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Human cationic trypsinogen (*PRSS1*) variants and chronic pancreatitis

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Németh BC, Sahin-Tóth M. Human cationic trypsinogen (*PRSS1*) variants and chronic pancreatitis. *Am J Physiol Gastrointest Liver Physiol* 306: G466–G473, 2014. First published January 23, 2014; doi:10.1152/ajpgi.00419.2013.—Variations in the serine protease 1 (*PRSS1*) gene encoding human cationic trypsinogen have been conclusively associated with autosomal dominant hereditary pancreatitis and sporadic nonalcoholic chronic pancreatitis. Most high-penetrance *PRSS1* variants increase intrapancreatic trypsin activity by stimulating trypsinogen autoactivation and/or by inhibiting chymotrypsin C-dependent trypsinogen degradation. Alternatively, some *PRSS1* variants can cause trypsinogen misfolding, which results in intracellular retention and degradation with consequent endoplasmic reticulum stress. However, not all *PRSS1* variants are pathogenic, and clinical relevance of rare variants is often difficult to ascertain. Here we review the *PRSS1* variants published since 1996 and discuss their functional properties and role in chronic pancreatitis.

The PRSS1 Gene

The serine protease 1 (*PRSS1*, *PR*oteaSe *S*erine *1*) gene in humans is located on chromosome 7q35, within the T cell receptor beta locus (43). The association of trypsinogen genes with this locus is important historically, because DNA sequencing of this region in 1996 and the fortuitous finding of eight trypsinogen genes intercalated here greatly facilitated the discovery of the susceptibility gene for hereditary pancreatitis. *PRSS1* codes for human cationic trypsinogen, the precursor for the most abundant digestive enzyme secreted by the human pancreas (45). Besides *PRSS1*, the locus also contains five trypsinogen pseudogenes, a relic gene, and *PRSS2*, that encodes anionic trypsinogen, the other major human trypsinogen isoform. The *PRSS3* gene coding for mesotrypsinogen, the relatively minor third human isoform, is found on chromosome 9p13.

Copy Number Mutations in PRSS1

Heterozygous triplication and duplication of a 605-kb segment containing the trypsinogen genes on chromosome 7 was found in French patients with hereditary and sporadic idiopathic chronic pancreatitis (5, 29, 33). Presumably similar heterozygous duplications of *PRSS1* were reported in two U.S. families with hereditary pancreatitis (26). Such copy number mutations should result in higher trypsinogen expression through a gene-dosage effect, although this has not been demonstrated directly. Higher trypsinogen concentrations, in turn, would increase the likelihood of autoactivation and development of intrapancreatic trypsin activity. A unique duplication event that resulted in an extra copy of a hybrid *PRSS2/PRSS1* trypsinogen gene was also described in a French family with hereditary pancreatitis (34).

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Gene Conversions Within Trypsinogen Genes

Human trypsinogen genes exhibit a high level of sequence identity, which may facilitate gene conversion events. In fact, gene conversion was suggested as a mechanism for the evolutionary origin of the most common hereditary pancreatitis-associated mutations (6). A gene conversion event was identified in a 6-yr-old German girl with sporadic chronic pancreatitis, which replaced exon 2 in *PRSS1* with that from *PRSS2* (60). A gene duplication event in a French family with hereditary pancreatitis resulted in a similar hybrid gene, containing exons 1–2 from *PRSS2* and exons 3–4–5 from *PRSS1* (34). Since exon 1 codes for part of the signal peptide which is removed in the endoplasmic reticulum, only changes in exon 2 affect the mature trypsinogen protein. The amino acids encoded by exon 2 are nearly identical between the two isoforms, with the exception of Ile29 and Ser54 found in anionic trypsinogen (*PRSS2*). Consequently, the hybrid genes described in the German and French studies encode cationic trypsinogen with mutations p.N29I and p.N54S. Whereas the p.N54S variation is functionally innocuous (60), the p.N29I mutation causes hereditary pancreatitis.

