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Carboxyl ester lipase hybrid 1 (*CEL-HYB1*) haplotypes confer varying risk for chronic pancreatitis

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The *CEL-HYB1* hybrid allele of the carboxyl ester lipase (*CEL*) gene and its pseudogene (*CELP*) has been associated with chronic pancreatitis (CP). Recent work indicated that amino acid positions 488 and 548 in *CEL-HYB1* determined pathogenicity. Haplotype Thr488-Ile548 was associated with CP while haplotypes Thr488-Thr548 and Ile488-Thr548 were benign. However, functional analysis revealed that Thr488 is the primary determinant of CEL-HYB1 misfolding and associated endoplasmic reticulum (ER) stress. To address this contradiction, we analyzed a cohort from Hungary and found significantly increased *CEL-HYB1* carrier frequency in CP cases (9/319, 2.8%) *versus* controls (5/618, 0.8%), yielding an odds ratio of 3.6 (95% confidence interval 1.2–10.7, $P = 0.024$). All *CEL-HYB1* positive carriers from Hungary had the Thr488-Thr548 haplotype. We analyzed the haplotype distribution of reported *CEL-HYB1* carriers from three European cohorts and found that 14/29 CP cases from Germany and 2/6 CP cases from Poland carried the Thr488-Ile548 haplotype, which was absent in *CEL-HYB1* positive controls from Germany (n = 13) and Poland (n = 8). All patients (n = 17) and controls (n = 9) from France carrying *CEL-HYB1* contained the Thr488-Thr548 haplotype. Functional studies using transfected cells indicated that both *CEL-HYB1* haplotypes induced significant ER stress and the Thr488-Ile548 haplotype had a stronger effect. We conclude that the Thr488-Thr548 haplotype of *CEL-HYB1* is widespread in Europe and increases CP risk by almost fourfold. In contrast, the Thr488-Ile548 haplotype is regionally restricted, but confers markedly stronger CP risk.

Keywords Pancreatitis, Genetic association study, Lipase, Misfolding, Endoplasmic reticulum stress

Genetic variants of digestive enzymes may increase risk for chronic pancreatitis (CP), the relapsing, progressive inflammatory disease of the pancreas[1]. There are two major mechanistic groups of risk variants; one is associated with increased intrapancreatic activity of the digestive protease trypsin (trypsin-dependent pathway of genetic risk)[2], the other is with proteotoxicity due to mutation-induced misfolding and consequent endoplasmic reticulum (ER) stress (misfolding-dependent pathway)[3]. Susceptibility genes in the trypsin-dependent pathway include *PRSS1*, *PRSS2*, *SPINK1*, *CTRC*, and *CTRB1-CTRB2*, while genes associated with misfolding variants comprise *CPA1*, *PRSS1*, *PNLIP*, *CTRC*, and *CEL*. The *CEL* codes for carboxyl ester lipase, a high-abundance

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enzyme expressed in the pancreas and the lactating mammary gland[4]. Single-nucleotide deletions in the first and fourth repeat of the C-terminal variable number of tandem repeats (VNTR) region of *CEL* cause the exceedingly rare autosomal dominant genetic disorder named "maturity-onset diabetes of the young 8" (MODY8), which is characterized by not only diabetes but also by exocrine pancreatic insufficiency due to CP and adipose replacement of the acinar tissue[5]. More recently, MODY8 has been considered as a particular subgroup of hereditary pancreatitis, which is typically associated with *PRSS1* variants[6].

