Published in final edited form as:

Hum Mutat. 2006 August ; 27(8): 721–730. doi:10.1002/humu.20343.

Mutations of Human Cationic Trypsinogen (*PRSS1*) and Chronic Pancreatitis

Niels Teich¹, Jonas Rosendahl¹, Miklós Tóth², Joachim Mössner¹, and Miklós Sahin-Tóth³

¹Medizinische Klinik und Poliklinik II, Universität Leipzig, Germany

²Department of Medical Chemistry, Pathobiochemistry and Molecular Biology; Semmelweis University, Budapest, Hungary

³Department of Molecular and Cell Biology, Boston University Goldman School of Dental Medicine, USA

Abstract

Ten years ago, the groundwork for the discovery of the genetic basis of chronic pancreatitis was laid by linkage analyses of large kindreds with autosomal dominant hereditary chronic pancreatitis. Subsequent candidate gene sequencing of the 7q35 chromosome region revealed a strong association of the c.365G>A (p.R122H) mutation of the *PRSS1* gene encoding cationic trypsinogen with hereditary pancreatitis. In the following years, further mutations of this gene were discovered in patients with hereditary or idiopathic chronic pancreatitis. In vitro the mutations increase autocatalytic conversion of trypsinogen to active trypsin and thus probably cause premature, intrapancreatic trypsinogen activation in vivo. The clinical presentation is highly variable, but most affected mutation carriers have relatively mild disease. In this review, we summarize the current knowledge on trypsinogen mutations and their role in pancreatic diseases.

Keywords

cationic trypsinogen; *PRSS1*; hereditary pancreatitis; chronic pancreatitis

INTRODUCTION

The human pancreas produces the digestive pro-enzyme trypsinogen in three highly similar isoforms. The isoenzymes are encoded by separate genes, *PRSS1* (protease, serine, 1; OMIM +276000), *PRSS2* (OMIM *601564) and *PRSS3*. On the basis of their relative isoelectric points and electrophoretic mobility, these are commonly referred to as cationic trypsinogen (*PRSS1*), anionic trypsinogen (*PRSS2*), and mesotrypsinogen (*PRSS3*). Normally, cationic trypsinogen represents circa 2/3 of total trypsinogen in the pancreatic juice, while anionic trypsinogen makes up approximately 1/3 (Guy et al., 1978; Rinderknecht et al., 1979, 1985). Mesotrypsinogen is a minor species, accounting for 2–10 % of human trypsinogens (Rinderknecht et al., 1984). Trypsinogens are synthesized as pre-pro-enzymes containing a signal peptide of 15 amino acids, followed by the 8 amino acid long pro-peptide, the trypsinogen activation peptide (TAP). The signal-peptide is removed upon entry into the endoplasmic reticulum lumen and the pro-enzymes (also called zymogens) are packaged into zymogen granules and eventually secreted into the pancreatic juice. Activation of trypsinogen

to trypsin occurs in the duodenum by the brush-border localized protease, enteropeptidase (enterokinase), which removes the TAP. Trypsin also activates trypsinogen (autoactivation), which in the duodenum may have a physiological role in facilitating zymogen activation, whereas ectopic autoactivation in the pancreas may result in pancreatitis. Genetic variants of the cationic trypsinogen (*PRSS1*) have been identified in patients with hereditary pancreatitis (OMIM #167800), familial or sporadic chronic pancreatitis. In contrast, mutations of anionic trypsinogen (*PRSS2*) or mesotrypsinogen (*PRSS3*) have not been found in association with chronic pancreatitis to date (Chen et al., 1999a; Nemoda et al., 2005). Classic hereditary pancreatitis follows an autosomal dominant inheritance pattern with incomplete penetrance and highly variable disease expression (Comfort and Steinberg, 1952; Le Bodic et al., 1996a; Sossenheimer et al., 1997; Keim et al., 2001; Howes et al., 2004). The mutations are located in three clusters within the trypsinogen sequence: in the TAP, in the N-terminal part of trypsin or in the longest peptide segment not stabilized by disulfide bonds between Cys64 and Cys139 (fig. 1). Thus, all pancreatitis-associated mutations discovered to date seem to cluster in the N-terminal half of the molecule encoded by exons 2 and 3. It is important to note, however, that investigation of the *PRSS1* gene in patients with suspected genetically determined chronic pancreatitis is restricted to these exons in most laboratories and possible C-terminal mutations may have been missed. The discovery of pancreatitis-associated cationic trypsinogen mutations in 1996 demonstrated that trypsinogen plays a central role in the pathogenesis of human pancreatitis (Whitcomb et al., 1996a). Here we review the wealth of genetic and biochemical data that has accumulated over the past ten years of research on the role of trypsinogen mutations in chronic pancreatitis.

MUTATIONS AND THEIR BIOLOGICAL RELEVANCE

The R122H and N29I mutations in *PRSS1* are commonly associated with hereditary pancreatitis

After genome wide linkage analyses, three independent groups reported an association of the hereditary pancreatitis phenotype with the long arm of chromosome 7 (Le Bodic et al., 1996b; Pandya et al., 1996; Whitcomb et al., 1996b). Within the same year, the c.365G>A mutation (p.R122H; p.R117H in the chymotrypsin numbering system) of the cationic trypsinogen gene was identified in all hereditary pancreatitis affected individuals and obligate carriers from five kindreds, but not in individuals who married into the families nor in 140 unrelated individuals (Whitcomb et al., 1996a). In the early literature, the mutation nomenclature was based on the chymotrypsin numbering commonly used by biochemists and crystallographers. The genetic numbering system, which designates the initiator methionine as position 1, was widely adopted in 2000 and has been in use since (Chen and Férec, 2000a; Teich, Hoffmeister and Keim, 2000).

R122H and increased trypsin stability

To explain why the R122H mutation might cause pancreatitis, David Whitcomb proposed that the Arg122-Val123 autolytic peptide bond in trypsin plays an important role in the degradation of prematurely activated trypsin in the pancreas. Destruction of this “failsafe mechanism” by the R122H mutation would increase intrapancreatic trypsin activity and disturb the protease-antiprotease equilibrium and eventually precipitate pancreatitis (Whitcomb et al., 1996a). Biochemical evidence supports the notion that Arg122 is important for autolysis of trypsin and mutations of this amino-acid result in increased trypsin stability (Higaki and Light 1986; Gaboriaud et al., 1996; Várallyai et al., 1998, Sahin-Tóth and Tóth, 2000). A study using cerulein-induced zymogen activation in isolated rat acini demonstrated that autodegradation of trypsin mitigates cathepsin B-mediated trypsinogen activation, suggesting that a failsafe mechanism might be indeed operational in the mammalian pancreas (Halangk et al., 2000). The Whitcomb model in its original form has remained very popular over the years; even

though more detailed biochemical analysis indicated that the R122H mutation results not only in increased trypsin stability but also in increased zymogen stability and increased autoactivation (Sahin-Tóth and Tóth, 2000, Kukor et al., 2002b). A weak trypsin-inhibitory activity associated with the Arg122 site is also lost in the R122H mutant (Kukor et al., 2002b). Thus, the pleiotropic biochemical effect of R122H raises the possibility that the pathogenic alteration is unrelated to trypsin stability. More importantly, the model fails to explain how the other pancreatitis-associated *PRSS1* mutations might work, as the majority of these do not affect trypsin stability.

