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Endoplasmic stress-inducing variants in *CPB1* and *CPA1* and risk of pancreatic cancer; a case-control study and meta-analysis

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Abstract

Gene variants that encode pancreatic enzymes with impaired secretion can induce pancreatic acinar endoplasmic reticulum (ER) stress, cellular injury and pancreatitis. The role of such variants in pancreatic cancer risk has received little attention. We compared the prevalence of ER stress-inducing variants in *CPA1* and *CPB1* in patients with pancreatic ductal adenocarcinoma (PDAC cases), enrolled in the National Familial Pancreas Tumor Registry, to their prevalence in non-cancer controls in the Genome Aggregation Database (gnomAD). Variants of unknown significance were expressed and variants with reduced secretion assessed for ER stress induction. In vitro assessments were compared with software predictions of variant function. Protein variant software was used to assess variants found in only one gnomAD control ("n-of-one" variants). A meta-analysis of prior PDAC case/control studies was also performed. Of the 1385 patients with PDAC, 0.65% were found to harbor an ER stress-inducing variant in *CPA1* or *CPB1*, compared to

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Study concept and design; MG, AK; acquisition of data; MK, AK, NR; analysis and interpretation of data; MK, AK, MG; drafting of the manuscript; MK, MG; critical revision of the manuscript for important intellectual content; MK, AK, MG; revised the manuscript and agreed with the manuscript's results and conclusions; all the authors; obtained funding; MG, AK; technical, or material support; MK, NR, AK, MG; study supervision; MG. All authors read and approved the final manuscript.

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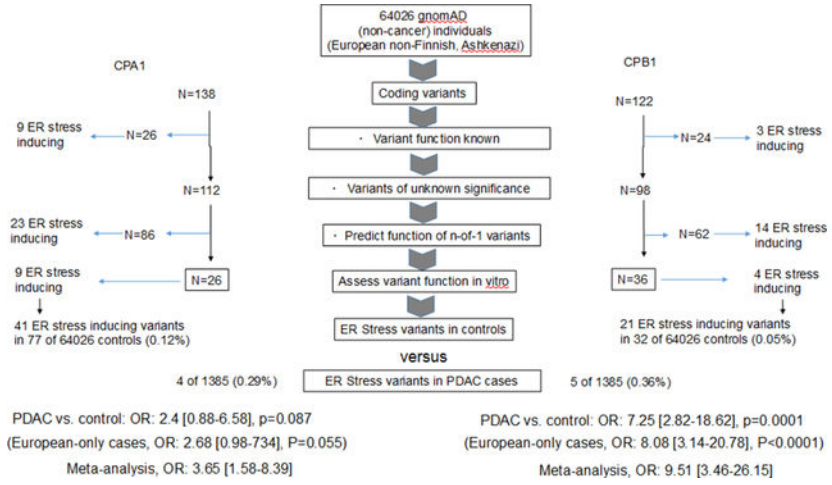
Ethics Statement: This study was approved by the Johns Hopkins Institutional Review Board with written informed consent provided by all case participants. The control variant data was obtained from the gnomAD database, a publicly available database of de-identified variant information.

0.17% of the 64,026 controls (Odds Ratio (OR): 3.80 [1.92–7.51] P=0.0001). ER stress-inducing variants in the *CPA1* gene were identified in 4 of 1385 PDAC cases versus 77 of 64,026 gnomAD controls (OR: 2.4 [0.88–6.58], p=0.087), and variants in *CPB1* were detected in 5 of 1385 cases versus 33 of 64,026 controls (OR: 7.02 [2.74–18.01], p=0.0001). Meta-analysis demonstrated strong associations for pancreatic cancer and ER-stress inducing variants for both *CPA1* (OR: 3.65 [1.58, 8.39], p<0.023) and *CPB1* (OR: 9.51 [3.46, 26.15], p<0.001). Rare variants in *CPB1* and *CPA1* that induce ER stress are associated with increased odds of developing pancreatic cancer.

Graphical Abstract



Keywords

pancreatic cancer; CPA1; CPB1; Endoplasmic reticulum stress; variant

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is the 3rd most common cause of cancer death in the United States with a 5-year survival of only ~10% (1). Early detection of pancreatic cancer and its precursors may be the most effective way of reducing mortality as a result of the disease (2, 3). Some patients are candidates for pancreatic surveillance because they carry a deleterious germline pathogenic variant in a known familial pancreatic cancer susceptibility gene (including in order of prevalence *BRCA2*, *ATM*, *BRCA1*, *PALB2*, *CDKN2A*, *MLH1*, *MSH2*, *MSH6*, *STK11* and *TP53*) (4–11). Pathogenic variants in high-penetrant pancreatic cancer susceptibility genes contribute to the familial clustering of pancreatic cancer. Most carriers of these variants have apparently sporadic pancreatic cancer, lacking a family history suggestive of a classic inherited cancer syndrome (12–15). Identifying new gene variants contributing to pancreatic cancer susceptibility will not only improve our understanding of the etiology of the disease but will also help inform the selection of subjects for pancreas surveillance.