CEL is highly polymorphic, prone to genomic rearrangements such as duplication, deletion, and recombination with its adjacent pseudogene *CELP*[4]. In 2015, a hybrid allele (*CEL-HYB1*) was discovered that consisted of exons 1–10 of *CEL* fused with exon 11' of *CELP*. *CEL-HYB1* encoded a functional enzyme but with a shortened and altered VNTR sequence[7]. When expressed in transfected cells, the CEL-HYB1 protein exhibited characteristic signs of misfolding, such as reduced secretion, intracellular retention and aggregation, cellular ER stress, and caused CP in transgenic mice[7–11]. Initial analysis of a small CP cohort with familial disease indicated significant enrichment of *CEL-HYB1* in CP cases (14%) relative to healthy controls (1%), suggesting that *CEL-HYB1* might be a strong risk factor for CP, as judged by the odds ratio (OR) of 15.5[7]. Replication studies in 3 European cohorts from Germany and France demonstrated smaller but still impressive effect sizes with an average OR of 5.2[7].

Follow-up studies, however, painted a more complex picture. First, it became apparent that *CEL-HYB1* was not found in East-Asian populations where a different fusion, *CEL-HYB2* was prevalent[12]. The *CEL-HYB2* allele, in which exons 1–9 of *CEL* are fused with exons 10'-11' of *CELP,* showed no association with CP, likely due to the degradation of its transcript via nonsense-mediated mRNA decay[12,13]. Second, a replication study in a pediatric cohort from Poland found no association of *CEL-HYB1* with CP, although a twofold enrichment in CP cases was observed with no statistical significance[14,15]. Interestingly, this analysis also found higher carrier frequency (2.4%) in the control population than the original report (range 0.7–1%). Third, a more recent study demonstrated that *CEL-HYB1* occurs as three haplotypes defined by amino-acids 488 and 548[8]. Remarkably, the distribution of the Thr488-Thr548 haplotype was similar in CP patients (34/55) and controls (18/20), whereas the Thr488-Ile548 haplotype was found only in CP patients (20/55) and never in controls. Haplotype Ile488-Thr548 was detected both in patients (1/55) and controls (2/20). Haplotypes Thr488-Thr548 and Thr488-Ile548 showed a similarly strong misfolding phenotype in cell culture experiments, while the Ile488-Thr548 haplotype had a minor effect, suggesting that Thr488 is the crucial determinant of misfolding. Introduction of Thr488 to full-length CEL also resulted in reduced secretion. The authors proposed that the Thr488-Ile548 haplotype of *CEL-HYB1* was pathogenic while the Thr488-Thr548 haplotype was benign or associated with much lower risk.

Given the somewhat incongruent recent observations, new replication studies are required to resolve apparent contradictions concerning the effect size and haplotype distribution of the *CEL-HYB1* allele in CP. In this study, we performed a case–control study and haplotype analysis on a genetically well-characterized, ethnically homogenous CP cohort from Hungary, and determined the haplotypes of all available *CEL-HYB1* carriers in the published cohorts from Germany, Poland, and France.

Methods

Study participants

For the case–control study, de-identified genomic DNA samples were obtained from the Hungarian National Pancreas Registry (ethical approval: TUKEB 36305-1/2016/EKU, biobanking approval: IF702–19/2012). Participants 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. In total, 319 unrelated patients with CP, including 134 with nonalcoholic CP (age at recruitment 58.66 ± 13.6 years, mean ± SD, range 22–85) and 185 with alcoholic CP (55.52 ± 9.9 years, range 23–79 years), and 618 control participants (40.98 ± 14.7 years, range 11–89 years) with no pancreatic disease were enrolled. Diagnosis of CP was based on the history of recurrent acute pancreatitis or recurrent abdominal pain typical for CP with pathological imaging findings consistent with CP, such as pancreatic calcifications, duct dilatation or irregularities, with or without exocrine pancreatic insufficiency or diabetes. Alcoholic CP was diagnosed when the patient's history included alcohol consumption of more than 80 g/day (men) or 60 g/day (women) for at least two years.

For analyses of haplotype distribution, *CEL-HYB1* carriers from Germany (cases n = 29, controls n = 13), France (cases n = 17, controls n = 9), and Poland (cases n = 6, controls n = 8) were used. Except for 4 CP cases from Germany and 8 CP cases from France, these *CEL-HYB1* positive participants were reported previously[7,14].