N29I and enhanced trypsinogen autoactivation

A few months after the R122H mutation was reported, a second hereditary pancreatitis associated mutation was identified within the N-terminal region of the cationic trypsinogen molecule. The c.86A>T mutation (p.N29I; p.N21I according to chymotrypsin numbering) was initially found in 4 families, 2 from the USA and 2 from Germany (Gorry et al., 1997; Teich, Mössner and Keim, 1998). A different c.86A>C mutation affecting the same amino-acid (p.N29T) was also reported in 2002 (Pfützer et al., 2002). Biochemical characterization of the N29I mutation using recombinant trypsinogen found no effect on trypsin or trypsinogen stability. On the other hand, moderately increased autoactivation was observed by two independent laboratories in 4 published studies (Sahin-Tóth 2000; Sahin-Tóth and Tóth 2000; Szilágyi et al., 2001; Teich et al., 2005). The N29T mutant exhibited a phenotype similar to that of R122H, both increased trypsin stability and enhanced autoactivation were documented (Sahin-Tóth 2000). Because increased autoactivation was observed with the R122H, N29I and N29T mutations, whereas N29I had no effect on trypsin stability, the logical conclusion was put forth that enhanced autoactivation is the common pathogenic mechanism of hereditary pancreatitis associated *PRSS1* mutations (Sahin-Tóth and Tóth, 2000).

The R122H and the N29I mutations are the most common *PRSS1* mutations worldwide. They have been frequently reported from Europe, North America and Asia (Nishimori et al., 2001) and R122H was also recently found in a family of Aboriginal descent in Australia (McGaughan et al., 2004). Neither mutation was detected in two hereditary pancreatitis families from Brazil (Bernardino et al., 2002) and no hereditary pancreatitis cases have been reported from Africa. So far, no trypsinogen mutations have been found in animals. Curiously, there is a relatively high incidence of pancreatitis in miniature Schnauzer dogs. Bishop and colleagues sequenced all exons of the canine cationic trypsinogen gene in four Schnauzers but no mutation was found (Bishop et al., 2004).

Arg122 in *PRSS1* is a mutational hotspot

In addition to the originally reported frequently found R122H mutation (c.365G>A), a rare double nucleotide substitution variant was also found (c.365–366 GC>AT), which also results in the R122H mutation at the amino-acid level. The routinely used *Afl-III* restriction digestion assay for the *PRSS1* R122H mutation does not detect this variant, therefore, DNA-sequencing is recommended to screen patients from hereditary pancreatitis families (Chen et al., 2000b; Howes et al., 2001). The R122H double nucleotide substitution variant was the first convincing example of a possible gene conversion event leading to a pancreatitis-causing trypsinogen mutation (see below).

A third mutation of Arg122, c.364C>T resulting in p.R122C, was discovered in 2001 by three independent groups (LeMaréchal et al., 2001; Pfützer et al., 2002; Simon et al., 2002a) and was also found in one patient from our cohort. The recombinantly expressed R122C mutant showed a complex phenotype. *PRSS1* contains 10 cysteine residues, which form 5 disulfide bridges in the correctly folded protease. The introduction of the extra unpaired cysteine in the R122C mutant results in partial misfolding during expression and purification of the

recombinant protein. As a result, the R122C preparation exhibits reduced activity, autolysis and autoactivation. On the other hand, when the data are normalized to the active trypsinogen fraction, the phenotype becomes very similar to that of R122H, exhibiting reduced autolysis (i.e. increased trypsin stability) and increased autoactivation (Simon et al., 2002a). The low-activity phenotype of R122C spawned the theory that a loss of trypsin function caused by a mutation could cause pancreatitis by eliminating a protective trypsin-dependent mechanism. This model has been favored by Markus Lerch's group and received additional support from their studies indicating that trypsin might play a protective role in cerulein-induced zymogen activation (Halangk et al., 2000). Although this model is thought-provoking, from a genetic point-of-view no evidence exists to support it; as so far no clear loss-of-function mutations (e.g. nonsense, splice-site or frameshift) have been found in association with chronic pancreatitis. Furthermore, results from the large majority of *in vitro* experiments using recombinantly expressed trypsinogen mutants favor enhanced trypsinogen activation as the key event in the pathogenesis of genetically determined chronic pancreatitis.

In addition to Arg122, mutations were also identified in the neighboring residues, Ala121 and Val123. The c.367G>A mutation, which results in p.V123M was found in a patient with chronic pancreatitis by Jian-Min Chen and Claude Férec and their co-workers (Chen et al., 2001). The c.361G>A mutation, which results in p.A121T was detected in association with hereditary pancreatitis in a German family (Felderbauer et al., 2005). Also in the vicinity of Arg122, the c.346C>T mutation resulting in p.R116C was reported by four research groups. Arg116 is unique to human cationic trypsinogen, however, its function is unclear. Unlike Arg122, it does not appear to be important for autolysis (M. S.-T., unpublished observation). The mechanism of the "Arg122-flanking" mutations described above has not been studied yet.

Taken together, the high frequency and variability of mutations in or close to Arg122 suggests that this sequence is particularly prone to mutations, for reasons that are not readily apparent. The "mutational hot-spot" idea is consistent with the worldwide distribution of R122H and and it is also supported by the direct identification of a "de novo" R122H mutation in a German patient (Simon et al., 2002b).

Gene conversion events can result in pancreatitis-associated trypsinogen mutations

Gene conversion – the substitution of genetic material from another gene – is recognized as the underlying cause of a growing number of genetic diseases, as Gaucher disease (OMIM #230800), von Willebrand disease (OMIM #193400), polycystic kidney disease (OMIM #601313) and Shwachman-Diamond syndrome (OMIM #260400). In the majority of cases, the donor gene is a duplicated pseudogene, which accumulated mutations over time and recombination of the mutated sequences with the normal gene results in the pathogenic genotype. In contrast, there is growing evidence, that gene conversion between two functional paralogous trypsinogen genes can occur and cause genetically determined chronic pancreatitis. Trypsinogen genes are tandemly repeated within the T-cell-receptor beta (TCR-beta) locus (Rowen, Koop and Hood, 1996). This is a hot spot for gene conversion events to generate a broad variety of TCR-beta genes (Flajnik, 2002). Therefore, conversion mutations within the interpolated trypsinogen gene family are very likely. This mechanism is evident in case of the R122H mutation associated with the c.365–366 GC>AT di-nucleotide substitution (c.365_366conNM_002770.2:c.365_366), but remains speculative for the single-nucleotide substitutions causing mutations R122H (c.365G>A), N29I (c.86A>T) and A16V (c.47C>T) (Chen and Férec, 2000b; Chen and Férec, 2000c; Chen et al., 2000). Recently, we described the clinical, genetic and biochemical properties of a large gene conversion event between the anionic trypsinogen gene (donor gene) and the cationic trypsinogen gene (acceptor gene) in a six-year old girl with chronic pancreatitis. In this patient, the conversion created 22 heterozygous nucleotide changes affecting mostly exon 2 of the *PRSSI* gene (c.41-34_c.200

+236conNM_002770.2:c.41-34_c.200+236). This resulted in the N29I and N54S mutations at the amino acid level. Biochemical characterization of the N29I–N54S double mutant revealed no difference from the previously investigated N29I mutant, indicating that the N54S mutation has no additional deleterious effect. This finding provided proof for the concept, that gene conversion is a possible mechanism for the development of pancreatitis associated *PRSS1* mutations (Teich et al., 2005).