Common Polymorphisms in PRSS1

Polymorphic variations with a population frequency >5% are relatively rare in *PRSS1*. Variant c.486C>T (p.D162= or p.D162D; dbSNP rs6666) in exon 4 and variant c.738C>T (p.N246= or p.N246N, dbSNP rs6667) in exon 5 are the only two variations within the coding region. The two variants are typically found in linkage disequilibrium and the C allele has a slightly higher frequency in Europeans (0.6), whereas it is less frequent (0.25) in subjects of Asian origin or subjects from India (0.1). No disease association has been demonstrated for either variant. Two additional common variants can be found in the 5′ region upstream of the ATG start codon: c.−204C>A (dbSNP rs4726576; C allele frequency is ~0.7 in Europeans

and ~0.4 in Asians) and c.−408C>T (dbSNP rs10273639; C allele frequency is ~0.6 in Europeans and ~0.3 in Asians). In a recent genomewide association study, variant c.–408C>T (i.e., the T allele) was demonstrated to have a small protective effect against chronic pancreatitis presumably by lowering trypsinogen expression (64). The C allele of the same variation (erroneously reported as −409C/T) was previously claimed to offer protection against pancreatic cancer in a Chinese population; however, independent confirmation is lacking (31).

PRSS1 Variants in the General Population

Published accounts indicate that sequencing the *PRSS1* gene of 200 French (7), 82 German (58), 420 Chinese (4, 68), 28 Korean (30), and 150 Brazilian (3) control subjects revealed only the p.E79K exon 3 variant in a French and a Brazilian individual. More recently, exon 3 of 1,000 healthy controls of German origin was sequenced and only the p.V123L variation was identified in a single subject (46). These observations indicate that *PRSS1* variants, other than the common polymorphisms, are exceedingly rare in the general population.

PRSS1 Mutations in Hereditary Pancreatitis

Autosomal dominant hereditary pancreatitis was first reported by Comfort and Steinberg in 1952 (10). Using genetic linkage analysis, three independent research groups localized the susceptibility gene to chromosome 7 in 1996 (27, 39, 62). In the same year, Whitcomb et al. (63) used candidate gene sequencing to identify the most common causative mutation p.R122H in *PRSS1*. Genetic heterogeneity underlying hereditary pancreatitis was soon recognized when the second most common *PRSS1* mutation, p.N29I, was described by two groups in 1997–1998 (16, 56). These two heterozygous mutations are found in ~90% of hereditary pancreatitis families worldwide, with p.R122H accounting for ~65% and p.N29I for ~25% of the cases. In the remaining 10% of the cases, *PRSS1* mutations p.A16V, p.D21A, p.D22G, p.K23R, p.K23_I24insIDK, p.N29T, p.V39A, p.R116C, and p.R122C were identified, always in the heterozygous state (2, 12, 22, 28, 40, 48, 55, 57, 65, 67). Mutations p.D21A, p.D22G, p.K23R, p.K23_I24insIDK, and p.V39A were found only in a single family each. Penetrance of *PRSS1* mutations in hereditary pancreatitis families is incomplete; it is estimated between 80 and 90% for carriers of p.R122H, although smaller pedigrees may exhibit lower apparent penetrance (21, 62, 41, 49). Note that mutations p.A16V and p.R116C exhibit variable penetrance and were also found in sporadic cases with no family history.

Hereditary pancreatitis-associated *PRSS1* mutations exert their effect via a so-called trypsin-dependent pathological pathway, which involves increased autoactivation of mutant trypsinogens resulting in elevated intrapancreatic trypsin activity. Rare mutations in the activation peptide of trypsinogen (p.D21A, p.D22G, p.K23R, and p.K23_I24insIDK) directly stimulate autoactivation (8, 15, 22, 37). In contrast, the more common hereditary pancreatitis-associated *PRSS1* mutations alter the regulation of activation and degradation of cationic trypsinogen by chymotrypsin C (CTRC), a digestive enzyme that controls trypsin levels generated through autoactivation of human trypsinogens (51) (Fig. 1). CTRC promotes degradation of trypsinogen by cleaving the Leu81-Glu82 peptide bond in the calcium binding loop and thereby attenuates trypsin levels during autoactivation (51, 52, 53). Importantly, trypsin-mediated autolytic cleavage of the Arg122-Val123 peptide bond is also required for CTRC-dependent degradation and inactivation of trypsinogen (51, 53). Paradoxically, CTRC also stimulates trypsinogen activation by processing the activation peptide at the Phe18-Asp19 peptide bond to a shorter form, which is cleaved by trypsin at a higher rate, resulting in increased autoactivation (38, 51).

