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Transgenic expression of human PRSS2 exacerbates pancreatitis in mice

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We read with great interest the study by Hegyi *et al*¹ which reported that a commonly occurring haplotype spanning the PRSS1-PRSS2 locus (encoding human cationic and anionic trypsinogens) is associated with chronic pancreatitis. While PRSS1 is the major focus in many studies,^{2,3} PRSS2 is also a major trypsinogen isoform synthesised in human pancreas. In normal human, the PRSS1/PRSS2 ratio is approximately 2:1.⁴ Chronic alcoholism increases the risk of pancreatitis. Strikingly, in these patients, total trypsinogen secretion was increased with selective upregulation of PRSS2, reversing the PRSS1/PRSS2 ratio.⁵ In vitro studies have shown human PRSS2 more sensitive to autocatalytic degradation and with lower autoactivation than PRSS1.⁶ In test tube assays under conditions of intracellular pathological trypsinogen activation, mixtures of PRSS1 and PRSS2 with increasing ratios of PRSS2 had markedly decreased rates of trypsinogen activation and yields of active trypsin.⁶ These observations led to a hypothesis that upregulation of PRSS2 may play a protective role against pancreatitis.⁶

In order to elucidate the role of human PRSS2 in the pathogenesis of pancreatitis in vivo, transgenic mice expressing human PRSS2 were developed using a bacterial artificial chromosome containing the full length human PRSS2 gene (see online supplementary methods). Two lines of PRSS2 mice were developed. PRSS2 expression level was higher in line #2 than that in line #1. Focal spontaneous pancreatitis (area <10%) was observed in line #2 but not in line #1 mice (see online supplementary results and online supplementary figure 1). To further elucidate the increased sensitivity to pancreatitis by PRSS2 expression, control and PRSS2 line #1 mice were challenged with a single dose of caerulein (100 µg/kg) and acute pancreatitis was evaluated at 24 hours. We observed minimal pancreatitis in control C57BL6 mice; however, all PRSS2 mice developed severe pancreatic oedema (figure 1A) with elevated pancreatic oedema and serum amylase levels (figure 1B,C). Histological examination revealed more severe pancreatitis in PRSS2 mice compared to

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control mice (figure 1D). Using six doses of caerulein (100 µg/kg/hour), more severe pancreatitis, with massive acinar cell death and inflammatory cell infiltration, developed (online supplementary figure 2A,B). At day 7 and day 21 after a single caerulein induction, all control mice showed full recovery from acute pancreatitis. Strikingly, all PRSS2 mice progressed to chronic pancreatitis with pancreatic atrophy, acinar destruction, increased markers of fibrosis and inflammatory cell infiltration (figure 2, online supplementary figure 3). With high dose of caerulein, we observed similar changes in PRSS2 line #2 mice. Inhibition of trypsin ameliorated pancreatitis in these mice (online supplementary figure 4), supporting the idea that increased trypsin activity is key to the severity of pancreatitis.

Collectively, our data demonstrated that increased human PRSS2 is in fact detrimental in pancreatitis. These findings are in agreement with a clinical study showing that a G191R variant of PRSS2 mitigates intrapancreatic trypsin activity and thereby protects against chronic pancreatitis in human.⁷ This study is of clinical importance in that the expression of PRSS2 surpasses PRSS1 and becomes the major isoform in the pancreas of human population with high risk of pancreatitis.⁵ It is believed that the evolutionary rationale for the existence of multiple trypsinogen isoforms is that their differences in inhibitor sensitivity may be advantageous for digestion of foods containing natural trypsin inhibitors.⁶ Therefore, inhibitors developed for pancreatitis therapy shall be tested for their sensitivity to all trypsin isoforms. Our novel animal model provides such an opportunity for testing the isoform-specific efficacy in vivo.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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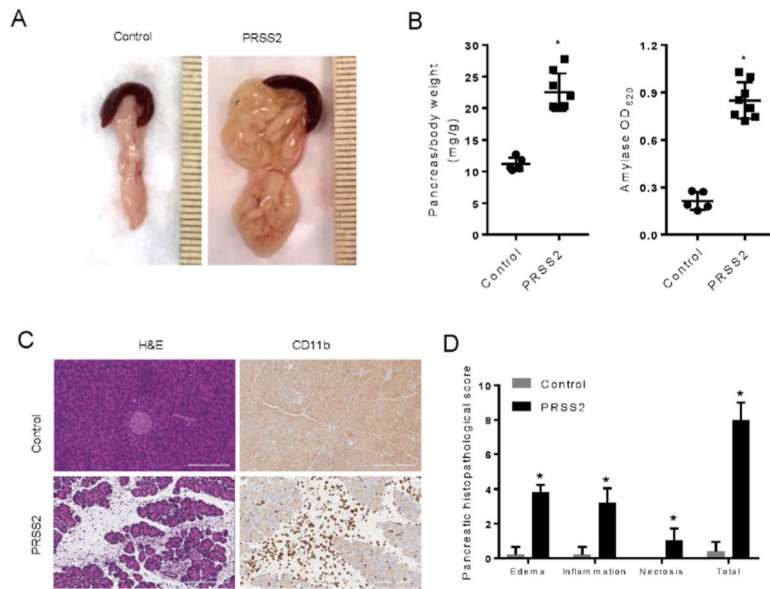