Chronic pancreatic injury from pancreatitis, especially hereditary young-onset pancreatitis, predisposes to the development of pancreatic cancer (16, 17). Most deleterious inherited

variants affecting the pancreatitis-susceptibility gene *PRSS1* cause premature trypsin activation (18). Pancreatitis can also be caused by other mechanisms. Due to the large amounts of protein they synthesize, acinar cells are susceptible to the accumulation of misfolded proteins and subsequent induction of ER stress (19), which has been implicated as a cause of pancreatitis (reviewed in (20)). Deleterious variants in *CPA1* and some coding variants in *CTRC* and *PRSS1,* though not *CPB1* (21), have been associated with hereditary recurrent acute and chronic pancreatitis (22–25), but not idiopathic chronic pancreatitis (26); these variants are thought to result in protein misfolding-induced endoplasmic reticulum (ER) stress. A *CPA1* variant that induces pancreatic acinar ER stress has been shown to cause pancreatitis in a mouse model (27) that is exacerbated by alcohol feeding (28). A prior GWAS identified a locus near *CTRB2*, encoding another pancreatic secretory enzyme, as a pancreatic cancer risk locus (29), another study found the locus associated with pancreatitis risk (30), and a recent paper implicated ER stress as the mechanism by which a common deletion variant in *CTRB2,* (average allele frequency of 8.2%), associates with pancreatic cancer risk (Odds ratio 1.36)(31).

ER stress-inducing variants encoding pancreatic enzymes can be deleterious without inducing attacks of acute pancreatitis. For example, ER stress-inducing variants in *CEL* can cause maturity-onset diabetes of the young (MODY8) and result in pancreatic atrophy without a history of pancreatitis (32). The mechanism of pancreatic insufficiency in Shwachman-Diamond syndrome may involve ER stress in part; it is caused by loss-of-function mutations in *SBDS* and other related genes involved in ribosomal biogenesis causing translational and other cellular defects (33–35). A case of Shwachman-Diamond syndrome with a young-onset pancreatic cancer has been described (36).

In a prior study, we found that rare ER stress-inducing variants in genes coding for pancreatic secretory enzymes *CPA1* and *CPB1* were more common in patients with pancreatic cancer than in control individuals (10). Because of the rarity of ER stress-inducing variants in these genes, a large cohort of controls is needed to inform risk estimates.

We therefore conducted an independent case-control study comparing the prevalence of deleterious germline variants in *CPA1* and *CPB1*, defined as variants that induce ER stress, in pancreatic cancer cases vs. controls in the gnomAD database (37).

MATERIALS AND METHODS

Description of case cohort and controls

Cases consisted of 1385 individuals with a diagnosis of pancreatic cancer enrolled in the National Familial Pancreas Tumor Registry (89.7% Caucasian, 4.2% African-American). Of these, 26.6% had familial pancreatic cancer (at least a pair of first-degree relatives in the family with PDAC). These cases were not previously included in our prior study of *CPA1* and *CPB1* variants (10). The mean age at pancreatic cancer diagnosis for the cases was 64.1 +/− 20.2 years. Individuals with a known pathogenic variant in a pancreatic cancer susceptibility gene were excluded. Subjects participated at enrollment by consenting to join the family registry whose aim is to identify and quantify the genetic contributions to

pancreatic cancer susceptibility so as to improve the outcome of individuals affected by the disease.

The gnomAD database (v2.1.1)(https://gnomad.broadinstitute.org/)(37) was filtered to include non-cancer controls who were either European (non-Finnish) (n=59095) or Ashkenazi Jewish (n=4931) to match the racial background of our cases. This created a representative control population of 64,026 which was used to estimate the prevalence of deleterious *CPA1* and *CPB1* variants in controls.

Next-generation sequencing (NGS)

DNA was extracted as previously described (10, 11). DNA was sequenced using an AmpliSeq Custom Panel designed to amplify the coding regions of *CPA1* and *CPB1*. NGS was performed with 540 chips (Ion S5 system, single-read, 200 bp read-length) and reads from the Ion Torrent Server were mapped (HG19) and variants called using NextGENe Software (Softgenetics, LLC, State College, PA) according to manufacturer's protocols as previously described (38). Median depth of coverage for *CPA1* and *CPB1* reads was 262 reads per amplicon. A minimum percentage coverage of reads of ≥10 was required for at least 85% of amplicons. Samples below this coverage were re-sequenced to obtain sufficient coverage. An amplicon depth of coverage of 15 reads was required to call a variant, and candidate variant reads of unknown significance were visualized with Integrated Genomics Viewer (IGV) software. Candidate variants of unknown significance were confirmed by Sanger sequencing, performed at the Johns Hopkins DNA sequencing core.

In vitro mutagenesis and transfection

To create variant expression constructs for *CPA1* and *CPB1* cDNA was amplified from normal pancreatic tissue using the gene-specific primers. PCR products were Sanger sequenced, restricted, and subcloned into pcDNA 3.1 vectors (ThermoFisher Scientific). Variants were generated by the overlap extension PCR method and cloned as previously reported and plasmid DNA (4 μg) was transfected into HEK 293T/17 cells (RRID: CVCL_1926) obtained from the American Type Culture Collection (Rockville, MD, USA) as previously described (10). The HEK293T cell line has been authenticated using short tandem repeat profiling within the last three years. All experiments were performed with mycoplasma-free cells. Each variant clone sequence was confirmed by Sanger sequencing.

Measurement of proCPB1, proCPA1 secretion and western blots to determine ER stress

Amounts of secreted proCPB1/CPA1 protein in the conditioned medium was measured by SDS-PAGE and densitometry as previously reported (10). Western blots were probed with rabbit polyclonal anti-Grp78 (BiP) antibody (ab21685; 1:2,000 dilution; Abcam). Rabbit monoclonal anti–β-actin antibody (#4970, 1:1,000 dilution; Cell Signaling Technology) was used an internal loading control. For BiP, rabbit polyclonal anti-Grp78 (BiP) antibody (ab21685; 1:2,000 dilution; Abcam) was used. A variant was classified as deleterious (ER stress-inducing) if there was a statistically significant increase in BiP protein (BiP/β-actin) bands quantified with the Chemidoc Touch Imaging System and Image Lab (v5.2.1) software (Bio-Rad) levels after transfection of the variant vs. the wild-type gene (mean of three independent transfections). Briefly, total proteins were extracted from whole cell

lysate using RIPA buffer (Sigma-Aldrich). For SDS- PAGE, 15 μg of cell lysate protein was separated by gel electrophoresis on 12% NuPAGE Novex Bis-Tris Protein Gel in MOPS running buffer (Invitrogen) and transferred to PVDF membrane using an iBlot2 Gel Transfer Device (Life Technologies). The secondary antibodies used were anti-rabbit IgG HRP-linked IgG (#7074, Cell Signaling Technology) used at 1:2000 dilution. Immunoblot detection was performed using chemiluminescence with a Chemidoc Touch Imaging system (Bio-Rad).