PRSS1 mutations interfere with the CTRC-mediated cleavages described above and render trypsinogen resistant to degradation and/or increase processing of the activation peptide (Fig. 1). Typically, a combination of two or more effects results in the common phenotype of increased activation (51). Thus mutations p.R122H and p.R122C completely block cleavage at Arg122 by trypsin but also decrease cleavage at Leu81 by CTRC. Mutation p.N29I increases cleavage of the activation peptide and reduces cleavage both at Leu81 and Arg122. Mutations p.V39A and p.N29T decrease cleavage at Leu81 and Arg122, whereas mutation p.A16V increases processing of the activation peptide by CTRC (38, 51). Regardless of mechanistic details, the unifying biochemical phenotype of all hereditary pancreatitis-associated mutants is increased rates of autoactivation, with markedly elevated final trypsin activity levels, relative to wild-type cationic trypsinogen.

The only exception to the unifying pathological mechanism described above is mutation p.R116C, which does not change trypsinogen activation but causes misfolding, which results in intracellular aggregation and degradation and consequently reduced secretion (25). Mutation-induced misfolding can elicit endoplasmic reticulum stress, which is probably responsible for the increased disease risk in carriers of p.R116C, although the exact mechanism is unknown. Mutation p.R116C exhibits variable penetrance and is often found in sporadic cases, suggesting that the misfolding-dependent pathological pathway may confer relatively smaller risk.

PRSS1 Variants in Sporadic Nonalcoholic Chronic Pancreatitis

The first indication that *PRSS1* variants can cause chronic pancreatitis with lower penetrance came from the identification of the p.A16V variant by Witt et al. (65) in four children with chronic pancreatitis. Only one child had a positive family history consistent with hereditary pancreatitis, whereas in three children the disease was sporadic with no family history, even though inheritance from unaffected parents was demonstrated. Subsequent studies based on the EUROPAC database (18) confirmed the variable penetrance of this variant, demonstrating that p.A16V was found in six families with hereditary pancreatitis, in one family with familial (single-generation) chronic pancreatitis, and in three cases of chronic pancreatitis with no family history. The biochemical phenotype of the p.A16V explains its genetic properties: the mutation causes increased autoactivation in the presence of CTRC; however, the rate of autoactivation and the final trypsin levels attained are much lower than those observed with the highly penetrant *PRSS1* mutations such as p.R122H (51). Increased autoactivation is due to faster processing of the mutant trypsinogen activation peptide by CTRC (38, 51). As noted above, mutation

