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Uncertainties in the classification of human cationic trypsinogen (*PRSS1*) variants as hereditary pancreatitis-associated mutations

Richárd Szmola and **Miklós Sahin-Tóth***
Department of Molecular and Cell Biology, Boston University Henry M. Goldman School of Dental Medicine, Boston, Massachusetts, 02118

Abstract

Autosomal dominant hereditary pancreatitis has been conclusively linked with cationic trypsinogen (PRSS1) mutations p.R122H and p.N29I, which can be found in ~90% of mutation-positive cases. To date, 35 additional rare or private *PRSS1* variants have been identified in subjects with hereditary or sporadic, idiopathic chronic pancreatitis. Despite the lack of sufficient genetic and functional evidence, many of these rare variants have been labeled as pancreatitis-associated. This problematic trend is notably illustrated by two recent studies that classified the p.A121T *PRSS1* variant as pancreatitis-associated, in large part owing to its intimate proximity to arginine-122, the residue affected by the disease-causing p.R122H mutation. Here we demonstrate that the p.A121T variant is functionally innocuous and shows no verifiable association with hereditary pancreatitis, on the basis of the available inconclusive data. This case cautions that assignment of clinical relevance to rare *PRSS1* variants should not be based on a perceived analogy with genuine disease-causing *PRSS1* mutations and further studies are required to prove or rule out possible low-penetrance causality of rare *PRSS1* variants.

Keywords

trypsinogen mutation; chronic pancreatitis; autoactivation; autolysis

Hereditary pancreatitis is a rare form of chronic pancreatitis inherited in an autosomal dominant fashion with incomplete penetrance (70–80%) and variable expressivity [1–2]. A significant (25–80%), but geographically variable, number of cases are caused by heterozygous mutations in the *PRSS1* gene which codes for human cationic trypsinogen [2–6]. Approximately 70% of the affected families carry the c.365G>A (p.R122H) mutation, and circa 20% the c.86A>T (p.N29I) mutation. In rare cases mutation p.R122H is caused by a dinucleotide change (c. 365_366GC>AT), possibly due to a gene-conversion mechanism [7,8], whereas mutation p.N29I has been also found in functional hybrid genes composed of segments of *PRSS1* and *PRSS2*, the gene encoding human anionic trypsinogen [9,10]. Evidence that p.N29I and p.R122H are causative for hereditary pancreatitis came from linkage analysis establishing chromosomal localization (7q35), the observed co-segregation of the mutations with pancreatitis in multiple kindreds worldwide, and functional studies suggesting a plausible mechanism of action which involves increased autoactivation (p.N29I, p.R122H) and resistance to degradation (p.R122H) [see 2,11,12 and references therein]. In addition to these two frequently found mutations, 10 other relatively rare disease-causing mutations have been identified in subjects with hereditary pancreatitis or idiopathic chronic pancreatitis without a

*Correspondence to Miklós Sahin-Tóth, 72 East Concord Street, Evans-433; Boston, MA 02118;Tel: (617) 414-1070; Fax: (617) 414-1041; miklos@bu.edu.

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family history (Table 1). Association of p.A16V, p.E79K, p.R116C, and p.R122C with chronic pancreatitis has been replicated in multiple families or patients and their pathogenic role is supported by functional analysis [13–16]. Penetrance of p.A16V is variable and family-dependent, which may hold true for some of the other rare mutations as well. Another subset of mutations (p.D19A, p.D22G, p.K23R, p.N29T, p.V39A, and p.C139S) has been found only in very few patients so far (Table 1), but clear segregation with disease and/or the biochemical and cell-biological properties of the mutant proteins are consistent with disease association [15,17,18]. Finally, 23 additional *PRSS1* variants, consisting of very rare or private mutations of unknown significance, have been identified in patients with chronic pancreatitis (Table 2); including a surprisingly large number of missense variants (p.P36R, p.G83E, p.I88N, p.K92N, p.Q98K, p.D100H, p.L104P, p.A121T, p.V123M, p.T137M, p.C139F, p.K170E, and p.G208A). Despite the lack of functional evidence, these variants are often described as (hereditary) pancreatitis-associated mutations or disease-causing mutations. In the present study we examine a recent example of this undesirable trend, variant p.A121T, which has been classified as a hereditary pancreatitis-associated mutation by two recent reports [19,20].

