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The common truncation variant in pancreatic lipase related protein 2 (PNLIPRP2) is expressed poorly and does not alter risk for chronic pancreatitis

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Abstract

A nonsense variant (p.W358X) of human pancreatic lipase related protein 2 (PNLIPRP2) is present in different ethnic populations with a high allele frequency. In cell culture experiments, the truncated protein mainly accumulates inside the cells and causes endoplasmic reticulum stress. Here, we tested the hypothesis that variant p.W358X might increase risk for chronic pancreatitis through acinar cell stress. We sequenced exon 11 of PNLIPRP2 in a cohort of 256 subjects with chronic pancreatitis (152 alcoholic and 104 non-alcoholic) and 200 controls of Hungarian origin. We observed no significant difference in the distribution of the truncation variant between patients and controls. We analyzed mRNA expression in human pancreatic cDNA samples and found the variant allele markedly reduced. We conclude that the p.W358X truncation variant of PNLIPRP2 is expressed poorly and has no significant effect on the risk of chronic pancreatitis.

Introduction

Recurrent acute pancreatitis and chronic pancreatitis are inflammatory diseases of the pancreas with significant health and economic burdens [1, 2]. After an initial episode of acute pancreatitis, 10 to 30% of adults and children have additional episodes and, of those, a large fraction develop chronic pancreatitis (CP) [3, 4]. Progression of a single episode to chronic pancreatitis often associates with genetic risk factors in genes encoding digestive enzymes expressed in pancreatic acinar cells [5–7]. Since the discovery that a genetic variant in PRSS1 (cationic trypsinogen) causes hereditary pancreatitis, most investigations to identify additional genetic risk factors focused on proteases and their inhibitors [8, 9].
More recent studies linked genetic variants in pancreatic lipases to increased risk for CP. The first report described variants in the gene encoding carboxyl ester lipase (CEL) that result in a form of autosomal dominant CP characterized by early-onset pancreatic insufficiency and diabetes [10]. A subsequent study found that a hybrid allele resulting from recombination of CEL and a neighboring pseudogene, CELP, increased risk for CP in northern Europeans [11]. Additionally, a report of two brothers who had a deficiency of pancreatic lipase (PNLIP) and evidence of CP showed they were homozygous for a missense mutation in PNLIP [12]. Follow-up studies indicated that the genetic variants of CEL and PNLIP likely cause disease through increased protein misfolding and maladaptive activation of unfolded protein response pathways [11, 13–15]. Importantly, these studies suggest that genetic variants in other pancreatic lipases, such as the pancreatic lipase related protein 2 (PNLIPRP2), might increase the risk for CP.

PNLIPRP2 is homologous with PNLIP and both belong to the same large lipase gene family [16–18]. Unlike PNLIP, which only digests triglycerides, PNLIPRP2 has lipase activity against triglycerides, phospholipids and galactolipids [16]. In newborn mice, PNLIPRP2 plays a critical role in fat digestion [19]. Its role in humans remains unclear. Intriguingly, a nonsense variant (p.W358X) in human PNLIPRP2 is present in different ethnic populations at a high allele frequency of 0.3 to 0.5 [20]. When expressed in transfected HEK 293T cells, the truncated protein largely accumulated inside the cells as a detergent-insoluble aggregate and only a small amount was secreted into the medium [21]. The intracellular aggregates activated the unfolded protein response. The findings show that p.W358X PNLIPRP2 can alter cellular physiology through two mechanisms. First, the secretory defect results in a loss of function that might affect dietary fat digestion. Second, the intracellular aggregates of truncated PNLIPRP2 may result in a gain of function by placing pancreatic acinar cells at increased risk for injury through a maladaptive unfolded protein response. In combination with other stressors, the presence of PNLIPRP2 aggregates can activate cell death and inflammatory pathways leading to pancreatitis. A similar mechanism was reported for misfolding PRSS1 and carboxypeptidase A1 (CPA1) mutants, which appear to cause pancreatitis through endoplasmic reticulum stress [22]. Herein, we investigated whether the p.W358X PNLIPRP2 allele is a genetic risk factor for CP in patients with alcohol-related and non-alcohol-related CP.