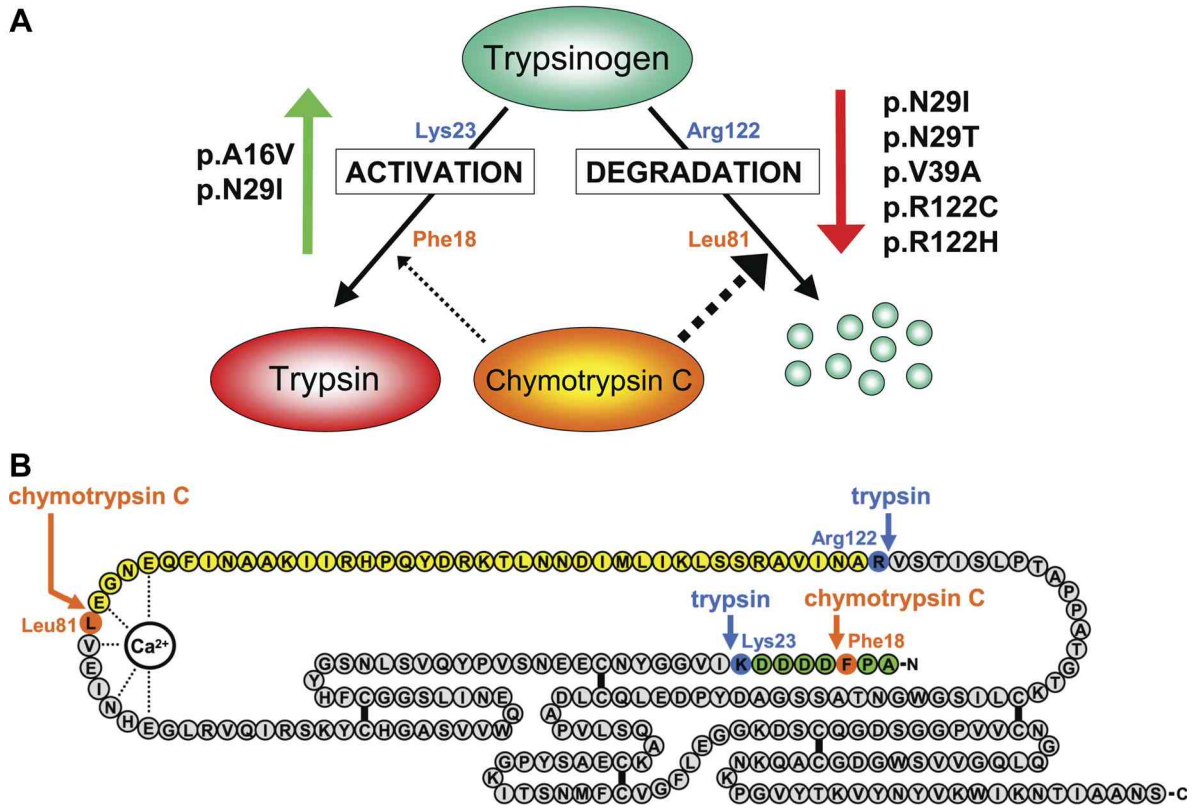

Fig. 1. Effect of pancreatitis-associated *PRSS1* mutations on the chymotrypsin C (CTRC)-dependent activation and degradation of human cationic trypsinogen. CTRC cleaves the Leu81-Glu82 peptide bond and trypsin cleaves the Arg122-Val123 peptide bond; these 2 cleavages result in the eventual degradation of trypsinogen. CTRC also stimulates autoactivation of cationic trypsinogen by cleaving the Phe18-Asp19 peptide bond in the activation peptide. The shortened activation peptide is more susceptible to trypsin-mediated activation at the Lys23-Ile24 peptide bond. The dominant effect of CTRC is degradation. *A*: *PRSS1* mutations can increase conversion of trypsinogen to trypsin by inhibition of CTRC-dependent trypsinogen degradation (red arrow) or by increasing CTRC-dependent stimulation of autoactivation (green arrow). See text for further details. *B*: proteolytic cleavage of human cationic trypsinogen by CTRC and trypsin. Primary structure of trypsinogen with disulfide bonds is shown. CTRC cleavage sites are highlighted in orange and trypsin cleavage sites are shown in blue. The activation peptide is in green. Note the yellow peptide segment not stabilized by disulfide bonds between the Leu81 and Arg122 cleavage sites. *B* is modified from Ref. 53, copyright by the National Academy of Sciences of the United States of America.

p.R116C is another example of a hereditary pancreatitis-associated mutation with variable penetrance. A recent study demonstrated that variant p.G208A was associated with ~4% of idiopathic and alcoholic chronic pancreatitis patients of Japanese origin, and increased disease risk by ~15- to 20-fold (32). This variant has no effect on trypsinogen activation but causes a moderate (~60%) reduction in trypsinogen secretion from transfected cells (46). Thus, as seen with variant p.R116C, mutation-induced misfolding and endoplasmic reticulum stress may be the pathologically relevant mechanism for variant p.G208A as well. It is also noteworthy that p.G208A is the first *PRSS1* variant for which an association with alcoholic pancreatitis has been demonstrated.