Protein variant software

Variants with characterized protein secretion either from functional assessments performed in this study or from the literature were included to determine how well three programs (MutationAssessor, Polyphen-2 and SIFT) predicted which variants induced ER stress. Most of the variants included in this analysis demonstrated to have reduced protein secretion were tested for ER stress effects (BiP measurement by western blot). For *CPA1*, a minority of variants classified in the literature as deleterious were so classified based on having a major impact on protein secretion alone (secretion ≤20% of normal)(22). Four CPA1 variants with borderline secretion (20–30% of normal) without known effect on ER stress were classified as indeterminate and not used for the software evaluation. All *CPB1* variants classified as ER stress-inducing had undergone an ER stress assay. Variants classified with the software program MutationAssessor as “high” were classified as deleterious, all others were classified as benign (39). With PolyPhen software, variants were predicted deleterious if they had “probably damaging” scores (≥0.85) (40). Inversely, SIFT scores classified as deleterious (0–0.05) were considered deleterious (41). In Supplemental tables S1 and S2 we provide a summary of the results used to evaluate protein variant software (variant, secretion, Bip level; 96 variants for *CPA1*, 76 variants for *CPB1*). Nonsense and frameshift insertion/deletion variants, which could result in defective enzymatic function, were evaluated for their likelihood of being subject to non-sense mediated decay (NMD) (42); (most such variants are likely to be subject to NMD unless they are in the terminal exon or near the start codon). Variants predicted to undergo NMD were classified as benign with respect to their potential to induce ER stress.

Statistics

Odds ratios were computed and Fisher’s exact test was used to test the hypothesis of a difference in the number of cases with ER-stress inducing variants in *CPA1* and *CPB1* variants in pancreatic cancer cases compared to controls. Meta-analysis of these findings with prior studies was conducted (in STATA v17) using both fixed-effect and random-effects methods, given there no evidence of heterogeneity (I=0.00), results are presented using fixed-effects across studies (43). Inverse-variance was used to weight each study. For counts of zero in prior studies, a value of 0.5 was used to compute odds ratios. To determine if BiP levels increase after cells are transfected with variant compared to wild-type gene, one-sided Student’s t-test was employed; (one-sided as the experiments test the hypothesis that BiP levels increase only, not decrease). To compare ages at diagnosis of cases with/without variants (two-sided). Apart from the meta-analysis, analyses were performed using JMP software v.14.1.0 (SAS Institute, Cary, CA).

RESULTS

The list of variants identified by functional analysis as ER stress-inducing in cases and controls is provided in Table 1. Table 2 provides a summary of the meta-analysis. Figures 1 and 2 show results of variants tested in vitro; Figure 3 displays the correlation between variant protein secretion and Bip levels and variant prediction software scores. Figure 4 provides a flow-chart summary of the results. Additional results are provided in Supplemental materials.

Variants in PDAC cases

Of 38 *CPA1/CPB1* variants identified in patients with PDAC (Supplementary Table S3), 3 were known to be ER stress-inducing, 20 were known to be benign with respect to their protein secretion/ER stress potential and fifteen were VUS that were functionally evaluated in HEK293T cells.

For *CPA1*, an ER stress-inducing germline variant was identified in four of the 1385 patients with PDAC. Two of these four PDAC cases were VUS (p.D51N, p.R181W) that were among the variants tested and found to have absent protein secretion and to induce ER stress (elevated BiP levels (44))(Figure 1). The other two patients with PDAC had variants previously shown to be ER stress-inducing (p.R237C, p.R386C)(10).

For *CPB1*, five of 1385 patients with PDAC had an ER stress-inducing germline variant, including three with previously uncharacterized variants (p.P55S, p.C186F and p.Tyr373PhefsTer47, a terminal exon variant, Figure 1), and two patients with a known ER stress-inducing variant (p.*418W)(10). Table 1 lists the variants identified as ER stress-inducing by in vitro analysis in PDAC cases and controls.

Variants in GnomAD controls

Among the gnomAD controls, twelve variants were known ER stress-inducing variants and 210 were VUS (112 *CPA1*, 98 *CPB1*). Sixty-two of the 210 VUS were found in two or more of controls and were tested *in vitro* (26 *CPA1* variants (Table S4), 37 *CPB1* variants)(Table S5). For the remaining 148 VUS that were each reported in only one of the 64026 controls, (termed “n-of-one” variants), software tools were used to predict the variant’s likelihood of being ER stress-inducing; (further described below). Supplementary Table S6 lists variants where protein secretion had been reported in the literature and includes our own evaluation of protein secretion and BiP induction of these variants.

For *CPA1*, 9 variants found in the controls were known to be ER stress-inducing variants (Table 1), and an additional nine of the 26 VUS characterized *in vitro* exhibited reduced protein secretion (Supplementary Figure S1) and ER stress induction (Figure 2) (Table 2). These 18 ER-stress inducing variants (by *in vitro* assessment) were found in 54 gnomAD controls (Table 1).