Reference sequence

Nucleotide numbering corresponds to the coding DNA sequence of *CEL*, with the first nucleotide of the ATG translation initiation codon denoted as + 1. Amino-acid numbering starts with the initiator methionine of the primary translation product. *CEL* genomic (chromosome 9) reference sequence: NC_000009.12 (ENSG00000170835). Note that prior studies used the AF072711.1 reference sequence, which differs from NC_000009.12 at several positions.

Detection of the *CEL-HYB1* allele

Screening for the *CEL-HYB1* allele was performed using the LightCycler-based assay reported in[7] on a CFX96 Touch Real-Time PCR Detection System. The reaction mix contained 1 × LC FastStart DNA Master^{PLUS} HybProbe Master Mix (Roche Diagnostics), 0.5 µM LC-forward primer, 0.5 µM LC-reverse primer, 0.18 µM LC-probe (TIB Molbiol), in a final volume of 10 µL. Cycle conditions were as follows: initial denaturation for 10 min at 95 °C; 45 cycles of 10 s denaturation at 95 °C, 10 s annealing at 60 °C and 15 s extension at 72 °C; followed by melting curve analysis. Confirmation of *CEL-HYB1* positive samples was carried out by long-range, duplex PCR, using the L11F, IAR, and CELP-VNTR-R primers, as detailed in[7].

Haplotype identification

The *CEL-HYB1* allele was amplified with the L11F and CELP-VNTR-R primers. The PCR amplicons (5 µL) were treated with 1 µL FastAP thermosensitive alkaline phosphatase and 0.5 µL exonuclease I (Thermo Fisher Scientific) 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 S10F and K11F sequencing primers, as given in[7].

Genotyping the *CEL* c.1463 T > C (p.Ile488Thr) variant

We used a restriction fragment length polymorphism (RFLP) assay to genotype the p.Ile488Thr variant in *CEL*. Briefly, exon 10 and flanking intronic sequences of the *CEL* gene were amplified using 0.6 U HotStarTaq DNA Polymerase (Qiagen), 0.2 mM dNTP, 0.5 µM CEL-10-F1 primer (5'-TAA GGC CAG ACA CAG TAG CTC-3'), S10R[7] primer, 10 × PCR buffer (Qiagen), and 10–50 ng genomic DNA template, in a final volume of 20 µL. The 1116 bp amplicon was digested with DpnII restriction enzyme (New England Biolabs), and the digestion products were visualized on a 2% agarose gel with ethidium bromide staining. The wild-type *CEL* amplicon was digested into 4 fragments (663, 216, 176, and 61 bp), while the variant *CEL* amplicon was cleaved into 3 pieces (663, 392, and 61 bp) by DpnII. We found that in some samples that contained the c.1296-48C > T *CEL* variant the digestion pattern changed and the wild-type *CEL* was digested into 3 fragments (879, 176, and 61 bp) while the variant amplicon was cleaved into 2 fragments (1055 and 61 bp). Amplicons containing the p.Ile488Thr variant were also verified by Sanger sequencing using the CEL-10-F1 and S10R primers.

Functional studies

The pcDNA3 plasmids containing different *CEL-HYB1* haplotypes were reported previously[8]. Relative to the *CEL* reference sequence, the expression plasmids contained the synonymous variant c.1497C > T (p.Asp499 =). HEK 293T cells were cultured and transfected with 4 µg total plasmid DNA (4 µg *CEL-HYB1* expression plasmid or 1 µg expression plasmid plus 3 µg empty vector, as indicated) and 5 µL of Lipofectamine 2000 (Life Technologies), as reported recently[16]. Cells were incubated overnight (~ 24 h), and the transfection mix was replaced with 1.5 mL of OptiMEM reduced serum medium (Life Technologies). Cells were collected for analysis 48 h later. Total RNA was isolated, reverse-transcribed, and *HSPA5* levels were measured by quantitative PCR, as reported previously[16].