Screening of various patient populations with sporadic idiopathic chronic pancreatitis has led to the identification of a large number of rare missense variants (4, 7, 14, 23, 30, 35, 42, 55, 58). The clinical significance of such variants has been unclear because their low frequency did not allow statistical determination of genetic association with pancreatitis. Nevertheless, authors often described these as causative or pancreatitis-associated simply by analogy with well-characterized disease-relevant *PRSS1* mutations. Characterization of the functional phenotype of these variants revealed that only the activation peptide variant p.D19A increased autoactivation in a manner similar to the hereditary pancreatitis-associated activation-peptide mutations (8, 15, 37). A handful of mutants showed a marked (p.D100H, p.C139F, and p.C139S) or moderate (p.K92N and p.S124F) secretion defect, which suggested that these mutations exerted their pathogenic effect through a mechanism that involves mutation-induced misfolding and endoplasmic reticulum stress, as described above for mutations p.R116C and p.G208A (25, 46). Another subset of mutants showed no phenotypic alterations compared with wild-type trypsinogen (p.L81M, p.Q98K, p.A121T, p.T137M, and p.S181G) or suffered increased degradation by CTRC (p.P36R, p.G83E, p.I88N, and p.V123M) (46, 52, 54). These variants are likely harmless and their identification in patients with chronic pancreatitis may be accidental. One variant (p.K170E) caused slightly increased trypsinogen secretion, which, similarly to the copy number mutations, may translate to increased risk for pancreatitis (46).

Variant p.E79K should be highlighted because this is the only rare *PRSS1* variant that was found not only in patients but also in unrelated controls (3, 7, 20, 23, 41, 50, 59). Early biochemical studies demonstrated an increased propensity of this mutant to transactivate anionic trypsinogen; however, this small phenotypic change is unlikely to be of pathological consequence (59). More recent studies indicated that the rate of