For *CPB1*, 3 variants found in controls were known ER stress-inducing variants (p.Gly146Arg, p.Ala366Pro and *418W)(10). An additional 4 VUS were variants found to have reduced secretion (Supplementary Figure S2) and to be ER stress-inducing by in

vitro analysis (Figure 2); three found only in controls (p.Arg195Cys, p.Arg231Gln, and p.Cys268Tyr) and one was also identified in a PDAC case (p.Tyr373PhefsTer47). These 7 variants determined to be ER stress-inducing in vitro assessment were found in 18 gnomAD controls (Table 1).

Among variants classified as deleterious in Table 1, there was no significant difference in the severity of the defect among PDAC cases versus gnomAD controls with respect to secretion defect, though the numbers of deleterious variants overall were small for such a comparison. But for all variants, there was a strong linear correlation between the magnitude of the secretion defect and the extent of BiP expression (Figure 3A, 3B).

Software tools to predict protein variant function

Software tools that evaluate protein variant function are not specifically designed to predict whether or not a variant can induce ER stress. So we compared *in silico* predictions of *CPA1* and *CPB1* variant function to *in vitro* measures of protein secretion and ER stress induction. For *CPA1,* 96 missense variants were identified that had had been evaluated *in vitro* (including published data and the variants tested in this study), for *CPB1,* 76 missense variants were identified. Of the 3 software tools evaluated, MutationAssessor was more accurate than PolyPhen-2 and SIFT at predicting if a variant was correctly classified as ER stress-inducing; (85.4% accuracy, [sensitivity 75%, specificity 91.7%]; Table S7, Figure 3A, Supplementary Figure 3). MutationAssessor was also the most accurate for *CPB1* variants (89.5% accuracy, [sensitivity 71.4%, specificity 91.3%]; Table S7, Figure 3D, Supplementary Figure S4).

n-of-one variants in GnomAD controls

Next, we used MutationAssessor to classify the n-of-one VUS in our gnomAD controls. There were 86 such *CPA1* variants (82 missense; 4 frameshift/in-frame variants were predicted to be benign). Using MutationAssessor, we estimate 23 *CPA1* n-of-one variants in the gnomAD controls are ER stress-inducing (Supplementary Table S8). For *CPB1* variants, of 62 n-of-one variants in the gnomAD controls (58 missense), 14 of these are predicted to be ER stress-inducing using MutationAssessor (Supplementary Table S9).

ER stress variant totals in PDAC cases vs. gnomAD controls

Overall, for *CPA1*, 54 gnomAD controls had one of the 18 ER stress-inducing variants as determined by *in vitro* assessment, 23 had an n-of-one variant predicted to be ER stress-inducing *in silico*, yielding a total of 77 controls (0.12% of the 64026 gnomAD controls). The odds of having an ER stress-inducing *CPA1* variant was non-significantly higher in PDAC cases (4 of 1385, (OR: 2.4 [0.88–6.58], p=0.087); European-only cases, 4 of 1242, (OR: 2.68, [0.98–734], P=0.055). A summary of the results is provided in Figure 4.

For *CPB1*, the corresponding numbers were 18 controls with an ER stress-inducing variant (by in vitro assessment) and 14 with an n-of-one variant predicted *in silico* to be ER stress-inducing, a total of 32 controls (0.05% of 64026 controls). The odds of having an ER stress-inducing *CPB1* variant was higher in PDAC cases (5 of 1385, (OR: 7.25 [2.82–18.62], p=0.0001; European-only cases, 5 of 1242, OR: 7.84 [3.05–20.11], P<0.0001)

(Figure 4). There were more individuals with an ER stress-inducing variant involving *CPA1* than involving *CPB1* in the gnomAD controls (P<0.00001).

In total, 0.65% of pancreatic cancer cases and 0.17% of controls had an ER stress-inducing variant in *CPA1* or *CPB1* (Odds Ratio (OR) 3.84 [1.94–7.58], P=0.0001; European-only cases, 0.72%, OR 4.28 [2.16–8.47], P<0.0001).

Meta-analysis

In our prior report (10), a two-stage case vs. control analysis was performed, including a set of sporadic PDAC cases and a set of familial cases that yielded a combined prevalence of ER stress-inducing variants in PDAC cases of 7 of 1546 for *CPA1* and 9 of 1546 for *CPB1*. The mean age at diagnosis of the patients with PDAC having a *CPA1/CPB1* ER-stress variant from that study and the current study was 64.8 +/− 10.4 years, not significantly different from the PDAC cases overall. There was also evidence of enrichment of deleterious variants in familial vs. sporadic PDAC cases, though the overall numbers were too small for such a comparison. The overall prevalence of ER stress-inducing variants among PDAC cases in the combined studies was 11 of 2931 (0.37%) for *CPA1* and 14 of 2964 (0.47%) for *CPB1,* (0.84% total).

Meta-analysis of these results with the prior case/control studies resulted in significant associations for both *CPA1* (OR: 3.65 [1.58, 8.39], p=0.023) and *CPB1* (OR: 9.51 [3.46, 26.15], p<0.001)(Table 2).

DISCUSSION

In this study we found that ER stress-inducing variants in the pancreatic secretory enzymes *CPB1* and in *CPA1* were significantly more common in patients with pancreatic cancer, suggesting these variants play an important role in pancreatic cancer risk and implicating variant-induced ER stress of pancreatic acinar cells in pancreatic cancer susceptibility. Further support for the role of ER stress in pancreatic cancer susceptibility comes from a recent post-GWAS case-control analysis which identified variants in the ER stress pathway gene *XBP1* as associated with pancreatic cancer risk (45). The prevalence of ER stress-inducing variants in *CPA1* and *CPB1* among non-cancer gnomAD controls was only 0.17%. We limited gnomAD controls to those of European and Ashkenazi background as the vast majority of our cases (~90%) were of reported European Ancestry.