RESULTS

Association of *CEL-HYB1* with chronic pancreatitis in Hungarians

We investigated the *CEL-HYB1* allele in 319 CP patients from Hungary (185 alcoholic, 134 non-alcoholic) and 618 ethnically matched controls without pancreatic disease. We found a significant overrepresentation of *CEL-HYB1* in patients *versus* controls (carrier frequency 9/319 vs. 5/618, OR = 3.6, 95% CI 1.2–10.7, $P = 0.024$) (Table 1). Subgroup analysis of alcoholic (6/185, 3.2%) and non-alcoholic (3/134, 2.2%) patients showed no substantial difference in *CEL-HYB1* carrier frequency (Table 1). The lack of significance in case of NACP may be explained by the lower number of patients in this subgroup. All *CEL-HYB1* positive participants were heterozygous. Sequencing exons 2 and 3 of *PRSS1*, exon 3 of *SPINK1*, exons 2, 3, and 7 of *CTRC,* exons 7, 8, and 10 of *CPA1*, and exons 4 and 11 of *CFTR* in the *CEL-HYB1* carriers revealed no CP risk variants, aside from the commonly occurring c.180C > T (p.Gly60 =) synonymous *CTRC* variant, which was found in 2/9 patients (1 homozygous, 1 heterozygous) and 3/5 controls (heterozygous).

CEL-HYB1 haplotype analysis

To assess the haplotype distribution in the cohort from Hungary, we sequenced exons 10 and 11' of the *CEL-HYB1* allele in all carriers (Table 2). Unexpectedly, we found that all cases and controls carried the Thr488-Thr548 haplotype of *CEL-HYB1*. No other *CEL-HYB1* haplotype was detected. To compare the *CEL-HYB1* haplotype distribution in Hungarians to other European populations, we sequenced the previously reported *CEL-HYB1* alleles in cohorts from Germany, France, and Poland (Table 2) ref[7,14]. We found that approximately half of the CP patients from Germany (14/29) and one third of the cases from Poland (2/6) carried the Thr488-Ile548 haplotype, while all patients from France (17/17) carried the Thr488-Thr548 haplotype. All controls from Germany (n = 13), Poland (n = 8), and France (n = 9) carried the Thr488-Thr548 haplotype. Thus, as reported previously[8], the Thr488-Ile548 haplotype was never found in *CEL-HYB1* positive controls. Of the 29 patients from Germany, family history was available for 21 participants. At least one other family member was affected in 8 of these patients. Interestingly, we found the Thr488-Ile548 haplotype in 75% (6/8) of patients with a family

Cohorts	Affected/Total (%)	OR [95% CI]	*P*
CP	9/319 (2.8%)	3.6 [1.2–10.7]	0.024
control	5/618 (0.8%)		
Subgroups			
ACP	6/185 (3.2%)	4.1 [1.2–13.6]	0.021
NACP	3/134 (2.2%)	2.8 [0.7–11.9]	0.161

Table 1. Carrier frequency of the *CEL-HYB1* allele in the cohort from Hungary. All participants were heterozygous. *CP* chronic pancreatitis, *ACP* alcoholic chronic pancreatitis, *NACP* non-alcoholic chronic pancreatitis, *OR* odds ratio, *CI* confidence interval.

Haplotype	Hungary		France		Germany		Poland	
	CP	control	CP	control	CP	control	CP	control
Thr488 Ile548	0/9 (0%)	0/5 (0%)	0/17 (0%)	0/9 (0%)	14/29 (48.3%)	0/13 (0%)	2/6 (33.3%)	0/8 (0%)
Thr488 Thr548	9/9 (100%)	5/5 (100%)	17/17 (100%)	9/9 (100%)	15/29 (51.7%)	13/13 (100%)	4/6 (66.7%)	8/8 (100%)

Table 2. Haplotype distribution of *CEL-HYB1* in European cohorts. *CP* chronic pancreatitis.

history but only in 23% (3/13) without a family history. We did not identify the previously reported Ile488-Thr548 haplotype[8,17] or the putative Ile488-Ile548 haplotype in any of the *CEL-HYB1* alleles sequenced.