Materials and methods
Nomenclature
Nucleotide numbering follows coding DNA numbering with the first nucleotide of the ATG translation initiation codon designated as +1. Amino acids are numbered starting with the initiator methionine of the primary translation product of PNLIPRP2. The NCBI genomic reference sequence for PNLIPRP2 (NC_000010.11, Homo sapiens chromosome 10, GRCh38.p12 primary assembly) and the NCBI coding DNA reference sequence (NM_005396.4) correspond to the minor truncation allele. In the present study, we used the major full-length PNLIPRP2 allele as reference for the designation of all PNLIPRP2 variants. In this manner, the nonsense p.W358X variant becomes the “effect” allele, which is the only biologically meaningful representation. Table 1 compares PNLIPRP2 variant designations using the two different reference sequences and lists the dbSNP numbers for unambiguous identification.

Study subjects
This study used de-identified genomic DNA samples from the registry of the Hungarian Pancreatic Study Group (ethical approval number TUKEB 22254-1/2012/EKU; biobanking approval number IF702-19/2012). Subjects were recruited from 11 Hungarian centers between
2012 and 2018 and all gave informed consent according to the ethical guidelines of the Declaration of Helsinki. The current study was also approved by the Institutional Review Board at Boston University (“Analysis of susceptibility genes in patients with chronic pancreatitis”; IRB number H-35382). A total of 256 unrelated patients with CP, including 152 with alcoholic CP and 104 with non-alcoholic CP and 200 control subjects with no pancreatic disease were analyzed. The CP study cohort included patients with a history of recurrent acute pancreatitis and/or pathological imaging findings consistent with CP, such as pancreatic calcifications, duct dilatation or irregularities, with or without exocrine pancreatic insufficiency or diabetes. Patient characteristics are described in Table 2. Alcoholic CP was diagnosed in CP cases with alcohol consumption of more than 80 g/day (men) or 60 g/day (women) for at least two years. De-identified pancreatic cDNA and matching genomic DNA samples (n = 9) from cadaveric donors were obtained from the University of Szeged, Hungary.

### DNA sequencing

Primer sequences and amplicon sizes are listed in Table 3. PCR reactions were performed using 1.0 U HotStar Taq DNA polymerase (Qiagen, Valencia, CA), 0.2 mM dNTP, 2.0 μL 10x PCR buffer (Qiagen), 0.5 μM primers, and 10–50 ng genomic DNA or cDNA template in a total volume of 20 μL. Cycling conditions were as follows: 15-min initial heat activation at 95 °C; 40 cycles of 30 s denaturation at 94 °C, 30 s annealing at 60 °C, and 60 s extension at 72 °C; and final extension for 5 min at 72 °C. Products were verified by 1.5% agarose gel electrophoresis. PCR amplicons (5 μL) were treated with 1 μL FastAP Thermosensitive Alkaline Phosphatase and 0.5 μL Exonuclease I (Thermo Fisher Scientific, Waltham, MA) for 15 min at 37 °C and the reaction was stopped by heating the samples to 85 °C for 15 min. Sanger sequencing was performed using the forward PCR primers as sequencing primer. Amplicons containing the heterozygous c.1070-379delG variant were also sequenced with the reverse primer.

### Table 1. Designation of PNLIPRP2 variants with respect to the NCBI reference sequence corresponding to the minor truncation allele and the full-length major allele used as the reference in this study.

<table>
<thead>
<tr>
<th>PNLIPRP2 region</th>
<th>dbSNP number</th>
<th>NCBI reference minor truncation allele</th>
<th>Reference used in this work major full-length allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intron 10</td>
<td>rs4751994</td>
<td>c.1070-379delG</td>
<td>c.1070-379delG</td>
</tr>
<tr>
<td>Intron 10</td>
<td>rs4751995</td>
<td>c.1070-321C&gt;T</td>
<td>c.1070-321T&gt;C</td>
</tr>
<tr>
<td>Exon 11</td>
<td>rs4751996</td>
<td>c.1074A&gt;G</td>
<td>p.X358W</td>
</tr>
<tr>
<td>Exon 11</td>
<td>rs10885997</td>
<td>c.1084A&gt;G</td>
<td>p.I362V</td>
</tr>
<tr>
<td>Exon 11</td>
<td>rs7910135</td>
<td>c.1181+55C&gt;A</td>
<td>c.1181+55A&gt;C</td>
</tr>
</tbody>
</table>

The truncation variant is highlighted in bold type.