The gnomAD controls are younger than our PDAC cases on average by about a decade and include a much younger age range. While we cannot rule out that some controls, particularly those with ER stress-inducing variants could eventually develop pancreatic cancer, misclassification of controls would be expected to increase the true effect size over that observed. The gnomAD database does not provide individual level data such as age or follow-up information about health or disease.

Several studies have found an association between deleterious variants in *CPA1* and hereditary pancreatitis (22, 24); the only study that failed to find an association with *CPA1* evaluated cases with idiopathic, not hereditary pancreatitis (26). One study of *CPB1* variants

and pancreatitis did not find an association (21). This study may have been underpowered to detect such an association given our data indicating ER stress-inducing variants in *CPB1* are rare in the general population. Misclassification of a variant in most circumstances will bias effects towards the null. Although classifying variants based on protein secretion without checking for evidence of ER stress can result in misclassification, MutationAssessor was found to be an accurate classification tool (*CPA1* 85.4%; *CPB1* 89.5%) for predicting variants that induced ER stress. Although we found a strong linear correlation between protein variant scores and the level of protein variant secretion (and Bip induction), the relationship may not be strictly linear, additional data would be needed to determine this, but highlight a strong relationship between the extent of the protein secretion defect and the level of ER stress.

Without a recognized clinical syndrome of pancreatitis or pancreatic insufficiency, the deleterious effects of ER stress-inducing variants involving pancreatic secretory enzyme genes will go unrecognized. Once pancreatic cancer develops, ductal obstruction generally obscures any precancerous phenotype. One relative undergoing pancreas surveillance because of their family history of pancreatic cancer with mild pancreatic atrophy was found to have an ER stress-inducing *CPA1* variant (38), but patients with these variants are not currently enrolled in pancreatic surveillance.

It is not surprising we did not find enrichment of deleterious *CPA1* or *CPB1* variants in patients with familial compared to those with sporadic pancreatic cancer. Familial pancreatic cancer is defined as two first-degree relatives affected by the disease, and while a useful practical definition, many such individuals probably do not harbor a high-penetrant pancreatic susceptibility gene (46). A challenge for investigators studying inherited susceptibility to pancreatic cancer is the late-onset and low incidence of the disease means that it is difficult to obtain DNA from multiple affected members of the same family (47). Even when DNA is available, low disease penetrance, a common feature of pancreatic cancer susceptibility gene variants, makes it challenging to study segregation (11).

Certain limitations of this study should be noted. We chose Bip protein induction in HEK293T cells as a marker of ER stress, as it is a master regulator of ER stress (44, 48) and a widely used marker for this purpose. Other markers of ER stress, such as alternate splicing of Xbp1 RNA, are also commonly used, but there is no consensus in vitro test to define ER stress. Although many ER stress-inducing variants are reported to have clinical consequences, the level of ER induction likely to be deleterious is not known. ER stress-inducing gene variants can be expected to interact with other factors that predispose to ER stress, such as alcohol (28, 49, 50) and smoking (50) to cause cellular dysfunction in vivo and clinical phenotypes. Having found that software tools could be used to accurately estimate ER stress, we used a combined functional and in *silico* approach to determine the prevalence of ER stress-inducing variants in controls. Although we used only a functional-only approach for the evaluation of variants in PDAC cases, because we limited the *in silico* evaluations of control variants to the rarest variants only (n-of-1 of ~64000 controls), and adjusted the final estimate of ER stress variant prevalence by taking into account the accuracy of the software, we do not believe this approach significantly affected our prevalence estimates.

Overall, this study confirms the association between ER stress-inducing variants involving *CPA1* and *CPB1* and pancreatic cancer risk. Based on our results, consideration should be given to including *CPA1* and *CPB1* as genes included in panels testing for pancreatic cancer susceptibility, recognizing that VUS in these genes require functional evaluation to identify variants associated with ER stress. The high odds ratios for deleterious variants in these genes indicate the lifetime risk of pancreatic cancer in affected carriers may be sufficiently high to warrant their inclusion in pancreatic surveillance programs, but additional studies are needed to better refine risk estimates. The International Cancer of the Pancreas Screening Consortium recommends selective screening for high-risk individuals with an estimated 5% or higher lifetime risk of developing pancreatic cancer (51).

In conclusion, our analysis of pancreatic cancer cases and gnomAD controls finds an excess of ER stress-inducing *CPA1* and *CPB1* variants among cases, lending support for the hypothesis these variants contribute to pancreatic cancer susceptibility.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments.

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Data Availability Statement:

The gnomAD variant is publicly available at https://gnomad.broadinstitute.org/. Individual level data from PDAC cases is available from the corresponding author for meritorious collaborative studies. Further details and other data that support the findings of this study are available from the corresponding author upon request.

Abbreviations:

ER	endoplasmic reticulum stress
gnomad	Genome Aggregation Database
IGV	Integrated Genomics Viewer
NMD	non-sense mediated decay
PDAC	pancreatic ductal adenocarcinoma

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Novelty and Impact:

We find a strong association between having an ER stress-inducing variant in *CPB1* and pancreatic cancer risk and a more moderate association for *CPA1* variants, implicating ER stress as a mechanism of pancreatic cancer susceptibility.