CEL variant c.1463 T > C (p.Ile488Thr) in Hungary

Since both pathogenic *CEL-HYB1* alleles contained Thr488, we tested whether this variation might increase CP risk when present in the full-length *CEL*, which typically contains Ile488. Using RFLP analysis, we investigated CP patients and controls that were negative for the *CEL-HYB1* allele and found the *CEL* variant p.Ile488Thr in 1/309 CP cases (0.3%) and in 2/611 controls (0.3%). Thus, the presence of Thr488 in *CEL* is rare and shows no association with CP (OR 1, 95% CI 0.1–11, $P=0.993$).

Functional analysis of *CEL-HYB1* haplotypes

A previous study analyzed the functional effects of the Thr488-Ile548 and Thr488-Thr548 haplotypes in transfected cells and found largely comparable effects concerning defective secretion, intracellular aggregation, and induction of ER stress[8]. Using the same expression constructs, we re-analyzed the effects of the *CEL-HYB1* haplotypes on the expression of the ER master chaperone *HSPA5* (BiP) in transiently transfected HEK 293T cells. We used reverse-transcription quantitative PCR instead of the previously employed Western blotting because of its higher sensitivity to detect smaller differences. Relative to cells transfected with empty vector, both *CEL-HYB1* haplotypes increased *HSPA5* expression significantly (Fig. 1). Using either 1 µg or 4 ug *CEL-HYB1* expression plasmid for transfection, we consistently observed significantly higher *HSPA5* expression in cells transfected with the Thr488-Ile548 haplotype *versus* the Thr488-Thr548 haplotype, although the difference was small.

Discussion

In the present study, we investigated the association of the *CEL-HYB1* allele with CP in Hungary and characterized the haplotypes responsible for disease risk. The experiments were inspired by recent observations suggesting that only the Thr488-Ile548 haplotype might be pathogenic whereas other *CEL-HYB1* haplotypes, the Thr488-Thr548 haplotype in particular, are probably less severe or innocuous variants [8]. Since most previously published studies on *CEL-HYB1* did not analyze the haplotype distribution, new replication studies and re-analysis of previously published cohorts were warranted. In our case–control study, we found a clear enrichment of *CEL-HYB1* in CP cases from Hungary with similar frequency in alcoholic and non-alcoholic CP. The effect size, as judged by the OR, was 3.6-fold, which is comparable to the impact of other important CP risk genes, such as heterozygous *CTRC* variants, or heterozygous severe *CFTR* variants such as p.Phe508del[1,2,18]. The OR obtained in our study was slightly smaller than those reported by Fjeld et al. (2015)[7] for 2 non-alcoholic CP cohorts from Germany (OR 5 and 6.6), but it replicates the OR of the cohort from France (OR 3.5). Taken together, our observations confirm that *CEL-HYB1* is an important risk variant and argue that genetic testing of CP patients should include screening for this allele.

Haplotype analysis of *CEL-HYB1* carriers from Hungary indicated that only the Thr488-Thr548 haplotype was present in our cohort. This finding was surprising in light of the recent proposal that the Thr488-Thr548 haplotype was not associated with CP risk[8]. To resolve this contradiction, we sequenced all available *CEL-HYB1* alleles from the published carriers from Germany, France, and Poland[7,14]. The results indicated that about half of the participants from Germany and one third of the participants from Poland carried the Thr488-Ile548 haplotype whereas carriers from France were harboring the Thr488-Thr548 haplotype only. As reported previously, the Thr488-Ile548 haplotype was never found in *CEL-HYB1* positive controls, indicating that this variant is a strong risk factor and may be considered disease-causing. Since cohorts from Hungary and France carry only the Thr488-Thr548 haplotype and disease association of *CEL-HYB1* was clearly demonstrated in both cohorts, the observations confirm that the Thr488-Thr548 haplotype is pathogenic with a smaller but still respectable effect size. The discrepancy in haplotype distribution likely explains the previously measured higher OR values in the cohorts from Germany *versus* the cohort from France [7], which was almost identical to the Hungarian value.

