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Most unambiguous loss-of-function *CPAI* mutations are unlikely to predispose to chronic pancreatitis

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Abbreviations: CP, chronic pancreatitis; ER, endoplasmic reticulum; LoF, loss-of-function; NMD, nonsense-mediated RNA decay; PTC, premature termination codon

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We have read with interest the recent publication of Hegyi and Sahin-Tóth¹ reporting that chronic pancreatitis (CP)-predisposing *CPA1* mutations function through the misfolding pathway rather than through loss of CPA1 protein/activity. Herein, we explore an additional insight gleaned from this study beyond those discussed in an accompanying Editorial.²

In the original study reporting the association of *CPA1* variants with CP, all unambiguous loss-of-function (LoF) variants (e.g., nonsense mutations) were lumped together with missense mutations that functionally impaired the CPA1 protein.³ However, unlike missense mutations, unambiguous LoF variants often result in transcripts that contain premature termination codons (PTC) and are thus prone to nonsense-mediated RNA decay (NMD). NMD detects and degrades PTC-containing transcripts, thereby preventing the accumulation of truncated proteins.^{4,5} This implies that most unambiguous LoF *CPA1* variants would not be able to elicit ER stress and hence, in the light of the Hegyi and Sahin-Tóth study, will not predispose to CP. Indeed, the most frequently observed LoF variant, c.79C>T (p.Arg27*), was present at a lower frequency in European CP patients than in controls (Table 1). Additionally, we evaluated the pLI score for *CPA1* in the Genome Aggregation Database (gnomAD; <http://gnomad.broadinstitute.org/>). The pLI score indicates the probability that a gene is intolerant to heterozygous LoF variants, ranging from 0 (completely tolerated) to 1.0 (extremely intolerant).⁶ The pLI score for *CPA1* is 0. For the sake of comparison, *PRSSI* and *SPINK1* have pLI scores of 0 and 0.33 respectively; unambiguous LoF variants in the *PRSSI* gene actually protect against CP whereas unambiguous LoF variants in the *SPINK1* gene predispose to CP.⁷

Of the *CPA1* variants so far reported,⁸ five may be regarded as unambiguous LoF variants by virtue of their mutation type and location (Table 1). We surveyed the clinical significance of these five LoF variants⁸. It would appear that only the classification of c.79C>T (p.Arg27*) as benign is strongly supported by genetic epidemiological data (Table 1). In

order to investigate the classifications of these five variants from a mechanistic standpoint, we tested whether the three coding variants that were predicted to generate PTCs, c.79C>T (p.Arg27*), c.357C>A (p.Tyr119*) and c.954_955delCA (p.Tys318*), would generate mutant transcripts that would be degraded by the NMD pathway. This was found to be the case for all three variants ([figure 1](#)). We also elucidated the precise splicing consequences of the two splice site mutations (i.e., c.148-1G>A and c.1072+1G>T) in a minigene system (see [online supplementary figure S1](#)); both gave rise to transcripts that were prone to NMD. All experimental details are described in [online supplementary material, table S1 and figures S2 and S3](#).

In summary, prompted by the recent Hegyi and Sahin-Tóth study,¹ we provide evidence to suggest that most of the unambiguous LoF *CPAI* variants reported to date may not predispose to CP. Following the same line of reasoning, some of the previously characterized LoF *CPAI* missense mutations² may also not predispose to CP. In other words, the pathology may be confined to a small subset of *CPAI* mutations that are capable of eliciting ER stress. This may help to explain why rare functionally defective *CPAI* variants were not found to be associated with CP in a large Chinese cohort study.⁹

Contributors J-HL, AB and EM performed the functional assay. J-MC, ZL, Z-SL and CF designed the study. J-MC drafted the paper. DNC critically revised the manuscript. All authors analysed the data, contributed to revision of the manuscript and approved the final manuscript.

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Competing interests None declared.