The A16V mutation is associated with idiopathic pancreatitis

In 1999, the c.47C>T mutation, which results in p.A16V was reported in 4 out of 44 pediatric patients with chronic pancreatitis (Witt, Luck and Becker, 1999). This transition gained particular interest, as it was the first indication, that trypsinogen mutations may be associated with idiopathic chronic pancreatitis. Initially, the mutation was reported in 10 percent of chronic pancreatitis patients without a family history. In subsequent European and North American studies, only a minority of patients with idiopathic chronic pancreatitis carried the *PRSS1*-A16V mutation (Chen et al., 1999b; Pfützer and Whitcomb, 1999; Chen et al., 2001; Howes et al., 2001; Truninger et al., 2001; Teich et al., 2002; Howes et al., 2004). This discrepancy might be explained by the strict selection of pediatric patients in the initial report, which decreased the effect of environmental risk factors of chronic pancreatitis to a large extent (Witt, Luck and Becker, 1999). Even though it has been reported in less than 20 families worldwide, the A16V mutation represents the 3rd most common cationic trypsinogen gene mutation. In contrast to the considerably more prevalent N29I and R122H mutations, A16V was almost exclusively detected in patients without a family history of chronic pancreatitis. Unfortunately, thorough clinical and functional characterization of this mutation is lacking so far. The A16V mutation affects the first amino acid of the activation peptide and thus forms part of the signal peptide cleavage site. It was proposed that enhanced trypsinogen activation or altered intracellular transport might be the relevant pathogenic phenotype. The reduced cellular viability of AR42J cells transfected with A16V-trypsinogen might support either model (Gaiser et al., 2005). On the other hand, a recent study using recombinant human cationic trypsinogen failed to demonstrate increased autoactivation in the A16V mutant (Király et al., 2006). Furthermore, secretion is typically not influenced by the amino-acid distal to the signal-peptide cleavage site and valine is frequently found in this position in other secretory proteins, including human mesotrypsinogen.

Rare *PRSS1* mutations

Other genetic variants of the *PRSS1* gene have been found in families with suspected hereditary pancreatitis, or in chronic pancreatitis patients without a family history (table 1). Some have been reported only once (–28delTCC, D19A, D22G, K23R, N29I+N54S, P36R, V39A, G83E, K92N, D100H, L104P, A121T, V123M, C139F) or in only a few families (N29T, E79K, R116C, R122C). Despite their limited frequency, the biochemical properties caused by some of these mutations offered interesting insights into the pathophysiology of genetically-determined chronic pancreatitis. The most interesting findings will be reviewed here.

Mutations of the trypsinogen activation peptide

The trypsinogen activation peptide (TAP) is hydrolyzed as the first step of the trypsinogen activation process. In vertebrates, this typically 8–10 amino-acid long peptide contains an unusual and highly conserved tetra-aspartate sequence, which precedes the scissile peptide bond Lys23-Ile24 (figure 1). This acidic motif serves as a specific recognition site for enteropeptidase, the physiological activator of trypsinogen. In addition, the acidic stretch was shown to contribute to the suppression of autoactivation. Interestingly, a recent study found that in human cationic trypsinogen the tetra-aspartate motif *per se* is not required for enteropeptidase recognition, whereas it is essential for maximal inhibition of autoactivation

(Nemoda and Sahin-Tóth, 2005). In addition to the A16V mutation (see above), 3 pancreatitis-associated TAP mutations have been detected to date. The c.68A>G and c.65A>G mutations (p.K23R and p.D22G, respectively) were identified in families with putative hereditary pancreatitis, while the c.56A>C (p.D19A) mutation was found in a patient with idiopathic chronic pancreatitis (Férec et al., 1999; Teich et al., 2000; Chen et al., 2003a). When analyzed using model peptides or recombinant trypsinogens, all 3 activation peptide mutations significantly increased autoactivation of cationic trypsinogen; particularly the D22G and K23R mutations, which lie closer to the trypsinogen activation site Lys23-Ile24 (Teich et al., 2000; Chen et al., 2003a). These data are consistent with earlier peptide studies showing that neutralization of any of the 4 aspartate residues in the activation peptide increases autoactivation of trypsinogen (Delaage et al., 1967; Abita, Delaage and Lazdunski, 1969; Radhakrishnan, Walsh and Neurath, 1969). This notion was also confirmed recently by Ala-scanning mutagenesis of the tetra-Asp motif in human cationic trypsinogen (Nemoda and Sahin-Tóth, 2005). The markedly increased autoactivation of the K23R mutant is explained by the preference of trypsin to cleave after Arg rather than Lys at the P1 position of substrates.

Conceptually, the activation peptide mutations are important, because they do not affect trypsin structure or function, indicating that pancreatitis-associated mutations exert their effect through altering the properties of the pro-enzyme trypsinogen and not the active enzyme trypsin. In this context, properties of the characterized mutants confirmed that increased autoactivation of cationic trypsinogen is a relevant pathomechanism in hereditary pancreatitis.

The E79K mutation

In one German family with putative hereditary pancreatitis and two patients with sporadic chronic pancreatitis from France (Chen et al., 2001) and Belgium, we detected the c.235G>A mutation, which results in p.E79K (Teich et al., 2004a). This mutation was also reported in a patient with alcoholic chronic pancreatitis and one healthy control from Brasil as well as 2 healthy subjects from France (Chen et al., 2001; Bernardino et al., 2003). The affected individuals in the German family exhibited late-onset chronic pancreatitis, suggesting that this mutation might have a relatively low penetrance (Teich et al., 2004a). The wild type and E79K mutant *PRSSI* alleles are transcribed in the pancreas to comparable levels. *In vitro* analysis of recombinant cationic trypsinogen carrying the E79K mutation revealed unaltered catalytic activity, autolysis, and inhibition by pancreatic secretory trypsin inhibitor. In contrast to previously studied mutations that increased autoactivation of cationic trypsinogen, autoactivation of E79K-trypsinogen was markedly decreased. Instead, E79K-trypsin proved to be an at least 2-fold better activator of anionic trypsinogen than wild type cationic trypsin. Thus, the E79K mutation appears to conform to the common mechanism of pancreatitis-associated mutations insofar as it leads to increased trypsinogen activation. What sets E79K apart from the other mutations is that increased trypsinogen activation is the result of transactivation of anionic trypsinogen instead of autoactivation (Teich et al., 2004a). However, it remains controversial whether the decreased autoactivation or the increased transactivation is the disease-relevant phenotype of E79K.

Can "loss of function" trypsinogen mutations protect against chronic pancreatitis?

Chen et al described the heterozygous nonsense mutation c.111C>A (p.Y37X) and the splicing site mutation IVS2+1G>A in the *PRSSI* gene of alcoholics without the development of chronic pancreatitis, but not in alcoholics with chronic pancreatitis and patients with hereditary or idiopathic chronic pancreatitis. They proposed that such "loss of function" mutations might protect against chronic pancreatitis in persons with increased pancreatitis risk (Chen et al., 2003b).