Several studies reported family pedigrees where pancreatitis seemed to segregate with *CEL-HYB1*[7,9,14]. Fjeld et al. (2015) found an OR value of 15 in their discovery cohort of familial CP cases[7]. Similarly, the *CEL-HYB1* allele frequency in the familial subgroup of CP patients from Poland was relatively high (3/20)[14]. It is possible, even likely, that these families carried the Thr488-Ile458 haplotype, resulting in the relatively high penetrance observed.

The carrier frequency of *CEL-HYB1* with the Thr488-Thr548 haplotype in controls from Hungary (0.8%) was similar to those reported in controls from Germany and France (range 0.7–1%)[7]. Similarly, a recent US study investigated the association of *CEL-HYB1* with pancreatic cancer and reported 4/1045 (0.4%) carriers with Thr488 in their control population of largely European origin[17]. This allele likely corresponded to the Thr488-Thr548 haplotype,

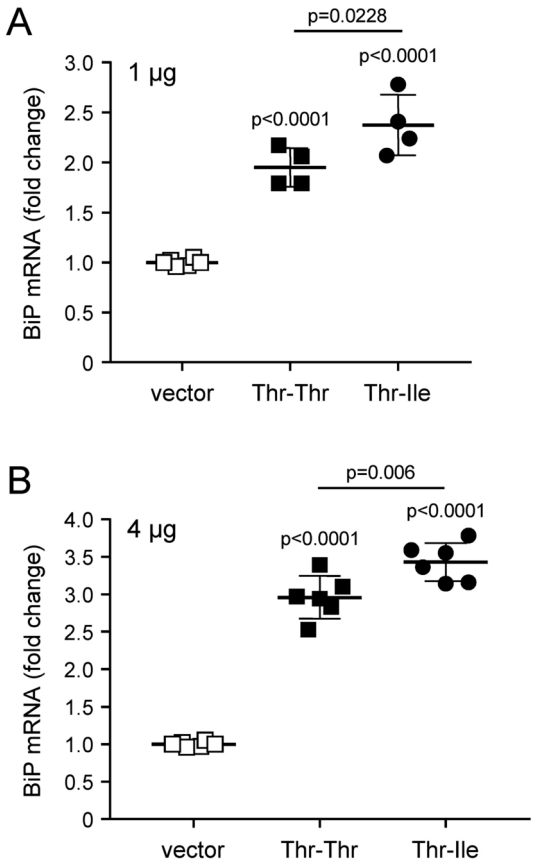

Fig. 1. Effect of *CEL-HYB1* haplotypes Thr488-Thr548 and Thr488-Ile548 on the endoplasmic reticulum stress marker HSPA5 (BiP) in transfected HEK 293T cells. Expression of *HSPA5* mRNA was measured by reverse transcription quantitative PCR and expressed as fold change relative to the mean value from cells transfected with empty vector. The difference of means was analyzed by one-way ANOVA followed by Tukey's post-hoc test. (**A**), Transfections were performed with 1 µg expression plasmid and 3 µg empty vector (4 µg total plasmid DNA). (**B**), Transfections were performed with 4 µg expression plasmid.

although the p.548 position was not analyzed. Curiously, the carrier frequency in controls from Poland was much higher (2.4%), which might explain the lack of significant association of *CEL-HYB1* with CP in that study, assuming the high detection rate in controls was somehow erroneous. We did not find the previously reported Ile488-Thr548 haplotype[8] in any of the *CEL-HYB1* positive samples analyzed in this study, which raises the possibility that this variant might not exist. However, the US study on pancreatic cancer also reported 12/1045 (1.1%) *CEL-HYB1* carriers with Ile488 in their control group, which appears to represent the Ile488-Thr548 haplotype[17]. The contradictory results suggest that the complex and polymorphic nature of the *CEL* locus might result in technical errors when genotyping *CEL-HYB1* variants. Finally, we note that the putative Ile488-Ile548 *CEL-HYB1* haplotype has never been found, although its functional properties have been reported[8].

