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Novel *PRSS1* mutation p.P17T validates pathogenic relevance of CTRC-mediated processing of the trypsinogen activation peptide in chronic pancreatitis

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To the editor

Mutations in *PRSS1* cause hereditary pancreatitis by reducing chymotrypsin C (CTRC)-dependent degradation of cationic trypsinogen and thereby promoting trypsinogen autoactivation [1, 2]. An interesting exception is mutation p.A16V, which does not affect trypsinogen degradation. Instead, it increases CTRC-mediated processing of the trypsinogen activation peptide to a shorter form (Figure 1A), which, in turn, results in accelerated autoactivation [1–3]. Mutation p.A16V exhibits variable penetrance, indicating that disease onset requires additional risk factors [4, 5]. Here we report the discovery of the novel p.P17T mutation that mimics the effects of the p.A16V mutation and corroborates the pathological relevance of N-terminal trypsinogen processing by CTRC.

The index patient was a 30 year old woman who was referred to the Hungarian Pancreatic Study Group for genetic testing and counselling. She was diagnosed with chronic pancreatitis at the age of 15 and suffered at least 13 documented acute attacks since that

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Informed patient consent: The index patient and her parents gave informed consent to genetic testing and publication of clinical data.

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time. Diagnostic signs of chronic pancreatitis included pancreas atrophy, parenchymal calcifications, Wirsung duct stones and dilatation visualized by transabdominal US, abdominal CT and therapeutic ERCP. Neither exocrine nor endocrine insufficiency was observed. She has smoked at least 7 pack years since age 23 whereas alcohol-drinking history was unremarkable. For treatment of intractable pain and non-resolving common bile duct stenosis, she underwent multiple endoscopic pancreatic and biliary duct stent placements followed by combined surgical drainage and resection (Frey's procedure) of the pancreas and cholecystectomy at the age of 30. After obtaining informed consent, we performed Sanger-sequencing of all exons and flanking intronic regions of the *CTRC, CPA1*, *PRSS1* and *SPINK1* genes and exons 4 and 11 of the *CFTR* gene. We found a novel heterozygous c.49C>A (p.P17T) variant in exon 2 of *PRSS1* (Figure 1B). With the exception of a heterozygous (p.N34S) variant in *SPINK1*, no other pathogenic variants were detected. Sequencing of *PRSS1* exon 2 in the parents showed only wild-type sequence, indicating that mutation p.P17T arose *de novo* in the index patient. The *SPINK1* p.N34S mutation was inherited from the unaffected mother.

Functional analysis of the p.P17T mutant was performed using recombinant trypsinogen according to published protocols [2]. For comparison, wild-type and p.A16V mutant trypsinogen were also included. Rates of N-terminal processing by CTRC were increased 3.3-fold by mutation p.A16V and 1.7-fold by mutation p.P17T (Figure 1C), relative to wild type. As a consequence of stronger N-terminal processing, mutants p.A16V and p.P17T exhibited increased initial rates of autoactivation and developed higher trypsin levels in the presence of 25 nM CTRC (Figure 2). Autoactivation in the absence of CTRC showed no significant differences between wild-type and the two mutants.

In conclusion, in an early-onset case of chronic pancreatitis we identified the *de novo PRSS1* mutation p.P17T in the activation peptide of human cationic trypsinogen. Mutation p.P17T showed similar biochemical characteristics as the known pathogenic mutation p.A16V and thus offers independent validation for the notion that accelerated N-terminal processing of the trypsinogen activation peptide by CTRC is a relevant pathogenic mechanism in chronic pancreatitis.

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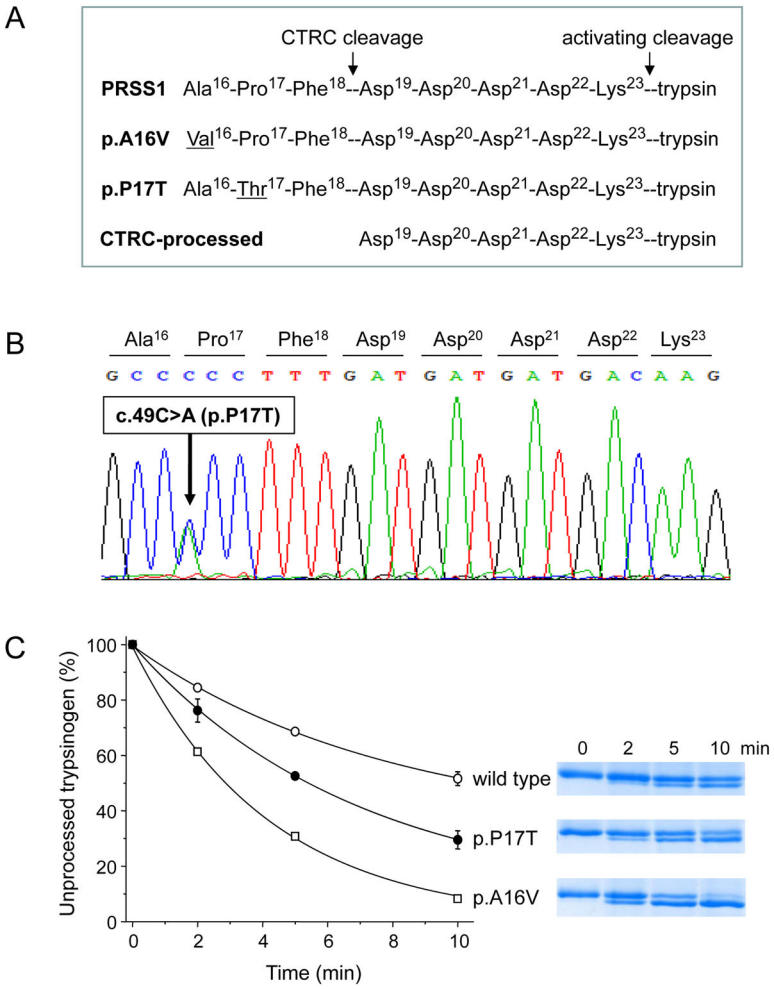