Table 1. *Variants in the PRSS1 gene encoding human cationic trypsinogen*

Region	Nucleotide Change	Amino Acid Change	Number of CP Carriers Reported	Number of Non-CP Carriers Reported	Clinical Significance
	PRSS1 duplication		7		Pathogenic
	PRSS1 triplication		26	2	Pathogenic
5 prime	c.−408C>T		common polymorphism	common polymorphism	Protective
5 prime	c.−204C>A		common polymorphism	common polymorphism	Nonpathogenic
5 prime	c.−36G>A			1	Unknown
5 prime	c.−30_−28delTCC		1		Unknown
intron 1	c.40+1G>A			1	Protective
intron 1	c.40+40delC		1		Unknown
intron 1	c.41–49C>T		1		Unknown
exon 2	c.47C>T	p.A16V	39	24	Pathogenic
exon 2	c.56A>C	p.D19A	1		Pathogenic
exon 2	c.62A>C	p.D21A	3		Pathogenic
exon 2	c.65A>G	p.D22G	2	1	Pathogenic
exon 2	c.68A>G	p.K23R	2		Pathogenic
exon 2	c.63_71dup	p.K23_I24insIDK	3		Pathogenic
exon 2	c.86A>T	p.N29I	285	18	Pathogenic
exon 2	*PRSS1-PRSS2* hybrid	p.N29I + p.N54S	7	1	Pathogenic
exon 2	c.86A>C	p.N29T	5		Pathogenic
exon 2	c.107C>G	p.P36R	2		Nonpathogenic
exon 2	c.111C>A	p.Y37X		1	Protective
exon 2	c.116T>C	p.V39A	7		Pathogenic
intron 2	c.200+1G>A			1	Protective
exon 3	c.235G>A	p.E79K	13	6	Pathogenic?
exon 3	c.241C>A	p.L81M	4		Unknown
exon 3	c.248G>A	p.G83E	1		Nonpathogenic
exon 3	c.263T>A	p.I88N	1		Nonpathogenic
exon 3	c.273C>A	p.A91=	1		Unknown
exon 3	c.276G>T	p.K92N	1		Pathogenic
exon 3	c.292C>A	p.Q98K	1		Nonpathogenic
exon 3	c.298G>C	p.D100H	2	3	Pathogenic
exon 3	c.310C>G	p.L104V		4	Unknown
exon 3	c.311T>C	p.L104P	1	3	Pathogenic
exon 3	c.346C>T	p.R116C	16	4	Pathogenic
exon 3	c.361G>A	p.A121T	14	6	Nonpathogenic
exon 3	c.364C>T	p.R122C	35	23	Pathogenic
exon 3	c.365G>A	p.R122H	793	69	Pathogenic
exon 3	c.365_366GC>AT	p.R122H	3		Pathogenic
exon 3	c.367G>A	p.V123M	1		Nonpathogenic
exon 3	c.367G>T	p.V123L		1	Nonpathogenic
exon 3	c.371C>T	p.S124F	1		Pathogenic
exon 3	c.403A>G	p.T135A		1	Unknown
exon 3	c.410C>T	p.T137M	2	1	Nonpathogenic
exon 3	c.415T>A	p.C139S	11		Pathogenic
exon 3	c.416G>T	p.C139F	2	1	Pathogenic
exon 3	c.417C>T	p.C139=		1	Unknown
exon 3	c.443C>T	p.A148V		1	Unknown
intron 3	c.454+10A>C		5		Unknown
intron 3	c.454+36T>C			1	Unknown
intron 3	c.454+75A>G		24	4	Unknown
intron 3	c.454+127A>T		1		Unknown
intron 3	c.454+157C>A		1		Unknown
intron 3	c.454+157C>G		1	2	Unknown
intron 3	c.454+172C>T		4		Unknown
intron 3	c.455–192T>A		1		Unknown
exon 4	c.486C>T	p.D162=	common polymorphism	common polymorphism	Nonpathogenic
exon 4	c.508A>G	p.K170E	2		Pathogenic?
exon 4	c.541A>G	p.S181G	1	1	Nonpathogenic
intron 4	c.592–79G>A		1		Unknown
intron 4	c.592–78G>A		1		Unknown
intron 4	c.592–24C>T		1		Unknown
intron 4	c.592–11C>T		1		Unknown
intron 4	c.592–8C>T		1		Unknown

Continued

Table 1.—*Continued*

Region	Nucleotide Change	Amino Acid Change	Number of CP Carriers Reported	Number of Non-CP Carriers Reported	Clinical Significance
exon 5	c.623G>C	p.G208A	22	3	Pathogenic
exon 5	c.738C>T	p.N246=	common polymorphism	common polymorphism	Nonpathogenic

Adapted from www.pancreasgenetics.org, February 2, 2014. For a complete list of citations please visit the database website. The number of reported chronic pancreatitis (CP) carriers is an approximation for the most frequent variants, because authors often published the same patients in multiple publications without indicating the repetitive entries. Non-CP carriers include nonpenetrant family members, unrelated healthy control subjects or individuals with conditions other than pancreatitis. The clinical significance field indicates our interpretation of the available genetic and functional data with respect to the pathogenic potential of *PRSS1* variants. The following genetic evidence was considered as indicative of pathogenic nature: segregation with disease, multiple reports worldwide of affected carriers, and absence in unrelated controls. In case of rare variants, phenotypic similarity to well-characterized, hereditary pancreatitis-associated mutations served as the basis for classification. Thus variants were considered pathogenic if they caused *1*) increased autoactivation in the absence or presence of chymotrypsin C (CTRC), *2*) reduced secretion, indicative of potential misfolding, or *3*) increased trypsinogen expression or secretion. Conversely, variants that are expected to reduce trypsinogen expression were designated protective.

autoactivation of mutant p.E79K is suppressed in the presence of CTRC; however, the mutant reaches higher final trypsin levels than wild-type trypsinogen, because of its resistance to CTRC-mediated degradation (A. Szabó and M. Sahin-Tóth, unpublished observations). Thus the biochemical phenotype is inconclusive but, together with the genetic data, may be cautiously interpreted as p.E79K being a mild pathogenic variant with low penetrance.