The reason for the large difference in the clinical impact of the two *CEL-HYB1* haplotypes is not readily apparent. *CEL-HYB1* has been postulated to exert its pathogenic effect via the so-called misfolding-dependent pathological pathway of CP genetic risk[2]. Risk variants of this pathway induce misfolding of abundantly expressed digestive enzymes, and cause reduced secretion, intracellular retention with aggregation, and ER stress. Carboxypeptidase A1 (*CPA1*) variants, and a subset of *PRSS1* (cationic trypsinogen) variants are the most frequently found examples of this group[2,16,19,20]. Cassidy et al. (2020) elegantly demonstrated that CEL-HYB1 exhibits all characteristics of a misfolding risk variant, however, the authors found only minimal differences between the effects of the Thr488-Thr548 and Thr488-Ile548 haplotypes[8]. Here, we confirmed this notion, as both haplotypes caused marked ER stress in transfected cells with a slightly higher effect recorded for the Thr488-Ile548 haplotype. It is difficult to explain how such a small functional difference might lead to the more severe clinical picture. Recent studies on the functional determinant of pathogenicity of *CPA1* variants found a surprising threshold effect with respect to secretion defect and ER stress[16,19]. Thus, only *CPA1* variants that caused essentially complete loss of secretion and high ER stress were associated with CP, whereas variants that exhibited low but measurable secretion and variable magnitude of ER stress were considered benign. Based on this paradigm, it seems possible that even minor functional differences between variants might result in dissimilar clinical outcomes. As a caveat, we and others[8] note that the haplotypes might affect mRNA expression of *CEL-HYB1* in the human pancreas, resulting in altered levels of the toxic protein products and associated ER stress.

Wild-type *CEL* contains Ile488, whereas both pathogenic *CEL-HYB1* haplotypes carry Thr488. To investigate whether the presence of Thr488 alone might render *CEL* pathogenic, we genotyped CP cases and controls for the p.Ile488Thr variant and found that the variant was rare (0.3%) and evenly distributed in the patient and control groups. The results indicate that Thr488 becomes pathogenic only in the context of *CEL-HYB1*. Kawamoto et al. (2022) reported the same variant in 5/1045 (0.5%) in a US population without pancreatic disease[17].

In summary, we demonstrated that the Thr488-Thr548 haplotype of *CEL-HYB1* increases CP risk by 3.6-fold in Hungarians. The results confirm that this haplotype is pathogenic and should be screened for during genetic testing of CP cases. We also replicated the observation that the Thr488-Ile548 haplotype is a stronger risk factor for CP. Finally, we note that while the Thr488-Thr548 haplotype is widespread in Europe, the Thr488-Ile548 haplotype has been identified only in Poland and Germany so far.

Data availability

The data that support the findings of this study are available from the corresponding author upon request.