MATERIALS AND METHODS

Plasmid construction and mutagenesis, expression and purification of wild-type and p.A121T mutant human cationic trypsinogen, trypsin activity measurements and protocols for cell culture and transfection are described in the online Supplementary Material.

RESULTS AND DISCUSSION

The p.A121T variant does not segregate with pancreatitis in the published pedigrees

In the pedigree presented by Liu et al. (2008) only the index patient was verified to carry the p.A121T variant *and* suffer from chronic pancreatitis [19]. The index patient's son was an unaffected carrier, whereas the father, who apparently suffered from chronic pancreatitis, had died and was not available for genetic testing. A remarkably similar pedigree was presented by Felderbauer et al. (2008) [20]. Again, only the index patient was confirmed to carry the p.A121T variant *and* have chronic pancreatitis. The index patients' brother and first-degree cousin both carried the p.A121T variant and suffered from recurrent abdominal pain apparently associated with cholelithiasis, whereas his niece and her mother were asymptomatic carriers. The index patients' deceased father and both grandparents had a history of abdominal pain and gall stone disease but no clinically diagnosed pancreatitis. Thus, the p.A121T variant seemed to segregate with cholelithiasis rather than chronic pancreatitis in this family. Whether or not abdominal pain in some of these patients was due to pancreatitis but was erroneously attributed to cholelithiasis cannot be ascertained from the available clinical information.

The p.A121T variant is functionally identical to wild-type cationic trypsinogen

The study by Felderbauer et al. (2008) presented functional experiments, in which bovine trypsin was used to digest model peptides corresponding to human cationic trypsinogen between Val118 and Leu128. The authors found that tryptic cleavage of the p.A121T-containing peptide was somewhat *faster* than digestion of the wild-type peptide, whereas, as expected, the p.R122H-containing peptide was not cleaved. The authors concluded that the p.A121T variant might enhance trypsin degradation and thereby cause pancreatitis, whereas the p.R122H mutation would cause the same disease through inhibition of trypsin degradation.

To test the functional effect of p.A121T in the context of the entire human cationic trypsinogen molecule, we expressed and purified this variant and compared its properties to wild-type cationic trypsinogen. As shown in Supplementary Fig S1, autoactivation of wild-type and p.A121T trypsinogens was identical at pH 5.0 or at pH 8.0. This result stands in contrast to the

documented increase in autoactivation caused by *PRSS1* mutants p.D19A, p.D22G, p.K23R, p.N29I, p.N29T, and p.R122H [11 and references therein]. During autoactivation, some of cationic trypsinogen becomes cleaved at the Arg122-Val123 peptide bond, which results in two characteristic bands on polyacrylamide gels. When wild-type and p.A121T trypsinogens were compared, no difference was detectable in the kinetics of appearance of these two bands or their intensity, indicating that the Arg122-Val123 peptide bond was cleaved at similar rates (Supplementary Fig S1B, lower panel). Although data are not shown, there was no difference in the activation of wild-type and p.A121T trypsinogens by human enteropeptidase at pH 8.0 or by human cathepsin B at pH 4.0.

To test whether degradation of cationic trypsin was affected by the p.A121T variant, first we followed autolysis of wild-type and p.A121T trypsins. Because autolysis of cationic trypsin is a very slow process, these experiments were performed in the absence of added calcium and in the presence of 10 mM K-EDTA. Autolysis was followed up to 5 hours, during which time approximately 50% of the initial trypsin activity was lost (Supplementary Fig S2A). However, no difference was observed between the autolysis kinetics of wild-type and p.A121T trypsins. We recently demonstrated that human cationic trypsin is specifically degraded by chymotrypsin C by a mechanism that involves cleavage of the Leu81-Glu82 peptide bond followed by tryptic cleavage of the Arg122-Val123 peptide bond [12]. Mutation p.R122H protects against chymotrypsin C mediated cleavage [12]. In contrast, variant p.A121T had no such effect and chymotrypsin C degraded wild-type and p.A121T trypsins at identical rates (Supplementary Fig S2B).