https://doi.org/10.1371/journal.pone.0206869.t001

### Table 2. Study population.

<table>
<thead>
<tr>
<th></th>
<th>All CP n = 256</th>
<th>NACP n = 104</th>
<th>ACP n = 152</th>
<th>Controls n = 200</th>
</tr>
</thead>
<tbody>
<tr>
<td>number</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td></td>
<td>194</td>
<td>62</td>
<td>60</td>
<td>44</td>
</tr>
<tr>
<td>mean age at recruitment</td>
<td>56±10</td>
<td>56±14</td>
<td>57±12</td>
<td>57±16</td>
</tr>
<tr>
<td>mean age of disease onset</td>
<td>48±12</td>
<td>48±16</td>
<td>47±12</td>
<td>48±18</td>
</tr>
</tbody>
</table>

Age values indicate mean ± S.D. in years. CP, chronic pancreatitis, NACP, non-alcoholic chronic pancreatitis, ACP, alcoholic chronic pancreatitis.

https://doi.org/10.1371/journal.pone.0206869.t002
Results

A common truncation variant in PNLIPRP2

The common truncation variant c.1074G>A (p.W358X) in PNLIPRP2 was first described in 2003 as W357X in European, African and Chinese populations with allele frequencies of 0.53, 0.55 and 0.33, respectively [20]. A 2010 study on the association of common gene variants and human dietary habits described the variant as W358X (rs4751995) with similar allele frequencies [23]. The discrepancy in numbering is because the original cloning study of PNLIPRP2 missed one of the two consecutive Met codons at the start of the coding sequence [18]. Interestingly, the first Met is encoded by a separate upstream exon, which should be counted as exon 1 of the PNLIPRP2 gene; placing the truncation variant in exon 11. The NCBI reference sequence for PNLIPRP2 corresponds to the minor truncation allele. To describe the truncation variant in a biologically meaningful manner, in this study we used the major full-length PNLIPRP2 allele as reference (Table 1).

DNA sequence analysis of exon 11 of human PNLIPRP2

We genotyped 152 subjects with alcoholic CP, 104 subjects with non-alcoholic CP and 200 control subjects, recruited from the registry of the Hungarian Pancreatic Study Group. We used direct DNA sequencing after PCR amplification of exon 11 and flanking intronic regions of PNLIPRP2. Within the amplified 793 nt sequence, we found 6 nucleotide variants, which included three intronic variants (c.1070-379delG, c.1070-321T>C and c.1181+55A>C), one synonymous variant (c.1161G>A, p.S387 = ), one missense variant (c.1084G>A, p.V362I) and the truncation variant c.1074G>A (p.W358X) (Fig 1). The commonly occurring variants c.1070-321T>C, p.W358X, p.V362I, p.S387 = and c.1181+55A>C were found in linkage disequilibrium as a conserved haplotype (CAAAC in Fig 1). Another common haplotype (CGGAA in Fig 1) was formed by variants c.1070-321T>C and p.S387 = .

When allele frequency was considered, distribution of the variants between patients and controls showed no significant difference (Table 4). Subgroup analysis for alcoholic and non-alcoholic CP patients versus controls revealed no association either (Tables 5 and 6). We also analyzed genotypes using dominant and recessive models but found no significant differences in genotype frequencies between all CP patients or the alcoholic and non-alcoholic cohorts versus controls (Tables 7, 8 and 9). Finally, comparison of the three haplotypes between patients and controls yielded no significant differences with the exception of the CGGAA haplotype (see Fig 1), which was overrepresented in the non-alcoholic CP cohort relative to controls (OR 1.6, P = 0.04) (Tables 10, 11 and 12). We consider this a spurious association due to limited sample size and chance.

Expression of the PNLIPRP2 truncation allele

To estimate the relative mRNA expression of the full-length and truncation alleles of PNLIPRP2, we used direct sequencing of pancreatic cDNA after PCR amplification of a 732 nt
fragment of the coding DNA. We obtained nine de-identified cDNA samples with matching genomic DNA from cadaveric donors. Sequencing of the genomic DNA revealed five heterozygous samples and one sample homozygous for the truncation allele. The electropherograms of the heterozygous genomic sequences showed two signals at the position of variants p.W358X and p.V362I, with comparable peak heights (Fig 2A). Surprisingly, when heterozygous cDNA samples were sequenced, only one peak was visible at these positions, which corresponded to the major full-length allele, whereas no signal was apparent for the minor truncation allele (Fig 2B). PCR amplification of the pancreatic cDNA sample with the homozygous truncation allele confirmed the absence of detectable mRNA expression (Fig 2C).

