR* on epithelial cells, *e.g.*, in the pancreatic duct. The *CFTR* protein functions as an anion channel directly regulating  $\text{Cl}^-$  efflux and, indirectly,  $\text{HCO}_3^{2-}$  secretion. Furthermore, this anion secretion regulates  $\text{H}_2\text{O}$  and  $\text{Na}^+$  efflux. A loss-of-function *CFTR* mutation thus results in reduced bicarbonate and fluid secretion and may support pancreatic duct obstruction by protein plugs (36). While normal pancreatic juice is alkaline and contains large amounts of  $\text{Ca}^{2+}$  the combination of hypercalcemia and impaired pancreatic juice flow might facilitate duct obstruction by crystallization of pancreatic stones.

To date, more than 1,500 mutations have been identified in the *CFTR* gene, and even in CF cohorts or patients with chronic pancreatitis the sequencing of all exons remains an exception. Thus, there are only limited data about *CFTR* mutations in the normal population. While  $\Delta\text{508F}$  is the most common mutation, it was published that the R553X mutation, which, was identified in 2 of our 24 (8.3%) patients, is rare even in families with cystic fibrosis (~1.8%) (37).

As for *SPINK1*, it is noteworthy that the first study of patients with CP that were analyzed for *CFTR* mutations contained two patients with pHPT (18). One of them had a  $\Delta\text{F508}$  mutation. While this certainly does not strengthen our study statistically, it underlines the relevance of our hypothesis.

Synergistic genetic effects have been proposed for *SPINK1* and *CFTR* mutations and different studies detected several combinations in ICP patients (20, 21, 38–40). We are the first to study the coincidence of hypercalcemia and genetic risk factors in pHPT patients. The transheterozygous state (*SPINK1*: N34S/*CFTR*: R553X) in one patient, suffering from pHPT and pancreatitis at an early age, may indicate that a cumulation of genetic risk factors even increases the risk for pancreatitis.

Nonetheless, we have detected *SPINK1* or *CFTR* mutations (including the 5T allele) in 9 of 25 patients and more comprehensive testing would likely have detected more rare mutations. Although the majority of our patients had no genetic alteration, our results indicate a genetic background. In addition, there might be other yet-to-define genetic or environmental risk factors that promote the development of pancreatitis on the background of pHPT-related hypercalcemia.

In conclusion, our findings of an association of N34S *SPINK1* and *CFTR* mutations and pancreatitis in patients with pHPT-associated hypercalcemia support the hypothesis that hypercalcemia may only moderately increase the risk

for pancreatitis. In fact, 36% of patients in this cohort who were characterized as pHPT-related pancreatitis had hypercalcemia and a genetic background. So, other genetic factors such as the N34S *SPINK1* or *CFTR* mutations might act as disease modifiers or inducers in order to substantially increase the probability for the development of pancreatitis in pHPT. Further studies will have to confirm this result while genetic testing for *CFTR* and *SPINK1* mutations may be useful in patients with pHPT and pancreatitis.

## STUDY HIGHLIGHTS

### What Is Current Knowledge

- Primary hyperparathyroidism is associated with pancreatitis.
- Hypercalcemia is the pathophysiological mechanism.
- Serine protease inhibitor Kazal type I (*SPINK1*) and cystic fibrosis transmembrane conductance regulator (*CFTR*) mutations are known risk factors for idiopathic pancreatitis.

### What Is New Here

- Pancreatitis is rare in primary hyperparathyroidism (4.6% in this study).
- If pancreatitis occurs, there seems to be a strong association with mutations in *SPINK1* and *CFTR* (36% in this study).
- Rather than hypercalcemia alone, additive risk factors such as genetic or environmental factors seem to increase pancreatitis susceptibility in pHPT patients.
- Mutational analysis of pancreatitis-associated genes seems relevant in those patients.

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## CONFLICT OF INTEREST

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**Specific author contributions:** Peter Felderbauer: conception and experimental design of the study, data analysis, and manuscript preparation; Elias Karakas: sample collection, evaluation, and characterization of pHPT cohorts, manuscript preparation; Volker Fendrich: sample collection and characterization of pHPT cohorts; Kerem Bulut: data analysis and manuscript preparation; Tilman Horn: establishing of PCR analysis, performed PCR, and data analysis of all probes; Rainer Lebert: establishing of PCR analysis; Tim Holland-Letz: statistical consultation; Frank Schmitz: conception and manuscript preparation; Detlef Bartsch: collection of probes and characterization of pHPT cohorts; Wolfgang E. Schmidt: conception and experimental design of the study, manuscript preparation.

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