CLINICAL AND DIAGNOSTIC ASPECTS

Hereditary pancreatitis vs. idiopathic chronic pancreatitis

Autosomal dominant hereditary chronic pancreatitis was first described after long-term follow-up of 36 members from 4 generations of a family, in which 6 members were affected (Comfort and Steinberg, 1952). The investigation of such large families was the most important prerequisite for successful genetic linkage studies, which have localized the hereditary pancreatitis gene on chromosome 7 (Le Bodic et al., 1996b; Pandya et al., 1996; Whitcomb et al., 1996b). However, in the daily clinical setting establishing such extensive pedigrees is not feasible and the inheritance pattern cannot be determined in some cases. Consequently, the criteria of hereditary pancreatitis have been changing over the years and may be somewhat different in the various clinical centers. In the recently published Europac study, the diagnosis of hereditary pancreatitis 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 these strict criteria were not met, but more than one affected family members were identified, mostly within the same generation, were classified as familial chronic pancreatitis. The diagnostic value of this classification is questionable, however, and at our center we diagnose hereditary pancreatitis if the patient has no other detectable cause of chronic pancreatitis and if he/she has one first or second degree relative with proven chronic pancreatitis. Furthermore, because the *post-hoc* analysis reveals a positive or putative family history of chronic pancreatitis in almost all "idiopathic chronic pancreatitis" patients with the common R122H or N29I mutations (Creighton et al., 1999, Teich, Hoffmeister and Keim, 1999), we classify all chronic pancreatitis patients with these mutations as highly susceptible of hereditary pancreatitis, even if a family history is not readily available. Based on Jane Creighton's data, that a susceptible family history for chronic pancreatitis is found *post-hoc* in almost all patients with typical hereditary pancreatitis-associated *PRSS1* mutations, one may assume that the presence of a mutation compels clinicians to obtain a full family history *post-hoc*, whereas in the absence of such a mutation no *post-hoc* analysis of the family history is done. This may misclassify chronic pancreatitis in patients with hereditary pancreatitis as "idiopathic". Therefore, exact evaluation of anamnestic and clinical data has to precede genetic testing to avoid misclassification and unnecessary molecular investigations. In most patients with a rare or unique *PRSS1* mutation, the family size is small or not reported (Chen et al., 2001; Teich et al., 2002) and the inheritance pattern of chronic pancreatitis associated with these mutations remains uncertain (table 1).

Idiopathic chronic pancreatitis has been a traditional clinical diagnosis describing the lack of an identifiable cause behind the disease. Clearly, as more and more genes (*PRSS1*, *SPINK1*, *CFTR*) and/or mutations are identified that cause or predispose to chronic pancreatitis, the number of patients with "true idiopathic" or "familial idiopathic" (Threadgold et al., 2002) disease keeps shrinking. As recent data suggest that chronic pancreatitis may be inherited in an autosomal dominant, autosomal recessive or multigenic fashion, the differentiation between hereditary and idiopathic chronic pancreatitis also becomes obsolete.

PRSS1 mutations and incomplete penetrance of chronic pancreatitis

Hereditary pancreatitis is associated with *PRSS1* mutations in more than 50 percent of affected families. However, in hereditary pancreatitis families with the *PRSS1* mutations N29I or R122H, only 80% of the mutation carriers suffer from chronic pancreatitis. The cause of this incomplete penetrance is uncertain. In addition to further genetic factors, smoking, alcohol consumption or the lack of antioxidants were assumed as manifestation factors (Sibert 1978; Sossenheimer et al., 1997; Amann et al., 2001; Keim et al., 2001). On the basis of a study of 60 affected and 25 non-affected persons carrying the *PRSS1* mutations N29I or R122H, we

suggested that the *TNF*-238A promotor variant could be a relevant manifestation factor for chronic pancreatitis in hereditary pancreatitis-families (Beranek et al., 2003).

Clinical characteristics

As to the clinical picture, both alcoholic and *PRSS1* mutation-associated chronic pancreatitis exhibit essentially identical clinical laboratory results, histopathology or morphological changes in imaging studies. On the other hand, *PRSS1* mutation associated pancreatitis manifests typically at an earlier age and pancreatic calcification and diabetes are less frequent complications (Keim et al., 2003). In the first large investigation of clinical criteria in hereditary pancreatitis, the mean age of onset was 12.9 years (Sossenheimer et al., 1997). Our own investigations revealed no difference in the age of onset between carriers of the N29I or R122H *PRSS1* mutations. The median was 13 years in each group, respectively. Only 4% of our patients had severe chronic pancreatitis with exocrine and endocrine insufficiency, pancreatic calcification and duct dilation as well as hospitalizations due to pancreatitis. In general, half of the mutation carriers had little or no complaints or complications (Keim et al., 2001). A recent European study revealed a mean age of onset of 10 years and 14.5 years for affected carriers of the *PRSS1* mutations R122H and N29I, respectively (Howes et al., 2004), but showed no mutation-dependent differences in complications such as exocrine or endocrine insufficiency or increased pancreatic cancer risk.

Hereditary pancreatitis and pancreatic cancer

As shown in an investigation of 8 patients with pancreatic cancer in a cohort of 246 hereditary pancreatitis patients, the lifetime risk of pancreatic cancer is about 50-fold higher than in the control population and corresponds with 1 per 1066 person-years. It is only 20-fold elevated in patients with chronic alcoholic pancreatitis (Lowenfels et al., 1997; Malka et al., 2002). In our cohort of 101 hereditary pancreatitis patients, pancreatic cancer was diagnosed in 3 patients with the R122H mutation with a median of 23 years after the onset of pancreatitis. This corresponds to a rate of about 1 per 1200 person-years among affected R122H-carriers (Keim et al., 2001). However, the data basis for the estimation of the pancreatic cancer risk in patients with *PRSS1*-associated hereditary pancreatitis is small. The largest clinical investigation in the pre-genetic era, however, revealed no pancreatic cancer in 72 patients from 7 families (Sibert 1978). Taken together, the pancreatic cancer risk in hereditary pancreatitis patients with a *PRSS1* mutation seems to be elevated - with an uncertain relative risk increase. Today, there is no generally accepted protocol for screening hereditary pancreatitis patients for early pancreatic cancer. However, affected mutation carriers should be strongly advised to stop smoking, as it is an additional risk factor for pancreatic cancer (Lowenfels et al., 2000).

The *PRSS1* mutation database

Since the first description of a trypsinogen mutation in hereditary pancreatitis (Whitcomb et al., 1996a), the experimental and clinical information on genetic alterations in chronic pancreatitis has been rapidly growing resulting in a more and more complex data set. To address this issue, in early 2001 we implemented a continuously updated database, which contains all genetic variants of the *PRSS1* and *SPINK1* genes (www.uni-leipzig.de/pancreasmutation). In addition to exact genetic data, this database contains PubMed links to the clinical characterization of patients with different mutations and to in vitro studies with mutant molecules. Since 2002, this database has been part of the integrated database project of the Human Genome Variation Society (HGVS). This ambitious project fosters the discovery and characterization of genomic variations including population distribution and phenotypic associations. Backed by the HGVS, the novel WayStation has been inaugurated recently (www.centralmutations.org, Teich et al., 2004b). This is a web-accessible centralized structure for the submission, peer review, publication and release of genetic variation data.