Table 4. Allele frequency of PNLIPRP2 variants in patients with chronic pancreatitis (CP) and controls without pancreatic disease.

<table>
<thead>
<tr>
<th>PNLIPRP2</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
<th>CP patient alleles</th>
<th>Control alleles</th>
<th>OR</th>
<th>P value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intron 10</td>
<td>c.1070-379delG</td>
<td></td>
<td>2/512 (0.4%)</td>
<td>1/400 (0.3%)</td>
<td>1.6</td>
<td>0.72</td>
<td>0.14–17.3</td>
</tr>
<tr>
<td>Intron 10</td>
<td>c.1070-321T&gt;C</td>
<td></td>
<td>319/512 (62.3%)</td>
<td>240/400 (60%)</td>
<td>1.1</td>
<td>0.48</td>
<td>0.84–1.4</td>
</tr>
<tr>
<td>Exon 11</td>
<td>c.1074G&gt;A</td>
<td>p.W358X</td>
<td>245/512 (47.9%)</td>
<td>192/400 (48%)</td>
<td>0.99</td>
<td>0.97</td>
<td>0.77–1.3</td>
</tr>
<tr>
<td>Exon 11</td>
<td>c.1084G&gt;A</td>
<td>p.V362I</td>
<td>245/512 (47.9%)</td>
<td>192/400 (48%)</td>
<td>0.99</td>
<td>0.97</td>
<td>0.77–1.3</td>
</tr>
<tr>
<td>Exon 11</td>
<td>c.1161G&gt;A</td>
<td>p.S387=</td>
<td>321/512 (62.7%)</td>
<td>240/400 (60%)</td>
<td>1.1</td>
<td>0.4</td>
<td>0.86–1.5</td>
</tr>
<tr>
<td>Intron 11</td>
<td>c.1181+55A&gt;C</td>
<td></td>
<td>246/512 (48%)</td>
<td>192/400 (48%)</td>
<td>1</td>
<td>0.99</td>
<td>0.77–1.3</td>
</tr>
</tbody>
</table>

The truncation variant is highlighted in bold type. OR, odds ratio; CI, confidence interval.
together, our observations indicate that the truncation allele is not expressed at the mRNA level to a significant extent, in all likelihood due to nonsense-mediated mRNA decay.

We also consulted the Genotype-Tissue Expression (GTEx) Portal (www.gtexportal.org/home) and found that all five common variants within the truncation haplotype were
Table 8. Genotype distribution of *PNLIPRP2* variants in patients with non-alcoholic chronic pancreatitis (NACP) and in controls.

<table>
<thead>
<tr>
<th><em>PNLIPRP2</em></th>
<th>Nucleotide change</th>
<th>Genotype</th>
<th>NACP patients</th>
<th>Controls</th>
<th>OR</th>
<th>P value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intron 10</td>
<td>c.1070-379delG</td>
<td>GG</td>
<td>103/104 (99%)</td>
<td>199/200 (99.5%)</td>
<td>1.9</td>
<td>0.64</td>
<td>0.12–31.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>delG</td>
<td>0/104 (0%)</td>
<td>1/200 (0.5%)</td>
<td>1.9</td>
<td>0.75</td>
<td>0.04–97.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>deldel</td>
<td>12/104 (11.5%)</td>
<td>27/200 (13.5%)</td>
<td>1.2</td>
<td>0.63</td>
<td>0.58–2.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>39/104 (37.5%)</td>
<td>106/200 (53%)</td>
<td>1.2</td>
<td>0.49</td>
<td>0.73–2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>82/104 (80.0%)</td>
<td>67/200 (33.5%)</td>
<td>1.0</td>
<td>0.063</td>
<td>0.58–1.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1/200 (0.5%)</td>
<td>27/200 (13.5%)</td>
<td>1.0</td>
<td>0.17</td>
<td>0.33–1.2</td>
</tr>
</tbody>
</table>

Exon 11    
c.1074G>A  
Exon 11    
c.1084G>A  
Exon 11    
c.1161G>A  
Intron 11  
c.1181+55A>C

Data were analyzed assuming dominant (shown in italics) or recessive models of inheritance. The truncation variant is highlighted in bold type. OR, odds ratio; CI, confidence interval.

https://doi.org/10.1371/journal.pone.0206869.t008

Table 9. Genotype distribution of *PNLIPRP2* variants in patients with alcoholic chronic pancreatitis (ACP) and in controls.