PRSS1 Variants in Conditions Unrelated to Pancreatitis

Chen et al. (9) reported two loss-of-function *PRSS1* variants, a nonsense variant p.Y37X and a splice-site mutation c.200+1G>A in intron 2 found in chronic alcoholics without pancreatic disease. The authors suggested that the variants should be protective against chronic pancreatitis. Gullo et al. (19) investigated *PRSS1* variants in benign pancreatic hyperenzymemia and found variant p.A148V and the splice-site mutation c.40+1G>A in intron 1. Variant p.A148V has no functional consequences (A. Schnúr and M. Sahin-Tóth, unpublished observations), whereas variant c.40+1G>A should result in decreased trypsinogen expression, which might be protective against chronic pancreatitis. Two studies described *PRSS1* variants in Chinese patients with pancreatic cancer (p.T135A, p.T137M, c.454+36T>C, and c.454+157C>G in intron 3), which are in all likelihood incidental findings unrelated to pathology (13, 68). The same group proposed a strong protective effect for the C allele of the common polymorphic variant c.−408C>T (erroneously reported as −409C/T) against pancreatic cancer (31). Variant p.L104V was reported in two female members of a Chinese family with familial solid pseudopapillary tumor of the pancreas and in two healthy male relatives (17). Finally, patients with

Table 2. *Mechanism of action of pathogenic* PRSS1 *variants*

Region	Nucleotide change	Amino acid change	Pathogenic mechanism	Notes	Citations
	PRSS1 duplication		increased secretion	no direct evidence	
	PRSS1 triplication		increased secretion	no direct evidence	
exon 2	c.47C>T	p.A16V	increased activation	CTRC dependent	38, 51
exon 2	c.56A>C	p.D19A	increased activation		8, 15, 24, 37
exon 2	c.62A>C	p.D21A	increased activation		37
exon 2	c.65A>G	p.D22G	increased activation		8, 15, 22, 24, 57
exon 2	c.68A>G	p.K23R	increased activation		8, 15, 24, 57
exon 2	c.63_71dup	p.K23_I24insIDK	increased activation		15, 22
exon 2	c.86A>T	p.N29I	increased activation	CTRC dependent	51
exon 2	*PRSS1-PRSS2* hybrid	p.N29I + p.N54S	increased activation	CTRC dependent	51, 60
exon 2	c.86A>C	p.N29T	increased activation	CTRC dependent	51
exon 2	c.116T>C	p.V39A	increased activation	CTRC dependent	51
exon 3	c.235G>A	p.E79K	increased activation*	CTRC dependent	unpublished
exon 3	c.276G>T	p.K92N	misfolding		46
exon 3	c.298G>C	p.D100H	misfolding		46
exon 3	c.311T>C	p.L104P	misfolding		unpublished
exon 3	c.346C>T	p.R116C	misfolding		25
exon 3	c.364C>T	p.R122C	increased activation	CTRC dependent	51
exon 3	c.365G>A	p.R122H	increased activation	CTRC dependent	51
exon 3	c.365_366GC>AT	p.R122H	increased activation	CTRC dependent	51
exon 3	c.371C>T	p.S124F	misfolding		46
exon 3	c.415T>A	p.C139S	misfolding		25
exon 3	c.416G>T	p.C139F	misfolding		46
exon 4	c.508A>G	p.K170E	increased secretion		46
exon 5	c.623G>C	p.G208A	misfolding		46

See Table 1 for inclusion criteria. Mutations in *PRSS1* can increase activation of cationic trypsinogen via 4 independent but mutually nonexclusive mechanisms: *1*) inhibition of CTRC-dependent trypsinogen degradation, 2) increasing CTRC-dependent stimulation of autoactivation; *3*) direct stimulation of autoactivation; and *4*) increased trypsinogen secretion. Alternatively, *PRSS1* mutations can cause misfolding and endoplasmic reticulum stress. See Fig. 1 for CTRC-dependent mechanisms of trypsinogen activation and degradation. *Note that the biochemical phenotype of p.E79K is ambiguous; see text for details. Citations refer to functional studies.