FUTURE PROSPECTS

The large majority of *PRSSI* variants associated with chronic pancreatitis have been catalogued and the likelihood of finding novel *PRSSI* mutations that affect a significant number of patients is diminishing. Nonetheless, as most laboratories have focused their studies on exons 2 and 3, it is still possible that new variants will be identified in exons 1, 3 and 5, or in the intronic and promoter regions. From a genetic aspect, a more interesting quest in the near future will be for modifier genes that might explain the penetrance problem, i.e. why some carriers of *PRSSI* mutations remain healthy, whereas their relatives with the same mutation exhibit severe disease. Despite intensive research, the disease mechanism remains poorly understood. The biochemical alterations caused by the mutations have been mostly clarified, but the significance of these phenotypic changes in the disease remains to be determined. In this respect, future development of cellular and animal models of hereditary pancreatitis will be particularly valuable.

Acknowledgments

The authors thank Cédric Le Maréchal for providing the nationalities of patients with rare *PRSSI* mutations, as reported in table 1.

REFERENCES

- Abita JP, Delaage M, Lazdunski M. The mechanism of activation of trypsinogen. The role of the four N-terminal aspartyl residues. *Eur J Biochem* 1969;8:314–324. [PubMed: 5816755]
- Amann ST, Gates LK, Aston CE, Pandya A, Whitcomb DC. Expression and penetrance of the hereditary pancreatitis phenotype in monozygotic twins. *Gut* 2001;48:542–547. [PubMed: 11247900]
- Arduino C, Salacone P, Pasini B, Brusco A, Salmin P, Bacillo E, Robecchi A, Cestino L, Cirillo S, Regge D, Cappello N, Gaia E. Association of a new cationic trypsinogen gene mutation (V39A) with chronic pancreatitis in an Italian family. *Gut* 2005;54:1663–1664. [PubMed: 16227369]
- Beranek H, Teich N, Witt H, Schulz HU, Mössner J, Keim V. Analysis of tumor necrosis factor alpha and interleukin 10 promoter variants in patients with chronic pancreatitis. *Eur J Gastroenterol Hepatol* 2003;15:1223–1227. [PubMed: 14560157]
- Bernardino AL, Guarita DR, Mott CB, Pedroso MR, Machado MC, Laudanna AA, Tani CM, Almeida FL, Zatz M. *CFTR*, *PRSSI* and *SPINK1* mutations in the development of pancreatitis in Brazilian patients. *JOP* 2003;4:169–177. [PubMed: 14526128]
- Bishop MA, Steiner JM, Moore LE, Williams DA. Evaluation of the cationic trypsinogen gene for potential mutations in miniature schnauzers with pancreatitis. *Can J Vet Res* 2004;68:315–318. [PubMed: 15581228]
- Chen JM, Audrézet MP, Mercier B, Quéré I, Férec C. Exclusion of anionic trypsinogen and mesotrypsinogen involvement in hereditary pancreatitis without cationic trypsinogen gene mutations. *Scand J Gastroenterol* 1999a;34:831–832. [PubMed: 10499487]
- Chen JM, Raguénes O, Férec C, Deprez PH, Verellen-Dumoulin C, Andriulli A. The A16V signal peptide cleavage site mutation in the cationic trypsinogen gene and chronic pancreatitis. *Gastroenterology* 1999b;117:1508–1509. [PubMed: 10610343]
- Chen JM, Férec C. Wanted: a consensus nomenclature for cationic trypsinogen mutations. *Gastroenterology* 2000a;119:277. [PubMed: 10928834]
- Chen JM, Férec C. Molecular basis of hereditary pancreatitis. *Eur J Hum Genet* 2000b;8:437–439.
- Chen JM, Férec C. Gene conversion-like missense mutations in the human cationic trypsinogen gene and insights into the molecular evolution of the human trypsinogen family. *Mol Genet Metab* 2000c;71:463–469. [PubMed: 11073713]
- Chen JM, Raguénes O, Férec C, Deprez PH, Verellen-Dumoulin C. A CGC>CAT gene conversion-like event resulting in the R122H mutation in the cationic trypsinogen gene and its implication in the genotyping of pancreatitis. *J Med Genet* 2000;37:e36. [PubMed: 11073545]