<table>
<thead>
<tr>
<th><em>PNLIPRP2</em></th>
<th>Nucleotide change</th>
<th>Genotype</th>
<th>ACP patients</th>
<th>Controls</th>
<th>OR</th>
<th>P value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intron 10</td>
<td>c.1070-379delG</td>
<td>GG</td>
<td>151/152 (99.3%)</td>
<td>199/200 (99.5%)</td>
<td>1.3</td>
<td>0.85</td>
<td>0.08–21.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>delG</td>
<td>1/152 (0.7%)</td>
<td>1/200 (0.5%)</td>
<td>1.3</td>
<td>0.89</td>
<td>0.03–66.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>deldel</td>
<td>6/152 (4.0%)</td>
<td>67/200 (33.5%)</td>
<td>0.79</td>
<td>0.44</td>
<td>0.44–1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>12/152 (7.9%)</td>
<td>106/200 (53%)</td>
<td>1.3</td>
<td>0.2</td>
<td>0.86–2.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>12/152 (7.9%)</td>
<td>67/200 (33.5%)</td>
<td>1.3</td>
<td>0.2</td>
<td>0.86–2.1</td>
</tr>
</tbody>
</table>

Exon 11    
c.1074G>A  
Exon 11    
c.1084G>A  
Exon 11    
c.1161G>A  
Intron 11  
c.1181+55A>C

Data were analyzed assuming dominant (shown in italics) or recessive models of inheritance. The truncation variant is highlighted in bold type. OR, odds ratio; CI, confidence interval.

https://doi.org/10.1371/journal.pone.0206869.t009

Table 10. Distribution of common *PNLIPRP2* haplotype alleles in patients with chronic pancreatitis (CP) and in controls.

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>All CP patients</th>
<th>Controls</th>
<th>OR</th>
<th>P value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAAAC</td>
<td>244/512 (47.7%)</td>
<td>191/400 (47.8%)</td>
<td>1.0</td>
<td>0.98</td>
<td>0.77–1.3</td>
</tr>
<tr>
<td>CGGAA</td>
<td>73/512 (14.3%)</td>
<td>48/400 (12.0%)</td>
<td>1.2</td>
<td>0.32</td>
<td>0.83–1.8</td>
</tr>
<tr>
<td>TGGGA</td>
<td>191/512 (37.3%)</td>
<td>160/400 (40.0%)</td>
<td>0.89</td>
<td>0.41</td>
<td>0.68–1.2</td>
</tr>
</tbody>
</table>

The truncation haplotype is highlighted in bold type. OR, odds ratio; CI, confidence interval. See Fig 1 for more details.

https://doi.org/10.1371/journal.pone.0206869.t010
associated with diminished PNLIPRP2 mRNA expression (Fig 3). The GTEx database is an open-access public resource to study tissue-specific gene expression and its relationship to genetic variation. The project analyzes global RNA expression within individual human tissues from deeply genotyped donors and correlates variations in gene expression with genetic alterations.

Discussion

Physicians have increasingly recognized that CP is a complex disorder associated with multiple risk factors [24]. For many, particularly children, genetic variants in genes encoding pancreatic digestive enzymes contribute to the pathophysiology of CP [6]. In this study, we sought to determine if a common genetic variant in PNLIPRP2 increased the risk for CP. The variant introduces a premature stop codon, p.W358X, resulting in a truncated protein and in vitro evidence suggests the expressed protein misfolds and activates the unfolded protein response [21]. We found no correlation of variant p.W358X with CP as a group or sub-grouped into alcoholic CP or non-alcoholic CP. This finding demonstrates that p.W358X is not a significant genetic risk factor for CP. We identified additional variants within exon 11 and the flanking intronic regions of PNLIPRP2, which formed conserved haplotypes. When these haplotypes were analyzed for disease association, we observed enrichment of the CGGAA haplotype (see Fig 1) in the non-alcoholic CP cohort. However, statistical significance was barely reached and we interpret this finding as fortuitous association due to the small sample size.