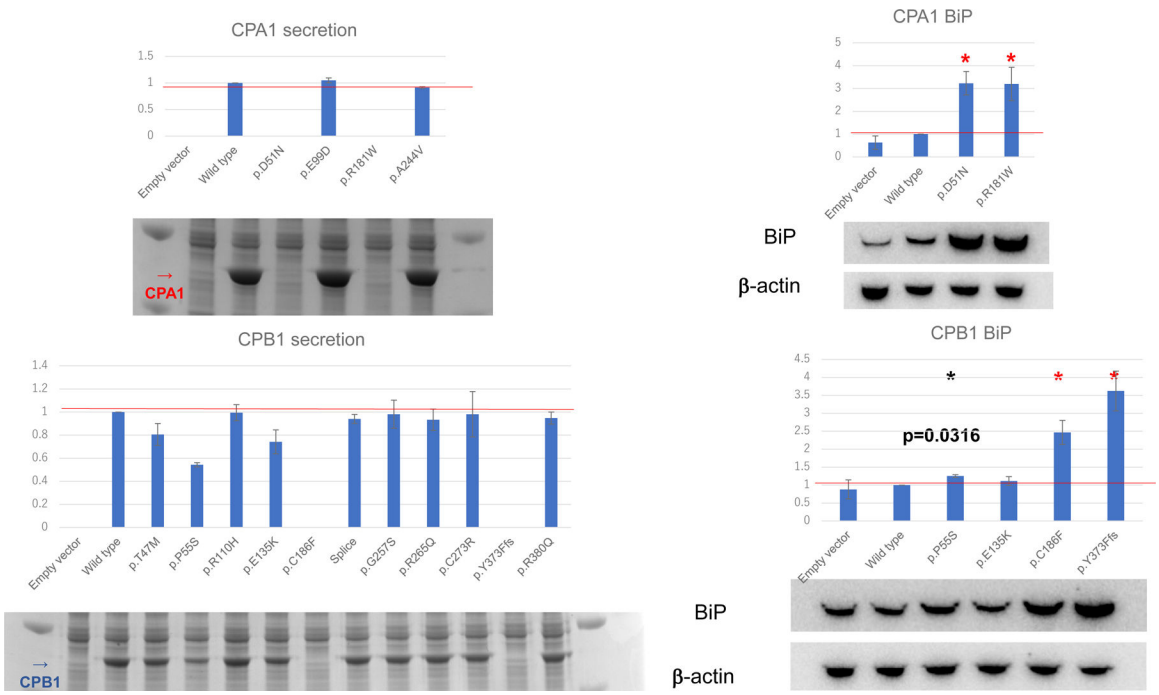

Figure 1:

Protein secretion and BiP induction (marker of ER stress) of *CPA1* and *CPB1* variants identified in pancreatic cancer cases expressed in HEK293T cells. Red * indicated statistically significant induction (p≤0.01). The black * refers to a variant with a significant Bip induction (p value of p=0.0316).

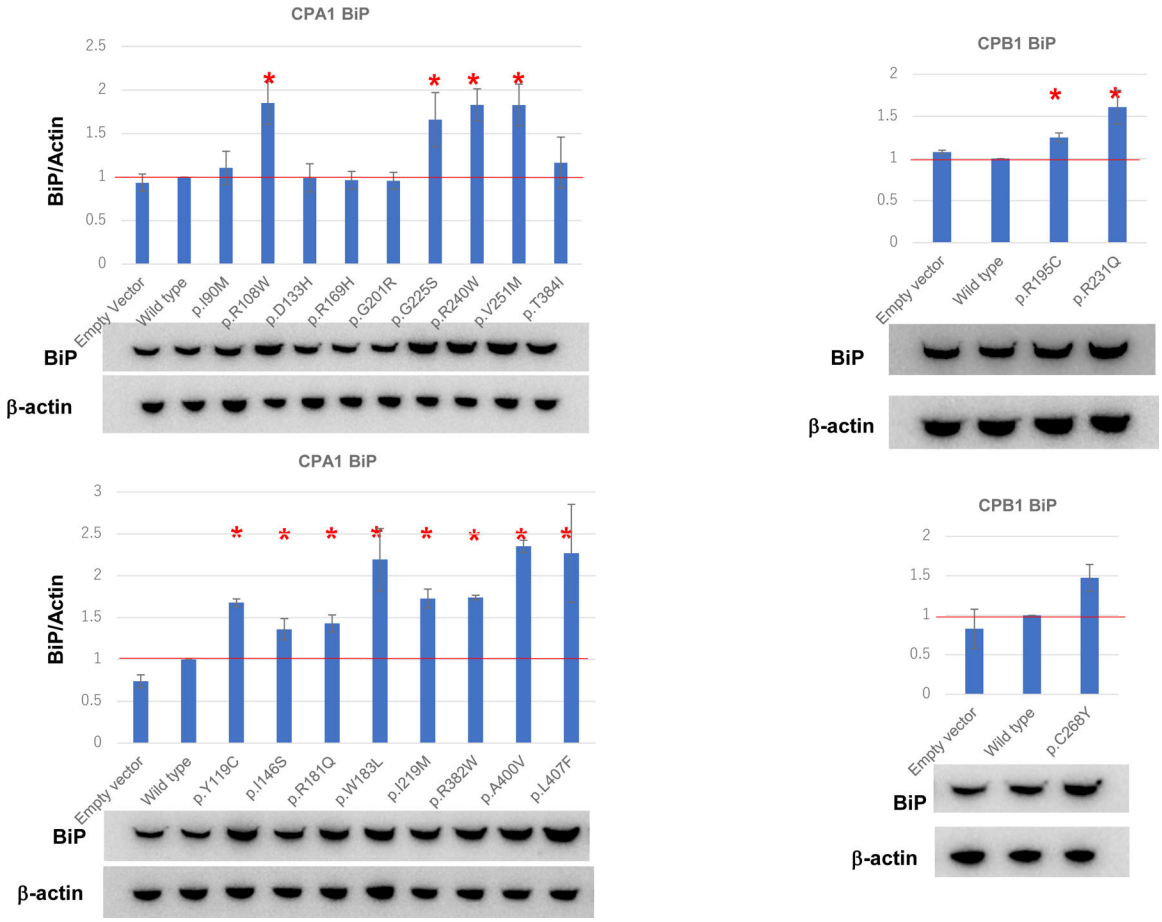

Figure 2:

BiP induction (marker of ER stress) of *CPA1* and *CPB1* variants identified in gnomAD controls expressed in HEK293T cells. * indicated statistically significant induction ($p \leq 0.01$).