Figure 1. Caerulein-induced severe acute pancreatitis in mice with transgenic expression of human PRSS2. (A) Wild type (WT) C57BL6 control and transgenic mice was injected with a single dose of caerulein (100 μ g/kg) intraperitoneally. Macroscopic appearance of pancreas 24 hours after caerulein induction in control and PRSS2 line #1 mice was shown. (B) Pancreatic oedema and serum amylase were measured and compared in control and PRSS2 line #1 mice (n=5–8). (C) Pancreas histology (H&E) and CD11b-positive inflammatory cell staining. (D) Pancreatitis histopathological scores were evaluated.

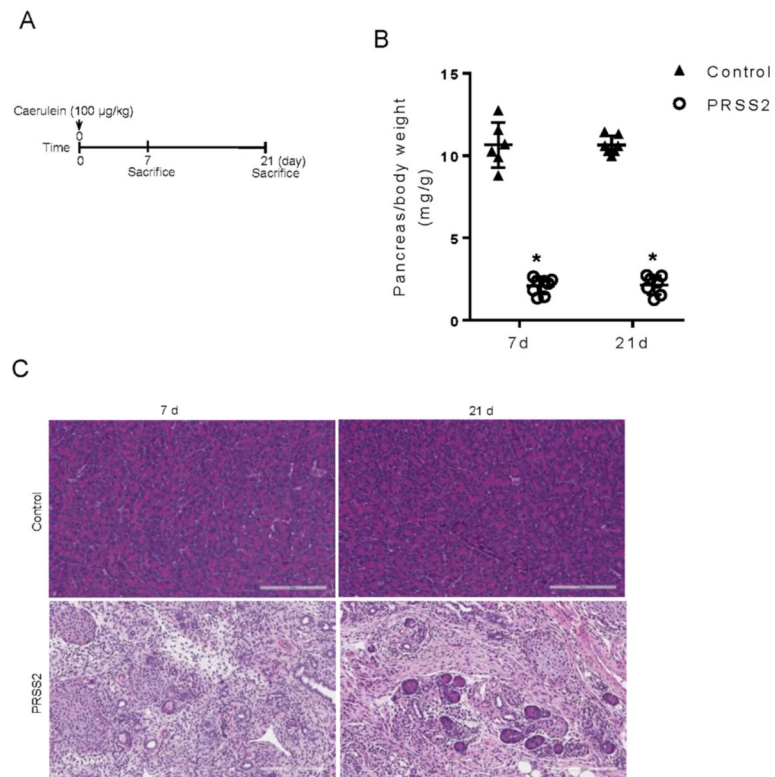


Figure 2. Transgenic PRSS2 mice but not WT C57BL control mice developed chronic pancreatitis after one episode of acute pancreatitis. (A) Schema of pancreatitis induction and tissue harvest time points. (B) Measurements of pancreas size at day 7 and day 21 after acute pancreatitis induction (n=6–8). (C) Pancreas histology (H&E) at day 7 and day 21 after caerulein.