We also compared the enzyme kinetic parameters of wild-type and p.A121T cationic trypsins and found no appreciable difference (Supplementary Table S1). Similarly, both trypsins digested bovine beta casein with comparable efficiency (Supplementary Fig S3). Finally, both wild-type and p.A121T trypsins were inhibited by human pancreatic secretory trypsin inhibitor (SPINK1) stoichiometrically and with equal affinity (not shown).

We recently found that *PRSS1* variants p.R116C and p.C139S cause trypsinogen misfolding and induce endoplasmic reticulum stress [15]. As a consequence of misfolding and intracellular retention, these mutants are secreted poorly from transfected mammalian cells. To test for this possibility, we transfected HEK 293T cells with wild-type and p.A121T trypsinogens and measured trypsinogen secretion up to 48 hours. Supplementary Fig S4 demonstrates that the two trypsinogens were secreted at similar rates and accumulated to comparable levels in the conditioned medium.

Concluding remarks

Taken together, the inconclusive genetic evidence and our new experimental results indicate that *PRSS1* variant p.A121T might not be associated with hereditary pancreatitis and exerts no effect whatsoever on the functional properties of human cationic trypsinogen and trypsin. Variants identified in the *PRSS1* gene are often assigned clinical relevance solely based on a perceived analogy with the genuine disease-causing *PRSS1* mutations. Because misclassification of clinically harmless genetic variants can directly affect the lives of the carriers and their relatives, further studies are required before rare *PRSS1* variants are declared pancreatitis-associated.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Chronic pancreatitis associated *PRSS1* mutations. Disease association is supported by genetic evidence showing segregation of the mutation with pancreatitis in two or more generations and/or functional studies pointing to a plausible mechanism of action. The p.D19A and p.C139S mutations were found only in idiopathic cases without a family history so far. Although the p.E79K mutation was identified in three separate families, none of the published pedigrees satisfy the formal criteria for hereditary pancreatitis. See Supplementary Table S2 for a complete list of citations used to assemble this table.

Nucleotide change	Amino acid change	Number of affected carriers reported
c.47C>T	p.A16V	35
c.56A>C	p.D19A	1
c.65A>G	p.D22G	2
c.68A>G	p.K23R	2
c.86A>T	p.N29I	>200
c.86A>T + c.161A>G	p.N29I + p.N54S	7
c.86A>C	p.N29T	3
c.116T>C	p.V39A	7
c.235G>A	p.E79K	8
c.346C>T	p.R116C	11
c.365G>A	p.R122H	>700
c.365_366GC>AT	p.R122H	3
c.364C>T	p.R122C	29
c.415T>A	p.C139S	3

Table 2

PRSS1 variants of unknown significance; found in subjects with chronic pancreatitis. The table does not show two *PRSS1* polymorphisms [c.486C>T (p.D162=) and c.738C>T (p.N246=)]; commonly found in subjects with chronic pancreatitis and healthy controls alike. Also unlisted are *PRSS1* variants c.111C>A (p.Y37X) and c.200 +1G>A, which were identified in two subjects, respectively, with chronic alcoholism but without pancreatic disease; and variants c.443C>T (p.A148V) and c.40+1G>A that were described in two subjects, respectively, with benign pancreatic hyperenzymemia. See Supplementary Table S2 for a complete list of citations used to assemble this table.

Nucleotide change	Amino acid change	Number of affected carriers reported
c.-30_-28delTCC		1
c.107C>G	p.P36R	1
c.248G>A	p.G83E	1
c.263T>A	p.I88N	1
c.276G>T	p.K92N	1
c.292C>A	p.Q98K	1
c.298G>C	p.D100H	1
c.311T>C	p.L104P	4
c.361G>A	p.A121T	2
c.367G>A	p.V123M	1
c.410C>T	p.T137M	2
c.416G>T	p.C139F	1
c.508A>G	p.K170E	1
c.632G>C	p.G208A	1
c.40+40delC		1
c.41-49C>T		1
c.454+157C>A + c.455-192T>A		1
c.592-79G>A		1
c.592-78G>A		1
c.592-24C>T		1
c.592-11C>T + c.592-8C>T		1