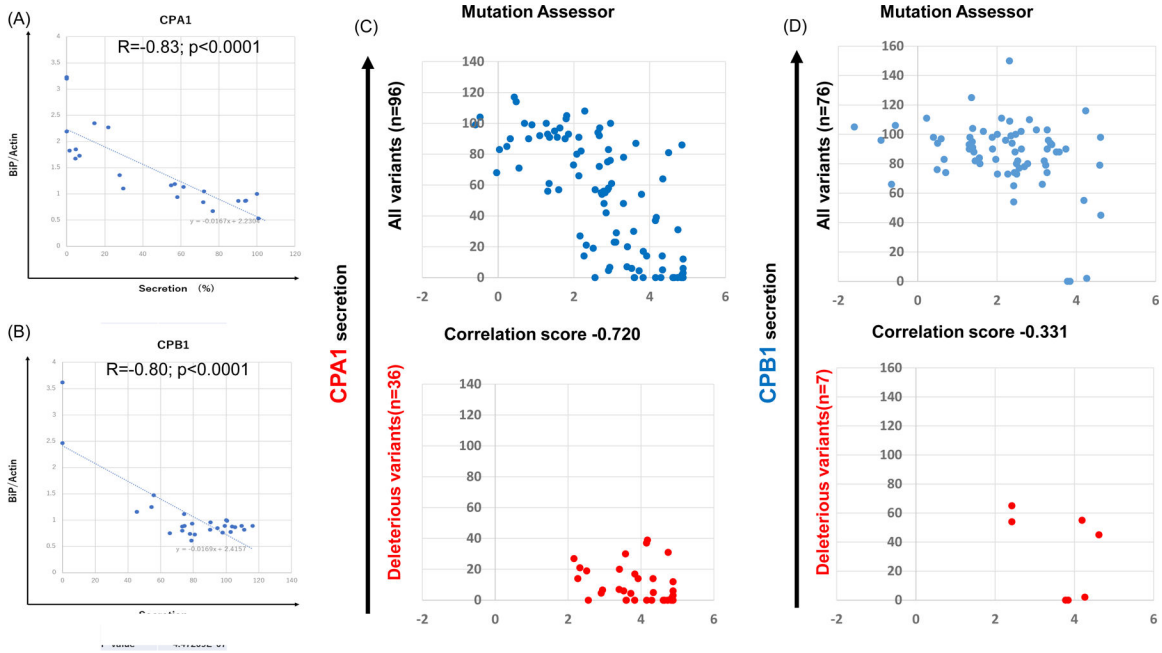

Figure 3:

Correlation between protein variant secretion (y-axis) and BiP induction for (A) CPA1, (B) CPB1. MutationAssessor scores (on the x-axis) and protein variant secretion for (C) *CPA1,* (D) *CPB1* variants; (top-half, all variants), bottom-half), deleterious-only variants (i.e. ER stress-inducing).

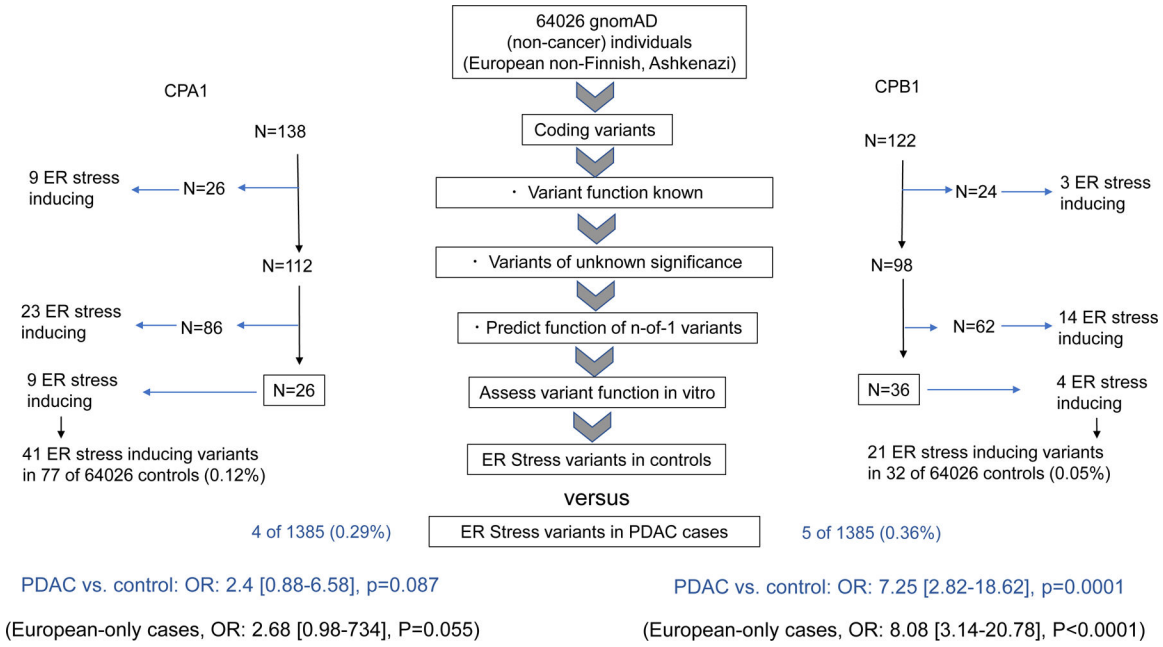

Figure 4:

Summary of the steps used to identify ER stress-inducing variants detected in PDAC cases vs. gnomAD controls.