Because the presumed mechanism whereby variant p.W358X would contribute to CP is by activating maladaptive unfolded protein response and cell death pathways, we sought to determine if expression of the p.W358X allele was lower than expression of full length PNLIPRP2. If so, the levels of truncated protein may not be sufficient to activate the unfolded protein response. We accomplished this goal in two ways. First, we PCR amplified PNLIPRP2 from pancreatic cDNA of heterozygous and homozygous p.W358X carriers and analyzed expression by DNA sequencing and agarose gel electrophoresis. Second, we interrogated the GTEx Portal database. Both methods confirmed that the amount of mRNA encoding p.W358X PNLIPRP2 is quite low compared to the mRNA amounts for full-length PNLIPRP2. The results suggest that the mRNA encoding the p.W358X variant undergoes nonsense-mediated decay [25]. In the previous study that characterized the cellular effects of the p.W358X variant the authors

Table 11. Distribution of common PNLIPRP2 haplotype alleles in patients with non-alcoholic chronic pancreatitis (NACP) and in controls.

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>NACP patients</th>
<th>Controls</th>
<th>OR</th>
<th>(P) value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAAAC</td>
<td>92/208 (44.2%)</td>
<td>191/400  (47.8%)</td>
<td>0.87</td>
<td>0.41</td>
<td>0.62–1.2</td>
</tr>
<tr>
<td>CGGAA</td>
<td>38/208 (18.3%)</td>
<td>48/400  (12.0%)</td>
<td>1.6</td>
<td>0.040*</td>
<td>1.0–2.6</td>
</tr>
<tr>
<td>TGGGA</td>
<td>77/208 (37.0%)</td>
<td>160/400 (40.0%)</td>
<td>0.88</td>
<td>0.48</td>
<td>0.62–1.3</td>
</tr>
</tbody>
</table>

The truncation haplotype is highlighted in bold type. OR, odds ratio; CI, confidence interval. See Fig 1 for more details. The asterisk indicates significant association.

https://doi.org/10.1371/journal.pone.0206869.t011

Table 12. Distribution of common PNLIPRP2 haplotype alleles in patients with alcoholic chronic pancreatitis (ACP) and in controls.

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>ACP patients</th>
<th>Controls</th>
<th>OR</th>
<th>(P) value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAAAC</td>
<td>152/304 (50.0%)</td>
<td>191/400  (47.8%)</td>
<td>1.1</td>
<td>0.55</td>
<td>0.81–1.5</td>
</tr>
<tr>
<td>CGGAA</td>
<td>35/304 (11.5%)</td>
<td>48/400  (12.0%)</td>
<td>0.95</td>
<td>0.84</td>
<td>0.60–1.5</td>
</tr>
<tr>
<td>TGGGA</td>
<td>114/304 (37.5%)</td>
<td>160/400 (40.0%)</td>
<td>0.90</td>
<td>0.5</td>
<td>0.66–1.2</td>
</tr>
</tbody>
</table>

The truncation haplotype is highlighted in bold type. OR, odds ratio; CI, confidence interval. See Fig 1 for more details.

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used artificial cDNA expression constructs, which lacked introns [21]. Consequently, the

PNLIPRP2 mRNA encoding the truncation variant did not suffer degradation and protein
expression levels achieved were high enough to induce the unfolded protein response. The present data strongly argue that this cannot be the case when variant p.W358X is expressed from its native gene in the acinar cells.

Given the low levels of mRNA expression, it is unlikely that p.W358X PNLIPRP2 causes disease through gain-of-function as suggested by studies in transfected tissue culture cells [21]. In retrospect, it seems reasonable to have predicted that p.W358X PNLIPRP2 should not be a significant risk factor for CP or another disease since it is so prevalent. More likely, any effect of p.W358X PNLIPRP2 on human health should result from loss-of-function. Humans harbor many genetic variants predicted to cause loss-of-function [26]. Homozygosity for loss-of-function variants either results in a non-fatal phenotype or represent benign variations in redundant genes. A non-fatal loss-of-function phenotype was found in Pnliprp2-deficient mice [19]. Suckling Pnliprp2-deficient mice had fat malabsorption and poor growth but survived to adulthood and were fertile. It is not known if a similar effect occurs in human infants homozygous for p.W358X PNLIPRP2. In humans, p.W358X PNLIPRP2 may represent a loss-of-function tolerant genetic variant with the loss of its lipase activity compensated by other lipases [16]. Alternatively, p.W358X PNLIPRP2 may represent a protective or disease modifying allele [27]. That is, homozygosity for this allele may confer protection against disease or modify adaptations to diet [23]. Determination of the importance of the common p.W358X PNLIPRP2 allele in human health will require additional investigations.

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**References**