- Chen JM, Piepoli Bis A, Le Bodic L, Ruszniewski P, Robaszkiewicz M, Deprez PH, Raguénes O, Quéré I, Andriulli A, Férec C. Mutational screening of the cationic trypsinogen gene in a large cohort of subjects with idiopathic chronic pancreatitis. *Clin Genet* 2001;59:189–193. [PubMed: 11260229]
- Chen JM, Kukor Z, Le Maréchal C, Tóth M, Tsakiris L, Raguénes O, Férec C, Sahin-Tóth M. Evolution of trypsinogen activation peptides. *Mol Biol Evol* 2003a;20:1767–1777. [PubMed: 12832630]
- Chen JM, Le Maréchal C, Lucas D, Raguénes O, Férec C. “Loss of function” mutations in the cationic trypsinogen gene (*PRSSI*) may act as a protective factor against pancreatitis. *Mol Genet Metab* 2003b;79:67–70. [PubMed: 12765848]
- Comfort MW, Steinberg AG. Pedigree of a family with hereditary chronic relapsing pancreatitis. *Gastroenterology* 1952;21:54–63. [PubMed: 14926813]
- Creighton J, Lyall R, Wilson DI, Curtis A, Charnley R. Mutations of the cationic trypsinogen gene in patients with chronic pancreatitis. *Lancet* 1999;354:42–43. [PubMed: 10406366]
- Delaage M, Desnuelle P, Lazdunski M, Bricas E, Savrda J. On the activation of trypsinogen. A study of peptide models related to the N-terminal sequence of the zymogen. *Biochem Biophys Res Commun* 1967;29:235–240. [PubMed: 6066284]
- Felderbauer P, Schnekenburger J, Lebert R, Bulut K, Werner I, Schmitz F, Domschke W, Schmidt WE. The detection of a novel PRSS1 mutation (A121T) indicates a different yet unreported phenotype in hereditary pancreatitis. *Pancreas* 2005;31:A441.
- Férec C, Raguénes O, Salomon R, Roche C, Bernard JP, Guillot M, Quéré I, Faure C, Mercier B, Audrézet MP, Guillausseau PJ, Dupont C, Munnich A, Bignon JD, Le Bodic L. Mutations in the cationic trypsinogen gene and evidence for genetic heterogeneity in hereditary pancreatitis. *J Med Genet* 1999;36:228–232. [PubMed: 10204851]
- Flajnik MF. Comparative analyses of immunoglobulin genes: surprises and portents. *Nat Rev Immunol* 2002;2:688–698. [PubMed: 12209137]
- Gaboriaud C, Serre L, Guy-Crotte O, Forest E, Fontecilla-Camps JC. Crystal structure of human trypsin 1: unexpected phosphorylation of Tyr151. *J Mol Biol* 1996;259:995–1010. [PubMed: 8683601]
- Gaiser S, Ahler A, Gundling F, Kuse ML, Savkovic V, Selig L, Teich N, Tomasini R, Dagorn JC, Mössner J, Keim V, Bödeker H. Expression of mutated cationic trypsinogen reduces cellular viability in AR4-2J cells. *Biochem Biophys Res Commun* 2005;334:721–728. [PubMed: 16036133]
- Gorry MC, Gabbazadeh D, Furey W, Gates LK Jr, Preston RA, Aston CE, Zhang Y, Ulrich C, Ehrlich GD, Whitcomb DC. Mutations in the cationic trypsinogen gene are associated with recurrent acute and chronic pancreatitis. *Gastroenterology* 1997;113:1063–1068. [PubMed: 9322498]
- Guy O, Lombardo D, Bartelt DC, Amic J, Figarella C. Two human trypsinogens. Purification, molecular properties, and N-terminal sequences. *Biochemistry* 1978;17:1669–1675. [PubMed: 656395]
- Halangk MW, Lerch MM, Brandt-Nedele B, Roth W, Ruthenburger M, Reinheckel T, Domschke W, Lippert H, Peters C, Deussing J. Role of cathepsin B in intracellular trypsinogen activation and the onset of acute pancreatitis. *J Clin Invest* 2000;106:773–781. [PubMed: 10995788]
- Higaki JN, Light A. Independent refolding of domains in the pancreatic serine proteinases. *J Biol Chem* 1986;261:10606–10609.
- Howes N, Greenhalf W, Rutherford S, O'Donnell M, Mountford R, Ellis I, Whitcomb D, Imrie C, Drumm B, Neoptolemos JP. A new polymorphism for the RI22H mutation in hereditary pancreatitis. *Gut* 2001;48:247–250. [PubMed: 11156648]
- Howes N, Lerch MM, Greenhalf W, Stocken DD, Ellis I, Simon P, Truninger K, Ammann R, Cavallini G, Charnley RM, Uomo G, Delhaye M, Spicak J, Drumm B, Jansen J, Mountford R, Whitcomb DC, Neoptolemos JP. European Registry of Hereditary Pancreatitis and Pancreatic Cancer (EUROPAC). Clinical and genetic characteristics of hereditary pancreatitis in Europe. *Clin Gastroenterol Hepatol* 2004;2:252–261. [PubMed: 15017610]
- Keim V, Bauer N, Teich N, Simon P, Lerch MM, Mössner J. Clinical characterization of patients with hereditary pancreatitis and mutations in the cationic trypsinogen gene. *Am J Med* 2001;111:622–626. [PubMed: 11755505]
- Keim V, Witt H, Bauer N, Bödeker H, Rosendahl J, Teich N, Mössner J. The course of genetically determined chronic pancreatitis. *JOP* 2003;4:146–154. [PubMed: 12853682]

- Kukor Z, Mayerle J, Krüger B, Tóth M, Steed PM, Halangk W, Lerch MM, Sahin-Tóth M. Presence of cathepsin B in the human pancreatic secretory pathway and its role in trypsinogen activation during hereditary pancreatitis. *J Biol Chem* 2002a;277:21389–21396. [PubMed: 11932257]
- Kukor Z, Tóth M, Pál G, Sahin-Tóth M. Human cationic trypsinogen. Arg(117) is the reactive site of an inhibitory surface loop that controls spontaneous zymogen activation. *J Biol Chem* 2002b;277:6111–6117. [PubMed: 11748242]
- Király O, Guan L, Szepessy E, Tóth M, Kukor Z, Sahin-Tóth M. Expression of human cationic trypsinogen with an authentic N terminus using intein-mediated splicing in aminopeptidase P (*pepP*) deficient *Escherichia coli*. *Prot Express Purif* 2006;48:104–111.
- Le Bodic L, Schnee M, Georgelin T, Soulard F, Férec C, Bignon JD, Sagniez M. An exceptional genealogy for hereditary chronic pancreatitis. *Dig Dis Sci* 1996a;41:1504–1510. [PubMed: 8689932]
- Le Bodic L, Bignon JD, Raguénes O, Mercier B, Georgelin T, Schnee M, Soulard F, Gagne K, Bonneville F, Muller JY, Bachner L, Férec C. The hereditary pancreatitis gene maps to long arm of chromosome 7. *Hum Mol Genet* 1996b;5:549–554. [PubMed: 8845851]
- Le Maréchal C, Chen JM, Quéré I, Raguénes O, Férec C, Auroux J. Discrimination of three mutational events that result in a disruption of the R122 primary autolysis site of the human cationic trypsinogen (*PRSSI*) by denaturing high performance liquid chromatography. *BMC Genet* 2001;2:19. [PubMed: 11734061]
- Lowenfels AB, Maisonneuve P, Di Magno EP, Elitsur Y, Gates LK Jr, Perrault J, Whitcomb DC. Hereditary pancreatitis and the risk of pancreatic cancer. International Hereditary Pancreatitis Study Group. *J Natl Cancer Inst* 1997;89:442–446. [PubMed: 9091646]
- 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. [PubMed: 10872414]
- Malka D, Hammel P, Maire F, Rufat P, Madeira I, Pessione F, Lévy P, Ruzsniwski P. Risk of pancreatic adenocarcinoma in chronic pancreatitis. *Gut* 2002;51:849–852. [PubMed: 12427788]
- McGaughan JM, Kimble R, Upton J, George P. Hereditary pancreatitis in a family of Aboriginal descent. *J Paediatr Child Health* 2004;40:487–489. [PubMed: 15265195]
- Nemoda Z, Sahin-Tóth M. The tetra-aspartate motif in the activation peptide of human cationic trypsinogen is essential for autoactivation control but not for enteropeptidase recognition. *J Biol Chem* 2005;280:29645–29652. [PubMed: 15970597]
- Nemoda Z, Teich N, Hugenberg C, Sahin-Tóth M. Genetic and biochemical characterization of the E32del polymorphism in human mesotrypsinogen. *Pancreatol* 2005;5:273–278. [PubMed: 15855826]
- Nishimori I, Kamakura M, Fujikawa-Adachi K, Morita M, Onishi S, Yokoyama K, Makino I, Ishida H, Yamamoto M, Watanabe S, Ogawa M. Mutations in exons 2 and 3 of the cationic trypsinogen gene in Japanese families with hereditary pancreatitis. *Gut* 1999;44:259–263. [PubMed: 9895387]
- Ochi F, Fujii M, Sakai T, Sugano M, Oshiro K, Okabayashi Y, Mita M, Kido Y. A case of acute pancreatitis associated with cationic trypsinogen N29T mutation. *Pancreas* 2003;27:199–201. [PubMed: 12883271]
- Otsuki M, Nishimori I, Hayakawa T, Hirota M, Ogawa M, Shimosegawa T. Research Committee on Intractable Disease of the Pancreas. Hereditary pancreatitis: clinical characteristics and diagnostic criteria in Japan. *Pancreas* 2004;28:200–206. [PubMed: 15028953]
- Pandya A, Blanton SH, Landa B, Javaheri R, Melvin E, Nance WE, Markello T. Linkage studies in a large kindred with hereditary pancreatitis confirms mapping of the gene to a 16-cM region on 7q. *Genomics* 1996;38:227–230. [PubMed: 8954806]
- Pho-Iam T, Thongnoppakhun W, Yenchitsomanus PT, Limwongse C. A Thai family with hereditary pancreatitis and increased cancer risk due to a mutation in *PRSS1* gene. *World J Gastroenterol* 2005;21:1634–1638.
- Pfützer RH, Whitcomb DC. Trypsinogen mutations in chronic pancreatitis. *Gastroenterology* 1999;117:1507–1508. [PubMed: 10610342]
- Pfützer RH, Myers E, Applebaum-Shapiro S, Finch R, Ellis I, Neoptolemos J, Kant JA, Whitcomb DC. Novel cationic trypsinogen (*PRSSI*) N29T and R122C mutations cause autosomal dominant hereditary pancreatitis. *Gut* 2002;50:271–272. [PubMed: 11788572]