Table 1:

ER stress-inducing variants in CPA1 + CPB1 in PDAC cases + gnomAD controls[^]

Chr/ position	rsID	AA change	Nucleotide Change	Variant annotation	Secretion (%)	ER stress	n	Mutation Assessor
CPA1, PDAC cases							1385	
7/130021474	rs267601284	p.D51N	c.151G>A	missense	0	Yes	1	medium
7/130023289	rs782248213	p.R181W	c.541C>T	missense	0	Yes	1	high
7/130024389	rs184981267	p.R237C	c.709C>T	missense	37	Yes	1	high
7/130027748	rs201750163	p.R386C	c.1156C>T	missense	30	Yes	1	high
						Total	4	
CPA1, gnomAD controls *							64026	
7/130021645	rs141886698	p.Arg108Trp	c.322C>T	missense	4.6	Yes	3	medium
7/130021679		p.Tyr119Cys	c.356A>G	missense	4.5	Yes	2	high
7/130022004	rs782716199	p.Ile146Ser	c.437T>G	missense	27	Yes	2	medium
7/130023289	rs782248213	p.Arg181Trp	c.541C>T	missense	0	Yes	3	high
7/130023290	rs377042960	p.Arg181Gln	c.542G>A	missense	39, 23 *	Yes *	2	high
7/130023296	rs781963872	p.Trp183Leu	c.548G>T	missense	0	Yes	2	high
7/130024389	rs184981267	p.Arg237Cys	c.709C>T	missense	37	Yes	4	high
7/130024399	rs781968100	p.Arg240Gln	c.719G>A	missense	0	Yes	4	high
7/130023596	rs1057524778	p.Ile219Met	c.657C>G	missense	6.6	Yes	2	medium
7/130024398	rs200036285	p.Arg240Trp	c.718C>T	missense	1.5	Yes	2	high
7/130027791		p.Ala400Val	c.1199C>T	missense	14	Yes	2	medium
7/130027811		p.Leu407Phe	c.1219C>T	missense	21	Yes	2	medium
7/130024381	rs782582120	p.Arg234His	c.701G>A	missense	0	Yes	3	high
7/130023612	rs782112003	p.Gly225Ser	c.673G>A	missense	12, 3.5 *	Yes *	8	high
7/130024431	rs782158803	p.Val251Met	c.751G>A	missense	0, 0.4 *	Yes *	2	high
7/130027736	rs782689631	p.Arg382Trp	c.1144C>T	missense	31, 0 *	Yes *	2	high
7/130027748	rs201750163	p.Arg386Cys	c.1156C>T	missense	30	Yes	6	high
7/130027818	rs782329019	p.Ile410_ His417dup	c.1228_1251dupATCATGGAGCACACCCTGAATCAC	inframe_ insertion	15	Yes	3	-
						Total	54	
CPB1, PDAC cases							1385	
3/148552300	rs1275676207	p.P55S	c.163C>T	missense	54	Yes	1	medium
3/148559692	-	p.C186F	c.557G>T	missense	0	Yes	1	high
3/148577788	rs201519774	p.*418Wext*37	c.1253A>G	No-stop	0	Yes	2	-
3/148577653	-	p.Y373Ffs	c.1118delA	Frameshift	0	Yes	1	-
						Total	5	

Chr/ position	rsID	AA change	Nucleotide Change	Variant annotation	Secretion (%)	ER stress	n	Mutation Assessor
CPB1, gnomAD controls *							64026	
3/148558724	rs143479075	p.Gly146Arg	c.436G>A	missense	2	Yes	3	high
3/148577631	rs1263242327	p.Ala366Pro	c.1096G>C	missense	0	Yes	2	high
3/148562271	rs759210795	p.Arg195Cys	c.583C>T	missense	65	Yes	2	medium
3/148562468	rs370907426	p.Arg231Gln	c.692G>A	missense	45	Yes	3	high
3/148563235	rs759488110	p.Cys268Tyr	c.803G>A	missense	55	Yes	2	high
3/148577788	rs201519774	p.*418Wext*37	c.1253A>G	No-stop	0	Yes	4	-
3/148577653	rs1482955493	p.Tyr373Phefs	c.1118delA	Frameshift (terminal exon)	0	Yes	2	-
						Total	18	

^ N-of-one variants found in controls predicted to be ER stress inducing by variant software are listed in Supplemental Tables.

*: where multiple numbers are listed, first numbers are from the literature, the last number is data from this study.

Table 2:

Meta-analysis of PDAC CPA1/CPB1 case-control studies

CPA1	*ER stress variants*					
	Cases		Controls		OR (95% CI)	Weight (%)
	Yes	No	Yes	No		
Study 1	3	950	0	1045	7.70 [0.40, 149.26]	10.77
Study 2	4	589	1	966	6.56 [0.73, 58.83]	16.82
Study 3	4	1381	77	63949	2.41 [0.88, 6.58]	72.41
Overall					3.65 [1.58, 8.39]	
CPB1	*ER stress variants*					
	Cases		Controls		OR (95% CI)	Weight (%)
	Yes	No	Yes	No		
Study 1	5	981	0	1045	11.72 [0.65, 212.13]	21.84
Study 2	4	589	1	966	14.77 [0.79, 274.84]	17.07
Study 3	5	1380	32	63994	7.25 [2.82, 18.62]	61.08
Overall					9.51 [3.46, 26.15]	

Fixed effects Mantel-Haemszel model