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References

1. Mayerle, J. et al. Genetics, cell biology, and pathophysiology of pancreatitis. *Gastroenterology* **156**, 1951–1968 (2019).
2. Hegyi, E. & Sahin-Tóth, M. Genetic risk in chronic pancreatitis: The trypsin-dependent pathway. *Dig Dis. Sci.* **62**, 1692–1701 (2017).
3. Sahin-Tóth, M. Genetic risk in chronic pancreatitis: the misfolding-dependent pathway. *Curr. Opin. Gastroenterol.* **33**, 390–395 (2017).
4. Johansson, B. B. et al. The role of the carboxyl ester lipase (*CEL*) gene in pancreatic disease. *Pancreatology* **18**, 12–19 (2018).
5. Raeder, H. et al. Mutations in the *CEL* VNTR cause a syndrome of diabetes and pancreatic exocrine dysfunction. *Nat. Genet.* **38**, 54–62 (2006).
6. El Jellas, K. et al. Two new mutations in the *CEL* Gene causing diabetes and hereditary pancreatitis: How to correctly identify MODY8 cases. *J. Clin. Endocrinol. Metab.* **107**(4), e1455–e1466 (2022).
7. Fjeld, K. et al. A recombined allele of the lipase gene *CEL* and its pseudogene *CELP* confers susceptibility to chronic pancreatitis. *Nat. Genet.* **47**, 518–522 (2015).
8. Cassidy, B. M. et al. Single nucleotide polymorphisms in CEL-HYB1 increase risk for chronic pancreatitis through proteotoxic misfolding. *Hum. Mutat.* **41**, 1967–1978 (2020).
9. Tjora, E. et al. Protein misfolding in combination with other risk factors in *CEL-HYB1*-mediated chronic pancreatitis. *Eur. J. Gastroenterol. Hepatol.* **33**, 839–843 (2021).
10. Mao, X. T. et al. The *CEL-HYB1* hybrid allele promotes digestive enzyme misfolding and pancreatitis in mice. *Cell Mol. Gastroenterol. Hepatol.* **14**, 55–74 (2022).
11. Fjeld, K. et al. The genetic risk factor CEL-HYB1 causes proteotoxicity and chronic pancreatitis in mice. *Pancreatology* **22**, 1099–1111 (2022).
12. Zou, W. B. et al. No association between *CEL-HYB* hybrid allele and chronic pancreatitis in Asian populations. *Gastroenterology* **150**, 1558–1560 (2016).
13. Molven, A., Fjeld, K. & Lowe, M. E. Lipase genetic variants in chronic pancreatitis: When the end is wrong, all's not well. *Gastroenterology* **150**, 1515–1518 (2016).
14. Oracz, G. et al. The hybrid allele 1 of carboxyl-ester lipase (*CEL-HYB1*) in Polish pediatric patients with chronic pancreatitis. *Pancreatology* **19**, 531–534 (2019).
15. Hegyi, E. Carboxyl ester lipase (*CEL*) hybrid genes and chronic pancreatitis. *Saga Continues. Pancreatol.* **19**, 479–480 (2019).
16. Sándor, M. et al. Novel p. G250A mutation sssociated with chronic pancreatitis highlights misfolding-prone region in carboxypeptidase A1 (CPA1). *Int. J. Mol. Sci.* **23**, 15463 (2022).
17. Kawamoto, M. et al. Endoplasmic stress-inducing variants in carboxyl ester lipase and pancreatic cancer risk. *Pancreatology* **22**, 959–964 (2022).
18. Hegyi, P. et al. CFTR: A New horizon in the pathomechanism and treatment of pancreatitis. *Rev. Physiol. Biochem. Pharmacol.* **170**, 37–66 (2016).
19. Sándor, M. & Sahin-Tóth, M. Functional predictors of pathogenicity of missense *CPA1* variants in chronic pancreatitis. *Gut* **73**, 1589–1590 (2024).
20. Hegyi, E. & Sahin-Tóth, M. Human *CPA1* mutation causes digestive enzyme misfolding and chronic pancreatitis in mice. *Gut* **68**, 301–312 (2019).

Author contributions

EH and MST conceived the study. EH directed the study. GB performed the genotyping and sequence analyses, and MS performed the functional studies. ME, EM, BCN, AMR, JR, JMC and HW provided genotyping data. XKX and MEL designed and provided pcDNA3 plasmids. PH planned and organized the collection of Hungarian clinical data and biological research samples used in this study. All other co-authors recruited study subjects, collected clinical data and/or provided genomic DNA samples. MST wrote the manuscript. GB prepared the tables and figures. All authors approved the final manuscript.

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Declarations

Competing interests
The authors declare no competing interests.

Additional information
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