- Radhakrishnan TM, Walsh KA, Neurath H. The promotion of activation of bovine trypsinogen by specific modification of aspartyl residues. *Biochemistry* 1969;8:4020–4027. [PubMed: 5388145]
- Rinderknecht H, Renner IG, Carmack C. Trypsinogen variants in pancreatic juice of healthy volunteers, chronic alcoholics and patients with pancreatitis and cancer of the pancreas. *Gut* 1979;20:886–891. [PubMed: 533700]
- Rinderknecht H, Renner IG, Abramson SB, Carmack C. Mesotrypsin: a new inhibitor-resistant protease from a zymogen in human pancreatic tissue and fluid. *Gastroenterology* 1984;86:681–692. [PubMed: 6698368]
- Rinderknecht H, Stace NH, Renner IG. Effects of chronic alcohol abuse on exocrine pancreatic secretion in man. *Dig Dis Sci* 1985;30:65–71. [PubMed: 3965275]
- Rowen L, Koop BF, Hood L. The complete 685-kilobase DNA sequence of the human beta T cell receptor locus. *Science* 1996;272:1755–1762. [PubMed: 8650574]
- Sahin-Tóth M. Hereditary pancreatitis-associated mutation asn(21) --> ile stabilizes rat trypsinogen in vitro. *J Biol Chem* 1999;274:29699–29704. [PubMed: 10514442]1999
- Sahin-Tóth M, Tóth M. Gain-of-function mutations associated with hereditary pancreatitis enhance autoactivation of human cationic trypsinogen. *Biochem Biophys Res Commun* 2000;278:286–289. [PubMed: 11097832]
- Sahin-Tóth M. Human cationic trypsinogen. Role of Asn-21 in zymogen activation and implications in hereditary pancreatitis. *J Biol Chem* 2000;275:22750–22755.
- Sibert JR. Hereditary pancreatitis in England and Wales. *J Med Genet* 1978;15:189–201. [PubMed: 671483]
- Simon P, Weiss FU, Sahin-Tóth M, Perry M, Nayler O, Lenfers B, Schnekenburger J, Mayerle J, Domschke W, Lerch MM. Hereditary pancreatitis caused by a novel *PRSSI* mutation (Arg-122--> Cys) that alters autoactivation and autodegradation of cationic trypsinogen. *J Biol Chem* 2002a; 277:5404–5410. [PubMed: 11719509]
- Simon P, Weiss FU, Zimmer KP, Rand S, Brinkmann B, Domschke W, Lerch MM. Spontaneous and sporadic trypsinogen mutations in idiopathic pancreatitis. *JAMA* 2002b;288:2122. [PubMed: 12413370]
- Sossenheimer MJ, Aston CE, Preston RA, Gates LK Jr, Ulrich CD, Martin SP, Zhang Y, Gorry MC, Ehrlich GD, Whitcomb DC. Clinical characteristics of hereditary pancreatitis in a large family, based on high-risk haplotype. The Midwest Multicenter Pancreatic Study Group (MMPSG). *Am J Gastroenterol* 1997;92:1113–1116. [PubMed: 9219780]
- Szilágyi L, Kénesi E, Katona G, Kaslik G, Juhász G, Gráf L. Comparative in vitro studies on native and recombinant human cationic trypsins. Cathepsin B is a possible pathological activator of trypsinogen in pancreatitis. *J Biol Chem* 2001;276:24574–24580. [PubMed: 11312265]
- Tautermann G, Ruebsamen H, Beck M, Dertinger S, Drexel H, Lohse P. R116C mutation of cationic trypsinogen in a Turkish family with recurrent pancreatitis illustrates genetic microheterogeneity of hereditary pancreatitis. *Digestion* 2001;64:226–232. [PubMed: 11842279]
- Teich N, Bauer N, Mössner J, Keim V. Mutational screening of patients with nonalcoholic chronic pancreatitis: identification of further trypsinogen variants. *Am J Gastroenterol* 2002;97:341–346. [PubMed: 11866271]
- Teich N, Bödeker H, Keim V. Cathepsin B cleavage of the trypsinogen activation peptide. *BMC Gastroenterol* 2002;2:16. [PubMed: 12102727]
- Teich N, Hoffmeister A, Keim V. Mutations of cationic trypsinogen in chronic pancreatitis. *Lancet* 1999;354:1302. [PubMed: 10520663]
- Teich N, Hoffmeister A, Keim V. Nomenclature of trypsinogen mutations in hereditary pancreatitis. *Hum Mutat* 2000;15:197–198. [PubMed: 10649497]
- Teich N, Keim V, Mössner J, Cotton RG, Horaitis O. The WayStation: the novel way for the collection of pancreatitis-associated mutations. *Pancreatol* 2004b;4:249–250. [PubMed: 15148444]
- Teich N, Le Maréchal C, Kukor Z, Caca K, Witzigmann H, Chen JM, Tóth M, Mössner J, Keim V, Férec C, Sahin-Tóth M. Interaction between trypsinogen isoforms in genetically determined pancreatitis: mutation E79K in cationic trypsin (*PRSSI*) causes increased transactivation of anionic trypsinogen (*PRSS2*). *Hum Mutat* 2004a;23:22–31. [PubMed: 14695529]