PRSS1-related hereditary pancreatitis have a 40–55% lifetime risk of developing pancreatic cancer (Ref. 66 and references therein).

The PRSS1 Database

The first database for *PRSS1* variants associated with chronic pancreatitis was created by Dr. Niels Teich at the University of Leipzig, Germany (61). Although the link is still active (http://www.uni-leipzig.de/pancreasmutation/db.html), the website has not been updated since 2006. To track the increasing number of *PRSS1* variants in the literature and to help with classification of their clinical relevance, in 2012 we created a new online database. Currently, the database lists 64 *PRSS1* variants: 2 copy number mutations, 34 missense variants, 4 synonymous variants, 1 nonsense variant, 1 microinsertion, 1 hybrid gene, and 21 variants in noncoding regions (Table 1). With respect to clinical significance, 25 variants are pathogenic (Table 2), 14 are nonpathogenic, 4 variants are protective, and 21 (mostly intronic) variants have unknown significance. The database can be accessed at www.pancreasgenetics.org.

Animal Models of PRSS1 Related Pancreatitis

Although considerable progress has been made in clarifying the mechanism of *PRSS1* mutations at the biochemical level, animal models that recapitulate salient features of human hereditary pancreatitis are still lacking. At the time of writing this review, only two publications documented attempts to generate such models. Selig et al. (47) created transgenic mice with the coding DNA of human *PRSS1* containing the p.R122H mutation. The animals did not develop spontaneous pancreatitis, and cerulein caused only slightly more severe pancreatitis in transgenic mice relative to controls. Archer et al. (1) described the spontaneous development of acute and chronic pancreatitis in a transgenic line with the p.R122H mutation introduced into the coding DNA of mouse trypsinogen isoform T8. Unfortunately, independent replications or additional studies on this promising model have not been published since 2006. It is also unclear whether the observed phenotype was related to the expression of the mutant trypsinogen. Nevertheless, this study focused attention to the question whether the biochemical effects human *PRSS1* mutations would be similar in the context of mouse and human trypsinogens and whether we can make use of mouse trypsinogens to model the human disease. Recently, we demonstrated that the mouse pancreas expresses four trypsinogen isoforms to high levels (T7, T8, T9, and T20), and mouse Ctrc strongly inhibits autoactivation of isoforms T8 and T9 through cleavage of the autolysis loop (36). In sharp contrast to the human situation (see Ref. 51 and Fig. 1), mutation p.R122H had no appreciable effect on the autoactivation of T8 trypsinogen in the presence of mouse Ctrc (36). These observations argue that human pancreatitis-associated mutations may not recapitulate the pathogenic biochemical phenotype in the context of mouse trypsinogens.

Genetic deletion of mouse T7 was recently shown to abolish intra-acinar trypsinogen activation in response to hyperstimulation with cerulein, whereas severity of acute pancreatitis was somewhat decreased but not diminished (11). Furthermore, development of cerulein-induced chronic pancreatitis was unaffected by the absence of T7 (44). These observations seem to call into question the direct role of trypsinogen in the development of pancreatitis and seem to be at odds with the preponderance of human genetic and biochemical data discussed in this review. However, a more likely explanation for the apparent contradiction is that the hyperstimulation model employed in these studies does not mimic the pathological pathway associated with human hereditary pancreatitis. Future studies will be needed to shed more light on this intriguing problem.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

B.C.N. and M.S.-T. conception and design of research; B.C.N. and M.S.-T. prepared figures; B.C.N. and M.S.-T. drafted manuscript; B.C.N. and M.S.-T. edited and revised manuscript; B.C.N. and M.S.-T. approved final version of manuscript.

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