Evaluation of the pathogenic significance of the novel p.T58M chymotrypsin C (CTRC) variant in recurrent acute pancreatitis

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To the editor:

Chymotrypsinogen C (*CTRC*) gene (OMIM: 601405) encodes the digestive enzyme chymotrypsin C (CTRC) expressed by the pancreas [1]. CTRC enzyme has a significant role in regulation of trypsinogen activation by cleaving and degrading human cationic trypsinogen, therefore it protects from early, intrapancreatic trypsinogen activation [2]. Loss-of function mutations in the *CTRC* gene that reduce secretion or catalytic activity of CTRC are associated with chronic pancreatitis [3]. Independently from the trypsin dependent-pathway, gain-of-function mutations of the *CTRC* gene cause endoplasmatic reticulum stress that also predispose the development of chronic pancreatitis [4]. However, genetic susceptibility plays a predominant role in the etiology of childhood onset pancreatitis [5, 6], recent publications showed the significance of genetic background in some unusual cases of late onset pancreatitis [7, 8]. Biochemical characterisation of novel mutations of known susceptibility genes associated with pancreatitis might help us to evaluate the clinical significance of unknown variants.

A Hungarian man with late onset recurrent acute pancreatitis was referred to the Hungarian Pancreatic Study Group for genetic testing and counselling. The first episode of acute pancreatitis was diagnosed at the age of 46. The index patient suffered 3 clinically documented acute attacks. Each acute episode was diagnosed according to the IAP/APA definition of acute pancreatitis [9]. Two more suspected attacks of acute pancreatitis were also documented. A single pancreatic pseudocyst as a complication of acute attacks was visualized by endoscopic ultrasound and therapeutic ERCP. Exocrine insufficiency was not observed and the patient was not diabetic. There was no sign of calcification, atrophy or Wirsung duct dilatation on abdominal CT and ultrasound. Except for smoking (30 packyear) no other environmental risk factors predisposing to pancreatitis were identified in the index patient's history. After obtaining informed consent, we performed Sanger-sequencing of all exons and flanking intronic regions of the *PRSS1, CTRC, CPA1*, and *SPINK1* genes and exons 3, 4, 10, 11 and 12 of the *CFTR* gene. We found a novel heterozygous missense c.173C>T (p.T58M) variant in exon 3 of *CTRC* gene (Figure 1A). No other pathogenic variant was detected. According to the family

history no other family member developed pancreatitis. Unfortunately, we could not recruit the family members for genetic testing, therefore we have no information whether other family members carried the novel missense mutation.

In vitro studies of catalytic activity and secretion in HEK293T cells of the wild type and p.T58M mutant chymotrypsinogen C was studied using recombinant enzymes according to published protocols [10]. There was no difference between the expression rate of wild type and mutant p.T58M chymotrypsingen C using transiently transfected HEK293T cells (Figure 1B). Wild type and mutant p.T58M CTRC activated at simultaneous rate by human cationic trypsin (*data not shown*). In the presence of the mutant CTRC the rate of N-terminal processing (Figure 1C) and autoactivation of human cationic trypsinogen (*data not shown*) were comparable with the effect of wild type CTRC. The mutant p.T58M cleaved large substrates β-casein (Figure 1D) or human cationic trypsinogen (Figure 1E) somewhat slower compared to wild type CTRC. Interestingly, we found that the p.T58M mutant chymotrypsin C (K_m = 10.5 ± 0.4 uM, K_{cat} = 24.0275 1/sec) cleaved the small substrate AAPF-pNA faster than the wild type CTRC (K_m = 13.4 ± 0.8 uM, K_{cat} = 11.7429 1/sec).

To summarize our findings we detected the novel c.173C>T (p.T58M) mutation in a late-onset case of recurrent acute pancreatitis. The novel p.T58M mutant CTRC has comparable biochemical characteristics to wild type CTRC on large substrates. Therefore, we conclude that this novel missense variant does not have significant role in the development of pancreatitis.

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LEGENDS TO FIGURES

Figure 1. Identification, expression and biochemical characteristics of the novel chymotrypsinogen C (CTRC) mutant c.173C>T (p.T58M). **(A)** Electropherogram showing the heterozygous c.173C>T (p.T58M) mutation in the *CTRC* gene of the index patient. Amino acid sequence of the wild type CTRC enzyme between residues Arg56 and Gly61 is also indicated. **(B)** 1.5 x 10^6 HEK293T cells/well were transiently transfected pcDNA3.1(-) plasmid DNA containing wild type or p.T58M CTRC gene in 2 mL total volume. As a negative control we used HEK293T cell medium without prior transfection. From each indicated medium 200 uL was precipitated in 10% TCA and loaded on SDS gel under reducing conditions. **(C)** N-terminal processing of p.L81A human cationic trypsinogen by 25 nM wild type and p.T58M CTRC. The experiments contained 20 nM SPINK1 trypsin inhibtor and 1 mM $CaCl_2$. The small shifts indicate the cleavage of p.L81A trypsinogen by CTRC between amino acid residues Phe18 and Asp19. **(D)** 0.2 mg/mL β-casein was cleaved by 5 nM wild type and p.T58M mutant CTRC at 37 ^oC in 0.1 M Tris (pH 8.0). **(E)** Cleavage of 2 uM human cationic trypsinogen by 10 nM wild type and p.T58M mutant CTRC at 37 ^oC in 0.1 M Tris (pH 8.0). 20 nM SPINK1 were added to experiment "**E"**. At the indicated time points 100 uL aliqouts were removed from the total volume of 450 uL assay and precipitated in 10% TCA than loaded on SDS gel under non-reducing **(C)** or reducing **(D, E)** conditions.