- Teich N, Mössner J, Keim V. Mutations of the cationic trypsinogen in hereditary pancreatitis. *Hum Mutat* 1998;12:39–43. [PubMed: 9633818]
- 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. [PubMed: 10026330]
- Teich N, Nemoda Z, Köhler H, Heinritz W, Mössner J, Keim V, Sahin-Tóth M. Gene conversion between functional trypsinogen genes *PRSS1* and *PRSS2* associated with chronic pancreatitis in a six-year-old girl. *Hum Mutat* 2005;25:343–347. [PubMed: 15776435]
- Teich N, Ockenga J, Hoffmeister A, Manns M, Mössner J, Keim V. Chronic pancreatitis associated with an activation peptide mutation that facilitates trypsin activation. *Gastroenterology* 2000;119:461–465. [PubMed: 10930381]
- Threadgold J, Greenhalf W, Ellis I, Howes N, Lerch MM, Simon P, Jansen J, Charnley R, Laugier R, Frulloni L, Olah A, Delhaye M, Ihse I, Schaffalitzky de Muckadell OB, Andren-Sandberg A, Imrie CW, Martinek J, Gress TM, Mountford R, Whitcomb D, Neoptolemos JP. The N34S mutation of SPINK1 (PSTI) is associated with a familial pattern of idiopathic chronic pancreatitis but does not cause the disease. *Gut* 2002;50:675–681. [PubMed: 11950815]
- Truninger K, Kock J, Wirth HP, Muellhaupt B, Arnold C, von Weizsäcker F, Seifert B, Ammann RW, Blum HE. Trypsinogen gene mutations in patients with chronic or recurrent acute pancreatitis. *Pancreas* 2001;22:18–23. [PubMed: 11138965]
- Várallyai E, Pál G, Patthy A, Szilágyi L, Gráf L. Two mutations in rat trypsin confer resistance against autolysis. *Biochem Biophys Res Commun* 1998;243:56–60. [PubMed: 9473479]
- Whitcomb DC, Gorry MC, Preston RA, Furey W, Sossenheimer MJ, Ulrich CD, Martin SP, Gates LK Jr, Amann ST, Toskes PP, Liddle R, McGrath K, Uomo G, Post JC, Ehrlich GD. Hereditary pancreatitis is caused by a mutation in the cationic trypsinogen gene. *Nat Genet* 1996a;14:141–145. [PubMed: 8841182]
- Whitcomb DC, Preston RA, Aston CE, Sossenheimer MJ, Barua PS, Zhang Y, Wong-Chong A, White GJ, Wood PG, Gates LK Jr, Ulrich C, Martin SP, Post JC, Ehrlich GD. A gene for hereditary pancreatitis maps to chromosome 7q35. *Gastroenterology* 1996b;110:1975–1980. [PubMed: 8964426]
- 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. [PubMed: 10381903]

Figure 1.

Linear map of pancreatitis-associated mutations within the primary structure of the human cationic trypsinogen. The 18 amino-acid positions affected by the pancreatitis-associated *PRSSI* variants (see Table 1) are denoted by asterisks. The positions of the most frequent N29I and R122H mutations are indicated. The gray call-out demonstrates the sequence of the trypsinogen activation peptide (TAP) and the mutations found in this region. The highly conserved tetra-aspartate motif in the activation peptide is underlined.

The pancreatitis mutation database (www.uni-leipzig.de/pancreasmutation). The sequence numbering is based on GenBank accession number U66061.1.

Table 1

*c.41-34 c.200+236conNM_002770.2:c.41-34 c.200+236		Number amino acid change		nucleotide change		localization		unrelated patients reported worldwide		disease association		first description		clinical characterisation		nationality of patients		In vitro studies	
1		p.A16V		c.1-28del/TCC		5'UTR3		1		IP		Ferec et al. 2000		Howes et al. 2004		North Africa		Gaiser et al. 2005;	
2				c.47C>T		exon 2		>10		IP		Witt, Luck and Becker, 1999				Caucasian		Király et al. 2006	
3		p.D19A		c.56A>C		exon 2		1		HP		Chen et al. 2003a		Chen et al. 2003a		French		Chen et al. 2003a;	
4		p.D22G		c.65A>G		exon 2		1		HP		Teich et al. 2000		Teich et al. 2000		Ukrainian		Nemoda and Sahin-Tóth 2005	
5		p.K23R		c.68A>G		exon 2		1		HP		Ferec et al. 1999				French		Teich et al. 2000;	
6		p.N29I+p.N54S		large conversion from anionic trypsinogen*		exon 2		1		IP		Teich et al. 2005		Teich et al. 2005		German/Italian		Teich, Bödeker and Keim 2002; Chen et al. 2003a; Gaiser et al. 2005	
7		p.N29I		c.86A>T		exon 2		>50		HP		Gorry et al. 1997		Amann et al. 2001; Keim et al. 2001; Keim et al. 2003; Howes et al. 2004; Otsuki et al. 2004		diverse		Sahin-Tóth 1999;	
8		p.N29T		c.86A>C		exon 2		1		HP		Pfützer et al. 2002		Pfützer et al. 2002; Ochi et al. 2003		diverse		Sahin-Tóth 2000;	
9		p.P36R		c.107C>G		exon 2		1		IP		Chen et al. 2001				French		Sahin-Tóth and Tóth 2000;	
10		p.Y37X		c.111C>A		exon 2		1 alcoholic without pancreatitis		IP protective?		Chen et al. 2003b				French		2000; Szilágyi et al. 2001; Kukor et al. 2002a; Teich et al., 2005; Gaiser et al. 2005	
11		p.V39A		c.116T>C		exon 2		1		HP		Arduino et al. 2005		Arduino et al. 2005		Italian		Sahin-Tóth 2000;	
12				IVS2+1G>A		intron 2		1 alcoholic without pancreatitis		HP protective?		Chen et al. 2003b				French		Kukor et al. 2002a	
13		p.E79K		c.235G>A		exon 3		4		HP, IP, AP		Chen et al. 2001		Teich et al. 2004a		diverse		Teich et al. 2004a	
14		p.G83E		c.248G>A		exon 3		1		IP		Chen et al. 2001				Italian			
15		p.K92N		c.276G>T		exon 3		1		IP		Chen et al. 2001				French			
16		p.D100H		c.298G>C		exon 3		1				Tautermann et al. 2002				German			
17		p.L104P		c.311T>C		exon 3		1		IP		Teich et al. 2002		Teich et al. 2002		German			
18		p.R116C		c.346C>T		exon 3		5		IP, HP		Le Maréchal et al. 2001		Tautermann et al. 2002; Teich et al. 2002; Pho-lam et al. 2005		diverse			
19		p.A121T		c.361G>A		exon 3		1		IP		Felderbauer et al., 2005				Caucasian		Simon et al. 2002a	
20		p.R122C		c.364C>T		exon 3		3				Le Maréchal et al. 2001		Simon et al. 2002a; Pfützer et al. 2002					

*c.41-34_c.200+236conNM_002770.2:c.41-34_c.200+236

Number	amino acid change	nucleotide change	localization	unrelated patients reported worldwide	disease association	first description	clinical characterisation	nationality of patients	In vitro studies
21	p.R122H	c.365G>A	exon 3	>100	HP	Whitcomb et al. 1996a	Sossenheimer et al. 1997; Amann et al. 2001; Keim et al. 2001; Simon et al. 2002a; Keim et al. 2003; Howes et al. 2004; Otsuki et al. 2004; McGaughan et al. 2004	diverse	Várallyay et al. 1998; Sahin-Tóth 1999; Sahin-Tóth and Tóth 2000; Kukor et al. 2002a; Kukor et al., 2002b; Gaiser et al. 2005
22	p.R122H	c.365G>A, c.366C>T	exon 3	2	IP	Chen et al. 2000		Caucasian	
23	p.V123M	c.367G>A	exon 3	1	IP	Chen et al. 2001		French	
24	p.C139F	c.416G>T	exon 3	1	IP	Teich et al. 2002b	Teich et al. 2002	German	
25	p.D162D	c.486G>T	exon 4	polymorphism		Teich et. al. 1998			
26	p.N246N	c.738C>T	exon 5	polymorphism		Teich et. al. 1998			