Figure 1.

N-terminal processing of the activation peptide of human cationic trypsinogen (PRSS1) by chymotrypsin C (CTRC) and the effect of mutations p.A16V and p.P17T. **A**, Amino-acid sequence of wild-type and mutant activation peptides with sites of CTRC processing and activating cleavage indicated. Activation can be catalyzed by enteropeptidase, cathepsin B or trypsin. **B**, Electropherogram showing the heterozygous c.49C>A (p.P17T) mutation in the *PRSS1* gene of the index patient. The amino-acid sequence of the activation peptide is also indicated. **C**, Effect of mutations p.A16V and p.P17T on the N-terminal processing of trypsinogen by CTRC. Trypsinogen (2 μM) was incubated with 25 nM CTRC in 0.1 M Tris-HCl (pH 8.0), 1 mM $CaCl_2$ and 20 nM SPINK1 trypsin inhibitor (final concentrations) at 37 °C. Reactions were terminated by precipitation with trichloroacetic acid (10% final concentration) and analyzed by non-reducing SDS-PAGE and Coomassie Blue staining. Representative gels from three replicates show the small mobility shift due to N-terminal processing. The graph shows the changes in the intensity of the unprocessed trypsinogen band expressed as percent of the total intensity of the processed and unprocessed bands, as determined by densitometry. Error bars indicate standard deviation. Note that the PRSS1 constructs used for these experiments contained the p.L81A mutation to allow for selective analysis of N-terminal processing without interference from CTRC-mediated degradation.

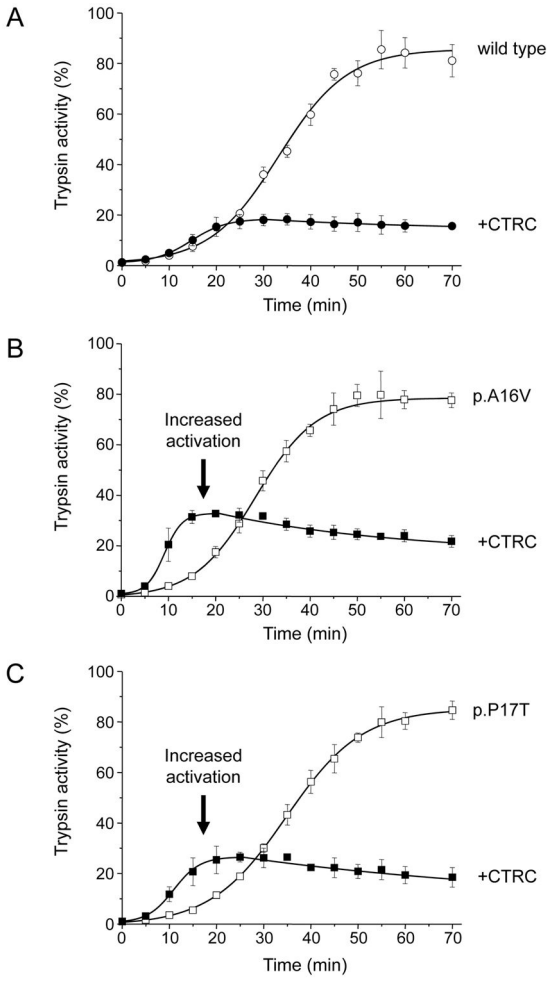

Figure 2.

Effect of mutations p.A16V and p.P17T on the autoactivation of human cationic trypsinogen (PRSS1) in the absence and presence of chymotrypsin C (CTRC). Trypsinogen (1 μM) was incubated with 10 nM initial trypsin in 0.1 M Tris-HCl (pH 8.0), 1 mM $CaCl_2$ and 0.05% Tween 20 (final concentrations) at 37 °C in the absence (empty symbols) or presence of 25 nM CTRC (solid symbols). At the indicated times, 2 μL aliquots were removed and trypsin activity was measured. Activity was expressed as percent of the maximal activity after full activation with enteropeptidase determined in separate assays. Data points show the average of three replicates with standard deviation. **A**, wild-type, **B**, mutant p.A16V, **C**, mutant p.P17T.