REFERENCES

1. Hegyi E, Sahin-Tóth M. Human *CPA1* mutation causes digestive enzyme misfolding and chronic pancreatitis in mice. *Gut* 2019;68:301-12.
2. Rosendahl J. Unfolding the mystery of digestive enzyme misfolding in chronic pancreatitis. *Gut* 2019;68:181-2.
3. Witt H, Beer S, Rosendahl J, *et al.* Variants in *CPA1* are strongly associated with early onset chronic pancreatitis. *Nat Genet* 2013;45:1216-20.
4. Lykke-Andersen S, Jensen TH. Nonsense-mediated mRNA decay: an intricate machinery that shapes transcriptomes. *Nat Rev Mol Cell Biol* 2015;16:665-77.
5. Goetz AE, Wilkinson M. Stress and the nonsense-mediated RNA decay pathway. *Cell Mol Life Sci* 2017;74:3509-31.
6. Lek M, Karczewski KJ, Minikel EV, *et al.* Analysis of protein-coding genetic variation in 60,706 humans. *Nature* 2016;536:285-91.
7. Chen JM, Férec C. Chronic pancreatitis: genetics and pathogenesis. *Annu Rev Genomics Hum Genet* 2009;10:63-87.
8. Sahin-Tóth M, Nemeth B. Genetic risk factors in chronic pancreatitis.
<http://www.pancreasgenetics.org/index.php> (accessed 12 January 2019).
9. Wu H, Zhou DZ, Berki D, *et al.* No significant enrichment of rare functionally defective *CPA1* variants in a large Chinese idiopathic chronic pancreatitis cohort. *Hum Mutat* 2017;38:959-63.
10. Roberts NJ, Norris AL, Petersen GM, *et al.* Whole genome sequencing defines the genetic heterogeneity of familial pancreatic cancer. *Cancer Discov* 2016;6:166-75.

FIGURE LEGEND

Figure 1 Quantitative reverse transcriptase-PCR analysis of HEK293T cells transfected with *CPAI* cDNA expression constructs carrying respectively the wild-type and variant sequences.

(A) mRNA expression levels of the three variant sequences relative to that of the wild-type sequence. (B) Relative mRNA expression levels of the variant sequences in transfected cells with (grey) and without (black) cycloheximide (an NMD inhibitor) treatment.

Table 1 The five unambiguous LoF *CPAI* variants discussed in this study

Region	Nucleotide change	Amino acid change	Cases (%) (<i>n</i> =1544) ^a	Controls (%) (<i>n</i> =6370) ^b	Current classification of clinical significance ⁸	Functional analysis findings (this study)
Exon 2	c.79C>T	p.Arg27*	1 (0.06)	7 (0.11)	Benign	mRNA expression analysis demonstrated that the mutant transcript was subject to NMD.
Intron 2	c.148-1G>A	p.Leu50Hisfs*16 (previously termed p.Leu50_Glu127del) ²	0 (0)	1 (0.02)	Likely pathogenic	Minigene splicing analysis revealed that the mutation primarily activated a cryptic 3'-splice site located 63 bp downstream of the normal one, resulting in the loss of the first 65 bp of exon 3 from the transcript. This would lead to a frameshift starting at amino acid position 50, with the new reading frame ending in a stop at position 16.
Exon 3	c.357C>A ^c	p.Tyr119*	—	—	Uncertain	mRNA expression analysis demonstrated that the mutant transcript was subject to NMD.
Exon 8	c.954_955delCA	p.Tyr318*	2 (0.13)	0 (0)	Pathogenic	mRNA expression analysis demonstrated that the mutant transcript was subject to NMD.
Intron 9	c.1072+1G>T	p.Asp330Ilefs*51	0 (0)	1 (0.02)	Likely pathogenic	Minigene splicing analysis confirmed that exon 9 was skipped. This would lead to a frame-shifting change starting at amino acid position 330, with the new reading frame ending in a stop at position 51.

^{a,b}Combined European data from reference 2 unless otherwise specified.^cDetected in a pancreatic cancer patient.¹⁰