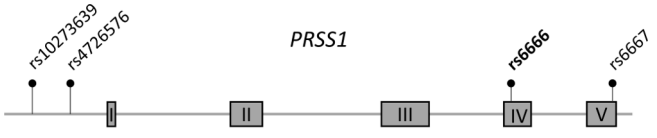
Alcohol-dependent effect of *PRSS1-PRSS2* haplotype in chronic pancreatitis

We read with great interest the studies by Derikx *et al*,[1] Boulling *et al*[2] and Masson *et al*,[3] in which the authors report that a commonly occurring haplotype spanning the *PRSS1-PRSS2* locus (encoding human cationic and anionic trypsinogen) is associated with chronic pancreatitis with an allelic OR of 0.7 in European cohorts (figure 1). Tagged by the c.-408C>T variant (rs10273639), this haplotype was first identified in a GWAS by the Whitcomb laboratory.[4] The small but significant protective effect is likely due to the c.-204C>A promoter variant (rs4726576) in *PRSS1*, which decreases trypsinogen expression and thereby reduces the risk of premature trypsin activation in the pancreas.[2] Curiously, Derikx *et al*[1] found a clear association of the *PRSS1-PRSS2* haplotype with alcoholic pancreatitis only, whereas no association was evident with non-alcoholic disease. Whitcomb *et al* also noted that the effect of the haplotype seemed to be amplified by alcohol.[4] The French study did not specify disease aetiology.[2] We were intrigued by the possible alcohol-dependent effect of this haplotype on pancreatitis risk because this was counterintuitive. The pathogenic role of intrapancreatic trypsin activation is believed to be predominant in non-alcoholic chronic pancreatitis and to play a lesser role in alcoholic pancreatitis. Therefore, one would expect to see a stronger protective effect in the non-alcoholic cohort,



LDmatrix (R^2)	rs10273639 (C/T)	rs4726576 (C/A)	rs6666 (C/T)	rs6667 (C/T)
rs10273639 (C/T)	X	0.996	0.992	0.996
rs4726576 (C/A)	0.996	X	0.988	0.992
rs6666 (C/T)	0.992	0.988	X	0.996
rs6667 (C/T)	0.996	0.992	0.996	X

Figure 1 Linkage of variants of the *PRSS1-PRSS2* haplotype within the *PRSS1* promoter and coding region. Linkage disequilibrium between the four SNPs, rs10273639 (c.-408C>T), rs4726576 (c.-204C>A), rs6666 (c.486C>T) and rs6667 (c.738C>T) in European populations was calculated. Data were taken from phase 3 (version 5) of the 1000 Genomes Project using the LDlink website (https://ldlink.nci.nih.gov/).

Table 1 Allele frequency and genotype distribution of the *PRSS1* c.486C>T (rs6666) variant in patients with CP and controls without pancreatic disease

	Allele frequency						
	C	T		OR	P value	95% CI	
Total CP (n=223)	298 (67%)	148 (33%)		0.67	0.002	0.52 to 0.86	
ACP (n=120)	168 (70%)	72 (30%)		0.57	0.0007	0.42 to 0.79	
NACP (n=103)	130 (63%)	76 (37%)		0.78	0.14	0.57 to 1.09	
Controls (n=296)	339 (57%)	253 (43%)					
	Genotype frequency						
	CC	CT	TT	OR	P value	95% CI	HWE
Total CP (n=223)	103 (46%)	92 (41%)	28 (13%)	*0.58* 0.63	*0.0025* 0.06	*0.41 to 0.83* 0.39 to 1.02	0.3
ACP (n=120)	59 (49%)	50 (42%)	11 (9%)	*0.52* 0.44	*0.0022* 0.0173	*0.33 to 0.79* 0.22 to 0.87	0.9
NACP (n=103)	44 (43%)	42 (41%)	17 (16%)	*0.66* 0.87	*0.08* 0.64	*0.42 to 1.04* 0.48 to 1.56	0.2
Controls (n=296)	98 (33%)	143 (48%)	55 (19%)				0.8

Genotype data were analysed, assuming dominant (shown in italics) or recessive models of inheritance for the minor T allele.
Calculations were performed using χ^2 test (GraphPad Prism V.8).
ACP, alcoholic chronic pancreatitis; CP, chronic pancreatitis; HWE, Hardy-Weinberg disequilibrium; NACP, non-alcoholic chronic pancreatitis.

which was not the case. To investigate this phenomenon, we genotyped 223 Hungarian cases with chronic pancreatitis (120 alcoholic and 103 non-alcoholic) and 296 controls for the c.486C>T variant (rs6666), which is part of the *PRSS1-PRSS2* haplotype showing a strong linkage disequilibrium with c.-408C>T (R^2=0.992) in Europeans (figure 1). The c.486C>T (p.Asp162=) variant is located in exon 4 of *PRSS1*. We PCR amplified this region from genomic DNA and used Sanger sequencing to identify the variant. When allele frequencies were considered, we replicated the previously reported association with chronic pancreatitis with an OR of 0.67 (p=0.002, 95% CI 0.52 to 0.86) (table 1). Remarkably, subgroup analysis revealed that the disease association was exclusively derived from the alcoholic cohort (OR 0.57, p=0.007, 95% CI 0.42 to 0.79), whereas the non-alcoholic cases showed no association (OR 0.78, p=0.14, 95% CI 0.57 to 1.09). Genotype distribution analysis using dominant and recessive models confirmed the allelic findings (table 1).

Thus, the *PRSS1-PRSS2* haplotype modifies chronic pancreatitis risk only in the presence of alcohol consumption. The reason for this phenomenon may be related to the effects of alcohol on trypsinogen expression. In humans, alcohol increases expression of all three trypsinogen isoforms, while pancreatic trypsin inhibitor expression remains relatively unchanged.[5] Due to these changes, the alcoholic pancreas is less well protected against premature trypsin activation and the risk-modifying effect of the *PRSS1-PRSS2* haplotype becomes pathologically relevant.

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Competing interests None declared.

Patient consent for publication Not required.

Ethical approval. Experiments were performed at Boston University. Deidentified genomic DNA samples were obtained from the registry of the Hungarian Pancreatic Study Group (ethical approval: TUKEB 22254-1/2012/EKU, biobanking approval: IF702-19/2012). Individuals in the registry were recruited from 11 Hungarian centres between 2012 and 2016, and all gave informed consent according to the ethical guidelines of the Declaration of Helsinki. The study was approved by the institutional review board (IRB) at Boston University ('Analysis of susceptibility genes in patients with chronic pancreatitis', IRB number H-35382).

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